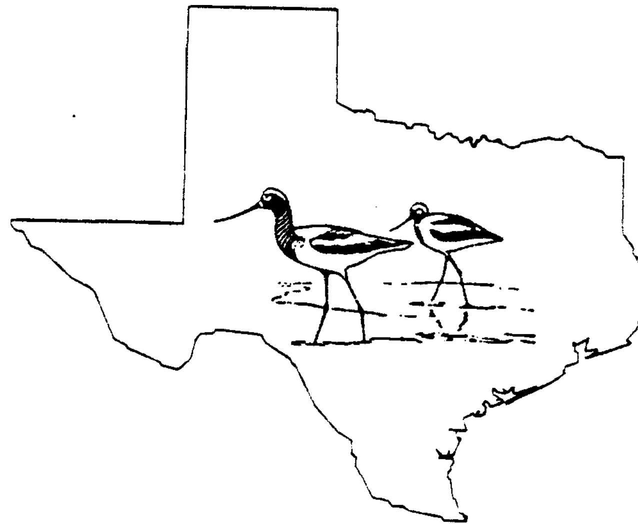
U.S. Fish and Wildlife Service
Region 2
Contaminants Program

CONTAMINANTS INVESTIGATION OF IRRIGATION DRAINWATER IN THE LOWER RIO GRANDE VALLEY, TEXAS

by
Stephen M. Robertson
and
Lawrence R. Gamble



U.S. Fish and Wildlife Service
Fish and Wildlife Enhancement
Corpus Christi Field Office
Campus Box 338, 6300 Ocean Drive
Corpus Christi, Texas 78412

December 1991

CONTAMINANTS INVESTIGATION OF IRRIGATION DRAINWATER IN THE LOWER RIO GRANDE VALLEY, TEXAS, 1990

Regional Study ID
R2-90-2-102

Prepared By

U.S. Fish and Wildlife Service
Ecological Services
Corpus Christi, Texas

Authors

Stephen M. Robertson
Lawrence R. Gamble

Reviewed By

Thomas W. Schultz
Field Contaminants Specialist

Under the Supervision of

Rogelio Perez
Field Supervisor

December 1991

ABSTRACT

An extensive study focusing on irrigation drainwater was carried out in the vicinity of Laguna Atascosa National Wildlife Refuge in the summer of 1990. During three sampling periods of 5 days each, up to 20 samples per day were collected for Microtox[R] testing, along with basic water quality measurements from each regular site. Sampling periods coincided with intensive aerial applications of pesticides and herbicides on cotton and other crop fields in this predominately agricultural region. Rainfall, and consequently runoff, was lower than anticipated, and perhaps accordingly, no dramatic indications of toxicity were observed for any of the 257 samples screened. The most commonly observed effect was a modestly to substantially greater light output in samples relative to controls. This increased light output may be an indication of a nutrient effect, or it may be a result of stimulatory effects (hormesis) caused by exposure to low levels of potentially toxic agents. An additional study is recommended to more accurately assess the water and sediment quality of these agricultural drains.

Key words: Agricultural drainwater, bioluminescent bacterial assay, Lower Rio Grande Valley, Texas.

INTRODUCTION

The Lower Rio Grande Valley of south Texas is a rich agricultural area supporting intensive production of vegetables, fruit, grain sorghum, and cotton. Current practices involve the combined use of irrigation, with the application of large amounts of fertilizers, pesticides, and herbicides, to maximize crop yields. The principal source of water for irrigation is the Rio Grande River, and irrigation runoff is collected in drainage ditches that eventually discharge into the Laguna Madre. Although such drainage ditches on the fringes of farm fields may accumulate significant levels of agricultural chemicals, they are frequently overgrown with brush, forming protective corridors for wildlife movement, nesting, and foraging, and thus provide significant wildlife habitat.

Previous studies have shown elevated levels of organochlorine pesticide residues and other contaminants in the study area (White et al. 1983, Gamble et al. 1988, Wells et al. 1988). Although many chemicals of concern are hydrophobic and will quickly bind to sediments, the contaminants are frequently redistributed by biological activity and by resuspension of sediments during flood events. Ahr (1973) found elevated levels of DDT in sediments over 1 meter deep at Laguna Atascosa National Wildlife Refuge, which receives large amounts of irrigation drainwater.

