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Paraquat Toxicity to Different Green Algae

M. E. Sáenz,[1] J. L. Alberdi,[1] W. D. Di Marzio,[1] J. Accorinti,[2] M. C. Tortorelli[1]

[1]Ecotoxicology Research Program, Department of Basic Sciences, National University of Luján, P.O. Box 221, 6700 Luján (B), Argentina
[2]Department of Biological Sciences, Faculty of Exact and Natural Sciences, University of Buenos Aires, Buenos Aires, Argentina

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Paraquat (l-1' dimethyl 4-4' bipyridylium dichloride) is a non-selective contact broad-spectrum post emergent herbicide, recognized for use in aquatic and terrestrial weed control and as an herbage desiccant non-volatile and insoluble in fat. Since its introduction in the 60's, paraquat has been used extensively in about 130 countries on a wide variety of agricultural crops. When a dipyridil is present it replaces the ferrodoxin, one of the electron transports of the photoact I (Akhavein and Linscott 1968). In this way Paraquat is reduced forming free radicals. This leads to the formation of hydroxyl radicals which have been implicated in the initiation of membrane damaging by lipid peroxidation, inactivation of proteins and damage to DNA. Depletion of NADPH formation, one of the most important ferrodoxin dependent reactions, may disrupt important NADPH - requiring biochemical processes (WHO 1984).

Paraquat is the active ingredient of the commercial formulation Osaquate commercialized by OSA, which is widely used in Argentina. The National Institute of Agricultural Technology (INTA) recommends its use for aquatic weed control with an application rate of 0.1 to 2 ppm of active ingredient in water (Calderbank 1972; Eisler 1990) and 4 lts. of Osaquate® formulation/ha in agricultural practices. Paraquat is strongly adsorbed in soils and sediment and can persist for at least 6 month without significant degradation (Eisler 1990). In freshwater ecosystems, the loss in the water column is rapid; initial applications of 1-5 mg/L are usually not detectable under field conditions after 8 to 27 days (Summers 1980).

There is scarce information about toxic effects of paraquat on microalgae. Butler (1977) reported that 0.1 ppm of paraquat inhibited growth of *Navicula osteraria,* while 100 ppm was needed to stop the growth of *Phaeodactylum tricornutum.* Ware and Roan (1970) found a decrease of 53% in carbon fixation of estuarine phytoplankton after 4 hr of exposure to 1 mg of paraquat /L.

It is very important to expand the knowledge about harmful effects of toxic substances on population growth of planktonic algae, since they are an important component of aquatic systems.

Correspondence to: M. E. Sáenz

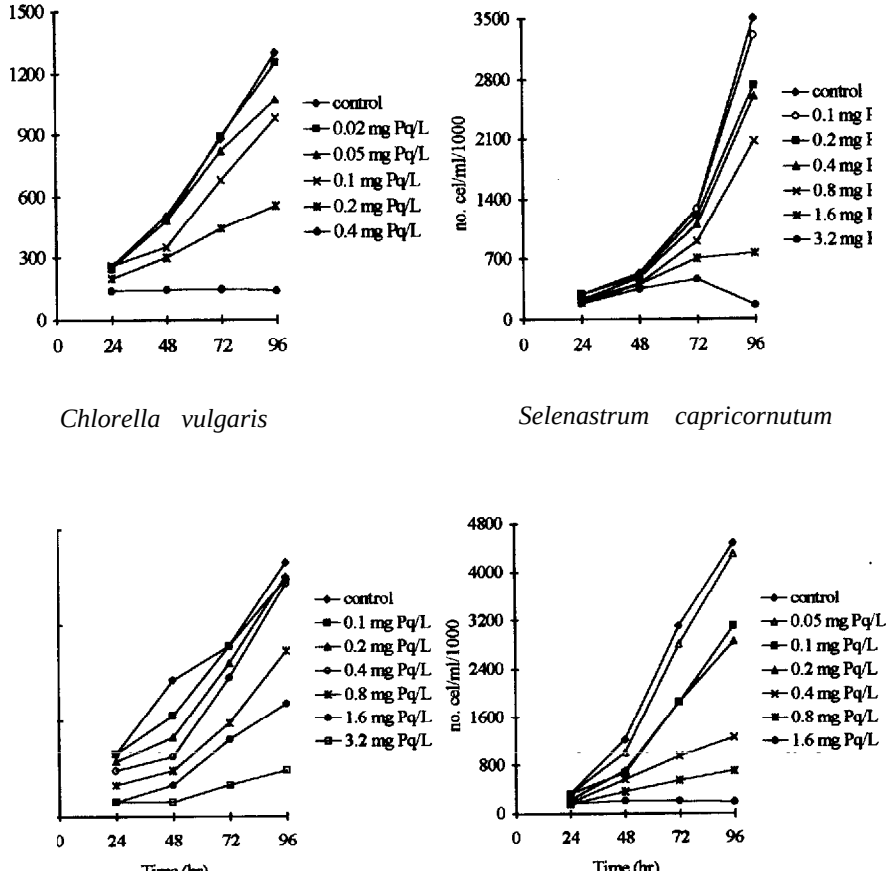
This study reports the results about the effects of paraquat upon population growth of the green algae, *Scenedesmus quadricauda, Scenedesmus acutus, Selenastrum capricornutum* and *Chlorella vulgaris.*

