EFFECT OF HEAVY METALS AND SULFUR DIOXIDE ON PHYTOPLANKTON

FINAL REPORT

Submitted to the Minnesota Regional Copper-Nickel Study Group

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ABSTRACT

Field and laboratory bioassays were conducted on four lakes near Ely, Minnesota, to examine the effects of copper, nickel and sulfur dioxide inputs on phytoplankton communities. These lakes represent a range of TOC concentrations and are likely to be affected by copper-nickel mining developments in the Ely area.

Algal responses were generally more extreme in laboratory than in field bioassays. In some cases, laboratory bioassays showed toxicity while concurrent field bioassays showed no effect. Concentrations of 50 and 100 µg/l Cu and 100 µg/l Ni were sometimes stimulatory and sometimes toxic in Birch and Greenwood lakes. Toxic effects were only slightly more pronounced in Birch (16 mg/l TOC) than in Greenwood Lake (25 mg/l TOC). Stimulatory effects (i.e., increases in chlorophyll a) were observed frequently in both field and laboratory and were rather unpredictable in their occurrence. Communities showing a stimulatory response were not observed to have higher P/B ratios than control communities. Since higher concentrations of these metals were invariably toxic, it is likely that stimulation represents a response to a mild level of stress. Nutrient limitation and food chain interactions are considered to be unlikely explanations for algal stimulation at these metal concentrations.

Laboratory bioassays with Clearwater (7 mg/l TOC) and South McDougal (38 mg/l TOC) lakes showed that Clearwater Lake was far more sensitive to copper and slightly more sensitive to nickel than South McDougal Lake. In Clearwater Lake 50 µg/l Cu was severly toxic.

No evidence was found to support the idea that copper and nickel act synergistically in these lakes. The effect of copper-nickel inputs on total algal biomass is dependent on the species present, their relative abundance, and the physical-chemical characteristics of the lake water. In many cases

in both field and laboratory bioassays, a tendency was observed for chlorophyll levels to recover after showing an initial toxic response. Occasionally, recovery was not evident until after the second week of the test.

In Birch Lake lowering the pH to 6.2, 5.0 and 4.6 seemed to have little effect on copper and nickel toxicity. However, in cultures which were not treated with metals, a pH drop to 5.0 caused an increase in chlorophyll while a pH drop to 4.6 was apparently toxic.

With few exceptions, phaeopigment/chlorophyll a ratios agreed with the assessments of toxicity made using only chlorophyll a values. Thus, in treatments in which chlorophyll a was lower than controls, the phaeopigment/chlorophyll a ratio was higher; and in treatments in which chlorophyll a indicated stimulation, the ratio was lower. In addition, this ratio was useful as an indicator of the degree of toxicity; that is, there was a positive correlation between the degree of toxicity and the ratio.

The results of algal cell counts may be summarized as follows:

1) Diatoms showed both stimulation and inhibition in response to metal treatments. Frequently, stimulation at low concentrations was accompanied by inhibition at high concentrations.

2) Blue-green algae were probably the most sensitive of the algal groups. They were never stimulated by low metal concentrations and were often inhibited by levels as low as 20 µg/l Cu and 100 µg/l Ni.

3) Among the Chrysophytes, Dinobryon and Hyalobryon were sometimes strongly stimulated by 50 and 100 µg/l Cu but were not stimulated by a combination treatment of 100 µg/l Cu + 800 µg/l Ni.

4) Some species of algae (especially blue-greens) were more inhibited by 100 µg/l Cu than by 800 µg/l Ni, but for other species the reverse was true.

5) Although the total number of species present in Birch and Greenwood lakes was similar, generally more species were affected by the metal treatments (positively or negatively) in Birch than in Greenwood Lake.

6) Low level metal treatments usually resulted in the stimulation of some algal species and the inhibition of others. Consequently, significant shifts in species composition occurred at low metal concentrations, and these shifts persisted even after chlorophyll concentrations recovered to control levels.

7) Inhibition and/or stimulation of particular species frequently occurred without detectable differences in chlorophyll between treatment and control bottles. In fact, some species often showed stimulation when the chlorophyll response was negative, and vice versa.

These findings shed doubt on the practices of using single species of test algae and total community biomass measurements as indicators of toxicity in algal bioassays, especially if the bioassays are to be used in setting water quality standards.

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INTRODUCTION

The Minnesota Regional Copper-Nickel Study is currently studying the potential impact of the development of copper-nickel mining in Northeastern Minnesota. If mining operations develop, lakes in the area to the south of Ely, Minnesota, may receive both geological and meteorological inputs of copper, nickel and sulfur dioxide (sulfuric acid). The lake phytoplankton (algae), which form the basis of the lake food web, are a very important component of these aquatic systems. Additions of metals may stimulate algal growth or have toxic effects. It is necessary to perform a series of algal bioassays, using the waters of the study area lakes, in order to predict the potential effects of inputs of metals and sulfuric acid on phytoplankton production, biomass and community structure.

The toxicity of copper and nickel to algae is dependent on many factors, including alkalinity, pH, ability of the water to complex metals, syngergistic and antagonistic interactions among metal ions, species composition of the phytoplankton community, and prior exposure of the community to copper and nickel. Since the study area contains many lakes which will vary in all of these characteristics, the issue becomes an immensely complicated one. It is clearly impossible to test all of the lakes and to make detailed predictions for each lake under each set of circumstances. However, the matter is somewhat simplified since most of the lakes fall within rather narrow ranges of alkalinity and pH and since the ability of lake waters to complex metal ions appears to be a factor of overriding importance in determining metal toxicity. The latter fact is central to the present study because many of the lakes of Northeastern Minnesota are

highly colored and contain relatively large quantities of dissolved organic matter. It is probable that the toxicity of metals in these lakes will largely depend on the degree to which metals are complexed by organic materials. A major goal of the present effort, therefore, was to elucidate these relationships by choosing lakes for study which fall on a spectrum of total organic carbon content. By focusing on this issue, it is hope that it will be possible to make predictions of metal toxicity of a rather general nature which will encompass most lakes of the study area.

While our aim is to develop a predictive capability, there are limitations inherent in all bioassay work, and these must be recognized. Firstly, all bioassays (with the exception of those employing an entire lakes as the experimental unit) are conducted for a period of time which is short in relation to the seasonal and yearly changes which occur in a natural lake ecosystem. Algal bioassays may be as short as 3 hours or as long as a month or more; but even in the latter case, extrapolation of the results to predictions of long-term changes in the lake phytoplankton community must be done with extreme caution. Phytoplankton algae may adapt to new conditions or change in biomass or species composition over an extended period of time in ways which are not fully understood. Secondly, the interpretation of changes in species composition of algae or of the presence or absence of a given algal species is somewhat obscure. Changes in species composition in an algal bioassay may have little relationship to the long-term changes which might occur in a lake. Indeed, the meaning of the seasonal species composition changes in a natural lake are, more often than not, a matter for conjecture. Thus, the algal bioassay work to be undertaken in this study cannot be expected to predict with absolute certainty the long-term changes in lakes which may be influence by copper-nickel mining, nor can it fully predict or assess the meaning of changes in phytoplankton species composition which may occur.

In spite of these limitations the use of algal bioassays is the only method available to assess the effects of toxic materials on phytoplankton. Because of the critical ecological role of the phytoplankton, it would be folly to ignore them in any impact assessment of copper-nickel mining. The use of long-term (i.e., 3-5 week) in situ bioassays employing the natural lake phytoplankton community may lessen considerably the uncertainty of extrapolating bioassay results to predict changes in lakes. Likewise, a combination of bioassay methods may be employed to reduce the possibility that one method does not accurately represent lake conditions. When such precautions are taken and when the data are interpreted critically, algal bioassays may prove invaluable in assessing the impact of pollutants on aquatic ecosystems.

Effects of Copper, Nickel, and pH on Phytoplankton

Evidence that certain trace metals may act as limiting factors for algal populations has increased steadily over the past decade (e.g., Goldman 1972, Dunstan 1975, Patrick et al. 1975, Shapiro and Glass 1975). Copper, an essential micronutrient, may stimulate algal growth at low concentrations but more typically acts as a poison. Thus, while Steemann Nielsen and Laursen (1976) found that 25 µg/l Cu^{++} stimulated growth in one lake, the same concentration inhibited growth in three other lakes. Other workers have similarly found that quite low concentrations of copper ion may be toxic to algae. Goldman (1972) reported that 79 µg/l Cu^{++}, a level only 1.25 times the natural copper concentration, severely inhibited photosynthesis in Clear Lake, California. In this lake 110 µg/l essentially stopped growth. Hutchinson and Stokes (1975) found that Chlorella vulgaris continued to grow actively at 40 µg/l Cu^{++} but that cells grown at 100 µg/l and higher levels failed completely within a week. At 200 µg/l the cells appeared to be

initially inhibited, then showed slow growth, followed by final death. Horne and Goldman (1974) reported that the nitrogen-fixing blue-green algae Aphanizomenon and Anabaena were adversely affected at concentrations of 5 to 10 µg/l.

Much work remains to be done regarding the physiological mechanism of action of copper. Copper acts at various sites of the electron transport system of photosynthesis and photoreduction, and it has come to be accepted that all algae have a micronutrient copper requirement (O'Kelley 1974). Copper toxicity, however, appears to be related to changes in cell membrane permeability and an efflux of potassium from the cell (McBrien and Hassall 1967). Potassium efflux is reciprocal (but non-stoichiometric) to copper binding. In Chlorella pyrenoidosa copper toxicity was reduced by increasing the potassium level within the medium (Steemann Nielsen et al. 1969). Other factors influencing copper toxicity are light intensity, cell concentration, stage of growth, and the presence of other ions.

It has become increasingly clear that the toxicity of an element cannot be discussed without regard to its chemical state and transformations in the environment (Wood 1974). The amelioration of copper toxicity by organic matter is of special importance (Horne and Goldman 1974, Steemann Nielsen and Laursen 1976, Hutchinson and Stokes 1976, Stokes and Hutchinson 1976. These studies indicate that copper which is bound to organic complexing agents is not toxic and is not taken up by algal cells. Free copper (Cu^{++}), on the other hand, is taken up and concentrated. In general, the toxicity of a given amount of copper added to lake water will depend on the amount of free copper which is complexed. Brown water lakes of high humus content

appear to have higher thresholds for copper toxicity than do clear water lakes, and copper is more likely to stimulate growth in stained lakes than in other lakes. However, growth stimulation of algae by copper has seldom been reported and has apparently never been observed to cause appreciable increases in algal biomass.

In addition to the problem of complexation, several other factors complicate the problem of copper toxicity measurements:

a) pH - The influence of pH on copper toxicity appears to be somewhat controversial. Hutchinson and Stokes (1975) suggest that toxicity may be ameliorated at high pH, since heavy metals such as copper and nickel are less soluble, and therefore less available for uptake, at higher pH's. On the other hand, Steemann Nielsen and Kamp-Nielsen (1970) found that in Chlorella the toxic effect of copper decreases with decreasing pH. Andrew (1976) believes that two different relationships are involved in copper toxicity. While copper ion activity increases with decreasing pH, it is also true that lowering the pH protonates exchange groups on the surface proteins of the organism, and a lower pH actually decreases the uptake of copper at those sites.

b) Calcium - Calcium has been strongly implicated as an ameliorating factor for heavy metal toxicity (Hutchinson and Stokes 1975).

c) Species specific responses - Different test algal species often respond quite differently to the same copper concentrations in the same test waters (Hutchinson 1973, Hutchinson and Stokes 1975). The blue-green algae as a group have been found to be especially sensitive to low concentrations of copper (Horne and Goldman 1974, Steemann Nielsen and Laursen 1976).

d) Tolerance - Algal strains isolated from lakes having elevated concentrations of copper and nickel were found to be tolerant of these metals

when compared to laboratory strains of similar algae (Stokes et al. 1973). Mechanisms of heavy metal tolerance apparently include incorporating the metal in dense inclusions in the cell nucleus (Ferstenberg et al. 1975) and exclusion of the metal from the cell (Foster 1977). Tolerance is an especially difficult factor to account for when attempting to extrapolate algal bioassay results to long-term responses of lake phytoplankton communities.

e) Synergism - Copper and nickel have been found to act synergistically, each enhancing the other's toxicity to test species of algae (Hutchinson 1973, Hutchinson and Stokes 1975). For example, 50 µg/l Cu^{++} reduced the growth of Chlorella to 95% of the control, while 100 µg/l Ni^{++} had no inhibitory effect. Yet, in combination, growth was completely inhibited.

Less information is available on nickel toxicity than on copper toxicity, but the data indicate that nickel is less toxic to algae. Indeed, it may be the major hazard from nickel pollution is to enhance the toxicity of copper. Of four algal species tested by Hutchinson (1973), Scenedesmus was the least tolerant, being largely killed at 100 µg/l. The other three species grew relatively well up to 300 µg/l. Other experiments with nickel indicate that the anion with which it is associated seems to have an effect on its toxicity (Patrick et al. 1975). Scenedesmus sp. had a threshold toxicity of 90 µg/l if nickel was in the form of nickel ammonium sulfate, whereas nickel chloride was less toxic with a threshold concentration of 1.5 mg/l.

Much of the toxicity work cited above is difficult to interpret in terms of natural algal communities. Most of the tests have employed laboratory test species of algae growing in artificial media. However, Patrick et al. (1975) have performed an interesting series of experiments with nickel using natural communities of periphyton algae growing in greenhouse streams. In these tests effects on species composition and

community structure were found at much lower concentrations of nickel than are indicated by tests with single species of laboratory algae. Nickel concentrations of only 4 to 9 µg/l significantly reduced diatom diversity and caused a shift in algal populations to species of blue-greens. These trends were confirmed at higher nickel concentrations. Patrick et al. conclude that nickel is deleterious to the growth of diatoms even at very low concentrations, but that greens and blue-greens are more tolerant. The blue-greens seem to be much more tolerant of higher concentrations than the greens. Accumulation of nickel was also found to affect C-14 uptake. Some C-14 uptake was observed at concentrations as high as 500 µg/l and 1 mg/l although it was significantly lower than uptake in the control. In light of these results it is unfortunate that more workers have not employed diatoms as test species of algae.

The pH of lake water may also have effects on the species composition of phytoplankton communities. Blue-green algae tend to dominate in lakes with relatively high pH's, perhaps because of their ability to utilize low concentrations of CO_2 (Shapiro 1973). This observation is consistent with the work of Yan and Stokes (1976) who experimentally manipulated pH in in situ cylinders in Carlyle Lake, Ontario. They found that greens and blue-greens dominated at pH 5 and above, while dinoflagellates and cryptomonads became dominant at pH 4. They also report that the number of algal species found in midsummer samples from acidified lakes decreased with decreasing pH. The decrease in diversity is most marked at pH values below 5.8 in Scandinavian lakes. Phytoplankton biomass may also be reduced in acidified lakes.

