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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.

Keywords: paraquat, deltamethrin, planktonic organisms, reservoirs, West Africa

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. While 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, compare to enhanced knowledge on temperate organisms, have been claimed by several authors (e.g. Wiktelius et al. 1999, Chapman et al. 2006)

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 is 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 semi-official data available has been provided by the AFAMIN resource (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, etc. are found in various forms. Contamination of similar ecosystems, such as the one reported on the bordering Ghana (Osafo and Frempong 1998, Ntow 2001, Ntow 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 may 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 (Wilhelm et al. 1996, Baun et al. 1998, Christensen et al. 2006, 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 prevent the nerves sodium channels from closing in arthropods, resulting in continuous nerve impulse transmission. A suspected action of paraquat on 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 Reservoir, 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 solid-phase extraction (SPE) cartridges.

Material and methods

Sampling and collection of target organisms

This study was focused on three reservoirs located in 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 analysing 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 the 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 solid phase extraction (SPE cartridges Oasis® HLB, Waters Corp.) to test the potential toxic effect of the water concentrate (Baun et al. 1998). For all bioassays, sub-surface 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-(1R,3R)-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 three days. All concentrations stated further are nominal, and all tests were performed in triplicate with solvent controls.

Solid phase extraction from natural samples

Water sample of 5 L was taken from surface in Loumbila reservoir in an acid-cleaned polyethylene bag, and immediately processed. Water was filtered through 0.8 / 0.2 µm porosity filter cartridge (AcroPak 500, Pall Corp.) at a flow rate of 2 L/h and immediately treated by solid phase extraction (SPE). The filtrate was acidified to pH 4 using concentrated HCl. An Oasis HLB 500 mg cartridges (Waters Corp., Milford, Massachusetts, USA) was conditioned using 5 mL MeOH:water (60:40 v/v). The 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. 2000) and deltamethrin (e.g. with C18 cartridges, Hengel et al. 1997) was intended, due to laboratory limitations on site. The acidified filtrate (1 to 4 L) was passed gently through the cartridge using a peristaltic pump (flow around 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 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 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 section was to test the sensitivity of complex bacterial communities. Isolation of bacterial strains, although possible, was not included since isolation media are prone to exert a strong selection among initial diversity of bacterioplankton. Sub-surface water samples from Koubri and Bam reservoirs were processed within 1 hour 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 hours and 24 hours. 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 SYBR-green I (Molecular Probes) bacterial cell stain 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 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 hours of treatment if significant differences were obtained in terms of abundance and thymidine incorporation. The non-parametric 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 few available, for their tropical origin (West Africa) and known occurrence in the local area (unpublished data), the cyanobacteria *Cylindrospermopsis raciborskii* and the chlorophyte *Monoraphidium* sp.. *In vivo* fluorescence (IVF) has been chosen as endpoint for measuring toxicity effects on microalgae, as it encompasses both modification of photosynthetic metabolism in short term experiments (Seguin et al. 2002). The algal strains were cultured on Z8X 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 hour light / 12 hour dark cycle. Cultures were renewed each week to ensure that cells were in exponential growth when the assays were carried out.

Cultures of each strain (1 mL inoculum added to 5 mL fresh media) were placed in 10 mL borosilicate glass tubes and received five concentrations of contaminant and a pure control. All samples were run in triplicates. Tubes were monitored non-destructively for two days using *in vivo* fluorescence (IVF) of chlorophyll *a* (Leboulanger et al. 2006). The tubes were dark adapted for 20 minutes before measuring the IVF using a TD 700 fluorimeter (Turner Designs). All 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 hours 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 two 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 non-limiting conditions.

