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Hexavalent Chromium Effects on Carbon Assimilation in *Selenastrum capricornutum*

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One of the difficulties in assessing toxic substances such as metals and complex organic compounds is the duration of the necessary tests. It would be beneficial to have available standardized tests of short duration that would allow a reduction in necessary manpower and overall cost as well as provide a method to evaluate chemicals that are readily degraded (i.e., within a few hours). One method available is the short-term photosynthetic response of algae to a given toxicant. Photosynthesis is not only a critical physiological response but is also one for which standard, accurate methods are available. Stadnyk et al. (1971) used the ^{14}C method to study the effects of pesticides on a freshwater alga. This technique has also been used to study the effects of chromium on algae (Wium-Anderson 1974). More recently, Giddings et al. (1983) proposed that the ^{14}C method be used to measure short-term photosynthetic response as a standard algal bioassay. Delistraty (1986) studied growth and photosynthetic response of Selenastrum capricornutum to oil shale by-product water. The present study was designed to further evaluate, via photosynthetic response, the potential effects of hexavalent chromium on Selenastrum capricornutum.

MATERIALS AND METHODS

The test organism, Selenastrum capricornutum Printz, was obtained from a culture maintained at North Texas State University, Institute of Applied Sciences. A large glass carboy containing nutrient media was inoculated with approximately 1000 cells/mL. The culture was allowed to grow for several days prior to initiation of the experiment. The algae were in the log growth phase when used. Two separate experiments

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were performed. In the first, cell counts indicated a density of 1 x 10^6 cells/mL. A denser culture was used in the second experiment.

Productivity was determined using the ^{14}C method (Schindler et al. 1972). Thirty-six 300-mL BOD bottles (18 light and 18 dark) were filled with the media containing S. capricornutum. A set of 6 bottles (light and dark, in triplicate) was filled with each of the nominal concentrations of chromium: 0.0 (control), 0.02, 0.04, 0.06, 0.08, and 0.10 mg/L. The bottles were inverted several times to distribute the chromium. The bottles were then injected with 1 mL of $NaH^{14}CO_3$ (10 µCi/mL, Amersham) and inverted a second time to mix. The bottles were allowed to incubate under fluorescent lights (400 ft-c) for 4 h.

Following incubation, 1.0 mL of 0.1 N HCL was injected into each bottle. A 5-mL subsample was withdrawn from each bottle and placed in a scintillation bottle. Following bubbling, 15 mL of Aquasol, a scintillation fluor (New England Nuclear) was added to each vial. The vials were allowed to dark-adapt for 24 h prior to being counted (Beckman LS-100). Inorganic carbon was determined from alkalinity, pH, and temperature (Lind 1979). Counts were converted directly to mg $C/m^3/h$. Values were analyzed for significant difference using Kruskal-Wallis analysis of variance (α=0.05).

RESULTS AND DISCUSSION

In the first experiment, primary productivity remained relatively constant through time with the exception at a concentration of 0.02 mg/L where there was a decrease in productivity (Fig. 1). However, this decrease was not significant (p=0.0827). The decline was due to both a decrease in carbon uptake in the light bottles and a sharp increase in the dark bottles. This initial increase in ^{14}C uptake in the dark bottles was followed by a constant and linear decrease (Fig. 2). Carbon uptake in the dark bottles was significantly different among treatments (p=0.0064).

The second experiment generally confirmed the results of the first test. Unlike the latter, however, there was no sharp decline in productivity at 0.02 mg/L (Fig. 3). Apparent dark bottle uptake did not increase at 0.02 mg/L, though it did show a subsequent decrease (Fig. 4). This decrease was not, however, significant (p=0.1045) due to wide replicate variance. Carbon uptake in both the light and dark bottles was considerably higher in all concentrations in the second experiment as a result of higher initial algal density.