Algal Bioassays: Limitations of Particular Methods

Three major methods of conducting algal bioassays are in common use: a) small volume batch cultures, b) continuous cultures, and c) large in situ enclosures constructed of polyethylene. A brief discussion of the limitations

of these methods follows.

a) Small volume batch cultures - These tests may be conducted either with a laboratory test species of alga or with the natural lake phytoplankton community, and they may use C-14 uptake, cell counts or chlorophyll concentration as a measure of growth. They are usually run in 125 to 250-ml bottles or flasks and are seldom continued for a period longer than several days. Many workers have discussed the limitations of these methods (e.g., Dugdale 1967; Schindler 1971, 1977; Cairns et al. 1972; Rice et al. 1973; Gerhart and Likens 1975; Klotz et al. 1975). The objections are perhaps most severe when a test species of laboratory alga is employed since the test species may have enjoyed luxury consumption of nutrients while growing in artificial media or may not be appropriate for the water to be tested. Such culture studies involve simple, stable environments lacking organismic interactions, succession, and other complex natural phenomena. Even when natural communities are used, the results of batch culture bioassays are frequently at odds with those of longer term continuous culture or field studies. The reasons for these discrepancies may include exhaustion of nutrients within the culture, buildup of waste products, or failure to account for longer-term adaptation of cells to toxic materials. In addition, chemical additions to batch cultures may alter the short-term photosynthetic rate in ways which are unrelated to the nutritional or toxic properties of the materials being tested.

The problem of adaptation to toxicants is an especially difficult one. Stockner and Antia (1976) present evidence indicating that algal exposures as long as 20 to 40 days to the pollutant may be required for successful adaptation and that phytoplankters tolerating initially only a low level of a pollutant may be trained to accept severalfold higher levels by repeated exposure to gradually increasing concentrations. Such adaptation or accomodation to toxic metals is also known in Escherichia coli

(Mitra et al. 1975). In light of these studies it seems wise to avoid small volume batch cultures where the goal is to predict the long-term effects of environmental perturbations. They may, however, prove of some value if the goal is to rapidly assess the ability of different lake waters to ameliorate metal toxicity on a short-term basis.

b) Continuous cultures - Continuous, chemostat-type cultures may be conducted using either test algal species (Rice et al. 1973, Goldman 1976) or the natural phytoplankton community (Barlow et al. 1973, Gerhart and Likens 1975). Continuous cultures of phytoplankton allow the substitution of medium flow for nutrient manipulations, making long-term assays with natural water feasible when it is experimentally desirable. They also offer the operational advantage of permitting work with steady state cell cultures, a situation which presumable simulates the natural one more closely than traditional batch cultures (Maddux and Jones 1964). Disadvantages of chemostat-type bioassays include artificial conditions of light and turbulence and the potential for growth of attached algae and bacteria in longer-term experiments. In addition, species successions in continuous cultures probably have little relationship to those which might occur in the lake environment.

A more serious problem concerns the gradual decline of many species populations in unenriched continuous cultures and the possible relationship of these declines to the manner in which the culture medium is prepared (Gerhart 1973). Some species may not be suited to survive the conditions of light and turbulence in the cultures; hence, some species changes are expected. However, it may also be significant that two sources of nutrient regeneration are generally reduced or eliminated by the culture design.

Firstly, zooplankters are largely excluded from the culture vessels, and zooplankton grazing is replaced by the non-regenerative "grazing" of the chemostat overflow. Secondly, if filtered lake water is used as the nutrient medium, water flowing into the culture will contain no detritus, and nutrient regeneration from the bacterial decomposition of detritus may be expected to gradually decline in the cultures. Thus, in spite of a continuous input of lake water, algal cells in chemostat cultures may be more nutrient starved than those in natural waters.

The potential effect of metal complexing in the brown-stained waters of Northeastern Minnesota creates added difficulties in this regard. Reservoir medium water for the cultures must be stored in these experiments, perhaps for several weeks, prior to use. To avoid biological change during the storage period and also to simplify measurements of biomass in the cultures, the water must be sterilized. There are two practical methods of sterilization: heat and filtration. If the former is chosen, the organic component of the lake water is changed in some unknown way, which may affect algal growth or the ability of the water to complex metals. If the latter is chosen, the particulate fraction of the water is removed; this removal may have important effects on both nutrient regeneration and the complexing capacity of the water (and hence the toxicity of metals to the algae). Filtration becomes an especially dubious procedure when it is considered that generally more than half of the copper in natural waters is associated with the particulate fraction (Stiff 1971, Kubota et al. 1974). On the other hand, if heat sterilization is employed, a procedure must be devised to replenish concentrations of dissolved gases which are lost as the water is heated.

The pH of the reservoir medium water must also be adjusted to pre-sterilization levels.

In spite of these limitations continuous culture bioassays offer important advantages over small volume batch cultures. Chief among these is the ability to perform relatively long-term tests (e.g., three week) with algae indigenous to the lake under study. They also permit many more tests and replicates to be performed than would be possible in a study employing strictly in situ field methods.

c) Large in situ enclosures - Enrichments of large, in situ glass or polyethylene enclosures are likely to provide the most reliable information when one is concerned with potential changes in phytoplankton biomass and species composition resulting from environmental perturbation (Goldman 1962, Schindler et al. 1971, Stoermer et al. 1971). These studies minimize the effects of enclosure while utilizing the natural light and temperature regime. Since they involve minimal alteration of the natural environment, there are fewer risks in interpreting the results. They are, however, relatively expensive both in terms of time and manpower and do not lend themselves to large numbers of treatments and replicates. Species changes in the enclosures are also difficult to interpret in a long-term sense. A two or three week experiment with periodic additions of chemicals cannot possibly simulate the complex patterns of enrichment which might occur over extended time periods as a result of copper-nickel mining. In this regard, the desirability of conducting relatively extended tests cannot be over-emphasized. For example, Yan and Stokes (1976) found changes in species composition in in situ cylinders after 28 days which had not been apparent after only 7 days of incubation.

METHODS

Two types of algal bioassays were used to assess the potential effects of inputs of copper, nickel and sulfuric acid on the lakes of the study area. From mid-June through mid-December, 1977, seven tests were conducted using continuous-flow laboratory culture bioassays. In addition,five tests using in situ enclosures were run between mid-June and early October. All but one of the in situ tests were run concurrently with continuous culture bioassays using identical treatment schemes, thus allowing a comparison of the two methods. Ultimately, the appropriateness of the laboratory tests as a means of assessing impact of the treatments was determined through this comparison. Both methods used naturally occurring phytoplankton communities as test algae. The duration of the tests varied from two to three weeks, and all treatments and controls were run in duplicate. To facilitate comparisons between lakes, the majority of runs involved simultaneous testing of two lakes using identical treatments.

With one exception, all of the bioassays were performed with water from Birch Lake and Greenwood Lake (Figure 1). These lakes were chosen for several reasons. Birch Lake is typical in water chemistry of many of the lakes in the copper-nickel study area; and, because of its location, there is a high probability it would be impacted if mining is initiated. Greenwood Lake was selected on the basis of its high total organic carbon (TOC) content and thus suspected high complexing capacity. Data compiled in late summer, 1977, by another team within the copper-nickel study indicated that in fact there is no marked difference in the complexing capacity of these two lakes. Consequently, in order to obtain at least one set of

bioassay data from lakes differing in this regard, continuous culture Run 5 investigated the effects of metals on two additional lakes, Clearwater and South McDougal (Figure 1). Clearwater Lake is a truly clear water lake of low complexing capacity, and South McDougal Lake is a brown water lake of high complexing capacity. A summary of some physical and chemical characteristics of these four lakes is presented in Table 1.

A summary of all bioassays performed is presented in Table 2. All metal additions were made as sulfate salts.

Analyses and Measurements

The effects of copper, nickel and sulfuric acid on the growth of algae was measured primarily through fluorometric determinations of chlorophyll a (corrected for phaeopigments) using 90% acetone extractions (Strickland and Parsons 1968). The samples were filtered through Whatman GF/F glass fiber filters. Acetone extracts from field bioassays and some laboratory bioassays were stored in the dark in a freezer for periods of up to 10 days prior to fluorometric analysis. Most measurements were made with a Turner Model 111 fluorometer. However, when this instrument failed, an Aminco-Bowman spectrophotofluorometer was used during a portion of Run 3 and all of Run 4 for both field and laboratory bioassays. Excitation and emission wavelengths on this instrument were set at 430 and 672 nm, respectively. Both fluorometers were calibrated with acetone extracts whose chlorophyll content was determined with a Hitachi Model 191 spectrophotometer using the trichromatic method (Strickland and Parsons 1968). The extracts for calibration were derived from a laboratory culture of Selenastrum capricornutum. In all bioassays chlorophyll determinations were made on alternate days three days a week on all cutlures and enclosures.

Two additional measures of treatment effect, algal species cell counts and rates of carbon-14 assimilation, were employed. Samples for cell counts,

consisting of 100-ml aliquots preserved with 1% acid Lugol's solution, were taken from one of each replicate set of enclosures at the beginning and end of each run. Counts were performed by Ecology Consultants, Inc., Fort Collins, Colorado. (Details of the counting method are included in the Aquatic Biology Operations Manual). Carbon-14 uptake was used to determine the relative productivity of algae in field enclosures. For these measurements 150-ml samples were removed from selected enclosures and inoculated with 1 ml aqueous $NaH^{14}CO_3$ solution (5 μCi/ml). Incubations were conducted at ambient air temperature in a shady location for approximately 2 hours. After transporting the samples to Duluth in the dark, sub-samples were filtered through HA Millipore filters (0.45 μ) and the filters were stored in a dessicator for two days. Carbon-14 uptake was measured with a Beckman LS 8000 automated scintillation counter using a toluene scintillator (Lind and Campbell 1969). Relative productivity was generally determined for both replicates of those treatments selected for productivity measurements. Replicate measurements were made on some samples to estimate the error of the technique. The mean disintegrations per minute (DPM) was calculated for each treatment, and a relative production/biomass (P/B) ratio was determined using chlorophyll *a* as an indicator of algal biomass.

Total alkalinity and pH were monitored on a weekly basis, the former using bromcresol green / methyl red indicator. A Hellige Lilliput pH meter was used in the field, while a Radiometer Model 29 pH meter was used for laboratory bioassays. In field Run 5 bacteria populations were measured using a standard plate count technique (American Public Health Association 1971).

Samples for total and dissolved copper, nickel and organic carbon were collected at the end of each week during field tests. Composite samples

were collected from laboratory cultures by combining aliquots of equal volume collected weekly. In general, samples were taken from only one of each set of replicate treatments. Dissolved fractions were determined on samples filtered through HA Millipore filters. The filters were pre-rinsed with 200 ml of double-distilled, deionized water. All samples were acidified with 1.5 ml of ultrapure 10% HNO_3 for every 100 ml of sample and stored in acid-washed polyethylene bottles supplied by the Minnesota State Department of Health. Samples were refrigerated and kept in the dark until shipped to the Department of Health for analysis.

Field Bioassays

Field operations were headquartered at the U.S. Forest Service North Central Research Station located on Birch Lake (Figure 1). Chlorophyll extractions and routine chemical measurements were performed at this location. Acetone extracts were stored in blackened, air-tight vials in a freezer and transported weekly to Duluth for fluorometric analysis.

In situ bioassays were conducted on Birch and Greenwood lakes. In the first four tests identical treatments of copper, nickel, and copper-nickel combinations were assayed concurrently on the two lakes (Table 2). The fifth bioassay examined interactions of copper and zooplankton populations in determining algal growth in Greenwood Lake. One site in the pelagic zone of each lake was selected for in situ work (Figure 1). The site on Birch Lake was in the vicinity of "Birch Lake Sample Site #1" used by aquatic team of the copper-nickel study group. The site on Greenwood Lake was located near the southwest end of the lake. Eight to twelve enclosures were placed in each lake depending upon the number of treatments being assayed.

Field Run 1 was a preliminary test using polyethylene bags as enclosures. These bags proved unsatisfactory as holes developed in the polyethylene under

the continued stress of wave action, and subsequent experiments employed Pyrex enclosures. The design and sampling procedures for the bags were similar to those described below for the Pyrex carboys.

The Pyrex enclosure system is illustrated in Figures 2 and 3. The entire system was held in place with a cement block attached to a surface float. The anchor block was enclosed with a plastic bag to avoid contamination of surrounding waters with calcium leached from the cement. The actual enclosure was a 21-liter Pyrex carboy suspended from the float with a line tied around its neck. The carboys were fitted with neoprene stoppers held tightly in place with metal clamps from canning jars. Two tubes passed through each stopper: an air exhaust/inlet tube and a sampling tube. The air exhaust/inlet tube was simply a small-diameter Tygon tube approximately 1 m in length which was sealed at its top end with a screw-type tubing clamp. During sampling, the clamp was removed, allowing air to flow into the carboy as water was pumped out through the sampling tube. The sampling tube consisted of (1) a length of rigid 1.9-cm PVC plumbing pipe which extended from the stopper to about 2/3 of the distance to the base of the carboy on the inside, and (2) a 1-m length of Tygon tubing clamped to the PVC pipe just above the stopper and sealed tightly at its top end with another neoprene stopper. A Guzzler 400 hand pump (Dart Union Co., Providence, RI) was fitted with polypropylene tubing and PVC elbows at each end and used both to withdraw and return water to the carboys. This scheme permitted all sampling operations to take place without removing carboys from the lake.

For sampling, the elbow on the output end of the pump was inserted in the sampling tube of the carboy, the air exhaust/inlet tube was opened, and 20 pump strokes of air were pumped into the carboy to mix its contents thoroughly. (This last procedure was not performed in Runs 1 and 2.) The pump was then immediately reversed, and six liters of water (33% of the

carboy's contents) were removed. A portion of this water was stored in a 1-liter polyethylene bottle in a cooler and used as sample water for all analyses. After removal of the sample water, 200 ml of metal solution was poured into the carboy (treatment carboys only) through the sampling tube, the pump was again reversed, and lake water was pumped from a depth of 0.5 m into the carboy to restore the original volume (18 liters). Final carboy volume was determined by noting when the carboy achieved slightly greater than neutral buoyancy. In essence, the carboys were operated as partial flow-through systems. On each sampling day 1/3 of the total enclosure volume was removed and replaced with fresh lake water with appropriate metal additions.

Since the enclosure volume was maintained at 18 liters, there was a 3-liter air space in the carboy. This allowed some mixing to take place and also helped to prevent CO_2 and oxygen depletion. Dissolved oxygen levels in select carboys were checked periodically (Winkler titration) and compared to lake levels. Oxygen depletion was never observed.

All hardware in contact with sample water (e.g., carboy, sample tubing, etc.) was acid washed with 50% HCl and rinsed with double-distilled, deionized water prior to each run.

Sampling was conducted during the morning hours (9:00 to 10:30 A.M.) on alternate days three days a week. During those runs in which two lakes were involved, schedules were staggered so that one lake was sampled each day. Daily records were kept regarding general weather conditions, precipitation, air and water temperature, and percent cloud cover. Temperature profiles were obtained weekly. Light penetration in both lakes was measured in early June and again in mid-July using a Secchi disk and a submersible

photometer. The June measurements indicated that 10% of the incident light penetrated to a depth of 0.9 m in Birch Lake and 0.6 m in Greenwood Lake. As a result, the enclosures were suspended with their upper water level at a depth of 0.5 m in both lakes.

Special mention must be made regarding Run 5 in which zooplankton populations within the enclosures were altered. Procedures were identical to those already described with the following exceptions:

1) In treatment bottles in which it was desired to eliminate the larger zooplankters, a 200-μ mesh net was fitted over the sampling tube prior to initial filling and during all subsequent additions of lake water.

2) In treatment bottles in which it was desired to increase zooplankton populations, 18 liters of water were filtered through the 200-μ net immediately after the initial filling, and the zooplankters collected were added to the bottle via the sampling tube. Thus, the populations of large zooplankters were doubled in these treatments. At subsequent sampling periods a volume of lake water equal to that removed during sampling (6 liters) was filtered through the net, and these zooplankters were also added to the bottle.