For acute toxicity experiment, newborn juveniles (less than 24 hours old) were collected from the culture and put into 40 mL flasks (four replicates per treatment) filled with 10 mL of the medium (5 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 hours and 48 hours. 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 (3 individuals per flask). Incubation lasted five 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 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 the 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 surface temperature varied from 27.3 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 µmole/L. Dissolved orthophosphate concentrations showed lower values in Koubri and the highest in Loumbila (1.42 µmole/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 hours ($p = 0.029$) 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 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 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 control value after 24 hours (Fig. 1C), whereas 57.7 and 577 µg/L paraquat resulted in CFU/mL accounting for 42 and 61% of control after 24 h respectively. A negative effect on bacterial activity (^{3}H-thymidine incorporation), with no significant recovery after 24 hours at any concentration (Fig. 1E) was also observed with paraquat, activity decreasing dramatically down to 4% of 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 hours at increasing concentrations showing a dose-dependent negative effect down to 30% of control activity for the sample exposed to 442 µg/L deltamethrin. In this latter case, after 24 hours, 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 *in vivo* fluorescence measurements. After 6 hours of exposure, IVF from *Monoraphidium* sp. and *Cylindrospermopsis raciborskii* cultures increased significantly (200 and 115% of control, respectively) at paraquat concentrations of 57 µg/L and higher (Fig. 2A). The patterns for the two species differed after 40 hours exposure, with the *Monoraphidium* sp. apparently recovering (Fig. 2B) except at the highest paraquat concentration (577 µg/L) where IVF was 238% of control value. However, *C. raciborskii* cultures exposed to 57 and 183 µg/L showed a significant decrease of IVF (70 and 35% of control value respectively) compared to the control, while 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 control value) after 40 hours 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, *Diaphanosoma excisum* (Fig. 3A) with a LOEC of 57.7 µg/L, and *Moina 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 hours 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 the paraquat concentrations tested at this moment. Nevertheless for this latter species, all individuals finally died after 48 hours incubation at 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 hours 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, D) and cultivable (Fig. 4B, E) bacteria abundance and bacterial activity (from ^{3}H-thymidine incorporation, Fig. 4C, F) were lower in the concentrated extract than in the control solution. Abundance of cultivable bacteria for the two communities appeared to increase after 24 hours exposure compared to the low response obtained after 12 hours exposure. The results obtained by flow cytometry showed a separation into 3 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 the

Loumbila reservoir, the cytograms, representing the FL1 and SSC distributions for the bacterial populations, were very different after 12 hours and 24 hours exposure. After 12 hours 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 hours 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

IVF measurements on *C. raciborskii* cultures were made after 6 and 24 hours incubation with concentrated water extracts (Fig. 6A and B respectively). Changes in IVF values of the *C. raciborskii* cultures were observed both after 6 and 24 hours incubation with serial dilutions of the extract. The IVF significantly decreased in the lower concentrations, 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 lowest observable effect concentration (LOEC, the lowest concentrations used in the test that caused significant changes compared with the control) 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 hours exposure at all concentrations (Fig. 7A, 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 know to rapidly adsorb on suspended particulate material and glassware. As previously reported (Wong 2000, Relyea 2005), 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 *Microcystis 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 LOEC of 0.05 to 0.8 mg/L for three strains of *Scenedesmus*, 0.05 mg/L, and 0.2 mg/L for *Chlorella vulgaris* and *Selenastrum capricornutum* respectively. Differences between chlorophytes and cyanobacteria should however be linked to the respective density of PSII and PSI in photosynthetic apparatus, that 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 EC50 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 of exposure; this is contrasting with the results of Perschbacher and Ludwig (2004), who applied aerially 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 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 endpoint 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 endpoints 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 of 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 microorganisms 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 hours and 24 hours exposure. However, despite a decrease near zero for ^{3}H thymidine incorporation rates (activity), culturable bacteria showed a clear recovery after 24 hours, 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 hours, with higher nucleic acid content and larger apparent size (FL1 and SSC curves in Fig. 5). The three different bacterial populations, dominant at the beginning of the experiment, were strongly affected by the toxicants during the twelve first hours of exposure with the disappearance of 3 groups compared to the controls owing to cell degradation. However, after 24 hours exposure, a distinct bacterial population appeared on the cytogram (Fig. 5C), suggesting either an adaptation and / or a selection of bacterial cells. A minor pre-existing 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, since the effects of toxicants on natural competition between microorganisms could have relevant effects on ecosystemic functions.

