THE PHOTOSYNTHETIC/RESPIRATORY RESPONSE OF A PERIPHYTIC POPULATION (*Selenastrum capricornutum*) TO PARAQUAT AS A BIOMARKER

ROCHA, T.R.P.*, WATANABE, T.**, SASSI, R.** & COLER, R.***

* Bolsista UFPB, PIBIC/CNPq

** Professor UFPB/CCEN/DSE
Campus I, João Pessoa, PB, Brasil

*** Visiting professor UFPB/CCEN/DSE
Campus I, João Pessoa, PB, Brasil

ABSTRACT: The photosynthetic/respiratory response of a periphytic population (*Selenastrum capricornutum*) to paraquat as a biomarker. The authors present data to demonstrate the sensitivity of a gravity-feed flow-through regime and its potential for application as a monitoring tool. The photosynthetic response of the periphyton *Selenastrum capricornutum* to varied concentrations of paraquat, was measured by determining the oxygen differential at both ends of a colonized glass tube through which the water sample flowed. Oxygen "spikes" were observed at 0.1, 0.01, 0.05, and 0.001 $\mu g.l^{-1}$ of paraquat. No such enhanced response was elicited at a concentration of 0.001 $\mu g.l^{-1}$. Respiration in both treatments increased more than 300% over the controls, but there was no correlation within treatments.

Key-words: Paraquat, biomonitoring.

RESUMO: Resposta fotossintética/respiratória da população perifítica (*Selenastrum capricornutum*) ao "paraquat" como um bioindicador. Os autores apresentam dados que demonstram a sensibilidade de um regime alimentado pela gravidade, com fluxo contínuo, e sua possível aplicação como ferramenta para biomonitoramento. A resposta fotossintética de *Selenastrum capricornutum* (perifiton) a concentrações variadas de "paraquat" foi obtida pela diferença nas quantidades de oxigênio dissolvido medidas em ambas extremidades de um tubo de vidro colonizado, através do qual percorreu a amostra de água. Picos de oxigênio foram observados nas concentrações de 0,1, 0,01, 0,05 e 0,001 $\mu g.l^{-1}$ de "paraquat". Houve leve diminuição da resposta na concentração de 0,001 $\mu g.l^{-1}$. A respiração aumentou mais de 300% em ambos os tratamentos quando comparada com o controle, mas não houve correlação entre o aumento e os tratamentos.

Palavras-chave: "Paraquat", biomonitoramento.

INTRODUCTION

The impoundments of Northeast Brazil are principally exploited as sources of potable water, irrigation water and fish protein. These uses are often incompatible, because intensive farming is predicated on the continuous application of fertilizers, insecticides and herbicides. Runoff carries these additives to the water body where they impact on the ecology and perhaps the public health. When one considers that in Northeast Brasil there are over 70,000 impoundments larger than 1,000 m^2 (Molle, 1991), the scale of potential insult becomes apparent. There is then a compelling urgency to monitor the quality of our surface waters; not only in Brazil but wherever population density and agriculture mandate.

In this regard, emphasis has shifled in the USA and Europe from chemical to biological criteria (bioassay and biological inventories). Adherence to chemical criteria alone failed to improve water quality significantly because pollution is fundamentally a biological phenomenon and as such requires biological end points. Unfortunately a species diversity index or EC_{50} requires 2-3 orders of magnitude more time to generate than a "$mg.l^{-1}$" type assessment. Consequently a growing priority has been placed on time, spuring the search for biological "quickies" (cytochrome, P-450, metallothionein, hormones, hepatic lesions etc) that indicate stress. Such biomarkers may lack validity (Chapman, 1996; Schlend, 1996), however, indicating merely minor internal adaptative adjustments to accommodate shifting external environmental pressures. What has relevance in an ecological risk assessment is a measure of the extent of mobilization of the organism's homeostatic response to a limiting environment or the direct impact of some stress on a vital physiological process. The authors are pursuing this quest at a physiological level, rather than from a biochemical or histological perspective where cause and effect relationships can be more readily and economically (albeit less rapidily) traced. One such process is photosynthesis/respiration. To qualify as a tool to be incorporated into the armory of a water quality laboratory the technique must be sensitive and generate reproducible meaningful data with a minimum investment of time, space, expertise and cost.