3) Zooplankton samples were collected from each enclosure on each sampling day. Four liters of enclosure water were filtered through an 80-μ mesh Wisconsin net and the entrapped organisms preserved with 2% formalin. The relative density of zooplankters in each enclosure was determined subjectively through visual inspection of the samples.

Continuous Culture Bioassays

Seven continuous culture bioassays were conducted at the University of Minnesota, Duluth, Limnological Research Station. Generally, waters from

two lakes were studied simultaneously, testing identical treatments on each lake. In addition to tests involving copper and nickel, two bioassays were conducted in which the pH of the culture water was experimentally lowered (Table 2).

Chemostats similar in design to those described by Carpenter (1968) were housed in a large walk-in incubator. The cultures were grown in 6-liter Florence flasks (4-liter culture volume). Two eight-channel Gilson peristaltic pumps were used to deliver reservoir medium and metals to sixteen cultures. During the first four runs the flow to each culture was maintained at 0.7 ml/min (dilution rate = 0.25/day), while during the last three the flow was 0.4 ml/min (dilution rate = 0.15/day). The flow rate was reduced during the latter portion of the study because growth rates and chlorophyll levels were lower during the winter months. Lighting was provided by four banks of wide-spectrum Grolux fluorescent lights on an 18:6 light/dark photoperiod. Light intensity was approximately 3120 lux (5.0 watts/m^2). Culture water temperature was maintained at lake temperature at 0.5 m depth except in Runs 6 and 7. During these runs culture water temperature was held 5-6^{o}C above lake temperature. All cultures were mixed continuously by bubbling with filtered air.

All hardware in contact with culture or reservoir water was acid-washed with 50% HCl and rinsed with double-distilled, deionized water prior to each test. Silicone tubing was used for all sample and delivery tubes.

During the initial set-up of Run 5, an assessment of bias in terms of culture placement and lighting was made. The initial cultures were placed in the incubator for a three day period without receiving metal additions or flow from the reservoirs. At the end of this period chlorophyll a

was determined in all sixteen cultures. Chlorophyll levels were identical (±10%) in each set of eight cultures containing water from the same lake.

Perhaps the most critical methodological problem associated with the culture experiments was that of reservoir water sterilization. Two preliminary tests using water from Cloquet Lake (high TOC) and Birch Lake were run to compare algal response to 50 µg/l Cu^{++} with heat-sterilized and filtered (0.45 µ) water as the reservoir medium. These tests indicated no difference in algal growth (measured by chlorophyll a) using the two types of media. Consequently, the heat-sterilization method, being less expensive and time-consuming, was chosen for the study.

Water was heat-sterilized by raising its temperature to approximately 98°C (steaming) for 20 minutes. The water was then cooled to room temperature and bubbled with HA Millipore filtered gas (5.1% CO_2, 11.8% O_2, 83.1% N_2) until the initial pH was restored. Additions of metals, phosphorus and nitrogen were then added directly to the reservoirs. Five µg/l P (as monobasic sodium phosphate) and 50 µg/l N (as sodium nitrate) were added in Runs 2-7. In Run 1 the reservoirs were not enriched with N or P.

The reservoir waters were kept in 21-liter Pyrex bottles covered with black plastic bags and sealed with neoprene stoppers. Each reservoir served as a medium source for a replicate pair of cultures. Reservoir water was replaced weekly with fresh, sterilized lake water. The reservoirs were housed in the incubator with the cultures.

Initial culture and reservoir water was collected in the pelagic zone at a depth of 0.5 m. In the case of Birch and Greenwood lakes, the collection sites coincided with the location of in situ enclosures, thus, assuring that similar waters were tested in laboratory and field during

coordinated studies. Water for initial cultures was filtered through 200-μ mesh cloth to remove the larger zooplankters and immediately placed in the incubator. During all but the last two tests, metals were not added directly to the initial cultures. Instead, metal concentrations in the cultures increased exponentially to the test levels as reservoir medium was pumped in. In Runs 6 and 7 the test concentrations of metals were added to the initial cultures as well as to the reservoirs.

The pH was adjusted in Runs 6 and 7 by adding appropriate amounts of 0.02 N H_2SO_4. In Run 6 pH was adjusted in reservoirs only, and it was expected that the pH in the cultures would shift gradually to the desired level. However, as a result of the spontaneous rebound of reservoir water pH, the desired change was not attained until acid was added directly to the cultures (see results). In Run 7 the pH was lowered 0.5 pH units every sampling day by direct addition of acid to the cultures until the desired pH was attained. Thereafter, daily additions of acid to the cultures were necessary to maintain this pH.

RESULTS

Temperatures and chemical data for the four study lakes are presented in Table 3 for each run. Both Greenwood and Birch lakes maintained temperatures near 20°C during the first three runs. During Run 4 (mid to late August) the weather was unusually cold, and temperatures began to fall. Lake temperatures continued to drop throughout the autumn season. Temperature profiles of the lakes indicated that neither lake stratified.

The total organic carbon present in Birch Lake was relatively constant at 15-18 mg/l during the summer, increasing to 22-23 mg/l in late autumn. TOC in Greenwood Lake remained near 25 mg/l until late September when it increased to 34 mg/l. This increase coincided with heavy rains and extremely high lake levels. Clearwater Lake (6 mg/l) and South McDougal Lake (35 mg/l) represent the extreme ranges of TOC for lakes of the study area. The organic carbon analyses show that nearly all carbon in the lakes was present in dissolved (<0.45 μ) form.

All of the lakes were slightly acid with low total alkalinities. Concentrations of copper and nickel were quite low and insignificant compared to treatment levels.

The measured treatment concentrations of metals were generally slightly lower but within 10% of the nominal (desired) concentrations (Tables 4a and 4b). The maximum difference between metal concentrations in replicate pairs was 8.9%. Measurements of total and dissolved metal concentrations indicated that the metals were present almost entirely in dissolved (<0.45 μ) form.

The calculated concentrations of chlorophyll *a* and phaeopigments present in all treatments are listed in Appendix A. A summary of all bioassay results is presented in Table 5.

Run 1: Effects of copper on Birch and Greenwood lakes.

1) In situ tests. Data from this experiment are limited since many of the polyethylene bags developed leaks. After one week only six of the original sixteen bags remained intact. In Birch Lake only 50 and 200 µg/l Cu were adequately tested, but both appeared to depress chlorophyll levels (Figure 4). Exposure to 200 µg/l Cu reduced chlorophyll a to approximately 20% of the control, and the apparent toxic effect of this concentration was observed on the first day of sampling (day 2). In the case of the 50 µg/l treatment, the effect is less certain since replicate treatments were not available to provide an estimate of experimental error. Chlorophyll in the control bag was comparable to lake levels.

In Greenwood Lake the effects of 100 and 200 µg/l Cu were assessed, and both reduced chlorophyll levels appreciably (Figure 5). At the end of the test the chlorophyll level in the bag treated with 200 µg/l was approximately 40% of the control bag. Thus, the same treatment (200 µg/l) appeared to be more inhibitory in Birch than in Greenwood Lake.

2) Continuous culture tests. In general, the results of the laboratory tests were similar to those obtained in the field during this run. In Birch Lake all metal concentrations tested (50, 100 and 200 µg/l Cu) were toxic, reducing chlorophyll a to levels significantly lower than controls (Figure 6). Cultures treated with 100 and 200 µg/l were virtually indistinguishable on the basis of chlorophyll concentrations, while the effect of 50 µg/l was perhaps less pronounced. The abrupt rise on day 13 in the relative chlorophyll in the 50 µg/l cultures is worth mention since similar chlorophyll peaks were observed in subsequent tests. The reduction in chlorophyll levels was apparent in all treatments on the first sampling day (day 4).

In Greenwood Lake, as in Birch Lake, all metal concentrations tested reduced chlorophyll concentrations significantly (Figure 7). However, in this case, 200 µg/l Cu appeared to be more toxic than either 50 or 100 µg/l. The latter two treatments were indistinguishable. The degree to which chlorophyll was reduced was similar to that observed in Birch Lake, although the effects of the treatments were not apparent as early in Greenwood Lake as in Birch Lake.

Run 2: Effects of copper on Birch and Greenwood lakes

1) In situ tests. This run was the first using glass carboys as enclosures. Replication of treatments was poor, especially in Birch Lake where the data points are so erratic that it is impossible to draw any conclusions (Figure 8). It is likely that these poor results were due to inadequate mixing of the water in the enclosures, since no effort was made to homogenize the contents of the enclosures prior to withdrawing samples. In subsequent runs the enclosures were mixed by bubbling with air (see METHODS) and replication of treatments was good. This suggests that algal cells in the carboys tend to settle and/or adhere to the carboy walls.

Replication of treatments was somewhat better in Greenwood Lake, where 100 µg/l Cu was clearly inhibitory (Figure 9). However, by day 12 chlorophyll levels in these enclosures had risen to equal those of the controls. Treatments of 20 and 50 µg/l Cu had no discernible effect on algal biomass in this experiment.

2) Continuous culture tests. In Birch Lake the three treatments (20, 50 and 100 µg/l Cu) had three distinctly different effects (Figure 10). The effect of 20 µg/l Cu is rather difficult to interpret, although identical in the two replicate cultures. While a chlorophyll peak (above controls) occurred on day 13, the final chlorophyll concentrations on day 17 were only 50% of the

controls. The 50 µg/l treatment was dramatically stimulatory with a chlorophyll peak on day 10. The peak in one culture was nearly 9 times that of the controls. Subsequently, chlorophyll levels in these cultures declined, and by day 17 they were less than twice the control values. The 100 µg/l treatment was clearly toxic; by day 13 chlorophyll concentrations in these cultures were less than 10% of control levels.

The results for Greenwood Lake (Figure 11) were similar to those for Birch. The 20 µg/l treatment was at first mildly stimulatory, then toxic. Markedly elevated chlorophyll levels were observed in the 50 µg/l cultures on days 13 and 15. Once again, this stimulatory effect was short-lived; on day 17 there was little difference between these cultures and the controls. Treatment with 100 µg/l Cu resulted in reduced chlorophyll levels, and this effect was evident even in the beginning of the experiment. By day 17, chlorophyll in these cultures was approximately 10% of controls.

Run 3: Effects of nickel on Birch and Greenwood lakes.

1) In situ tests. Birch and Greenwood lakes responded similarly to additions of nickel (Figures 12 and 13). Treatments of 100 and 400 µg/l Ni had no effect on algal biomass, except perhaps in Birch Lake where the 400 µg/l enclosures were slightly lower in chlorophyll content than the controls during the last three sampling days. The 1000 µg/l treatment significantly reduced chlorophyll concentrations in both lakes, an effect which was first noticed on day 7 in each case. The extent of the reduction was similar in the two lakes. In Greenwood Lake chlorophyll levels in the enclosures were similar to those in open lake water. In Birch Lake chlorophyll levels in the enclosures were significantly lower than open lake values, probably because of a phytoplankton bloom which began in the lake after the experiment had started.

2) Continuous culture tests. In Birch Lake the treatment of 1000 µg/l Ni was severely toxic (less than 5% of controls by day 13), and the 400 µg/l treatment was perhaps slightly toxic (Figure 14). These results are in qualitative agreement with the in situ test results. In contrast, the treatment of 100 µg/l Ni exhibited a definite tendency to cause temporary chlorophyll peaks (growth stimulation) much as did low concentrations of copper in previous runs. This stimulation by nickel was not observed in the field or in later laboratory tests (which, however, employed much larger nickel concentrations).

In Greenwood Lake 1000 µg/l Ni was immediately and severely toxic (Figure 15). The magnitude of the chlorophyll reduction was comparable to that in Birch Lake cultures but much greater than that observed in the field in either lake. Cultures treated with 400 µg/l maintained chlorophyll levels similar to the controls until day 10, after which they dropped sharply. This drop in chlorophyll coincided in time with a drop in Birch Lake for this same treatment (Figure 14). Unlike Birch Lake, cultures treated with 100 µg/l Ni also showed reduced chlorophyll. This effect was apparent even in the early days of the run. However, on day 13 a chlorophyll peak occurred in one of the cultures of this treatment, again suggesting that low concentrations of metals may have stimulatory effects.

Run 4: Effects of copper, nickel, and copper-nickel combinations on Birch and Greenwood lakes.

1) In situ tests. In Birch Lake all of the treatments were toxic (Figure 16). The effects of 50 µg/l Cu were only slightly less than those of 100 µg/l Cu, and both of these treatments recovered to control levels by the end of the run. Interestingly, this recovery was not yet apparent on day 16 of the test. Although showing about the same toxicity as the copper treatments, the 800 µg/l Ni treatment had not recovered by the end of the test (day 21).

The most toxic treatment was the combination of 100 µg/l Cu and 800 µg/l Ni. The result of this treatment appeared to be an additive effect of the two metals; neither synergism nor antagonism was evident. No tendency of this treatment to recover to control levels was observed.

A drop in chlorophyll concentration was observed in all enclosures immediately after the start of the experiment, and chlorophyll in the enclosures remained lower than that in open lake water for the duration of the test. This phenomenon was not observed in other field bioassays and is probably related to the fact that Birch Lake chlorophyll levels were unusually high at the time of the experiment. Apparently, whatever factor(s) was maintaining this bloom was at least partly excluded from the environment of the test bottles.

The response of Greenwood Lake was quite different than that of Birch Lake (Figure 17). Consistent and prolonged increases in chlorophyll content over controls were observed in treatments receiving both 50 and 100 µg/l Cu. The increases were somewhat greater in the 50 than the 100 µg/l treatments. Both the nickel and the copper-nickel combination treatments were inhibitory, the latter slightly more so than the former. There was also a consistent trend towards recovery in these treatments. It is of interest that despite the fact that 100 µg/l Cu was stimulatory by itself, adding it to 800 µg/l Ni resulted in a further reduction in chlorophyll. In general, however, the nickel and copper-nickel additions used in this run were more toxic in Birch Lake than in Greenwood Lake.

Production/biomass ratios were calculated for the control and copper-treated enclosures in Greenwood Lake on days 17 and 22 (Table 6). The ratios for the copper-treated enclosures were not significantly different from those for the controls.

2) Continuous culture tests. The two lakes responded similarly to the treatments, all of which were toxic (Figures 18 and 19). The combination of 100 µg/l Cu and 800 µg/l Ni was the most inhibitory. Additions of 100 µg/l Cu and 800 µg/l Ni by themselves resulted in equal reductions in chlorophyll. Although the treatments produced effects of similar magnitude in Birch and Greenwood lakes, inhibition occurred more quickly in Birch Lake.

Run 5 (Field): Interaction between copper and zooplankton in Greenwood Lake.

The apparent stimulation of algae by copper in Greenwood Lake in Run 4 seemed to warrant further investigation. If copper was a limiting nutrient in that experiment, one would expect to find higher P/B ratios in copper-treated enclosures. Since this was not the case, other hypotheses must be considered. One attractive hypothesis is that zooplankton are more sensitive to the copper additions than are the algae and are either killed or unable to graze normally in copper-treated bottles. Since Greenwood Lake was observed to have large populations of copepods and cladocerans, inhibition of zooplankton grazing might be expected to result in accumulation of algal biomass. Field Run 5 was designed to test this hypothesis.

