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NOTES

Effect of Heavy Metals on Short-Term Photosynthetic Rates in *Potamogeton amplifolius*

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INTRODUCTION

Comparatively little is known about the effects of heavy metals on aquatic vascular plant physiology. Studies that have been reported focus on relatively long term effects. In 8-day exposure experiments, chromium in concentrations exceeding 1.0 mg/l reduced the growth rates of the duckweed genera *Lemna* and *Spirodela* (Staves and Knaus 985). Glycolate metabolism in *Potamogeton pectinatus* L. was stimulated by 3-day exposure to cadmium (0.1 to 1.0 mM) and copper (0.01 to 10 mM), whereas *Hydrilla verticillata* (Lf.) Royle was inhibited by the same concentrations (Jana and Choudhuri 1981). Filbin and Hough (1979) demonstrated that chlorophyll *a* content, photosynthetic carbon assimilation, dark glucose uptake, and several other metabolic functions were inhibited upon incubating plants for 20 days in media in which chromium levels were increased from 2.50 to 3.94 mg/l. Kay et al. (1984) found that reduced shoot and root growth rates and leaf chlorosis could be elicited in water hyacinth (*Eichhornia crassipes*) (Mart.) Solms) by exposure to chromium and copper (threshold values to 2 mg/l and 0.5 mg/l, respectively) for several weeks.

We are aware of no reports describing the short term (i.e. minutes to a few hours) effects of heavy metals on important physiological parameters in aquatic vascular plants. Leaf disk and section bioassays have previously been employed to examine the effects of pH and various organic chemicals on short-term photosynthetic ^{14}C-assimilation (PCA) rates in submersed macrophytes (Beer et al. 1982; Francko and Wetzel 1984; Francko 1986). In this investigation, we used a leaf disk bioassay method to conduct a preliminary analysis of the effect of short-term (15 min to 4 h) chromium and copper exposure on PCA rates in the pondweed *Potamogeton amplifolius* Tuckerm.

MATERIALS AND METHODS

Specimens of intact *P. amplifolius* were collected from the littoral zone of Sanborn Lake, an oligotrophic spring-fed lake located about 5 km from the university campus. Plants relatively free from epiphytic-calcium carbonate encrustations were returned to the laboratory immediately after collection. Leaves were gently surface disinfested with wet Kimwipes and 0.75-cm disks were cut using a cork borer. Disks were placed in artificial medium (Medium II of Forsberg 1965, modified by Wetzel 1969; pH 8.2) prepared with water purified by reverse osmosis, deionization, organic removal, and submicron filtration.

Three disks each were placed into beakers containing 25-ml Medium II. At time zero, sterile stock solutions of $CuSO_4$ or hexavalent chromium (as potassium dichromate) were used to amend medium solutions with each metal, producing final concentrations of 0.5 μg/l to 10 mg/l of added copper or chromium, respectively. Medium II prepared as above contained 3.7 1.6 μg/l (mean ± SD; N = 2) total chromium prior to amendment (HGA AA analysis conducted in the Oklahoma State University Water Quality Research Laboratory, using EPA reference standards). Flame AA analyses conducted on Medium II aliquots amended with 0, 5, 25, and 100 mg/l of added chromium indicated that the difference between added and analyzed metal never exceeded 12%. Comparisons between the amount of copper added versus copper detected in incubation media were not conducted due to instrumentation malfunctions.

Leaf disks were incubated in chromium or copper containing media for 5 min, 1 h, or 4 h (70 E μE m^{-2} s^{-1} illumination, 22 ± 1 C). At the conclusion of the incubation period, 50 μl of a ^{14}C-bicarbonate solution (0.2μCi) were added to each flask and 15-min photosynthetic bioassays were performed (Beer et al. 1982; Francko and Wetzel 1984; Francko 1986). Thus, the total exposure period to metals in our protocol was 20, 75 and 255 min. Leaf disks were then removed from bathing media, rinsed in weakly acidic distilled water, and placed into glass scintillation vials (one per vial) containing 0.5 ml of quaternary ammonium tissue solubilizer. Vials were tightly capped, heated for 1 h at 60C, cooled, amended with 10 ml of scintillation cocktail, and incubated overnight in the dark to reduce chemoluminescence. Samples were then assayed via liquid scintillation spectrometry (Beckman Model 7500 in the automatic quench control mode). Radioactive carbon assimilation in metal-treated variants, (N = 6 disks per metal concentration) corrected for background counts and dark assimilation, was compared to assimilation in control disks lacking metal addition and was expressed as the percent deviation from control assimilation rates in each experiment.

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RESULTS AND DISCUSSION

Data on the effect of chromium addition on leaf disk PCA rates are shown in Figure 1. With two exceptions (25 and 100 μg/l), 20-min chromium exposure in the range of 0.5 μg/l to 1 mg/l induced statistically significant reductions in leaf disk PCA rates. Disks incubated for 75 min, however, were unaffected by chromium addition with the exception of 1 and 100 μg/l addition variants, which displayed significantly higher PCA rates than controls. In 255 min incubations, chromium concentrations between 10 and 200 μg/l significantly inhibited PCA rates. No effects on PCA rates were noted at the two highest chromium additions employed (5 and 10 mg/l levels in the medium.

