Evaluation of a Battery of Toxicity Tests for Use in the Assessment of Water Quality in a Costa Rican Laboratory

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ABSTRACT: The Laboratory for Ecotoxicological Studies (ECOTOX) of IRET evaluated the following toxicity tests: *Daphnia magna* (daphnia test), *Hydra attenuata* (hydra test), *Allium* sp. (onion test), *Panagrellus redivivus* (nematode test), *Lactuca sativa* (seed test), and the Fluctuation kit. Selection criteria were that the tests should be sensitive to a range of different toxicants, reliable, and preferably of low cost. The tests were evaluated for their reproducibility and sensitivity with 24 blind samples which contained metals, pesticides, and other organic compounds. The hydra, seed, and onion tests were more reproducible when evaluated with a set of samples of a mixture of cadmium and metolachlor. Daphnia and hydra were the most sensitive organisms in our laboratory using mortality or reduction in growth as endpoints. Sublethal effects in the hydra test were useful to detect additional effects. Lettuce seeds and onions performed better than the nematode test for pesticides and other organic compounds. For metals the nematode test was more sensitive than the seed and onion tests. Environmental water samples collected in a banana plantation area were tested with the hydra and seed assays. The hydra test was more sensitive to the pollutants present in these samples. Reproduction in the hydra test was measured as an additional endpoint and differences with the control were observed. This study concluded that short-term bioassays such as daphnia, hydra, seed, and onion tests are promising for screening water quality. Environ Toxicol 15: 312–321, 2000

Keywords: short-term bioassays; reproducibility; sensitivity; environmental samples; water quality

INTRODUCTION

Toxicity testing has long been considered a fundamental tool in the evaluation of water quality. Costa Rica has important water resources, but surface and ground water sources are exposed to a number of pollutants from agricultural, domestic and industrial sources (Astorga and Coto, 1996; Reynolds, 1996). Reynolds (1996) identified agrochemicals and urban waste as major risks for ground water sources in Costa Rica. An average of 16 kg of pesticides is used per hectare of agricultural land in Costa Rica, and many of the most widely used pesticides have been identified in surface waters (De la Cruz, 1998; Castillo et al., 1997; Abarca and Ruepert, 1992; Von Düszeln, 1988). The country has developed facilities to monitor microbiological quality of water but not toxicity testing. Therefore it is important to develop and standardize a battery of toxicity tests for water quality assessment. These tests should be sensitive to different toxicants, reliable, and preferably of low cost. Accordingly the Laboratory for Ecotoxicological Studies (ECOTOX) of IRET joined the

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WaterTox project, sponsored by the International Development Research Centre (IDRC), to engage in an evaluation of a battery of toxicity test for assessment of water quality. A battery of tests was selected for evaluation based on a number of factors, including simplicity, low technological requirements, short duration, and low cost. Additionally the battery should include organisms from different trophic levels. The bioassays selected were the *Daphnia magna* assay (Dutka, 1989), the hydra assay (Trottier et al., 1997), and the nematode toxicity assay (Samoiloff, 1990) which evaluate mortality of the organisms exposed as well as other sublethal endpoints; the *Allium* test (Fiskesjö, 1993) and the seed toxicity assay with *Lactuca sativa* (Dutka, 1989) which evaluate root elongation; and the Muta-ChromoPlate test kit (Fluctuation kit) that evaluates mutagenicity (Rao and Lifshitz, 1995). More details are provided in the first article in this issue.

METHODOLOGY

Six short-term bioassays were evaluated with 24 blind samples, sent by IDRC. The list of compounds evaluated and their dilutions are presented in Appendix 1. The blind samples were analyzed over a 1-year period divided into sets of four samples each for a total of six sets). The compounds tested included heavy metals (arsenic, cadmium, copper, chromium, mercury, and zinc), pesticides (aldrin, *p,p′*-DDT, lindane, metolachlor, and pentachlorophenol) and other organic compounds (aniline, 2,4-dinitrophenol, 4-nitroquinoline-*N*-oxide, and nonylphenol). A group of samples with the same chemical mixture [2 mg/L Cd^{2+} as chloride (Fisher Scientific, anhydrous, in deionized water) and 10 mg/L metolachlor (Riedel-de Haën, 97% in methanol)] were included to evaluate reproducibility of test results. Positive and negative controls were included for the different organisms in each batch of samples; the compounds and concentrations used as positive controls for each test are included in Table I. Each sample was tested at three different concentrations except for the nematode test which used only two concentrations per sample and the fluctuation test which used one or two concentrations per sample. Reconstituted water was prepared with salts provided by IDRC and deionized water from a Milli-Q water purification system (Millipore). This water was used to prepare all chemical solutions and the required dilutions as well as the negative controls. The stock solution used to prepare the seed positive control was prepared using Milli-Q water.

