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Effect of Aniline on *Chlorella vulgaris*

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Release of hazardous waste into the aquatic ecosystem produces a variety of complex responses beyond lethality to specific organisms. (Christman et al. 1973) Hidden injury, such as inhibition of photosynthesis, or changes in oxygen consumption, must significantly affect the role that a primary producer plays in the phytoplankton -- zooplankton -- copepod -- minnow -- sunfish -- bass pyramid. When sublethal concentrations exist, a sudden elimination of organisms will not occur, but as growth and reproduction of a species are affected over a period of time, the final result could well be the same (Cairns 1971 and 1972).

Chlorella vulgaris is the chief representative of primary producers in this particular food chain. A direct correlation between concentration of waste effluent, including aniline, released by a dye company into a waterway in Eastern North Carolina, and the rise and fall of populations of Chlorella, was demonstrated by Witherspoon (1980). The present study establishes threshold concentrations of aniline which affect growth of these algae, but also shows that physiologic parameters within the organism, such as the rate of photosynthesis, were decreased as sub-threshold concentrations of toxicant.

MATERIALS AND METHODS

Unialgal asenic cultures of Chlorella vulgaris Beijerinck, obtained from Carolina Biologic Algal Collection, Burlington, NC, were incubated in millipore-filtered and autoclaved Bold's Basal Medium (BBM). Inocula of approximately 103 cells/ml were added to 100

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ml aliquots of medium and the cultures were incubated at 24°C±1° in Percival growth chambers, for 14 days. Cell counts were correlated with Absorbance (A_{750}) on a Beckman DU spectrophotometer. Cool white light at an intensity of 280 foot candles illuminated the cultures during the incubation period.

RESULTS AND DISCUSSION

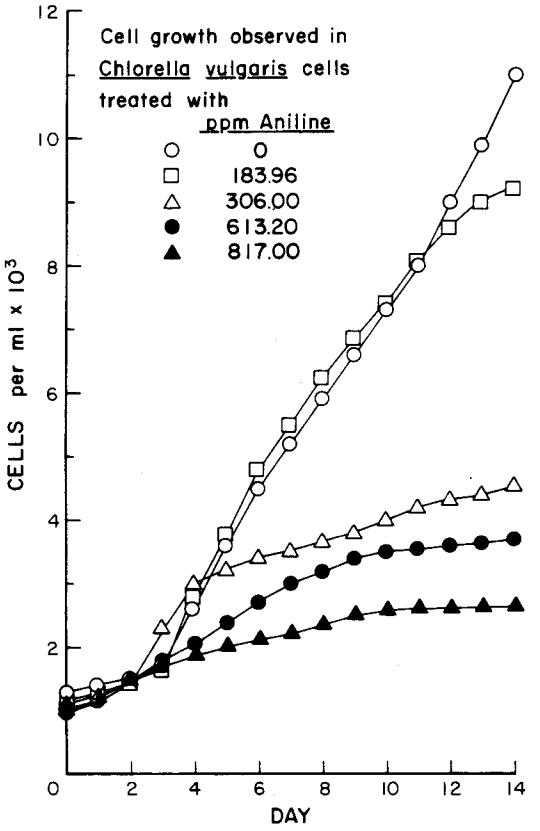

Figure 1. Cell growth observed in Chlorella vulgaris cells treated with ppm Aniline

For purposes of establishing deviation from equilibrium populations of controls, nine experimental replicates were pooled for each data point and Student's T test was done. Concentrations of aniline above 183.9 ppm were significant at the 0.5 level and this was defined as the critical or threshold level. Decline in experimental populations at this concentration occurred on day 10 of incubation. (Fig. 1). Threshold concentration was

measured as lethal concentration 50 (LC_{50}) between day 12 and 13.

Threshold concentration of aniline, defined as that concentration of aniline that produced a significant difference from equilibrium population A_{750}, was determined by observing the effect of cell population increase over a range of concentration of toxicant from 102 to 1022 ppm, in increments of 10 ppm, over 14 days.