The objective ofthe present investigation then is to assess the sensitivity and practicality of a flow-through gravity-feed delivery system used in conjunction with periphyton colonized (*Selenastrum capricornutum*) glass tubes to routinely process suspect waters through monitoring the photosynthetic response to herbicides. *Selenastrum capricornutum* has been adapted by U.S.E.P.A. as a standard planktonic assay organism. Consequently its environmental prerequisites are well defined (Lewis, 1995). In addition, because of its reportedly thin cell well and lower activity of many enzymes, it is considered a relatively sensitive species (Brown, 1993). The herbicide paraquat was selected because, according to Goellner (1985), it is commonly applied in sugar cane plantations, and is reported to lose its toxicity rapidly through absortion and degradation and is immobile in soil. Any test to detect this compound in the water column would necessarily have to be sensitive.

REVIEW OF THE LITERATURE

The continuous measurement of O_2 production by the same algal population provides a kind of time-lapse photography perspective. However, when static photosynthetic activity assessments were compared to 4-5 day algal population growth studies, the latter were considered less sensitive. This has been observed for heavy metais, pesticides, surfactants, phenols and effuents (Lewis, 1995). In all of these instances, however, the tests were of short duration with the majority lasting 2-4 hours. In restrospect these observations are not

surprising. By definition, acute toxicity tests are less sensitive stress indicators than chronic toxicity tests. Further Shonwanck et al. (1996) do not consider the standard algal growth inhibition test as well suited to the assessment of algal toxicity of strong chelators. A more valid assessment would be to compare growth with photosynthesis over the same time interval.

It is difficult to imagine that a process so fundamental as photosynthesis and so energy demanding would not more readily reflect the added homeostatic burden of stress particularly with a flow-through delivery system. Flowing waters are physiologically more productive then standing waters (Hynes, 1970) and thus would have a greater response range to reflect perturbation. However, while evaluation of periphytic primary productivity in colonized tubes has been a valid field protocol (Tease et al., 1983; Tease & Coler, 1984; Coler & Rockwood, 1989), it has been adapted to the laboratory in only one instance (Coler et al., in press).

Paraquat, a bipyridine herbicide, is a class two pesticide (Goellner, 1981) similar in mechanism of toxicity to diquat generating hydrogen peroxide within the plant in the presence of light. However, because it degrades rapidly and combines readily with soil and colloids (Goellner, 1981), there is minimal accumulation of the parent molecule in the water column. The degradation productions however may persist up to six months and little is noted about their toxicity. In this regard, Brooker & Edwards (*apud* Hellawell, 1986) report that in a field application only 6% of the paraquat applied was absorbed by macrophytes while 36% was not accounted for. No data were offerred regarding the toxicity of the decomposition products. It may be added parenthetically that this choice was motivated as well by an unconfirmed report that paraquat appeared to stimulate photosynthesis in light-dark bottle productivity assessments (Watanabe et al., 1994).

METHODOLOGY

The investigators adopted the protocol of Coler et al. (in press) to determine photosynthetic rates of periphyton (*Selenastrum capricornutum*) colonizing illuminated glass tubes through which water flowed at a controlled rate. Tube colonization was accomplished by transferring culture and medium (Walne, 1966) to illuminated agar lined, stoppered tubes for 10-15 days. After a faint green color was observed the culture medium was permitted to flow through at a rate of 0.5 $ml.min^{-1}$. Testing began when the DO differential reached an asymptote. Test concentrations of paraquat were administered via a 1 l reservoir with paraquat exposure being maintained for six hours before returning to infusion with standard culture media. A new treatment was not initiated until the DO differential returned to within the control range (0.47 ± 0.05). A DO bottle flanking each side of the tube providing a "before" and "affer"' perspective. DO concentrations were determined by the Alsterberg-Azide modification of the Winkler method (Eaton et al., 1995). The difference in DO concentrations between the two bottles was interpreted as primary productivity and respiration (with and without light, respectively). Data are reported as the difference between the bottles and not as $mg.l^{-1}$ O_2 generated per hour per square meter, because operating parameters (culture media, flow, substrate, illumination) were not altered.

To minimize the investment of time, space, labor, and supplies (culture, medium, reagents, sample), flow rate was maintained at 3 $ml.min^{-1}$. Tube diameter and length respectively were 0.5 and 100 cm. To conserve sample volume (a critical consideration when transporting over a distance), flow was maintained only during the experiment. [This practice did not diminish consistency of response]. Instead of incorporating the standard experimental design (concurrently maintained controls and replicates) we resorted to multiple (2-4) successive analyses 2-3 times per week waiting until productivity returned to overlapping confi-

dence intervals of pre-stress values before initiating a new treatment. Each value's successor serves as its predecessor's replicate thusly providing a measure of consistency of response over an extended period of time.