All tests were carried out at ambient temperature (24–28°C) except for the fluctuation test which was conducted in an incubator (35–38°C). A brief description of the specifics of the different tests follows.

TABLE I. Response to reference toxicants for the different bioassays

	Response (in %) to Reference Toxicants							
			Hydra[c]			Nematode[e]		
Sample Number	Onion[a]	Daphnia[b]	Survival	Sublethality	Fluctuation[d]	Survival	Total Fitness	Seed[f]
1	nt[g]	100	92	17	nt	100	70	17
2	57	100	100	28	70	79	85	15
3	65	100	78	100	70	87	87	32
4	55	100	100	0	73	88	91	9
5	41	93	100	33	76	70	0[h]	42
6	nt	100	100	0	81	100	77	25
Mean	54.5	98.8	95	29.7	74	84.8	82	23.3
SD	10	2.9	8.9	37.1	4.6	11.8	8.4	12.2
CV (%)	18	3	9	124	6	14	10	52

[a] Reduction in growth (%) calculated from measurements of root elongation (mm) in each treatment compared with that of the controls, 72 h, 0.5 mg/L Cu^{2+} ($CuSO_4 \cdot 5H_2O$).

[b] Lethality, 48 h, 0.16 mg/L Cr^{6+} ($K_2Cr_2O_7$).

[c] 96 h, 1 g/L NaCl.

[d] Number of positive wells (those showing change in color indicative of a reverse mutation), 120 h, 25 μg/L NaN_3.

[e] 96 h, 5 mg/L Zn^{2+} ($ZnSO_4 \cdot 7H_2O$).

[f] Reduction in growth (%) calculated from measurements of root elongation (mm) in each treatment compared with that of the controls, 120 h, 10 mg/L Zn^{2+} ($ZnSO_4 \cdot 7\ H_2O$).

[g] nt, not tested.

[h] No organisms reached the adult stage.

Daphnia magna Assay (Daphnia Test)

The 48-h static renewal daphnia test (Dutka, 1989) was conducted with a stock of *D. magna* already acclimatized to the ambient temperature conditions in our laboratory. The stock complied with quality parameters such as no ephippia, $\leqslant 10\%$ mortality, time to first brood $\leqslant 10$ days, average number per brood $\geqslant 10$. Only $\leqslant$ 24-h-old neonates were used for testing. Three replicates of 10 organisms per vessel were used for each sample and for the control. Exposures were conducted in 30-mL plastic cups containing 25 mL of solution. Mortality, defined as lack of movement after gentle prodding, was recorded at 24- and 48-h intervals.

Allium Test (Onion Test)

The onion bulbs were provided by Carolina Biological Supply Company (Burlington, NC, USA) and stored in a ventilated area at room temperature (24–28°C) until the tests were begun. The test described in Fiskesjö (1993) was carried out in 15-mL test tubes; six tubes were used for each test concentration and control. Small onion bulbs were carefully unscaled and cultivated on top of the test tubes filled with the solutions. Every 24 h sample dilutions were added to the testing tubes to maintain the proper level. A total renewal of water was carried out after 48 h. One onion of each treatment with the most poorly growing root was removed. Root growth (mm) was recorded after 72 h. The onion test was only conducted for samples 5 to 20. An irregular growth of the onion roots was observed in the controls as well as in the treatments. The onions were possibly treated with a fungicide previous to their exportation to Costa Rica to comply to the country's import regulations. Although this did not seem to affect the results, it would be better for future assays to grow the onions organically in Costa Rica.