Uptake of aniline by cells was determined by bringing standard cell cultures to threshold concentrations of aniline, removing 5 ml aliquots daily, filtering the cells and reading the A_{300} of the filtrate. The difference between concentrations of aniline in the cultures initially and that remaining in the filtrate was assumed to have been taken up by cells. (Light exposure causes aniline to darken, but this effect, as measured by absorption with the spectrophotometer, was not observed below concentrations of 225.5 ppm.)

Photosynthesis and respiration were determined on test and control cultures by measuring oxygen release and uptake with a Clark electrode in a Gilmex observation chamber. The procedure is a modification of the Light-Dark Reaction flask assay. (Boss 1980 NC State University unpublished technique).

Respiration, as measured by oxygen consumption, was not significantly affected at threshold concentration, but was reduced by day 11, at a concentration of 255.5 ppm (Fig. 2).

Photosynthesis, measured in terms of oxygen produced and consumed in the light, accumulated over 15 minute intervals for data points, was initially affected significantly at the threshold concentration on day 3 of incubation, and the ability to photosynthesize continued to decline until day 14. At concentration of toxicant below threshold levels (173.74 ppm), photosynthesis began to decrease on day 7 and continued to decline through day 14 (Fig. 3).

Games and Hites (1977) reported aniline as one of the byproducts released in the first and final effluent from a particular dye manufacturing plant. Analysis of the effluent showed aniline present in a concentration range of 36-488 ppm before treatment. Aniline and related compounds are released by other industries as well. In some cases, they were found in higher concentration <u>after</u> treatment in holding ponds, due to oxidation-reduction reactions occurring there (Games and Hites 1977). The study of Witherspoon (1980) also indicated

aniline to be a major compound of dye plant effluent and that the rise and fall in population density of Chlorella vulgaris fluctuated with corresponding decreases and increases in waterway aniline concentration.

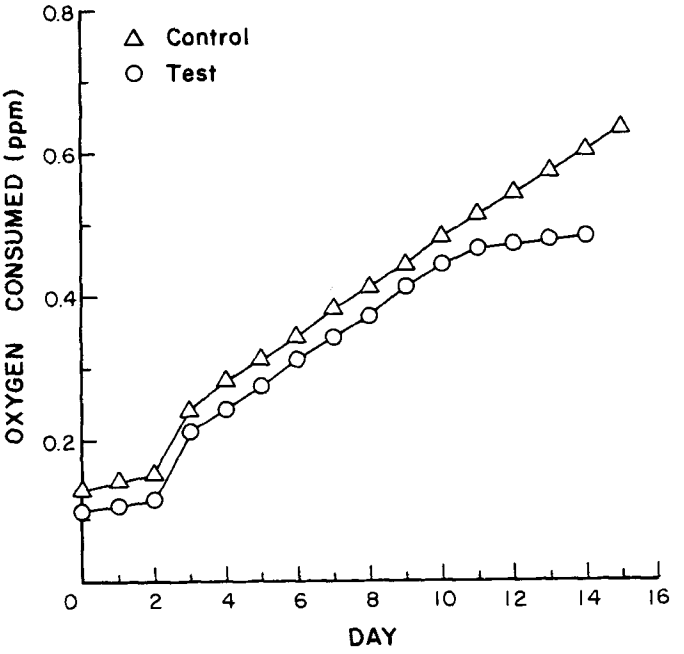

Figure 2. Oxygen consumed by Control vs Test Cultures containing 255.5 ppm Aniline

Results from the present study show that aniline not only significantly affects growth of Chlorella vulgaris, but also affects respiration and photosynthesis. Comparison of the growth curve (Fig. 1) with oxygen consumption at 255.5 ppm (Fig. 2) may explain decreased oxygen consumption as a factor of decreased population density. However, the decline in rate of photosynthesis occurred at an aniline concentration (173.44 ppm) below that considered critical for growth. Since federal standards emphasize effects on growth as a primary determinant in establishing exposure limits for aquatic organisms to chemical compounds, it seems obvious then, that this is not the only parameter to be measured if aquatic ecosystems are to be preserved. Primary producers would be eliminated over time through sub-lethal exposures, with subsequent severe damage, if not elimination of entire food pyramids.