MATERIALS AND METHODS

Two different strains of *Scenedesmus quadricauda* was used. The standard strain (CCAP 276/21) was obtained from the Culture Collection of Algae and Protozoa, England. A wild strain was obtained from Luján river water samples, a lowland watercourse that rises in the North West of Buenos Aires Province, by enrichment techniques and plate selection by micromanipulation. The strain was cleaned by transferring the cells to fresh liquid nutrient medium periodically. It was maintained for a year in the Ecotoxicology Laboratory Culture Collection, National University of Luján, before its use as test organism. *Scenedesmus acutus* (SAG 276-3a) was obtained from the Culture Collection of Göttingen University, Germany. *Selenastrum capricornutum* (CHL1) was provided by CETESB (Companhia de Tecnologia de Saneamento Ambiental, São Paulo) Brazil. *Chlorella vulgaris* was obtained from the Culture Collection of Plant Physiology, University of Buenos Aires.

Algal toxicity test of 96 hr of exposition was conducted following the general design of U. S. Environmental Protection Agency (USEPA 1989). Algal stock cultures from each specie were maintained in modified Detmer's nutrient medium (pH: 7.5) (Accorinti 1962), under controlled conditions in a climatized room at 22°C +/- l°C, 3000 lux/cm^2 of continuous "cool-white" fluorescent lighting and at 100 excursions/mm on a shaker (Sáenz et al. 1992; 1993). The inocula were prepared from these cultures to provide an initial cell density of 5 x 10^4 cell/ml of each specie in treated and control flasks. The test solutions consisted in modified Detmer's nutrient medium with the addition of different concentrations of paraquat. Nominal concentrations were prepared from the commercial formulation Osaquate which contained 27.6 % of active ingredient. Control cultures were incubated in the same medium without the herbicide. Control and treated cultures were grown under the same conditions of temperature, photoperiod and shaking that of the stock cultures and were done in triplicate. Each toxicity test was done twice. Cell counts were correlated with absorbance (750 nm) on a Shimadzu MUV 240 spectrophotometer (Walsh 1988; Lorenzetti 1989).

A one-way statistical analysis of variance ($p < 0.05$) in conjunction with Dunnett's test and Tukey's multiple range test was performed in the assessment of the observed differences between control and cultures exposed to different concentrations of the herbicide (Steel and Torrie 1960). These analysis were done with TOXSTAT version 3.5 (WEST Inc and Gulley 1996). The EPA probit analysis version 1.4 was employed to calculate the EC_{50} values at each time and 95 % confidence intervals (Finney 1971). Dunnett's test was used to calculate the NOEC and LOEC value (USEPA 1989; Calow 1993). Chronic values (ChV) were calculated according U.S Environmental Protection Agency (USEPA 1989) and American Public Health Association (APHA 1992). The EPA program version



Chlorella vulgaris *Selenastrum capricornutum*

Scenedesmus quadricauda (CCAP 276/21) *Scenedesmus quadricauda*

Figure 1. Growth curves of algae species exposed to different concentrations of paraquat.

1.61 was used in the statistical assessment of 96 hr EC_{50} values obtained from different species (USEPA 1985).