The response of the cyanobacterium *Cylindrospermopsis raciborskii* to the extract could support several hypotheses with respect to the time evolved before measuring the toxicity endpoint. There was clearly a time-dependant reaction of *C. raciborskii*, with the dose-response curves similar for six and twenty-four hours of incubation. Significant changes in the IVF relative to the controls were observed after six hours exposure but changes in dose-response patterns were noticed after 24 hours. 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 while 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 hours and 24 hours and a noticeable bleaching of the cultures was observed with the highest concentration after 24 hours. 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 hours and 40 hours exposure.

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 *Diaphanosoma excisum* and *Moina 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 re-diluted 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 GC-MS and LC-MS (Dr. 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, taking in mind that contamination, if any, could be detrimental to sustaining aquatic life and economical values of these ecosystems, but also to human health.

Conclusions

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 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 non target microorganisms 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 towards 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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Table 1: Main environmental parameters and physico-chemical characteristics of the three reservoirs studied in May 2005 (one sample per reservoir, taken at the central station). Temp: temperature; SRP: soluble reactive phosphorus.

	Basin population (2004)	*max. volume (Mm^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*	27964	43.0	360	6.82	61.9	15.5	6.5	28.4	58.4	1.42	3.5	9.59
Bam#,§	62347	31.0	210	7.12	85.8	59	7.3	27.3	18.0	0.10	3.2	18.64
Koubri#,£	43750	3.5	130	6.90	81.7	63.5	7.2	30.4	17.3	0.04	0.7	14.51

*: reservoir sampled for extraction and concentration of putative toxic compounds.
#: reservoirs sampled for bacterial populations used in ecotoxicological tests.
§: reservoir from which *Monoraphidium* sp. was isolated.
£: reservoir from which zooplankton test organisms were isolated.

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.

Table 3. Reported LOECs (lowest concentrations resulting in significant effects relative to controls) in µg/L for short-term effects of paraquat and deltamethrin on the planktonic microorganisms during this study. n.e.o.: no effect observed. *: maximal concentration assayed was 4.6 µg/L.

	bacterioplankton (12h)			*C. raciborskii*		*Monoraphidium* sp.		*M. micrura*		*D. excisum*
endpoint	bacterial density	CFU	thymidine incorporation	IVF 6 h	IVF 40 h	IVF 6 h	IVF 40 h	survival	growth	survival
paraquat	577	57.7	5.7	183	57.8	57.8	578	577	n.e.o.*	57.7
deltamethrin	4.4	4.4	4.4	n.e.o.	n.e.o.	n.e.o.	443	4.4	2.21	4.4

Figure captions

Figure 1: Effects of paraquat and deltamethrin on bacterioplankton isolated from the Koubri reservoir. A and B: effects of paraquat and deltamethrin respectively, on mean and SD of cell density. Black bars: initial densities (0 h); light grey: after 12 h incubation; dark grey: 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 (CFU). Black bars: initial CFUs (0 h); light grey: after 12 h incubation; dark grey: 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 grey: after 12 h incubation; dark grey: after 24 h of incubation.

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

Figure 3: Acute toxic effects of paraquat (A, B) and deltamethrin (C, D) on *Diaphanosoma excisum* (A, C) and *Moina 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; ** = 1 % confidence level). Chronic toxic effects of paraquat (E) and deltamethrin (F) on *Moina micrura* growth rate after five days of incubation. Significant changes relative to control are marked with asterisks (* = 5 % confidence level; ** = 1 % confidence level).

Figure 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), compare to control without extract additions. Black bars: initial values (0 h); light grey: after 12 h incubation; dark grey: after 24 h of incubation.

Figure 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.

Figure 6: Effects of Loumbila extracts on *in vivo* fluorescence (IVF) of *Cylindrospermopsis 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).

Figure 7: Acute toxic effects of Loumbila extracts on *Diaphanosoma excisum* (A) and *Moina micrura* (B) survival after 24 h incubation (open circles). C: chronic toxic effects of Loumbila extracts on *Moina micrura* growth rate after five days of incubation. Significant changes relative to control are marked with asterisks (*** = 0.1 % confidence level).