When the large zooplankters were experimentally removed in two of the bottles, chlorophyll levels increased significantly above control levels (Figure 20, with plastic overlay). In bottles in which the zooplankters were removed and which received 50 µg/l Cu, chlorophyll levels were even higher. However, 50 µg/l Cu alone did not stimulate chlorophyll levels in this experiment, nor did the addition of extra zooplankters have any effect. The remaining treatment, 50 µg/l Cu plus added zooplankters, appeared to have some stimulatory effect. Unfortunately, several bottles were lost during this run as a result of vandalism.

Production/biomass ratios for this run show, with one exception, lower ratios in treatments which included zooplankton removal (Table 7). Bottles receiving only 50 µg/l Cu did not show lower P/B ratios.

Bacterial plate counts showed higher bacteria populations in all bottles than in open lake water (Table 8). Variability among the counts was high, and there was no apparent relationship between bacteria populations and metal treatments.

Algal populations and water temperatures were both considerably lower in this run than in Run 4, making direct comparisons between these run hazardous.

Run 5 (Continuous Culture): Effects of copper and nickel on Clearwater and South McDougal lakes.

All of the treatments (50 and 100 µg/l Cu, 1000 µg/l Ni) were severely toxic in Clearwater Lake, reducing chlorophyll concentrations to about 10% of the controls (Figure 21). No differences among the treatments were evident. There was some suggestion that low concentrations of these metals might also be stimulatory in Clearwater Lake since a rise in chlorophyll was detected in all treated cultures on the first sampling day when metal concentrations were still quite low.

In South McDougal Lake the treatments were also toxic, but much less so than in Clearwater (Figure 22). The nickel treatment was most toxic, reducing chlorophyll to about 20% of the controls. The 100 µg/l Cu treatment was initially more toxic than the 50 µg/l Cu treatment, but between days 5 and 8 both of these cultures recovered, finally achieving chlorophyll values of about 65% and 80% of the controls. In contrast, the 50 µg/l cultures had values of only 30% and 50% of controls at the end of the experiment.

Run 6 (Continuous Culture): Effect of lowered pH in combination with copper and nickel on Birch Lake.

All of the metal treatments tested in this run were toxic (Figure 23, with plastic overlay). Their ranking in order of increasing toxicity was: 100 µg/l Cu < 600 µg/l Ni < 100 µg/l Cu + 600 µg/l Ni. This ranking was the same for both normal-pH and pH-altered cultures. It was not possible to test for synergism in this experiment since the mono-metal treatments alone reduced chlorophyll to less than 50% of controls. Thus, the combined effect of the metals was necessarily less than additive. Cultures receiving 100 µg/l Cu showed some tendency to recover.

Lowering the pH to 6.2 had no appreciable effect on the treatments, but the sudden drop in pH to 4.6 on day 11 resulted in a sharp decline in chlorophyll in those cultures which were not treated with metals. In contrast, the metal-treated cultures showed no abrupt declines at this time.

Run 7 (Continuous Culture): Effect of lowered pH in combination with copper and nickel on Birch Lake.

Run 7 was identical to Run 6 except that 400 instead of 600 µg/l Ni was tested and the pH of one set of cultures was adjusted to 5.0 on a daily basis. The 100 µg/l Cu treatment was somewhat less toxic in this run than in Run 6 (Figure 24, with plastic overlay). The lowest chlorophyll concentration recorded for this treatment was approximately 50% of the controls, and recovery was complete by the end of the run (day 14). Similar remarks apply to the cultures receiving 400 µg/l Ni. The cultures receiving the combined copper-nickel treatment were consistently lower in chlorophyll than the cultures receiving only a single metal, and some recovery occurred in only one of the two cultures. The two metals did not appear to act synergistically in this run.

In those cultures in which pH was manipulated, the pH rebounded to some extent after each addition of acid, so that the pH actually fluctuated between 5.0 and 6.0. The cultures which received acid but no metals actually increased in chlorophyll above the controls, in one case to as much as 200%. In the metal-treated cultures lowering the pH seemed to have little effect on toxicity, except that recovery trends were less pronounced and no treatment had returned to control levels by the end of the run.

Phytoplankton Cell Counts

In continuous culture Run 2 diatoms apparently accounted for the chlorophyll peaks observed in both Birch and Greenwood lakes (Table 9). It is of interest that while these diatoms were more abundant in the 50 µg/l Cu treatments than in the controls, they were severely inhibited in the 100 µg/l Cu treatments.

Cell count data for field Runs 2-5 are presented in Tables 10-13. These results may be summarized as follows:

1) Diatoms showed both stimulation and inhibition in response to metal treatments. Frequently, stimulation at low concentrations was accompanied by inhibition at high concentrations.

2) Blue-green algae were probably the most sensitive of the algal groups. They were never stimulated by low metal concentrations and were often inhibited by levels as low as 20 µg/l Cu and 100 µg/l Ni. However, the genus Agmenellum, often abundant in these lakes, was resistant to even high concentrations of metals.

3) Among the Chrysophytes, Dinobryon and Hyalobryon were sometimes strongly stimulated by 50 and 100 µg/l Cu. This stimulatory effect vanished when 800 µg/l Ni was added to the copper treatment in Run 4.

4) Some species of algae (especially blue-greens) were more inhibited by 100 µg/l Cu than by 800 µg/l Ni, but for other species the reverse was true.

5) Although the total number of species present in Birch and Greenwood lakes was similar, generally more species were affected by the metal treatments (positively or negatively) in Birch than in Greenwood Lake.

6) Low level metal treatments usually resulted in the stimulation of some algal species and the inhibition of others. Consequently, significant shifts in species composition occurred at low metal concentrations, and these shifts persisted even after chlorophyll concentrations recovered to control levels.

7) Inhibition and/or stimulation of particular species frequently occurred without detectable differences in chlorophyll between treatment and control bottles. In fact, some species often showed stimulation when the chlorophyll response was negative, and vice versa.

Phaeopigments

With few exceptions, phaeopigment/chlorophyll a ratios agreed with the assessments of toxicity made using only the chlorophyll a values. Thus, in treatments in which chlorophyll a was lower than controls, the phaeopigment/chlorophyll a ratio was higher; and in treatments in which chlorophyll a indicated stimulation, the ratio was lower. In cases which were exceptions to these trends, the ratios of the treatments were similar to those of the controls. In addition, the phaeopigment/chlorophyll a ratio was useful as an indicator of the degree of toxicity; that is, there was a positive correlation between the degree of toxicity and the ratio.

Phaeopigment/chlorophyll a ratios in natural lake water generally ranged from 0 - 0.2. However, in Greenwood Lake in late summer (latter portion of Run 3 and all of Run 4) phaeopigment concentrations increased markedly

as chlorophyll a levels dropped. During this time the ratio reached a peak value of 0.42. The decline in algal populations at this time corresponded to rapidly dropping lake temperatures. In the field bioassays control enclosures exhibited ratios similar to those of lake water. In control laboratory cultures, however, phaeopigment/chlorophyll a ratios were typically low at the start of an experiment (0 - 0.1) but rose during the middle of the test to values of about 0.3 - 0.6. Subsequently, the ratios again declined.

In treatments which appeared toxic (as indicated by depressed chlorophyll a levels) the ratios were often as high as 3.0 - 7.0 in laboratory cultures and as high as 0.6 in the field enclosures. These results are in agreement with chlorophyll a measurements in indicating that algae in the continuous cultures were far more sensitive to the toxins than algae in the field enclosures.

DISCUSSION

The levels of copper and nickel found to be toxic in this study are consistent with the findings of other workers. Our study also agrees with published information which indicates that copper is less toxic to algae in waters of high humus content. For example, Clearwater and South McDougal lakes differ greatly in TOC, complexing capacity, and in response to copper additions. In this regard, our data suggest that complexing capacity may be a better measure of a lake's resistance to copper poisoning than TOC. Birch and Greenwood lakes differ significantly in TOC but overall showed similar responses to copper additions, Birch being only slightly more sensitive than Greenwood. This latter observation correlates well with measurements of complexing capacity on the two lakes (Table 1). More data are needed to establish complexing capacity as a valid predictive tool in algal toxicity tests.

Algae in continuous cultures generally showed more extreme responses to metal additions than algae in field enclosures. Both stimulatory and toxic responses were usually of greater magnitude in the laboratory. Several factors might be involved: 1) Algal densities in the continuous cultures were always much lower than in the field. Low cell densities mean low concentrations of excreted metabolic products, some of which may be important in complexing metals. 2) The reason for declining cell densities in the cultures is not clear. It is possible that the algae are already stressed in the culture environment and, consequently, that metals are even more toxic in the cultures than they would be under natural conditions. 3) The complexing capacity of the lake water may have been lowered when the reservoir water was heat-sterilized. At the moment, we cannot choose among these theories.

It seems certain, however, that the use of continuous culture bioassays would lead to more conservative management decisions than the use of field bioassays.

The longest bioassay conducted in this study was field Run 4, which lasted 22 days. The copper-treated enclosures in this test were observed to recover, approaching control chlorophyll values, on day 19 in Birch Lake (Figure 16). Numerous other instances of recovery or partial recovery of treated cultures or enclosures were recorded. Thus, our data support the contention of Stockner and Antia (1976) that relatively long term bioassays are essential to adequately assess the potential of algae to adapt to pollutants. This recovery phenomenon is almost ceratinly a biological response of the algae themselves (whether physiological or genetic) rather than a result of chemical changes occurring through time in the test water (e.g., see Stokes et al. 1973), and its occurrence rather seriously complicates the application of bioassay results to practical management decisions and the setting of water quality standards. For example, while 100 µg/l Cu was usually toxic to algae in the lakes we studied, it seems quite possible that on a time scale of months many algae could successfully adapt to this copper concentration. Indeed, Stokes et al. (1973) have isolated a strain of Scenedesmus which grows at concentrations of 400 µg/l Cu and 1500 µg/l Ni. On the other hand, it is also likely that, were such concentrations allowed to persist in the environment, important changes in phytoplankton species composition and food web relationships would occur. We have almost no ability to predict these changes with bioassay experiments.

Little evidence was found to support the idea that copper and nickel act synergistically in these lakes (see Hutchinson 1973). When copper and nickel were tested individually and in combination, the effect of the combination treatment appeared to be an additive effect of the two metals administered

singly. Our data on this question are not extensive, however, and more information would be desirable.

A somewhat unexpected finding was the rather frequent occurrence of chlorophyll increases, or peaks, above controls in metal-treated enclosures and cultures. Stimulatory responses were observed with concentrations of 50 and 100 µg/l Cu and 100 µg/l Ni. Few instances of stimulation of algae by copper are reported in the literature, and in no case has a mechanism for the stimulation been proved. In the marine environment Reeve et al. (1976) found increases of chlorophyll a in copper-containing experimental ecosystems and suggest that decreases in zooplankton grazing pressure in these enclosures are responsible. Working off the south coast of Finland, Niemi (1972) found that low concentrations of copper (2.5 and 10 µg/l), phenol and potassium cyanide were stimulatory to phytoplankton. At a certain concentration of each chemical an inhibitory effect became apparent. Niemi does not discuss these results in detail, but since C-14 uptake was used as the measure of phytoplankton response and since phenol and potassium cyanide are not known micronutrients, they clearly suggest that phytoplankton may respond to low levels of chemical stress with a generalized increase in productivity. Some support for this idea is provided by Ferstenberg et al. (1975) whose growth rate experiments with laboratory and metal-tolerant strains of algae showed initially higher rates of cell division compared to controls in cultures treated with low levels of copper (50 and 100 µg/l). These authors suggest that, since the initiation of cell division appears to be correlated with a critical cell volume, cells taking up copper may reach this critical volume sooner and thus divide more rapidly than cells which are not taking up metal. Few studies concerned with natural lake waters have dealt with this phenomenon. Steemann Nielsen and Laursen (1976) have observed increases

in the photosynthetic rate in a brown water lake treated with 25 µg/l Cu. However, they do not propose a reason for the stimulatory effect, except to note that copper may be more completely complexed in this lake than in other lakes where this same concentration was inhibitory.

Field Run 5 in this study was designed to test the hypothesis, suggested by the field data from Greenwood Lake in Run 4, that chlorophyll increases in copper-treated enclosures were a result of reduced grazing pressure in these enclosures. Our data fail to confirm this hypothesis, which predicts that enclosures from which large zooplankters were removed would have chlorophyll levels greater than or equal to enclosures from which the zooplankton were removed but which also received 50 µg/l Cu. Instead, these latter enclosures showed an added stimulation above the former (Figure 20). The suggestion is that copper has a direct stimulatory effect on the algae. The fact that enclosures receiving only copper did not exhibit stimulation above control enclosures is somewhat mystifying, but may be related to the fact that conditions encountered in Run 5 (temperature, TOC, chlorophyll, lake water level) were considerably different from those of field Run 4. Finally, it is well known that one effect of zooplankton grazing is to increase the carbon fixation rates of algal cells, and our data confirm this point (Table 7). The fact that low P/B ratios did not occur in field Run 4 or in enclosures treated with only 50 µg/l Cu in Run 5, but only where zooplankters were removed, makes it appear unlikely that the zooplankton grazing hypothesis is correct.

Nutrient limitation is also an unattractive hypothesis to account for these stimulatory effects. In continuous culture experiments chlorophyll peaks were usually not observed until considerable buildup of metal concentrations had occurred in the cultures. In addition, abrupt chlorophyll

increases were typically followed by equally abrupt declines. These observations do not suggest nutrient limitation but are much more readily accounted for if one assumes that the algae respond to low stress situations by an increase in cell division rate. Thus, as concentrations of the metal build up in the cultures, a point is reached several days into the experiment when chlorophyll levels rise in response to mild stress. Then, as concentrations continue to increase, toxicity quickly ensues. (Note: In the first five continuous culture runs metals were not added directly to the cultures, but only to the reservoir water. Buildup of metal concentrations was therefore exponential. In Runs 1-4 buildup, as a percent of the final concentration, was as follows: Day 1 - 22%, day 2 - 40%, day 3 - 53%, day 4 - 64%, day 5 - 72%, day 6 - 78%, day 7 - 83%, day 8 - 87%, etc. This method of administering metals in gradually increasing concentrations in the laboratory may help to explain some of the differences in algal response noted in concurrent field and laboratory bioassays.)

Obviously, our data are inconclusive regarding the reasons for stimulation of algae by low metal concentrations. Two more observations are worth mentioning, however: 1) In continuous culture Run 7 a pH drop to 5.0 resulted in an increase in chlorophyll, while in Run 6 a drop to pH 4.6 was apparently toxic. Is it possible that low, marginally toxic pH's stimulate algae in a manner similar to low concentrations of metals? 2) In field Run 4 on Greenwood Lake 100 µg/l Cu stimulated chlorophyll, while 800 µg/l Ni was inhibitory. Yet, when the two metals were added in combination, chlorophyll values were even lower than in the 800 µg/l Ni treatment. This observation provides strong support for the notion that the algae are responding to 100 µg/l Cu as an environmental stress, not as a growth stimulant, and, indeed that the two metals may have similar, additive physiological effects.

In this study measurements of chlorophyll a alone proved inadequate in assessing the extent of phytoplankton community responses to metal additions. Our data suggest that changes in algal species composition may occur in lakes receiving metal inputs and that these changes cannot be detected by measures of total biomass, but only by species cell counts. At the moment we cannot say whether these species composition shifts would be of a temporary or permanent nature. If permanent, they could undoubtedly have important effects on aquatic food webs.