Unlike organochlorines, most pesticides currently in use are relatively short lived in the environment. As a result, chemical analysis of environmental samples rarely provides sufficient information to determine whether these chemicals are having an impact on fish and wildlife resources. In addition, due to the highly toxic nature of many of these new pesticides, wildlife managers need a tool to rapidly evaluate water quality. Such a tool could possibly allow them to divert irrigation drainwaters away from important wetlands at critical times. The objective of this study was to evaluate a method of cost effective, rapid evaluation of water-borne contamination that could serve as a management tool for refuge managers in the Lower Rio Grande Valley.

The method evaluated in this study was a bacterial bioluminescent bioassay which measures light output inhibition of the luminescent bacterium *Photobacterium phosphoreum* using Microtox[R1] methodology. Microtox[R] testing is a simple, inexpensive, and rapid means to measure potential toxicity of solutions, making it particularly useful as a screening tool. Since the introduction of the Microtox[R] system for assessing complex industrial effluents in 1979, its application has been extended to determine the toxicity of aquatic pollutants, wastewaters, fossil fuel process waters, mycotoxins and other chemicals. Some governmental regulatory agencies employ Microtox[R] screening tests to monitor for environmental problems (Somasundaram et al. 1990). In this study, only Microtox[R] screening and basic water quality measurements were carried out. No additional bioassays nor chemical residue analyses were included in this study.

---

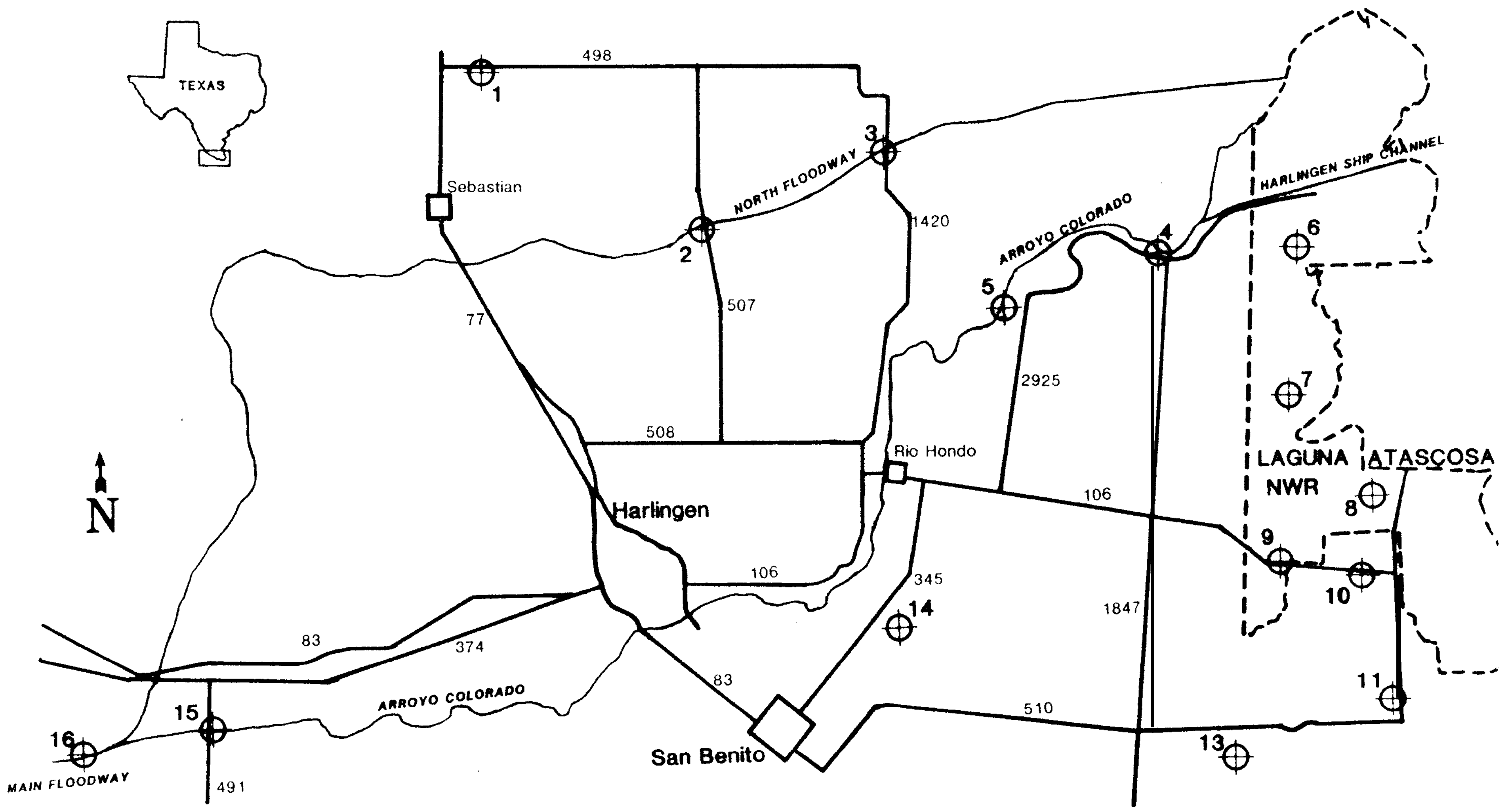
[1] Mention of the Microtox[R] tradename does not constitute endorsement of the product by the U.S. Fish and Wildlife Service.