Figure 1

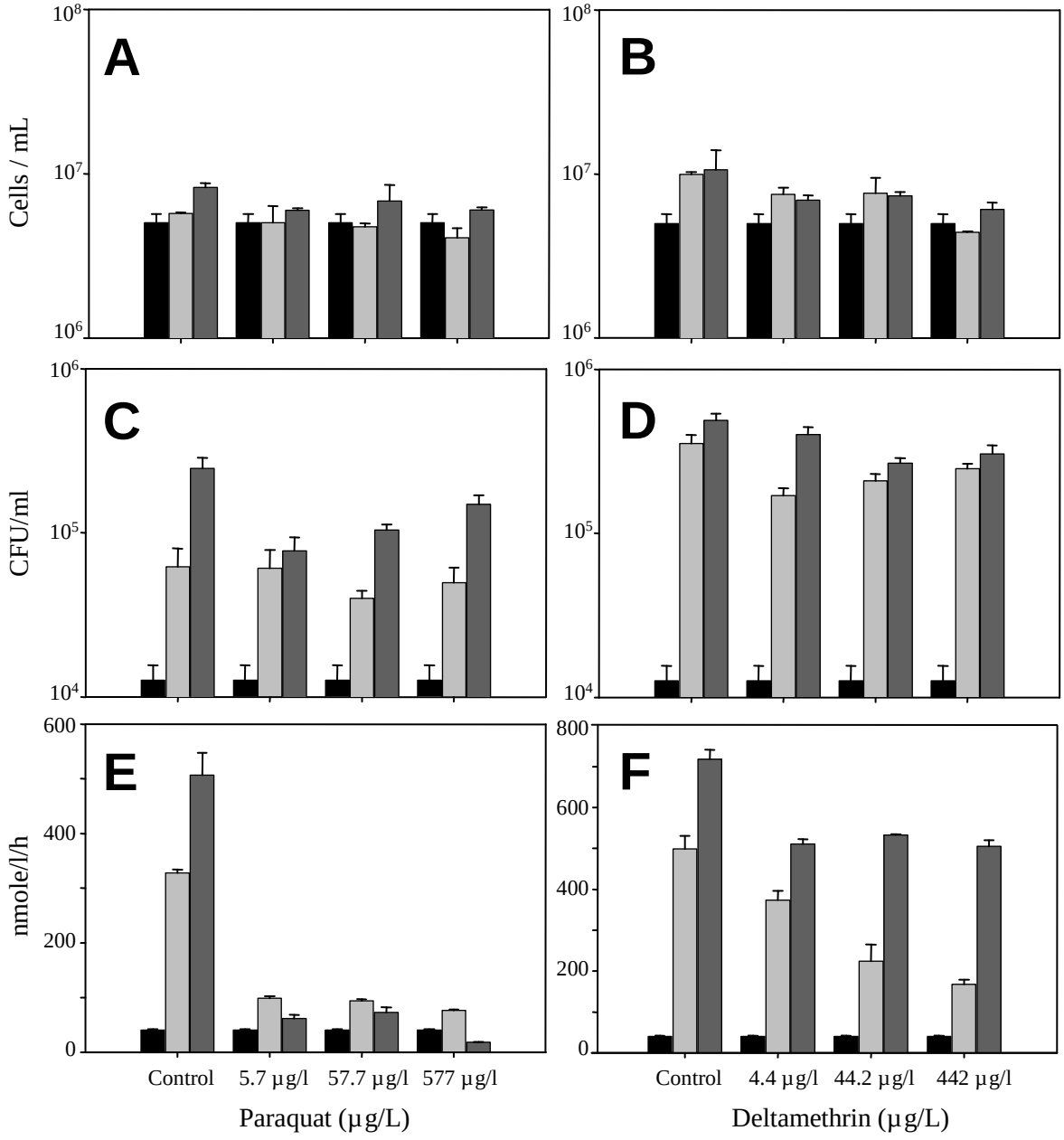
A
B
C
D
E
F
Cells / mL
CFU/ml
nmole/l/h
10^8
10^7
10^6
10^5
10^4
600
400
200
0
800
Control
5.7 µg/l
57.7 µg/l
577 µg/l
Paraquat (µg/L)
Control
4.4 µg/l
44.2 µg/l
442 µg/l
Deltamethrin (µg/L)

