RAPPORT SCIENTIFIQUE

ECOMET : "ETUDE COMPAREE DES EFFETS DE PHYTOSANITAIRES ET DE PRODUITS DE LUTTE ANTI-VECTORIELLE SUR LES COMMUNAUTES MICROBIENNES AQUATIQUES D'ECOSYSTEMES TROPICAUX "

PROGRAMME EVALUATION ET REDUCTION DES RISQUES LIES A L'UTILISATION DES PESTICIDES – AO 2006

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1. PREAMBULE

Le projet ECOMET a été construit pour contribuer à combler un déficit de connaissances : le manque de données scientifiques dans le domaine de l'écotoxicologie aquatique tropicale, par comparaison à celles acquises en milieu tempéré. Ces connaissances préalables sont nécessaires pour doter les organismes en charge de la réglementation et de la gestion des risques liés aux usages des pesticides dans la zone tropicale, et notamment dans les territoires et départements de l'Outre-mer français. Ce travail représente une première étape en ce sens, puisqu'il démontre l'intérêt de s'approprier des modèles spécifiques aux milieux tropicaux pour l'évaluation du risque, et propose des protocoles et outils opérationnels pour une mise en place sur site des expérimentations nécessaires. Les résultats acquis ont fait et feront l'objet d'une valorisation la plus complète possible, cependant ils devront faire l'objet par la suite d'une transposition dans le domaine réglementaire et de compléments expérimentaux si la pertinence de l'approche comparative tempéré / tropical est retenue.

Le choix de la démarche mise en œuvre consistait à travailler sur des modèles biologiques de complexité croissante, allant de la population (culture, élevages) aux communautés naturelles (expérimentations en microcosmes sur le terrain), en passant par un degré intermédiaire sur les groupes fonctionnels (expérimentation sur phytoplancton et zooplancton naturels isolés du milieu). Le présent rapport respectera donc cette gradation dans la complexité des objets d'étude, présentant d'abord les résultats obtenus dans les conditions de laboratoire, et ensuite les expérimentations menées sur le terrain. Enfin, une présentation de l'état de contamination anthropiques des deux retenues collinaires de Mayotte est proposée, non seulement au niveau de la contamination par les phytosanitaires (rapport BRGM) mais également concernant les contaminants bactériens, qui marquent de façon parallèle aux pesticides la pression humaine sur les écosystèmes. Pour chaque chapitre, les références citées sont placées à la fin de la section, pour faciliter la lecture.

2. MODELES BIOLOGIQUES TROPICAUX AU LABORATOIRE

2.1. MODELES BACTERIENS

De nombreuses études ont été menées sur les relations entre bactéries du sol ou des sédiments et contaminants chimiques (par ex. Ducrocq et al. 1999, Widenfalk et al. 2008), que ce soit dans des perspectives agricoles, d'évaluation environnementale ou de remédiation des sites contaminés (biodégradation des contaminants). Par contraste, les connaissances sur les bactéries aquatiques dans un contexte comparable sont encore parcellaires. Et pourtant, les bactéries hétérotrophes présentes dans la colonne d'eau des écosystèmes aquatiques jouent un rôle majeur dans les cycles biogéochimiques des éléments dissous (Amblard et al. 1998): le bactérioplancton contribue a leur recyclage, en assimilant le carbone organique dissous, et entre en compétition avec le phytoplancton pour l'azote et le phosphate dissous qui sont des éléments majeurs pour le contrôle du fonctionnement trophique (Kirchman 2008).

Quelques études font état des effets de contaminants chimiques sur le bactérioplancton analysé au sein du plancton naturel. Elles reposent généralement sur des expérimentations en microcosmes où l'analyse porte sur la structure (Stachowski-Haberkorn et al. 2008) ou sur la diversité métabolique (Brandt et al. 2004) des populations bactériennes exposées au toxique. Certaines font état d'effets à court terme sur l'activité globale du bactérioplancton (Leboulanger et al. 2009), mais en général les répercussions sur les bactéries sont déduites des effets observés directement sur les autres compartiments biologiques (Van den Brink et al. 2009).

Concernant les tests écotoxicologiques proprement dits, et mis à part le test Microtox™ commercialisé et dont l'organisme modèle est la bactérie marine luminescente *Vibrio fischeri* (Johnson 2005), une tentative de modèle "standardisé" a été proposée par Wadhia et coll. (cf. Gabrielson et al. 2003, Wadhia & Thompson 2007, Wadhia 2008). Ce dernier repose sur une gamme de plusieurs microorganismes exposés simultanément en microplaques, et dont l'activité est mesurée à l'aide d'indicateurs colorés.

Durant cette étude, des comparaisons et mises au point techniques ont été réalisées. Des bactéries issues des retenues de Mayotte ont été employées, avec comme références externes une souche marine isolée de l'étang de Thau et des souches d'Afrique de l'Ouest. Plusieurs méthodes d'évaluation de la toxicité ont été employées, la

plus simple reposant sur une mesure de la croissance de la souche bactérienne en culture en fonction de la quantité de pesticide appliquée. Pour gagner en sensibilité, notamment dans le cas de souches bactériennes à croissance lente, deux méthodes ont également été abordée, l'une basée sur la microrespirométrie avec adaptation de la méthode Microresp™ développée pour les bactéries du sol (Campbell *et al*. 2003), l'autre sur l'utilisation de chromogènes similaire au protocole MARA (Gabrielson *et al*. 2003) également commercialisé depuis.

2.1.1. Effets des pesticides sur la croissance bactérienne

Quatre souches de la bactérie *Escherichia coli* ont été testées : une souche classique de laboratoire référencée (O126B16), une isolée de Méditerranée (Méd), une isolée de Mayotte (May), la dernière isolée de l'estuaire du Sénégal (Sén). Les souches ont été régulièrement ré-isolées par étalement sur gélose nutritive, puis exposées aux pesticides en milieu liquide (bouillon trypticase soja TSB), en microplaques. Les microplaques ont été incubées 16 h à 36°C et la croissance des bactéries estimée par mesure de l'absorbance à 590 nm (Dasilva 2007).

Les trois souches isolées du milieu (Méd, May et Sén) présentaient des taux de croissance plus élevés qu'O126B16 en milieu TSB (0,30 à 0,33 h-1 et 0,23 h-1 respectivement). Les trois pesticides testés (diuron, téméphos et fénitrothion) n'ont pas montré de toxicité marquée même aux concentrations les plus fortes (respectivement 1100 µM, 560 µM et 9000 µM). Seules les souches May et Sén présentaient une sensibilité significative au diuron à 1100 µM, la plus forte concentration testée. L'analyse statistique des résultats montrait par ailleurs une légère augmentation du taux de croissance avec la plus forte dose de fénitrothion pour les trois souches May, Sén et O126B16, et avec la plus forte dose de téméphos pour Méd.

2.1.2. Effets des pesticides sur la respiration bactérienne

La microrespirométrie consiste à mesurer l'activité respiratoire (ici le dégagement de dioxyde de carbone) de microorganismes en conditions contrôlées, et en petits volumes (quelques centaines de µL). Dans le contexte de notre travail, elle devait permettre d'évaluer des effets de type toxique en quelques heures, pourvu que la toxicité ait pour effet immédiat une réduction de l'activité métabolique, donc de la respiration, des bactéries mises en présence de molécule toxiques. Cette technique a fait l'objet de mises au point dédiée à l'étude de l'activité biologique de la microflore des sols (Rowell 1995)

aboutissant à un système standardisé et commercialisé (Microresp™, cf. Campbell et al. 2003). Son utilisation a également été couronnée de succès en écotoxicologie terrestre (Kaufmann et al. 2006) mais à notre connaissance n'a jamais été transposée aux bactéries des milieux aquatiques.

La mesure repose sur la quantification relative du dégagement de CO_2 par les suspensions de bactéries (Fig. 1A). Les bactéries en croissance exponentielle dans leur milieu de culture sont distribuées dans les 96 puits d'une plaque de 3 cm de hauteur (1 mL par puits). Une microplaque standard contenant la gélose de détection est posée de façon hermétique (joint silicone et serrage par vis) sur la première plaque (Fig. 1B). La gélose est rose foncé en l'absence de CO_2, et se décolore progressivement au fur et à mesure que le dioxyde de carbone est dégagé par l'activité respiratoire des organismes. La mesure de densité optique se fait à 590 nm, longueur d'onde par défaut conseillée par le fabricant (le pic d'absorbance étant plus proche de 570 nm); la relation entre la $[CO_2]$gaz et la DO_{590} n'est pas linéaire mais prend la forme d'une exponentielle décroissante (Fig. 1C).

Figure 1 : Schéma de principe de la microrespirométrie (A: image du site http://www.microresp.com) et mise en œuvre (B: tiré de Campbell *et al.* 2003). La réponse attendue en fonction du dégagement de CO_2 n'est pas linéaire (C: tiré de Campbell et al. 2003). Dans notre cas, la fraction de sol est remplacée par une culture de bactéries en milieu liquide.

En l'absence d'une mesure exacte de la teneur en CO_2 dans l'atmosphère des microplaques, il n'est pas possible de quantifier le CO_2 de manière absolue ; la mesure de DO est donc utilisée de manière relative, en incluant dans chaque plaque expérimentale des témoins non contaminés par un toxique, et en exprimant les effets des différents traitements en % du témoin.

Nous nous sommes heurtés à de grosses difficultés matérielles pour adapter cette méthode aux cultures bactériennes en milieu liquide. Pour nous assurer de sa sensibilité, des toxiques de référence ont été employés, le bichromate de potassium et les antibiotiques pénicilline et streptomycine. Sur l'ensemble des traitements, seul le

mélange streptomycine+pénicilline provoque une diminution significative (test ANOVA : $p = 4{,}64*10^{-4}$) de l'activité respiratoire des bactéries, aucun des pesticides testés (paraquat, fénitrothion, téméphos, DDT, diuron, chlorpyrifos) n'ayant provoqué d'effet mesurable même aux concentrations les plus élevées (de l'ordre de 500 à 1000 µg/L pour chaque composé). Cette approche ne semble donc pas adaptée à l'évaluation des effets toxiques des pesticides sur les bactéries en cultures liquides, et *a fortiori* au bactérioplancton naturel dont la biomasse est encore plus diluée.

2.1.3 Références

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Wadhia K, Thompson KC (2007) Low-cost ecotoxicity testing of environmental samples using microbiotests for potential implementation of the Water Framework Directive. *Trends Anal Chem* 26: 300-307

2.2. MODELES PHYTOPLANCTONIQUES

Le phytoplancton est un groupe d'organismes (auquel nous incluons par convention les cyanobactéries) dont le rôle en tant que producteurs primaires dans les écosystèmes aquatiques n'est plus à souligner, et qui présente de nombreux avantages pour l'expérimentateur au laboratoire, puisque de nombreuses souches sont facilement diffusées et cultivées, ce qui en fait des organismes de choix pour les tests écotoxicologiques en conditions contrôlées.

Le diuron, le paraquat, le téméphos et le fénitrothion ont été testés sur différentes souches de phytoplancton tropical et tempéré, incluant des cyanobactéries, actuellement en culture au MNHN (série PMC) et à l'INRA de Thonon les Bains (série TCC) :

- les cyanobactéries *Anabaena solitaria* PMC 200-01 et PMC 200-03, *Anabaena flos-aquae* PMC 207-03, *Anabaena sphaerica* var. *tenuis* PMC 188-03, *Cylindrospermopsis raciborskii* PMC 118-02 isolées du Sénégal, *Cylindrospermopsis raciborskii* PMC 235-07 isolée de Mayotte, et *Anabaena flos-aquae* TCC 79 du Bourget ;
- les chlorophycées *Monoraphidium* sp. PMC BF6 isolée du Burkina Faso, *Selenastrum capricornutum* TCC 81, *Scenedesmus acutus* TCC 325 isolées du Léman ;
- les diatomées *Fragilaria crotonensis* TCC131 et *Nitzschia palea* TCC139-1 du Léman ;
- la chrysophycée *Synura* sp. TCC 167 du Bourget.

Le point final d'évaluation de l'effet toxique était la biomasse après 72 h (microalgues eucaryotes) ou 6 et 8 jours (cyanobactéries, à croissance moins rapide). La biomasse était évaluée par mesure de la densité optique à 650 nm, après vérification de la linéarité de la relation entre DO_{650} et densité cellulaire mesurée par comptage des cellules au microscope. D'autre part, la fluorescence *in vivo* brute de la chlorophylle *a* était mesurée de façon quasi-simultanée avec le même appareil, en excitant les

échantillons à 450 nm et mesurant la fluorescence résultante à 680 nm : cette mesure est d'une part un proxy de la biomasse en l'absence d'effet toxique, et peut révéler un effet sur le contenu cellulaire en chl a et/ou le blocage de l'activité photosynthétique en présence de certains composés (Seguin et al. 2002, Leboulanger et al. 2009). Le blocage de la photosynthèse a pour résultante habituelle une augmentation de la fluorescence. L'analyse des données se fait d'une part par ajustement à un modèle dose-effet sigmoïde à l'aide de la macro REGTOX développée par E. Vindimian du Cemagref Montpellier (http://www.normalesup.org/~vindimian/fr_index.html) (détermination des CE50), et d'autre part par ANOVA (détermination de la LOEC et de la NOEC) à l'aide du logiciel libre PAST.

Comme la deltamethrine (cf. rapport d'avancement 2008), le fénitrothion et le téméphos ne se sont pas avérés suffisamment toxiques pour permettre le calcul d'une CE50. En effet, dans les rares cas où un effet inhibiteur de la croissance était observé, la concentration nécessaire était proche de la solubilité maximale théorique de la molécule (voire supérieure, Leboulanger et al. 2009). L'essentiel des comparaisons entre souches est donc effectué sur les deux herbicides diuron et paraquat. Toutes les CE50 calculées au cours de ce projet sont récapitulées dans le Tableau 1, montrant 1) une gamme de CE50 de 2,9 (chlorophycée tropicale *Monoraphidium* sp. à 25°C) à 104 (chrysophycée tempérée *Synura* sp. à 25°C) µg/L pour le diuron, et 2) une gamme de CE50 de 24,6 (cyanobactérie tropicale *Anabaena sphaerica* var. *tenuis* à 25°C) à 974,4 (chrysophycée tempérée *Synura* sp. à 25°C) µg/L pour le paraquat.

Tableau 1. Récapitulatif des CE50 (en µg/L) calculées pour les cultures de phytoplancton et de cyanobactéries. n.d. non déterminé. * concentrations sans intervalle de confiance (effet maximal non atteint). Les données ont été calculées en utilisant REGTOX, avec au moins 6 replicats pour chaque concentration testée.

		DIURON		PARAQUAT		FENITROTHION
Souche		20°C	25°C	20°C	25°C	25°C
TCC81	*Selenastrum capricornutum*	36,3	58,4	687,4	391,0	n.d.
TCC325	*Scenedesmus acutus*	26,7	88,2	81,3	114,3	n.d.
TCC139-1	*Nitzschia palea*	144,7	93,3	60,9	93,9	n.d.
TCC79	*Anabaena flos-aquae*	79,2	51,9	114,3	30,2	n.d.
TCC167	*Synura sp.*	23,1	104,0	192,1	974,3	n.d.
PMC200.01	*Anabaena solitaria*	42,8 (33,3-51,0)	18,2 (14,5-18,9)	202,6 (188,4-224,5)	60,4 (55,4-69,8)	244,8*
PMC200.03	*Anabaena solitaria*	**n.d.**	36,3 (28,3-47,0)	188,2 (102,4-291,0)	47,4 (42,2-53,1)	107,8*
PMC118.02	*Cylindrospermopsis raciborskii*	6,1 (3,9-8,3)	8,4 (3,5-9,6)	48,8	33,6 (29,3-36,5)	1058*
PMC235.07	*Cylindrospermopsis raciborskii*	12,2 (8,8-17,0)	9,6 (8,6-11,2)	44,2 (39,1-45,6)	41,0 (37,7-44,0)	n.d.
PMC188.03	*Anabaena sphaeria var. tenuis*	36,8 (29,5-41,5)	24,1 (21,5-29,5)	126,8 (100,0-158,7)	24,6 (22,5-26,7)	3400*
PMC207.03	*Anabaena flos-aquae*	66,6 (52,2-75,0)	46,2 (39,8-54,3)	207,1 (188,3-226,0)	51,2 (47,1-56,0)	n.d.
PMCBF06	*Monoraphidium sp.*	5,6 (4,7-8,8)	2,6 (0,9-6,1)	222,6 (215,4-239,0)	302,8 (256,0-351,8)	2850*

Le fénitrothion ne s'est pas avéré suffisamment toxique pour calculer des CE50 avec un intervalle de confiance raisonnable ; les données présentées ont été obtenues sans qu'un

effet maximal (absence de croissance des cultures) n'aie pu être observé et utilisé pour caler la gamme d'effet minimal / maximal dans le processus d'analyse des données.

L'effet de la température n'est pas univoque, puisque l'application d'une température de culture de 20 ou 25°C lors de la procédure de test modifie la CE50 mesurée sans direction constante pour le diuron alors que dans 9 cas sur 12 la CE50 du paraquat diminue quand la température augmente (Fig. 2).

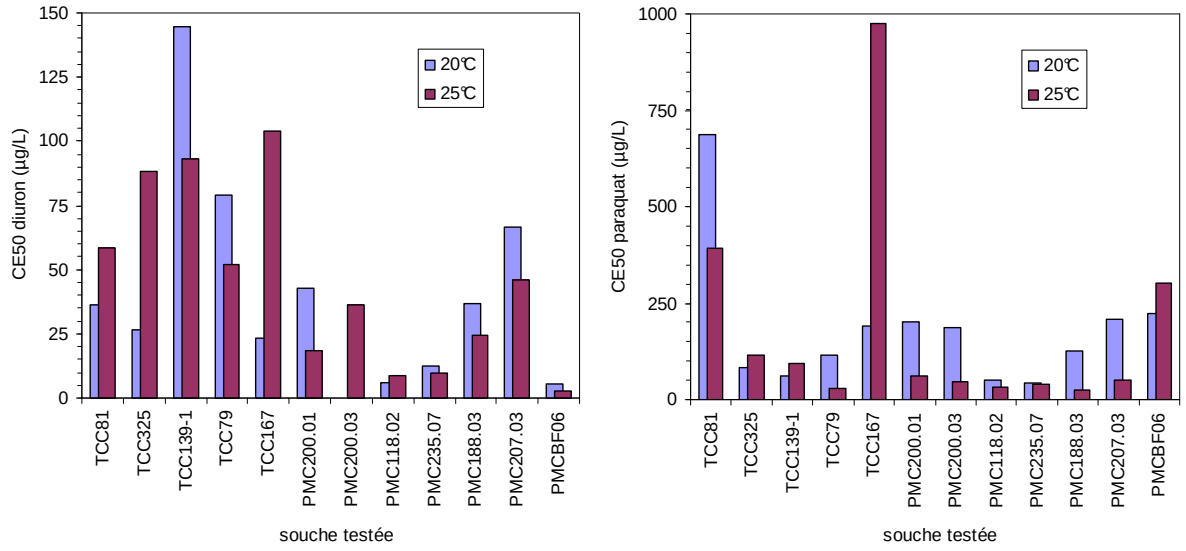

Figure 2. Effet de la température de culture sur la CE50 calculée pour le diuron (gauche) et le paraquat (droite).

En comparant ces données à celles issues de la base EPA, nous avons tracé les courbes de distribution de sensibilité des espèces pour le diuron et le paraquat (Fig. 3).

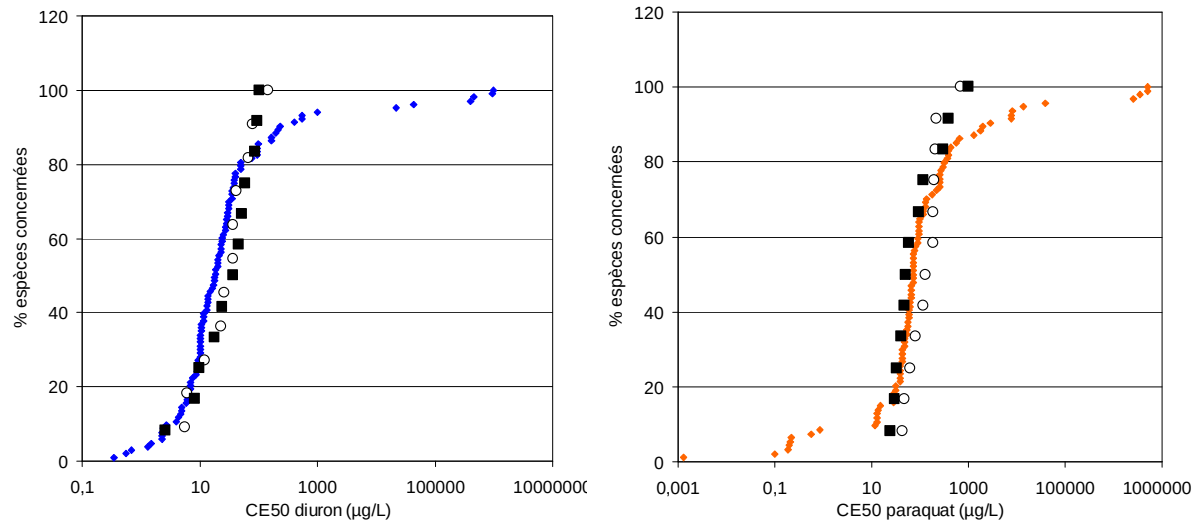

Figure 3. Courbes de distribution de sensibilité des espèces de phytoplancton pour le diuron (gauche) et le paraquat (droite). Losanges colorés : données issues de l'US-EPA ; autres symboles issus de ce travail. Carrés noirs : mesures effectuées à 25℃ ; cercles blancs : mesures effectuées à 20℃.

Les données acquises au cours du projet ECOMET concernant la sensibilité des espèces phytoplanctoniques et cyanobactériennes en culture ne diffèrent pas de la gamme de valeurs de CE50 déjà disponibles dans la littérature scientifique. A partir d'une douzaines de souches en culture, près de 80% de la gamme présente dans la base EPA est balayée, ne permettant pas de suggérer une sensibilité intrinsèquement différente entre souches issues d'un milieu tropical ou tempéré.

2.3. MODELES ZOOPLANCTONIQUES

Ce travail a été entrepris initialement sur deux espèces isolées de Mayotte, *Diaphanosoma excisum* (cladocère) et *Thermocyclops decipiens* (cyclopide). Les élevages n'ont pu être conservés au cours du déménagement du laboratoire d'un des partenaires, et un nouveau travail d'isolement a été entrepris à la suite de la seconde mission expérimentale à Mayotte en juin 2009 ; les aléas d'un tel travail nous ont conduits à travailler sur un autre cyclopide, *Mesocyclops aspericornis*. Les résultats obtenus font l'objet d'une publication en cours de finalisation, à soumettre début 2011 à la revue *Bulletin of Environmental Contamination and Toxicology* :

Sensitivity of Two *Mesocyclops* (Crustacea, Copepoda, Cyclopidae) from Tropical and Temperate Origins, to the Herbicides Diuron and Paraquat, and the Insecticides Temephos and Fenitrothion

(Section: Aquatic Toxicology)

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Abstract

Ecotoxicological risk assessment in the tropics is mostly based on knowledge gained from temperate organisms' models in laboratory testing, whereas numerous studies enlightened the necessity for regionally designed model organisms. We compared the relative toxicities of two herbicides and two insecticides, to nauplii of two freshwater zooplankton species reared in laboratory conditions, *Mesocyclops aspericornis* (Daday 1906) isolated from a tropical reservoir and *Mesocyclops* sp. from a temperate pond. Both strains were sensitive to paraquat and temephos, more slightly to fenitrothion and diuron, *Mesocyclops aspericornis* being generally more tolerant than *Mesocyclops* sp. Mortality of nauplii increased as exposure time for both species. The insecticide fenitrothion was toxic only for the temperate *Mesocyclops* sp. Further isolation of putative model organisms from tropical ecosystems is needed, that will require international cooperation, to fulfil the requirements for a tropical risk assessment scheme.

Keywords: zooplankton, herbicide, insecticide, tropical and temperate model organisms

Experimental bioassays involving cultured and captive organisms are keystone steps in environmental risk assessment of pesticides and chemicals, especially regarding aquatic ecosystems. Inadequacies of classical models, that are mostly organisms of temperate origins, are now well recognized when applied to subtropical and tropical regions (Castillo et al. 1997, Kwok et al. 2007) and a rising concern of ecotoxicology is the search for new, native species to be included in environmental risk assessment of chemicals (Moreira-Santos et al. 2005, Leboulanger et al. 2009, Menchaca et al. 2010). Due to the relative unbalance of knowledge about chemical sensitivity for tropical species and environments, relative to temperate

counterparts often used as surrogates, further studies regarding sensitivity of organisms from various origins are to perform (Daam and Van den Brink 2010).

Freshwater cyclopoids inhabits almost all kinds of freshwater habitats, and *Mesocyclops aspericornis* have been reported form many water bodies in the Tropical and Sub-Tropical zones (Zehra and Altaff 2002). The latter can reach sizes up to 1.5 mm and, as a predator in pelagic systems, have been proposed as a biological agent for control of mosquito larvae (Kosiyachinda et al. 2003, Ramanibai and Kanniga 2008). Laboratory breeding of captive populations is easy, with a generation time close to a week (Kosiyachinda et al. 2003). Provided that *M. aspericornis* is not a complex of cryptic species, it is thus a good candidate as a model zooplankton fro freshwater risk assessment; such ubiquitous properties were prone to support the implementation of tropical daphniids to replace the temperate *Daphnia magna*, as for example species belonging to *Ceriodaphnia* genus (Do Hong et al. 2004).

Lineages of cyclopid zooplankton belonging to the genus *Mesocyclops* were isolated from a temperate and a tropical water bodies, in order to tests the toxicity of four commonly used pesticides, two herbicides and two insecticides to these two closely related species. The nauplius stages were exposed to increasing concentrations of the pesticides for up to three days, to assess their acute sensitivity and avoid indirect effect by diet exposure.

Material and Methods

Collection of living tropical zooplankton was performed on March, 2009 at Combani Reservoir, Mayotte (12°46’39”S; 45°08’29”E), whereas temperate strain was collected on July, 2009, at Borély City Park, Marseilles (43°15’38”N; 5°22’57”E). Animals were collected by vertical hauls using 20 µm mesh plankton net, immediately sorted under binocular magnification, and *Mesocyclops* picked up from the whole community using plastic pipetting. Sorted zooplankton was then raised in 5 L glass cylindrical aquaria, with de-chlorinated tap water at a two weeks renewal rate. Food was supplied weekly as commercial aquarium flakes

(Tetra Min), and livestock was kept at 25°C in a climatic chamber with 12h light – 12h dark cycle at 20 µmol photons.m^{-2}.s^{-1} for one year before the experiment.

Pure pesticides (Sigma-Aldrich) were weighed and dissolved on HPLC-grade methanol, stored at -20°C before use. Semi-logarithmic dilutions were prepared on methanol, and tests solutions were prepared by diluting stock solution in filtered tap water. Effective concentrations of pesticides were determined using normalized methods; diuron was measured using LC/DAD/MS and ^{2}H-simazine as internal standard, paraquat by LC/DAD and UV detection, whereas fenitrothion and temephos were analyzed using GC/MS with 1-bromo, 2-nitrobenzene as internal standard.

At the beginning of the experiment, newborn nauplii were picked up from the aquaria, and 10-15 individuals distributed in 40 mL plastic vials containing 20 mL of 0.45 µm filtered water. Quadruplicates were performed for each test concentration, together with a control with the same amount of methanol as test vessels (0.05% v/v). Every 24h for three successive days, dead animals were counted and removed from the vessels.

Two-ways ANOVA were performed to test, for each molecule, the interaction effects between pesticides concentration and copepod strain. *Post hoc* comparisons, using Scheffé test, were also performed to test the differences between the controls and the different concentrations for each molecule and for each copepod strain. All the analyses were performed using Statistica V.6 software (StatSoft Inc., Tulsa, USA). When available, EC50 was evaluated using the REGTOX macro for Excel® developed by E. Vindimian (http://www.normalesup.org/~vindimian/fr_index.html) estimating upper and lower limits for parameters using Hill's model. This macro allows calculating EC50 and confidence intervals even in cases where death of all individuals is not achieved with the higher concentrations tested.

Available data on diuron, paraquat, fenitrothion, and temephos toxicity to zooplankton, were retrieved from U.S. EPA ECOTOX database (http://cfpub.epa.gov/ecotox/), and only results regarding ostracods and copepods presumably from freshwaters were kept for analysis. Species-sensitivity distribution curves (SSD) were drawn using EC50 available for each molecule.

Results and discussion

Laboratory rearing of both cyclopids in the laboratory was proven easy for several months. Both *Mesocyclops* strains were proven sensitive to each molecule tested, with nevertheless high differences regarding levels of sensitivity and time lag for toxicity appearance (summarized in Table 1). In all tests nauplii mortality appeared in control vessels after 72h incubations, revealing the sensitivity of the organisms to starvation during this early development stage (Fig. 1). This behaviour is consistent with the development stages of cyclopoids, that requires feeding for further molting and growth of nauplii. In such a case, exposure duration should not exceed 48h to ensure accurate routine evaluation of chemical toxicity, despite the evidence for late appearance of toxic effects for some compounds. Meanwhile, by following the mortality effect across time one could still perform reliable estimation of pesticide toxicity.

