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Use of *Daphnia* spp. for the Ecotoxicological Assessment of Water Quality in an Agricultural Watershed in South-Central Chile

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Abstract. Because of the importance of surface waters from the Chillán River watershed (Chile) for recreation, agricultural irrigation, and the production of drinking water, local concern about river water quality has increased considerably during the last decade. Agricultural and forestry activities in the watershed, characterized by an intensive use of pesticides, are thought to play an important role in the generation of non-point-source pollution, whereas the discharge of urban wastewater from the city of Chillán constitutes a major point source of pollution. In the present investigation, acute and chronic laboratory bioassays using *Daphnia* spp. were conducted on surface water samples from 17 river stations located throughout the watershed. Sampling occurred on 6 occasions during a 16-month period (2000 to 2001) and included both high and low flow conditions. Almost all toxic effects observed in summer were directly related to the discharge of urban wastewater, whereas toxicity in rural areas was mainly detected during the winter period when rainfall and river flow are high. Toxicity test results were compared with measured physicochemical water-quality data. Mortality and alterations in reproductive success of *Daphnia* spp. were not consistently reflected in detected chemical pollution. With only one exception (atrazine), detected pesticide concentrations were below known toxicity levels. However, additive and synergistic effects of the presence of a mixture of pesticides could not be excluded as a possible cause of observed toxicity. At several stations, filtering of the water sample led to a strong decrease in toxicity, which suggests the presence of xenobiotics attached to the smaller sediment fraction. Inclusion of sediment chemical analysis and sediment toxicity testing in future work should therefore be encouraged. The presented approach provided information about the adverse effects of human activities on surface water quality in the watershed, not easily obtained from classical monitoring schemes. In specific cases, the approach may represent an economically attractive alternative to physicochemical analyses. Modifications to the proposed methodology should be introduced if the effects of intrastorm and interstorm variability of water quality are to be analyzed.

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The watershed of the Chillán River (hereafter called "Chillán watershed") is located in South-Central Chile (36°33′ to 36°53′ S, 72°21′ to 71°24′ W). It has a total drainage area of about 757 km^2, of which 27% is located in the Andes Mountain Range, and the remaining 73% is situated within Chile's Central Valley (Figure 1). Altitude values in the watershed range from 3200 m above sea level (MASL) in the upper part, to 75 MASL at the outlet. The total length of the Chillán River from its headwaters down to its confluence with the Ñuble River, just east of the Coastal Mountain Range, is 105 km.

In the Andean part of the watershed, land cover consists of permanent snow fields in the uppermost locations and native forests in the middle and lower parts with some bare rock and grasslands in between. Although still restricted in extension, exotic species forestry plantations *(Eucalyptus* and *Pinus* spp) have been introduced recently. They are almost exclusively located in the foothills of the Andes (Figure 2).

Land use in the Central Valley is mainly agricultural with sugar beet, wheat, and pastures predominating. Horticulture, vineyards, and orchards occupy only a small percentage of the total watershed area. Forestry plantations of *Pinus radiata* and *Eucalyptus globulus* are becoming increasingly important. They are concentrated in an area of rolling hillslopes just southeast of the city of Chillán (175,000 inhabitants). The only other urban center of importance in the watershed is the much smaller village of Pinto, with 9000 inhabitants (Figure 2). During the dry season, irrigation is applied to approximately 45% of the total watershed area. Use of river water for recreation is common.

The present study focuses on the middle and lower parts of the Chillán watershed, which are completely located within the Central Valley. Here, the climate is Mediterranean with a dry, warm summer period and a cold, humid winter period, both of approximately equal length. The mean maximum temperature for January, the hottest month, is 28.8°C, and the mean minimum temperature for July, the coolest month, is 3.5°C (Uníversidad de Concepción 2002).