Hydra attenuata Acute Toxicity Test (Hydra Test)

H. attenuata (Pallas) is a freshwater microinvertebrate with ubiquitous occurrence in freshwater environments. Culture stock was provided by the Centre Saint-Laurent, Environment Canada (Montreal, Quebec, Canada). The 96-h static bioassay (Trottier et al., 1997) was carried out in 12-well microplates. Three replicates were used for each sample and for the control. Three hydras were transferred to each well containing 4 ml of solution. Sublethal and lethal endpoints were recorded daily. Five types of hydras are identified (Trottier et al., 1997); one is indicative of normal organisms, two are indicative of sublethal effects (clubbed and shortened tentacles), and two are indicative of lethal effects (tulip phase and the posttulip phase which leads to disintegration of the organism). A control of the *Hydra* growth rate (k) was conducted prior to the performance of each set of assays; this was within the normal parameters of $k = 0.3–0.4$.

Muta-ChromoPlate Test Kit (Fluctuation Kit)

This test is a modified version of the traditional Ames fluctuation assay (Rao and Lifshitz, 1995). The test employs a mutant strain of *Salmonella typhimurium*, carrying mutation(s) in the operon coding for histidine biosynthesis. When these bacteria are exposed to mutagenic agents, under certain conditions reverse mutation occurs. The Muta-ChromoPlate method is an alternative assay performed in liquid culture based on multiple yes/no color endpoints. The kits used in this study were provided by the WaterTox project (IDRC). The protocol is described in Rao and Lifshitz (1995), an aseptic working facility is required, but since ECOTOX does not have this facility, a homemade one was put in place with good results according to the sterility check. Samples and controls were dispensed into 96-well microtitration plates and incubated for 5 days at 37°C (35–38°C). The number of positive wells (those that had changed color) were counted after 120 h and compared to those in the background plate which contained only sterile water and bacterial test strain. A sample was considered positive when the number of positive wells in the treated plate was significantly higher than in the background plate. This test was used to evaluate samples 5 to 24.

Panagrellus redivivus Assay (Nematode Test)

Over a 96-h period each stage of the nematodes' four molts will fall within a size range that is a known characteristic of the *P. redivivus* strain bq1 (Samoiloff, 1990). Under adverse environmental conditions the animals will respond by death or a halt in growth. By monitoring a controlled population over their 96-h growth period, both lethal and sublethal effects of a tested sample can be assessed. The stock culture of *P. redivivus* bq1 was provided by the Aquatic Ecosystem Protection Branch of the National Water Research Institute (Burlington, Canada). Maintenance of cultures and the 96-h test were carried out according to Samoiloff (1990). The M9Y medium described in Samoiloff (1990) was used for controls and sample dilution. Exposure was carried out in 2.5-mL flat bottom plastic cups with 10 replicates for controls and samples. Ten organisms in second juvenile stage (J2) were placed in each cup. The organisms were identified by size and counted after 96 h with the aid of a Leica Stereozoom 7. Survival (S), maturation (M), growth (G),

and total fitness values were determined (Samoiloff, 1990) as follows.

$$M = 100 \times [A_{test}/(J4_{test} + A_{test})] \div [A_{control}/(J4_{control} + A_{control})]$$

$$G = 100 \times [(J4_{test} + A_{test})]/S_{test} \div [(J4_{control} + A_{control})/S_{control})]$$

and the total fitness value as

$$100 \times [(4 \times S_{test}) + (2 \times G_{test}) + M_{test}] \div 7$$

where A_{test} and $J4_{test}$ are the number of adults and fourth stage juveniles in the test sample, $A_{control}$ and $J4_{control}$ are the number of adults and fourth stage juveniles in the control, S_{test} is survival value in the tested sample (% of survivors), G_{test} is growth value in the tested sample, and M_{test} is maturation value in the tested sample.

Lactuca sativa Toxicity Assay (Seed Test)

This short term (120-h) root elongation test is carried out in Petri dishes without soils or sediments being included. This bioassay may be performed with any seeds of economically important species that are available and that germinate and grow rapidly. One of the more commonly used species is lettuce (*L. sativa*) which was used in this study. Seeds were provided by IDRC. Upon arrival they were stored in glass vials kept in a desiccator at room temperature. The test was performed in accordance with the protocol described by Dutka (1989). No attempt was made to select seeds of similar size for the assays. Twenty seeds were placed over filter paper moistened with 6–7 mL of samples or controls in Petri dishes. These were covered with aluminum foil and kept in the dark for the duration of the test. After 120 h the number of germinated seeds was counted and the roots were measured (node to tip measurement).