METHODS AND MATERIALS

The study area, located in the Lower Rio Grande Valley of south Texas, is shown in Figure 1. Water samples were collected from irrigation drainwater ditches and other bodies of water receiving agricultural runoff (Figure 2). During three, 5-day periods in the summer of 1990 (July 9-13, July 23-27, and August 6-10) samples were taken daily from up to 15 regular sites (Appendix 1) along with selected samples being taken to spot check additional sites of suspected contamination. Sampling periods coincided with heavy aerial applications of pesticides and herbicides on cotton and other crops. Chemically cleaned, 60 ml capacity, amber colored glass jars were used for sample containers. Water samples were collected by submersing an inverted jar, removing the lid underwater, and righting the vessel to allow it to fill at a depth of 10 to 15 cm below the water surface. Samples were then labeled and placed on ice for bioassay analysis later that same day. Time elapsed between sample collection and Microtox[R] screening was a maximum of 12 hours.

Basic water quality parameters were recorded from the sample site at the time the sample was collected. Dissolved oxygen, temperature, pH, salinity and conductivity were measured, and the hour of sample collection and relative water level were also noted. Relative water levels were recorded from a wooden yardstick implanted vertically in submersed mud at the sample site. Unfortunately, several of these yardsticks were taken, and the subsequent data lost, during the course of this study.

The Microtox[R] photobacterial bioassay was used to screen all water samples. Measurements were made with a Microtox[R] Model 500 toxicity analyzer, a form of temperature-controlled biophotometer. This assay, relying on the relative light outputs of Photobacterium phosphoreum, has been well-documented (Bulich 1979, Ribo and Kaiser 1987). Results are expressed as a median effective concentration (EC50), the concentration that causes a 50% reduction in light emission. A modified 100% concentration Microtox[R] procedure was used to streamline screening of samples (Appendix 2) while enhancing the sensitivity of the screen. However, the 100% screen is prone to pipetting 'noise' because no initial (prior to exposure to the test solution) light levels are taken. To reduce errors pertaining to technique variability, a system of replicated blanks and samples was used. These replications do not change the test, but merelv increase the reliability of the results. Of the 30 sample wells in the Microtox[R] unit, three sets of three blanks (at the beginning, middle and end of the network) were used. The light sensitivity was set by the first blank, and the blank reference value used was an average of the 9 blank readings. Readings for all samples were taken after exposing the bacteria to the test solution for 5 and 15 minutes, and most samples were also measured after 30 minutes. Samples were prepared at nominal 100% solution (actually 90% after Microtox Osmotic Adjustment Solution), in sets of three in series. Using the whole grid, 7 samples (with 3 replicates each) were tested at a time. Since no EC 50 data are generated with this procedure, results only indicate if a problem exists, as determined by the average of the blanks (n=9) compared to the average of each of the sample readings (n=3). A light output reduction was interpreted as an indication of some toxicity, at which point the sample was processed through the standard 100% Microtox assay with 4 dilutions to generate EC 50 data. This standard assay is described in Microbics instruction manuals (Microbic8 Corp. 1987a). When EC 20 was not calculable, or when EC 20 was above 100%, samples were considered non-toxic (Ankley et al. 1989). When EC 50 values below 45% concentration were found, the standard Microtox[R] procedure was followed to generate a more accurate effective concentration curve (Microbics Corp. 1987b). The standard procedure virtually eliminates pipetting 'noise' type errors, because before and after exposure readings are taken for all samples.