Effective concentration of pesticides varied depending on the molecule considered. Effective fenitrothion concentrations at the beginning of the exposure were reduced to 49.1±2.9% of the nominal amount (n = 3). This insecticide has already been proven extremely labile in our experimental conditions, with a further exponential decay during incubation (Leboulanger et al. in revision).

The highest paraquat dose applied (644 µg/L) resulted soon in nauplii mortality after one day exposure for the two strains. After further exposure this herbicide caused significant nauplii mortality at only 102 µg/L and 322 µg/L for temperate and tropical strains

respectively. Dose-response curves were drawn (example Fig. 2), and LOECs and EC50s were estimated from ANOVA results (Table 1) and REGTOX computing (Table 2) respectively. Paraquat EC50 for temperate *Mesocyclops* was far much lower than the one calculated for the tropical strain.

The other herbicide tested, diuron, is practically non toxic for the two zooplankton species, and dose-response curve was not relevant. Nevertheless, the highest concentration tested resulted in significant mortality of nauplii relative to control treatments, with a LOEC of 487 µg/L.

Temephos was toxic for the temperate *Mesocyclops* after one day of exposure, at 330 µg/L, whereas the tropical strain remained unaffected for two days by the insecticide. As for paraquat, dose-response curves were analyzed, and accurate estimation of EC50 was possible only for the temperate strain (45µg/L, see Table 2). For the tropical one, the calculated EC50 is comprised between 960 and 3044 µg/L, due to the low mortality achieved within the test.

Fenitrothion was almost non toxic for both zooplankton strains, since mortality was significant relative to controls only after 72h exposure, at 213 µg/L and 324 µg/L for the temperate and tropical organisms respectively. Calculated EC50s for both strains are similar, and close to the tenth of the reported water solubility for this insecticide.

The species sensitivity distribution (SSD) curve obtained for the four pesticides (Fig. 3) showed different patterns depending on the compound. For paraquat, lowest EC50s reported are very low, close to 0.001 µg/L, but most of the species are affected at concentrations exceeding 1000 µg/L. The EC50s values calculated from the present study for two *Mesocyclops* strains lie in the lower 25% of the data set and one should consider that this genus is sensitive to paraquat compare to other tested species, mainly daphniids. By contrast the two cyclopids we used are tolerant to diuron, and should be considered as comparable to other species regarding this herbicide. Both *Mesocyclops* strains are tolerant to fenitrothion

compare to other tested zooplankton, since EC50s are on the 5% of higher values reported. For temephos, the temperate strain is moderately sensitive, whereas the tropical one is among the most tolerant organisms.

In the light of these results, further studies are needed to implement the construction of a tropical-designed frame for environmental risk assessment of pesticides. This frame will benefit from field studies, which will help in selecting relevant organisms based on their known repartition throughout the entire tropical zone. Laboratory work will ensure the availability of tests organisms selected from the field by complete proficiency of life-cycle for long term, before ecotoxicological implementation of these models. Most of all, accelerated and efficient communication and international collaboration will only allow the definition of consensus model and test procedures, comparable to the one implemented in the temperate countries, to the *in fine* benefit of tropical freshwater ecosystems.

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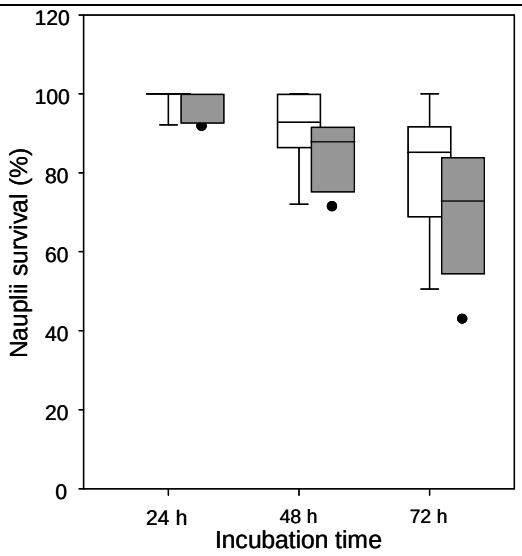

Figure 1. Whisker-box plot of cyclopoid nauplii survival in control vessels during incubations. Open bars: *Mesocyclops aspericornis* from Mayotte; shaded bars: *Mesocyclops* sp. from Borély. Median and outlier values are reported.

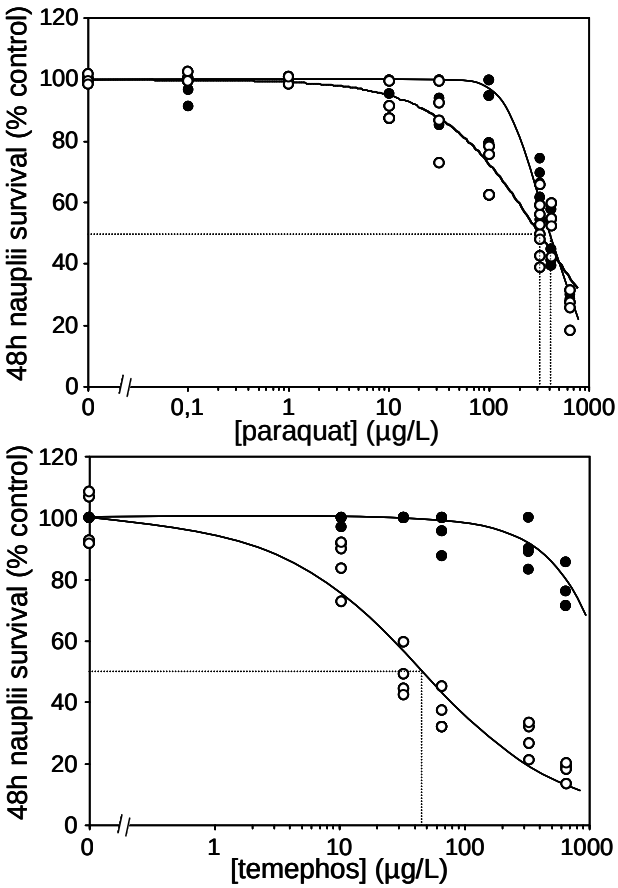

Figure 2. Dose-response curve of temperate (open symbols) and tropical (closed symbols) *Mesocyclops* nauplli to paraquat (upper panel) and temephos (lower panel) exposure. Survival rate is estimated relative to controls after 48 h.

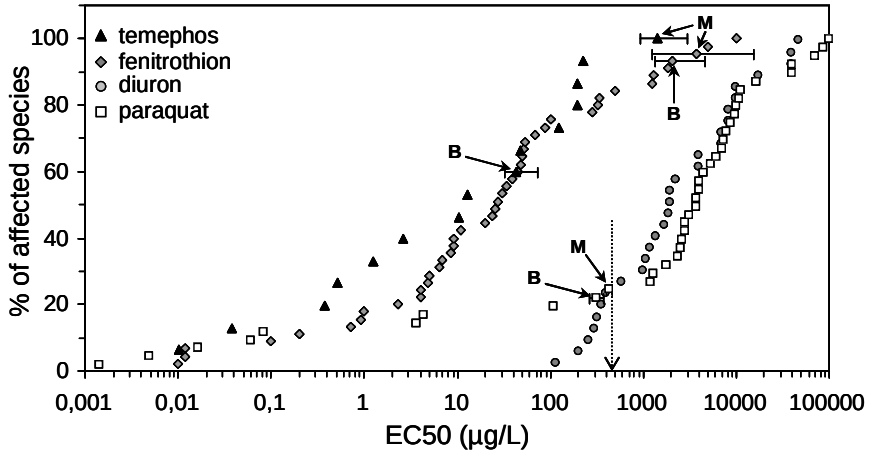

Figure 3. Species-sensitivity distribution curve for temephos, fenitrothion, paraquat, and diuron toxicity for freshwater zooplankton, including data from the present study with confidence intervals. M: Mayotte, tropical strain of *Mesocyclops aspericornis*; B: Borély, temperate strain of *Mesocyclops* sp. Dashed arrow indicates the LOEC of diuron for both strains.

3. EXPERIMENTATIONS SUR GROUPES FONCTIONNELS NATURELS

3.1. BACTERIOPLANCTON

L'objectif de ces expériences était de déterminer l'impact des deux pesticides, le paraquat et le fénitrothion, sur la dynamique des communautés bactériennes, en termes structurel et fonctionnel. L'action de ces deux molécules a été évaluée sur les taux de croissance des bactéries avec des concentrations croissances de pesticides et ceci dans deux communautés bactériennes isolées des deux retenues collinaires de Combani et Dzoumogné.

Les populations bactériennes ont été isolées par filtration sous 1 µm, puis les populations ont été diluées (25:75) avec de l'eau du milieu filtrée sous 0,22 µm (37,5 ml de 0,22 µm et 12,5 ml de 1 µm). Cette dilution permettait l'induction une croissance des populations bactériennes en raison de la pression de prédation moindre. Quatre concentrations de pesticides ont été utilisées : 0 ; 5,37 ; 53,75 et 503 ,7 µg/l de paraquat et 0 ; 0,73 ; 7,33 et 73,3 µg/l de fénitrothion. Un témoin pour chaque molécule utilisée a été incubé en présence du solvant seul (méthanol).

Tous les traitements ont été réalisés en duplicats. Ainsi 16 sacs de marque WhirlPak ont été incubés par réservoir pour les deux pesticides à l'obscurité, et à température contrôlée (4 concentrations x 2 temps x 2 molécules). L'incubation durait 24 heures. Un ensemble d'échantillonnages a été réalisé pour analyser :

- - la notion d'activité par incorporation de la thymidine tritiée
- - l'abondance et la biomasse des bactéries totales par microscopie en épifluorescence
- - l'abondance et la mise en évidence des différentes populations (et sous-populations) bactériennes et phytoplanctoniques (fluorescence et taille des cellules) par cytométrie
- - les profils structurels de communautés bactériennes par l'analyse en DGGE des fragments de 16S rDNA.
- - les profils fonctionnels par les mesures de potentialités métaboliques des communautés bactériennes par l'utilisation des tests BIOLOG.

Les analyses en composantes principales (Fig. 4) ont permis de structurer les réponses métaboliques des différentes communautés bactériennes naturelles et impactées par les deux pesticides, le paraquat (P) et le fénitrothion (F). Pour la retenue de Combani, les profils des communautés impactées sont bien différenciés de ceux du milieu naturel (F1-F11, fénitrothion ; P1-P11, paraquat). Un impact significatif des deux molécules à fortes doses est donc observé pour les communautés bactériennes de Combani.

Pour la retenue de Dzoumogné, seuls les profils suite au fénitrothion (DF1-DF11) sont bien différenciées des autres, en particulier des profils témoins DE1-DE2 et du paraquat (DP1-DP11). Un impact significatif du fénitrothion est mis en évidence sur les communautés bactériennes de Dzoumogné, alors que le paraquat ne semble pas déstructurer les communautés bactériennes.

Tous ces résultats montrent que l'utilisation des microplaques BIOLOG fournit des informations pertinentes sur les profils métaboliques des communautés. Les résultats d'incorporation de thymidine tritiée fournissent également des informations sur l'activité des communautés bactériennes en présence-absence des pesticides testés.

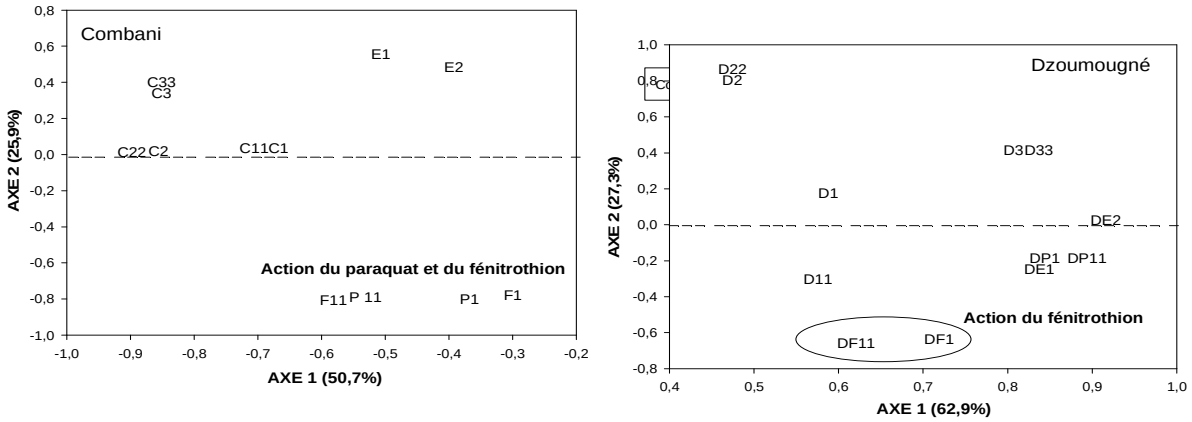

Figure 4. Analyse en composantes principales des résultats des profils métaboliques (BIOLOG ECO) en duplicats pour les deux réservoirs. C1-C33, D1 – D33 (structure naturelle des communautés), E1-E2, DE1-DE2 : profils non impactés ; F1-F11, DF1-DF11 profils impactés par le fénitrothion ; P1-P11, DP1-DP11 profils impactés par le paraquat.

3.2. PHYTOPLANCTON

Les tests ont été réalisés sur les communautés phytoplanctoniques des deux retenues collinaires de Combani et Dzoumogné. L'eau tamisée sur 200 µm a été répartie dans des tubes en verre de 50 mL de contenance, placés sur une roue en plexiglas à moitié immergée, permettant une homogénéisation des températures et des éclairements pour tous les échantillons (Leboulanger et al. 2006). Des concentrations croissantes en

atrazine (emportée par erreur à la place du diuron) paraquat et fénitrothion ont été ajoutées, chaque concentration en triplicat. La fluorescence *in vivo* a été mesurée à l'aide d'un fluorimètre TD700 après 6 h d'exposition puis chaque jour pendant quatre jours, comme descripteur de la biomasse et d'éventuels effets toxiques sur le photosystème II. Les résultats montraient une différence importante de sensibilité entre le phytoplancton de Combani (globalement peu ou pas sensible) et celui de Dzoumogné. Une CE50 pour le paraquat a pu être calculée, de 70 et 123 µg/L pour le phytoplancton des deux retenues respectivement, après 24 h d'exposition. Il faut noter que la toxicité de ce composé présentait un accroissement au cours du temps d'exposition (Fig. 5). Le fénitrothion a eu un effet plus marqué (CE50 = 650 µg/L) sur le phytoplancton de Dzoumogné uniquement, et au bout de deux jours d'exposition (Fig. 6).

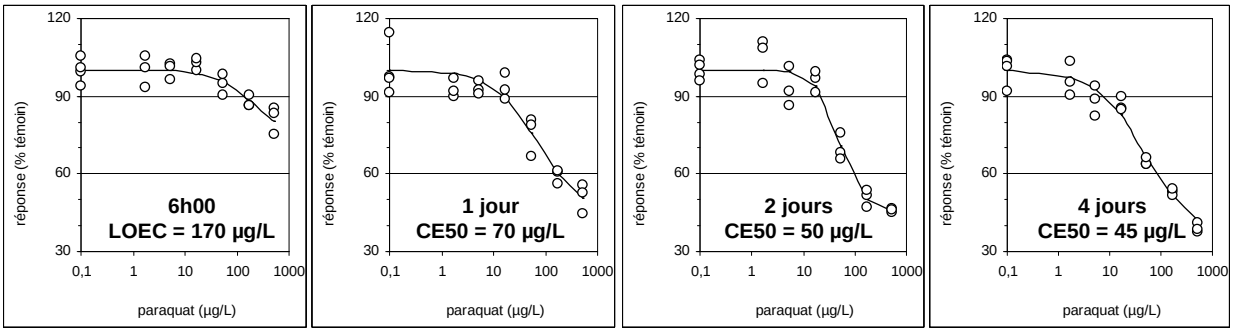

Figure 5. Evolution de la toxicité du paraquat au cours du temps, évaluée par la mesure de fluorescence *in vivo*, sur le phytoplancton de Dzoumogné (courbe = ajustement au modèle de Hill).

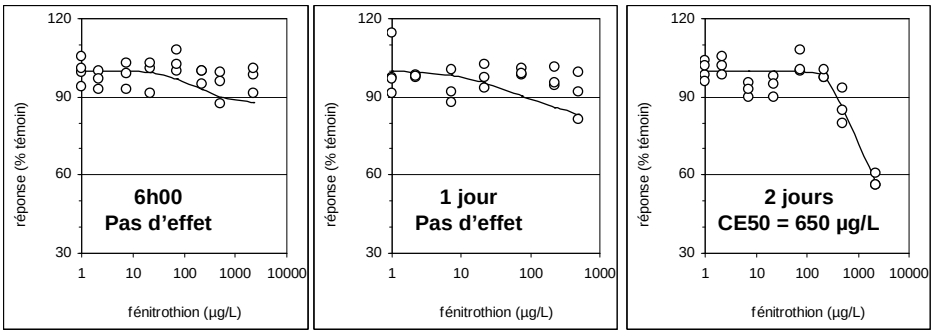

Figure 6. Evolution de la toxicité du fénitrothion au cours du temps, évaluée par la mesure de fluorescence *in vivo*, sur le phytoplancton de Dzoumogné.

La mesure de la fluorescence *in vivo* brute de la chlorophylle *a* s'est avérée un bon descripteur de l'effet toxique. Cependant, avec l'atrazine (et tout les inhibiteurs du photosystème II dont le diuron), l'effet observé est opposé, c'est-à-dire qu'une augmentation de la FIV est constatée. Il est alors nécessaire d'effectuer une

transformation mathématique des données pour obtenir des courbes plus conformes aux relations dose-effet classiques (Fig. 7).

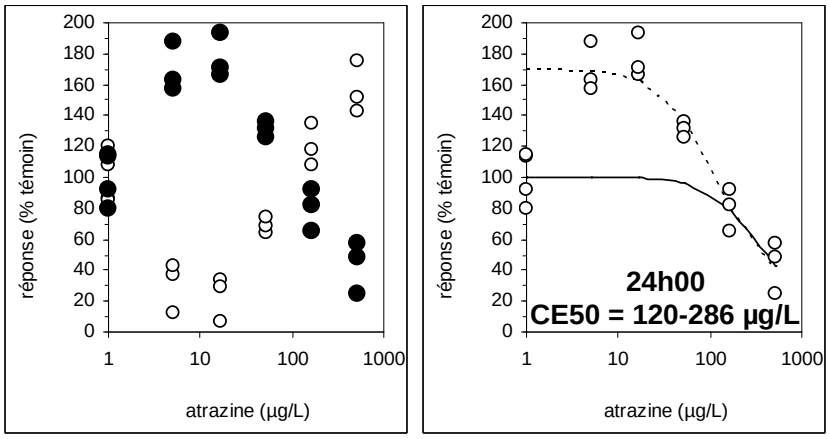

Figure 7. Effet de l'atrazine, évalué par la mesure de fluorescence in vivo, sur le phytoplancton de Combani. Les données brutes exprimées en % des témoins (cercles blancs, graphique de gauche) doivent être transformées (cercles noirs) avant tout traitement. La répartition des points ainsi obtenue montre clairement un effet hormétique (graphique de droite) qui rend le calcul de la CE50 plus difficile.

On constate cependant une augmentation de la FIV dès la première dose d'atrazine appliquée, traduisant probablement un blocage du PSII ; nous sommes encore actuellement à la recherche d'un modèle de courbe dose-effet applicable à ce type de données.

En parallèle, les effets des trois molécules sur l'activité photosynthétique potentielle ont été évalués à l'aide du Phyto-PAM. Ces tests sont réalisés à court terme (24 heures d'exposition) et n'intègrent pas les effets sur la croissance, au contraire de ceux exposés ci-dessus. La LOEC pour l'atrazine est de l'ordre de 85 µg/L, variant de 40 à 150 µg/L en fonction du facteur mesuré (rendement de fluorescence, vitesse du transfert d'électrons, intensité de lumière saturante…). Le paraquat provoque un effet de type hormétique sur le rendement de fluorescence pour les concentrations comprises entre 0,5 et 17 µg/L, et diminue cette variable au-delà de 54 µg/L. Le fénitrothion seul est sans effet, mais semble augmenter la toxicité apparente du paraquat.

3.3. PUBLICATION

Une première publication synthétise une partie des travaux sur le bactérioplancton, le phytoplancton et le zooplancton (Leboulanger et al. 2009) et est reproduite ici in extenso dans sa version publiée.

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Responses of Planktonic Microorganisms from Tropical Reservoirs to Paraquat and Deltamethrin Exposure

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Abstract This study focused on the effects of two pesticides, paraquat (herbicide) and deltamethrin (insecticide), which are two common molecules used intensively in Burkina Faso. Natural bacterial populations, phytoplankton cultures (one cyanobacterium, *Cylindrospermopsis raciborskii*, and one chlorophycea, *Monoraphidium* sp.), and two species of zooplankton (*Diaphanosoma excisum* and *Moina micrura*) were isolated from aquatic communities and were used as biological targets in the experimental protocols. Paraquat was moderately toxic to bacteria and phytoplankton, whereas deltamethrin was significantly toxic only to the zooplankton species. Paradoxically, the chlorophycea *Monoraphidium* sp. exhibited a significant increase of in vitro fluorescence after 48 h at the highest doses. Preliminary tests were also performed from natural water extract of the main drinking water supply of the country (Loumbila Reservoir) by using solid-phase extraction. Obviously, the natural extract proved to be toxic to the same biological targets. Despite the absence of any determination of pesticides in the natural extract, the question of contamination and toxicity of these waters affects concerns about the safety of water supply and the effect of human pressure on the dynamics of planktonic communities of freshwater reservoirs in arid regions of western Africa.

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Introduction

The many ecological disturbances in aquatic ecosystems linked to anthropogenic pressures (e.g., loss of biodiversity, harmful algal blooms, hypoxia, disease, and decline in fisheries) have been well documented (Conley 1999; Paerl et al. 2003). In freshwater ecosystems, deterioration of water quality might be attributable to excess nutrients, leading to excessive primary production or eutrophication (Paerl 1997). Among the aquatic biota, microorganisms are generally highly sensitive and their dynamics can be seriously affected by environmental perturbation. Bacteria, phytoplankton, and zooplankton have fast growth rates and, therefore, can provide meaningful and quantifiable indicators of ecological change on short timescales (Paerl et al. 2003). On the other hand, these organisms can respond to low levels of pollutants such as pesticides, which constitute a major anthropogenic stress on natural communities (Relyea 2005).

The indiscriminate use of pesticides for chemical control of pests might strongly disturb biological functioning in aquatic systems (Lürling and Roessink 2006; Relyea 2005). Experiments to understand the real effects of pesticides on a broad range of taxa seem to be the best way of obtaining information about patterns of diversity and productivity in aquatic systems subjected to pesticides. Whereas phytoplankton and zooplankton have often been used as test

organisms to follow the effects of pesticides in aquatic systems, responses of natural bacterial communities have rarely been studied for this purpose in aquatic systems, unlike in terrestrial systems (e.g., Nicholson and Hirsch 1998). Due to their rapid growth rate, bacteria could exhibit rapid functional recovery after toxic exposure, resulting in a different community structure (Knapp et al. 2005). Furthermore, needs for assessment of tropical species sensitivity, compared to enhanced knowledge on temperate organisms, have been claimed by several authors (e.g., Chapman et al. 2006; Wiktelius et al. 1999).

Scarcity of water resources and continuous degradation of quality are current hazards for human beings in the West Africa Sahelian zone. Among the multiple sources of degradation, pollution can be directly linked to human activities (e.g., intensified agriculture and urban expansion) in an area where the environmental policy is generally weak. In Burkina Faso, thousands of reservoirs of various sizes have been created in the last 50 years to cope with the seasonal variations in rainfall and increasing demand. Very little data are available on the use of pesticides in the Nakambé basin (formerly White Volta), the main central basin in Burkina Faso, although most of the reservoirs lie in this region with a high water demand for human purposes. The only semiofficial data available has been provided by the AFAMIN resource (http://www.afamin.net, discontinued to date) that listed the compounds authorized for use in agriculture in western Africa for the 2002–2005 period. Thus, a wide range of products appears to be available in Burkina Faso for controlling insects and pests, but, in most cases, no information about their composition is given by the local suppliers. Nevertheless, among the compounds that are listed, highly toxic chemicals such as thiram, paraquat, cypermethrin, deltamethrin, and so forth are found in various forms. Contamination of similar ecosystems, such as the one reported on the bordering Ghana (Osafo and Frempong 1998, Ntow 2001, 2005), suggests that impact on surface waters is likely to occur in Burkina Faso.

Even when the target compounds have been identified and quantified, their toxicity to aquatic life is usually unpredictable owing to the lack of data on tropical ecosystems and organisms (Wiktelius et al. 1999). This problem might be resolved by using bioassays on water concentrates to give a direct functional response to the pollutant on a given organism (or community). Man-made chemicals and their metabolites can be concentrated using solid-phase extraction (SPE) (Baun et al. 1998; Christensen et al. 2006; Wilhelm et al. 1996; Xue et al. 2006) to achieve toxic levels that are high enough to have a significant effect on the target organisms in experiments.

This study aimed at evaluating the toxic effects of two pure pesticides (an herbicide, paraquat, and an insecticide, deltamethrin) on tropical planktonic organisms. Paraquat targets the PSI system of photosynthetic organisms and leads to intracellular oxidative stress, whereas deltamethrin prevents the nerve's sodium channels from closing in arthropods, resulting in continuous nerve impulse transmission. A suspected action of paraquat on the muscular system of zooplankton have been documented (Gagneten 2002), although no mechanistic explanation for direct effects of deltamethrin on phytoplankton or bacterioplankton have been proposed to date. The selected test organisms were isolated from two reservoirs on the Nakambé River for bacteria communities and zooplankton species. Cultures of phytoplankton species isolated from other West Africa reservoirs (Lake Guiers and Dakar Bango reservoirs, North Senegal) were also used. This first toxicological report in West Africa was completed by preliminary experiments on natural water toxic potential (the Loumbila Reservoir, dedicated to drinking water supply of the Ouagadougou city) using SPE cartridges.

Materials and Methods

Sampling and Collection of Target Organisms

This study was focused on three reservoirs located in the Nakambé Basin in central Burkina Faso, western Africa. The environmental parameters of each reservoir during the survey are listed in Table 1. Standard methodologies were applied for analyzing the environmental parameters (see details in Bouvy et al. 2006).

Two reservoirs were sampled in March, 2005, for isolation of test organisms, Koubri (12°12'59" N; 1°19'4" W) and Bam (13°19′53″N; 1°30′50″ W). These reservoirs were created by damming natural permanent wetlands, resulting in permanent water bodies. No strong anthropogenic pressure, as effluent sewage or industrial discharge, was observed in situ, and pollution, if any, should be diffuse. Bacterial communities were sampled from these two reservoirs. Zooplanktonic cladoceran species (*Moina micrura* and *Diaphanosoma excisum*) were collected from the Koubri Reservoir, using a 200-μm mesh net. Phytoplankton cultures were used for testing photosynthetic organisms: a heterocystous cyanobacterium (*Cylindrospermopsis raciborskii* PMC 118–02) from Lake Guiers, Senegal (Berger et al. 2006) and a chlorophycea (*Monoraphidium* sp. PMC BF6) from Bam Reservoir, Burkina Faso. Loumbila Reservoir (12°29′38″ N; 1°24′8″ W) is located in an area of intensive farming, with a strong eutrophication process with high load of suspended material and orthophosphate concentrations (Table 1). This reservoir, which is also the main drinking water for the capital, Ouagadougou, was sampled for SPE (SPE

Table 1 Main environmental parameters and physicochemical characteristics of the three reservoirs studied in May 2005 (one sample per reservoir, taken at the central station)

	Basin population (2004)	Max. volume (M/m^3)	Max. depth (cm)	pH	Conductivity (µS/cm)	Secchi depth (cm)	$[O_2]$ (mg/L)	Temp. (°C)	Sestonic load (mg/L)	SRP (µM)	NH_4^+ (µM)	Chl-*a* (µg/L)
Loumbila[a]	27,964	43.0	360	6.82	61.9	15.5	6.5	28.4	58.4	1.42	3.5	9.59
Bam[b,c]	62,347	31.0	210	7.12	85.8	59.0	7.3	27.3	18.0	0.10	3.2	18.64
Koubri[b,d]	43,750	3.5	130	6.90	81.7	63.5	7.2	30.4	17.3	0.04	0.7	14.51

Note: SRP: soluble reactive phosphorus

[a] Reservoir sampled for extraction and concentration of putative toxic compounds

[b] Reservoir sampled for bacterial populations used in ecotoxicological tests

[c] Reservoir from which *Monoraphidium* sp. was isolated

[d] Reservoir from which zooplankton test organisms were isolated

cartridges Oasis®; HLB, Waters Corp.) to test the potential toxic effect of the water concentrate (Baun et al. 1998). For all bioassays, subsurface water samples were collected using acid-washed polyethylene bottles and then immediately transported to a temporary field laboratory near Koubri.