Environmental Samples

A limited number of environmental samples from a banana plantation area in the North Atlantic zone of Costa Rica were tested for toxicity with the seed and hydra tests. Water samples were collected at two sites. The first sampling site (P1) was located in an open canal 100 m from the packing plant just before it flowed into a natural stream. The plant uses approximately 12 L of water per second to cushion the fruit during handling and to clean the fruit and processing facilities (Hernandez and Witter, 1996). Postharvest fungicides are sprayed on the fruit before packing. The plant is equipped with a trap for solid wastes. The second site (S5) was located in a natural stream that receives waters from the drainage canals of a banana plantation. Sediments were collected in S5 and at the conjuction of the Suerte River with the Tortuguero Lagoon in the Conservation Area of Tortuguero (R3) which is located approximately 10 km downstream of the banana production area. Water and sediment samples were also collected from a stream located in the La Selva Biological Station (LS) to be used as controls. Water samples were collected in 4-L glass bottles, transported to the laboratory in an ice chest, and refrigerated upon arrival until the beginning of the tests. Sediments were transported in ice and kept at 4°C until extraction of pore water was carried out in the laboratory.

Interstitial water was extracted from sediments by centrifugation at 1520*g* (Beckman TJ-6). The supernatant was placed in 1 L glass containers and maintained at 4°C until the next day when the assays were started.

In addition to the regular lethal and sublethal endpoints included in the hydra test (Trottier et al., 1997) the total number of organisms (including buds with visible tentacles) was recorded to observe possible reproductive effects. Where mortality was observed the LC_{50} was estimated by the probit method (Stephan, 1977). Results of the reproductive parameter were compared using a one-way analysis of variance (ANOVA) followed by Tukey's test (Statgraphics for Windows, 3.1).

RESULTS AND DISCUSSION

Reproducibility of Each Bioassay Toward Their Reference Toxicants

No mortality above 10% occurred in any of the negative controls. Results for the positive controls are shown in Table I. The lethal response of the hydra to 1 g/L NaCl was low and would indicate the need to adjust the concentration of this compound or change the positive control chemical. Sublethal response of the hydra was in general also low, ranging between 0 and 33%. The result was very different during the test carried out in May 1998, with 100% sublethal effects. At the moment we have no explanation for this response since growth rate for hydra appeared to be normal. The daphnia test showed an almost constant 100% mortality when exposed to 0.16 mg/L chromium (from $K_2Cr_2O_7$), also indicating the need for adjustment of the positive control in our laboratory.

The response of the lettuce seeds to the reference toxicant was in general also low with only one value close to 50% in growth reduction. The results of the onion bioassay showed responses closer to 50%. The variability of the seed bioassay calculated with the values of growth reduction is very high (Table I); however, the variability [coefficient of variation (CV)] calculated using the root length (mm) is 10% for the positive control and 12.7% for the negative control. A high variability in root elongation within each treatment was observed in the lettuce seed root elongation assay, and this could influence the variability and sensitivity of the test. The intrinsic variability between seeds could be better controlled with a selection of seeds of similar size to carry out the assay. Additionally the assay could start with 22–24 seeds instead of 20 and eliminate the extreme results (smallest and largest).

The nematode test measured three different endpoints: growth, survival, and maturation, the three are combined in a single measure, total fitness. The smallest CVs were obtained with the survival endpoint and the total fitness integrated value. The growth and maturation endpoints showed highly variable results (CV = 64.1 and 81%, respectively). Technical difficulties in counting the organisms and assessing their size could have influenced this result.

The fluctuation test had a good response to its positive control and a low variability (CV = 6%). All of the tests with the reference toxicant were significantly different from the background plates.