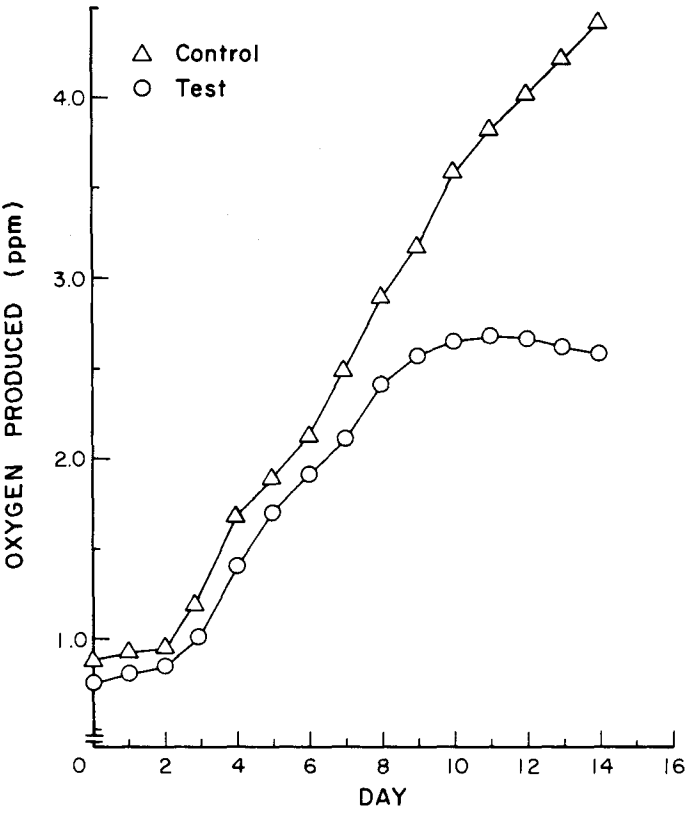

Figure 3. Oxygen produced by Control vs Test Cultures containing 173.74 ppm Aniline

Since aniline is absorbed by these algae, it would be interesting to note what persistence this chemical has within organisms, if any, or what kind of detoxifying reactions of aniline and related compounds occur.

If the chemicals or their metabolites are persistent, then biological amplification may seriously affect the zooplankton which feed on the algae, as well as those organisms nearer the apex of the food pyramid.

REFERENCES

Batterton J, Winters K (1978) Aniline's selected toxicity on aquatic plant species. Science 199: 1068-70

Cairns J (1971) The effects of major industrial spills upon stream organisms. The 26th Purdue Industrial Waste Conference. Purdue University Eng Bull 140 (I): 156-70
Cairns J (1972) Pollution related structural and functional changes in aquatic communities worth emphasis on fresh water algae and protozoa. Proc Acad Sci Phila 124(5):79-127
Christman RF, Man BW, Welch EB (1973) The natural environment: waste and control. Goodyear Pub Co, Pacific Palisades, California
Games W, Hites RA (1977) Composition, treatment efficiency, and environmental significance of dye manufacturing plant effluents. Analyt Chem 49:1433-40
Maloney TE, Palmer CM (1956) Toxicity of six chemical compounds to thirty cultures of algae. Water and Sewage Works 509-13
Whitford LA, Schumacher GT (1969) A manual of the fresh water algae in North Carolina. Tech Bull 188:4,36,45
Witherspoon AM (1980) Water resources report. NC State University/UNC Chapel Hill

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