Pure Pesticide Solutions

Analytical-grade deltamethrin ((*S*)-α-cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylate, a synthetic pyrethroid in use for insect control) and paraquat (1,1′-dimethyl-4,4′-bipyridinium, a systemic herbicide) were purchased from Sigma-Aldrich. These compounds were chosen as model pesticides, among commercially available ones in Burkina Faso (paraquat as Gramoxone® or Calloxone®) and deltamethrin personally observed on sale at Koubri local market (as K-othrine®). Five different concentrations and one control solution were prepared by serial dilution (steps of $10^{1/2}$) of an initial methanol solution (Table 2) and then stored for few days at –20°C. Working solutions were made by diluting the methanol solutions in pure water in acid-washed 20-mL glass scintillation vials and stored in the dark below 4°C for less than 3 days. All concentrations stated further are nominal, and all tests were performed in triplicate with solvent controls.

SPE from Natural Samples

A 5-L water sample was taken from the surface in Loumbila Reservoir in an acid-cleaned polyethylene bag and immediately processed. Water was filtered through a 0.8/0.2-µm porosity filter cartridge (AcroPak 500; Pall Corp.) at a flow rate of 2 L/h and immediately treated by SPE. The filtrate was acidified to pH 4 using concentrated HCl. An Oasis HLB 500-mg cartridge (Waters Corp., Milford, MA, USA) was conditioned using 5 mL MeOH:water (60:40 v/v). The

Table 2 Concentration of toxicants (reservoir extracts and pure pesticides) assayed on the planktonic microorganisms during this study

	Used concentration during the test					
Extracts (mL/L) Loumbila	0	64.5[a]	102[b]	645[a]	1020[b]	2041[a]
		3.2[c]	12.8[c]	32[c]		6123[d]
Pure pesticides (µg/L)						
Paraquat	0	5.7[a]	18.3[b]	57.8[a]	183[b]	578[a]
		1.2[c]	2.9[c]	4.6[c]		
Deltamethrin	0	4.4[a]	14.0[b]	44.3[a]	140[b]	443[a]
		0.22[c]	0.89[c]	2.21[c]		
Methanol content (% v/v)	0.05					

[a] Tested on all organisms

[b] Tested only on phytoplankton cultures

[c] Tested only on zooplankton during chronic toxicity assessment

[d] Tested only on natural bacterioplankton of Koubri and Bam reservoirs

cartridge model was chosen as the most generalist one according to the manufacturer's manual, to ensure extraction of as many compounds as possible. No specific extraction for paraquat (e.g., with MCX cartridges; Nunez et al. 2002) and deltamethrin (e.g., with C18 cartridges; Hengel et al. 1997) was intended, due to laboratory limitations on site. The acidified filtrate (1–4 L) was passed gently through the cartridge using a peristaltic pump (flow ~1 L/h). The cartridge was then rinsed with 10 mL MeOH:water (5:95 v/v), and the compounds retained were eluted by gently passing 10 mL of analytical-grade methanol using a syringe. The methanol extract was collected and evaporated in moving air at room temperature (between 25°C and 30°C). The final volume of extract was adjusted to 2 mL to give a concentration factor close to 4000 depending on the initial volume of water (which was determined by the filtering capacity of the system within a few hours and the particulate matter in the sample). Five different

concentrations and one control solution were prepared by serial dilution (steps of $10^{1/2}$) of the initial methanol solution (Table 2). Working solutions were made by diluting the methanol solutions in pure water in acid-washed 20-mL glass scintillation vials. For all toxicity tests, the concentrations of the reservoir extracts were expressed as volume/volume (mL/L or L/L depending on the case); a value of 1 L/L means a concentration equivalent to that in the original environment (Baun et al. 1998).

Bioassays

Bacterial Bioassay

The purpose of this subsection was to test the sensitivity of complex bacterial communities. Isolation of bacterial strains, although possible, was not included because isolation media are prone to exert a strong selection among initial diversity of bacterioplankton. Subsurface water samples from Koubri and Bam reservoirs were processed within 1 h after collection. Bacterial populations were obtained by filtration through 1-μm Nuclepore polycarbonate filters with a sterilized Nalgene filtering flask (vacuum <2 cm Hg) to remove all bacterial grazers and phytoplankton. Three different concentrations and the control solution were assayed for pure pesticides. Only one concentration (6.1 L/L) was tested for the concentrated water extract of Loumbila. All bioassays were based on bacterial growth measurements using a dilution technique. The ratio of original to filtered samples was 25:75 (v/v) using 0.22-μm-filtered water from the same reservoir. Diluted bacterioplankton were transferred into acid-washed 500-mL polycarbonate bottles. All treatments were performed in duplicate and the bottles were incubated in the reservoir at a depth of 1 m to mimic the in situ environmental conditions, especially temperature.

Subsamples were removed for bacterial analysis after incubation for 12 h and 24 h. Bacterial abundance was determined by epifluorescence microscopy after staining with DAPI fluorochrome (Porter and Feig 1980). Analysis by flow cytometry of the bacterial community were performed using the SYBR-green I (Molecular Probes) bacterial cell stain and using the method described by Marie et al. (1997). Subsamples were fixed with buffered formalin and immediately stored in liquid nitrogen until analysis, as described by Bouvy et al. (2004) and were then treated as reported by Troussellier et al. (1999). Cultivable heterotrophic bacterial counts were performed by plating 100 μL of untreated water or decimal dilutions onto nutrient agar (tryptic soy agar, TSA; AES Laboratory). Bacterial activity was estimated using (methyl-^{3}H) thymidine incorporation into cold trichloracetic acid (TCA) precipitate as described by Bouvy et al. (2004).

The pesticide molecules and the natural extract were considered to be toxic after 12 and 24 h of treatment if significant differences were obtained in terms of abundance and thymidine incorporation. The nonparametric Kruskal–Wallis test was used for analyzing significant differences in biological responses.

Algal Bioassay

Phytoplankton cultures were used as model organisms for planktonic photoautotrophs. Use of natural phytoplankton communities locally sampled was precluded by the high level of suspended material in reservoirs, which would have interfered with the chosen measuring protocol. Two species were selected among the few available for their tropical origin (West Africa) and known occurrence in the local area (unpublished data), the cyanobacteria *C. raciborskii*, and the chlorophyte *Monoraphidium* sp. In vivo fluorescence (IVF) has been chosen as the end point for measuring toxicity effects on microalgae, as it encompasses modification of photosynthetic metabolism in short-term experiments (Seguin et al. 2002). The algal strains were cultured on Z8X media for the cyanobacteria and Z8 media for the chlorophyceae (Briand et al. 2004), kept in a thermostatic chamber (28°C) and illuminated using fluorescence tubes providing light intensity of about 100 μE/m/s, with a 12-h light/12-h dark cycle. Cultures were renewed each week to ensure that cells were in exponential growth when the assays were conducted.

Cultures of each strain (1 mL inoculum added to 5 mL fresh medium) were placed in 10-mL borosilicate glass tubes and received five concentrations of contaminant and a pure control. All samples were run in triplicate. Tubes were monitored nondestructively for two days using IVF of chlorophyll-*a* (Leboulanger et al. 2006). The tubes were dark-adapted for 20 min before measuring the IVF using a TD 700 fluorimeter (Turner Designs). All of the results were expressed as a percentage of control IVF after zero correction. Toxicity was considered to be positive after 6, 18, 24, and 48 h of exposure if significant changes in IVF values were obtained, either decreasing or increasing relative to the control (Seguin et al. 2002). The results were analyzed using ANOVA and post hoc Tukey's pairwise comparison (Past freeware; Hammer and Harper, 2005).

Zooplankton Bioassay

Two zooplanktonic cladoceran species (*Moina micrura* and *Diaphanosoma excisum*) were collected using a 200-μm mesh net. The animals were acclimated to laboratory conditions in 5-L buckets for several hours. The ovigenous females of the two species were identified and separated using a binocular microscope. The animals were then raised

for 2 weeks in 1-L bottles, filled with Koubri Reservoir water initially filtered through a 30-μm mesh to remove other grazers. Each day, the filtered water was changed and the zooplankton cultures were diluted in order to ensure that reproduction stayed clonal under nonlimiting conditions.

For acute toxicity experiment, newborn juveniles (less than 24 h old) were collected from the culture and put into 40-mL flasks (four replicates per treatment) filled with 10 mL of the medium (five individuals per flask). The medium was made using 30-μm-filtered Koubri Reservoir water with 125 μL of the pesticide or concentrated extract. The flasks were checked after incubating for 24 and 48 h. Each time, motionless animals were considered to be dead and removed from the flasks, and eventual recovery was not recorded.

For chronic toxicity assessment, newborn juveniles were collected and put into 40-mL flasks (four replicates per treatment) filled with 30 mL of the medium (three individuals per flask). Incubation lasted 5 days, corresponding to the time taken to reach an optimal density just before the apparition of sexual forms and population collapse (Pagano et al. 2000). The flasks were checked each day by counting the animals in a 10-mL subsample of the homogenized culture using a binocular microscope. After counting, each living individual was collected and put back in the test vessel and the volume was made up to 30 mL by adding 10 mL of freshly prepared medium with the same concentration of toxicant. At the end of the experiments, all the animals (dead or alive) were counted. The population growth rate was calculated from the exponential growth phase between the beginning and the end of incubation time. The results were analyzed using ANOVA and post hoc Tukey's pairwise comparison to test the growth rate differences between concentrations.

Results

Environmental Context of the Reservoirs Studied

Values of the main physical and chemical features of each reservoir are presented in Table 1. All the reservoirs are shallow (mean depth: 230 cm) and very turbid (Secchi depth less than 63 cm). A high density of suspended matter (sestonic weight) was observed in the reservoirs, especially in Loumbila Reservoir (58.4 mg/L). Conductivity was generally low, between 61.9 and 85.8 μS/cm, pH values were between 6.82 and 7.12, and the surface temperature varied from 27.3°C to 30.4°C. Dissolved oxygen concentrations at the surface were close to saturation. The dissolved ammonium concentrations varied between 0.7 and 3.5 μmol/L. Dissolved orthophosphate concentrations showed lower values in Koubri and the highest in Loumbila (1.42 μmol/L). Chlorophyll-*a* concentrations showed large variations between reservoirs, between 9.6 and 18.6 μg/L.

Effects of Paraquat and Deltamethrin on Planktonic Microorganisms

Effects on Natural Bacterial Populations

For each pesticide tested on bacterial communities isolated from Koubri Reservoir, three concentrations and the control solution were used. Paraquat did not seem to have any significant effect on the total abundance of the bacteria (Fig. 1A) at any concentration (Mann–Whitney test, $p = 0.343$). On the contrary, deltamethrin caused a significant decrease of total bacterial abundance after 12 and 24 h ($p = 0.029$) with 76% (12 h) and 65% (24 h) of control value for 4.4 μg/L contamination and 77% (12 h) and 70% (24 h) of the control value for 44.2 μg/L. A partial recovery was noticed, showing enhancement of bacterial growth, for 442 μg/L deltamethrin, with an increase from 44% to 57% of the control value between 12 and 24 h (Fig. 1B). However, the effects were significantly different for other bacterial descriptors, which are considered to be realistic physiological indicators of bacterial growth. In the present study, activity expressed as thymidine incorporation was inhibited (definitively with paraquat, transitory with deltamethrin), whereas cell density was not affected. Paraquat had a significant effect on cultivable bacteria, especially at the lowest concentration (5.7 μg/L), which resulted in 31% of the control value after 24 h (Fig. 1C), whereas 57.7 and 577 μg/L paraquat resulted in colony-forming units (CFU)/mL, accounting for 42% and 61% of the control value after 24 h, respectively. A negative effect on bacterial activity (^{3}H-thymidine incorporation), with no significant recovery after 24 h at any concentration (Fig. 1E) was also observed with paraquat, the activity decreasing dramatically down to 4% of the control value for 577 μg/L paraquat after 24 h exposure. Deltamethrin did not show any significant difference in cultivable bacteria compared to the control (Fig. 1D); however, the bacterial activity was reduced after 12 h at increasing concentrations, showing a dose-dependent negative effect down to 30% of the control activity for the sample exposed to 442 μg/L deltamethrin. In this latter case, after 24 h, all deltamethrin concentrations resulted in the same ^{3}H-thymidine incorporation rate, reduced by 30% with respect to the control (Fig. 1F).

Effects on Cultured Microalgae

The herbicide paraquat was shown to be toxic to the two algal strains tested, based on IVF measurements. After 6 h

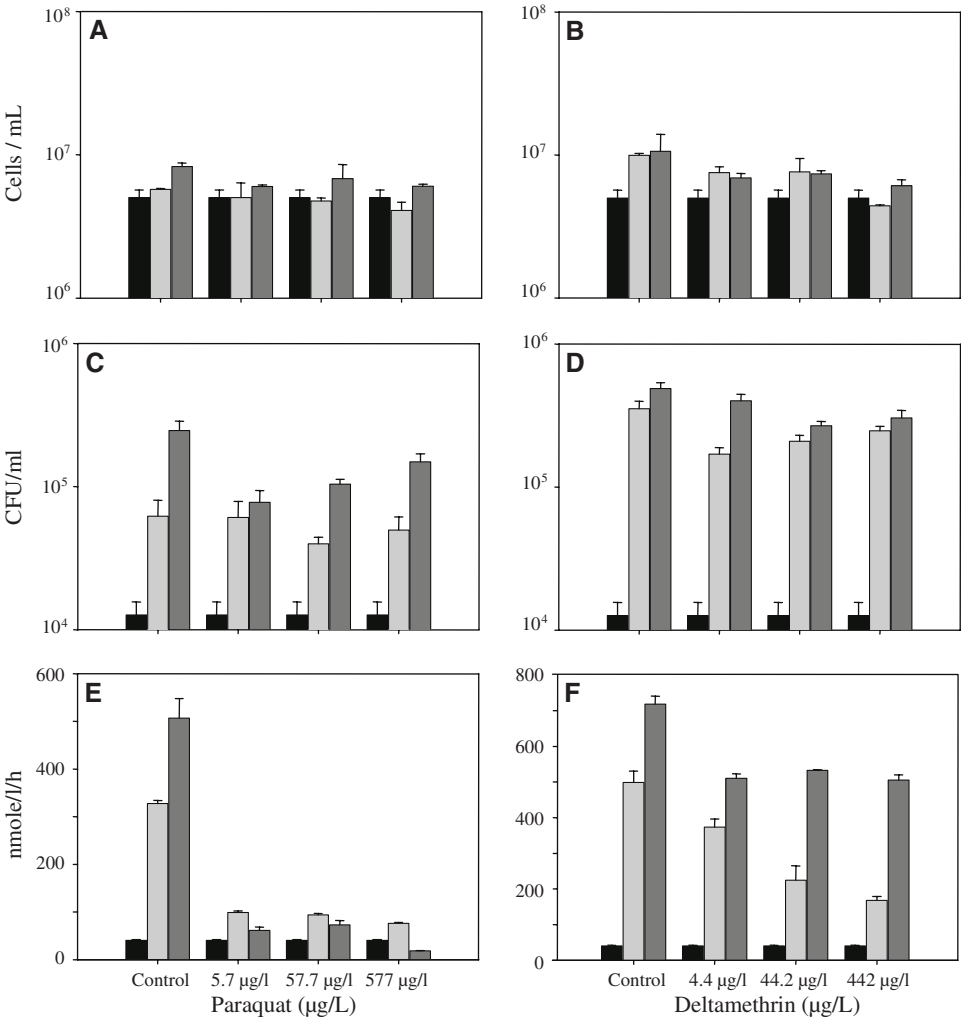


Fig. 1 Effects of paraquat and deltamethrin on bacterioplankton isolated from Koubri Reservoir. (**A**, **B**) Effects of paraquat and deltamethrin respectively, on mean and standard deviation (SD) of cell density. Black bars: initial densities (0 h); light gray: after 12 h incubation; dark gray: after 24 h of incubation. Effects of paraquat (**C**) and deltamethrin (**D**) on mean and SD of cultivable bacteria density expressed as colony forming units (CFUs). Black bars: initial CFUs (0 h); light gray: after 12 h incubation; dark gray: after 24 h of incubation. Effects of paraquat (**E**) and deltamethrin (**F**) on mean and SD of bacterial activity expressed as ^{3}H-thymidine incorporation rate (pmol/L/h). Black bars: initial activities (0 h); light gray: after 12 h incubation; dark gray: after 24 h of incubation

of exposure, IVF from *Monoraphidium* sp. and *C. raciborskii* cultures increased significantly (200% and 115% of the control, respectively) at paraquat concentrations of 57 µg/L and higher (Fig. 2A). The patterns for the two species differed after 40 h exposure, with the *Monoraphidium* sp. apparently recovering (Fig. 2B) except at the highest paraquat concentration (577 µg/L), for which IVF was 238% of the control value. However, *C. raciborskii* cultures exposed to 57 and 183 µg/L showed a significant decrease of IVF (70% and 35% of the control value, respectively) compared to the control, whereas the highest concentrations induced an increase up to 148% relative to the control (Fig. 2B), together with an apparent bleaching of the cultures.

The insecticide deltamethrin did not have any effect on any of the cultures for the first day of incubation (Fig. 2C). However, at the highest concentration (443 µg/L), there was a significant increase ($p < 0.01$) in IVF in *Monoraphidium* sp. cultures (220% of the control value) after 40 h exposure (Fig. 2D).

Effects on Zooplankton Cultures

In the acute toxicity tests (short time effect), a dose–effect relationship on zooplankton mortality was noticed for paraquat for the two species: *D. excisum* (Fig. 3A) with a LOEC (the lowest concentrations used in the test that caused significant changes compared with the control) of 57.7 µg/L and *M. micrura* (Fig. 3B) with a LOEC of 577 µg/L. On the other hand, deltamethrin was highly toxic to both these species (Fig. 3C and D) and all individuals died at the lowest concentration of toxicant (4.4 µg/L) so that no dose–response relationship could be determined for deltamethrin. Paraquat herbicide was more toxic to *D. excisum* than to *M. micrura*. After only 24 h of exposure, the third concentration (57.7 µg/L) was lethal for more than half of the individuals of *D. excisum*, whereas no significant effect was observed for *M. micrura* for all of the paraquat concentrations tested at this moment. Nevertheless, for this latter species, all individuals finally died after 48 h incubation at

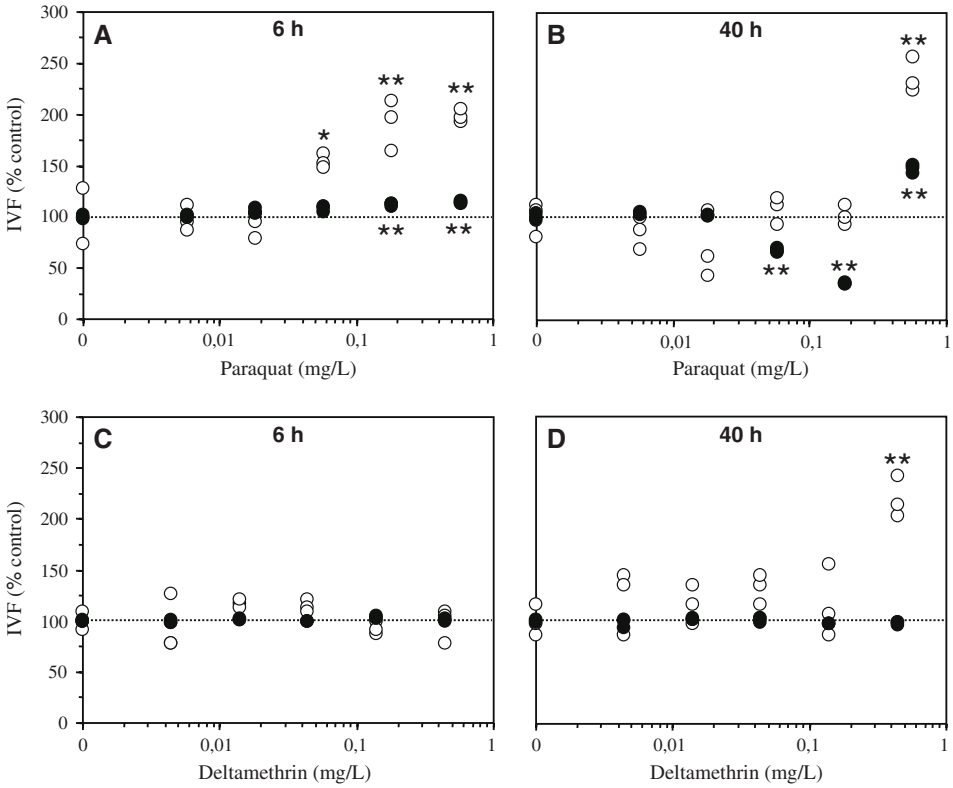


Fig. 2 Effects of pure pesticides on IVF of *Monoraphidium* sp. (open circles) and *C. raciborskii* (closed circles). Results are expressed as a function of incubation time (**A**) 6 h and (**B**) 40 h for paraquat; (**C**) 6 h and (**D**) 40 h for deltamethrin; significant changes relative to control are marked with asterisks (*5% confidence level; **1% confidence level)

578 μg/L paraquat, which was the only concentration that gave results significantly different from the control.

In the chronic toxicity tests with a long time effect, lower concentrations were used (up to 2.21 μg/L); paraquat did not have any significant effect on *M. micrura* ($F = 3.13$; Fig. 3E) although lower population growth rates were observed at the higher concentrations of paraquat. Deltamethrin had a significant effect on *M. micrura* ($F = 240.2$) with a noticeable reduction of the population growth rate at 2.21 μg/L (Fig. 3F).

Effects of Concentrated Water Extract from Loumbila on Planktonic Microorganisms

The final concentration of the water extract from Loumbila was close to 6.1 compared to the natural concentration. The test solution preparation (dilution in aqueous medium) diluted the initial concentrates and the effective maximum concentration applied to the organisms was 2.04 L/L (i.e., the used concentration of Loumbila extract in the most concentrated test conditions was twice the natural concentration).

Effects on Bacterial Populations

The bacterial populations tested were isolated from two reservoirs (Koubri and Bam) characterized by their trophic status (Table 1). Responses of growth rates obtained after 24 h exposure were similar for the two bacterial communities, with the water extracts from Loumbila Reservoir having an obvious negative effect (Fig. 4): Total (Fig. 4A and D) and cultivable (Fig. 4B and E) bacteria abundance and bacterial activity (from ^{3}H-thymidine incorporation; Fig. 4C and F) were lower in the concentrated extract than in the control solution. The abundance of cultivable bacteria for the two communities appeared to increase after 24 h exposure compared to the low response obtained after 12 h exposure. The results obtained by flow cytometry showed a separation into three groups (A1, A2, A3) for bacterial populations isolated from Koubri Reservoir that differed in the increase in DNA content level (dye fluorescence intensity; FL1) and apparent size (scatter values; SSC) (Fig. 5A). With the water extracts from Loumbila Reservoir, the cytograms, representing the FL1 and SSC distributions for the bacterial populations, were very different after 12 and 24 h exposure. After 12 h treatment, the three initial groups disappeared completely, leaving a single population B1 (Fig. 5B). This comprised small cells with low nucleic acid content. After 24 h exposure, cytometric analysis revealed a separation into two bacterial groups, C1 and C2, with different FL1 and SCC results compared with the control (Fig. 5C).

Effects on Cultured Microalgae

The IVF measurements on *C. raciborskii* cultures were made after 6 and 24 h incubation with concentrated water

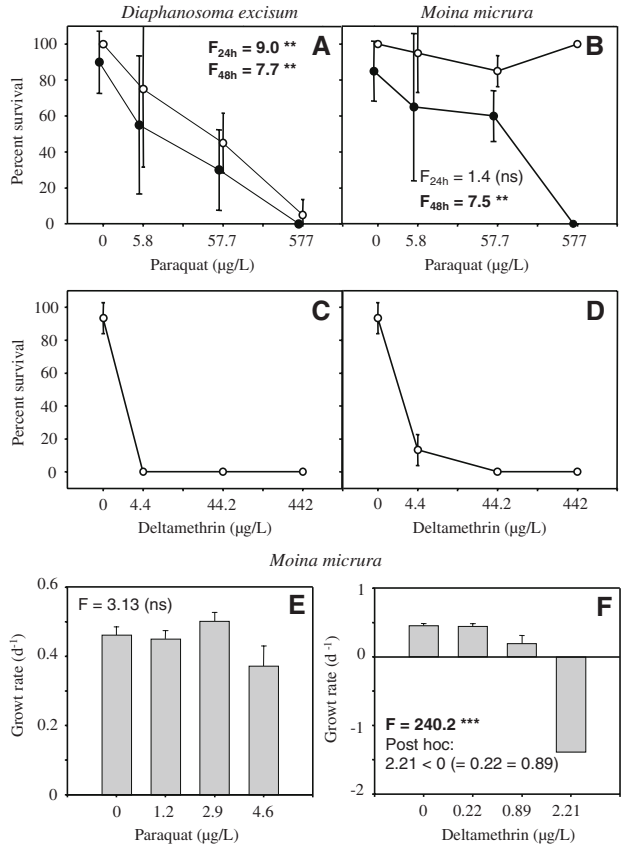


Fig. 3 Acute toxic effects of paraquat (**A**, **B**) and deltamethrin (**C**, **D**) on *D excisum* (**A**, **C**) and *M. micrura* (**B**, **D**) survival after 24 (open circles) and 48 h incubation (closed circles). Significant changes relative to control are marked with asterisks (*5% confidence level; ** % confidence level). Chronic toxic effects of paraquat (**E**) and deltamethrin (**F**) on *M. micrura* growth rate after 5 days of incubation. Significant changes relative to control are marked with asterisks (*5% confidence level; **1% confidence level)

extracts (Fig. 6A and B, respectively). Changes in IVF values of the *C. raciborskii* cultures were observed both after 6 and 24 h incubation with serial dilutions of the extract. The IVF significantly decreased in the lower concentrations and then increased significantly compared to the control for higher concentrations (2041 mL/L). The IVF significantly decreased at lower concentrations ($p < 0.01$ for 645 mL/L) but reached levels up to 150% of the control for the highest concentration (2040 mL/L). The LOEC was 645 mL/L for the concentrated extracts (two-thirds of the normal concentration).

Effect on Zooplankton Cultures

In the acute toxicity test, the concentrated water extract had a dramatic effect on the two zooplankton species, with a 100% mortality rate after 24 h exposure at all concentrations (Fig. 7A and B). In the chronic toxicity tests, the effect on population growth rates was significant for the extract: at concentrations of 12.8 and 32 mL/L; the extracts resulted in a negative population growth rate indicating a long-term mortality (Fig. 7C).

Discussion

The results of this study indicate that pesticides can have short-time toxic effects on the planktonic microorganisms of tropical freshwaters. The lag between inoculation in test vessels and appearance of significant effects ranged between 6 and 48 h, depending on the molecule and the test organisms. This was supposed to avoid major bias due to the relative fugacity of the two pesticides, which are known to rapidly adsorb on suspended particulate material and glassware. As previously reported (Relyea 2005; Wong 2000), the effects of the herbicide and the insecticide on the microorganisms tested were very different, as illustrated by the LOECs (Table 3). In our study, the herbicide paraquat was toxic to both phytoplankton species tested, whereas the insecticide deltamethrin was highly toxic to zooplankton, in accordance with their known biochemical modes of action (Lahr et al. 2000). Deltamethrin inhibits selectively the acetylcholine esterase of insects, whereas paraquat generates oxygen radicals by diverting electron flow in photosystem I of plants. Paraquat toxicity has been reported for the cyanobacterium *M. aeruginosa* isolated from Florida, and the toxicity resulted in an increase in synthesis and release of microcystin in surrounding waters (Ross et al. 2006). The LOEC reported for paraquat in the present study (57 µg/L) lies in the range observed with standard phytoplankton organisms; for example, Sáenz et al. (1997) reported an LOEC of 0.05–0.8 mg/L for three strains of *Scenedesmus* and 0.05 mg/L and 0.2 mg/L for *C. vulgaris* and *S. capricornutum*, respectively. Differences between chlorophytes and cyanobacteria should, however, be linked to the respective density of PSII and PSI in photosynthetic apparatus, which constitutionally differs in each taxa. On the other hand, unexpected effects were noticed for high-level contamination by paraquat on zooplankton, as previously reported by Gagneten (2002) or Alberdi et al. (1996) with an EC_{50} of 2.57 and 4.55 mg/L for two *Daphnia* species. Our results exhibited significant zooplankton mortality at 57.7 µg/L and death of all organisms at 577 µg/L after 48 h exposure; this contrasts with the results of Perschbacher and Ludwig (2004), who aerially applied paraquat in microcosms at a concentration of 120 µg/L, resulting in no effect on zooplankton. Paraquat, as a quaternary amine, is rarely considered to be toxic for animals, as this is not linked to its primary herbicide function.