Sample Sites for Microtox Investigation of Irrigation Drainwater

RESULTS AND DISCUSSION

Water quality averages and ranges for each site are presented in Table 1. All water **quality** data fell within appropriate ranges of **pH** and salinity for Microtox[R] testing **as** freshwater samples. Maximum salinity measured was 18 ppt, and **pH** ranged from 7.30 to 9.30 standard units. Dissolved oxygen (DO), **pH** and temperature varied with collection hour, as would be expected. Turbidity and **coloration** of water samples was negligible, so no adjustments were made prior to Microtox[R] testing.

Rainfall was lower than anticipated during the study period, and consequently **agricultural runoff** was below normal. A total of 257 samples were tested with the Microtox[R] assay from regular sites and the additional sites that were spot checked; eighteen resulted in relatively minor reductions **of** light output relative to controls, and were therefore re-tested using the standard 100% procedure with 4 dilutions. Only one of these was sufficiently toxic to generate EC 50 data, and only at the shortest time interval. This sample was from site 1 on July 11, 1990. An EC 50 value of 139.4% and EC 20 value of 63.3% were generated. Even though confidence intervals for the 100% test are expected to be about 10 times broader than for the standard test (Tarkpea and **Hansson 1989),** 95% confidence intervals for this particular sample were inordinately large, **0.13-147,038%** and **1.61-2,492%** for the EC 50 and EC 20, respectively, so we hesitate to draw inferences from this one 'hit'. Differences in light output at different time intervals (5, 15, and 30 minutes) were minor. Light output from samples generally declined only slightly overthesetime intervale, whereas blank samples always decreased substantially.

The most commonly observed result in our testing (81% of samples) was a consistent higher light output from samples relative to control blanks. There are two plausible explanations for this. One possibility is that the observed increased light emissions are the result of a nutrient effect, where in the absence of toxicante, substances in the sample enhance the media for increased bacterial metabolism. This same phenomenon was observed in 37% of samples from the Detroit River by Ribo et al. **(1985),** and they attributed it to possible nutrient enhancement; however, nutrients ehould not be a limiting factor to blanks. Another possibility is that of hormeeia (etimulatory effects) which are often caused by exposure to low levels of potentially toxic agents (Stebbing 1982, Microbic8 Corp. 1991). The bacteria may be raising their metabolic rate, and consequently, their light output, due to a tendency to overcorrect for low levels of inhibitory challenge. Under this hypothesis, it would appear that most samples tested in this study carried sub-effective concentrations of undetermined toxicants.

Microtox[R] was judged more sensitive than Daphnia magna 48-hour lethality and Chironomus **tentans 10-day** growth for evaluation of Detroit River **sediments** (Giesy et al. 1988). However, on testing of sediment pore waters, Microtox[R] **was** far **less** sensitive than either sea-urchin sperm cell or sea-urchin morphological development-tests (Carr and Chapman 1991). In **testing** of various **toxicant solutions,** Nacci et al. (1986) also found Microtox[R] to often be less sensitive than sea urchin sperm cell and early embryo growth **tests,** but felt that, given the ease with which the test is performed, Microtox[R] would be useful in monitoring relative toxicity changes in systems where it has been shown to be responsive to a particular toxicant.

Our study has no information from other types of bioassay8 and no complementary analytical data for verification. We reccomend another study be **conducted** which includes a battery of sensitive toxicity tests in addition to Microtox[R]. The assessment of sediment pore water and whole sediments should be included as a part of this study. Sediments should be the major focus because of the hydrophobic nature of many agriculture chemicals.

Table 1. Arithmetic means (and ranges) forwaterqualitydata.