Different algal species showed a remarkable range of responses to the same metal treatment under the same conditions. These varied responses shed some doubt on the practice of using single species algal assays as a means of determining realistic water quality standards. Indeed, the problem of setting standards is itself considerably complicated since one must choose some criterion for establishing adverse environmental impact. What is this criterion to be? Clearly, if the criterion is any change in community composition, much lower critical levels of pollutants will be established than if the criterion is a change in total algal biomass or the response of a test algal species. In this regard, much more work is needed to establish the long term meaning of species composition changes in algal bioassays.

REFERENCES

American Public Health Association. 1971. Standard Methods for the Examination of Water and Wastewater. 13th ed. A.P.H.A., Washington, D.C., 874 p.

Andrew, R.W. 1976. Current "state of the art" metal speciation determinations. p. 293. In Toxicity to Biota of Metal Forms in Natural Waters. I.J.C. Workshop, October 1975.

Barlow, J.P., W.R. Schaffner, F. deNoyelles, Jr. and B.J. Peterson. 1973. Continuous flow nutrient bioassays with natural phytoplankton populations. p. 299-319. In Symposium on bioassay techniques and environmental chemistry. Ann Arbor Science Publishers, Inc.

Cairns, J. Jr., G.R. Lanza and B.C. Parker. 1972. Pollution related structural and functional changes in aquatic communities with emphasis on freshwater algae and protozoa. Proc. Acad. Nat. Sci. Philadelphia 124: 79-127.

Dugdale, R.C. 1967. Nutrient limitations in the sea: dynamics, identification, and significance. Limnol. Oceanogr. 12: 685-695.

Dunstan, W.M. 1975. Problems of measuring and predicting influence of effluents on marine phytoplankton. Environ. Sci. and Technol. 9: 635-638.

Ferstenberg, L.B., P.M. Stokes and B. Silverberg. 1975. An electron microscope study of copper in Scenedesmus. Abstract of paper presented at the International Heavy Metals Conf., Toronto, October 1975.

Foster, P.L. 1977. Copper exclusion as a mechanism of heavy metal tolerance in a green alga. Nature 269: 322-323.

Gerhart, D.Z. 1973. Nutrient limitation and production of phytoplankton in Mirror Lake, West Thornton, New Hampshire. Ph.D. thesis, Cornell Univ. 160 p.

Gerhart, D.Z. and G.E. Likens. 1975. Enrichment experiments for determining nutrient limitation: four methods compared. Limnol. Oceanogr. 20: 649-653.

Goldman, C.R. 1962. A method of studying nutrient limiting factors in situ, in water columns isolated by polyethylene film. Limnol. Oceanogr. 7: 99-101.

Goldman, C.R. 1972. The role of minor nutrients in limiting the productivity of aquatic ecosystems. p. 21-33. In G.E. Likens (ed.), Nutrients and eutrophication: the limiting nutrient controversy. American Society of Limnology and Oceanography Special Symposia, Vol. 1.

Goldman, J.C. 1976. Identification of nitrogen as a growth-limiting nutrient in wastewaters and coastal marine waters through continuous culture algal assays. Water Research 10: 97-104.

Horne, A.J. and C.R. Goldman. 1974. Suppression of nitrogen fixation by blue-green algae in a eutrophic lake with trace additions of copper. Science 183: 409-411.

Hutchinson, T.C. 1973. Comparative studies of the toxicity of heavy metals to phytoplankton and their synergistic interactions. Water Poll. Res. Canada 8: 68-90.

Hutchinson, T.C. and P.M. Stokes. 1975. Heavy metal toxicity and algal bioassays. In Water Quality Parameters, p. 320-343. ASTM STP 573. American Society for Testing and Materials, Philadelphia.

Hutchinson, T.C. and P.M. Stokes. 1976. Organo-metal complexes and their bioavailability in some Ontario lakes. Report of project done under contract to Environment Canada with Canada Centre for Inland Waters, June 1974-April 1975.

Klotz, R.L., J.R. Cain and F.R. Trainor. 1975. A sensitive algal assay: an improved method for analysis of freshwaters. J. Phycol. 11: 411-414.

Kubota, J., E.L. Mills and R.T. Oglesby. 1974. Lead, Cd, Zn, Cu, and Co in streams and lake waters of Cayuga Lake Basin, New York. Environ. Sci. and Technol. 8: 243-248.

Lind, O.T. and R.S. Campbell. 1969. Comments on the use of liquid scintillation for routine determination of C-14 activity in production studies. Limnol. Oceanogr. 14: 787-789.

Maddux, W.S. and R.F. Jones. 1964. Some interactions of temperature, light intensity, and nutrient concentration during the continuous culture of Nitzschia closterium and Tetraselnis sp. Limnol. Oceanogr. 9: 79-86.

McBrien, D.C. and K.A. Hassall. 1967. The effect of toxic doses of copper upon the respiration, photosynthesis and growth of Chlorella vulgaris. Physiol. Plant. 20: 113-117.

Mitra, R.S., R.H. Gray, B. Chin and I.A. Bernstein. 1975. Molecular mechanisms of accomodation in Escherichia coli to toxic levels of Cd^{++}. J. Bacteriol. 121: 1180-1188.

Niemi, A. 1972. Effects of toxicants on brackish-water phytoplankton assimilation. Commentat. Biol. 55: 1-19.

O'Kelley, J.C. 1974. Inorganic nutrients. p. 619. In W. Stewart (ed.), Algal physiology and biochemistry, U. of Calif. Press, Berkeley, 989 p.

Patrick, R., T. Bott and R. Larson. 1975. The role of trace elements in management of nuisance growths. EPA-660/2-75-008.

Reeve, M.R., G.D. Grice, V.R. Gibson, M.A. Walter, K. Darcy and T. Ikeda. 1976. A controlled environmental pollution experiment (CEPEX) and its usefulness in the study of larger marine zooplankton under toxic stress. p. 145-162. In: A. Lockwood (ed.), Effects of pollutants on aquatic organisms. Cambridge Univ. Press, London.

Rice, H.V., D.A. Leighty, G.C. McLeod. 1973. The effects of some trace metals on marine phytoplankton. CRC Critical Reviews in Microbiology, Sept. 1973: 27-49.

Schindler, D.W. 1971. Carbon, nitrogen, and phosphorus and the eutrophication of freshwater lakes. J. Phycol. 7: 321-329.

Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes. Science 195: 260-262.

Schindler, D.W., F.A.J. Armstrong, S.K. Holmgren and G.J. Brunskill. 1971. Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario, by addition of phosphate and nitrate. J. Fish. Res. Bd. Canada 28: 1763-1782.

Shapiro, J. 1973. Blue-green algae: why they become dominant. Science 179: 382-384.

Shapiro, J. and G.E. Glass. 1975. Synergistic effects of phosphate and manganese on growth of Lake Superior algae. Verh. Internat. Verein. Limnol. 19: 395.

Steemann Nielsen, E. and L. Kamp-Nielsen. 1970. Influence of deleterious concentrations of copper on the growth of Chlorella pyrenoidosa. Physiol. Plant. 23: 828-840.

Steemann Nielsen, E., L. Kamp-Nielsen and S. Wium-Anderson. 1969. The effect of deleterious concentrations of copper on the photosynthesis of Chlorella pyrenoidosa. Physiol. Plant. 22: 1121-1123.

Steemann Nielsen, E. and H.B. Laursen. 1976. Effect of $CuSO_4$ on the photosynthetic rate of phytoplankton in four Danish lakes. Oikos 27: 239-242.

Stiff, M.J. 1971. The chemical states of copper in polluted fresh water and a scheme of analysis to differentiate them. Water Research 5: 585-599.

Stockner, J.G. and N.J. Antia. 1976. Phytoplankton adaptation to environmental stresses from toxicants, nutrients, and pollutants - a warning. J. Fish. Res. Bd. Canada 33: 2089-2096.

Stoermer, E.F., C.L. Schelske and L.E. Feldt. 1971. Phytoplankton assemblage differences at inshore versus offshore stations in Lake Michigan, and their effects on nutrient enrichment experiments. Proc. 14th Conf. Great Lakes Res. p. 114-118.

Stokes, P.M. and T.C. Hutchinson. 1976. Copper toxicity to phytoplankton, as affected by organic ligands, other cations and inherent tolerance of algae to copper. p. 159-185. In Toxicity to Biota of Metal Forms in Natural Waters. I.J.C. Workshop, October 1975.

Stokes, P.M., T.C. Hutchinson and K. Krauter. 1973. Heavy-metal tolerance in algae isolated from contaminated lakes near Sudbury, Ontario. Can. J. Bot. 51: 2155-2168.

Strickland, J.D.H. and T.R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Bd. Canada Bull. 167. 311 p.

Wood, J.M. 1974. Biological cycles for toxic elements in the environment. Science 183: 1049-1052.

Yan, N.D. and P.M. Stokes. 1976. The effects of pH on lake water chemistry and phytoplankton in a LaCloche Mountain lake. Presented at the 11th Symposium on Water Pollution in Canada, Burlington, Ontario, 1976.

APPENDIX A

Calculated Chlorophyll a and Phaeopigment Concentrations for Laboratory and Field Bioassays*

*Concentrations of chlorophyll a and phaeopigments are given in µg/l.

LABORATORY RUN 1
Birch Lake

Treatment		Day 4	6	9	11	13	16
control	chl.a	3.20	1.12	0.74	0.32	0.15	0.20
	phaeo.	0.20	0.12	0.05	0.17	0.07	0.05
	ratio	0.06	0.11	0.07	0.53	0.50	0.27
control	chl.a	2.85	1.23	0.41	0.25	0.07	0.09
	phaeo.	0.16	0.14	0.07	0.15	0.10	0.07
	ratio	0.06	0.12	0.17	0.62	1.39	0.71
50µg/1 Cu	chl.a	1.28	0.71	0.34	0.11	0.11	0.05
	phaeo.	0.20	0.15	0.05	0.07	0.13	0.05
	ratio	0.16	0.22	0.15	0.64	1.16	1.05
50µg/1 Cu	chl.a	1.63	0.69	0.28	0.09	0.12	0.05
	phaeo.	0.20	0.12	0.07	0.07	0.12	0.07
	ratio	0.12	0.19	0.28	0.85	1.00	1.34
100µg/1 Cu	chl.a	1.40	0.44	0.16	0.07	0.04	0.04
	phaeo.	0.26	0.05	0.04	0.11	0.11	0.07
	ratio	0.19	0.13	0.30	1.70	2.37	1.60
100µg/1 Cu	chl.a	1.98	0.44	0.13	0.08	0.04	0.04
	phaeo.	0.00	0.00	0.01	0.10	0.07	0.07
	ratio	0.00	0.00	0.11	1.20	1.88	1.60
200µg/1 Cu	chl.a	1.75	0.38	0.28	0.08	0.05	0.03
	phaeo.	0.08	0.09	0.07	0.10	0.09	0.08
	ratio	0.05	0.24	0.26	1.25	1.63	2.29
200µg/1 Cu	chl.a	2.33	0.58	0.18	0.06	0.04	0.02
	phaeo.	0.02	0.19	0.09	0.05	0.08	0.11
	ratio	0.01	0.33	0.50	0.92	2.00	5.24

LABORATORY RUN 1
Greenwood Lake

Treatment	Day	4	6	9	11	13	16
control	chl.a	3.88	2.17	1.20	0.47	0.24	0.12
	phaeo.	0.31	0.27	0.01	0.14	0.11	0.04
	ratio	0.08	0.13	0.01	0.31	0.47	0.39
control	chl.a	2.64	2.02	1.02	0.41	0.28	0.29
	phaeo.	0.03	0.19	0.05	0.09	0.09	0.03
	ratio	0.01	0.10	0.05	0.23	0.35	0.12
50µg/1 Cu	chl.a	4.35	2.17	0.59	0.21	0.13	0.06
	phaeo.	0.00	0.62	0.16	0.11	0.16	0.07
	ratio	0.00	0.30	0.27	0.53	1.25	1.15
50µg/1 Cu	chl.a	3.18	2.21	0.68	0.23	0.12	0.06
	phaeo.	0.25	0.29	0.14	0.13	0.12	0.04
	ratio	0.08	0.14	0.21	0.56	0.99	0.62
100µg/1 Cu	chl.a	3.57	2.41	0.13	0.20	0.14	0.06
	phaeo.	0.50	0.33	0.03	0.15	0.19	0.07
	ratio	0.14	0.14	0.23	0.77	1.40	1.15
100µg/1 Cu	chl.a	3.50	1.76	0.47	0.20	0.11	0.11
	phaeo.	0.00	0.27	0.04	0.11	0.15	0.07
	ratio	0.00	0.16	0.09	0.57	1.38	0.69
200µg/1 Cu	chl.a	2.48	1.31	0.20	0.07	0.04	0.04
	phaeo.	0.25	0.16	0.08	0.08	0.05	0.02
	ratio	0.10	0.13	0.43	1.15	1.23	0.45
200µg/1 Cu	chl.a	4.27	1.37	0.25	0.09	0.03	0.01
	phaeo.	0.33	0.17	0.03	0.11	0.10	0.07
	ratio	0.08	0.13	0.14	1.18	2.95	4.16

LABORATORY RUN 2
Birch Lake

Treatment	Day	1	3	6	8	10	13	15	17
control	chl.a	6.82	1.75	-	0.26	1.46	1.08	1.26	1.21
	phaeo.	0.00	0.47	-	0.17	0.00	0.00	0.00	0.10
	ratio	0.00	0.27	-	0.67	0.00	0.00	0.00	0.08
control	chl.a	3.68	0.44	0.26	0.26	0.31	1.68	0.94	1.30
	phaeo.	0.56	0.32	0.13	0.11	0.12	0.00	0.00	0.07
	ratio	0.15	0.73	0.50	0.42	0.39	0.00	0.00	0.06
20µg/l Cu	chl.a	12.40	0.94	0.22	0.20	0.49	2.15	0.89	0.53
	phaeo.	0.00	0.60	0.34	0.19	0.21	0.00	0.07	0.23
	ratio	0.00	0.64	1.55	0.93	0.43	0.00	0.09	0.44
20µg/l Cu	chl.a	10.60	0.98	0.35	0.29	0.40	2.62	1.08	0.58
	phaeo.	0.00	0.49	0.11	0.30	0.20	0.00	0.10	0.22
	ratio	0.00	0.50	0.31	1.02	0.50	0.00	0.09	0.38
50µg/l Cu	chl.a	11.20	1.66	0.53	0.56	3.84	5.18	3.36	2.10
	phaeo.	0.00	0.46	0.10	0.28	0.00	0.00	0.00	0.00
	ratio	0.00	0.28	0.19	0.50	0.00	0.00	0.00	0.00
50µg/l Cu	chl.a	11.50	1.66	0.53	1.40	7.89	4.71	2.42	1.63
	phaeo.	0.00	0.52	0.10	0.07	0.00	0.00	0.00	0.00
	ratio	0.00	0.32	0.19	0.05	0.00	0.00	0.00	0.00
100µg/l Cu	chl.a	12.40	0.80	0.31	0.23	0.20	0.13	0.11	0.11
	phaeo.	0.00	0.50	0.12	0.25	0.25	0.20	0.29	0.30
	ratio	0.00	0.63	0.39	1.06	1.25	1.50	0.38	2.75
100µg/l Cu	chl.a	14.30	0.98	0.35	0.17	0.13	0.09	0.09	0.09
	phaeo.	0.00	0.00	0.04	0.17	0.13	0.09	0.24	0.21
	ratio	0.00	0.00	0.13	1.00	1.00	1.06	2.70	2.37