The toxicity of deltamethrin to the phytoplankton *Monoraphidium* sp. was unexpected, and the concentration

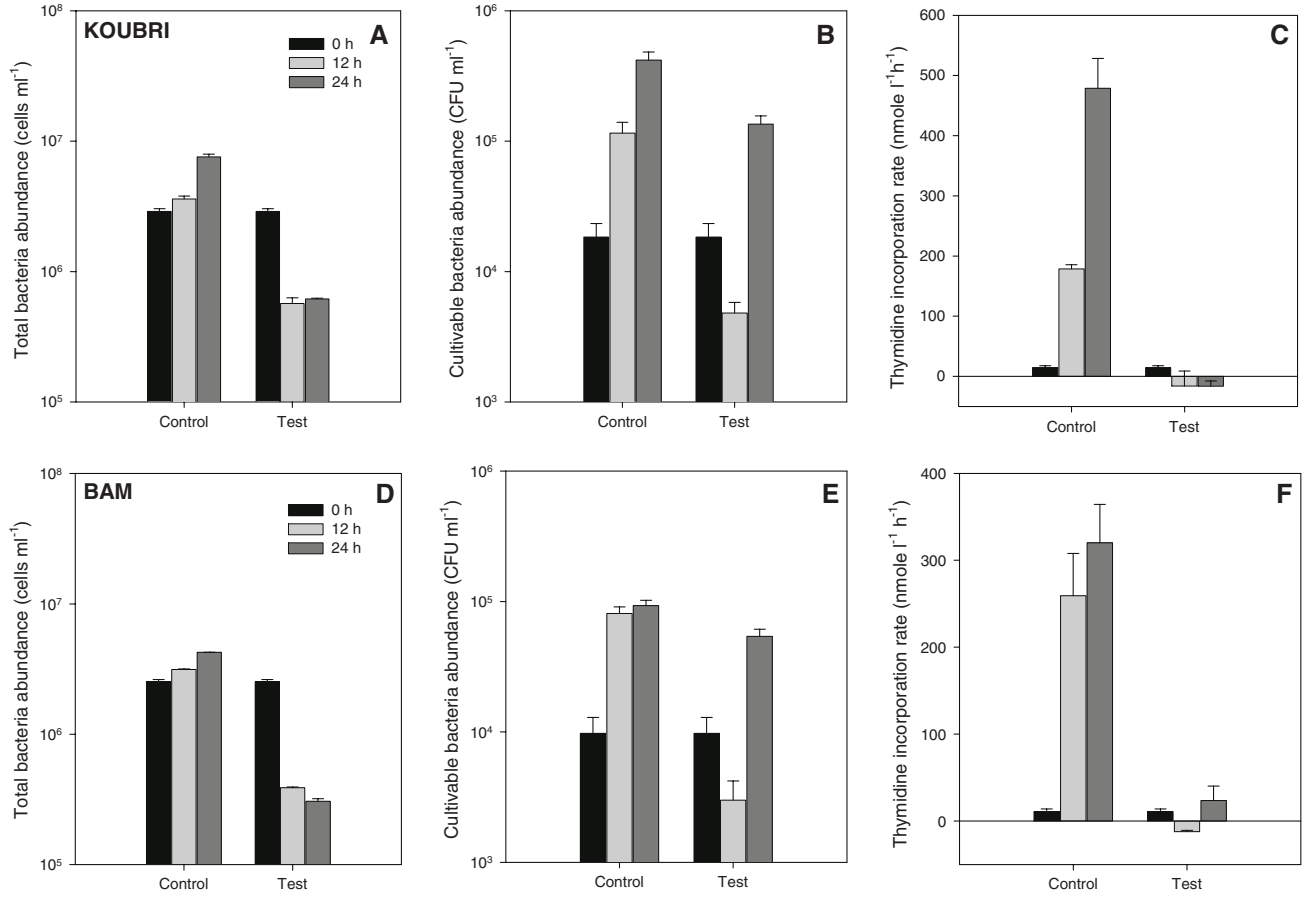
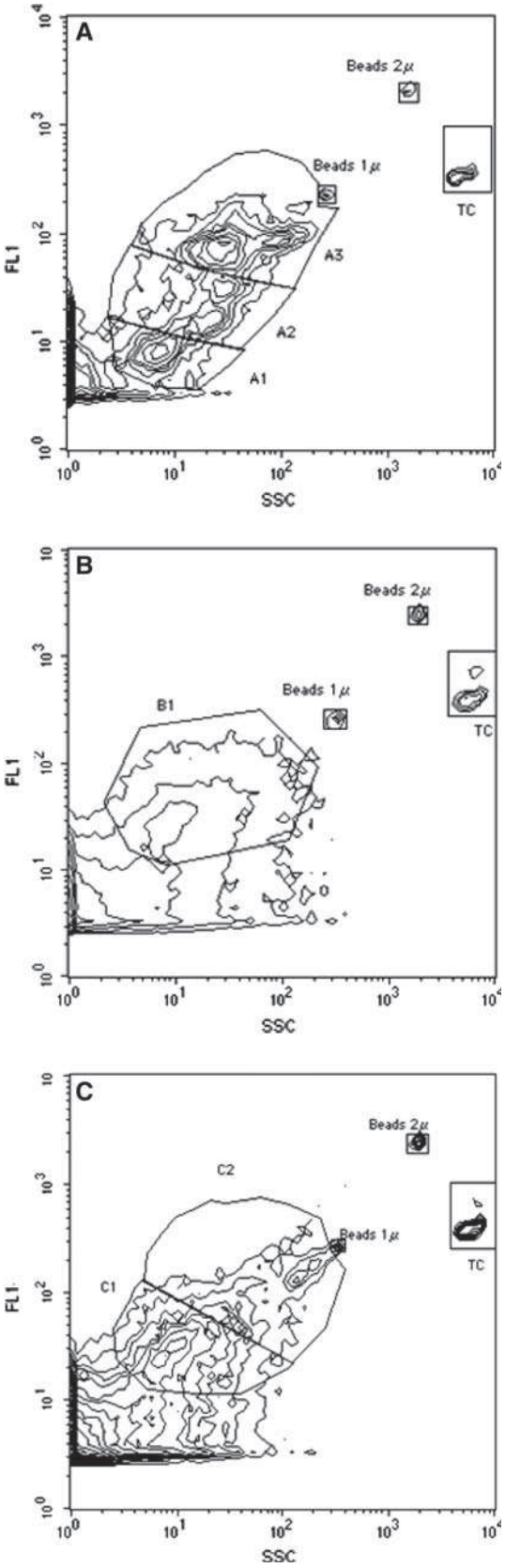


Fig. 4 Effects of extracts from Loumbila ("Test") on mean and SD of bacterial abundance **(A, D)**, cultivability **(B, E)**, and activity **(C, F)** of natural bacterioplankton isolated from reservoirs of Koubri (upper panel) and Bam (lower panel) compared to control without extract additions. Black bars: initial values (0 h); light gray: after 12 h incubation; dark gray: after 24 h of incubation

required for adverse effects (LOEC of 442.5 μg/L) was extremely high compared with the LOEC for zooplankton (the lethal dose was 4.42 μg/L; chronic LOEC was 2.21 μg/L; see Table 3). The precise mechanism of deltamethrin toxicity to chlorophycea is unknown to date, the rise in IVF clearly not being linked to cell disruption and chlorophyll-*a* release in the medium but probably resulting from a modification of the PSII function (Seguin et al. 2002). Bacterial communities, used in this study as non-target microorganisms, which hitherto have rarely been included in aquatic ecotoxicological studies, were shown to be sensitive to the two pesticides tested, especially paraquat. Depending on the end point chosen for assessing toxicity, the effects of paraquat were varied with a bacterial cell density remaining unaffected, whereas cultivable bacteria and bacterial activity (estimated from ^{3}H-thymidine incorporation rates) decreased significantly at a 4.4-μg/L concentration of paraquat in the medium. Our results suggest that the activity and viability end points provide a good proxy for metabolically active bacteria in the presence of pesticides. The influence of paraquat on nitrifying bacteria in aqueous systems has been studied by Gadkari (1998), which shows a complete inhibition of ammonium and nitrite oxidation pathways at low concentrations of paraquat (1 μg/L). Among the rare studies treating the interaction bacteria–pesticide, Knapp et al. (2005) showed a "top-down" effect of deltamethrin on natural microbial communities in outdoor mesoscoms, rather than the direct negative effect on bacteria as suggested in this study.

Concentrated extracts of water from Loumbila Reservoir showed clear toxic effects for all types of microorganism assayed. The two natural bacterial populations (from the Koubri and Bam reservoirs) responded in a similar way to the extract, with a marked negative effect on the bacterial abundance after 12 and 24 exposure. However, despite a decrease near zero for ^{3}H-thymidine incorporation rates (activity), culturable bacteria showed a clear recovery after 24 h, suggesting that a fraction of the bacterial populations was able to cope with the Loumbila extracts. This result was confirmed by flow cytometry analysis with the rise of a new bacterial population after 24 h, with higher nucleic acid content and larger apparent size (FL1 and SSC curves



◀ **Fig. 5** Example of cytogram obtained on bacterial communities exposed to Loumbila extract at the beginning of exposure (**A**) and after 12 h (**B**) and 24 h incubation (**C**). Fluorescence intensity of stained DNA (FL1 channel) and apparent size (SSC channel) standards are represented by calibrated beads of 1 and 2 μm diameter, whereas a quantitative standard (TrueCount; TC) allowed precise determination of sample volume, thus bacterial density in the analyzed sample

in Fig. 5). The three different bacterial populations, dominant at the beginning of the experiment, were strongly affected by the toxicants during the first 12 h of exposure, with the disappearance of three groups compared to the controls owing to cell degradation. However, after 24 h exposure, a distinct bacterial population appeared on the cytogram (Fig. 5C), suggesting an adaptation and/or a selection of bacterial cells. A minor preexisting bacterial consortium, tolerant to the toxicants, was selected by the toxicants in the Loumbila extract. This corroborates the hypothesis postulated by Lopez et al. (2005), in a study on biodegradation, that it is possible for a selection of a bacterial consortium to degrade and transform pesticides. Further experiments will be needed to determine more clearly the type of bacterial populations involved in this phenomenon using genetic and molecular approaches, because the effects of toxicants on natural competition between microorganisms could have relevant effects on ecosystemic functions.

The response of the cyanobacterium *C. raciborskii* to the extract could support several hypotheses with respect to the time evolved before measuring the toxicity end point. There was clearly a time-dependant reaction of *C. raciborskii*, with the dose–response curves similar for 6 and 24 h of incubation. Significant changes in the IVF relative to the controls were observed after 6 h exposure, but changes in dose–response patterns were noticed after 24 h. With *Monoraphidium* sp. cultures (results not shown), the Loumbila extract showed a different pattern of responses than those reported for *C. raciborskii*. The two concentrations 645 and 1020 mL/L caused a significant decrease in IVF and the highest one (2041 mL/L) resulted in a significant increase of IVF relative to the control. These response types were quite similar for measurements taken after 6 and 24 h and a noticeable bleaching of the cultures was observed with the highest concentration after 24 h. Our results suggest that one or several compounds in the Loumbila extracts are rapidly and lethally toxic to the cyanobacterium *C. raciborskii*, resulting in cell death and photosynthetic pigment release in the surroundings, as visually observed. These effects were similar in appearance to the effects of paraquat on *C. raciborskii* after 18 h and 40 h exposure.

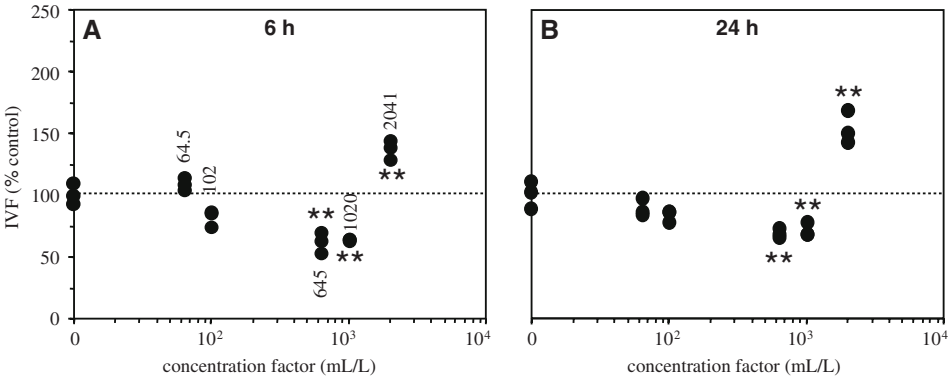


Fig. 6 Effects of Loumbila extracts on IVF of *C. raciborskii* (closed circles). Results are expressed as a function of incubation time (6 and 24 h) and significant changes relative to control are marked with asterisks (*5% confidence level; **1% confidence level)

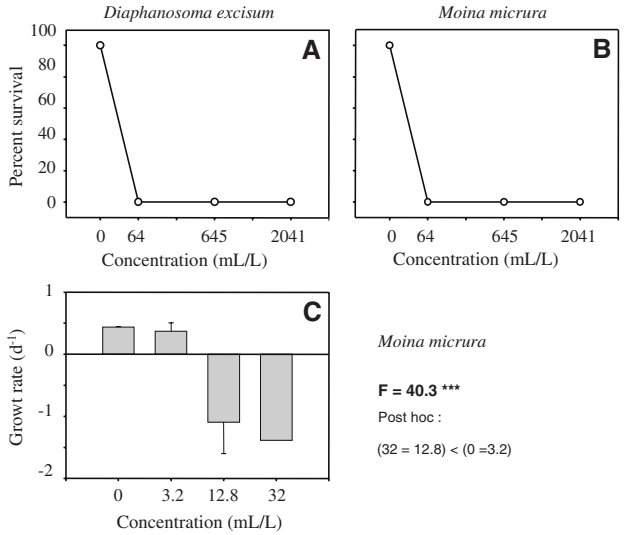


Fig. 7 Acute toxic effects of Loumbila extracts on *D. excisum* (**A**) and *M. micrura* (**B**) survival after 24 h incubation (open circles). Significant changes relative to control are marked with asterisks (*5% confidence level; **1% confidence level). (**C**) Chronic toxic effects of Loumbila extracts on *M. micrura* growth rate after 5 days of incubation. Significant changes relative to control are marked with asterisks (*5% confidence level; **1% confidence level).

Zooplankton populations responded dramatically to the Loumbila extracts. The lowest concentration assayed during the acute toxicity tests (64 mL/L) was fully lethal for the two species *D. excisum* and *M. micrura*; however, in the chronic toxicity tests, only the 12.8-mL/L dose was toxic to *M. micrura*. The effects of Loumbila extracts were similar to those observed with pure deltamethrin, with the death of all the individuals exposed to the weakest effective concentration during acute tests, whereas the rediluted samples resulted in a decrease in the *M. micrura* population growth rate.

The effects of the Loumbila extract on phytoplankton and zooplankton are similar to the combined effects of paraquat (on phytoplankton) and deltamethrin (on zooplankton). Despite the lack of chemical identification and quantification of compounds present in the extract using gas chromatography–mass spectrometry (MS) and liquid chromatography–MS (C. Mouvet, BRGM Orléans, personal communication), it is obvious that it contains a cocktail of pollutants and/or toxic natural compounds, affecting drastically the organisms living in the aquatic ecosystem. These results raise concerns about the actual chemical pressure exerted on these water bodies of Burkina Faso, keeping in mind that contamination, if any, could be detrimental not only to sustaining aquatic life and economical values of these ecosystems but also to human health.

Conclusion

This study was based on tropical planktonic microorganisms to assess successfully the ecotoxicological effects of known pesticides and an unknown water extract. By using "local" organisms rather than "standardized" laboratory cultured organisms (such as *Pseudokirchneriella subcapitata*, *Daphnia pulex*, etc.) this study highlights the

Table 3 Reported LOECs (in μg/L) for short-term effects of paraquat and deltamethrin on the planktonic microorganisms during this study

Endpoint	Bacterioplankton (12 h)			*C. raciborskii*		*Monoraphidium* sp.		*M. micrura*		*D. excisum* survival
	Bacterial density	CFU	Thymidine incorporation	IVF 6 h	IVF 40 h	IVF 6 h	IVF 40 h	Survival	Growth	
Paraquat	577	57.7	5.7	183	57.8	57.8	578	577	n.e.o.[b]	57.7
Deltamethrin	4.4	4.4	4.4	n.e.o.[a]	n.e.o.	n.e.o.	443	4.4	2.21	4.4

[a] n.e.o.: no effect observed

[b] Maximal concentration assayed was 4.6 μg/L

importance of experimental and local investigations to examine pesticide or unknown toxicant effects. Our results showed that both paraquat and deltamethrin were toxic to microalgae and zooplankton, respectively, in accordance with the mode of action. Moreover, paraquat was significantly toxic to nontarget microorganisms such as natural bacterioplankton. Toxicity testing of the Loumbila water extract resulted in apparent combination of effects, such as ones observed with paraquat (herbicide) and deltamethrin (insecticide), on phytoplankton and zooplankton from surrounding areas. These effects were not supported by analytical detection and quantification of any pesticide among a list of 80 searched for (not shown), and the precise nature of toxic compounds in the extracts is still unknown. Nevertheless, the approach reported here can be considered as a step toward making studies ecologically relevant (Lahr et al. 2000; Lopes et al. 2007), as part of the concern for regional testing programs in the tropics (Wiktelius et al. 1999). Effort will be pursued to enhance knowledge about sensitivity of tropical aquatic organisms, relative to their temperate counterparts. This will improve environmental assessments in the tropics, as the organisms used to assess toxic effects would be those that are exposed to contamination in the field or their close ecological and phylogenetic relatives. Furthermore, this could lead to more specific studies for individual freshwater bodies in West African countries, which are poorly known regarding the concern of environmental assessment.

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3.4. ZOOPLANCTON

3.4.1. Méthodologie

Les effets de trois molécules, le diuron, le paraquat et le fénitrothion, ont été évalués sur le zooplancton de deux retenues collinaires de Mayotte (Combani et Dzoumogné). Trois espèces et stades de zooplancton ont été utilisés : nauplii et adultes de cyclopides (*Thermocyclops* sp.) et juvéniles de *Diaphanosoma excisum*.

Les animaux été pêchés la veille des expériences avec un filet à plancton de 0.3m d'ouverture et 60 µm de vide de maille. Des lots de femelles adultes ovigères étaient constitués de retour au laboratoire et mis en incubation pendant une nuit et les nauplii fraîchement éclos issus de ces femelles ont été utilisées pour les expérimentations. Par ailleurs, des femelles adultes « sauvages » de *Thermocyclops* sp. et de *Diaphanosoma excisum* ont servi de support aux expériences sur animaux adultes.

Tableau 1 : Détails des expériences de toxicité réalisées avec les trois molécules.

Date (t0)	molécule	Concentrations en µg/L	Zooplancton	Site	eau
25/03/2007	**Paraquat**	0, 5.4, 53.8 et 503.7	Thermocyclops nauplii	Combani	<60 µm
28/03/2007		0, 53.8, 503.65, 1075	Thermocyclops nauplii	Dzoumogné	<60 µm
25/03/2007		0, 5.4, 53.8 et 503.7	Thermocyclops adultes	Combani	<60 µm
26/03/2007		0, 1075	Thermocyclops adultes	Combani	<60 µm
29/03/2007		0, 53.8, 503.65, 1075	Thermocyclops adultes	Dzoumogné	<60 µm
21/06/2008		0, 0.52 , 5.2 , 21.3, 52, 210	Diaphanosoma	Combani	<60 µm
19/06/2008	**Diuron**	0, 2.2, 11, 22, 110, 220	Thermocyclops nauplii	Combani	<60 µm
19/06/2008		0, 2.2, 11, 22, 110, 220	Thermocyclops adultes	Combani	<60 µm
25/03/2007	**Fenitrothion**	0, 0.7, 7.3 et 73.3	Thermocyclops nauplii	Combani	<60 µm
28/03/2007		0, 73.3, 503.7, 7500	Thermocyclops nauplii	Dzoumogné	<60 µm
28/03/2009		0, 9.3, 46.5, 93.3, 187, 466	Thermocyclops nauplii	Combani	<60 µm
28/03/2009		0, 9.3, 46.5, 93.3, 187, 467	Thermocyclops nauplii	Combani	<0,2 µm
25/03/2007		0, 0.7, 7.3 et 73.3	Thermocyclops adultes	Combani	<60 µm
26/03/2007		0, 220, 503.7, 2200, 7500	Thermocyclops adultes	Combani	<60 µm
29/03/2007		0, 73.3, 503.7, 7500	Thermocyclops adultes	Dzoumogné	<60 µm
28/03/2009		0, 9.3, 46.5, 93.3, 187, 468	Thermocyclops adultes	Combani	<60 µm
28/03/2009		0, 9.3, 46.5, 93.3, 187, 469	Thermocyclops adultes	Combani	<0,2 µm
30/03/2009		0, 933, 2800, 3733	Thermocyclops adultes	Combani	<60 µm
30/03/2009		0, 933, 2800, 3733	Thermocyclops adultes	Combani	<0,2 µm
20/06/2008		0, 4.2, 21, 42, 84, 210	Diaphanosoma	Combani	<60 µm

En début d'expérience des lots homogènes d'animaux (30-40 nauplii ; 15-20 adultes) étaient constitués et introduits dans des flacons contenant 20 ml d'eau du milieu tamisée à 60 µm. Quelques expériences ont également été réalisées avec de l'eau filtrée à 0.2 µm pour simuler des conditions de jeune. Les molécules étaient ensuite introduites à différentes concentration (t0). Pour chaque traitement (molécule - concentration) 4 réplications étaient réalisées. Une observation de chaque flacon était

effectuée chaque jour pendant trois jours successifs (t24, t48, t72). Les morts étaient comptés et enlevés du flacon. En fin d'expérience les animaux restant étaient comptés.

Plusieurs expériences ont ainsi été réalisées avec les trois molécules au cours des trois missions réalisées en 2007, 2008 et 2009 (Tableau 1)

3.4.1. Résultats

Effets chez le cyclopide *Thermocyclops* sp.

Diuron

Aucun effet n'a été noté après 72 heures d'incubation au cours des deux expériences réalisées sur les nauplii et adultes de *Thermocyclops*.

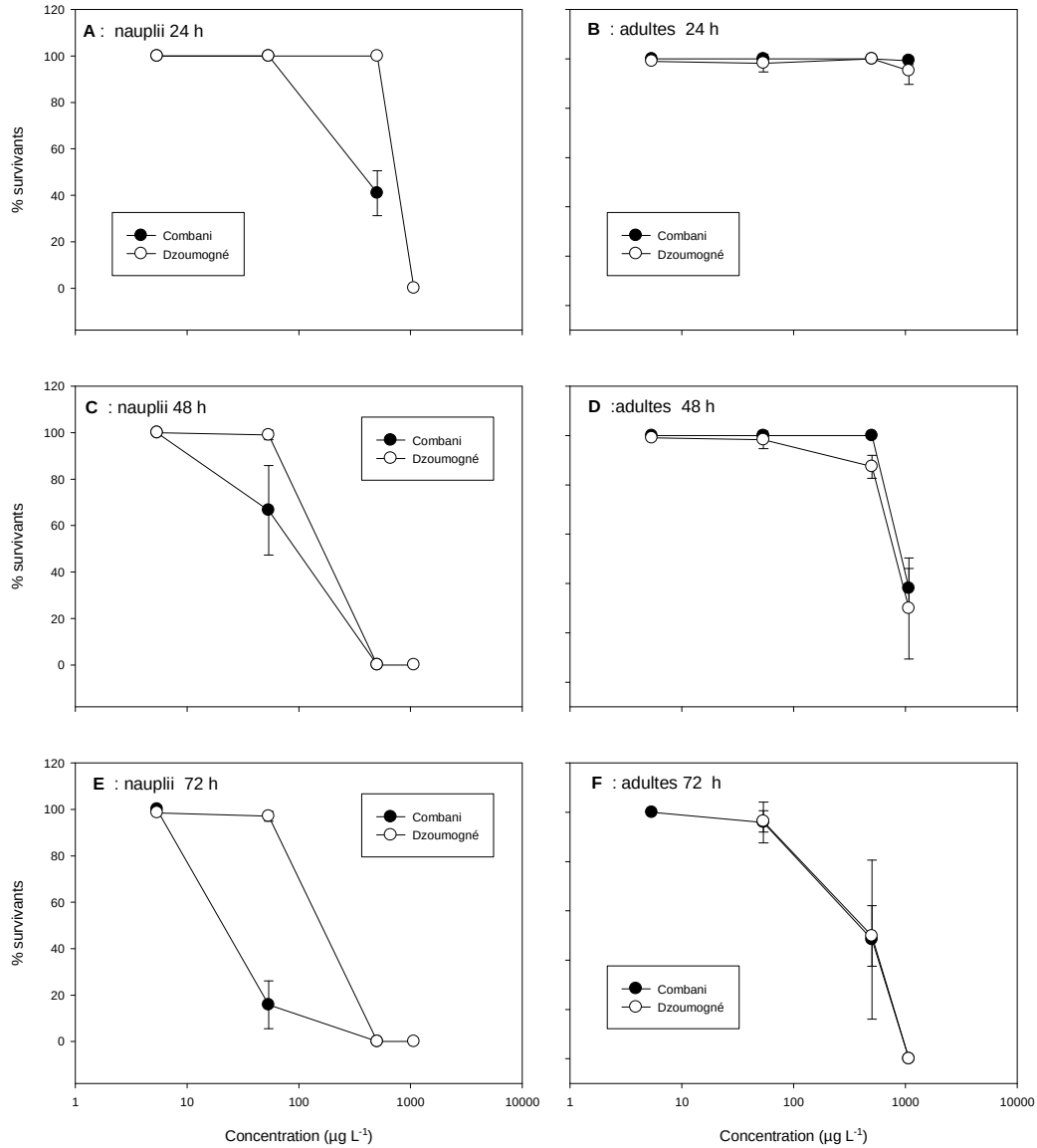

Figure 1. Expérience de toxicité pour tester les effets du Paraquat sur la survie de nauplii (A, C, E) et d'adultes (B, D, F) de *Thermocyclops* sp des lacs de Combani et de Dzoumogné : variation du pourcentage de survie après 24 (A, B), 48 C, D) et 72 h (E, F) en fonction de la concentration.

Paraquat (Fig. 1)

Chez les nauplii, les effets du paraquat sont significatifs (ANOVA ; Tableau 2) au bout de 24h (plus de 50 % de mortalité) à partir de la concentration 1.1 mg/L chez les individus de Combani et à partir de 6,8 mg/L chez ceux de Dzoumogné (100 % de mortalité). Après 72 h d'exposition, plus de 80% des nauplii sont tués par 5,4 µg/L de paraquat pour les individus de Combani, alors que 100% des nauplii issus de Dzoumogné sont encore vivants avec 54 µg/L et sont tués avec 340 µg/L.

Tableau 2. ANOVA à deux facteurs pour tester les effets du lac d'origine (Combani vs Dzoumogné) et de la concentration de paraquat ou de fénitrothion sur la mortalité des nauplii et des adultes de *Thermocyclops*. Valeurs de p après 24, 48 et 72 heurs d'incubation. Les valeurs significatives ($p<0.05$) sont en caractères gras.

	lac	concentration	lac x concentration
Paraquat			
Nauplii			
24 h	**0.000**	**0.000**	**0.000**
48 h	**0.004**	**0.000**	**0.001**
72 h	**0.000**	**0.000**	**0.000**
Adultes			
24 h	0.178	0.606	0.606
48 h	**0.000**	**0.001**	**0.001**
72 h	0.955	**0.000**	0.952
Fénitrothion			
Adultes			
24 h	0.719	**0.005**	0.758
48 h	0.071	**0.000**	**0.015**
72 h	0.068	**0.000**	0.107

Les adultes ne sont sensibles qu'au bout de 48 h (pas d'effet significatif à 24 h ; Tableau 2) toujours à partir de la concentration 1,1 chez les individus de Combani et à partir de la concentration 6,8 mg/L chez ceux de Dzoumogné. Dans les deux lacs les nauplii sont donc plus sensibles que les adultes et chez les deux stades de développement, les individus de Combani sont plus sensibles que ceux de Dzoumogné.

Les différences de sensibilité entre individus des deux lacs sont plus marquées chez les nauplii (effet lac significatif à 24, 48 et 72 heures) que chez les adultes (effet lac non significatif à 24 et 72 h) (Tableau 2).