Reproducibility of the Bioassay's Responses to a Metolachlor and Cadmium Mixture

The results of assays using the five samples containing the mixture of cadmium (2 mg/L) and metolachlor (10 mg/L) to the different assays are shown in Table II. The hydra and daphnia tests were the most sensitive to this mixture, whereas the nematode test was the least sensitive. The hydra test had total mortality at 50 and 100% concentration of the mixture. At the lowest concentration tested (10%) the results showed higher variability. Difficulties in correctly identifying one of the sublethal types (clubbed tentacles) could have had an influence on this variability. Improvement of the identification of sublethal types could increase the sensitivity of the test. In the daphnia test the first three repetitions of the mixture sample had similar results for the highest concentration tested (range 87–93% mortality) but showed lower mortality in the last two tests (40 and 60%). This test had the highest variability with the exception of the percentage of maturation of the nematode test which again had the highest variability (CV = 63%). However, the daphnia test showed a good dose-response curve for the three concentrations tested each time. Lettuce seeds were more sensitive than the onions to this mixture.

The nematodes did not show a consistent response to the mixture of cadmium and metolachlor, only samples 5 and 17 were sensitive in terms of mortality (35 and 42%) while the other repetitions showed a low

TABLE II. Reproducibility of the bioassay's responses to a metolachlor and cadmium mixture

	Response (in %) to Highest Sample Concentration[a]							
			Hydra (96 h)			Nematode (96 h)		
Sample Number[b]	Onion (72 h)[c]	Daphnia (48 h) Lethality	Lethality	Sub-lethality	Fluctuation(120 h)[d]	Total Fitness	Survival	Seed (120 h)[c]
5	28	93	100	100	−	72	65	63
9	47	87	100	100	−	81	85	55
13	43	93	100	100	+	86	84	54
17	39	40	100	100	−	68	58	56
22	nt[e]	60	100	100	+	67	91	49
Mean	39.3	74.6	100	100		74.8	76.6	55.4
SD	8.2	23.7	0	0		8.3	14.3	5
CV (%)	21	32	0	0		11	19	9

[a] The highest sample concentration was 100% for the onion, hydra, and seed tests, 50% for the nematode and fluctuation tests, and 10% for the daphnia test (Appendix 1).

[b] Mixture of 2 mg/L Cd^{2+} as chloride [Fisher Scientific (anhydrous) in deionized water] and 10 mg/L metolachlor (Riedel-de Haën, 97% in methanol).

[c] Reduction in root elongation (%) calculated from measurements of root elongation (mm) in each treatment compared with that of the controls.

[d] A sample is considered positive when the number of wells that change color in the treated plate is significantly higher than in the background plate. Sample number 22 was tested with a 25% dilution.

[e] nt, not tested.

mortality (9–16%). The growth parameter had a lower variability (22%) with the mixture metolachlor/cadmium than with the reference toxicant (64%). As mentioned previously technical difficulties might be responsible for these results.

The Fluctuation test also had a very low reproducibility and only samples 13 and 22 were positive. Since the response to the positive control was good and uniform, the variable response to the toxic mixture could be an indication of operational problems. However, many of the laboratories that participated in the evaluation had similar variable results (see articles in this issue).

Sensitivity to Different Toxicants

The six bioassays were ranked for sensitivity according to their response to the different compounds. The compounds were tested at different concentration levels (Appendix 1), and some bioassays showed no response to some compounds at the highest concentration tested. The best option was to rank tests according to response of organisms to the highest concentration tested. Dilution of samples in each particular case was accounted for. The response to the lower concentrations tested was used to differentiate between similar responses at the highest concentrations. Number 1 was used for the most sensitive assay and number 5 for the least sensitive assay; numbers were repeated for tests that showed similar sensitivities. The compounds were grouped in three categories: metals, pesticides, and organics. A ranking value was calculated for each group of chemicals. According to our results the daphnia test was the most sensitive to the largest number of chemicals in the three groups followed by the hydra test. Daphnia and hydra ranked first for metals and organics. For the pesticides tested, which included aldrin, DDT, lindane, metolachlor, and pentachlorophenol, the daphnia test was the most sensitive. The seed and hydra tests followed, although they were much less sensitive than the daphnia test. Daphnia organisms were not sensitive to dinitrophenol and hydra were not sensitive (using mortality as an endpoint) to aldrin, aniline, arsenic, DDT, dinitrophenol, pentachlorophenol, and zinc. However, sublethal effects were observed in the case of the hydra exposed to aldrin, aniline, arsenic, and zinc. The daphnia test showed good dose-response curves for all compounds at the three concentrations tested.

