Bull. Environ. Contam. Toxicol. (2001) 66:263–268

DOI: 10.1007/s001280000233

Bulletin of Environmental Contamination and Toxicology

Algal Growth Recovery Studies After Paraquat Exposure

M. E. Sáenz, W. D. Di Marzio, J. L. Alberdi, M. C. Tortorelli

Ecotoxicology Research Program, Department of Basic Sciences, National University of Luján, Post Office Box 221 (6700) Luján (B), Argentina

Received: 10 March 2000/Accepted: 16 October 2000

Paraquat (1-1' dimethyl 4-4' biyridylium dichloride) is a non selective contact broad-spectrum post emergent herbicide used in aquatic and terrestrial weed control and as an herbage desiccant. According to the National Register of Agrochemicals Products, paraquat is used in Argentina at a mean quantity of 1.196.000 liters of commercial formulation a year, both alone and in combination with other herbicides. This herbicide is mainly used in agricultural crops of potatoes and grapevines, while a minor use includes fruit trees, sugar cane, pastures and sunflowers. The recommended application rate is 0.1 to 2 ppm of active ingredient in water for aquatic weed control and 4 lts. of Osaquat® formulation/ha in agricultural practise (WHO 1984; Eisler 1990).

The biochemical mechanism of paraquat toxicity is due to the cyclic oxidation and reduction in tissues, leading to production of superoxide anion and other free radicals, and eventually the highly destructive hydrogen peroxide. Hydroxyl radicals have been implicated in the initiation of membrane damaging by lipid peroxidation, inactivation of proteins and damage to DNA. Photosynthesis reactions causes a depletion of NADPH formation at photoact I level, affecting important NADPH - requiring biochemical processes (WHO 1984).

There are some studies about the effects of paraquat on aquatic vascular plants, but few about the effects on microalgal species. Butler (1977) reported that 0.1 mg/L of paraquat inhibited growth of *Navicula osteraria*, while 100 mg/L was needed to stop the growth of *Phaeodactylum tricornutum*. Sáenz et al. (1993; 1997) found a 96 hr Effective Concentration 50 (EC_{50}) between 0.04 and 1.30 mg Pq a.i./L to different green algae species. Eisler (1990) mentioned that freshwater algae, in general, die at paraquat concentrations between 0.25 and 0.5 mg/L. He also reported that herbicidal effects have been found for three species of freshwater algae between 0.25 and 1.0 mg Pq/L, while this effect was noticed at paraquat concentration higher than 5.0 mg/L to the other two species.

The present study reports the results of an experiment performed for the definition of algistatic or algicidal effects of paraquat on the green algae *Scenedesmus quadricauda*, as well as the effects of this herbicide on primary productivity. *Scenedesmus quadricauda* is an important constitutive specie of the Luján River

Correspondence to: M. E. Sáenz

plankton community, in terms of abundance and frequency (del Giorgio et al. 1991). The Luján River is a planice system that runs across the North West of Buenos Aires province where there is important agricultural activity. From this point of view, these studies provides important basic information about the phytotoxicity of this widespread herbicide to natural algal populations.

MATERIALS AND METHODS

Unispecific culture of *Scenedesmus quadricauda* was obtained from the Luján River water samples using enrichment techniques and plate selection by micromanipulation. The strain was cleaned by transferring the cells to fresh liquid nutrient medium weekly. We did not use antibiotic or funguicidal products. The strain was maintained for a year in the Ecotoxicology Laboratory Culture Collection, National University of Luján, before its use as a test organism.

The 96 hr algal toxicity test exposure was conducted following the general design of the USEPA (1989). Algal stock cultures were maintained in modified Detmer's nutrient medium (pH: 7.5) (Accorinti 1962), under controlled conditions in a climatized room at 22°C +/- 1°C, 3000 lux/cm^2 of continuous "cool-white" fluorescent lighting and at 100 excursions/min on a shaker (Sáenz et al. 1992; 1993; 1997).