Fenithrotion (Fig. 2)

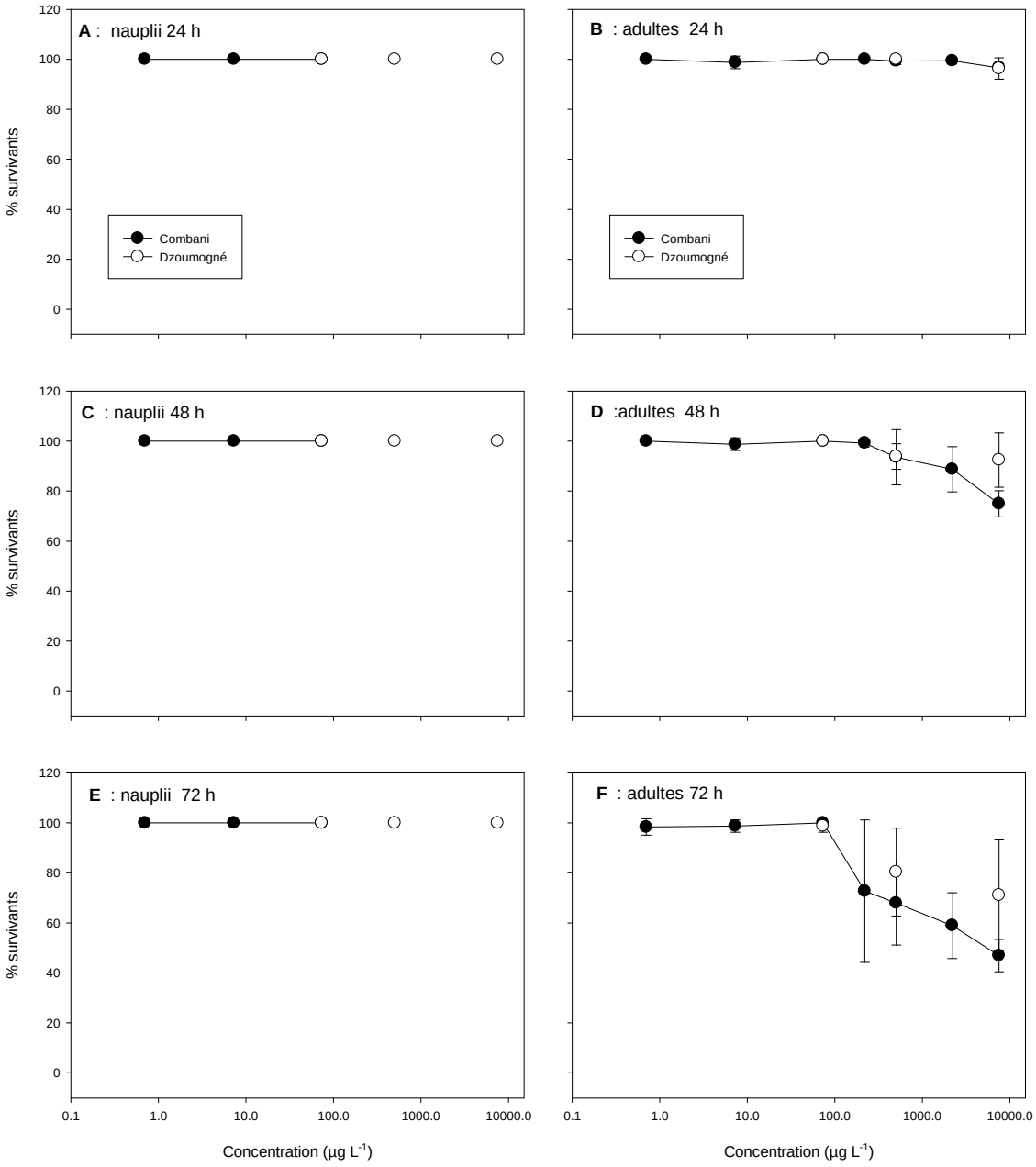

Figure 2. Expérience de toxicité pour tester les effets du fénitrothion sur la survie de nauplii (A, C, E) et d'adultes (B, D, F) de *Thermocyclops* sp. des lacs de Combani et de Dzoumogné : variation du pourcentage de survie après 24 (A, B), 48 C, D) et 72 h (E, F) en fonction de la concentration.

Contrairement au paraquat, les nauplii sont moins sensibles que les adultes, puisqu'on n'observe aucune mortalité quelle que soit la dose chez les individus des deux lacs. Par contre une mortalité significative est observée chez les adultes de Combani à partir de la dose 503 µg/L au bout de 48 h alors que chez les adultes de Dzoumogné, les effets ne se font sentir qu'au bout de 72 h. La plus grande sensibilité des adultes de Combani n'est

cependant pas confirmée par l'ANOVA au seuil de risque de 0.05, mais ce seuil est approché ($p < 0.1$) après 48 et 72 h (Tableau 2).

Il est donc intéressant de constater que pour les deux molécules la sensibilité des animaux de Combani est plus grande que celle de ceux de Dzoumogné qui seraient donc plus résistant, peut-être compte tenu d'une histoire de vie différente. Seuls les tests sur des animaux élevés en condition standardisée permettent de gommer les effets liés à l'histoire de vie.

L'absence d'effet aigu du fénitrothion sur les nauplii, alors que des mortalités sont observées chez les adultes peut paraître surprenante. Il y a peut-être un effet indirect lié à l'alimentation. En effet, les jeunes nauplii ne se nourrissent pas, alors que les adultes consomment du phytoplancton. Ainsi une altération du phytoplancton par le fénithrotion et une répercussion entraînant une dégradation de l'alimentation des adultes est envisageable

Pour tester cette hypothèse des expériences complémentaires ont été réalisées en 2009, au cours desquelles l'effet du fénitrothion a été testé comparativement chez des individus à jeun (incubation dans de l'eau filtrée à 0,2 µm dépourvue de particules nutritives) ou nourris (eau tamisée à 60 µm, contenant des particules sestoniques) (Fig. 3). Comme précédemment, aucun effet significatif n'a été observé chez les nauplii.

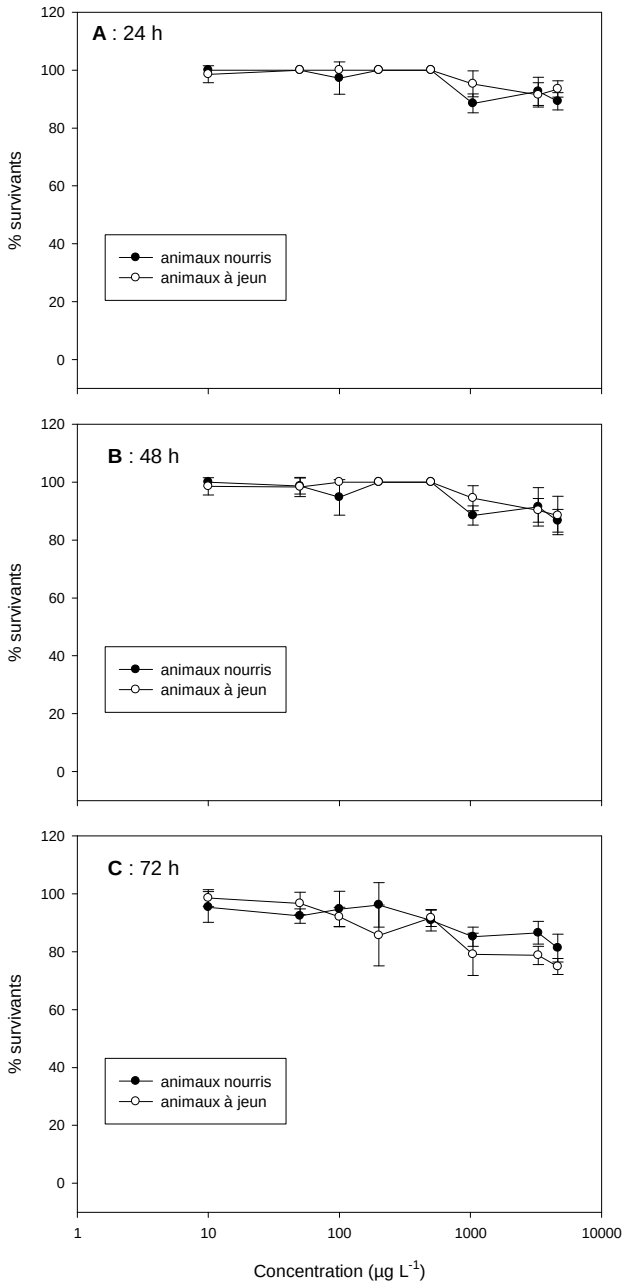

Figure 3. Expérience de toxicité pour tester les effets du fénitrothion sur la survie d'adultes de *Thermocyclops* sp nourris ou à jeun : variation du pourcentage de survie en fonction de la concentration après 24 (A), 48 (B) et 72 h (C).

Par contre chez les adultes les effets sont sensibles dès 24 heures à partir de la dose 500 µg/L aussi bien chez les individus nourris que chez les individus à jeun. L'effet des conditions trophiques n'est significatif qu'à partir de 72 heures (Tableau 3) avec une sensibilité plus importante chez les animaux à jeun. Cette expérience ne permet donc pas de confirmer l'hypothèse d'un effet indirect lié à une altération du phytoplancton par le fénithrotion et une dégradation corrélative des conditions trophiques.

Tableau 3. ANOVA à un et deux facteurs pour tester les effets des conditions trophiques (animaux nourris vs à jeun) et de la concentration de fénitrothion sur la mortalité des adultes de *Thermocyclops*. Valeurs de *p* après 24, 48 et 72 heurs d'incubation. Les valeurs significatives ($p<0.05$) sont en caractères gras.

	24 h	48 h	72 h
ANOVA 2 facteurs			
cond. Trophique	0.0561	0.1738	**0.0168**
concentration	**0.0000**	**0.0000**	**0.0000**
cond. Trophique x concentration	**0.0457**	0.2848	**0.0317**
ANOVA un facteur (concentration)			
animaux nourris	**0.000**	**0.000**	**0.000**
animaux à jeun	**0.000**	**0.000**	**0.000**

Effets sur le cladocère *Diaphanosoma excisum*.

Diaphanosoma est sensible au paraquat à partir de 5 µg/L et l'effet est net à partir de 21 µg/L (Figure 4). Ce cladocère a donc une sensibilité équivalente à celle observée pour *Thermocyclops*.

En revanche les expériences montrent que *Diaphanosoma* (Figure 2) est beaucoup plus sensible au fénitrothion que *Thermocyclops*. On observe en effet une mortalité du cladocère très importante dès la dose 4,1 µg/L (80% de morts après 48 h et 100% à 72h) , alors qu'aucun effet n'était observé chez les nauplii de copépode et que chez les adultes l'effet n'était sensible qu'à partir de 500 µg/L avec un pourcentage important de survivants (40-60%) jusqu'aux doses les plus élevées après 72 heures.

3.4.2. Conclusions : principaux résultats

- Pas d'effet du diuron

- Sensibilité au paraquat chez tous les animaux testés. Les nauplii de cyclopides sont plus sensibles que les cyclopides adultes et que les cladocères

- Sensibilité au fénitrothion des cladocères et des cyclopides adultes mais pas d'effet chez les nauplii. Les cladocères sont les plus sensibles.

- Effet du fénitrothion aussi bien chez les individus à jeun que nourris ce qui écarte l'hypothèse d'un effet indirect lié à une dégradation de la source de nourriture (phytoplancton)

- Effet de l'histoire de vie sur les réponses observées : pour les deux molécules (paraquat et fénitrothion) la sensibilité des cyclopides de Combani est plus grande que celle de ceux de Dzoumogné

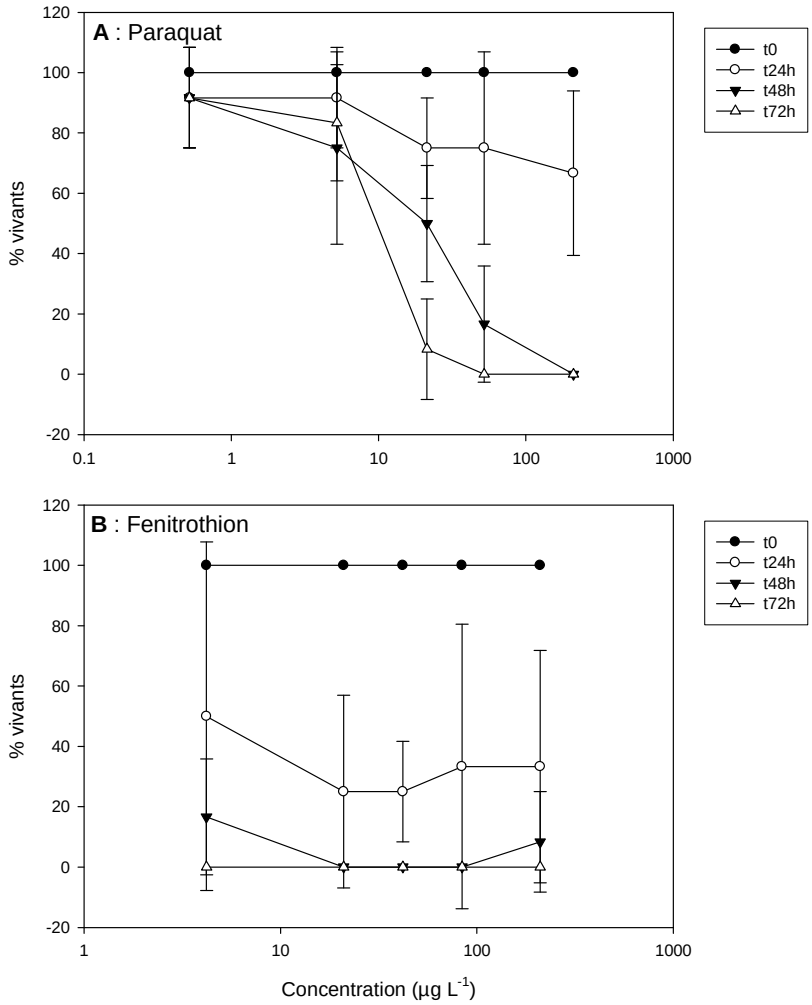

Figure 4. Expérience de toxicité pour tester les effets du fénitrothion sur la survie de *Diaphanosoma excisum* en fonction de la dose de paraquat (A) et de fénitrothion (B)

4. EXPERIMENTATIONS SUR LES COMMUNAUTES NATURELLES

4.1. EXPERIENCE EN MICROCOSMES DE 2008

Cette première série d'expérimentations a fait l'objet d'une publication scientifique actuellement en **révision mineure** pour le journal *Archives of Environmental Contamination and Toxicology* (courrier de l'éditeur du 10 décembre 2010), livré ici dans son intégralité sous son format de soumission. La version corrigée doit être retournée à l'éditeur courant janvier 2011.

A comparison of the effects of two herbicides and an insecticide on tropical freshwater plankton in microcosms

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Abstract: Natural plankton communities from a tropical freshwater reservoir (Combani Reservoir, Mayotte Island, Mozambique Channel) were exposed, in 20L nutrient-enriched microcosms, to two nominal concentrations of three pesticides, the herbicides, diuron (2.2 and 11 µg/L) and paraquat (10 and 40.5 µg/L), and the insecticide, fenitrothion (10 and 100 µg/L), commonly used in the tropics for agriculture and disease vector control. Bacterioplankton, phytoplankton and zooplankton communities were monitored for five days after exposure and the concentrations of toxicant and major nutrients were measured.

1) Bacterioplankton growth was noticeable in all systems and was slightly affected by pesticide in any concentration. A transitory increase in thymidine-based bacterial production was observed in diuron and fenitrothion-treated microcosms followed by a marked decrease in all microcosms after five days. The functional diversity of bacterioplankton, evaluated using BIOLOG ECO® microplates, was reduced by exposure to the highest pesticide concentrations,
2) Phytoplankton was affected by pesticides in different ways. Chlorophyll biomass and biovolumes were increased by diuron addition and decreased by paraquat, whereas

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fenitrothion-treated microcosms remained unaffected relative to the controls. Phytoplankton taxonomic diversity was decreased by paraquat and high doses of fenitrothion but was unaffected by the addition of diuron. The decrease in diversity was due to a reduction in the number of species, whereas the density of small cells increased, especially after the addition of paraquat.

3) Heterotrophic flagellates were sensitive to paraquat and to the highest diuron concentration: a reduction in biomass of up to 90% was observed for 40.5 µg/L paraquat. Zooplankton, dominated by *Thermocyclops decipiens* and *Diaphanosoma excisum*, was slightly sensitive to diuron, and very sensitive to paraquat. High concentrations of the insecticide fenitrothion were effective only on young stages.

The potential direct and indirect effects of pesticide contamination on such a simplified plankton food web, typical of newly constructed reservoirs, appear to differ significantly depending on the biological compartment considered. The overall sensitivity of tropical plankton is comparable to the sensitivity for temperate systems and direct and indirect effects appeared rapidly within five days of exposure.

Keywords: diuron, paraquat, fenitrothion, plankton, direct and indirect effects, microcosms, Mayotte Island

Introduction

In the general context of aquatic contamination, environmental risk assessment requires knowledge of the potential effects of chemicals on isolated organisms and artificial or natural communities (Schwarzenbach et al. 2006). For aquatic ecosystems, toxicity testing ranges from standard tests in the laboratory to field studies, including microcosm and mesocosm experiments (Caquet et al. 2000). However, as pointed out in recent papers (Daam et al. 2009, Daam & Van den Brink 2010, Freitas & Rocha 2010, Leboulanger et al. 2009) few studies have been carried out for tropical systems. These regions are facing a dramatic increase in anthropogenic pressures, including contamination of surface water bodies threatening the sustainability of water supply resources and aquatic ecosystems health and services (Lacher & Goldstein 1997).

The difference in sensitivity exhibited by organisms originating from different latitudes was highlighted, for example, for marine invertebrates or freshwater animals facing metal contamination (Chapman et al. 2005, Kwok et al. 2007) and tropical zooplankton (Freitas & Rocha 2010). To take these differences into account, Lacher & Goldstein (1997) and Wiktelius et al. (1999) advocated the evaluation of chemical side-effects in tropical ecosystems. This has still not been undertaken, despite emerging concerns such as the use of obsolete pesticides (e.g. Haylamicheal & Dalvie 2009) that affect both human and environmental safety in developing countries.

Among non-target organisms subject to pesticides in tropical freshwater bodies, plankton and its components (bacterio-, phyto-, and zooplankton sensu lato) are known to respond on a short timescale to low levels of pollutants (Daam et al. 2009, Knapp et al. 2005), mainly owing to their intrinsic sensitivity and high population turnover (Paerl et al. 2003, Relyea 2005). Plankton provides biomass and energy tunneling for higher consumers, initiates the turnover of nutrients in the water column, contributes to human waste disposal and is an indicator of the global quality of aquatic ecosystems. In addition to all these reasons for the widespread use of plankton species as test organisms for standard ecotoxicology procedures (Blaise & Férard 2005) many species can be easily

bred and maintained in laboratory conditions. Small size and short lifespan are also advantageous for enclosed studies on complex communities in microcosms, allowing both sample replication and scheduling in relatively small volumes and short times, making them ideal for community ecology tests (Drake et al. 1996). These microcosms can fill the gap between mesocosms, that are often complex and costly, and totally artificial experiments on cultures, at a reasonable cost (Brandt et al. 2004). Using microcosms for aquatic ecotoxicity studies is of great interest (Nyström et al. 2002) and is appropriate, for example, for species-directed studies even in complex communities (Bérard et al. 1999).

Numerous chemicals are potential contaminants of terrestrial surface waters and pesticides for agriculture and vector disease control are of particular interest, as they are used close to water bodies. In the tropics, the herbicides, diuron and paraquat, are used as weed control agents, whereas the insecticide, fenitrothion, is one of the chemicals used in *Aedes albopictus* (Asian tiger mosquito) eradication (for example in the Indian Ocean islands during the Chikungunya virus epidemics of 2005-2006). Diuron blocks the photosynthetic electron transfer in plants and algae and paraquat generates superoxide O_2^- that affects all the cellular components. As a consequence, they affect non-target organisms, such as micro algae, that are exposed to these herbicides. Diuron is recognized as more toxic than paraquat for phytoplankton (Cetin & Mert 2005, Perschbacher & Ludwig 2004, Schrader et al. 2003, Wong 2000). These herbicides may also be toxic in varying degrees for other non-photosynthetic microorganisms: environmental bacteria are sensitive to paraquat (Leboulanger et al. 2009) and zooplankton to diuron (Perschbacher & Ludwig 2004, Zimba et al. 2002). Fenithrothion is mutagenic for bacteria (Matsuhita et al. 2002) that may degrade and metabolize it (Hashizume et al. 1994, Nishihara et al. 1997). Phytoplankton is sensitive to varying degrees to this insecticide (Kent & Weinberger 1991), cyanobacteria being more tolerant than green algae (Sabater & Carrasco 2001). Although able to metabolize fenitrothion (Kashiwada et al. 1998), zooplankton is often sensitive to it when submitted to high exposures (Kaur & Ansal 1996, Lahr et al. 2000), which interfere with energy metabolism (Choi et al. 2001).

So far as we are aware, the effects of diuron, paraquat and fenitrothion on single components of a plankton food web and the possible emergence of indirect effects through trophic relationships and community dynamics have not been documented for freshwater tropical plankton. A simplified microcosm was, therefore, designed for a remote field study, to test the sensitivity of tropical plankton communities isolated from a pristine water body not affected by pesticides. The effects of each of the three pesticides were monitored for each biological component of the plankton community, using functional and structural measurements. Given the highly simplified trophic chain involved in this study to enhance compartment linking, a distinction was drawn between direct and possible indirect effects of chemicals during the experiment.

Material and Methods

Study site, microcosm design and results

Mayotte Island is part of the Comoros Archipelago, in the Mozambique Channel. Except for a semi-temporary pond (Dziani Karihani) and a soda crater lake (Dziani Dzaha), no natural limnic system exists on this island. Two freshwater reservoirs were constructed to meet the current and predicted drinking water requirements for a growing population: Combani reservoir, filled in 1998, and Dzoumogné reservoir, filled in 2003. The Combani Reservoir, 25 ha in surface, was totally invaded in 2005-2007 by the floating fern *Salvinia molesta*. The only fish species in the open waters of the reservoir was the guppy, *Poecilia reticulata*, introduced in Mayotte freshwaters in the 1980s to control mosquitoes. Surveys in 2006 and 2007 showed that the nutrient concentration in the Combani reservoir water was low, with typical nitrate concentrations of 0.1 – 0.7 µM, ammonium concentrations of 0.2 – 1 µM and soluble reactive phosphorus 0.1 – 0.7 µM in surface water. Alkalinity was between 550 and 650 µM depending on the season.

Using sterile, acid-cleaned light-density polyethylene (LDPE) containers, 450 L of raw water was collected from the surface at dusk from the Combani Reservoir dam (12°46’39”S, 45°08’29”E). The water was kept at outdoor temperature in low light for less than one hour and gently stirred in a

1000 L polyethylene tank after filtration through a 60 µm mesh on returning to the field laboratory. Larger cells and grazers were thus removed before controlled inoculation the following day (see zooplankton subsection below). Water was allowed to stabilize overnight and divided into 20 L aliquots the next morning in translucent LDPE 22 L semi-rigid bags (the light attenuation inside the bag had previously been determined as around 60% of incident PAR). Subsamples were taken at the same time to characterize the initial conditions (pesticide and nutrient concentrations, abundance of plankton). After treatment (inoculation with pesticide or not, nutrient enrichment, see below), the bags were gently agitated by hand and distributed at random in an outdoor inflatable pool under a sun screen (several layers of insect net). The system was designed to reduce fluctuations in light and temperature, thus providing semi-natural conditions for plankton incubation. The water temperature and irradiance (photosynthetically available radiation, PAR) were recorded inside the pool every minute using data loggers (HOBO temperature logger, Onset, and MkV/L AlecElectronics light recorder, Japan, respectively) for the duration of the experiment.

Contamination and nutrient enrichment procedures

Diuron (1-(3,4-dichlorophenyl)-1,1-dimethylurea), paraquat (1,1'-dimethyl-4,4'-bipyridinium), and fenitrothion (*O*,*O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate) were purchased from Sigma (SigmaAldrich, L'Isle d'Abeau, France), weighted at 0.01 mg using electronic scales, and kept below 4°C in 5 mL Parafilm-sealed calibrated bottles until use. They were dissolved in water (paraquat) or methanol (diuron and fenitrothion) just before use, and then diluted with GF/F filtered water from the reservoir to give the solutions used to inoculate the microcosms. Methanol was added to paraquat working solutions and methanol only was added to the controls to achieve the same methanol concentration in all the microcosms (final concentration of 0.05‰ v:v in water). The microcosms were inoculated once only on the first day of the experiment, at two nominal concentrations for each: diuron (2.2 and 11 µg/L), paraquat (10 and 40.5 µg/L) and fenitrothion (10 and 100 µg/L). All treatments were run in triplicate including controls, and are referred to as LD and

HD for low and high concentration diuron, LP and HP for low and high concentration paraquat, and LF and HF for low and high concentration fenitrothion throughout this paper.

The microcosms were enriched with nutrients daily using $NaNO_3$, NH_4Cl and NaH_2PO_4 (all analytical grade, purchased from Sigma-Aldrich), dissolved in double distilled water and kept refrigerated for the duration of the experiment. Each day the nutrients were added just after sampling in order to achieve a daily semi-continuous enrichment of 2.50 µM ammonium, 1.25 µM nitrate, and 0.25 µM phosphate. Microcosms were agitated thoroughly by hand and randomly replaced in the pool after each nutrient enrichment.

Analytical chemistry: pesticides and nutrient monitoring

100 mL samples of each of the contaminated microcosms were taken for pesticide concentration monitoring. Extraction was carried out immediately using Supelco systems: the diuron was extracted by solid phase extraction (SPE) using Oasis® HLB cartridges (Waters), paraquat was extracted by SPE using Oasis® MCX and fenitrothion was extracted by liquid:liquid extraction using dichloromethane. All extracts were kept frozen until analysis. Paraquat was detected and quantified by LC/DAD and UV detection, with a quantification limit (q.l.) of 0.1 µg/L (for 1 L sample). Diuron was analyzed with an internal standard (^{2}H-simazine) using LC/DAD/MS and UV detection, with a q.l. of 0.05 µg/L. Fenitrothion was analyzed using GC/MS with 1-bromo, 2-nitrobenzene as internal standard. *In situ* sampling was performed on the first day of the experiment to verify that there was no significant contamination of the reservoir that might interfere with the interpretation of the results.

Ammonium was determined in the hour following sampling (just before the daily addition of nutrient) by fluorimetry according to Holmes et al. (1999) and nitrate and nitrite were quantified colorimetrically after sample preservation according to Strickland & Parsons (1972). Soluble reactive phosphorus (SRP) was measured using the colorimetric method described by Murphy & Riley (1962). Dissolved oxygen was measured by Winkler iodometric titration where appropriate and total dissolved inorganic carbon (DIC) was calculated from direct pH measurements and alkalinity titration.

Bacterioplankton

Bacterioplankton samples (1.8 mL) were preserved using 100 µL of 0.22 µm-filtered 37% formalin in 2 mL cryovials and immediately frozen in liquid nitrogen. Flow cytometry was performed using a Becton Dickinson FACScalibur after SybrGreen staining. An accurate bacterial count was achieved using calibrated fluorescent beads (TrueCount, BD Biosciences). For bacterial activity, samples of 1.4 mL were put into 2 mL centrifuge tubes, 20 µL ^{3}H-thimidine was added (GE Healthcare, TRK 637) and incubated in the dark at ambient temperature for one hour. Incorporation was stopped by adding 70 µL TCA 100% (w:v in water). The samples were stored at 4°C until analysis.

The potential metabolic diversity of bacterioplankton was assessed using BIOLOG EcoPlate™ microplates, incubated with 150 µL of microcosm water in each well. Color development lasted for three days at ambient temperature (27-28°C) in the dark and the appearance and intensity of purple coloration was checked every 12 h using a BioRad 680 microplate reader equipped with a 590 nm filter. Data was normalized according to the "average well color development" (AWCD) method described by Garland and Mills (1991) and the substrate utilization efficiency was evaluated for carbon only, carbon and nitrogen and carbon and phosphorus compounds grouped by family.

Phytoplankton

Samples (50 mL) were preserved using 4% final concentration of buffered formalin for microscopic determination and enumeration at the beginning, middle and the end of the experiment. Phytoplankton was taxonomically assigned to the lowest possible level using standard flora, and the accuracy of counts was assumed to be 10% (Lünd et al. 1958). The biovolumes were calculated according to Hillebrand et al. (1999). Size classes were categorized arbitrarily as small (< 100 µm3), low-medium (100-500 µm^{3}), high-medium (500-2500 µm^{3}) and large cells (> 2500 µm^{3}). The diversity indices were calculated based on cell counts using PAST 1.82b freeware (Hammer et al. 2001); Shannon's H' and Margalef's D indices were chosen from the most commonly used indices, the latter being thought more effective for rare species (Barrantes and Sandoval 2009). Significant differences between the diversity indices for each triplicate set of microcosms were calculated using one-way

ANOVA: significance was determined by comparing replicates of treated microcosms with the replicate control microcosms at the same time.

Spectrophotometric determination of chlorophyll *a* was performed using 25 mm Whatman GF/F glass filters to collect phytoplankton, stored frozen after sampling, and processed as described by Strickland and Parsons (1968). *In vivo* fluorescence of chlorophyll *a* was used as a proxy of taxonomically-assigned phytoplankton biomass, measured daily using a multi-wavelength fluorometer (FluoroProbe, bbe-Moldaenke, Kiel FRG) that discriminated between the main pigment groups (Leboulanger et al. 2002). 25 mL of each microcosm were sampled using a syringe with a stopper, stored in the dark for 5 minutes and put into a 25 mL optical glass cuvette fitted into the FluoroProbe. A magnetic stirrer and a homemade plastic shading box were used to stabilize the fluorescence signal. The recording duration was 2 minutes for each sample, after which data was expressed as the mean equivalent concentration of chlorophyll *a* (µg eq. chl *a*/L) for each putative phytoplankton group identified from the fluorescence signals (Chlorophyceae, Bacillariophyceae, Cyanobacteria, and Cryptophyceae) and the total chlorophyll *a*.

Heterotrophic flagellates and zooplankton

Samples (15 mL) for heterotrophic nanoflagellates (HNF) were taken and fixed using buffered formalin at 2% final concentration and stored at 4°C. Counting was done using epifluorescence microscopy after staining with 4'6'-diamidino-2-phenylindole dihydrochloride (DAPI).

Zooplankton was collected on a 60 µm mesh nylon net during the early processing of the water used for inoculating. Another collection was made at dawn of the following day, using vertical hauls: six different collections were made from 8 m depth using a 60 µm mesh nylon net, corresponding roughly to 4,000 L of water, concentrated to 200 mL. Subsamples were preserved using buffered formalin for further examination. Living zooplankton was immediately identified, counted and homogeneously divided between each microcosm, at an initial concentration of individuals corresponding to 6 times the natural density of animals. At each sampling time during incubation, zooplankton was identified and counted by sampling 2 L after thoroughly mixing each

microcosm and passing through a 60 µm mesh net. Zooplankton was preserved using 5% final concentration or buffered formalin, the filtered water being replaced in the corresponding microcosm to keep the volume constant. On the final day the total volume of each microcosm was processed for total zooplankton enumeration.