LABORATORY RUN 2
Greenwood Lake

Treatment	Day	1	3	6	8	10	13	15	17
control	chl.a	10.00	-	1.70	0.94	0.71	0.50	0.94	0.78
	phaeo.	0.00	-	0.04	0.03	0.17	0.05	0.13	0.07
	ratio	0.00	-	0.03	0.03	0.25	0.10	0.14	0.09
control	chl.a	9.79	-	2.38	0.67	0.98	0.47	0.62	1.66
	phaeo.	0.00	-	0.00	0.90	0.04	0.12	0.11	0.00
	ratio	0.00	-	0.00	1.35	0.04	0.27	0.18	0.00
20µg/1 Cu	chl.a	9.79	-	2.29	1.26	0.83	0.44	0.35	0.24
	phaeo.	0.00	-	0.00	0.29	0.37	0.17	0.24	0.15
	ratio	0.00	-	0.00	0.23	0.45	0.40	0.69	0.64
20µg/1 Cu	chl.a	8.85	-	2.15	1.17	0.89	0.32	0.38	0.22
	phaeo.	0.00	-	0.00	0.31	0.35	0.32	0.20	0.24
	ratio	0.00	-	0.00	0.27	0.40	0.98	0.54	1.10
50µg/1 Cu	chl.a	6.99	-	2.11	1.17	0.80	1.79	2.96	0.94
	phaeo.	0.00	-	0.01	0.34	0.38	0.00	0.00	0.03
	ratio	0.00	-	0.00	0.30	0.47	0.00	0.00	0.04
50µg/1 Cu	chl.a	6.99	-	2.02	1.03	0.86	4.04	4.31	0.91
	phaeo.	0.17	-	0.00	0.31	0.45	0.00	0.00	0.05
	ratio	0.02	-	0.00	0.30	0.53	0.00	0.00	0.06
100µg/1 Cu	chl.a	5.36	-	1.30	0.44	0.32	0.20	0.17	0.13
	phaeo.	0.00	-	0.11	0.35	0.41	0.19	0.32	0.33
	ratio	0.00	-	0.09	0.80	1.25	0.93	1.82	2.49
100µg/1 Cu	chl.a	4.66	-	1.03	0.62	0.26	0.17	0.15	0.09
	phaeo.	0.14	-	0.14	0.46	0.35	0.26	0.34	0.38
	ratio	0.03	-	0.14	0.73	1.33	1.50	2.20	4.20

LABORATORY RUN 3
Birch Lake

Treatment	Day	1	3	6	10	13	15	17
control	chl. a	5.59	3.05	0.96	1.03	1.96	2.14	2.50
	phaeo.	0.87	0.24	0.24	0.00	0.00	0.00	0.00
	ratio	0.16	0.08	0.25	0.00	0.00	0.00	0.00
control	chl. a	6.06	2.69	0.91	0.65	1.10	1.48	2.04
	phaeo.	0.49	0.00	0.01	0.00	0.00	0.00	0.00
	ratio	0.08	0.00	0.01	0.00	0.00	0.00	0.00
100 µg/l Ni	chl. a	7.22	2.60	0.83	0.97	2.52	3.37	2.04
	phaeo.	0.29	0.35	0.17	0.00	0.00	0.00	0.00
	ratio	0.04	0.14	0.21	0.00	0.00	0.00	0.00
100 µg/l Ni	chl. a	6.29	2.87	0.88	0.65	4.69	4.57	2.30
	phaeo.	0.17	0.15	0.26	0.00	0.00	0.00	0.00
	ratio	0.03	0.05	0.30	0.00	0.00	0.00	0.00
400 µg/l Ni	chl. a	6.29	2.83	0.86	0.85	0.56	1.12	0.98
	phaeo.	0.17	0.16	0.02	0.00	0.00	0.00	0.00
	ratio	0.03	0.06	0.03	0.00	0.00	0.00	0.00
400 µg/l Ni	chl. a	7.57	3.50	0.96	1.00	0.92	2.18	2.54
	phaeo.	0.01	0.00	0.24	0.00	0.00	0.00	0.00
	ratio	0.00	0.00	0.25	0.00	0.00	0.00	0.00
1000 µg/l Ni	chl. a	5.94	1.84	0.32	0.12	0.06	0.08	0.08
	phaeo.	0.26	0.28	0.20	0.17	0.06	0.13	0.16
	ratio	0.04	0.15	0.63	1.40	1.00	1.63	2.00
1000 µg/l Ni	chl. a	6.52	2.06	0.43	0.17	0.06	0.08	0.04
	phaeo.	0.46	0.29	0.25	0.08	0.03	0.16	0.14
	ratio	0.07	0.14	0.59	0.50	0.50	2.00	3.50

LABORATORY RUN 3
Greenwood Lake

Treatment	Day	1	3	6	10	13	15	17
control	chl. a	7.92	3.23	1.51	1.15	4.81	*7.09	4.28
	phaeo.	0.81	0.77	0.55	0.10	0.00	0.00	0.00
	ratio	0.10	0.24	0.37	0.09	0.00	0.00	0.00
control	chl. a	7.69	2.87	1.40	1.00	1.20	*4.87	4.21
	phaeo.	0.87	0.76	0.53	0.27	0.00	0.00	0.00
	ratio	0.11	0.27	0.38	0.28	0.00	0.00	0.00
100 µg/l Ni	chl. a	8.15	2.78	1.08	0.52	1.50	1.40	1.75
	phaeo.	0.58	0.91	0.61	0.41	0.00	0.00	0.00
	ratio	0.07	0.33	0.57	0.79	0.00	0.00	0.00
100 µg/l Ni	chl. a	7.92	2.60	0.80	0.45	3.01	4.09	3.91
	phaeo.	0.29	0.96	0.80	0.56	0.00	0.00	0.00
	ratio	0.04	0.37	1.00	1.25	0.00	0.00	0.00
400 µg/l Ni	chl. a	7.11	2.78	1.13	0.95	1.44	1.92	1.17
	phaeo.	0.58	0.85	0.48	0.17	0.00	0.00	0.00
	ratio	0.08	0.31	0.43	0.18	0.00	0.00	0.00
400 µg/l Ni	chl. a	7.22	2.83	-	0.95	0.96	1.46	0.93
	phaeo.	0.64	0.74	-	0.32	0.00	0.00	0.06
	ratio	0.09	0.26	-	0.34	0.00	0.00	0.07
1000 µg/l Ni	chl. a	6.52	2.42	-	0.30	0.34	0.14	0.35
	phaeo.	0.64	1.08	-	0.45	0.00	0.31	0.02
	ratio	0.10	0.45	-	1.50	0.00	2.22	0.07
1000 µg/l Ni	chl. a	6.99	2.29	-	0.22	0.12	0.12	0.05
	phaeo.	0.69	0.90	-	0.60	0.00	0.30	0.36
	ratio	0.10	0.40	-	2.67	0.00	2.51	7.26

*2 filters used

LABORATORY RUN 4
Birch Lake

Treatment	Day	1	3	5	7	10	12	14
control	chl. a	12.30	7.13	3.75	1.00	1.42	1.28	1.28
	phaeo.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ratio	0.00	0.00	0.00	0.00	0.00	0.00	0.00
control	chl. a	12.00	8.33	6.70	1.12	2.14	1.22	1.50
	phaeo.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ratio	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100 µg/l Cu	chl. a	12.30	5.24	1.23	0.60	0.28	0.58	0.42
	phaeo.	0.00	0.00	0.00	0.07	0.01	0.14	0.42
	ratio	0.00	0.00	0.00	0.12	0.04	0.24	1.00
100 µg/l Cu	chl. a	12.60	5.50	1.23	0.25	0.31	0.28	0.16
	phaeo.	0.00	0.00	0.00	0.12	0.00	0.14	0.32
	ratio	0.00	0.00	0.00	0.50	0.00	0.50	1.99
800 µg/l Ni	chl. a	12.90	4.79	1.75	0.36	0.40	0.48	0.56
	phaeo.	0.00	0.00	0.00	0.00	0.00	0.06	0.00
	ratio	0.00	0.00	0.00	0.00	0.00	0.12	0.00
800 µg/l Ni	chl. a	12.60	5.84	1.77	0.25	0.44	0.14	0.08
	phaeo.	0.00	0.00	0.00	0.00	0.00	0.10	0.10
	ratio	0.00	0.00	0.00	0.00	0.00	0.71	1.25
100 µg/l Cu + 800 µg/l Ni	chl. a	12.00	2.66	0.47	0.13	0.08	0.04	0.04
	phaeo.	0.00	0.00	0.01	0.06	0.04	0.20	0.08
	ratio	0.00	0.00	0.03	0.50	0.50	5.00	2.00
100 µg/l Cu + 800 µg/l Ni	chl. a	12.00	3.52	0.60	0.13	0.12	0.08	0.04
	phaeo.	0.00	0.00	0.00	0.06	0.00	0.19	0.32
	ratio	0.00	0.00	0.00	0.50	0.00	2.38	8.03

LABORATORY RUN 4
Greenwood Lake

Treatment \ Day		1	3	5	7	10	12	14
control	chl. a	5.84	3.69	2.63	2.05	1.47	1.87	3.97
	phaeo.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ratio	0.00	0.00	0.00	0.00	0.00	0.00	0.00
control	chl. a	5.67	3.09	2.38	1.60	1.25	1.38	1.56
	phaeo.	0.00	0.00	0.00	0.00	0.00	0.00	0.78
	ratio	0.00	0.00	0.00	0.00	0.00	0.00	0.50
100 µg/l Cu	chl. a	5.50	3.09	1.80	0.72	0.89	0.64	0.72
	phaeo.	0.00	0.00	0.00	0.02	0.00	0.11	0.00
	ratio	0.00	0.00	0.00	0.03	0.00	0.17	0.00
100 µg/l Cu	chl. a	5.15	3.01	1.43	0.50	0.66	0.42	0.92
	phaeo.	0.00	0.00	0.00	0.10	0.00	0.12	0.00
	ratio	0.00	0.00	0.00	0.20	0.00	0.29	0.00
800 µg/l Ni	chl. a	5.58	3.18	1.60	0.51	0.53	0.54	0.44
	phaeo.	0.00	0.00	0.00	0.05	0.00	0.06	0.28
	ratio	0.00	0.00	0.00	0.11	0.00	0.11	0.64
800 µg/l Ni	chl. a	4.89	3.01	1.52	0.49	0.49	0.56	0.46
	phaeo.	0.00	0.00	0.00	0.04	0.00	0.07	0.74
	ratio	0.00	0.00	0.00	0.09	0.00	0.12	1.61
100 µg/l Cu + 800 µg/l Ni	chl. a	5.67	3.09	1.09	0.11	0.17	0.06	0.08
	phaeo.	0.00	0.00	0.00	0.15	0.02	0.36	0.40
	ratio	0.00	0.00	0.00	1.41	0.12	6.02	5.01
100 µg/l Cu + 800 µg/l Ni	chl. a	5.15	2.49	0.88	0.06	0.15	0.04	0.10
	phaeo.	0.00	0.00	0.02	0.20	0.04	0.38	0.35
	ratio	0.00	0.00	0.03	2.98	0.28	9.50	3.51

LABORATORY RUN 5
Clearwater Lake

Treatment	Day	initial	1	3	5	8	10	12
control	chl.a	2.20	2.60	2.20	2.20	1.50	1.00	0.54
	phaeo.	0.28	0.05	0.00	0.00	0.00	0.00	0.00
	ratio	0.13	0.02	0.00	0.00	0.00	0.00	0.00
control	chl.a		2.40	1.70	1.70	0.94	0.60	0.38
	phaeo.		0.00	0.00	0.00	0.04	0.00	0.04
	ratio		0.00	0.00	0.00	0.04	0.00	0.11
50µg/1 Cu	chl.a		3.00	0.45	0.17	0.15	0.07	0.04
	phaeo.		0.00	0.07	0.07	0.06	0.04	0.05
	ratio		0.00	0.16	0.41	0.40	0.57	1.30
50µg/1 Cu	chl.a		2.80	0.56	0.22	0.11	0.09	0.07
	phaeo.		0.00	0.06	0.03	0.05	0.03	0.03
	ratio		0.00	0.11	0.14	0.45	0.33	0.43
100µg/1 Cu	chl.a		3.00	0.40	0.15	0.12	0.07	0.04
	phaeo.		0.09	0.13	0.09	0.06	0.05	0.06
	ratio		0.03	0.33	0.60	0.50	0.71	1.50
100µg/1 Cu	chl.a		3.20	0.38	0.13	0.06	0.09	0.03
	phaeo.		0.00	0.09	0.06	0.04	0.03	0.03
	ratio		0.00	0.24	0.46	0.67	0.33	1.00
1000µg/1 Ni	chl.a		1.90	0.40	0.22	0.07	0.04	0.03
	phaeo.		0.00	0.05	0.06	0.01	0.03	0.04
	ratio		0.00	0.13	0.27	0.14	0.75	1.33
1000µg/1 Ni	chl.a		2.80	0.87	0.24	0.10	0.07	0.05
	phaeo.		0.00	0.00	0.04	0.00	0.00	0.01
	ratio		0.00	0.00	0.02	0.00	0.00	0.20

LABORATORY RUN 5
South McDougal

Treatment	Day	initial	1	3	5	8	10	12
control	chl.a	4.10	1.20	1.00	1.30	1.30	1.40	1.30
	phaeo.	0.09	0.40	0.07	0.05	0.00	0.00	0.00
	ratio	0.22	0.33	0.07	0.04	0.00	0.00	0.00
control	chl.a		1.40	1.20	1.40	1.00	1.20	0.94
	phaeo.		0.40	0.14	0.00	0.13	0.00	0.00
	ratio		0.29	0.12	0.00	0.13	0.00	0.00
50µg/1 Cu	chl.a		1.40	0.94	1.00	0.69	0.40	0.31
	phaeo.		0.36	0.14	0.07	0.04	0.00	0.00
	ratio		0.26	0.15	0.07	0.06	0.00	0.00
50µg/1 Cu	chl.a		1.10	0.94	1.10	0.96	0.78	0.54
	phaeo.		0.54	0.14	0.00	0.00	0.00	0.05
	ratio		0.50	0.15	0.00	0.00	0.00	0.09
100µg/1 Cu	chl.a		1.20	0.67	0.73	0.90	0.96	0.72
	phaeo.		0.41	0.13	0.00	0.00	0.00	0.00
	ratio		0.34	0.19	0.00	0.00	0.00	0.00
100µg/1 Cu	chl.a		1.10	0.78	0.78	0.92	1.00	0.90
	phaeo.		0.27	0.16	0.01	0.01	0.06	0.00
	ratio		0.25	0.21	0.01	0.01	0.06	0.00
1000µg/1 Ni	chl.a		1.20	0.45	0.29	0.24	0.46	0.22
	phaeo.		0.31	0.21	0.13	0.11	0.08	0.03
	ratio		0.26	0.47	0.45	0.33	0.33	0.14
1000µg/1 Ni	chl.a		1.30	0.38	0.22	0.12	0.21	0.22
	phaeo.		0.27	0.22	0.16	0.14	0.06	0.04
	ratio		0.21	0.58	0.73	1.17	0.29	0.18