Figure 2

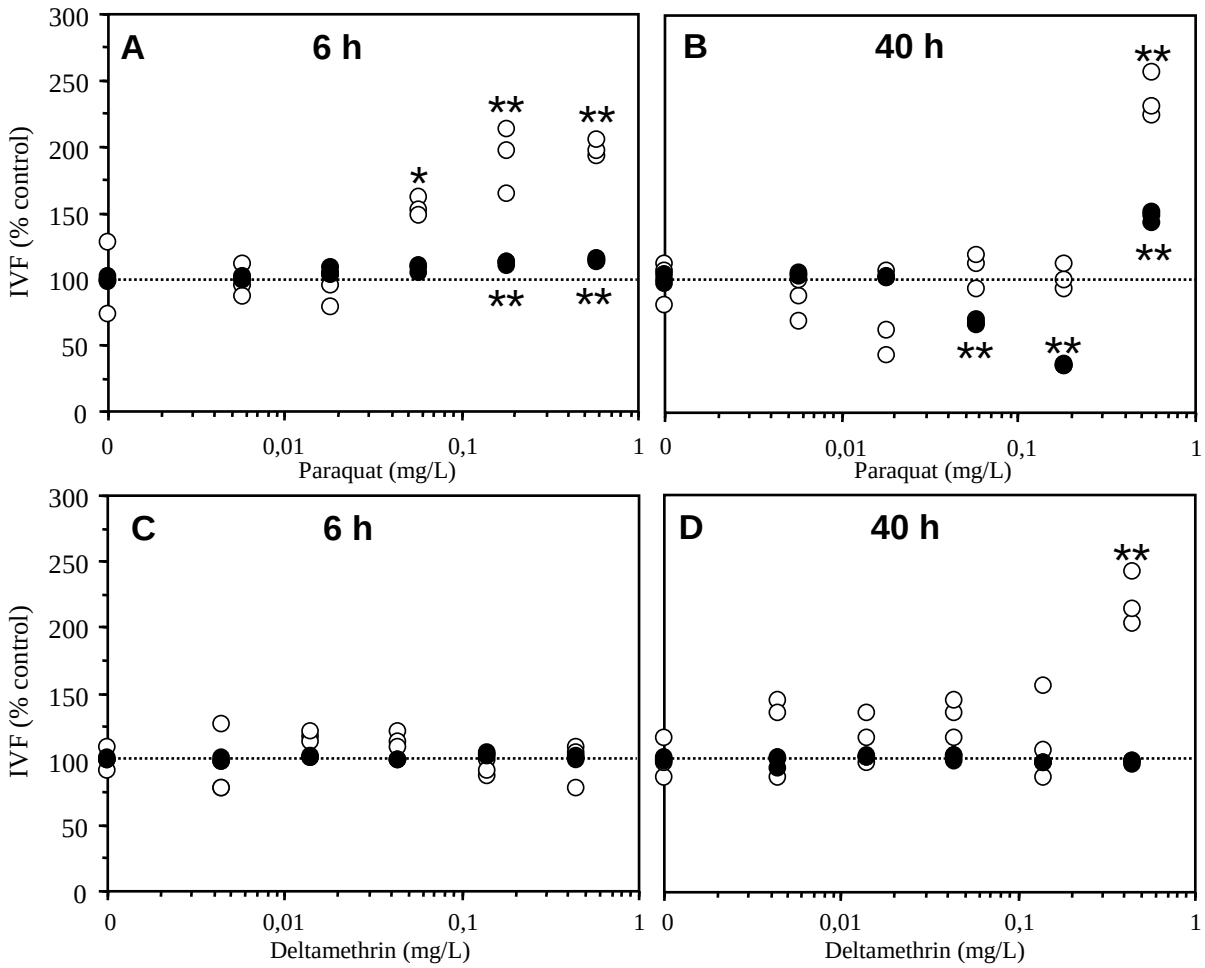
A
6 h
B
40 h
C
6 h
D
40 h
IVF (% control)
Paraquat (mg/L)
Deltamethrin (mg/L)
0
50
100
150
200
250
300
0,01
0,1
1
*
**