Results

Structure of the plankton community at the beginning of the experiment

Based on cell counts, microscopic identification and enumeration, and field records, the plankton food web in Combani reservoir appeared extremely simplified (Table 1). Phytoplankton biomass was low and dominated by green micro algae, especially euglenophyceae and zygophyceae, with a small number of coccoid cyanobacteria and pinnate diatoms and a significant number of small cells (picophytoplankton, mostly cyanobacteria). Zooplankton was dominated by the carnivorous cyclopoida *Thermocyclops decipiens*, with a high density of nauplii (up to 445 ind/L), copepodites and adult copepods being less abundant (25 and 2.5 ind/L respectively). Herbivorous grazers were dominated by the cladocera *Diaphanosoma excisum* and the rotifer *Brachionus falcatus*. The only top predator inhabiting the pelagic system was the guppy *Poecilia reticulata*, which school even in the open waters of the reservoirs and which was removed from the experimental systems.

TABLE 1

Temperature and irradiance during the experiment

The temperature within the microcosms varied during the experiment with a marked day / night cycle, to a greater extent than that reported *in situ*, ranging from 23.0°C at dusk to 27.3°C at dawn. However, these fluctuations were within the range of natural temperature changes in the Combani reservoir measured using immersed recorders in 2007, namely from 24.6 to 28.6°C during the austral summer period. Light attenuation by sunscreen and LDPE bags resulted in a PAR intensity of ca. 35% of incident light in the vessels. The light attenuation coefficient in the Combani Reservoir was calculated at 0.72 m^{-1} (using discrete measurements on the water column and regression to the Beer-

Lambert law), and so the irradiance for the microcosms was equivalent to a depth of between 1.5 and 1.75 m.

Fate of pesticides in the microcosms

Pesticides were monitored daily in each inoculated microcosm and the results for the start and the end of the experiment are summarized in Table 2. Diuron was the only pesticide added whose final concentration was almost the same as the initial concentration, with LD and HD at 1.46 ± 0.71 µg/L and 11.36 ± 1.00 µg/L respectively. Paraquat decreased immediately after application to approximately 50% of the initial concentration and remained stable thereafter in both sets of microcosms during incubation. Fenitrothion dropped to 45% of the initial concentration just after addition and the concentration decreased exponentially over the following five days with the final concentrations for LF and HF at 0.56 ± 0.05 µg/L and 5.42 ± 0.32 µg/L respectively, about 10% of the initial measured concentrations. A simple exponential decay model was applied to the data to calculate the half-life for fenitrothion in the microcosms, which was between 35 and 45 hours approximately.

TABLE 2

Nutrient dynamics

The initial nutrient concentrations in the sampled water from the Combani Reservoir were below the detection limit (0.5 µM) for ammonium, 0.12 µM for nitrate and 0.2 µM for SRP. Daily additions were, therefore, well above normal nitrogen nutrient concentrations and similar to the environmental concentration for SRP. It is probable that limitation by nutrients in the initial community, if any, was due to nitrogen rather than phosphorus. Spikes of nutrients were visible in the dissolved phase of the microcosms for only two to three days and the increase in uptake rates by organisms was certainly responsible for the almost complete disappearance of nitrogen during the last two to three days (Fig. 1). There were differences between some microcosms relative to the controls. The pesticide inoculation resulted in different patterns depending on the concentration and the pesticide, with no effect for fenitrothion, low to moderate effects for diuron and a marked

decrease in uptake for paraquat. For both herbicides the reduction in nitrogen uptake rates was dependent on the concentration.

- The LD inoculation had no effect on nutrient levels, whereas the HD inoculation inhibited the nitrogen uptake only for the three first days of the experiment (Fig. 1 LD & HD),
- The LP inoculation reduced nitrate assimilation slightly and the HP inoculation reduced both nitrate and ammonium (Fig. 1 HP),
- Neither LF nor HF inoculation had any noticeable effect on the assimilation of nutrients (Fig. 1 LF & HF).

None of the pesticides had a noticeable effect on SRP concentrations.

For all microcosms, the mean DIC concentration was 728±26 µM. Such a low variance suggests that none of the chemicals had an effect on gross inorganic carbon exchanges.

FIGURE 1.

Bacterioplankton structure, activity and metabolic diversity

Bacterioplankton cell density doubled in all the microcosms for the first day of incubation, the rate being higher in the microcosms with pesticide inoculation than in the controls, but for the remaining five days of exposure, only HP resulted in a higher bacterial cell count (Fig. 2a), which was always higher than in all other microcosms. There was a significant increase in thymidine incorporation rates in all microcosms during the incubation time. Thymidine incorporation started at 17.6 ± 1.4 pmol/L/h and peaked at 95 ± 35 pmol/L/h at day 4 in the control microcosms. HD, LP and HP exposure enhanced apparent bacterial activity relative to the controls during the first three days of incubation, whereas both LF and HF resulted in a lower bacterial activity after five days (Fig. 2b). It was clear that the relative increase in bacterial activity was transient in microcosms with added herbicide, whereas the reduction in activity in microcosms treated with fenitrothion occurred only after more than 100 h of exposure (Fig. 2b).

FIGURE 2.

The metabolic diversity was significantly modified by adding pesticide. Color development was not impaired by pesticide treatment, except for HP treatment. The utilization efficiency for all BIOLOG EcoPlate™ substrates was enhanced by low concentrations of all pesticides (Fig. 3), resulting from an increase in functional diversity. However, the higher concentrations decreased both AWCD and functional diversity, particularly with paraquat and fenitrothion (Fig. 3, insert) which caused a particularly low efficiency of CP substrate (carbon and phosphorus source) utilization by bacterioplankton.

FIGURE 3.

All the parameters for the structure and activity of bacterioplankton suggested that the addition of any of the pesticides stimulated the bacterial activity (thymidine incorporation) and abundance, with the exception of fenitrothion, and that the highest doses reduced the substrate utilization efficiency assessed by BIOLOG EcoPlate™.

Phytoplankton dynamics

The addition of nutrients during the experiment encouraged considerable phytoplankton growth, with biomass almost doubling during the incubation process.

Chlorophyll *a* measured by spectrometry decreased slightly from 13.3±2.4 µg chl *a*/L measured in the initial sample, to 5.3-9.2 µg chl a/L in all the microcosms at day 0 of incubation, and increased thereafter (Fig. 4), peaking at day 5 for the control. Communities exposed to diuron and fenitrothion evolved in a similar way and the chl *a* biomass remained similar to the controls for both doses except during the two last days of the experiment, where the control microcosms reached 52.4±11.1 µg chl *a*/L on day 4 and the biomass dropped to 24.6±2 µg/L for HD microcosms. Only paraquat reduced both the early peak of chlorophyll biomass and the final concentration (24.7±0.9 and 23.6±3.9 µg chl *a*/L on day 4, reduced to 17.8±1.5 and 15.3±1.3 µg chl *a*/L on day 5, for LP and HP treatments respectively).

FIGURE 4.

Unlike the spectrophotometer measurements, the total equivalent chl *a* measured by the fluorescence probe did not vary greatly throughout the experiment ($r^2 = 0.74$, n = 121) although it did depend on the treatment and the sampling date. *In vivo* fluorescence measurements of chlorophyll *a* increased in the total biomass in all the microcosms (Fig. 5). The total biomass in the controls varied from 7.5 to 28 µg eq. chl *a*/L, with almost 98% of the biomass-related fluorescence signal attributed to Chlorophyceae after 5 days. A stationary phase occurred starting on day 4, the biomass remaining stable for the next day (Fig. 5a). The diatom-related fluorescence did not change significantly (around 0.6 µg eq. chl *a*/L), whereas the cyanobacteria-related signal started to decrease towards zero at day 3 after peaking at 1.3 µg eq. chl *a*/L on day 2. Both LD and HD diuron concentrations resulted in an apparent increase in biomass, for total chl *a*, Chlorophyceae and diatoms, with higher concentration of 30-35 µg eq. chl *a*/L at days 5-6 for total biomass. Cyanobacteria also vanished from the microcosms after two days (Figs. 5b & 5c). With paraquat, the apparent growth of phytoplankton was reduced compared to the controls, diatoms were absent from the beginning of incubation and cyanobacteria survived for two days. The final biomass was 24 µg eq. chl *a*/L in the LP microcosms and 22 µg eq. chl a/L in the HP (Fig. 5d & 5e). Fenitrothion microcosms were similar, in terms of *in vivo* florescence of chlorophyll *a*, to the controls (Fig. 5f & 5g).

FIGURE 5.

Up to 83 different phytoplankton taxa were recorded during this experiment, most of which were assigned to a genus and several assigned to a species. The most abundant species was not determined, resembling a small euglenoid such as *Trachelomonas* sp. This organism, with a 6-8 µm size range, exceeded 10^7 cells/mL in microcosms with added paraquat and fenitrothion at the end of incubation. Other abundant taxa were *Teilingia* sp. (Desmidiaceae), *Tetraedron minimum* (Chlorophyceae) and *Staurastrum* sp. (Desmidiaceae). Green algae were the most abundant algae in number and biomass, *Nitzschia* sp. (Bacillariophyceae) and *Planktolyngbya* sp. (cyanobacteria) being the dominant non-green algae in the microcosms. Numerous taxa were only recorded once during the survey, in very small numbers and were, therefore, not considered in the discussion. Species

richness in microcosms varied depending on time and treatment, ranging from 29 to 52: in all cases species richness decreased in each microcosm during the incubation period. This was not strictly correlated with biomass and abundance, for which uncoupling was observed: in microcosms with added paraquat (LP and HP) and fenitrothion (HF), the resultant phytoplankton communities were dominated by smaller cells and increases in density were not supported by a concomitant increase in biomass (Fig. 6).

FIGURE 6.

The phytoplankton size structure was dominated by the 100-500 μm^3 group in the control microcosms at the beginning of incubation, whereas smaller and larger cells were partly removed during the set-up of the experimental system (Table 3). After five days, the number of cells with volume less than 100 μm^3 and more than 2500 μm^3 decreased in the control microcosms, with a similar change in microcosms with added diuron. Microcosms with added paraquat and fenitrothion-behaved differently and the 100-500 μm^3 size class increased to more than 60% of the total phytoplankton biomass.

TABLE 3.

The diversity of phytoplankton communities was also affected by inoculation with pesticide. The Shannon index H' remained fairly constant in the control microcosms and in LD, HD and LF microcosms but it decreased significantly over time in LP, HP and HF microcosms relative to the control microcosms at the same sampling times (Fig. 7). The calculated H' in HF microcosms for the last day was also significantly different from that for LP and HP treatments at the same time. The Margalef D index was highly variable in this study, probably owing to numerous rare species that increased the variance of results between replicated treatments. D decreased over time in all microcosms and only the addition of LP and HP resulted in transitory significant differences with respect to the controls. In these latter cases, there was no significant difference in D at the end of incubation in any of the microcosms inoculated with pesticide (data not shown).

FIGURE 7.

Heterotrophic nanoflagellates and zooplankton dynamics and size structure

The density of heterotrophic nanoflagellates (HNF) did not change significantly in the control microcosms from the start to the end of the experiment. However, these organisms were highly sensitive to paraquat and both LP and HP concentrations resulted in a decrease of HNF abundance. A high concentration of diuron had only a slight effect (Table 4). LD and fenitrothion had no significant effect.

TABLE 4.

Overall, zooplankton carbon biomass was insensitive to diuron (Table 4): total, nauplii or cyclopidae copepodites and adults did not differ in microcosms inoculated with diuron relative to the controls. However, the adult stages of the copepod *Diaphanosoma excisum* were slightly reduced compared to the control in the HD treatment. A reduction in rotifer biomass was also noticed but it was below significance level (p= 0.072). Low concentrations of paraquat (LP) caused a significant reduction in *Thermocyclops decipiens* copepodite biomass relative to the controls, whereas HP reduced the carbon biomass in all groups of zooplankton, mainly the cladocera and copepod nauplii. Total zooplankton, rotifers, and *T. decipiens* nauplii and adults were insensitive to fenitrothion, whereas copepodites decreased with the higher insecticide concentrations (p= 0.051). Despite the apparent conservation of total zooplankton carbon biomass in microcosms inoculated with fenitrothion, all life stages of *D. excisum* vanished for both insecticide concentrations. The only carnivorous zooplankton in the microcosms were large copepodite and adult stages of *T. decipiens*. The adults remained constant in all microcosms inoculated with pesticide. Copepodite stages increased whereas nauplii dropped from the start to the end of the experiment, a manifestation of the life-cycle of the species (Pagano et al. 2000).

Discussion

Direct effects of pesticides on plankton components and inference of indirect effects

The duration of the experiment was long enough to span more than a complete life cycle (ca. 4 days) of the cladocera *D. excisum* and equivalent to the minimum development time (ca. 6 days) for *T. decipiens* (Jana & Pal 1984, Pagano et al. 2000). This period was long enough for complete life cycles of smaller organisms, which are known to double in hours (bacteria) or days (phytoplankton and rotifers). This experiment was conducted as a short-term estimation of the toxic effects of pesticides, compared to long-term exposure in mesocosms (Daam et al. 2009, 2010, Knauer et al. 2010) that can detect changes in ecological successions of organisms spanning up to several months and require more complex statistical analyses. Of the pesticides tested, diuron did not result in any drop in chlorophyll biomass compared to the controls, even though the organisms are thought to be sensitive to this herbicide at the two concentrations tested (e.g. in the same range as accepted hazard concentrations, Knauer et al. 2010). Furthermore, the apparent biomass of phytoplankton expressed in chlorophyll *a* appeared to increase with the addition of LD and HD, a pattern which was not supported by microscopic examination of samples. Both phytoplankton biovolumes and numbers decreased, suggesting that the chlorophyll content of phytoplankton cells increased to cope with the photosynthesis inhibition caused by the herbicide. Bacterioplankton also responded by an increase in production rates, whereas neither HNF nor zooplankton were affected by the addition of diuron.

Paraquat exerted an inhibitory effect on all components of the plankton community except bacteria which had higher density and activity than in the control microcosms, and rotifers which were only slightly reduced in the HP microcosms. Small phytoplankton also increased more than larger phytoplankton and phytoplankton biovolumes of microcosms inoculated with paraquat remained close to those of the controls, with higher cell densities. Smaller organisms of all trophic categories are, therefore, enhanced under paraquat stress, which could be consistent with *r*-strategist behavior. Despite increasing in numbers, bacterial metabolic diversity assessed using BIOLOG EcoPlate™ was reduced in HP microcosms. A general reduction in plankton biomass, taxonomic diversity and metabolic potential appeared in the systems under paraquat stress. Copepodites of *Thamnocephalus decipiens* had dose-dependent sensitivity to paraquat (Table 3), and

slight sensitivity to fenitrothion at the higher concentration. Top-down control of the food chain is, therefore, expected to occur only by HP contamination among the pesticides tested but as this decreased the biomass of all organisms except bacteria, and specifically phytoplankton, there was no increase in primary producer density during the experiment.

The insecticide fenitrothion was clearly toxic only to *Diaphanosoma excisum* and eradicated this organism at the lower concentration (4.65 µg/L as measured at the beginning of incubation time, Table 1). *Thermocyclops* copepodites were nevertheless visibly reduced under HF treatment, although this was not supported by two-way ANOVA (p= 0.051). Bacterioplankton, phytoplankton and HNF numbers were not significantly affected by fenitrothion, whereas both bacterial productivity and metabolic diversity, and size structure of phytoplankton, did change under LF and HF treatments.

These results indicate a few indirect effects on the communities that are not explained by the direct toxicity of the pesticides on the organisms studied (Fig. 8).

FIGURE 8.

Some of the effects observed were expected given the known toxicity for related organisms, whereas others were not sufficiently supported by previous studies. Despite evidence in the literature of diuron being acutely toxic for phytoplankton at levels often below 20 µg/L, neither the raw chlorophyll biomass nor the phytoplankton size structure were affected by this herbicide in this experiment, whereas cell densities and biovolumes were reduced. Diuron toxicity is thus partly compensated by structural changes in the photosynthetic apparatus (Seguin et al. 2002) and the reduction in phytoplankton density favored bacterioplankton by increasing competition for nutrients (Fig. 8b). However, this reduction in the food supply did not modify the density of adult grazers and predators, which would have required a longer incubation time.

Paraquat was toxic to almost all compartments of the plankton community, leading to a reduction in biomass, numbers, and overall trophic functioning as illustrated by the one-day lag in nutrient (mainly nitrogen) depletion of the water in the microcosms (Fig. 8c). In such cases, paraquat was sufficiently toxic to exert a static effect without causing the plankton community to collapse.

Furthermore, phytoplankton biomass was maintained by a relative increase in smaller organisms, to the detriment of larger cells. Thus, paraquat could be considered as being able to favor r-strategists, that can benefit both from nutrient supply released from microbial reduction and from a reduction in predation owing to toxicity for zooplankton. Species that benefited from the addition of paraquat must be tolerant to this herbicide because of its stability during the experiment. This tolerance merits further research.

Fenitrothion was slightly toxic to zooplankton (Fig. 8d), mainly medium-sized grazers (*Diaphanosoma* and young stages of *Thermocyclops*). This resulted in a relative increase in small phytoplankton cell density. As fenitrothion indirectly stimulated phytoplankton growth, this resulted in an inhibition of bacterioplankton, probably owing to enhanced competition for nutrients.

Conclusion

This study showed that the freshwater plankton community of the Combani Reservoir, a tropical lake that is only ten years old, was sensitive to three of the pesticides that may be used in this area, with doses of 2.56 and 10.82 µg/L diuron, 5.32 and 21.73 µg/L paraquat, and 4.65 and 34.66 µg/L fenitrothion. The initial plankton community was reduced in complexity mainly owing to the reduction in zooplankton species, and green algae were dominant in the phytoplankton. As expected, diuron and paraquat had a dose-dependent effect on primary producers but their effects were different. Phytoplankton apparently compensated for the toxicity of diuron by changes in cellular composition and photosynthetic apparatus, but failed to maintain a cell density comparable to that of the controls. The reduction in population numbers was not accompanied by a significant change in phytoplankton diversity and therefore there was no evidence that diuron was selective. The reduction in the density of phytoplankton reduced the food availability for herbivorous zooplankton, but the experiment did not last enough for this phenomenon to result in changes in the density of grazers. However, the increase in bacterioplankton observed is attributable to higher nutrient availability. Toxic effects of paraquat on phytoplankton were balanced by the parallel reduction in zooplankton density, and therefore primary producers biomass was maintained in the microcosms

even at the highest dose. Fenitrothion has little effect on zooplankton and had an indirect effect on phytoplankton diversity during the experiment. These effects were observed as dose-dependent, and both herbicides remained stable in the systems during the experiment, whereas the insecticide fenitrothion decreased exponentially with a half-life close to two days. The experiments described here suggest that acute contamination of the tropical freshwater reservoirs could result in a significant impact on plankton communities and, in the case of a simplified trophic web or community, indirect effects could arise within the time span of several days.

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Figure captions

Figure 1. Nutrient concentrations (expressed in µM, mean ±SD of the three replicate microcosms) in the microcosms: control CONT (a); low diuron LD (b); high diuron HD (c); low paraquat LP (d); high paraquat HP (e); low fenitrothion LF (f); high fenitrothion HF (g). Samples were taken on the morning just before daily nutrient additions (see text).

Figure 2. Bacterioplankton density (mean of three replicate microcosms) during the exposure (panel a), and metabolic activity expressed as ^{3}H-thymidine incorporation rates relative to controls (panel b). Closed circles: CONT; open squares: LD; closed squares: HD; open diamonds: LP; closed diamonds: HP; open triangles: LF; closed triangles: HF. Solid lines represent controls and high pesticide doses, dashed lines represent lower pesticide doses.

Figure 3. Relative efficiency of Biolog EcoPlate™ substrate utilization by bacterioplankton sampled from the initial sample and each microcosm at the end of the exposure period (arbitrary units). Black bars: carbon only substrates; striped bars: carbon and nitrogen substrates; open bars: carbon and phosphorus substrates. Insert: gross bacterial growth in the microplates expressed as AWCD, symbols as in Fig. 2a.

Figure 4. Phytoplankton biomass in the microcosms expressed as chlorophyll *a* measured by spectrophotometry (µg/L). Mean of replicate microcosms.

Figure 5. Phytoplankton biomass in the microcosms expressed as chlorophyll *a* measured by fluorometry (µg/L) for total community and main groups. Mean of replicate microcosms.

Figure 6. Phytoplankton biomass in the microcosms expressed as cell density and biovolumes, at the beginning and the end of the incubation period.

Figure 7. Diversity of phytoplankton communities in the microcosms during the incubation period, expressed as mean (±SD) of Shannon index H' for the initial sample, at day 1 (closed symbols), 3

(shaded symbols) and 5 (open symbols). Significant differences are marked with an asterisk (ANOVA, $p<0.01$).

Figure 8. Upper panel: schematic view of the pelagic biome of Combani Reservoir, and the microcosm's ecosystem (inside the dotted box). Lower panel: community relations existing within the microcosms, for controls (a), diuron (b), paraquat (c), and fenitrothion (d) treated microcosms. Xenobiotic toxicants are marked X1 for diuron, X2 for paraquat, and X3 for fenitrothion. C: carnivores (*Thermocyclops decipiens*, adults); H1: large herbivores (*Diaphanosoma excisum* and cyclopidae nauplii); H2: small herbivores (rotifers, heterotrophic nanoflagellates); P1: large phytoplankton; P2: small phytoplankton; D: decomposers (bacterioplankton).

Figures 1 to 8
Click here to download Figure: Figs_1_8_Leboulanger.ppt

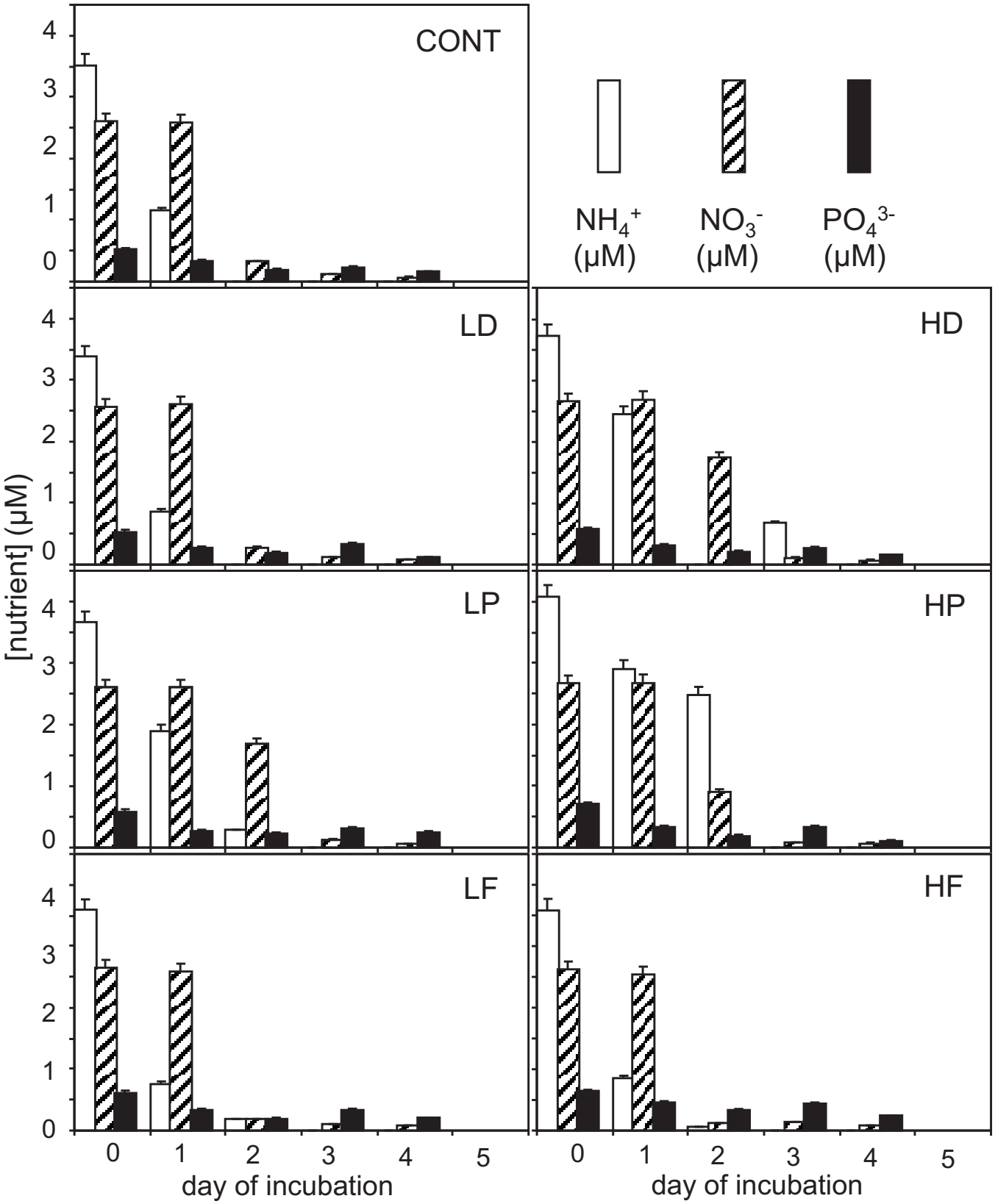
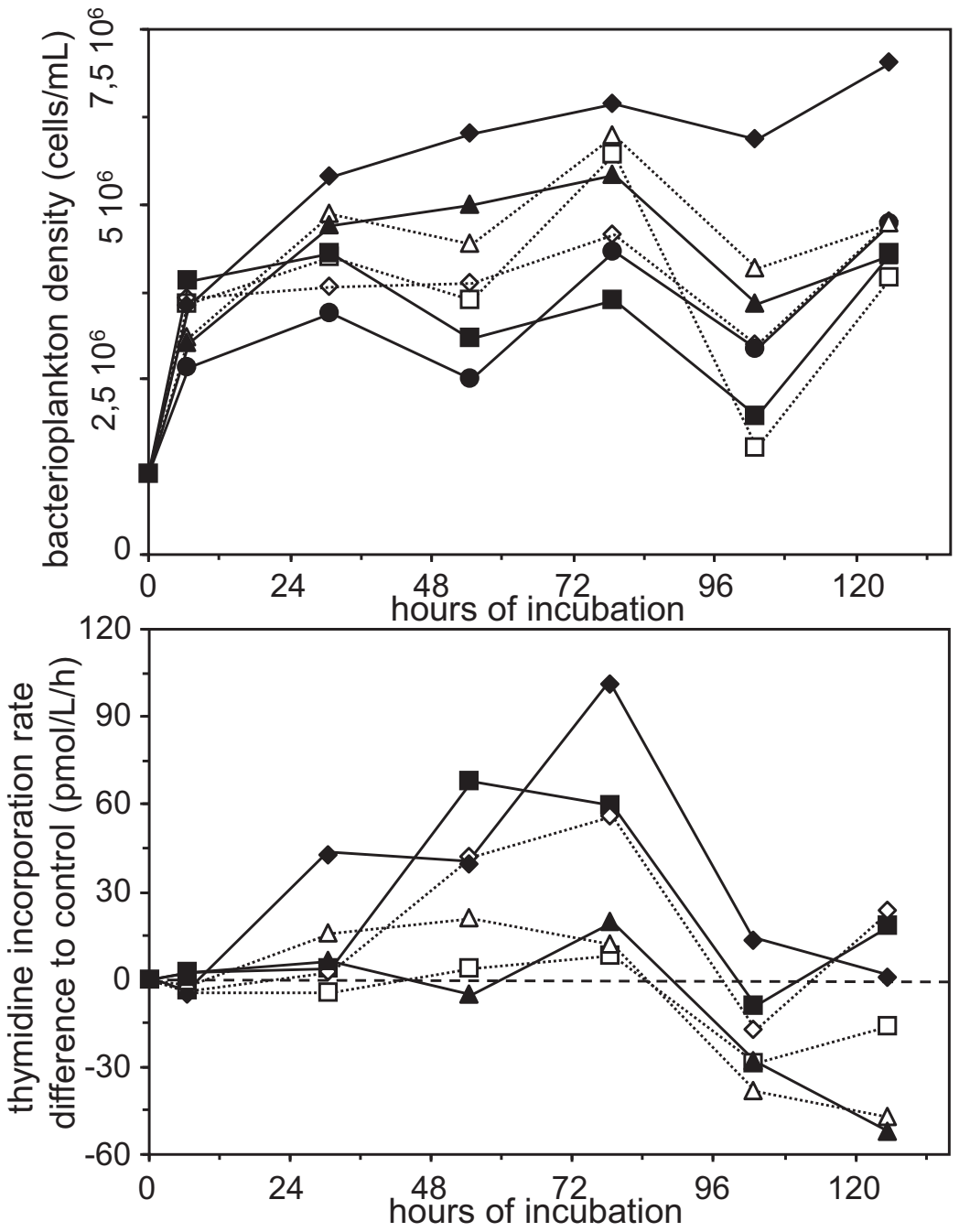