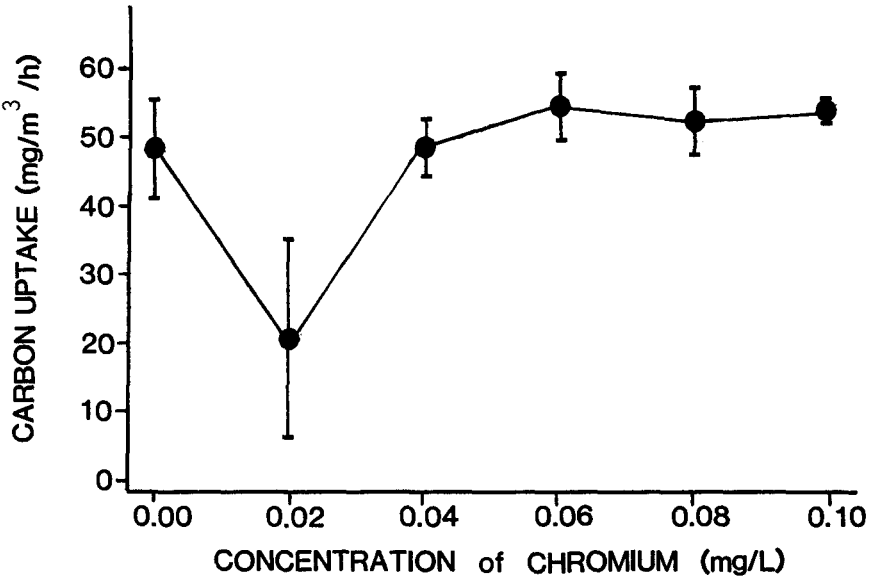

Figure 1. Primary productivity in Selenastrum capricornutum exposed to varying concentrations of chromium ($\bar{X}$ ± 1 S.D.) (Experiment #1).

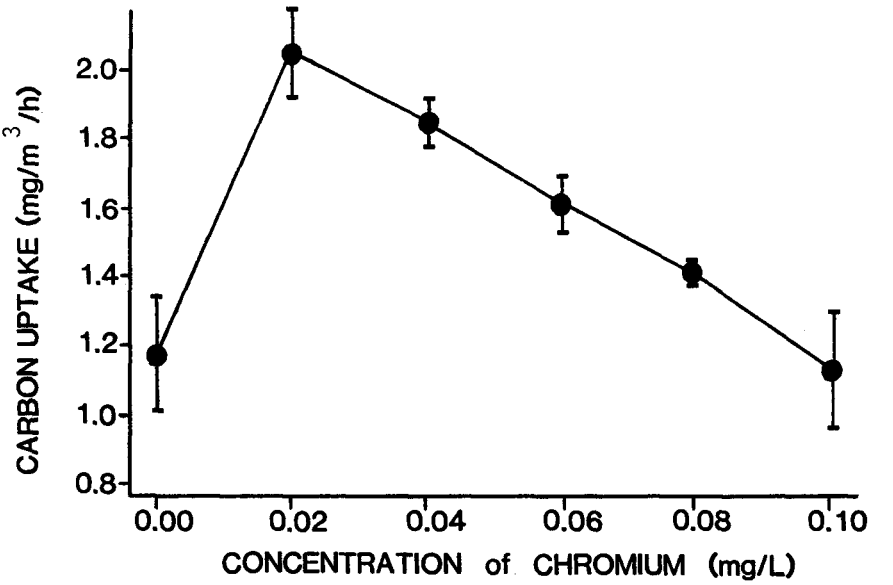

Figure 2. Carbon uptake in the dark bottles by Selenastrum capricornutum exposed to varying concentrations of chromium ($\bar{X}$ ± 1 S.D.) (Experiment #1).

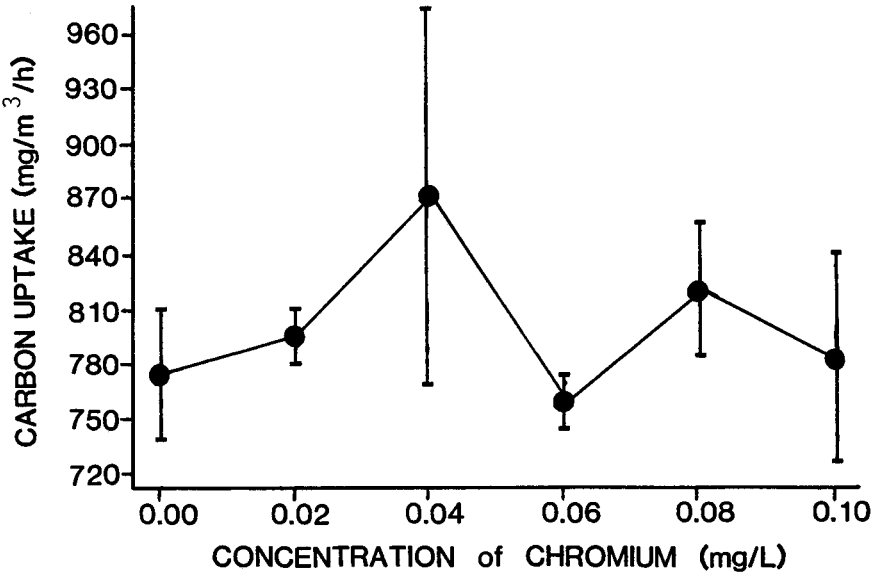

Figure 3. Primary productivity in Selenastrum capricornutum exposed to varying concentrations of chromium ($\bar{X}$ ± 1 S.D.) (Experiment #2).