Figure 3

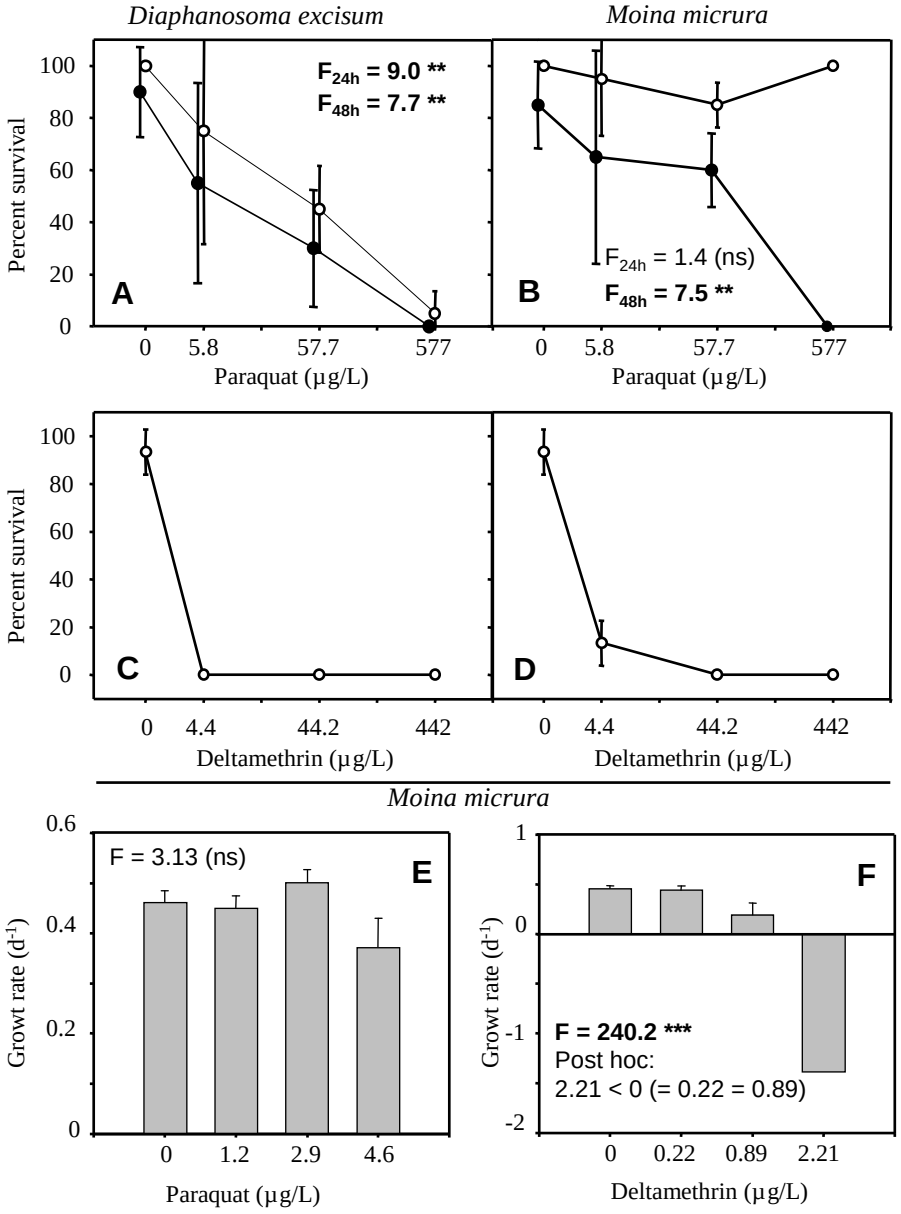
Diaphanosoma excisum
Moina micrura
Percent survival
100
80
60
40
20
0
A
F_{24h} = 9.0 **
F_{48h} = 7.7 **
B
F_{24h} = 1.4 (ns)
F_{48h} = 7.5 **
0
5.8
57.7
577
Paraquat (µg/L)
C
D
4.4
44.2
442
Deltamethrin (µg/L)
Moina micrura
Growt rate (d^{-1})
0.6
0.4
0.2
0
E
F = 3.13 (ns)
1.2
2.9
4.6
F
1
-1
-2
F = 240.2 ***
Post hoc:
2.21 < 0 (= 0.22 = 0.89)
0.22
0.89
2.21