RESULTS AND DISCUSSION

Paraquat affected each specie in a different manner. In Figure 1, the different pattern of growth of the cultures exposed to different concentrations of paraquat can be seen. In the case of *Chlorella vulgaris,* there was a significant inhibition of growth with respect to the control, of the cultures exposed above 0.1 mg Pq/L at 48 hr of exposition. At 96 hr, the cultures exposed from 0.05 mg Pq/L and higher, showed a significant inhibition of growth when compared with the control. The culture exposed to 0.02 mg Pq/L didn't show a significant inhibition of growth within the 96 hr of exposition. When a multiple comparison was performed, there

was no significant difference between the cultures exposed to 0.05 and 0.1 mg Pq/L, at 96 hr.

In Selenasrium *capricornutum* growth curves, there wasn't significant inhibition between control and cultures exposed to different concentrations of paraquat, at 48 hr. There was not a significant growth inhibition with respect to the control in the culture exposed to 0.1 mg Pq/L along the test. However, a significant decrease in growth with respect to the control was observed in those cultures exposed above 0.2 mg Pq/L at 96 hr. At this time, a significant difference on growth was observed among exposed cultures, except between those exposed to 0.2 and 0.4 mg Pq/L.

The effects of paraquat on *Scenedesmus quadricauda (CCAP* 276/21) were noticed when growth of all cultures exposed, decreased significantly with respect to the control at 48 hr of exposition. However at 96 hr, there was a growth recovery of those cultures exposed to 0.1, 0.2 and 0.4 mg Pq/L, giving no significant decrease in growth when compared to the control. The cultures exposed from 0.8 mg Pq/L and higher showed a significant inhibition growth respect to the control.

Considering *Scenedesmus quadricauda* strain isolated from Luján river samples, all paraquat concentrations exerted a significant decrease in growth with respect to the control, except that of 0.05 mg Pq/L, at 48 and 96 hr. In a multiple comparison at 96 hr, there was not a significant difference on growth inhibition between the cultures exposed to 0.1 and 0.2 mg Pq/L; this was also noticed between those exposed to 0.4 and 0.8 mg Pq/L. Nevertheless, there was a significant difference on growth inhibition between these two groups, The cultures exposed to 1.6 mg Pq/L was significantly inhibited from the beginning of the test.

Table 1. 96 hr toxicity endpoints (mg /L) of paraquat to different green algae species (in brackets 95 % confidence intervals)

	Scenedesmus quadricauda (CCAP 276/21)	*Scenedesmus quadricauda*	*Scenedesmus acutus* (SAG 276-3a)	*Chlorella vulgaris*	*Selenastrum capricornutum*
EC_{50}	1.30 (1.01-1.6)	0.22 (0.18-0.26)	0.047 (0.02-0.07)	0.14 (0.10-0.19)	0.67 (0.55-0.81)
NOEC	0.4	0.05	0.02	0.02	0.1
LOEC	0.8	0.1	0.05	0.05	0.2
ChV	0.56	0.07	0.03	0.03	0.14

The effects of paraquat on Scenedesmus *acutus* (SAG 276-3a) growth were reported in Sáenz et al. (1993). Significant inhibition growth with respect to the control was notice above 0.05 mg Pq/L. since 48 hr of exposition. Similar results were found respect to growth parameters such as growth rates and generation time

of cultures exposed at different concentrations of paraquat. An interesting stimulatory effect was found in cultures exposed to 0.02 mg Pq/L.

The 96 hr EC_{so} of *Scenedesmus acutus* (SAG 276-3a) was significantly different from those of the others species and it was the lowest value. However, *Chlorella vulgaris* was as sensitive as *Scenedesmus acutus* (SAG 276-3a) considering the NOEC and LOEC values.

There was not a notable difference between 96 hr EC^{50} values obtained from toxicity tests of *Scenedesmus quadricauda, Chlorella vulgaris* and Selenastrum *capricornutum*. Under our experimental conditions, the sensitivity towards paraquat of the two former species was comparable to that of the test organism widely used, *Selenastrum capriconutum*. As previously indicated (Sáenz et al. 1992), these results reinforce the use of *Scenedesmus acutus* (SAG 276-3a) and the native strain of *Scenedesmus quadricauda,* as test organisms in aquatic toxicological studies since they are common species in the aquatic environment of Buenos Aires province.