Seeds were more sensitive to arsenic, DDT, and zinc than the hydra. A reduction in root growth of 50% or more in the highest concentration tested (in general 100% of the sample) was also observed with compounds such as lindane, metolachlor, nitroquinoline, and potassium dichromate. According to our results, the seed test was not sensitive to dinitrophenol (1 mg/L), nonylphenol (20 mg/L), mercury, and cadmium (5 mg/L). In general the seed test and the onion test had similar results. The onion test responded better to compounds like pentachlorophenol and the seed test to DDT. The seed test was the most sensitive test to the mixture of arsenic and lindane. The onion test was sensitive to metals such as copper, mercury, and arsenic and to pesticides such as metolachlor and pentachlorophenol. It was also sensitive to organics such as aniline and nitroquinoline.

In general the nematode test (using mortality as an endpoint) was not sensitive to organic compounds (with the exception of dinitrophenol) nor to the pesticides. However, it showed more sensitivity to metals than the seed and the onion test. With more experience using this test, perhaps some of the other endpoints (growth or maturation) could be used and prove more sensitive to other toxicants.

Responses of the Hydra and Seed Tests to Environmental Samples

Results are shown in Tables III to VI. The water samples collected at the effluent of the banana packing plant (P1) in April and May 1997 were found to be toxic in the hydra test, the 50% lethal concentration (LC_{50}) was calculated as 9.23–9.96% of the effluent concentration (Table III).

No toxicity was observed when the seed test was used to evaluate one of the samples collected at the packing plant (Table IV) in May 1997. A sample collected at the same site at a different date (P1, February 1997) and tested at three different sample dilutions showed no significant growth reduction either (Table V). In a study conducted in the North Atlantic Region of Costa Rica, 97% of the samples collected at two packing plants (including P1) showed pesticide residues. Sixty-one percent of the samples had detectable levels of three to five pesticides, including compounds such as chlorpyrifos, imazalil, propiconazol, and thiabendazole (Castillo et al., 2000). Other compounds used in the packing plant are aluminum sulfate, disinfectants, and banana latex (Hernandez and Witter, 1996).

Interstitial water (pw) extracted from sediments collected at the Conservation Area of Tortuguero (R3) was toxic to the hydra (LC_{50} 57.4% of effluent concentration) and also affected seed growth (36.5% growth reduction) (Tables III and IV). Pesticides have been found in surface waters collected at this site (Castillo et al., 2000), but further toxicity and chemical analyses should be conducted.

The sublethal endpoint was able to detect toxicity in more diluted samples than the lethal endpoint (Table VI). The total number of organisms in sample P1 (May

TABLE III. Toxicity of various environmental samples as assessed by the hydra test

	Environmental Samples		
Sampling Site	Date of Sampling	LC_{50} as % of Sample[a]	95% Confidence Limits
P1	1-04-97	9.96	8.0–12.4
P1	15-05-97	9.23	8.3–10.3
S5 (pore water)	15-04-97	No observed sublethal or lethal effects	—
R3 (pore water)	15-04-97	57.4	43.3–76.1

[a] LC_{50} calculated with trimmed Spearman-Karber method, $\alpha = 10$.

TABLE IV. Toxicity of various environmental samples as assessed by the seed test

	Surface Water			Pore Water		
	Mean Root Elongation (mm)	SD	% of Growth Reduction	Mean Root Elongation (mm)	SD	% of Growth Reduction
Control[a]	49.4	13.8		43.3	19.6	
Sample:						
S5 (15-04-97)	44.7	18.9	9.5	46.7	12.6	7.9[b]
R3 (15-04-97)	nt[c]	—	—	27.5	8.3	36.5
P1 (15-05-97)	48.9	15.0	1.0	nt	—	—

[a] Control surface and pore water was obtained from a stream in La Selva Biological Station, located in the North Atlantic region of Costa Rica.
[b] Growth increase.
[c] nt, not tested.