For the shortest incubation period, copper additions between 5 and 25 μg/l consistently induced a strong stimulation of PCA, whereas concentrations of 50 μg/l and above strongly repressed PCA rates (Figure 1). At concentrations above 100 μg/l, repression exceeded 50 percent of the control assimilation rate. As in chromium experi-

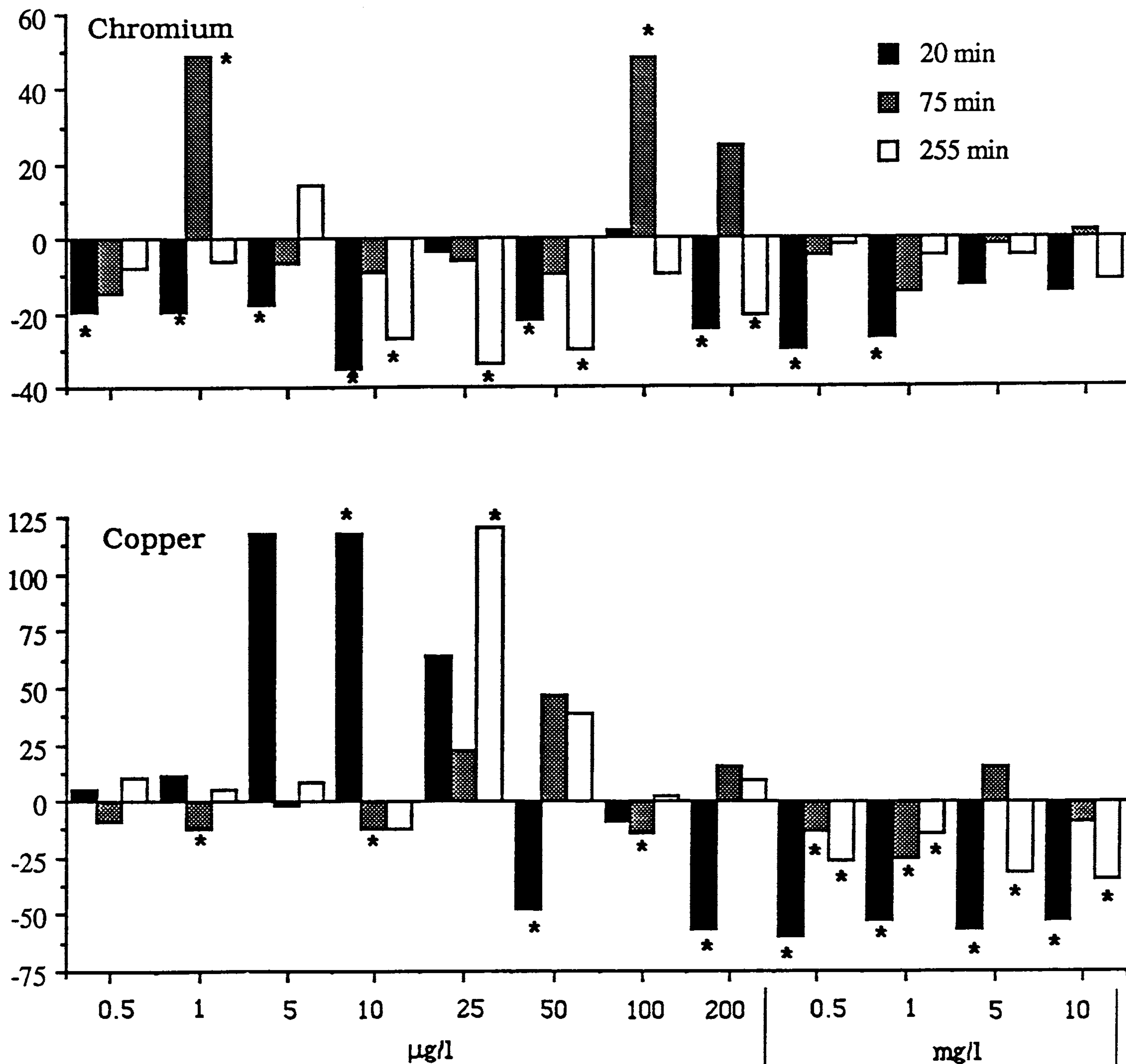

Figure 1. Time-series effects of hexavalent chromium (top) and copper (below) addition on photosynthetic ^{14}C- assimilation in *Potamogeton* leaf disks, pH 8.2. Data shown as mean percentage difference between treated disks and unamended control disks (N = 6). Astericks denote significant differences between control and treated disks (P < 0.05; t-test).

ments, longer term incubation generally produced less pronounced effects on PCA rates that did 20 min incubations.

Taken together, such data suggest that the *Potamogeton* leaf disk bioassay is relatively insensitive to chromium levels in the medium. Although low levels of chromium addition produced reproducible reductions in PCA rates during the shortest incubation period used, inhibition did not appear to be dose-specific and in no case was an EC-50 concentration detectable. Thus, while the effects on PCA rates we noted here may be ecologically significant in littoral waters containing chromium contamination, the data argue against the utility of this method as a metal-detection bioassay system for chromium.

Leaf disks appeared slightly more sensitive to low-dose treatment with copper than chromium, especially during 20-min exposure experiments. Although heavy metals are generally inhibitory to organismal metabolism, the stimulation of short-term PCA rates we noted with 5 to 25 μg/l copper is not without precedent. Copper-induced stimulation of *Potamogeton* physiological variables has previously been reported by Jana and Choudhuri (1981), who found elevated glycolate metabolism rates in *Potamogeton pectinatis* incubated for three days with 10 mg/l to 10 g/l copper.

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