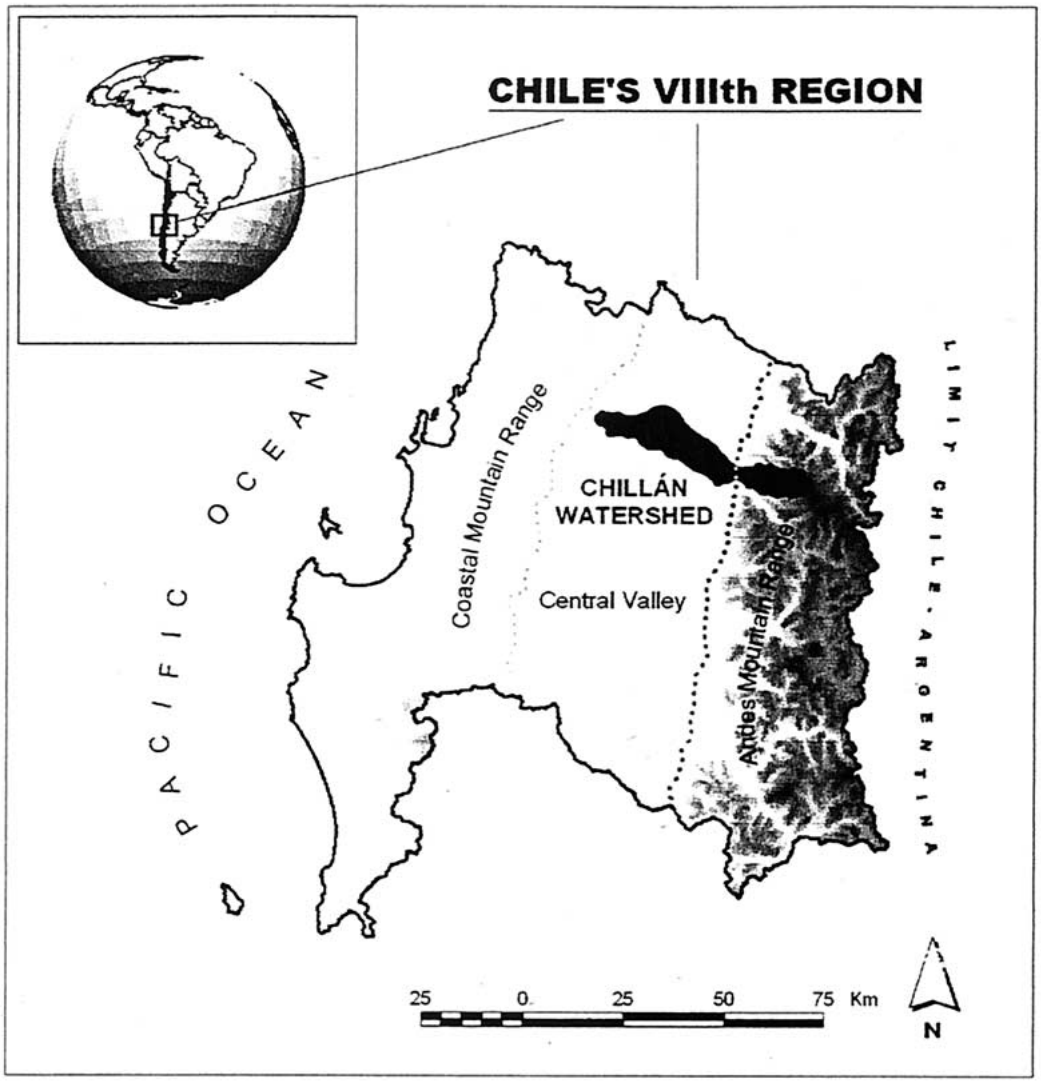

Fig. 1. Location of the Chillán watershed within Chile's 8th Region.

Average annual precipitation is 1624 mm, and most rainfall occurs between May and September with June being the wettest month. Mean annual river flow for the Chillán River is 22.9 $m^3\ s^{-1}$ at the outlet. However, flow rates can decrease well below 1 $m^3\ s^{-1}$ during the dry season when decreased discharge during the recession period is further depleted due to excessive water extraction for irrigation purposes and for production of drinking water.

Agrochemical use is widespread throughout the study area. Farmers claim a 30% to 50% yield loss if agrochemicals are not employed. Results from a recent survey (EULA 2002) indicate an estimated use of a total amount of approximately 29,450 kg y^{-1} of pesticides in the watershed. The agrochemicals applied are mainly herbicides such as MCPA (3340 kg y^{-1}), glyphosate (2880 kg y^{-1}), penmedipham (1590 kg y^{-1}), simazine (1040 kg y^{-1}), atrazine (930 kg y^{-1}), and others.

MCPA is used on crops such as wheat, oats, barley, and maize on an almost continuous basis. Glyphosate, penmediphan, and sulphur (3351 kg y^{-1}) are applied to industrial crops such as raps and sugar beet. A total of 1359 kg y^{-1} of organophosphate pesticides—including chlorpyrifos (58%), metamidofos (40%), diazinon, and dimetoate (together-2%)—are used to protect industrial crops (sugar beets, raps), vegetables (potatoes, beans, carrots), fruits (apples, cherries, raspberries). Nearly 90% of the total amount of simazine is applied to forestry plantations (pine and eucalyptus). Other applications include apple trees (2.4%), asparagus (2.1%), raspberries (3.6%), and maize (0.9%).

Because of the economic importance of the availability of good-quality surface water in the watershed, the environmental fate of the pesticides applied to the agricultural fields is of great interest. According to Moses *et al.* (1993), around 90% of pesticides applied in agriculture never reach their target organism but are instead dispersed through air, soil, and water. Streams within an agricultural catchment are expected to be susceptible to brief pesticide inputs after precipitation events (Wauchope 1978; Kreuger 1995). An additional problem in the study area appears to be the fact that the majority of empty cans are being cleaned in surface waters on farming land, and only a small amount is given back to the providers.

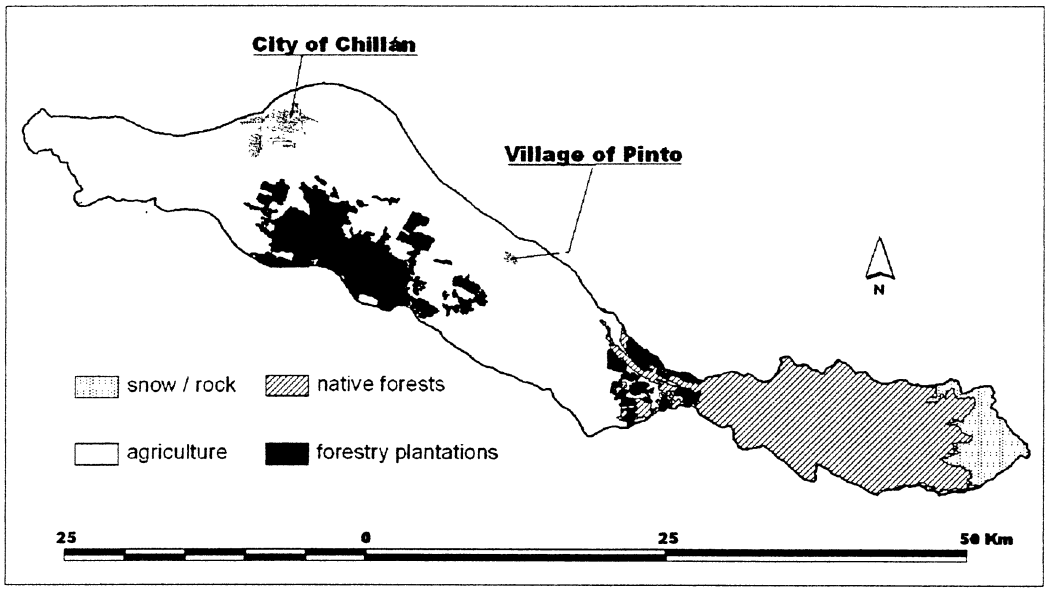

Fig. 2. Simplified land use map for the Chillán watershed

Considering the generalized use of different kinds of pesticides, the inadequate management of empty cans, and the pronounced rainy season in the study area—which is characterized by frequent, high-intensity rainfall—it has been suggested that there is an important potential for pesticide contamination of surface waters, especially during periods of application and rainstorm events occurring shortly after application. However, the sporadic nature of these phenomena makes detection of pesticide contamination in surface waters difficult when relying only on the classic periodic physicochemical monitoring schemes.