1997) also showed a decrease compared to the controls (Table VI) although the results were not statistically significant. No sublethal effect was observed at this same dilution, indicating that the reproductive endpoint could be more sensitive. The number of samples evaluated was too limited and additional assays should be conducted to assess the potential of this endpoint.

The seed test might prove more useful in assessing samples collected in agricultural areas such as in the rice culture, where more herbicides are being used. Studies conducted at IRET have reported the presence of herbicides in surface waters in areas influenced by rice production (De la Cruz et al., 1998; Castillo et al., 1997).

We are unaware of any study in banana plantation areas where bioassays were used to evaluate toxicity of surface waters and effluents of packing plants. The bioassays evaluated in this study could be useful in the assessment of environmental quality of waters, especially when a battery of organisms from different trophic

TABLE V. Results of seed bioassay with diluted series of an environmental sample

	Sample P1 (27-02-97)		
Sample Concentration	Mean Root Elongation (mm)	SD	% of Growth Reduction
Control[a]	45.0	14.4	
32%	37.9	15.9	15.9
56%	41.3	11.5	8.2
100%	39.2	15.5	12.9

[a] Control surface and pore water was obtained from a stream in La Selva Biological Station, located in the North Atlantic region of Costa Rica.

TABLE VI. Analysis of toxicity of environmental samples using *H. attenuata* (reproduction endpoint)

Sample	Sample Concentration (%)	Survival (%)	Sublethal Effects (%)	Reproduction (Total Number of Organisms)
Control LS (15-5-97)	100	100	0	15
Control (pw) LS (15-5-97)	100	100	0	16
S5−pw (15-4-97)	100	100	0	14
R3−pw (15-4-97)	100	0	100	0
	50	70	70	0
	25	100	0	15
	12.5	100	0	14
	6.25	100	0	12
	3.12	100	0	16
	1.56	100	0	12
Control	100	100	0	15
P1 (15-5-97)	100	0	100	0
	50	0	100	0
	25	0	100	0
	12.5	8	92	0
	6.25	100	0	9
	3.12	100	8	13
	1.56	100	0	13
Control	100	100	0	18
P1 (1-4-97)	100	0	100	0
	50	0	100	0
	25	0	100	0
	12.5	20	100	0
	6.25	100	0	13
	3.12	100	0	13
	1.56	100	0	12
Control	100	100	0	14

levels are used. With the results obtained in this study we propose a basic battery for environmental samples including the daphnia, hydra, and seed tests. The inclusion of relevant native organisms in a battery of test would be useful for environmental impact assessment purposes. This is especially true for those countries with climatic conditions different from those where the original tests were developed.

CONCLUSION AND RECOMMENDATIONS

Daphnia and hydra were the most sensitive test organisms in our laboratory when using mortality (daphnia, hydra, and nematode) or reduction in growth (onions and seeds) as endpoints. Sublethal effects in the hydra test proved useful for detecting toxic effects of certain chemicals. Seed and onion tests performed better than the nematode test for organics and pesticides. For metals the nematode test was more sensitive than seed and onion tests. The hydra, seed, and onion tests showed more reproducibility when evaluated using a blind set of samples of the same mixture of toxicants.

The onion, seed, and Fluctuation assays have the advantage of not involving any cultures. The daphnia, hydra, and nematode assays are more time-consuming and require culture of the organisms. However, considering the sensitivity of the daphnia test, it should be included in any battery of tests to evaluate water quality. The response of the hydra test to environmental samples proves its usefulness in the assessment of water quality. In particular the reproduction endpoint seems to be promising and thus additional assays should be carried out to further test its potential. Extending the duration of the assay to 120 h when using the reproduction endpoint should also be considered.

The seed assay should be included in a battery of test for water quality evaluation because of (a) the convenience of using seeds, (b) their sensitivity to some toxicants, and (c) information obtained from another trophic level. At this point the inclusion of the nematode test does not seem convenient. With more experi-

ence and further evaluation it might prove useful under some conditions, for example, in waters polluted with metals and in agricultural areas where nematicides are used.

Problems with the Fluctuation kit should be addressed before further testing. It is also an expensive test and we would not recommend its inclusion in a basic battery at this time. However, means to detect mutagenic substances should be considered, especially in areas with intensive use of potentially mutagenic compounds.