RESULTS AND DISCUSSION

A review of the data (Table 1) indicates the rapid generation of oxygen spikes upon exposure to paraquat concentrations of 0.10, 0.05, and 0.01 $\mu g.l^{-1}$. A concentration of 0.001 $\mu g.l^{-1}$, however, failed to provoke this response. A concentration of 0.1 $\mu g.l^{-1}$ of paraquat elicited a spike six times that of central which required 28 days to return normal. On the other hand, 0.05 $\mu g.l^{-1}$ produced a spike approximately twice that of central value and required only 9 days to attain control levels. A concentration of 0.01 $\mu g.l^{-1}$ produced a spike 3.5 times that of the control with a recovery period of 16 days. At 0.001 $\mu g.l^{-1}$ there was no observed increase in oxygen differential, but rather, a slight decrease. This observed enhanced oxygen differential could serve as a biomarker up to somewhere between 0.001 $\mu g.l^{-1}$ and 0.01 $\mu g.l^{-1}$. These limits of sensitivity, however, are 100,000 times greater than those concentrations generally applied in the field (Goellner, 1981) and could serve as a qualitative biomarker. From a gross inspection of the data the height of the peak O_2 values have no quantitative validity. This may be due to an additive effect of consecutive paraquat exposures. It may further be noted, that there was a trend to increasing erratic recoveries with larger DO differential oscillations during the recovery following each exposure sustained. If the tube was replaced more frequently with unstressed cultures, perhaps it could serve as a quantitative tool.

It is not clear from where this enhanced oxygen differential originates. While it could be a reflection of stimulated photosynthesis, it may be an interaction between the manganous hydroxide and the hydrogen peroxide produced by the reduction of paraquat to a free radical cation by the chloroplast and it's subsequent reaction with molecular oxygen. Such an event does not seem likely because the heightened oxygen production induced by 0,1 $\mu g.l^{-1}$ could be measured for weeks after only 6 hours of exposure. In any case, the fact that recuperation did occur serves to indicate that the interval of exposure was insufficient for the peroxide molecule to destroy the selectivity of the cell membranes. Respiration, on the other hand, varied markedly between controls (0,16 $mg.l^{-1}$) and treatments (0.5 $mg.l^{-1}$), but not among treatments. It seems likely that there would be a concommitant impact on respiration were we simply measuring photosynthesis. Neither the data base nor the expertise of the authors support further speculation.

It is our intent to demonstrate that this tool provides novel dimensions for applied research and water quality monitoring. In the latter application, the use of a single tube characterized with regard to productivity and respiration would suffice to identify levels of paraquat well below reportedly stressful concentrations (Hellawell, 1984). A 1 l sample could be processed in a day at virtually no cost. It remains to be seen, however, what level of sensitivity could be achieved with other herbicides.

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Table I - Average (replicate measurements) dissolved oxygen differentials (DO) with confidence intervals (CI) generated between two ends of a colonized (*S. capricornutum*) glass tube exposed to 0.1, 0.05, 0.01 and 0.001 $\mu g.l^{-1}$ of paraquat for indicated number of days.

	Treatment ($\mu g.l^{-1}$)	D.O.Diff. ($\mu g.l^{-1}$)	CI (Days)	Duration	% Control
	0 (control)	0.47 (11)	0.05	65	-
	0.05	0.4 (4)	0	0.5	85
P		0.85 (4)	0.08	1.0	181
		0.43 (3)	0.09	9	92
H	0.1	0.21 (4)	0.12	0.5	45
		2.82 (6)	0.11	1.0	600
O		2.5 (1)	-	24	532
		1.83 (3)	0.42	26	389
T		1.65 (2)	0.45	28	351
		0.7 (3)	0.2	33	162
O		0.52 (5)	0.09	34	111
		0.5 (3)	0.2	46	106
S		0.52 (3)	0.15	50	111
		0.43 (3)	0.12	55	92
Y		0.45 (5)	0.06	57	96
		0.44 (5)	0.06	62	94
N	0.01	1.68 (4)	0.07	1	357
		0.3 (2)	-	3	70
T		0.6 (2)	-	5	128
		0.63 (6)	0.05	7	134
E		0.3 (3)	-	12	64
		0.68 (4)	0.33	14	145
S		0.53 (3)	0.12	17	113
		0.63 (3)	0.12	19	134
I		0.67 (4)	0.06	21	142
		0.53 (3)	0.12	26	113
S		0.38 (5)	0.05	1	81
	0.001	0.42 (3)	0.06	5	89
		0.8 (4)	-	9	170
R	0 (control)	0.163 (8)	0.072	2	-
E	0.001	0.5 (2)	-	1	300
S	0.01	0.5 (2)	-	1	300
P					

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