Sample Site #	Temperature °C	DO mg/l	Salinity ppt	Conductivity µmho's	pH	# Samples n
1	30.1 (28-34)	8.5 (5-12.4)	2.1 (2-2.5)	4555 (4150-5200)	8.4 (7.8-8.9)	14
2	29.6 (28-33)	7.7 (5.2-10.8)	1.9 (1-2.5)	4114 (3500-4656)	8.2 (7.8-8.5)	14
3	29.2 (27.8-32)	8.4 (6.5-11)	1.9 (1-2.5)	4200 (3490-5000)	8.3 (7.9-8.6)	14
4	31 (39.3-34)	12.9 (9.1-17.6)	9.9 (6.5-16)	17970 (12500-27800)	8.7 (8.2-8.9)	14
5	31 (27.2-34)	9.0 (6.7-11.4)	9.5 (8-11.5)	17210 (11000-20800)	8.4 (7.7-8.8)	14
6	29.6 (27.3-32)	6.1 (1.3-10.5)	15.4 (14.2-18)	26750 (25000-30000)	8.1 (7.8-8.4)	4
7	28.3 (26.5-32.2)	4.6 (2.2-8.7)	10.7 (8.9-12.5)	18910 (15500-22500)	8.1 (7.4-9.0)	14
8	29.7 (27-34.5)	5.7 (2.2-10)	13.4 (10-16.5)	23570 (18800-31000)	8.6 (7.5-9.3)	13
9	28.7 (27.2-32.8)	4.8 (2.3-12)	2.1 (1.5-2.7)	4330 (3700-5200)	7.9 (7.3-8.3)	14
10	28.2 (26.3-32.0)	3.4 (0.5-7.7)	5.7 (4.5-6.5)	9600 (7000-13000)	8.0 (7.4-8.6)	14
11	27.5 (25.4-34.0)	3.6 (2.2-7.7)	5.0 (3.5-6.2)	9000 (5500-11300)	7.6 (7.3-7.8)	14
13	29.4 (28-33)	5.2 (2.5-10.1)	1.4 (0.5-2)	3390 (3050-3900)	8.2 (7.5-8.7)	14
14	29.1 (28-32)	6.0 (4.4-8.3)	0.6 (0-1)	1700 (1450-1950)	8.2 (7.8-8.5)	14
15	29.8 (28.5-32)	7.3 (5.2-8.2)	2.2 (1-2.6)	4630 (4100-5000)	8.2 (7.8-8.5)	14
16	31.7 (27.9-34.8)	9.2 (5.6-11.8)	1.6 (1-2.2)	3970 (2550-4900)	8.1 (7.4-8.5)	8

CONCLUSIONS

Results of Microtox testing of the Lower Rio Grande Valley irrigation drainwater under the low discharge conditions that occurred during the study period indicate little,. if **any,** sublethal effects to the phospholuminescent bacterium, Photobacterium phosphoreum. Further testing during both dry conditions and periods of heavy rainfall, and subsequent increased agricultural runoff, is warranted, however, to ascertain whether increased drainwater discharge may result in increased contaminant loading (and hence toxicity) into adjacent receiving waters. Such testing should include evaluation of the potential toxicity of the associated sediment pore water, since many agricultural contaminants tend to partition in sediments and not the overlaying water column.

ACKNOWLEDGEMENTS

We thank Steve Thompson and Steve Van Ryper of Laguna Atascosa National Wildlife Refuge for their assistance with field work in this project. We also thank **Allan** Strand, Bryan Pridgeon and Charlie Sanchez for reviewing the draft manuscript and providing suggestions that improved this report.

LITERATURE CITED

Ahr, W.M. 1973. Long lived pollutants in sediments from the Laguna Atascoea National Wildlife Refuge, Texas. Geol. **Soc.** America Bull. **84:2511-2516.**

Ankley, G.T., R.A. Hoke, J.P. Giesy and P.V. Winger. 1989. **Evaluation** of the toxicity of marine sediments and dredge spoils with the Microtox[R] bioassay. Chemosphere **18:(9-10):2069-2075.**

Bulich, A.A. 1979. Use of luminescent bacteria for determining toxicity in aquatic environments. Amer. **Soc.** Test. Mater. STP **667:98-106.**

Carr, R.S. and Chapman, D. 1991. Personal communication. US Fish And Wildlife Service, National Fisheries Contaminant Research Center, Corpus **Christi** Field Research Station, TX.