The only important point source of pollution in the watershed corresponds to the discharge of the urban wastewater from the city of Chillán on the Las Toscas Tributary. Although the presence of individual compounds in water samples can be determined by means of physicochemical analyses, other methods are needed to assess potential synergistic effects on both human and ecosystem health caused by the mixture of compounds typically present in wastewaters.

Recently, increased emphasis has been placed on the potential offered by the inclusion of toxicity tests in the determination of the water quality of aquatic ecosystems (Kikuchi *et al.* 2000; Hunt *et al.* 1999; Werner *et al.* 2000). In the present work, the surface waters of the Chillán watershed were screened for toxicity through direct measurement of biological responses of *Daphnia pulex* and *D. magna* caused by exposure to river-water samples. The results from the toxicity tests were compared with physicochemical water-quality data to identify factors influencing spatial and temporal patterns, as well as intensity of ambient toxicity. The utility of the applied approach is discussed.

Materials and Methods

River Water Sampling

Spatial variation of surface water toxicity in the watershed was characterized through the selection of a total of 17 sampling stations. Seven stations (E1 to E7) were located on the Chillán River, and the remaining 10 stations (T1 to TI0) were distributed over the tributaries (Figure 3). Samples were also taken directly from the municipal wastewater discharge, which is located on the Las Toscas Tributary shortly upstream of station T10. Station E1, situated on the transition between the Andes and the Central Valley, served as a reference station because only a limited amount of human activities takes place upstream of this point.

To assess temporal variations in river water toxicity, six different surveys were executed during a 16-month period. Surveys took place during March, July, and November 2000, and during January, April, and June 2001. The surveys included both high (winter) and low (summer) river-flow conditions. In March 2001, a biological treatment plant for the municipal wastewater became fully operational. Consequently, the last two surveys included samples of the treated municipal wastewater.

Water samples were collected using precleaned polyethylene (PE) and glass bottles (5 L). They were transported with ice in insulated coolers and stored in the laboratory under dark conditions at 4°C until the physicochemical analyses were performed. Subsamples for the toxicity tests were frozen until use.

Preparation of Daphnia *spp. Cultures*

D. magna and *D. pulex* were obtained from in-house cultures using the guidelines given by the United States Environmental Protection Agency (USEPA 1993). For cultures and bioassays, an ambient temperature of 20°C ± 2°C and a photoperiod of 16 hours light to 8 hours dark was maintained. Moderately hard dilution and control water was prepared according to standard methods (USEPA 1993). Hardness was 241.96 ± 16.98 mg L^{-1} and 124.92 ± 7.40 mg L^{-1} as $CaCO_3$ for *D. magna* and *D. pulex*, respectively. Dissolved-oxygen (DO) levels were between 8.22 and 10.13 mg L^{-1} and pH was between 7.74 and 7.96 at a temperature of 20°C.

Acute Toxicity Testing

Acute toxicity was determined by exposing *D. magna* and *D. pulex* juveniles (age <24 hours) to the river water samples. Results were

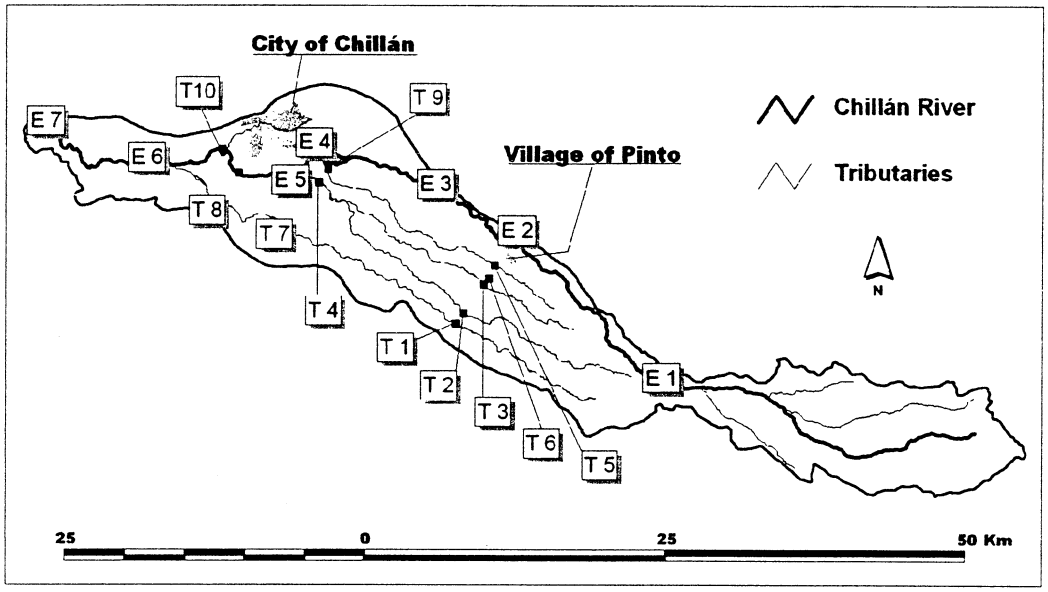

Fig. 3. Location of the different surface water sampling stations in the Chillán watershed. E = On main river, T = on a tributary

expressed as the strength of the solution (percent dilution of the original sample) that causes 50% mortality. Borosilicate cups (30 ml) filled to 25 ml served as test chambers. All tests (including a control) consisted of four replicates with each replicate containing five organisms. Test solutions were not renewed and the organisms not fed during the experiments. Mortality (defined as a lack of organism mobility when the vessel was shaken) was recorded for each sample both after 24 hours and at the end of the 48-hour exposure period. DO concentrations (Hanna HI 9142), pH (Schott CG 825), and conductivity (Hanna HI 8733) were measured at the beginning and end of each test. Samples showing acute toxicity were filtered using 0.70-µm and 0.45-µm Whatman paper filters and subsequently subjected to a new acute bioassay. The 24-h to 48-h LC_{50} values from both the filtered and the unfiltered samples were converted to toxic units ($TU = 100 / LC_{50}$) and then compared. The percentage of toxicity decrease (% TR) was calculated as follows:

$$\%TR = 100 \times [1 - (TU_{\text{after filtering}}/TU_{\text{before filtering}})] \qquad (1)$$

Static Renewal Chronic Toxicity Testing

The static renewal chronic toxicity tests were performed using only *D. pulex*, with the exception of the tests for the March 2000 survey, for which *D. magna* was used. A single daphnid (<24 hours old at the initiation of the test) was exposed for 21 days to 20 ml river sample using 30-ml cups. The tests were executed using a sample concentration of 100%, except for the stations where previously acute toxicity was detected. In these cases, successive dilutions were tested until mortality was no longer observed. Organisms were fed three times per week with a suspension of yeast, trout chow, and alfalfa; the equivalent carbon content was 7.2 mg C L^{-1}. The frozen original river-water subsamples were used to renew the test solutions each time feeding took place. Test conditions (pH, temperature, DO, photoperiod) were identical to those for the acute tests. Each day, the mortality and the number of neonates per cup were recorded. The time to the first brood was also noted, and neonates and dead organisms were removed from the cups.