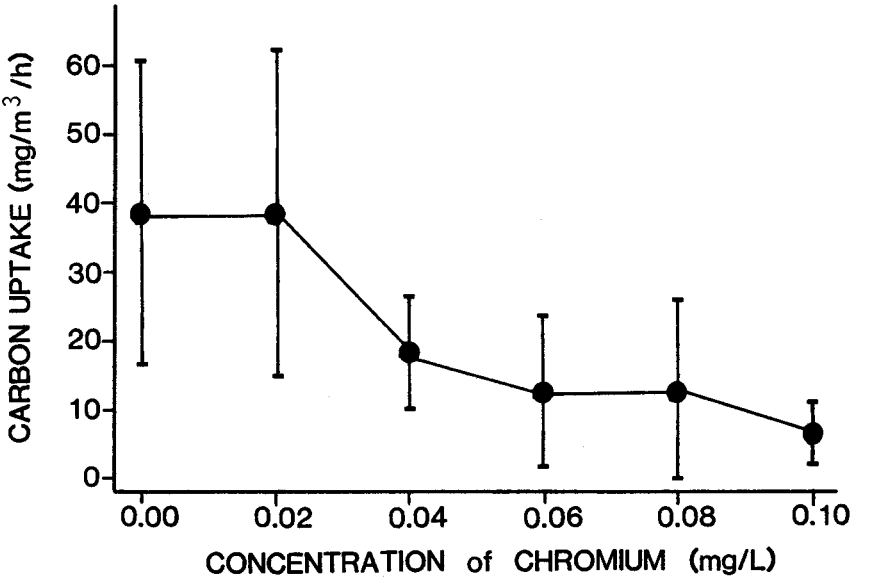

Figure 4. Carbon uptake in the dark bottles by Selenastrum capricornutum exposed to varying concentrations of chromium ($\bar{X}$ ± 1 S.D.) (Experiment #2).

It is apparent that a stimulatory effect (hormesis) by chromium is occurring in the dark bottles. Such heightened uptake, due to the effects of toxicants, has been noted by several researchers. Laughlin et al. (1981) found that low doses of mixed hydrocarbons (jet fuel) initially enhanced growth of algae, which was then followed by a decrease. Lederman and Rhee (1982) noted a similar reaction by Fragilaria crotonensis when exposed to HCB (hexachlorobiphenyl), as measured by ^{14}C assimilation. Initial growth enhancement has also been discovered in Scenedesmus quadricaudata upon exposure to Carbaryl (1-naphthyl methyl-carbamate) and Toxaphene (octachlorocamphene) (Stadnyk et al. 1971). Unlike organics, however, a stimulatory effect by chromium, or other metals, has not been well documented. One of the first studies completed on chromium found enhanced growth at concentrations ranging from 0.0001 - 0.32 mg/L (Hervey 1949). Wium-Anderson (1974), using the ^{14}C method, noted only inhibition of algal photosynthesis by chromium, and thus questioned Hervey's results. The former, however, used relatively high concentrationss, the lowest being 0.1 mg/L which, in the present study, did indeed result in carbon uptake below that of the control.

The fact that changes were apparent in the dark bottles but not in the light implies that either 1) photosynthesis in the light bottles is masking effects that are present or 2) chromium is affecting processes that are occurring primarily in the dark bottles. Although several processes may be occurring in the dark bottles (Peterson 1980), the two predominant activities are dark uptake of carbon and respiration. This is particularly true in laboratory studies where bacterial and zooplanktonic contamination is minimized or eliminated. The ^{14}C method of determining productivity assumes respiration in both light and dark bottles to be approximately equal. However, increasing light intensity has actually been shown to significantly inhibit CO_2 evolution (Brown and Weis 1959; Wetzel and Likens 1979). Reassimilation of intracellular CO_2 as a source of endogenous carbon during photosynthesis also occurs (Raven 1972). This process may also be affected by light intensity (Peterson 1980).

The appearance of significant changes in carbon assimilation in the dark bottles is due to effects upon the dark CO_2 pathways. Chromium has been found to stimulate CO_2 production in fungi (Burkeholder and Mertz 1967). In addition, other researchers have concluded that, in algae, it is the dark processes that are notably affected by chromium as well as by organics (Wium-Anderson 1974; Glooschenko and Glooschenko 1975). Therefore, there would appear to be a stimulation of

respiratory activity as well as a suppression of the dark CO_2-requiring photosynthetic reaction. The greater variance and amelioration of effects in the second experiment can be attributed to the greater algal density which can affect the action of a toxicant (Wium-Anderson 1974).

In summary, hexavalent chromium appears to act primarily on processes occurring in the absence of light in Chlorophyceae. Respiration is stimulated while dark carbon uptake may be suppressed. Low concentrations of chromium (< 0.1 mg/L) may have a stimulatory effect and thus improve apparent carbon uptake. However, this action is critically dependent upon initial cell density; very dense cultures may exhibit no effects whatsoever. Therefore, initial cell counts, as well as other test conditions, must be standardized if short-term ^{14}C productivity is to be used as a fast and effective means of toxicant evaluation.

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