An inoculum was prepared from this culture to provide an initial cell density of 5 x 10^4 cell/mL in treated and control flasks. The test solutions consisted of modified Detmer's nutrient medium with the addition of different concentrations of paraquat. Nominal concentrations of paraquat were prepared with a dilution factor of 0.5 from the commercial formulation Osaquat® containing 27.6% of active ingredient. Control and treated cultures were growth under the same conditions of temperature, photoperiod and shaking as that of the stock cultures.

The procedure of Payne and Hall (1979) was used for the determination of algistatic (reduction in growth rate) and algicidal (cell death) effects. After the contact period of 96 hr, each exposed culture was centrifuged (10 min, 2000 x *g*), the algal pellet was resuspended in fresh nutrient medium and recentrifuged. Cells were enumerated and then inoculated in sterile nutrient medium to give an initial inoculum of 5 x 10^4 cel/mL. A recovery period of ten days were performed at the same incubation conditions of the contact period. The enumeration of the cells were done on days 3, 5, 7, and 10 (Payne and Hall 1979; Rand 1995).

Cell counts were correlated with absorbance (750 nm) on a Shimadzu MUV 240 spectrophotometer (Walsh 1988; Lorenzetti 1989). Growth rates (r) and generation times (GT) of control and treated cultures were calculated as was indicated in Sáenz et al. (1993). Primary productivity was measured with the dark and light bottles method. Treated and controls bottles were incubated for four hours at 22 °C under a light intensity of 3000 Lux/cm^2. Dissolved oxygen was determinated by a Hanna oxygen electrode. A one-way statistical analysis of variance ($p < 0.05$) in conjunction with Tuckey's multiple range test and

Dunnett's test was perform (Sokal and Rohlf 1979). The TOXSTAT version 3.5 (WEST Inc and Gulley 1996) was used. The EPA probit analysis version 1.4 was employed to estimate the EC_{50} values at each time and 95% confidence intervals (Finney 1971).

The algistatic concentration was determined according to Payne and Hall (1979). Functional lineal regression analysis were performed according to Ricker (1973).

RESULTS AND DISCUSSION

The toxic effects of paraquat were noticed from the beginning of the test. There was an increase in growth inhibition as the concentrations of paraquat increased. EC_{50} values at each time of exposure are indicated in Table 1. At the end of the contact period of 96 hr, the population growth of those cultures exposed to concentrations equal and above 0.4 mg Pq a.i./L were severely affected (Table 2). There was a total growth inhibition of population exposed at 3.2 mg Pq a.i./L, as indicate the growth rates values (Table 2).

Table 1. EC_{50} (mg a.i./L) values to paraquat for *Scenedesmus quadricauda*.

Time (hr)	EC_{50}	95% IC*
24	0.87	(0.6 - 1.6)
48	0.21	(0.14 - 0.28)
72	0.19	(0.15 - 0.23)
96	0.22	(0.18 - 0.26)

* 95 % confidence intervals

The growth rates of populations exposed to 0.4 and 0.8 mg Pq a.i./L indicated an algistatic response, while populations exposed at 1.6 and 3.2 mg Pq a.i./L showed an apparent algicidal response. When the cultures were resuspended in free-herbicide nutrient medium during the recovery period, there was a recovery of the population exposed to 0.4 mg Pq a.i./L from the third day of incubation. *Scenedesmus quadricauda* was also recovered from concentrations 0.8, 1.6, and 3.2 mg Pq a.i./L, but with an expanded lag phase which was more extended in the case of the population exposed to 3.2 mg Pq/L (Figure 1). At day 10, growth rates of the cultures that were exposed from 0.05 to 1.6 mg Pq a.i./L did not show a significant decrease with respect to the growth rate of control culture (ANOVA - Dunnet, $p < 0.05$). In the case of cultures exposed to the highest concentration, this parameter was significantly lower with respect to the control value (ANOVA - Dunnet, $p < 0.05$) (Figure 1). The cultures exposed to 3.2 mg Pq a.i./L in the contact period had shown a 100% inhibition growth, but developed a growth rate of 0.42/day at the end of the recovery period. According to this evidence, paraquat would cause algistatic rather than algicidal effects. The algistatic concentration estimated was 0.75 mg Pq a.i./L.