Gamble, L.R., G. Jackson and T.C. Maurer. 1988. Organochlorine, trace element and petroleum hydrocarbon contaminants investigation of the lower Rio Grande Valley, Texas, 1985-1986. U.S. Fish Wildl. Serv., Ecological Servicea, Corpus **Christi,** TX. Unpublished document.

Gieay, J.P., R.L. Graney, J.L. **Newsted,** C.R. Rosiu, A. **Benda.** 1988. A comparison of three sediment bioassay methods for Detroit River sediments. Environ. Toxicol. Chem. **7:483-498.**

Microbics Corp. 1987a. Microtox 100% test procedure. Microtox application note M-111, 4. Microbic8 Corp. Carlsbad, CA.

Microbic8 Corp. 1987b. How to run a standard Microtox test. p 39. **Microbics** Corp. Carlsbad, CA.

Microbic8 Corp. 1991. Microtox Manual, p 5. Model 500 toxicity test system, preliminary release. Microbic8 Corp. Carlsbad, CA.

Nacci, D.E., E. Jackim and R. Walsh. 1986. Comparative evaluation of three rapid marine toxicity tests: sea urchin early embryo growth test, sea urchin sperm cell toxicity test, and Microtox. Environ. Toxicol. Chem. 5:521-525.

Ribo, J.M. and K.L.E. Kaiser. 1987. Photobacterium phosphoreum toxicity bioassay. I. Test procedures and applications. Toxicity Assess. 2:305-323.

Ribo, J.M., B.M. Zaruk, H. Hunter and K.L.E. Kaiser. 1985. Microtox toxicity test results for water samples from the Detroit River. J. Great Lakes Res. 11(3):297-304.

Somasundaram, L., J.R. Coats, K.D. Racke and H.M. Stahr. 1990. Application of the Microtox system to assess the toxicity of pesticides and their hydrolysis metabolites. Bull. Environ. Contam. Toxicol. 44:254-259.

Stebbing, A.R.D. 1982. Hormesis - the stimulation of growth by low levels of inhibitors. Science Tot. Environ. 22:213-234.

Tarkpea, M. and M. Hansson. 1989. Comparison between two Microtox test procedures. Ecotox. and Environ. Safety 18:204-210.

Wells, F.C., G. A. Jackson, and J. Rogers. 1988. Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Lower Rio Grande Valley and Laguna Atascoea National Wildlife Refuge, Texas, 1986-1987. U.S. Geological Survey, Water Resources Investigations Report 87-4277. 89 pp.

White, D.H., C.A. Mitchell, H.D. Kennedy, A.J. Krynitsky and M.A. Ribick. 1983. Elevated DDE and toxaphene residues in fishes and birds reflect local contamination in the Lower Rio Grande River Valley, Texas. Southwestern Naturalist. 28:325-333.

Appendix 1. Sample Site Description

A brief site by site description follows to indicate peculiarities of sample sites and which sites showed preliminary indications of light reduction relative to controls in the 100% screen. Unless otherwise indicated, sites were sampled 14 times each.

Site 1.
Sampling site 1 was located in the Big Ditch, at the first farm road bridge east (about 0.5 km) of highway 77. At this point, the water flow is fairly slow. Carp and shorebirds were occasionally seen at this site. The one sample which gave a hit was from this site, and 3 other samples taken in mid and late July had minor light reductions, though no EC50 data could be generated.

Site 2.
This site was below the highway 507 bridge over the North Floodway. only two samples in early July showed minor light reductions.

Site 3.
Also in the North Floodway, site 3 was below the Highway 1420 Bridge. Three samples in mid and late July showed minor light reductions.

Site 4.
This site was on the south bank of the Arroyo Colorado at Arroyo City Road, just upstream from Arroyo City. This is close to the water intakes for the shrimp farms. Mullet and other unidentified fish were frequently spotted here. Three samples, one in each sampling period, showed minor light output reductions.

Site 5.
Upstream from site 4, this site was also on the south bank of the Arroyo Colorado, near the county line. No water samples from here gave any indication of toxicity.

Site 6.
Located on the Laguna Atascoea National Wildlife Refuge, this site was at the second crossing of Laguna Atascosa, on the south side of the water control structure. The water level here was initially very low, and this site was completely dry by the beginning of the second sampling period. No light reduction was observed in any of the 4 samples from this site.