Determination of Physicochemical Water Quality

The following physicochemical water quality parameters were determined on samples taken at the different sampling stations during the different surveys: temperature, pH, conductivity, DO, total hardness, total dissolved solids, total suspended solids, chemical oxygen demand, biological oxygen demand, nitrate, nitrite, ammonia, phosphorous, chloride, residual chlorine, oil, and greases. All analyses were conducted according to standard methods (APHA 1985). Total Kjeldahl nitrogen was determined by sample digestion with sulfuric acid (Merck) and selenium reagent (Kjeldahl tablets, Merck). A Kjeldahl apparatus was used for the distillation and titration of samples with hydrochloric acid (Riedel-de Haën). DO, conductivity, and pH were directly measured in the field. The other analyses were performed as soon as possible after arrival at the laboratory but always well within 1 week after sampling took place. Priority was given (<24 hours) to those parameters most subject to changes as a result of storage.

Water samples were screened for the presence of the following pesticides: the organophosphate chlorpyriphos and the herbicides simazine, atrazine, and MCPA as well as the organochlorine pesticides heptachlor, endrin, endosulphan, and methoxychlor. For the first four, the following methodology was used: a 4-L subsample was processed using solid-phase extraction with Env+ cartridges; adsorbed pesticides were then eluted with 5 ml methanol for residue analysis (Merck), and 20 µl of extract was injected into a high-pressure liquid chromatograph (Agilent 1100 Series) with a diode array detector. Chromatographic conditions were as follows: column zorbax C-18; an isocratic mixture (methanol 70% and water 30%) was used as elution solvent with a continuous flux of 1 ml min^{-1}. Calibration curves were established for each analyte, using pure analytical standards (ChemService), dissolved in methanol. Detection limits were determined using a signal–noise relationship >3 and were 0.27 µg L^{-1} for MCPA, 0.065 µg L^{-1} for atrazine, 0.19 µg L^{-1} for simazine, and 0.1 µg L^{-1} for chlorpyriphos. The organochlorine pesticides were analyzed using liquid–liquid extraction with hexane and GC-ECD analysis. in this case, a 1-L sample was extracted three times with 30 ml hexan/p residue analysis. The extracts were combined, cleaned up with a florisil column, eluted with hexane, and concentrated up to 500 µl under nitrogen. A 2-µl aliquot was injected in a Perkin Elmer Autosystem 2000 gas chromatograph equipped with an electron-capture detector. A SBP-5

Table 1. Acute toxicity values (LC_{50}) for *D. magna* and *D. pulex* of ambient water samples from the Chillán River (July survey)[a]

	24-h LC_{50} (%)		48-h LC_{50} (%)	
Station	*D. magna*	*D. pulex*	*D. magna*	*D. pulex*
E1	ND[b]	68.04 (60.58–76.42)	ND ND	37.89 (31.89–45.18)
E2	ND	35.36 (24.95–50.10)	71.83 (53.44–114.52)	25
E4	ND	ND	62.13 (58.58–79.37)	100[c]
T2	ND	100[c]	ND	71.36 (68.07–74.84)
T4	95.27 (74.13–111.93)	ND	50[c]	ND
T5	85.72 (65.56–112.08)	ND	59.80 (50.15–71.32)	ND
T6	85.60 (65.56–112.08)	44.26 (34.34–57.04)	68.28 (51.37–82.94)	31.50 (24.84–39.94)
T9	84.56 (65.56–112.08)	ND ND	75.28 (53.02–115.02)	ND

Note. The 24-h and 48-h mean lethal values (LC_{50}) are expressed as percent sample strength causing 50% survival.
[a] Only data from those stations showing toxicity are presented.
[b] Not detected (100% survival in original sample.
[c] 95% CI not calcultated.
LC_{50} = Median lethal concentration.

capillary column (30 m × 0.25 mm i.d., 0.25-μm film thickness) was used. The chromatographic conditions were as follows. Carrier gas was helium, head column pressure was 190 kPa, and split-splitless injection mode was used. Injector temperature was 200°C, and detector temperature was 320°C. Oven temperature was programmed as follows: 100°C for 10 min, then to 280°C at a rate of 5°C min^{-1}, and then hold at 280°C during 20 min. Detection limits for the four analytes were established as 2 ng L^{-1} using a signal–noise ratio >3.

Statistical Analysis of Toxicity Results

For the acute toxicity tests, 24- and 48-h LC_{50} values, and 95% confidence intervals (CI) were calculated using Probit and Spearman-Karber analysis (Finney 1971, 1978). No correction was made for control mortality because control survival was ≥90% in all cases. Reproduction results from the 21-day tests on *D. pulex* were first examined for normal distribution and homogeneity of variance using χ^2 test and Bartlett's test, respectively. When data were found to be normal and homogeneous, the mean number of young per female animal was examined by one-way analysis of variance and compared with control results using Dunnett's *t*-test (Dunnett 1955) and Multiple Comparison of Tukey. Nonnormal data were analyzed by Kruskal-Wallis test followed by Mann-Whitney *U* test. All statistics were performed using the Statsoft 6.0 software package.

Results and Discussion

Acute Toxicity

Acute toxicity was observed only in water samples obtained during the March 2000 (summer) and July 2000 (winter) surveys. For the July 2000 samples, toxic effects were observed in both *D. magna* and *D. pulex. D. pulex* was not used for the March 2000 survey because the species culture was not available yet at that time.