Figure 4

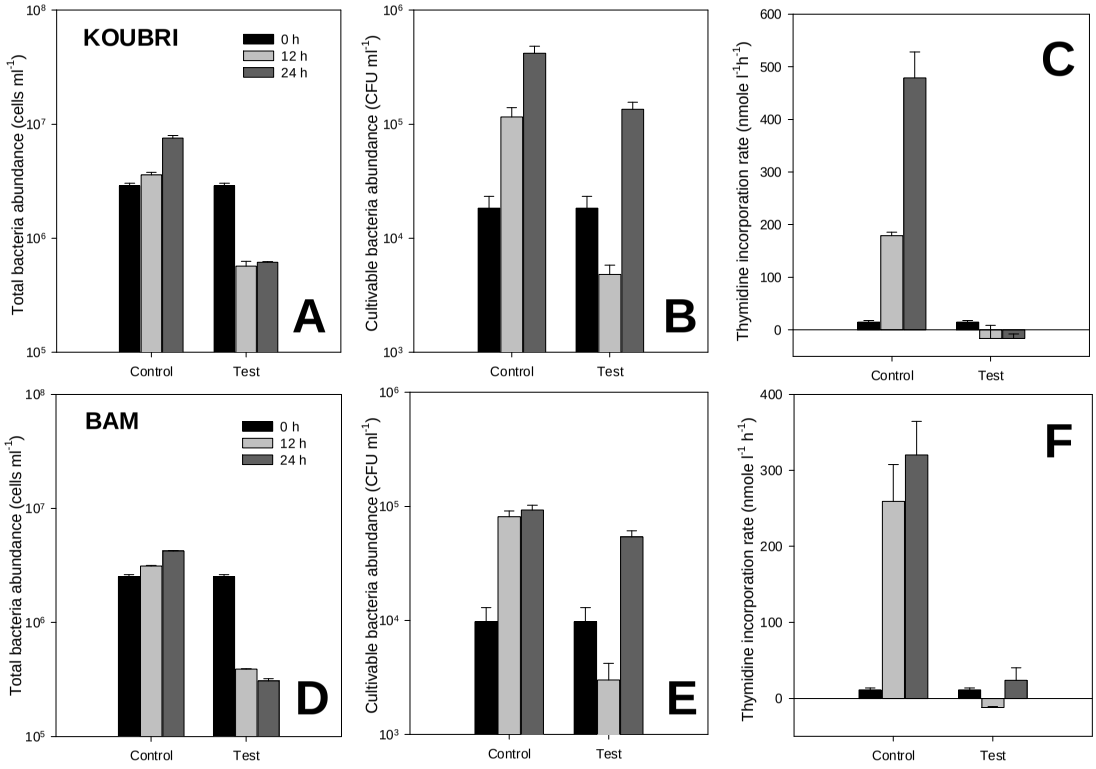
KOUBRI
0 h
12 h
24 h
A
B
C
BAM
D
E
F
Total bacteria abundance (cells ml-1)
Cultivable bacteria abundance (CFU ml-1)
Thymidine incorporation rate (nmole l-1 h-1)
Control
Test