LABORATORY RUN 6
Birch Lake

Treatment	Day	initial	3	5	8	11	14	16
control	chl. a	3.00	2.10	1.70	1.40	2.40	2.00	1.80
	phaeo.	0.00	0.02	0.02	0.00	0.00	0.00	0.00
	ratio	0.00	0.01	0.01	0.00	0.00	0.00	0.00
control	chl. a	2.70	2.00	2.30	1.30	1.70	1.50	1.90
	phaeo.	0.00	0.04	0.00	0.00	0.00	0.00	0.00
	ratio	0.00	0.02	0.00	0.00	0.00	0.00	0.00
pH 5.0	chl. a		1.70	1.40	1.30	2.00	0.50	0.34
	phaeo.		0.11	0.02	0.00	0.00	0.02	0.05
	ratio		0.06	0.01	0.00	0.00	0.04	0.15
pH 5.0	chl. a		1.70	1.90	1.10	1.50	0.54	0.34
	phaeo.		0.13	0.00	0.11	0.00	0.02	0.05
	ratio		0.08	0.00	0.10	0.00	0.04	0.15
100 µg/l Cu	chl. a		1.10	0.76	0.45	0.54	0.99	0.87
	phaeo.		0.05	0.01	0.06	0.00	0.00	0.00
	ratio		0.05	0.01	0.13	0.00	0.00	0.00
100 µg/l Cu	chl. a		1.30	1.20	0.58	0.52	0.65	1.10
	phaeo.		0.11	0.02	0.02	0.11	0.01	0.00
	ratio		0.08	0.02	0.03	0.21	0.02	0.00
600 µg/l Ni	chl. a		1.10	0.72	0.36	0.16	0.17	0.18
	phaeo.		0.11	0.04	0.06	0.10	0.06	0.04
	ratio		0.10	0.06	0.17	0.63	0.35	0.22
600 µg/l Ni	chl. a		0.94	0.65	0.36	0.22	0.20	0.22
	phaeo.		0.14	0.14	0.06	0.06	0.06	0.06
	ratio		0.15	0.22	0.17	0.27	0.30	0.27
100 µg/l Cu + pH 5.0	chl. a		1.20	0.85	0.92	0.96	0.69	0.52
	phaeo.		0.18	0.04	0.00	0.00	0.00	0.00
	ratio		0.15	0.05	0.00	0.00	0.00	0.00
100 µg/l Cu + pH 5.0	chl. a		1.20	1.00	0.56	0.50	0.32	0.40
	phaeo.		0.18	0.18	0.03	0.01	0.15	0.00
	ratio		0.15	0.18	0.05	0.02	0.47	0.00

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Treatment \ Day		initial	3	5	8	11	14	16
600 µg/l Ni + pH 5.0	chl. a		1.20	0.81	0.40	0.35	0.21	0.22
	phaeo.		0.09	0.07	0.08	0.04	0.07	0.02
	ratio		0.08	0.09	0.20	0.11	0.33	0.09
600 µg/l Ni + pH 5.0	chl. a		0.94	0.60	0.31	0.25	0.19	0.21
	phaeo.		0.14	0.07	0.07	0.04	0.05	0.03
	ratio		0.15	0.12	0.23	0.16	0.26	0.14
100 µg/l Cu +600 µg/l Ni	chl. a		0.49	0.31	0.17	0.10	0.08	0.05
	phaeo.		0.20	0.17	0.11	0.12	0.07	0.07
	ratio		0.41	0.55	0.65	1.20	0.88	1.40
100 µg/l Cu +600 µg/l Ni	chl. a		0.45	0.36	0.13	0.08	0.05	0.03
	phaeo.		0.22	0.13	0.08	0.06	0.03	0.03
	ratio		0.49	0.36	0.62	0.75	0.60	1.00
100 µg/l Cu +600 µg/l Ni + pH 5.0	chl. a		0.65	0.49	0.21	0.12	0.06	0.07
	phaeo.		0.14	0.11	0.10	0.09	0.10	0.07
	ratio		0.22	0.22	0.48	0.75	1.70	1.00
100 µg/l Cu +600 µg/l Ni + pH 5.0	chl. a		0.54	0.40	0.14	0.08	0.05	0.04
	phaeo.		0.12	0.13	0.05	0.03	0.04	0.04
	ratio		0.22	0.33	0.36	0.38	0.80	1.00

LABORATORY RUN 7
Birch Lake

Treatment \ Day		3	5	7	9	12	14
control	chl. a	1.40	1.10	1.10	0.52	0.37	0.33
	phaeo.	0.09	0.00	0.05	0.01	0.00	0.04
	ratio	0.06	0.00	0.05	0.02	0.00	0.12
control	chl. a	1.70	1.20	2.40	0.54	0.49	0.41
	phaeo.	0.00	0.02	0.04	0.08	0.00	0.00
	ratio	0.00	0.02	0.02	0.15	0.00	0.00
pH 5.0	chl. a	1.40	1.10	0.94	0.60	0.49	0.52
	phaeo.	0.09	0.00	0.09	0.05	0.00	0.05
	ratio	0.06	0.00	0.10	0.08	0.00	0.10
pH 5.0	chl. a	1.10	0.58	1.50	0.58	0.96	0.64
	phaeo.	0.00	0.13	0.00	0.03	0.00	0.00
	ratio	0.00	0.22	0.00	0.05	0.00	0.00
100 µg/l Cu	chl. a	0.94	0.86	0.80	0.47	0.33	0.41
	phaeo.	0.14	0.00	0.13	0.10	0.06	0.03
	ratio	0.15	0.00	0.16	0.21	0.18	0.07
100 µg/l Cu	chl. a	1.10	1.00	1.30	0.63	0.45	0.43
	phaeo.	0.27	0.02	0.05	0.06	0.02	0.00
	ratio	0.25	0.02	0.04	0.10	0.04	0.00
100 µg/l Cu +pH 5.0	chl. a	1.20	0.94	0.86	0.40	0.32	0.32
	phaeo.	0.13	0.09	0.00	0.07	0.04	0.02
	ratio	0.11	0.10	0.00	0.18	0.13	0.06
100 µg/l Cu +pH 5.0	chl. a	1.20	0.94	0.86	0.45	0.35	0.25
	phaeo.	0.04	0.04	0.05	0.06	0.00	0.00
	ratio	0.03	0.04	0.06	0.13	0.00	0.00
400 µg/l Ni	chl. a	1.30	1.20	0.80	0.29	0.45	0.37
	phaeo.	0.11	0.00	0.13	0.13	0.03	0.06
	ratio	0.08	0.00	0.16	0.45	0.07	0.16

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Treatment \ Day		3	5	7	9	12	14
400 µg/l Ni	chl. *a*	1.40	0.86	0.72	0.40	0.27	0.37
	phaeo.	0.04	0.11	0.09	0.03	0.03	0.00
	ratio	0.03	0.13	0.13	0.08	0.11	0.00
400 µg/l Ni +pH 5.0	chl. *a*	1.20	0.86	0.86	0.45	0.30	0.29
	phaeo.	0.07	0.05	0.06	0.09	0.06	0.02
	ratio	0.06	0.06	0.07	0.20	0.20	0.07
400 µg/l Ni +pH 5.0	chl. *a*	0.94	0.58	0.42	0.29	0.24	0.19
	phaeo.	0.09	0.14	0.00	0.09	0.02	0.04
	ratio	0.10	0.24	0.00	0.31	0.08	0.21
100 µg/l Cu +400 µg/l Ni	chl. *a*	0.86	0.45	0.27	0.15	0.30	0.29
	phaeo.	0.16	0.11	0.17	0.12	0.07	0.05
	ratio	0.19	0.24	0.63	0.80	0.23	0.17
100 µg/l Cu +400 µg/l Ni	chl. *a*	0.86	0.72	0.52	0.29	0.14	0.08
	phaeo.	0.16	0.04	0.11	0.07	0.07	0.10
	ratio	0.19	0.06	0.21	0.24	0.50	1.25
100 µg/l Cu +400 µg/l Ni +pH 5.0	chl. *a*	0.86	0.81	0.45	0.24	0.24	0.22
	phaeo.	0.16	0.12	0.04	0.07	0.04	0.03
	ratio	0.19	0.15	0.09	0.29	0.17	0.14
100 µg/l Cu +400 µg/l Ni +pH 5.0	chl. *a*	0.86	0.90	0.63	0.29	0.14	0.14
	phaeo.	0.16	0.06	0.00	0.06	0.05	0.02
	ratio	0.19	0.07	0.00	0.21	0.36	0.14

FIELD RUN 1
Birch Lake

Treatment	Day	initial	2	4	7
*control	chl.a		3.60	7.90	9.70
	phaeo.		0.50	0.47	0.00
	ratio		0.14	0.06	0.00
control	chl.a		2.70	3.40	4.20
	phaeo.		0.54	0.56	0.61
	ratio		0.20	0.02	0.15
*50µg/l Cu	chl.a		4.40	6.20	6.40
	phaeo.		0.50	0.56	0.46
	ratio		0.11	0.09	0.07
50µg/l Cu	chl.a		1.90	4,60	-
	phaeo.		0.58	0.20	-
	ratio		0.31	0,04	-
100µg/l Cu	chl.a		1.40	1.60	1.90
	phaeo.		0.40	0.75	0.56
	ratio		0.31	0.47	0.29
100µg/l Cu	chl.a		2.60	4.30	6.70
	phaeo.		0.54	0.00	0.00
	ratio		0.21	0.00	0.00
*200µg/l Cu	chl.a		1.60	1.80	1.60
	phaeo.		0.47	1.70	0.79
	ratio		0.29	0.94	0.49
200µg/l Cu	chl.a		1.40	1.30	1.10
	phaeo.		0.36	0.84	0.32
	ratio		0.29	0.65	0.29
LAKE	chl.a	-	6.00	8.80	7.00
	phaeo.	-	0.53	0.80	0.32
	ratio	-	0.09	0.09	0.05

* intact bags

FIELD RUN 1
Greenwood Lake

Treatment	Day	initial	2	5
*control	chl.a		3.20	9.00
	phaeo.		0.25	0.00
	ratio		0.08	0.00
control	chl.a		2.30	8.30
	phaeo.		0.29	0.00
	ratio		0.13	0.00
50µg/1 Cu	chl.a		3.50	10.50
	phaeo.		0.58	0.00
	ratio		0.17	0.00
50µg/1 Cu	chl.a		2.00	10.80
	phaeo.		0.50	0.00
	ratio		0.25	0.00
*100µg/1 Cu	chl.a		2.50	5.20
	phaeo.		0.58	0.79
	ratio		0.23	0.15
100µg/1 Cu	chl.a		3.80	4.70
	phaeo.		0.29	0.07
	ratio		0.08	0.01
*200µg/1 Cu	chl.a		1.60	3.60
	phaeo.		0.83	0.32
	ratio		0.52	0.09
200µg/1 Cu	chl.a		2.30	6.20
	phaeo.		0.29	0.29
	ratio		0.13	0.05
LAKE	chl.a	2.80	3.20	6.80
	phaeo.	-	0.43	0.00
	ratio	-	0.13	0.00

* intact bags

FIELD RUN 2
Birch Lake

Treatment	Day	initial	2	4	6	9	11	13	16
control	chl.a		11.90	6.30	6.80	7.50	6.50	4.20	5.60
	phaeo.		0.00	1.10	1.10	0.93	0.00	0.23	-
	ratio		0.00	0.17	0.16	0.12	0.00	0.06	-
control	chl.a		12.80	15.20	6.80	4.20	6.50	5.10	7.00
	phaeo.		1.70	0.58	1.50	0.70	0.00	0.47	-
	ratio		0.13	0.04	0.22	0.17	0.00	0.09	-
20µg/l Cu	chl.a		7.00	7.90	12.80	8.90	10.70	14.50	8.90
	phaeo.		1.10	0.82	1.20	0.00	0.00	0.00	-
	ratio		0.16	0.10	0.09	0.00	0.00	0.00	-
20µg/l Cu	chl.a		4.90	8.20	7.70	10.30	14.90	7.90	6.50
	phaeo.		0.88	0.76	1.20	1.50	0.58	0.00	-
	ratio		0.18	0.09	0.16	0.15	0.04	0.00	-
50µg/l Cu	chl.a		7.50	8.40	9.30	9.80	11.70	7.50	6.50
	phaeo.		1.30	0.70	1.20	1.10	1.60	0.70	-
	ratio		0.17	0.08	0.13	0.11	0.14	0.09	-
50µg/l Cu	chl.a		6.50	7.50	9.10	7.50	7.00	12.60	7.00
	phaeo.		0.82	0.23	1.40	0.23	0.35	0.18	-
	ratio		0.13	0.03	0.15	0.03	0.05	0.01	-
100µg/l Cu	chl.a		4.70	4.40	4.70	4.70	5.60	14.00	9.30
	phaeo.		0.58	0.47	1.30	0.18	0.35	0.35	-
	ratio		0.12	0.11	0.28	0.04	0.06	0.03	-
100µg/l Cu	chl.a		4.20	4.40	16.30	6.50	5.60	10.30	5.10
	phaeo.		0.53	0.29	0.00	1.10	0.70	0.82	-
	ratio		0.13	0.07	0.00	0.17	0.13	0.08	-
LAKE	chl.a	16.60	18.70	18.20	4.00	13.50	14.90	14.90	-
	phaeo.	0.00	0.00	1.75	0.23	0.00	0.00	0.00	-
	ratio	0.00	0.00	0.10	0.06	0.00	0.00	0.00	-

FIELD RUN 2
Greenwood Lake

Treatment	Day	initial	1	3	5	7	10	12	14	16
control	chl.a		10.50	14.50	9.60	12.10	13.50	10.70	10.70	10.70
	phaeo.		0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ratio		0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
control	chl.a		14.90	13.30	11.20	10.30	10.70	8.90	8.90	7.50
	phaeo.		0.00	1.90	0.00	0.58	0.00	0.23	0.90	-
	ratio		0.00	0.14	0.00	0.06	0.00	0.03	0.10	-
20µg/l Cu	chl.a		11.70	11.40	10.70	7.00	7.90	8.90	9.30	13.10
	phaeo.		0.58	0.47	0.29	0.70	0.47	0.23	0.12	-
	ratio		0.05	0.04	0.03	0.10	0.06	0.03	0.01	-
20µg/l Cu	chl.a		9.60	11.70	11.70	10.30	10.30	8.40	9.30	7.00
	phaeo.		0.76	0.93	0.06	0.76	0.00	0.35	0.12	-
	ratio		0.08	0.01	0.01	0.07	0.00	0.04	0.01	-
50µg/l Cu	chl.a		10.30	13.30	11.40	11.40	14.50	11.70	9.30	14.90
	phaeo.		0.58	0.88	0.64	0.99	0.00	0.00	0.12	-
	ratio		0.06	0.07	0.06	0.09	0.00	0.00	0.01	-
50µg/l Cu	chl.a		9.80	11.20	6.50	11.70	9.80	8.90	10.70	7.50
	phaeo.		0.70	0.35	0.29	0.58	0.00	0.23	0.47	-
	ratio		0.07	0.03	0.04	0.05	0.00	0.03	0.04	-
100µg/l Cu	chl.a		9.80	6.10	6.30	3.70	3.50	8.40	13.50	9.80
	phaeo.		0.70	0.93	1.20	1.50	0.82	1.10	0.82	-
	ratio		0.07	0.15	0.19	0.41	0.23	0.13	0.06	-
100µg/l Cu	chl.a		7.90	6.50	4.20	3.70	4.40	8.90	12.10	7.50
	phaeo.		0.82	1.20	0.88	1.90	0.82	0.23	1.20	-
	ratio		0.10	0.18	0.21	0.51	0.19	0.03	0.10	-
LAKE	chl.a	11.20	10.70	13.30	10.10	12.40	9.80	9.80	6.50	-
	phaeo.	0.35	0.48	0.00	0.00	0.00	0.35	0.00	1.20	
	ratio	0.03	0.04	0.00	0.00	0.00	0.04	0.00	0.18	-