For the March 2000 survey, only the samples taken from station T10 on the Las Toscas Tributary and from the municipal wastewater discharge showed acute toxicity; 48-h LC_{50} values for *D. magna* ranged between 63.1% and 85.4%. Because urban wastewater constituted >50% of the total discharge observed at station T10 during the summer period, acute toxicity at this point was almost certainly directly related to the discharge of municipal wastewater only a short distance upstream from this sampling location. These same stations did not exhibit acute toxicity during the other surveys.

Acute toxicity was recorded at different sampling sites during July 2000 (Table 1). However, only the stations E2 (downstream of the small village of Pinto), E4 (near the upstream limit of the city of Chillán), and T6 (Lipincura sector) caused acute toxicity in both species. Surprisingly, during this winter survey acute toxicity was also observed at reference station E1 despite the fact that human activities upstream of this point are restricted: some forestry plantations, subsistence agriculture, and 1 small-scale trout nursery. Of the 10 stations located on tributaries, 5 showed acute toxicity (50%), but this percentage was slightly lower for the main stream (43% or 3 stations of a total of 7).

Filtering the water samples from the July 2000 survey through a 0.45-μm filter caused acute toxicity decreases ranging from 52% to 100% (Fig. 4). Acute toxicity was completely removed for stations E1, E4, T2, T4, and T6. This fact could be related to sorption of hydrophobic toxicants from the water samples to the filter medium (Carr *et al.* 1995), however, toxicity decreases for these same samples using a 0.70-μm filter were not significant. Considering that smaller particles bind xenobiotics much more efficiently than the coarse fraction of the sediments and suspended solids, the results of these tests suggest that toxic compounds present in

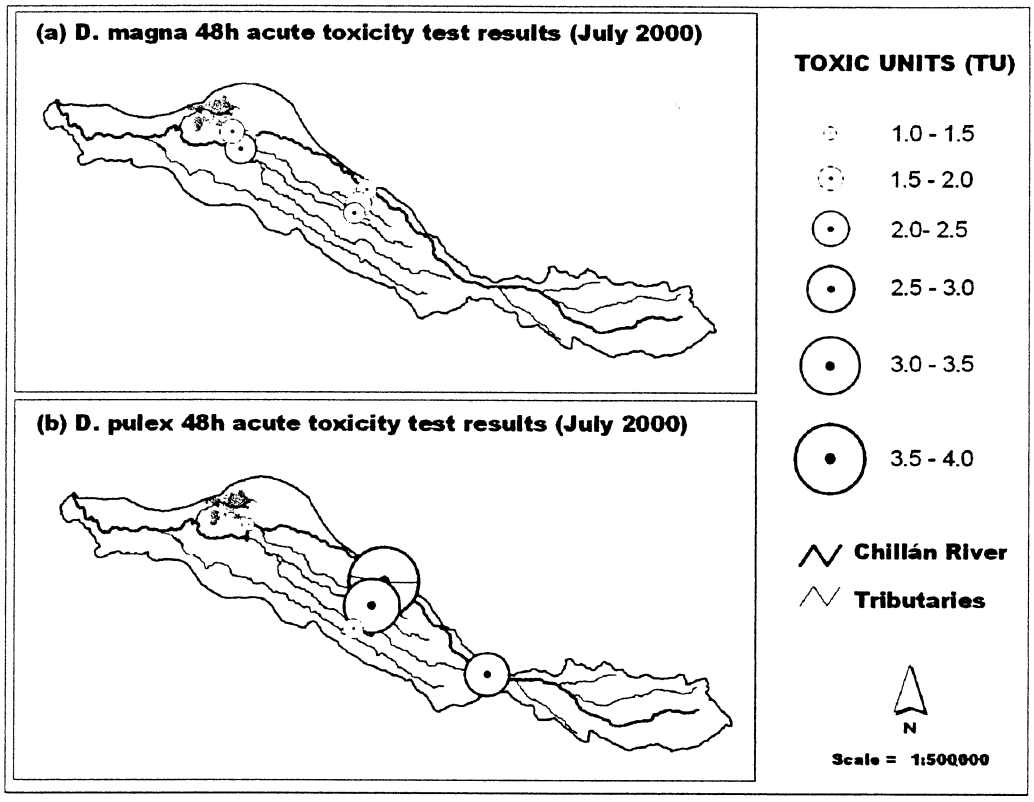

Fig. 4. Acute toxicity during July 2000 at the different sampling stations expressed as toxic units (the gray shading indicates the percentage of toxicity that was removed on filtering with a 0.45-μm filter; white color indicates toxicity was not removed)

the water samples were associated with particles between 0.45 and 0.70 μm in diameter. No direct relationship could be established between the spatial distribution of toxicity and the concentration of suspended solids (data not shown). The origin of runoff and sediments may be more important than the actual suspended solids concentration, as is suggested by the *D. pulex* data. The only station where toxicity could not completely be removed after filtration was E2, which is located downstream of the urban center of Pinto. All other stations are located in rural areas where runoff originates from agricultural or forested lands. This hypothesis, however, is only partially confirmed by the data from the *D. magna* tests (Figure 4). Suspended solids concentrations, as an indicator of the magnitude of the runoff phenomenon, did seem to be important for the temporal distribution of toxicity. With the exception of station T10, acute toxicity was observed only in winter, more specifically during the July 2000 survey, when the highest suspended solid concentrations of the study period were observed at almost all stations.

Based on ambient toxicity testing with *Ceriodaphnia dubia*, Kszos *et al.* (1997) stated that acute mortality could be caused by an interaction between pathogens and toxicants. Downey *et al.* (2000) indicated elimination of pathogens using 0.20-μm membrane filters. Therefore, care must be taken when interpreting the results from toxicity tests because manipulations, such as sample filtering, that eliminate toxicants can also remove pathogens or alter the sample. However, Downey *et al.* (2000) also indicated that 0.45-μm filters, which were used in the present study, were not consistently effective for the removal of pathogens. This consideration, together with the fact that all samples were frozen until use, suggests that the toxicity observed in the present study was not caused by the presence of pathogens.