Table 2. Growth rate (r) and generation time (GT) of *Scenedesmus quadricauda* cultures exposed to different concentrations of herbicide paraquat during contact and recovery period.

mg Pq a.i./L	r (day^{-1})		GT (day^{-1})	
	Contact	Recovery	Contact	Recovery
0	0.88	0.49	0.78	1.41
0.05	0.88	0.49	0.78	1.41
0.1	0.78	0.49	0.88	1.41
0.2	0.86	0.48	0.80	1.44
0.4	0.63	0.49	1.10	1.41
0.8	0.50	0.49	1.38	1.41
1.6	0.22	0.50	3.15	1.38
3.2	0	0.42	0	1.65

Primary productivity of *Scenedesmus quadricauda* populations was notably affected from paraquat concentrations of 0.2 mg Pq a.i./L, exerting a decrease in net primary productivity more than fifty per cent with respect to the control (Table 3).

Table 3. Effect of paraquat on primary productivity (mg O_2/l/h) of *Scenedesmus quadricauda* populations after four hr of incubation.

	Control	0.2 mg Pq a.i/L	0.4 mg Pq a.i./L	0.8 mg Pq a.i./L
PPG [a]	1.202	0.655	0.43	0.43
PPN [b]	0.882	0.335	0.11	0.11
R [c]	0.32	0.32	0.32	0.32
%I [d]	----	62	88	88

[a]Gross primary productivity. [b]Net primary productivity. [c]Respiration. [d] %I: inhibition of net primary productivity with respect to the control.

From the evidence of this study, paraquat recommended application rate for aquatic weed control would affect the population growth of *S. quadricauda.* According to our results, from 48 hs of exposure, the EC_{50}s values were within the application range of the herbicide. Furthermore, application doses of 1.6 mg Pq a.i./L caused a 90% reduction of population growth with respect to the control after 96 hs of exposure. The primary productivity of *S. quadricauda* populations was also affected at paraquat concentrations within recommended doses. This is an outstanding fact to consider because of the important role this specie play in the Luján River phytoplankton community. Considering the algistatic concentration, important damage would also be expected upon population recovery after paraquat applications. Growth rate were notably affected with the consistently slow recovery due to an increase of the generation time of algal populations. This fact could cause a variation in the composition of the natural phytoplankton community of the Luján River (Thompson and Couture 1991).

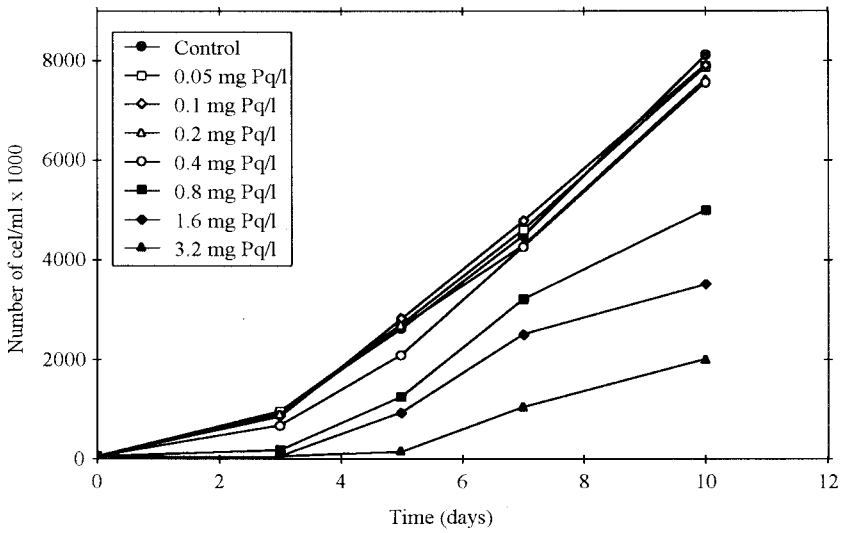

Figure 1. Growth curves of *Scenedesmus quadricauda* cultures during recovery period after paraquat exposition.