Fig. 1


bacterioplankton density (cells/mL)
7,5 10^6
5 10^6
2,5 10^6
0
0
24
48
72
96
120
hours of incubation
thymidine incorporation rate
difference to control (pmol/L/h)
120
90
60
30
0
-30
-60
0
24
48
72
96
120
hours of incubation

2a

2b

Fig. 2

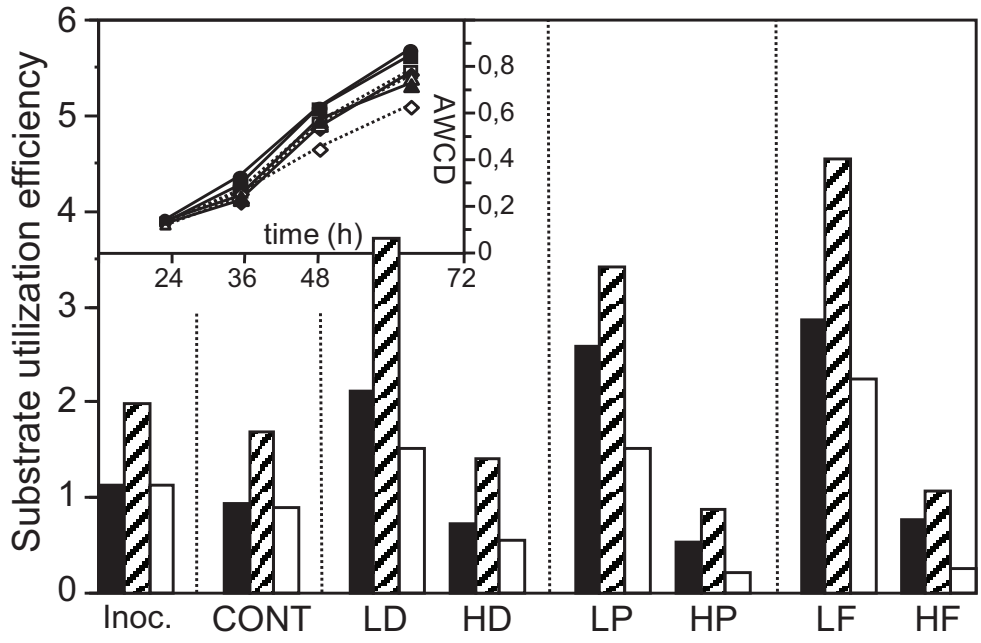

Substrate utilization efficiency
6
5
4
3
2
1
0
AWCD
0,8
0,6
0,4
0,2
0
time (h)
24
36
48
72
Inoc.
CONT
LD
HD
LP
HP
LF
HF

Fig. 3

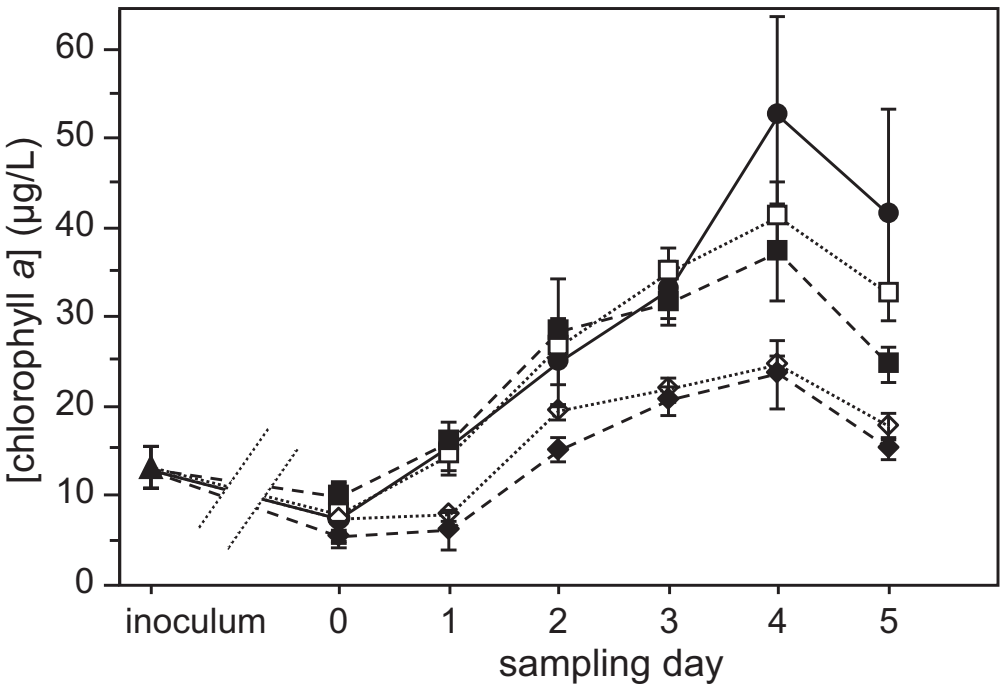

[chlorophyll a] (µg/L)
0
10
20
30
40
50
60
inoculum
0
1
2
3
4
5
sampling day

Fig. 4

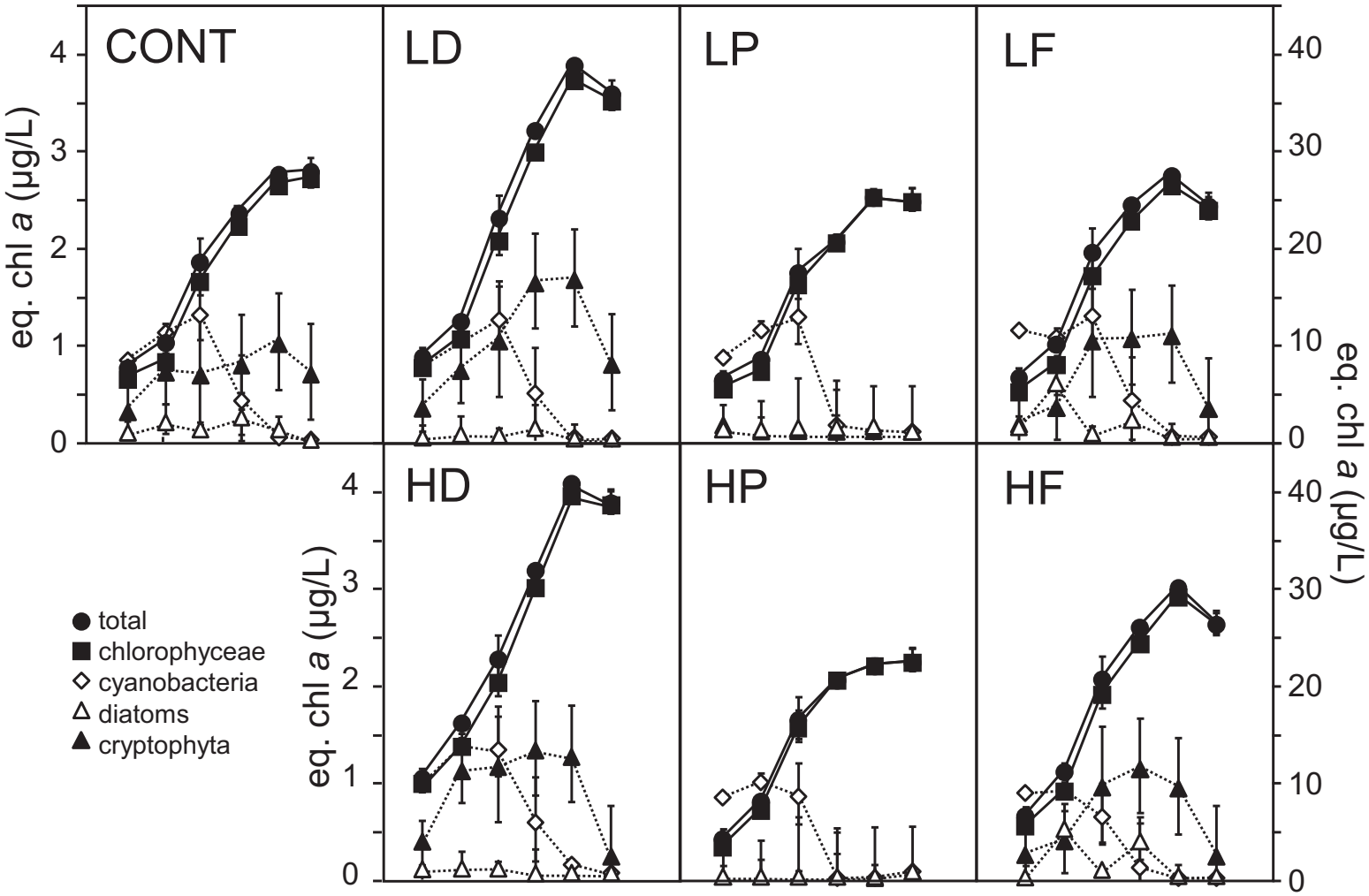

CONT
LD
LP
LF
HD
HP
HF
eq. chl a (µg/L)
eq. chl a (µg/L)
0
1
2
3
4
0
10
20
30
40
total
chlorophyceae
cyanobacteria
diatoms
cryptophyta

Fig. 5

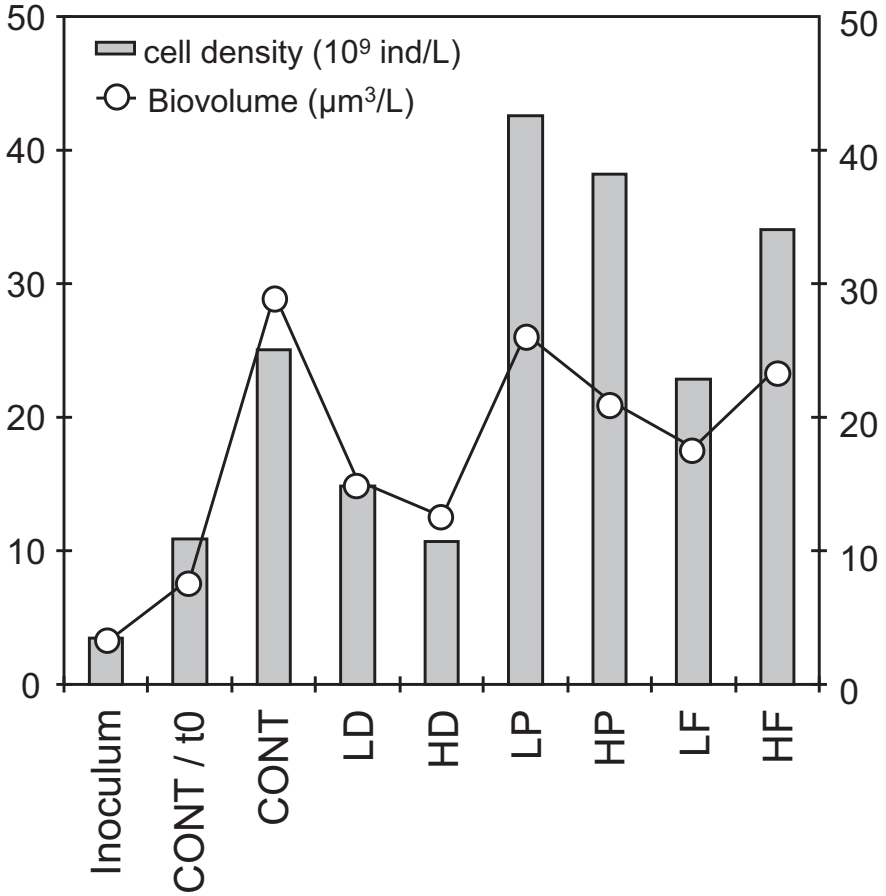

cell density (10⁹ ind/L)
Biovolume (µm³/L)
50
40
30
20
10
0
Inoculum
CONT / t0
CONT
LD
HD
LP
HP
LF
HF

Fig. 6

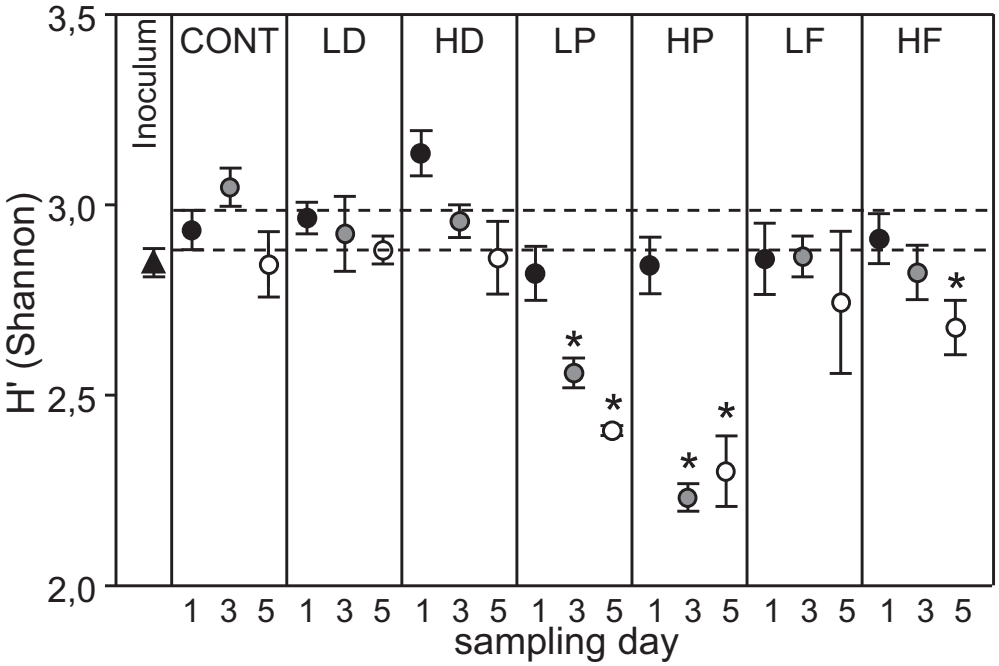

3,5
3,0
2,5
2,0
H' (Shannon)
Inoculum
CONT
LD
HD
LP
HP
LF
HF
1 3 5 1 3 5 1 3 5 1 3 5 1 3 5 1 3 5 1 3 5
sampling day
*
*
*
*
*

Fig. 7

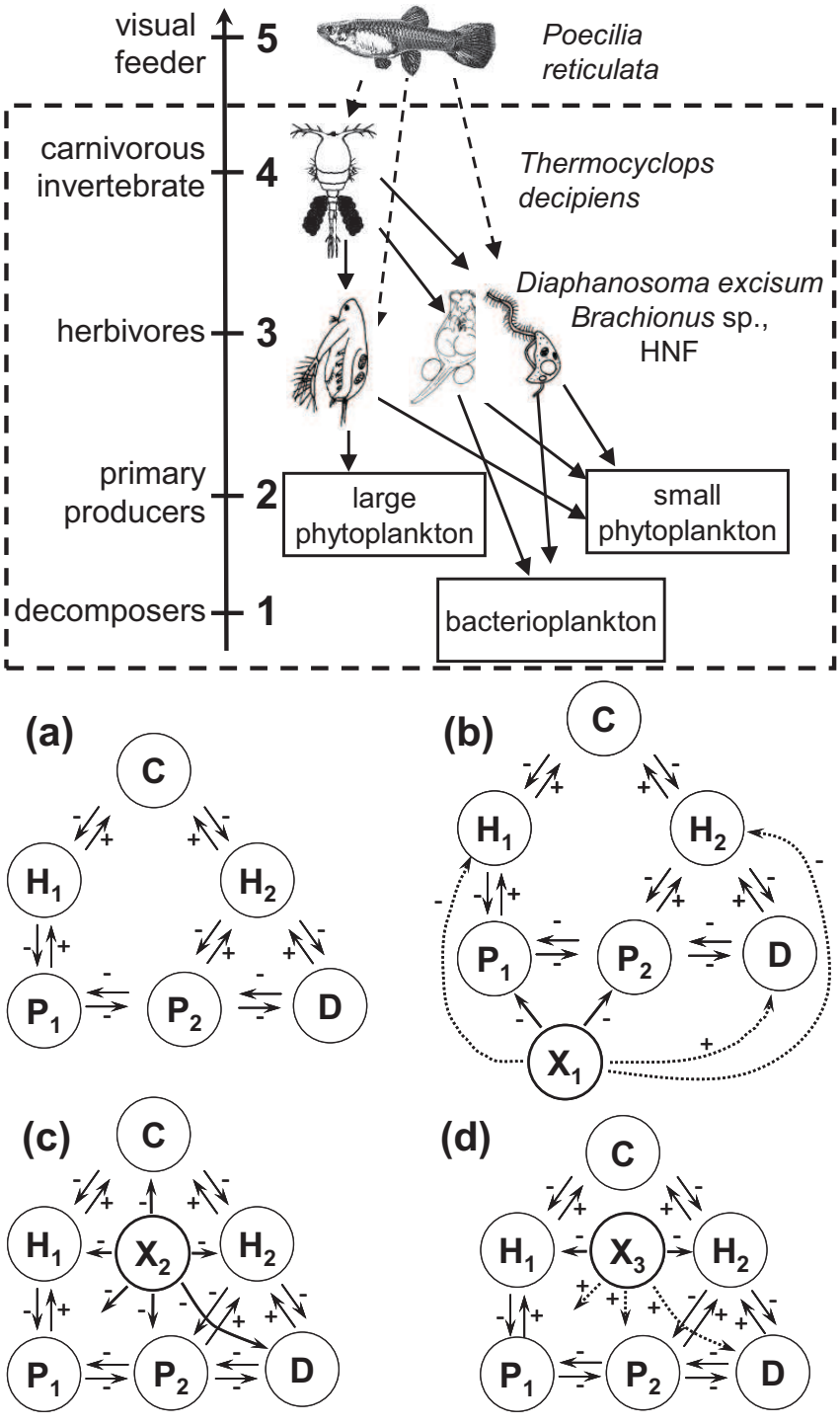

visual feeder
5
Poecilia reticulata
carnivorous invertebrate
4
Thermocyclops decipiens
Diaphanosoma excisum
Brachionus sp.,
HNF
herbivores
3
primary producers
2
large phytoplankton
small phytoplankton
decomposers
1
bacterioplankton
(a)
C
H1
H2
P1
P2
D
(b)
C
H1
H2
P1
P2
D
X1
(c)
C
H1
X2
H2
P1
P2
D
(d)
C
H1
X3
H2
P1
P2
D

Fig. 8

Table 1

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Table 1. Phytoplankton and zooplankton taxa identified in the planktonic community of Combani Reservoir, Mayotte, at the beginning of the microcosms experiment in June, 2008. Dominant phytoplankton species are those appearing at densities higher than 10^5 cell/mL, rare zooplankton at less than 2 ind. /L, in the inoculum.

PHYTOPLANKTON

Phylum	Class	Genus (species)	Remarks
Cyanobacteria	Chroococcales	*Aphanocapsa* sp.	dominant
		Aphanothece sp.	
		Chroococcus sp.	4 species
		Chroococcus limneticus	
		Coelosphaerium sp.	
		Microcystis sp.	
		Snowella sp.	
		Synechocystis sp.	
	Oscillatoriales	*Jaaginema* sp.	
		Planktolyngbya sp.	
	Pseudanabaenales	*Spirulina* sp.	
Euchlorophyceae	Chlorococcales	*Ankistrodesmus* sp.	
		Chlorella sp.	
		Chodatella sp.	
		Coelastrum reticulatum	
		Coelastrum microsporum	
		Crucigenia rectangularis	
		Crucigenia tetrapedia	
		Dictyosphaerium sp.	3 species
		Didymocystis sp.	
		Kirshneriella sp.	2 species
		Monoraphidium sp.	
		Oocystis sp.	
		Scenedesmus sp.	3 species
		Selenastrum sp.	
		Tetraedron minimum	dominant
		Tetraedron muticum	
		Tetraedron regulare	
		Tetraedron triangulare	
	Volvocales	*Phacotus* sp.	dominant

PHYTOPLANKTON (continued)

Phylum	Class	Genus (species)	Remarks
Zygophyceae	Zygnematales	*Cosmarium* sp.	4 species, dominant
		Euastrum sp.	
		Pandorina s p.	
		Staurastrum sp.	5 species, dominant
		Staurodesmus sp.	
Euglenophyceae	Euglenales	*Teilingia* sp.	
		Euglena sp.	
		Phacus sp.	2 species
		Trachelomonas sp.	2 species
		Unknown, cf. *Teilingia* sp.	dominant
Dinophyceae	Peridiniales	Unknown	2 species
		Peridinium sp.	
Bacillariophyceae	Coscinodiscales	centric diatom	
	Diatomales	*Synedra* sp.	2 species, dominant
	Naviculales	*Nitzschia* sp.	
	Pennatophycidales	Unknown	
Xanthophyceae	Mischococcales	*Tetraplektron* sp.	
Chrysophyceae	Chromulinales	*Chromulina* sp.	

ZOOPLANKTON

	Rotiferes	*Brachionus calyciflorus*	dominant
		Lecane sp.	rare
		Conochiloide sp.	rare
		Asplanchna sp.	rare
		Hexarthra sp.	
	Copepodes	*Thermocyclops* decipiens	dominant
		Mesocyclops sp.	very rare
		unidentified harpacticoïde	very rare
	Cladocera	*Diaphanosoma excisum*	dominant
		unidentified macrothricidea	very rare
		Chydorides sp.	very rare

Table 2

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Table 2. Concentration of pesticides measured at the beginning and the end of the incubations for each treatment (values in µg/L, standard deviation for 3 measurements between brackets). Range (95% confidence of exponential decay model fitting) of calculated half-life is given for fenitrothion (hours:minutes').

	Diuron		**Paraquat**		**Fenitrothion**	
	LD	HD	LP	HP	LF	HF
beginning	2.56 (0.18)	10.82 (0.43)	5.32 (0.72)	21.73 (1.03)	4.65 (0.79)	34.66 (0.82)
end	1.46 (0.71)	11.36 (1.00)	5.64 (0.47)	23.43 (1.15)	0.56 (0.05)	5.42 (0.38)
half-life (min-max)	-	-	-	-	34:57' - 44:30'	40:32' - 45:20'

Table 3
Click here to download Table: Table_3_AECT.xls

Table 3. Mean relative contribution of phytoplankton size classes to total biovolumes (expressed in % of total biovolume, averaged ion triplicates for each treatment / microcosm).

SIZE RANGE	start		at day 5 of incubation						
biovolume (μm^3)	inoculum	control	control	LD	HD	LP	HP	LF	HF
< 100	28	12	19	18	10	15	19	15	14
100-500	43	64	39	41	43	64	62	60	61
500-2500	16	14	27	30	40	17	15	17	19
> 2500	14	10	15	10	6	3	4	8	6

Table 4

Click here to download Table: Table_4_AECT.xls

Table 4. Summary of pesticides effects on heterotrophic nanoflagellates (HNF) cell density and zooplankton carbon biomass. Significant differences (two-way ANOVA) between treated microcosms and control at the end of the incubation period are indicated (bold type; * = 5%, ** = 1%, *** = 0.1%). Italicized values refers to discussed significance in the text.

			Zooplankton (µg C/L)						
						Thermocyclops decipiens			
	conditions	**HNF (cell/mL)**	total zooplankton	rotifers[$]	cladoceres[£]	total *T. decipiens*	nauplii	copepodites	adults
	inoculum	305000	50.36 ± 2.80	9.71 ± 0.37	3.43 ± 0.55	37.12 ± 2.94	24.91 ± 1.33	7.85 ± 1.25	4.35 ± 0.67
final	control	287901 ± 23756	53.78 ± 11.19	18.06 ± 8.90	2.60 ± 1.51	33.05 ± 0.88	6.23 ± 1.46	20.87 ± 1.55	5.95 ± 2.22
final	LD	260735 ± 70534	61.04 ± 12.56	26.14 ± 4.17	2.27 ± 0.31	32.62 ± 8.94	6.36 ± 1.91	20.31 ± 6.37	5.95 ± 0.89
final	HD	141295 ± 64834	41.12 ± 6.13	14.87 ± 3.92	0.77 ± 0.15	25.42 ± 3.24	5.04 ± 0.62	14.31 ± 2.45	6.08 ± 0.27
final	LP	**84496 ± 43394***	**33.26 ± 5,22***	9.12 ± 2.24	1.54 ± 0.91	22.57 ± 2.98	6.47 ± 1.04	**10.23 ± 1,76***	5.85 ± 0.40
final	HP	**25497 ± 5687*****	**7.59 ± 2,01*****	*1.39 ± 0.74*	**0.05 ± 0,05***	**6.14 ± 1,37*****	**0.62 ± 0,20****	**2.95 ± 0,97*****	2.58 ± 0.68
final	LF	316282 ± 35855	61.31 ± 8.28	32.38 ± 5.74	**0.00 ± 0***	28.91 ± 2.73	6.11 ± 1.00	17.72 ± 0.98	5.08 ± 1.64
final	HF	328575 ± 48186	54.48 ± 6.26	32.56 ± 7.06	**0.00 ± 0***	21.92 ± 1.34	5.00 ± 0.38	*11.63 ± 0.27*	5.30 ± 1.32

($: mainly *Brachionus calyciflorus* ; £: mainly *Diaphanosoma excisum* .)

4.2. EFFETS D'UNE EXPOSITION COURTE SUR LA DIVERSITE DES MICROORGANISMES

4.2.1. EXPERIENCE EN MICROCOSMES DE 2009

Le protocole expérimental employé au cours de la mission 2008 a été intégralement respecté pour 2009, avec cette fois comme inoculum biologique les communautés planctoniques de la retenue collinaire de Dzoumogné. De la même façon, les paramètres environnementaux, les concentrations en sels nutritifs et le suivi de la concentration en pesticides dans les microcosmes ont été réalisés. Comme en 2008, les expositions réelles des organismes, déterminées par la mesure analytique de la concentration en pesticides dans chaque microcosme, ne correspondaient pas totalement à la concentration nominale calculée à partir des pesées et dilutions préalables. Pour les herbicides, la concentration restait stable au cours du temps à 80% (diuron aux deux doses), 85,6% (faible dose de paraquat) et 76,6% (forte dose de paraquat) de la concentration nominale. Le fénitrothion a encore montré sa fugacité dans les systèmes expérimentaux, avec une concentration initiale de 21% de la concentration nominale, et une décroissance exponentielle au cours du temps (demi-vie de 44,7 heures).

4.2.2. Effets de l'exposition aux pesticides sur la diversité des microorganismes procaryotes et eucaryotes

Pour caractériser la diversité des communautés eucaryotes et procaryotes, nous avons choisi une méthode d'empreinte moléculaire (DNA fingerprinting) : la PCR-DGGE (Polymerase Chain Reaction - Denaturating Gradient Gel Electrophoresis). Cette méthode a pour objectif l'évaluation de la diversité dans une communauté complexe de micro-organismes sur la base de leurs génotypes. Une double filtration a permis de retenir dans un premier temps les communautés microalgales eucaryotes sur un filtre nucléopore de porosité 2µm. Le filtrat a ensuite été filtré sur 0.2 µm (nucléopore) pour le suivi de la communauté procaryote de la fraction 0.2-2µm. Les filtres ont été immédiatement congelés.

Extraction d'ADN

L'extraction à été réalisée sur des ½ filtres d'après la méthode décrite par Liu et al. (1997). L'ADN extrait a ensuite été dosé (nanodrop).

Amplification PCR

Après extraction de l'ADN, une réaction de polymérisation en chaîne (PCR) permet l'amplification spécifique d'une région cible du génome. A l'échelle d'une communauté, la spécificité de l'amplification par PCR permet d'évaluer la diversité génétique sur des critères taxonomiques et fonctionnels. Une région de l'ADN ribosomal (ADN codant pour l'ARNr 18S pour les eucaryotes et 16S pour les procaryotes) est amplifiée par PCR. La réaction de PCR est réalisée dans un thermocycler PTC-100 (MJ Research Inc.). Le mix de réaction contient : 5 µL de tampon 10X, 1,5 mM de MgCl2, 120 µM de chaque dNTP, 1µM de chaque amorce, 5 mg/mL de BSA et 1,25 U de Taq polymérase et de l'eau stérile qsp pour 50 µL.

Les amorces et les programmes PCR spécifiques utilisés pour chaque communauté :

- ARNr 16S : les amorces sont 328GC (GC clamp –CCTACGGGAGGCAGCAG) (Muyzer 1993) et 907RM (CCGTCAATTCMTTTGAGTTT) (Schauer 2003).

- ARNr 18S : les amorces sont GC516R (GC clamp- ACCAGACTTGCCCTCC) (Amann et al 1990) et 1AF (CTGGTTGATCCTGCCAG) (Medlin et al. 1988).

L'amplification des produits de PCR est ensuite vérifiée par électrophorèse sur gel d'agarose 0,5%.

Electrophorèse sur gel dénaturant

Les amplicons obtenus, de tailles identiques, sont ensuite séparés par électrophorèse à travers un gel vertical de polyacrylamide. Ce gel contient un gradient linéaire de substances dénaturantes qui permet de séparer les amplicons sur la base de leur séquence nucléotidique. En effet, la DGGE exploite la propriété de l'ADN à être dénaturé in vitro sous l'effet d'un traitement chimique (urée). Cette dénaturation est fonction de la composition en nucléotides de la séquence d'ADN étudiée. La stabilité de l'ADN est directement liée à sa composition en bases et plus particulièrement à la stabilité des liaisons permettant l'appariement de ces bases. Lors de l'étape de dénaturation, la séparation complète de la double hélice d'ADN est empêchée grâce à la présence d'un domaine à forte température de fusion créé artificiellement en incorporant à la molécule un domaine riche en GC (amorce avec queue GC). La migration du fragment est alors fortement ralentie voire stoppée du fait de l'encombrement de sa nouvelle structure (Muyzer et al. 1993). L'analyse DGGE a été effectuée sur 50 échantillons simultanément

grâce au système INGENYphorU (Ingeny International) selon le mode opératoire décrit par Muyzer et al. (1998) et les conditions expérimentales de Shauer et al. (2003).