Chronic Toxicity

Chronic toxicity test results indicate the existence of pollution pulses in the Chillán watershed, especially during the winter period when both rainfall and river flow are high. During the March 2000 survey, mean survival for *D. magna* was 100% for nearly all the samples including both the control and the reference (station E1) samples. The only exceptions were stations T10 and T3. At station T3, survival was 80% for a sample concentration of 100%. *Post hoc* analysis of Mann-Withney indicated significant effects on reproduction rates at stations T3 and T7 (a decrease of 94.51% and 91.56%, respectively) compared with the reference station (47.4 ± 10.5) (Table 2). Because acute toxicity was detected on *D. magna* for the March 2000 survey at station T10, chronic toxicity testing for this station was started using a 50% dilution of the original sample. At this concentration, mortality was no longer observed; instead, a significant increase in reproduction rates was obtained (+145%).

Meanwhile, mortality was the main effect observed on samples from the July 2000 survey (Figure 5). Early mortality, i.e., after only 5 to 12 days of exposure also caused decreased fecundity for *D. pulex* at stations E7, T2, and T3. New tests using a 25% sample strength for station E2 still caused 40% mortality, but reproduction was not significantly affected.

Table 2. Statistical significance (Mann-Witney p values) of changes in reproductive success of *D. pulex* for the different sampling sites and surveys

	Survey					
Station	March[a]	July[b]	November[b]	January[b]	April[b]	June[b]
E5	ND[c]	0.09524	0.84127	0.63889	0.84127	0.00032
E8	0.76066	0.00794	0.54762	0.00505	0.30952	0.05683
T1	ND	0.05175[d]	0.55555	0.01768	0.54762	0.12961
T2	ND	0.01587[e]	0.99997	0.75505	0.69048	0.05652
T3	0.00015	0.00794	0.54762	0.34343	ND	0.00129
T4	ND	0.42063[d]	0.84127	1	1	0.00226
T7	0.00015	ND	0.42063	0.26768	0.42063	0.79896
T10	0.00014[d]	0.00794[d]	0.00794[d]	ND	0.05555	0.00032

Note: Significant difference in reproduction of *D. pulex* ($p < 0.05$).
[a] Results for *D. magna*.
[b] Results for *D. pulex*.
[c] Not detected.
[d] Concentration of 50%.
[e] Concentration of 70%.

For the November 2000 spring survey, *D. pulex* fecundity and survival at all stations except T10 differed little from the control. Results from the summer survey (January 2001) indicated a significant decrease in fecundity at stations E7 and T1, whereas mortality (40%) was observed only at site T1 after an exposure time of 12 days.

During the June 2001 survey, stations E7, T7, and T2 showed mortality (>40%), although reproductive success did not significantly differ from that of the reference station. However, a significant increase in reproduction of *D. pulex* was detected for stations E5, T3, and T4 (Figure 5). A significant increase in reproduction rates (172% to 229%) of *D. pulex* was observed at station T10 throughout all seasons. This increase was assumed to be caused by the input of nutrients and/or xenoestrogens from domestic wastewater discharge. Even after introduction of the biological wastewater treatment plant, an increase in reproduction rates of approximately 50% to 60% was still observed, except for the April 2001 survey, during which 80% mortality was obtained.

Physicochemical Water Quality Data

With only a few exceptions (station T10 and E7), the physicochemical analyses of the water samples show no indices of the presence of high levels of pollutants in the water column: Table 3 gives an overview of the mean values and SDs for the classic water quality parameters measured at each sampling station. At station T10, ammonium concentrations were quite a bit higher than the reported acute toxic level of 2.94 mg L^{-1} for *D. magna* (Gersich and Hopkins 1986) and thus may be responsible for observed toxic effects.

All samples from the March, July, and November 2000 surveys, and a limited number of samples from the April and June 2001 surveys, were screened for pesticides. Samples from the 2001 surveys that were included were those from those stations E1 (reference station), E3 (intake of drinking water for the city of Chillán), and E6 (downstream of the urban wastewater discharge) as well as those stations on the tributaries where acute toxicity had been observed during the July 2000 survey (i.e., stations T2, T4, T5, T6, and T9). Detected pesticide concentrations in the water samples were low, generally in the order of nanograms per liter. Only atrazine was present at higher concentrations, namely, at station T7 (Quilmo subwatershed) during the July 2000 and the June 2001 surveys (0.216 μg L^{-1} and 1.32 μg L^{-1}, respectively). For the June 2001 survey, *D. pulex* mortality at station T7 for the chronic bioassay was 40%. Studies report that even short-term exposure to atrazine at concentrations ≥1.15 μg L^{-1} decreases *Daphnia* spp survival (Dodson *et al.* 1999), so the observed mortality may effectively have been influenced by the presence of this compound. Other compounds such as ch1orpyriphos may have been present in surface waters at levels below the detection limit; the reported toxicity levels are in the 0.12–0.23-μg L^{-1} range for *D. magna* (Kikuchi *et al.* 2000).

In April 2001, heptachlor was found in samples from stations T1, T5, and T6 at concentrations of 13.01, 11.93, and 13.79 ng L^{-1}, respectively. Endrin (5.06 ng L^{-1}), endosulfan (13.54 ng L^{-1}), and metoxychlor (35.58 ng L^{-1}) were detected in the sample taken at station E2. Endosulfan is known to affect the feeding rate of zooplankton and may result in decreased growth and reproduction rate. Compared with controls, a 50% decrease in filtration and ingestion rates was obtained for *D. magna* at concentrations of 0.44 and 0.61 mg L^{-1}, respectively (Fernández-Casalderrey *et al.* 1994). Nebeker et al. (1983) reported acute 48-h EC_{50} values of 343 and 271 μg L^{-1} on *D. magna*, and Barry *et al.* (1995) found a subchronic no-observed-effect concentration (NOEC) of 40 μg L^{-1} for *D. carinata.* In this study, detected endosulfan concentrations were well below the described toxic range for *Daphnia* spp. (Barry 1996). With the exception of the levels of atrazine at station T7 during July 2000, the concentrations of the detected pesticides in the watershed would not lead one to expect toxic effects on the *Daphnia* spp. used in the bioassays. Where toxicity did occur, it may have been caused by additive and/or synergistic effects, e.g., mixtures of pesticides that were either not analyzed or were present in concentrations below the