Under our experimental conditions, paraquat exerted algistatic and no algicidal effects upon populations of the freshwater alga *Scenedesmus quadricauda*, even at concentrations of 3.2 mg Pq a.i./L. The data reported in Eisler (1990) would not be applied for this specie since paraquat concentrations of 0.25 and 0.5 mg Pq a.i./L didn't cause the death of algal cells.

Since there is information about adverse effects of paraquat upon other common freshwater green algae species (Sáenz et al. 1997), care must be taken in the use of this herbicide for aquatic weed control.

Acknowledgments. We thank the Department of Basic Sciences of the National University of Luján for the financial support which made possible the realization of this study. We thank Gustavo Cifuentes for the technical assistance provided during this study.

REFERENCES

Accorinti J (1962) Efectos de los ácidos indolacético y giberélico sobre el crecimiento de *Scenedesmus obliquus*. Rev Museo de L P 39: 101-124

Butler GL (1977) Algae and pesticides. Residue Rev 66: 19-62

del Giorgio P, Vinocur AL, Lombardo RJ and Tell HG (1991) Progressive changes in the structure and dynamic of the phytoplankton community along a pollution gradient in a lowland river - a multivariate approach. Hydrobiology 224: 139-154

Eisler R (1990) Paraquat hazards to fish, wildlife and invertebrates: a synoptic review. Contaminant Hazard Reviews. U.S. Fish Wildlife Service Biological Report 85 (1.22) pp 20

Finney DJ (1971) Probit analysis. Cambridge Univ Press
Lorenzetti ML (1989) Avaliação da toxicidades de substancias químicas a *Chlorella vulgaris*. Procedimento Operacional Padronizado N° 014 CETESB, São Paulo, Brazil
Payne AG and Hall RH (1979) "A method for measuring algal toxicity and its application to the safety assessment of new chemicals". In: Marking L.L., R.A. Kimerle (eds) Aquatic Toxicology, ASTM STP 667, American Society for Testing and Materials pp 171-180
Rand GR (1995) Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment. Taylor & Francis, Washington, USA, pp1125
Ricker WE (1973) Linear regressions in fishery research. J Fish Res Board Canada 30: 409-434
Sáenz ME, Accorinti J and Tortorelli MDC (1992) *Scenedesmus acutus* clon 276-3a y su uso en tests toxicológicos algales. Brazilian Journal of Toxicol 5: 5-7
Sáenz ME, Accorinti J, Tortorelli MDC (1993) Toxicity of paraquat to a green algae *Scenedesmus acutus*. J Environ Sci Health B 28: 193-204
Sáenz ME, Alberdi JL, Di Marzio WD, Accorinti J and Tortorelli MDC (1997) Paraquat toxicity to different green algae. Bull Environ Contam Toxicol 58: 922-928
Sokal RR and Rohlf FJ (1979) Biometria - principios y métodos estadísticos en la investigación biológica. H Blume, Madrid
Thompson PA and Couture P (1991) Short- and long-term changes in growth and biochemical composition of *Selenastrum capricornutum* populations exposed to cadmium. Aquat Toxicol 21: 135-144
United States Environmental Protection Agency (US EPA) (1989) *Selenatrum capricornutum* growth test. In: Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms. Environmental Monitoring and Support Laboratory Office of Research and Development, EPA/600/4-89/014
Walsh GE (1988) Principles of toxicity testing with marine unicellular algae. Environ Toxicol Chem 7: 979-987
WEST Inc and DD Gulley (1996) TOXSTAT® V 3.5 Western Ecosystems Technology Inc. WY
WHO World Health Organization (1984) Paraquat and Diquat. Environ Health Crit 39 pp128