On the other hand, the strain *Scenedesmus quadricauda* (CCAP 276121) was the least sensitive (Table 1).

Considering the application range of paraquat (0.1 - 2 mg Pq/L), NOEC values of *Scenedesmus quadrticauda, Chlorella vulgaris, Selenastrum capricornutum* and *Scenedesmus acutus* (SAG 276-3a) were notably under this range, except that corresponding to *Scenedesmus quadricauda* (CCAP 276/21). It means that important effects would be caused on those algae populations after paraquat applications. In addition, chronic effects would be expected considering the respectively LOEC and ChV values (Table 1).

An important fact to consider is that *Scenedesmus quadricauada, Scenedesmus acutus* and *Chlorella vulgaris* are constitutive of the plankton community in Luján river (de1 Giorgio et al. 1991). The respectively 96 hr EC_{50} were the lowest values and they were within or under the recommended application range for this herbicide in aquatic weed control. It is worth mentioning that these algae species are trophically related to the native fish *Cnesterodon decemmaculatum* and the native cladoceran *Daphnia spinulata,* two important organisms of aquatic environments of Buenos Aires province. Moreover, Di Marzio et al. (1994) and Alberdi et al. (1996) have reported toxic effects towards these organisms caused by paraquat concentrations inside or near the used range in aquatic practices.

Because of the above results, the use of paraquat in the recommended way for aquatic weed control, would cause both acute and chronic damages to natural green algae populations commonly present in aquatic environments. This would also affect the development of others natural populations of non-target organisms closely connected, by means of its food relationships.

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REFERENCES

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

Akhavein Aq Linscott DL (1968) The dipyridylium herbicides, paraquat and diquat . Residue Rev 23 : 97- 146

American Public Health Association (1992) Standard methods for the examination of water and wastewater, 18th ed. Amer Publ Hlth Assoc, Washington, DC

Alberdi JL, Sáenz ME, Di Marzio WD, Tortorelli MDC (1996) Comparative Acute Toxicity of two Formulations Herbicides, Paraquat and Glyphosate, to *Daphnia magna* and *D. spinulata.* Bull Environ Contam Toxicol 57

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

Calderbank A (1972) Environmental considerations in the development of diquat and paraquat as aquatic herbicide. Out Agr 72: 51

Calow P (1993) Handbook of ecotoxicology. Blackwell Scientific Publ, L

de1 Giorgio P, Vinocur AL, Lombardo RJ, Tell HG (1991) Progressive changes in the structure and dynamics of the phytoplankton community along a pollution gradient in a lowland river - a multivariate approach. Hydrobiology 224: 129- 154

Di Marzio WD, Tortorelli MDC (1994) Effects of paraquat (PQ) on survival and total cholmesterase activity of fry *Cnesterodon decemmaculatus* (Pisces, Poeciliidae). Bull Environ Contam Toxicol 52: 274-278

Eisler R (1990) Paraquat hazards to fish, wildlife, and invertebrates: A synoptic review. Contaminant Hazard Reviews. US Fish and Wild Serv Biol Rep 85 (1.22) 28 pp

Finney DJ (1971) Probit analysis. 3rd Ed. Cambridge Univ Press

Lorenzetti ML (1989) Avaliação da toxicidades de substancias químicas a *Chlorella vulgaris*. Procedimento Operational Padronizado, 014 CETESB São Paulo Brazil

Sáenz ME, Accorinti J, Tortorelli MDC (1992) *Scenedesmus acutus* clon 276-3a y su uso en tests toxicológicos algales. Brazilian 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 (2): 193- 204

Steel RG, Torrie JH (1960) Principles and procedures of statistics with special reference to biological sciences. Mc Graw Hill, NY

Summers LA (1980) The bipyridilium herbicides. Academic Press

U S Environmental Protection Agency (1985) Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, Environmental Monitoring and Support Laboratory Office of Research and Development, EPA 600/4-85/013

U S Environmental Protection Agency (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
Ware GW, Roan CC (1970) Interaction of pesticides with aquatic microorganisms and plankton. Residue Rev 33: 15-45
WEST, Inc and DD Gulley (1996) TOXSTAT® V3.5. Western Ecosystems Technology Inc. WY
WHO World Health Organization (1984) Paraquat and diquat. Environmental Health Criteria 39. 128 pp