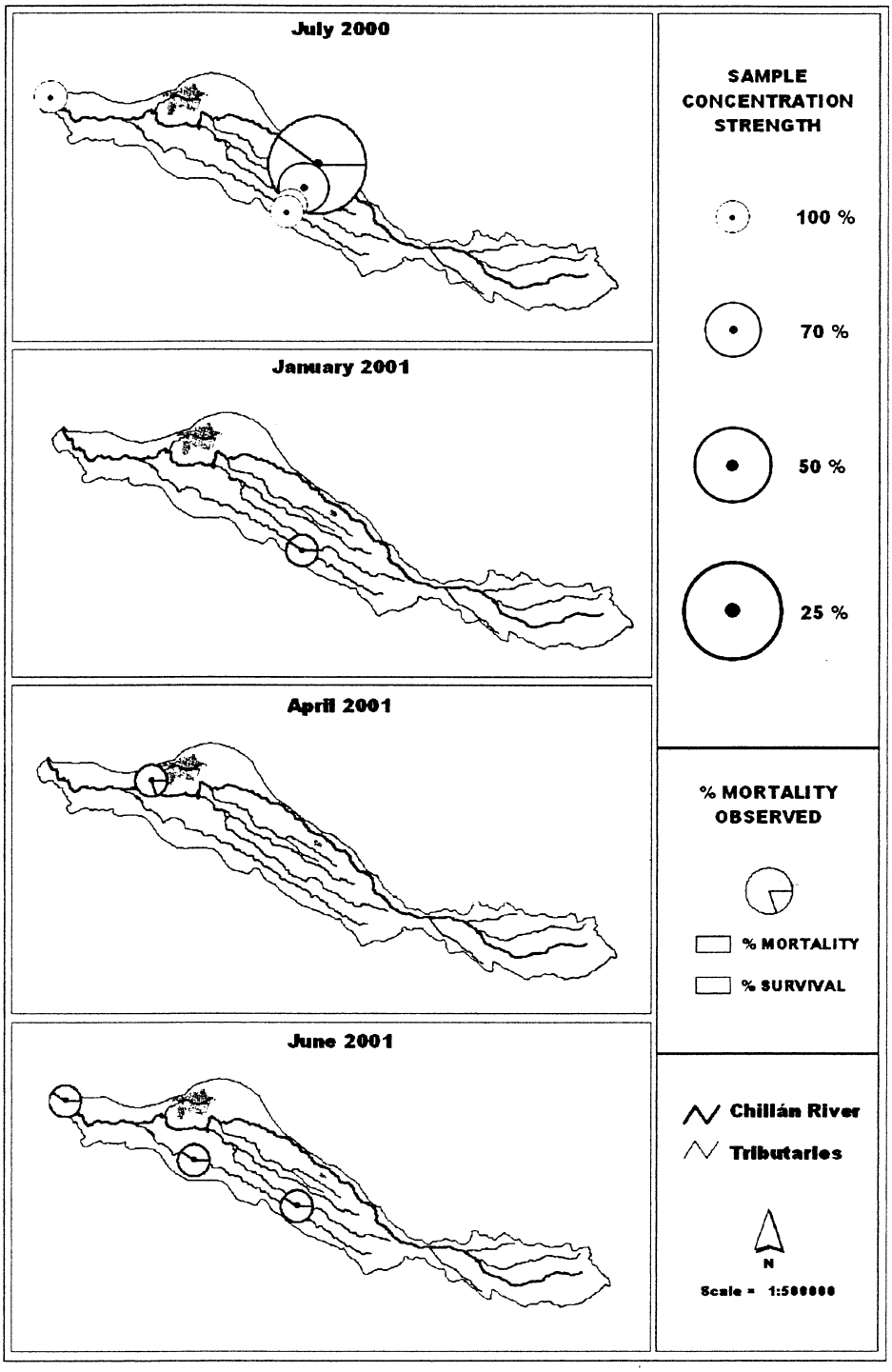

Fig. 5. Temporal and spatial distribution of mortality in chronic bioassays versus test sample strength in the Chillán watershed

detection limit. Additive and synergistic effects of multiple herbicides and insecticides on the aquatic biota are largely unknown (Bailey et al. 1997). Lack of correlation between observed toxicity and detected pesticide levels may also be related to the fact that the used techniques required filtration of the water samples previous to the analysis. This observation acquires special relevance considering the fact that in the case of the acute assays, toxicity was frequently removed after filtering of the sample water. Several pesticides easily bind suspended particulate matter. For example, such is the case of chlorpyriphos, which is typically found attached to particles in water samples (Dabrowskia *et al.* 2002). For such compounds, it is highly probable that the pesticide became retained in the filter during sample preparation. Unmeasured chemicals—such as hydrocarbons, heavy metals, or PCBs–could also have been responsible for the observed toxicity, although potential sources of these compounds in the watershed are rather scarce.