Site 7.
Upstream from site 6 and also on Laguna Atascoea NWR, this site was on the south side of the water control structure at crossing #1 on Laguna Atascoea. No indication of overt toxicity was detected here.

Site 8.
Athel pond near the headquarters of Laguna Atascosa NWR was site 8. Samples were taken in the vicinity of the large diameter culvert pipe passing under the road. On three occasions in late Julv and early August, samples showed minor reductions to light output in the Microtox® test. A total of thirteen samples were collected at this site.

Site 9.
This site was just downstream (north) of the highway 106 bridge over the upper Cayo Atascosa. Only one sample in August showed any light reduction as compared to controls in the Microtox® test.

Site 10.
This site, on Resaca de 105 Cuates, was just south of highway 106. None of the samples depressed light emission by the bacteria.

Site 11.
This site was at a small irrigation drainage ditch between the Port Isabel/Cameron Co. Airport and highway 510, near **Bayview.** No samples indicated toxicity.

Site 13.
Upstream from site 10, site 13 was also on Reeaca de 105 **Cuates, at** Share Rd. 28. No samples from this site reduced light output in the Microtox® assay.

Site 14.
Site 14 was on Resaca de 105 Freenos where it crosses Nelson Rd. No toxicity was detected here.

Site 15.
South of Mercedes, site 15 was in the Arroyo Colorado below the bridge at Highway 491. Of eight samples taken here, only one, in mid July, showed any reduction of light output as compared to controls.

Site 16.
Site 16 was at the end of the main floodway in Llano Grande Lake, between the boat launch and the bridge of highway 1015. Of eight samples analyzed, only **one** taken in late July reduced light emission as compared to blanks in the Microtox® test.

Appendix 2. Modified **Microtox**[R] 100% Procedure with Replicates

For marine samples (20 - 60 ppt salinity)

Analyzer & Sample Preparation
1) Add 1 **ml Recon** Solution to the cuvette in the reagent well.
2) Add 1 ml FSW[2] to cuvettes Al-Cl, **A3-C3,** and **A5-C5.**
3) Add 1 **ml** of sample to each of 3 cuvettes (Dl-Fl, **AZ-C2, D2-F2, D3-F3, A4-C4, D4-F4, D5-F5).**
4) Wait 5 minutes.

Reagent Preparation
1) Reconstitute a vial of reagent
2) Mix 20 times with 500 ul pipette
3) Wait 15 minute5 for reagent stabilization

Assay Procedure
1) Set/start timer for 5 and 15 minutes.
2) Add 20 ul of reagent to each cuvette in the following order: Al, **Bl,** Cl, **Dl,** El, **Fl,** A2, B2, C2, etc.
3) Mix each cuvette by shaking 2-3 times in the same order of reagent addition.
4) When the timer alarm sounds, read the (IT) light level for Al, **Bl,** Cl, etc.
5) Take averages and compare data.

For freshwater samples (O-20 ppt salinity) Note: actually this is a 90% procedure

Analyzer & Sample Preparation
1) Add 1 ml **Recon** Solution to the cuvette in the reagent well.
2) Add 1 **ml** diluent to cuvettes Al-Cl, **A3-C3,** and **A5-C5.**
3) Add 900 ul of sample to each of 3 cuvettes (Dl-Fl, **A2-C2, A3-C3, D3-F3, D4-F4, A5-C5, D5-F5).** This is easily accomplished by adding 1000 ul (2 x 500) and removing 100 ul with another pipette.
4) Add 100 ul **MOAS** to each sample cuvette.
5) Mix all samples thoroughly.
6) Wait 5 minutes.

Reagent Preparation
1) Reconstitute a vial of reagent
2) Mix **20 times** with a small pipette.
3) Wait 15 minute5 for reagent stabilization

Assay Procedure
1) Set/start timer for 5 and 15 minutes.
2) Add **20ul** of reagent to each cuvette in the following order: Al, **Bl,** Cl, **Dl,** El, **Fl,** A2, B2, C2, etc.3) Mix each cuvette by shaking **2-3 times** in the same order of reagent addition.
4) When the timer alarm sounds, read the (IT) light level for Al, **Bl,** Cl, etc.
5) Take averages and compare data.

---

[2] Filtered seawater free of toxicity