Traitement des données

Des profils multi-bandes sont révélés sous UV et les variations dans le nombre de bandes renseignent sur la richesse spécifique d'une communauté. La DGGE est une technique semi-quantitative qui informe sur l'abondance relative de chaque espèce au sein de la communauté liée à l'intensité de la bande observée. Les gels sont analysés avec le logiciel GelCompar (Bionumerics) qui permet d'identifier les différentes bandes et de quantifier leur intensité. Quand nécessaire, les échantillons de différents gels DGGE peuvent être intégrés dans une même analyse grâce à la présence d'échantillons de référence communs positionnés sur les différents gels (GelCompar, Bionumerics). On obtient ainsi pour chaque analyse une matrice d'intensité relative aux différentes bandes ou l'intensité totale par échantillon est rapportée à 100%. Après avoir testé la normalité des données, celles-ci sont analysées par Analyse en Composantes Principales (ACP) ou par Multi-Dimensional Scaling (MDS) en l'absence de normalité.

Résultats – plancton procaryote

Sur le plan de la richesse et de la diversité, analysées à partir du nombre de bandes présentes sur chaque ligne de migration en DGGE, on observe globalement une baisse des deux paramètres pour les deux plus fortes doses d'herbicides (diuron et paraquat) et pour les faibles et fortes doses de fénitrothion, les témoins restant stables au cours du temps (Fig. 1.) Le fénitrothion à la plus forte dose provoque à la fois une diminution de la richesse et de la diversité très marquée à la fin des incubations.

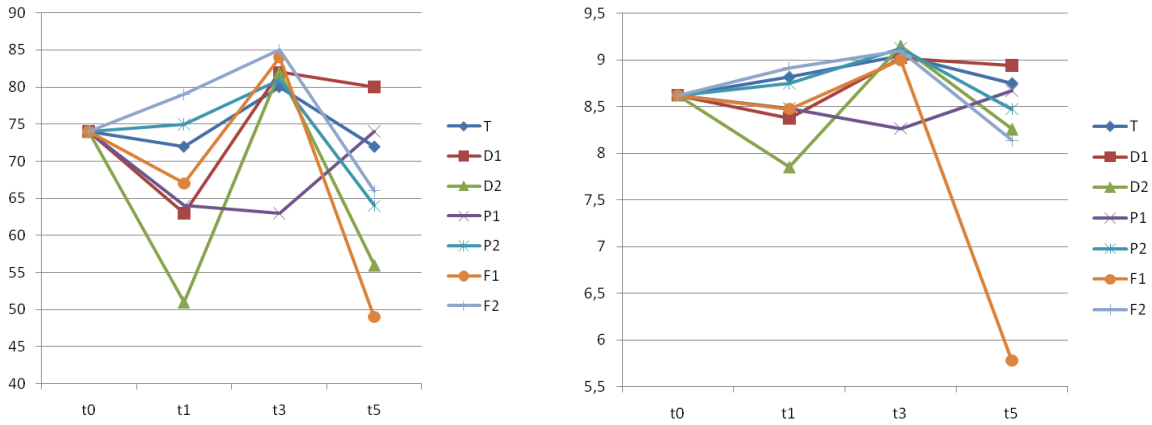


Figure 1. Evolution du nombre de bandes (richesse, gauche) et de la diversité (indice de Shannon, droite) des ADN codant pour l'ARNr 16S au cours du temps dans les microcosmes.

Lors des deux expérimentations de 2008 et 2009, nous avons observé une structuration des communautés procaryotes, sous l'effet du paraquat et du fénitrothion principalement, mais également du diuron en 2009 uniquement (Fig. 2).

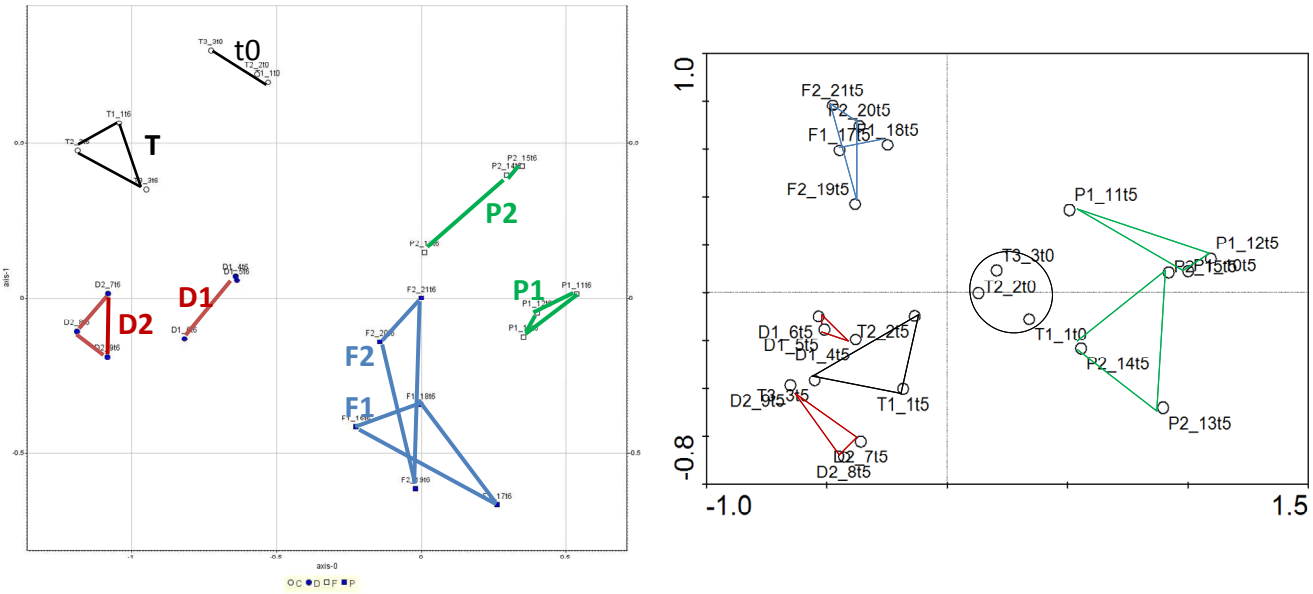


Figure 2. Analyses en Composantes Principales des communautés procaryotes lors des expérimentations de 2008 sur Combani (gauche) et 2009 sur Dzoumogné (droite).

On peut suivre plus précisément les trajectoires suivies par les communautés en fonction de chaque traitement, au cours du temps et par rapport aux microcosmes témoins. Les communautés exposées au diuron ne montrent pas de trajectoire différente à celle du témoin (Fig. 3), alors que le paraquat provoque une divergence dès le premier jour d'exposition, d'autant plus marquée que la dose est élevée (Fig. 4). L'effet du fénitrothion est visible après trois jours d'exposition, indépendamment de la dose appliquée (Fig. 5).

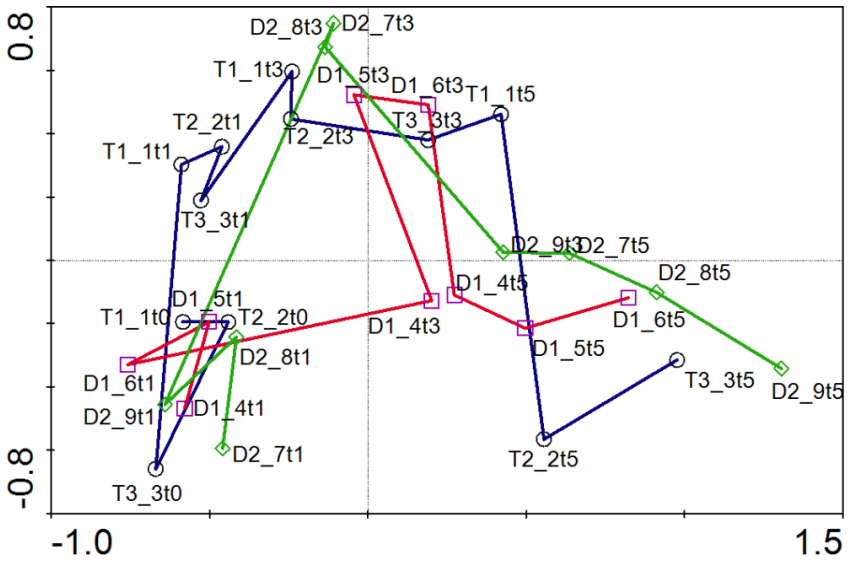


Figure 3. ACP quantitative ; effet du diuron au cours du temps, en fonction de la dose (D1 : faible, lignes rouges ; D2 : forte, lignes vertes) par rapport aux microcosmes témoins (lignes bleues).

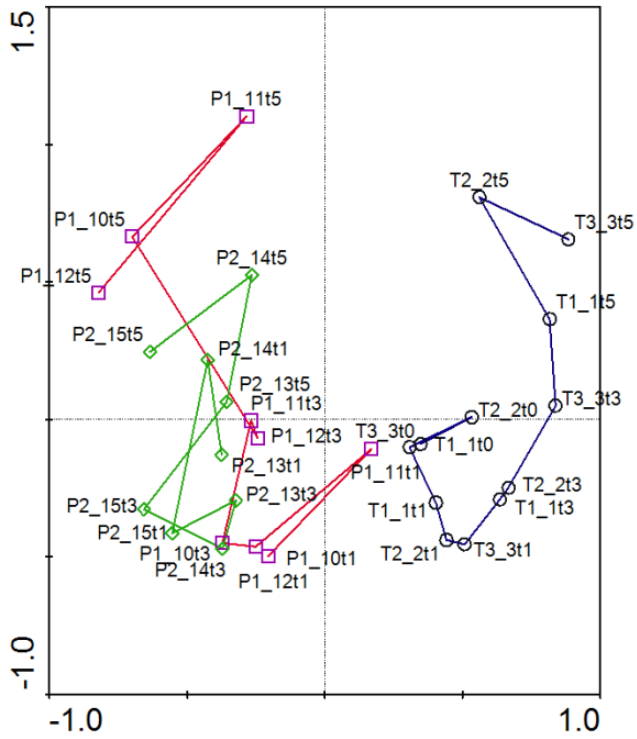


Figure 4. ACP quantitative ; effet du paraquat au cours du temps, en fonction de la dose (P1 : faible, lignes rouges ; P2 : forte, lignes vertes) par rapport aux microcosmes témoins (lignes bleues).

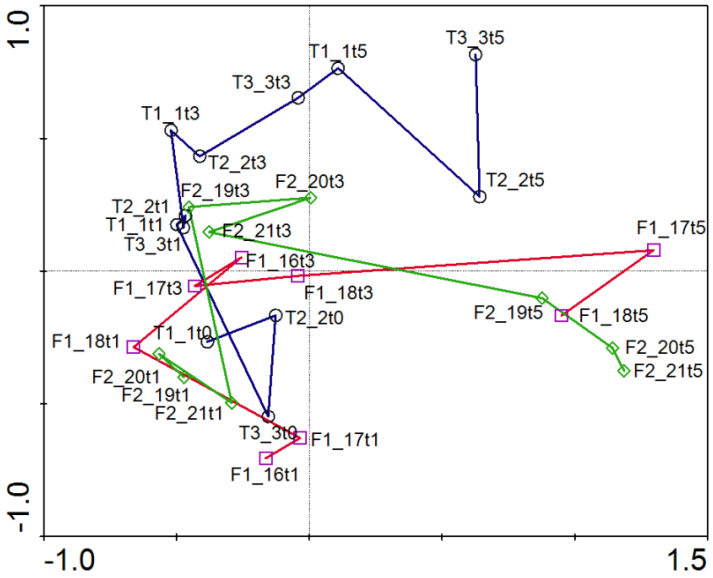


Figure 5. ACP quantitative ; effet du fénitrothion au cours du temps, en fonction de la dose (F1 : faible, lignes rouges ; F2 : forte, lignes vertes) par rapport aux microcosmes témoins (lignes bleues).

Résultats – plancton eucaryote

Comme pour les communautés planctoniques procaryotes, une baisse de richesse et de diversité est observée au cours du temps dans les microcosmes, en fonction du traitement. Tous les traitements pesticides provoquent une baisse de la richesse, exprimée en nombre de bandes présentes sur chaque ligne de migration en DGGE, cependant contrairement à ce qui a été observé avec l'ADN codant pour l'ARNr 16S, les témoins sont également touchés par une diminution globale de la richesse et de la diversité (Fig. 6).

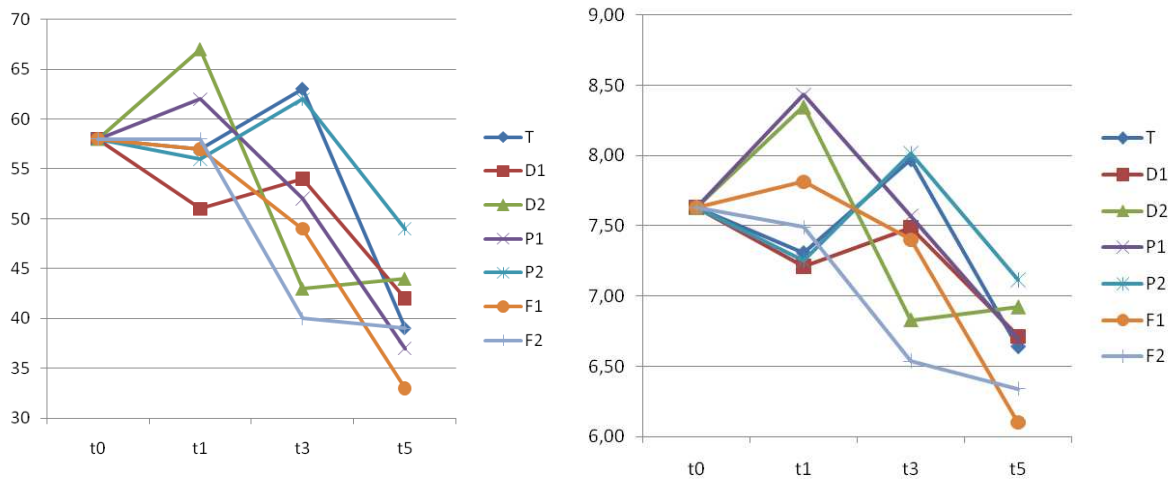


Figure 6. Evolution du nombre de bandes (richesse, gauche) et de la diversité (indice de Shannon, droite) des ADN codant pour l'ARNr 18S au cours du temps dans les microcosmes.

L'analyse en composantes principales au temps d'incubation final montre un effet structurant uniquement pour les communautés exposées au diuron et au paraquat en 2008, et au paraquat et au fénitrothion en 2009, avec un effet marqué dans ce dernier cas en fonction de la concentration appliquée.

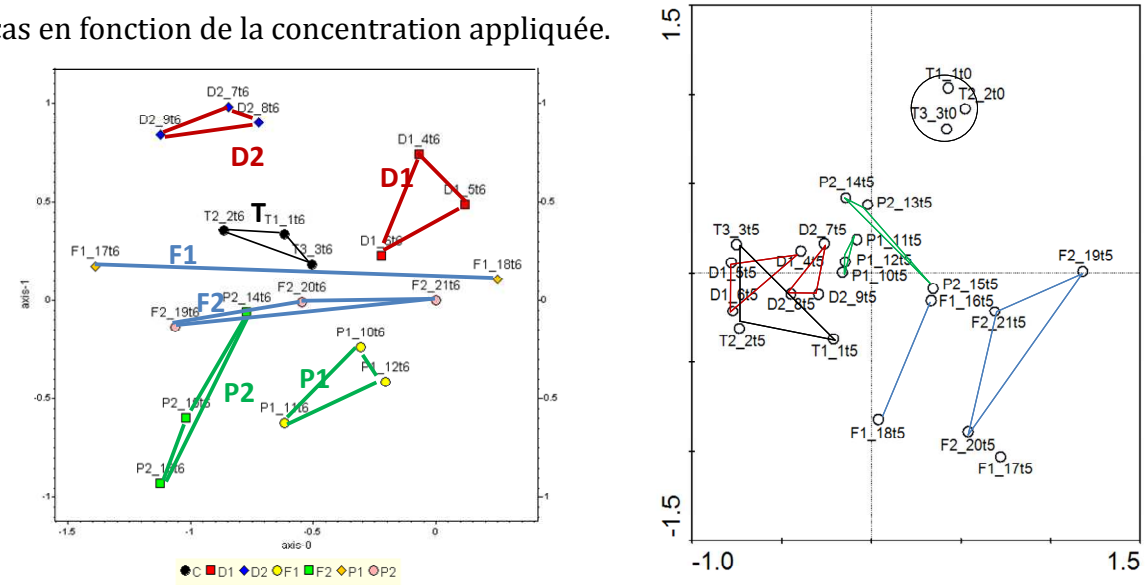


Figure 7. Analyses en Composantes Principales des communautés eucaryotes lors des expérimentations de 2008 sur Combani (gauche) et 2009 sur Dzoumogné (droite).

Les trajectoires des communautés eucaryotes sont moins marquées que pour les communautés procaryotes, et une ACP quantitative par molécule testée, avec l'ensemble des dates de prélèvement, permet seulement de montrer un effet pour le paraquat et le fénitrothion.

5. ETAT DE LA CONTAMINATION DES RETENUES DE COMBANI ET DZOUMOGNE

PUBLICATION EN COURS DE RÉDACTION, À SOUMETTRE À LA REVUE *KNOWLEDGE AND MANAGEMENT OF AQUATIC ECOSYSTEMS*

BACTERIOLOGICAL AND CHEMICAL WATER QUALITY IN TWO TROPICAL FRESHWATER RESERVOIRS (MAYOTTE ISLAND, MOZAMBIC CHANNEL): ECOLOGICAL AND HUMAN HEALTH RISKS.

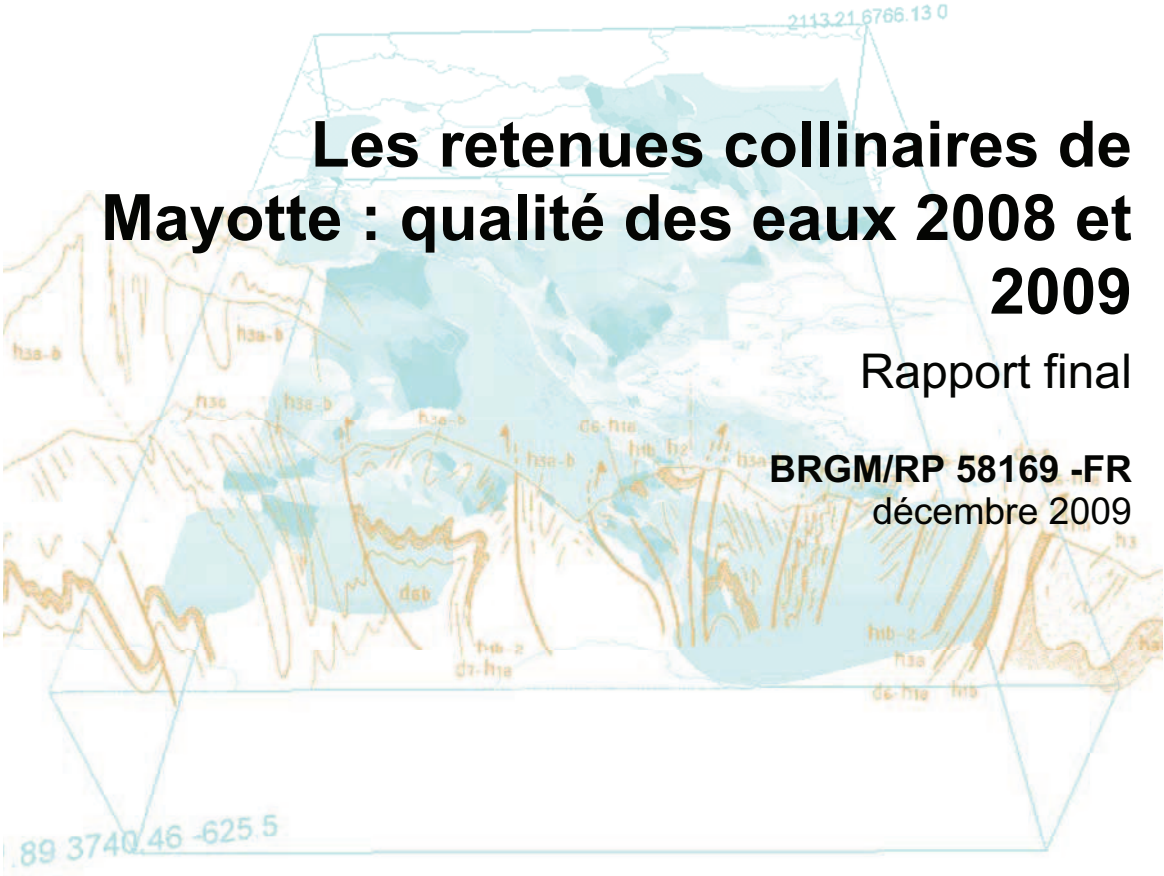
Bouvy M., Amalric L., Bouchez A., Carré C., Cecchi P., Pagano M., Sarazin G. & Leboulanger C.

Abstract:

Two freshwater reservoirs (Combani and Dzoumogné) located in the Mayotte Island are used as drinking water resource for local population. The drainage basin is under anthropogenic pressure, and the chemical and microbiological inputs are not well studied. In this context, the spatial patterns of the thermo-tolerant coliforms (TTC) and faecal streptococci (FS) abundances, together with the pesticide concentrations (including phyto-pharmaceuticals and mosquito control products) were studied at different seasons (November 2006, March 2007, June 2008 and March 2009). Using sterile bottle at a depth of 0.5 m, samples were taken at 5-6 stations per reservoir, and at each major river inlets. In the two reservoirs, pesticides concentrations were low (below 0.1 µg/l) except for the piperonyl butoxyde adjuvant in Combani reservoir. In contrast, concentrations in the river inflows were higher, especially for piperonyl butoxyde and chlorpyriphos ethyl in Dzoumogné reservoir. During the rainy and flow seasons, concentrations were undetectable probably due to the dilution by rainfall. The water quality of Combani reservoir characterized by lower concentrations of FIB (Faecal indicator bacteria) than values reported from Dzoumogné reservoir, generally inferior to those defined by the European Union bathing water quality directive. At a temporal scale, no significant difference of FIB concentrations were noticed in Combani reservoir, while higher concentrations were reported for Dzoumogné reservoir in March 2007 and June 2008. In March 2009, large concentrations were observed in the two river inlets with higher values of TTC and *Escherichia coli* suggesting a substantial microbiological pollution issuing from the drainage basin. The large concentrations of FIB raised the previous years in Dzoumogné could be explained by the direct access of the cattle to the edges of the reservoir. Since the recent awakening of the local authorities with respect to the environment protection (watering of the cattle, washing of cars), the public awareness campaigns allow to sensitize the local populations allowing a further reduction in contaminant pressure.

Sont également reproduites ci après la page de garde, l'introduction et les conclusions du rapport rédigé par L. Amalric sur la contamination des eaux superficielles de Mayotte par les pesticides.

Document public



Les retenues collinaires de Mayotte : qualité des eaux 2008 et 2009

Rapport final

BRGM/RP 58169 -FR
décembre 2009

Géosciences pour une Terre durable
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Les retenues collinaires de Mayotte : qualité des eaux - Bilan pesticides 2008 et 2009

Rapport final

BRGM/RP 58169-FR
décembre 2009

L. Amalric

Vérificateur :	**Approbateur :**
Nom : WINCKEL Anne	Nom : BOULMIER Jean-Luc
Date : 16/12/2009	Date : 18/12/2009
Original signé	Original signé

Le système de management de la qualité du BRGM est certifié AFAQ ISO 9001:2000.

Géosciences pour une Terre durable
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Mots clés : Mayotte – Phytosanitaires – Analyse – Retenues d'eaux – Eaux superficielles

En bibliographie, ce rapport sera cité de la façon suivante :
Amalric L. (2009) – Les retenues collinaires de Mayotte : qualité des eaux - bilan pesticides 2008 et 2009. BRGM/RP-58169-FR, (47p, 28 illustrations).

Synthèse

Le projet ECOMET « *Etude comparée des effets de phytosanitaires et de produits de lutte antivectorielle sur les communautés microbiennes aquatiques d'écosystèmes tropicaux* » (subvention CV070000783) d'une durée de 3 ans, financé par le Ministère de l'Ecologie et du Développement Durable, a pour objectif principal de comparer les réponses de microorganismes aquatiques tropicaux à celles de leurs équivalents en milieux tempérés, à des contaminants de type phytosanitaires ou produits de lutte anti-vectorielle.

Deux missions de terrain ont eu lieu à Mayotte en 2008 (pendant la saison sèche) et en 2009 (pendant la saison des pluies) afin de réaliser les essais en mésocosmes à partir de l'eau des deux sites de l'étude : les retenues d'eau de Combani et Dzoumogné. Dans ce cadre et afin de disposer d'un état de référence sur la contamination des ces retenues d'eaux destinées à l'alimentation en eau potable nécessaire à un suivi ultérieur de l'évolution de la qualité des eaux comme prévu par la Directive Cadre sur l'Eau [directive 2000/60/CE], des prélèvements ont été réalisés les deux années en vue de la recherche des composés phytosanitaires. Ces résultats complètent ceux obtenus en 2007 dans le cadre d'un premier projet « Les retenues collinaires de Mayotte : environnement et qualité biologique des eaux » financé par le Ministère de l'Outre-Mer.

Sur la base des trois prélèvements réalisés de 2007 à 2009, il apparait que les retenues d'eaux collinaires de Combani et Dzoumogné de Mayotte et leurs affluents ne contiennent que quelques composés phytosanitaires, utilisés soit en agriculture (difénoconazole, propiconazole), soit dans la lutte contre les moustiques (pipéronyl butoxyde et chlorpyriphos éthyle).

Dans les deux réservoirs d'eau Combani et Dzoumogné, les concentrations sont toutes inférieures à la limite de potabilité de 0,1 µg/l, à l'exception du pipéronyl butoxyde dans la retenue de Combani. Dans les affluents, en revanche, les teneurs peuvent être plus importantes notamment pour le pipéronyl butoxyde et le chlorpyriphos éthyle à Dzoumogné. En saison des pluies, ces concentrations deviennent inférieures aux limites de quantification en raison de la dilution due aux fortes précipitations. L'emploi d'un échantillonneur passif destiné à accumuler les composés polaires, lors des prélèvements en saison des pluies, montre également l'absence de composés quantifiables.

Il est difficile de relier ces valeurs à la nature et aux quantités de produits phytosanitaires utilisés sur Mayotte car elles ne sont pas connues dans le détail.

5. Conclusion

Dans le projet ECOMET les missions réalisées à Mayotte avaient pour objectif de décrire la qualité de l'eau en terme de salubrité publique. Les échantillonnages réalisés dans les deux réservoirs de Mayotte, Combani et Dzoumogné, en mars 2007, juin 2008 et mars 2009 permettent de dresser un état des lieux des caractéristiques de chaque plan d'eau, état de référence nécessaire à un suivi ultérieur de l'évolution de la qualité des eaux comme prévu par la Directive Cadre sur l'Eau [directive 2000/60/CE].

Dans ce cadre, la détermination des composés phytosanitaires présents dans les retenues d'eaux et leurs affluents a été réalisée.

Sur la base des trois prélèvements réalisés de 2007 à 2009, il apparait que les retenues d'eaux collinaires de Combani et Dzoumogné de Mayotte et leurs affluents ne contiennent que quelques composés phytosanitaires, utilisés soit en agriculture soit dans la lutte contre les moustiques. Cependant, le nombre de composés est faible, quatre composés seulement ayant été quantifiés. Dans les deux réservoirs d'eau destinés à l'alimentation en eau potable, les concentrations sont toutes inférieures à la limite de potabilité de 0,1µg/l, à l'exception du pipéronyl butoxyde dans la retenue de Combani. Dans les affluents, en revanche, les teneurs peuvent être plus importantes notamment pour le pipéronyl butoxyde et le chlorpyriphos éthyle à Dzoumogné, en période de saison sèche. En saison des pluies, ces concentrations deviennent inférieures aux limites de quantification en raison de la dilution due aux fortes précipitations. Ces données étayent le fait que ces sites sont considérés comme peu soumis à la pression agricole sur leurs bassins versants, faiblement anthropisés.

Ce bilan de la contamination des eaux des retenues ne concerne que les composés phytosanitaires ; il est donc partiel au regard des exigences de la Directive Cadre sur l'Eau et de l'utilisation des cours d'eaux par la population mahoraise. En effet des pratiques telles que le lavage des véhicules, le rejet des huiles de vidanges, des eaux sales et des déchets et le lavage du linge dans les cours d'eaux peuvent être constatées. Les autorités locales en ont pris conscience et ont mis en place des mesures de sensibilisation des populations locales sur les risques environnementaux en interdisant un certain nombre d'activités notamment autour des retenues d'eaux (panneaux d'interdiction de l'abreuvement du bétail, lavage des voitures, par exemple).

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