Table 3. Physicochemical water-quality data

Station	pH[a]	Cond[a]	N_{tot}[b]	$N\text{-}NH_3$[b]	P_{tot}[b]	COD[b]	BOD_5[b]	DO[b]	SS[b]
E1	7.48 ± 0.41	81.06 ± 46.20	0.04 ± 0.02	0.013 ± 0.004	0.037 ± 0.032	1.75 ± 0.96	1.43 ± 0.51	9.98 ± 0.51	4.47 ± 0.60
E2	7.70 ± 0.71	87.30 ± 31.50	0.11 ± 0.07	0.015 ± 0.004	0.014 ± 0.004	2.00 ± 0.82	1.10 ± 0.12	10.08 ± 0.12	5.23 ± 3.11
E3	7.78 ± 0.78	77.88 ± 45.13	0.12 ± 0.03	0.010 ± 0.002	0.011 ± 0.001	1.50 ± 0.58	1.10 ± 0.20	10.23 ± 0.20	4.00 ± 1.73
E4	7.66 ± 0.65	84.66 ± 33.78	0.32 ± 0.08	0.029 ± 0.028	0.018 ± 0.003	3.50 ± 1.73	2.13 ± 1.67	10.13 ± 0.61	3.33 ± 1.85
E5	7.49 ± 0.32	84.90 ± 37.99	0.25 ± 0.08	0.021 ± 0.005	0.019 ± 0.002	4.50 ± 2.65	1.75 ± 1.51	10.25 ± 1.89	4.17 ± 3.05
E7	7.32 ± 0.22	124.12 ± 88.28	2.28 ± 1.49	1.655 ± 1.852	0.919 ± 1.261	20.50 ± 16.50	5.18 ± 5.98	8.00 ± 2.79	6.53 ± 4.63
T1	6.64 ± 0.77	34.24 ± 21.33	0.31 ± 0.08	0.008 ± 0.002	0.009 ± 0.002	3.50 ± 1.29	1.30 ± 0.48	10.00 ± 1.38	10.93 ± 8.66
T2	6.88 ± 0.34	35.78 ± 20.01	0.33 ± 0.07	0.009 ± 0.003	0.010 ± 0.001	4.25 ± 2.06	0.98 ± 0.05	9.25 ± 1.00	9.27 ± 7.39
T3	6.78 ± 0.45	28.18 ± 5.42	0.33 ± 0.13	0.014 ± 0.002	0.013 ± 0.006	5.00 ± 1.73	0.93 ± 0.12	9.47 ± 1.29	10.20 ± 7.62
T4	6.96 ± 0.54	54.50 ± 33.65	0.20 ± 0.01	0.016 ± 0.003	0.030 ±0.015	3.75 ± 1.50	1.03 ± 0.05	8.90 ± 2.25	6.57 ± 4.52
T5	6.80 ± 0.42	44.32 ± 15.06	0.53 ± 0.05	0.018 ± 0.002	0.016 ± 0.008	3.75 ± 2.36	0.95 ± 0.33	9.08 ± 0.79	11.47 ± 2.87
T6	6.46 ± 0.18	41.18 ± 21.96	0.42 ± 0.23	0.019 ± 0.013	0.009 ± 0.002	4.00 ± 0.00	0.77 ± 0.25	8.03 ± 1.82	9.23 ± 6.72
T7	7.16 ± 0.42	55.38 ± 30.99	0.26 ± 0.14	0.011 ± 0.004	0.013 ± 0.006	2.67 ± 0.58	1.03 ± 0.45	9.87 ± 1.01	6.63 ± 7.70
T8	7.38 ± 0.37	62.70 ± 39.47	0.32 ± 0.17	0.018 ± 0.010	0.018 ± 0.009	4.25 ± 2.06	0.98 ± 0.05	8.95 ± 1.15	7.90 ± 5.47
T9	7.37 ± 0.57	87.34 ± 32.23	0.62 ± 0.37	0.015 ± 0.008	0.026 ± 0.008	5.00 ± 1.73	1.07 ± 0.46	10.00 ± 0.60	8.23 ± 4.92
T10	7.44 ± 0.21	390.25 ± 130.80	33.37 ± 6.87	20.189 ± 6.471	4.160 ± 1.365	216.13 ± 107.73	86,15 ± 54.02	2.25 ± 1.13	117.07 ± 54.51

Note. All parameters are expressed in mg/L except pH (–) and conductivity (µS/cm).
[a] Data represent five different sampling surveys.
[b] Data represent three different sampling surveys.
BOD = Biological oxygen demand, COD = chemical oxygen demand, Cond = Conductivity, DO = dissolved oxygen, N_{tot} = total nitrogen, P_{tot} = total phosphorus, SS = suspended solids.

Conclusions

Most sampling stations showing acute toxicity were located in rural areas where agricultural and forestry activities are intense. The bioassays seemed to indicate that especially during the winter period, when heavy rainfall causes soil particles and attached xenobiotics to reach the river network, toxic effects in aquatic organisms can be expected. During 1ow-flow conditions (summer), acute toxicity is detected only at and immediately downstream of the urban wastewater discharge.

Spatial and seasonal variations in chronic toxicity indicate that other than the strong influence of the urban effluents, there were detectable effects in the rural areas where intensive agricultural activities take place (grasslands and production of cereals). The results from the chronic toxicity tests also confirmed the previously observed relationship between rainfall events and toxicity in the rural part of the watershed.

Mortality and alterations in reproductive success of *Daphnia* spp. were not consistently reflected in detected chemical pollution. In general, measured concentrations of individual pesticides in surface waters were lower than acute toxicity levels. Only for the case of the herbicide atrazine did detected concentrations seem to be directly related to the observed chronic toxicity; however, atrazine was detected in only two samples. Additive and synergistic effects of the presence of a mixture of pesticides at low concentrations can not be excluded as a possible cause of observed toxicity. In contrast, other limitations of the analytical techniques—such as potential removal of pesticides during sample filtration previous to the analyses, the limited number of analytes, and detection limits higher than or close to toxic concentrations for some compounds (e.g., chlorpyriphos)—should be considered.

As a general conclusion, we can state that the used technique provided some interesting information about the adverse effects of human activities on water quality. In most cases, these impacts would not have been detected using only the classic monitoring scheme based on physicochemical parameters. The analysis of the spatial and temporal context of the observed toxicity also puts some light on the possible causes of aquatic contamination. In that sense, the presented technique seems useful for general screening purposes, especially when limited resources are available. However, once toxicity has been detected, complementary methods (e.g., sediment chemical analysis, sediment toxicity testing, analysis of suspended solids) are needed for complete identification of the cause–effect relationships in order to adequately remediate the problem.

Considering the high amounts of pesticides used in the studied watershed, more toxic effects were expected from the analysis. A possible limitation of the used approach may be its staticity. Even in the chronic toxicity tests, the sample water composition remained the same throughout the test period, whereas conditions in the field can vary continuously. During rainstorms, pesticides in running surface waters may be present at relatively high concentrations for only a short period of time, after which a rapid decrease in concentration takes place. As a consequence, when the proposed technique is to be applied in areas where intermittent contamination is important, adverse effects caused by peak runoff events can easily be missed unless specific field campaigns are set up. It may be of

great interest to take into account the temporal variability of local field conditions, the use of additional field techniques such as *in situ* bioassays and biomarkers, and the establishment of biotic indices.

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