Figure 5

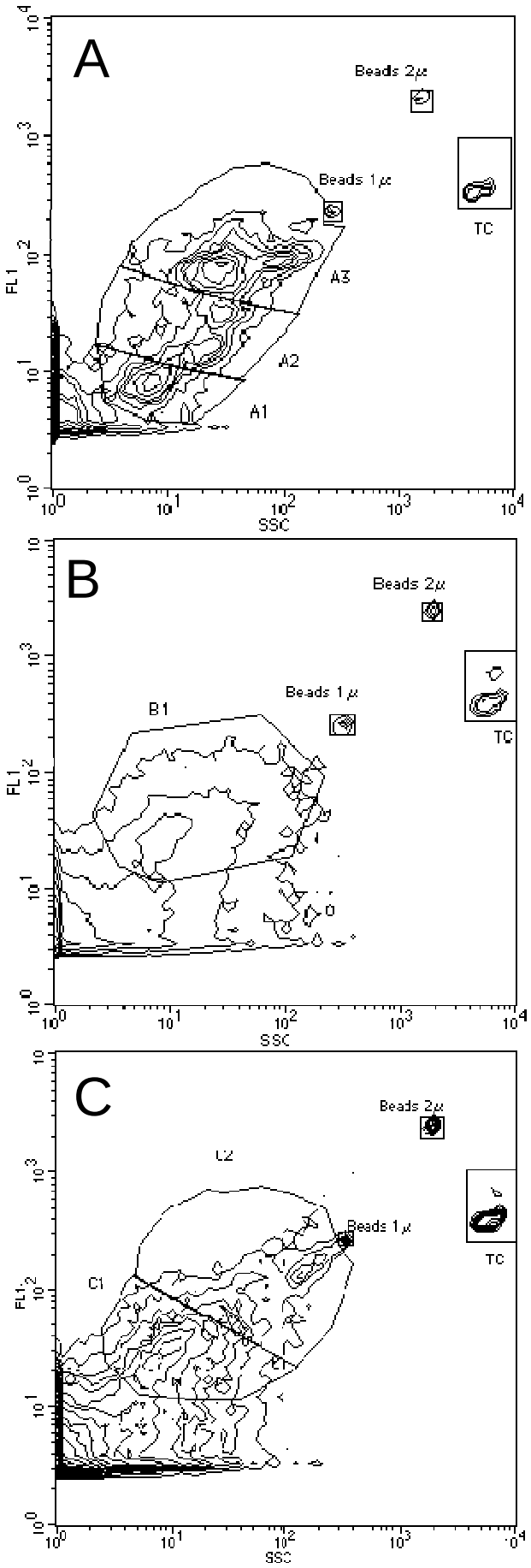
A
Beads 2μ
Beads 1μ
TC
A3
A2
A1
FL1
SSC
B
Beads 2μ
Beads 1μ
TC
B1
FL1
SSC
C
Beads 2μ
L2
Beads 1μ
TC
C1
FL1
SSC

Figure 6

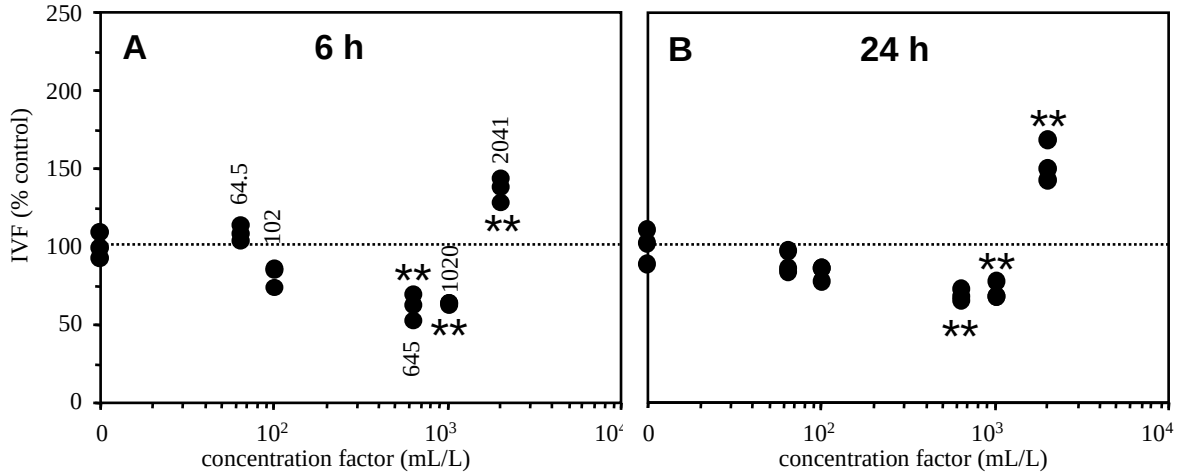

Figure 7