This study concluded that short-term bioassays such as daphnia, hydra, seed, and onion tests are promising for screening water quality. A further evaluation of the different assays with different types of environmental samples will be valuable. The inclusion of chronic tests to evaluate drinking water quality is necessary since toxicant levels present in these waters are expected to be low.

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Appendix 1. Sample concentrations (%) for the different bioassays[a]

Sample ID	Toxicant	Sample Concentrations (%)				
		Onion	Seed	Daphnia	Hydra	Fluctuation
1	2,4-Dinitrophenol	100, 10, 3	100, 60, 30	100, 50, 25	100, 50, 10	2.5, 12.5 mL
2	Cu^{2+} as sulfate	100, 10, 3	100, 60, 30	(1:100)[b] 100, 50, 10	(1:30)[b] 100, 50, 10	12.5 mL
3	Cd^{2+} as chloride	100, 10, 1	100, 60, 30	16, 12, 10	100, 50, 10	2.5, 15 mL
4	Cd^{2+} as chloride (1 mg/L) + metolachlor (5 mg/L)	100, 10, 3	100, 50, 10	40, 35, 30	100, 50, 10	2.5, 15 mL
5, 9, 13, 17 and 22	Cd^{2+} as chloride (2 mg/L) + metolachlor (10 mg/L)	100, 25, 5 100, 50, 20	100, 75, 50 100, 75, 50	10, 1, 0.1 10, 2, 0.5	100, 50, 10[c] 100, 50, 10	2.5, 10 mL 5 mL
6	K_2CrO_7 total chromium	100, 25, 5	100, 50, 25	5, 1, 0.1	100, 50, 10	(1:4)[b] 2.5 mL (1:5)[b] 2.5 mL
7	Aniline	100, 50, 5	100, 50, 10	10, 1, 0.1	100, 50, 25	(1:2)[b] 10 mL
8	Zn^{2+} as sulfate	100, 25, 5	100, 60, 30	2, 0.1, 0.02	100, 50, 25	2.5, 12.5 mL
10	Metolachlor	100, 5, 1.5	100, 50, 25	100, 80, 50	100, 35, 10	5, 12.5 mL
11	Cu^{2+} as sulfate	(1:2)[b] 100, 10, 2	100, 75, 50	1, 0.5, 0.1	(1:160)[b] 100, 50, 10	(1:2)[b] 2.5 mL
12	4-Nitroquinoline-*N*-oxide	100, 50, 5	100, 50, 30	80, 50, 10	(1:2)[b] 100, 50, 25	(1:50)[b] 2.5, 10 mL
14	Hg^{2+} as chloride	100, 50, 5	100, 75, 50	5, 1, 0.1	(1:64)[b] 100, 50, 25	(1:45)[b] 2.5, 7.5 mL
15	Nonylphenol	100, 50, 5	100, 75, 50	5, 1, 0.1	(1:16)[b] 100, 50, 25	2.5, 10 mL
16	Pentachlorophenol	100, 50, 5	100, 75, 50	70, 30, 10	100, 25, 5	2.5 mL
18	Aldrin	100, 50, 5	100, 75, 50	2, 0.5, 0.1	100, 50, 3.125	5, 15 mL
19	As^{2+}	50, 5, 1.5	100, 50, 10	40, 10, 1	100, 50, 25	5 mL
20	*p,p′*-DDT	100, 50, 1.5	100, 75, 25	25, 10, 1	100, 75, 50	5, 15 mL
21	4-Nitroquinoline-*N*-oxide	100, 50, 10	100, 50, 30	75, 40, 10	75, 50, 10	(1:50)[b] 2.5, 12.5 mL
23	Pentachlorophenol and As^{2+}	100, 50, 5	100, 50, 15	50, 10, 0.8	100, 50, 25	(1:2)[b] 2.5 mL Undiluted 5 mL
24	Lindane	100, 50, 10	100, 75, 40	50, 10, 0.8	100, 50, 25	2.5, 10 mL

[a] The nematode test used the same sample dilutions (10 and 50%) for every compound.
[b] Initial dilution.
[c] There was an initial dilution of 1:20 for sample 5.

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