FIELD RUN 3
Birch Lake

Treatment	Day	initial	2	5	7	9	12	14	16
control	chl.a		7.20	7.10	8.20	8.00	9.30	11.30	10.70
	phaeo.		0.54	1.72	1.88	0.10	0.24	0.58	0.00
	ratio		0.08	0.24	0.23	0.01	0.03	0.05	0.00
control	chl.a		7.40	6.90	9.30	8.00	8.30	9.20	8.90
	phaeo.		0.52	2.46	2.58	0.00	1.60	1.78	0.00
	ratio		0.07	0.36	0.28	0.00	0.19	0.19	0.00
100µg/l Ni	chl.a		7.40	7.30	9.90	8.90	8.60	8.88	11.90
	phaeo.		0.34	2.06	1.62	1.90	2.56	2.00	0.16
	ratio		0.03	0.28	0.16	0.21	0.30	0.23	0.01
100µg/l Ni	chl.a		7.80	7.10	10.20	10.20	8.80	8.60	8.10
	phaeo.		0.84	2.98	3.12	2.04	2.18	2.38	0.54
	ratio		0.11	0.42	0.31	0.20	0.25	0.28	0.07
400µg/l Ni	chl.a		6.90	7.60	9.30	9.70	7.90	7.70	6.50
	phaeo.		0.00	1.04	1.68	0.92	2.28	2.02	1.06
	ratio		0.00	0.14	0.23	0.09	0.29	0.26	0.16
400µg/l Ni	chl.a		7.90	7.80	8.10	8.60	7.10	7.00	6.30
	phaeo.		0.20	1.38	2.34	1.12	2.80	2.54	0.72
	ratio		0.03	0.18	0.29	0.13	0.39	0.36	0.11
1000µg/l Ni	chl.a		8.20	6.40	5.50	5.00	5.20	5.30	5.30
	phaeo.		0.00	0.80	1.52	0.58	1.40	1.36	0.46
	ratio		0.00	0.13	0.28	0.12	0.27	0.26	0.09
1000µg/l Ni	chl.a		7.70	6.90	5.70	4.80	3.80	4.30	4.90
	phaeo.		0.40	1.74	1.50	0.60	0.88	2.00	1.40
	ratio		0.05	0.25	0.26	0.13	0.23	0.47	0.29
LAKE	chl.a	7.70	10.50	11.20	14.10	21.60	18.60	17.10	18.00
	phaeo.	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00
	ratio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FIELD RUN 3
Greenwood Lake

Treatment	Day	initial	2	4	7	9	11	14	16	18
control	chl.a		12.00	10.60	13.40	14.60	14.40	9.60	6.50	6.10
	phaeo.		0.00	0.92	1.08	2.04	1.26	3.72	3.22	2.18
	ratio		0.00	0.09	0.08	0.14	0.09	0.39	0.50	0.34
control	chl.a		13.00	9.20	13.20	13.20	12.60	8.50	6.50	6.30
	phaeo.		1.30	1.24	1.38	3.00	3.60	3.92	3.40	1.62
	ratio		0.10	0.13	0.10	0.23	0.29	0.46	0.52	0.26
100µg/l Ni	chl.a		9.60	10.04	13.40	14.40	13.80	9.00	7.60	5.50
	phaeo.		0.40	0.50	1.08	2.34	2.94	3.60	2.66	2.24
	ratio		0.04	0.05	0.08	0.16	0.21	0.40	0.35	0.41
100µg/l Ni	chl.a		13.00	9.80	12.80	12.40	12.40	9.70	7.60	5.90
	phaeo.		0.84	1.36	2.76	2.82	2.82	4.88	4.10	2.92
	ratio		0.06	0.14	0.22	0.23	0.23	0.50	0.54	0.49
400µg/l Ni	chl.a		10.60	10.40	10.80	13.20	13.40	10.70	9.00	7.10
	phaeo.		0.30	0.71	1.08	1.92	2.16	3.34	2.34	2.26
	ratio		0.03	0.07	0.10	0.15	0.16	0.31	0.26	0.32
400µg/l Ni	chl.a		9.80	10.00	10.80	11.00	10.40	10.30	8.40	7.00
	phaeo.		0.70	0.52	2.16	2.40	3.00	3.56	3.66	2.36
	ratio		0.07	0.05	0.20	0.22	0.29	0.35	0.44	0.34
1000µg/l Ni	chl.a		11.00	10.40	10.20	8.60	8.80	7.00	5.90	5.30
	phaeo.		0.00	0.00	0.60	2.20	2.72	3.08	3.10	1.90
	ratio		0.00	0.00	0.06	0.26	0.31	0.44	0.53	0.36
1000µg/l Ni	chl.a		10.60	11.20	9.00	8.00	8.00	6.20	5.80	4.10
	phaeo.		0.00	0.48	1.80	1.72	4.34	3.88	3.20	1.30
	ratio		0.00	0.04	0.20	0.22	0.54	0.63	0.55	0.32
LAKE	chl.a	7.60	11.60	15.60	11.20	18.00	9.60	8.00	7.60	6.70
	phaeo.	-	0.00	0.00	0.00	0.00	2.28	2.62	1.58	2.84
	ratio	-	0.00	0.00	0.00	0.00	0.24	0.33	0.21	0.42

FIELD RUN 4
Birch Lake

Treatment	Day	initial	2	5	7	9	12	14	16	19	21
control	chl.a		11.80	8.00	8.50	10.30	13.30	13.80	12.60	12.30	13.50
	phaeo.		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ratio		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
control	chl.a		10.00	7.50	8.50	10.80	12.90	12.70	12.30	13.20	11.70
	phaeo.		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ratio		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50µg/l Cu	chl.a		6.50	5.40	6.30	6.30	6.90	7.50	6.90	11.50	12.60
	phaeo.		0.52	0.81	0.45	0.18	0.66	0.00	0.30	0.00	0.00
	ratio		0.08	0.15	0.07	0.03	0.10	0.00	0.04	0.00	0.00
50µg/l Cu	chl.a		6.10	4.40	5.30	6.40	7.50	8.20	9.00	12.70	12.00
	phaeo.		1.14	1.05	1.23	0.00	0.60	0.00	0.18	0.00	0.00
	ratio		0.19	0.24	0.23	0.00	0.08	0.00	0.02	0.00	0.00
100µg/l Cu	chl.a		5.50	3.90	4.00	5.50	6.60	6.10	6.10	11.50	10.50
	phaeo.		0.26	0.96	0.81	0.00	0.69	0.33	0.42	0.00	0.00
	ratio		0.05	0.25	0.20	0.00	0.10	0.05	0.07	0.00	0.00
100µg/l Cu	chl.a		5.20	3.80	4.00	5.40	7.50	6.90	7.30	10.50	10.50
	phaeo.		0.56	1.11	1.35	0.00	0.33	0.39	0.48	0.00	0.30
	ratio		0.11	0.29	0.34	0.00	0.04	0.06	0.07	0.00	0.03
800µg/l Ni	chl.a		10.10	6.00	6.00	7.50	8.40	8.60	8.20	7.50	8.10
	phaeo.		0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.06	0.00
	ratio		0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.00
800µg/l Ni	chl.a		9.50	6.20	5.70	7.00	8.00	8.20	7.20	7.50	7.50
	phaeo.		0.00	0.33	0.24	0.00	0.00	0.00	0.00	0.06	0.06
	ratio		0.00	0.05	0.04	0.00	0.00	0.00	0.00	0.01	0.01
100µg/l Cu + 800µg/l Ni	chl.a		4.20	1.70	2.10	4.00	4.30	4.80	4.50	4.50	4.90
	phaeo.		1.20	0.78	1.14	0.00	0.00	0.00	0.00	0.00	0.14
	ratio		0.29	0.46	0.54	0.00	0.00	0.00	0.00	0.00	0.03
100µg/l Cu + 800µg/l Ni	chl.a		4.30	1.70	2.10	3.60	3.60	5.20	4.20	4.10	4.90
	phaeo.		0.56	0.51	1.14	0.00	0.00	0.00	0.00	0.04	0.00
	ratio		0.13	0.33	0.54	0.00	0.00	0.00	0.00	0.01	0.00
LAKE	chl.a	18.00	17.10	14.60	16.20	19.80	16.80	17.70	16.30	18.90	18.30
	phaeo.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ratio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FIELD RUN 4
Greenwood Lake

Treatment	Day	initial	3	5	7	10	12	14	16	19	21
control	chl.a		4.80	5.40	6.00	7.10	6.90	4.10	6.80	6.30	8.00
	phaeo.		1.29	1.62	1.02	2.13	2.82	1.89	1.62	1.26	3.66
	ratio		0.27	0.30	0.17	0.30	0.41	0.46	0.24	0.20	0.46
control	chl.a		6.00	6.80	7.10	7.50	8.40	5.90	7.80	8.10	8.40
	phaeo.		1.29	1.08	1.05	2.22	2.94	1.71	2.46	1.08	2.94
	ratio		0.22	0.16	0.15	0.30	0.35	0.29	0.32	0.13	0.35
50µg/l Cu	chl.a		7.40	9.20	9.80	12.80	13.80	11.60	11.00	10.50	11.70
	phaeo.		1.02	0.84	1.05	1.83	2.94	1.41	1.74	1.38	3.69
	ratio		0.14	0.09	0.11	0.14	0.21	0.12	0.16	0.13	0.32
50µg/l Cu	chl.a		7.10	9.80	10.40	12.80	13.40	12.00	11.60	10.80	12.00
	phaeo.		1.32	0.78	1.26	2.64	2.58	2.04	1.14	1.08	3.39
	ratio		0.19	0.08	0.12	0.21	0.19	0.17	0.10	0.10	0.28
100µg/l Cu	chl.a		7.40	8.60	9.80	10.80	12.00	10.50	10.70	10.50	11.00
	phaeo.		1.29	1.44	1.05	3.78	3.12	2.46	2.85	3.00	4.44
	ratio		0.17	0.17	0.11	0.35	0.26	0.23	0.27	0.29	0.40
100µg/l Cu	chl.a		6.90	8.40	9.80	11.70	11.40	9.90	9.90	9.00	10.50
	phaeo.		0.93	0 .78	1.32	3.42	3.18	2.52	3.05	1.80	3.54
	ratio		0.13	0 .09	0.13	0.29	0.28	0.25	0.31	0.21	0.34
800µg/l Ni	chl.a		3.90	4.10	4.70	5.70	6.30	5.90	6.00	5.80	7.80
	phaeo.		0.92	0.81	0.21	1.32	1.26	0.24	1.02	3.66	2.46
	ratio		0.24	0.20	0.04	0.23	0.20	0.04	0.17	0.63	0.32
800µg/l Ni	chl.a		3.70	3.80	4.50	5.70	6.20	6.00	6.50	7.20	8.70
	phaeo.		0.98	1.11	0.90	1.32	1.14	2.10	1.92	1.22	2.64
	ratio		0.26	0.29	0.20	0.23	0.18	0.35	0.30	0.17	0.30
100µg/l Cu + 800µg/l Ni	chl.a		4.00	3.90	4.20	5.40	5.40	5.40	5.90	5.50	7.40
	phaeo.		1.18	1.50	0.66	1.08	1.35	1.08	1.98	1.34	2.64
	ratio		0.30	0.38	0.16	0.20	0.25	0.20	0.34	0.24	0.36
100µg/l Cu + 800µg/l Ni	chl.a		4.00	4.20	4.40	5.40	5.60	4.80	5.70	5.10	6.50
	phaeo.		0.95	1.20	1.05	1.62	0.93	0.87	1.32	1.56	2.46
	ratio		0.24	0.29	0.24	0.30	0.17	0.18	0.23	0.31	0.38
LAKE	chl.a	7.80	7.50	6.90	6.80	7.70	7.50	8.70	5.60	8.40	8.10
	phaeo.	-	0.33	0.12	0.54	1.26	1.14	0.00	0.66	0.24	5.40
	ratio	-	0.04	0.02	0.08	0.16	0.15	0.00	0.12	0.03	0.67

FIELD RUN 5
Greenwood Lake

Treatment	Day	initial	2	4	7	9	11
control	chl.a		2.40	2.10	2.00	2.00	2.10
	phaeo.		0.24	0.12	0.08	0.20	0.12
	ratio		0.10	0.95	0.04	0.10	0.06
control	chl.a		2.30	2.10	1.90	2.00	2.00
	phaeo.		0.20	0.12	0.28	0.08	0.32
	ratio		0.09	0.06	0.15	0.04	0.16
large zooplankton removed	chl.a		3.00	2.90	2.90	3.10	lost
	phaeo.		0.24	0.00	0.00	0.00	-
	ratio		0.08	0.00	0.00	0.00	-
extra zooplankton	chl.a		2.50	2.10	1.80	2.20	2.30
	phaeo.		0.28	0.24	0.12	0.16	0.20
	ratio		0.11	0.11	0.07	0.07	0.09
large zooplankton removed	chl.a		3.10	3.00	3.60	3.50	3.70
	phaeo.		0.16	0.00	0.00	0.00	0.00
	ratio		0.05	0.00	0.00	0.00	0.00
extra zooplankton	chl.a		2.70	2.20	1.80	2.10	2.00
	phaeo.		0.28	0.16	0.12	0.12	0.08
	ratio		0.10	0.07	0.07	0.06	0.04
50µg/1 Cu	chl.a		2.60	2.30	-	-	-
	phaeo.		0.32	.20	-	-	-
	ratio		0.12	0.09	-	-	-
50µg/1 Cu	chl.a		2.40	2.40	*1.80	*1.80	*1.70
	phaeo.		0.24	0.00	0.24	0.12	0.20
	ratio		0.10	0.00	0.13	0.07	0.12
zooplankton removed +50µg/1 Cu	chl.a		3.30	4.70	4.30	4.70	4.40
	phaeo.		0.00	0.00	0.00	0.00	0.00
	ratio		0.00	0.00	0.00	0.00	0.00
zooplankton added +50µg/1 Cu	chl.a		2.30	2.90	*1.80	*1.90	*1.80
	phaeo.		0.20	0.00	0.24	0.16	0.36
	ratio		0.09	0.00	0.13	0.08	0.20

continued on next page

FIELD RUN 5
Greenwood Lake

Treatment	Day	initial	2	4	7	9	11
zooplankton removed +50µg/l Cu	chl.a		3.30	4.60	4.80	4.70	4.60
	phaeo.		0.00	0.00	0.00	0.00	0.00
	ratio		0.00	0.00	0.00	0.00	0.00
zooplankton added +50µg/l Cu	chl.a		4.50	3.30	2.90	2.70	2.30
	phaeo.		0.04	0.00	0.00	0.00	0.08
	ratio		0.01	0.00	0.00	0.00	0.03
LAKE	chl.a		4.90	1.60	2.40	2.40	3.20
	phaeo.		0.00	0.12	0.00	0.00	0.00
	ratio		0.00	0.08	0.00	0.00	0.00

* bottle moved

Figure 1. Study Lakes and Enclosure Sites.

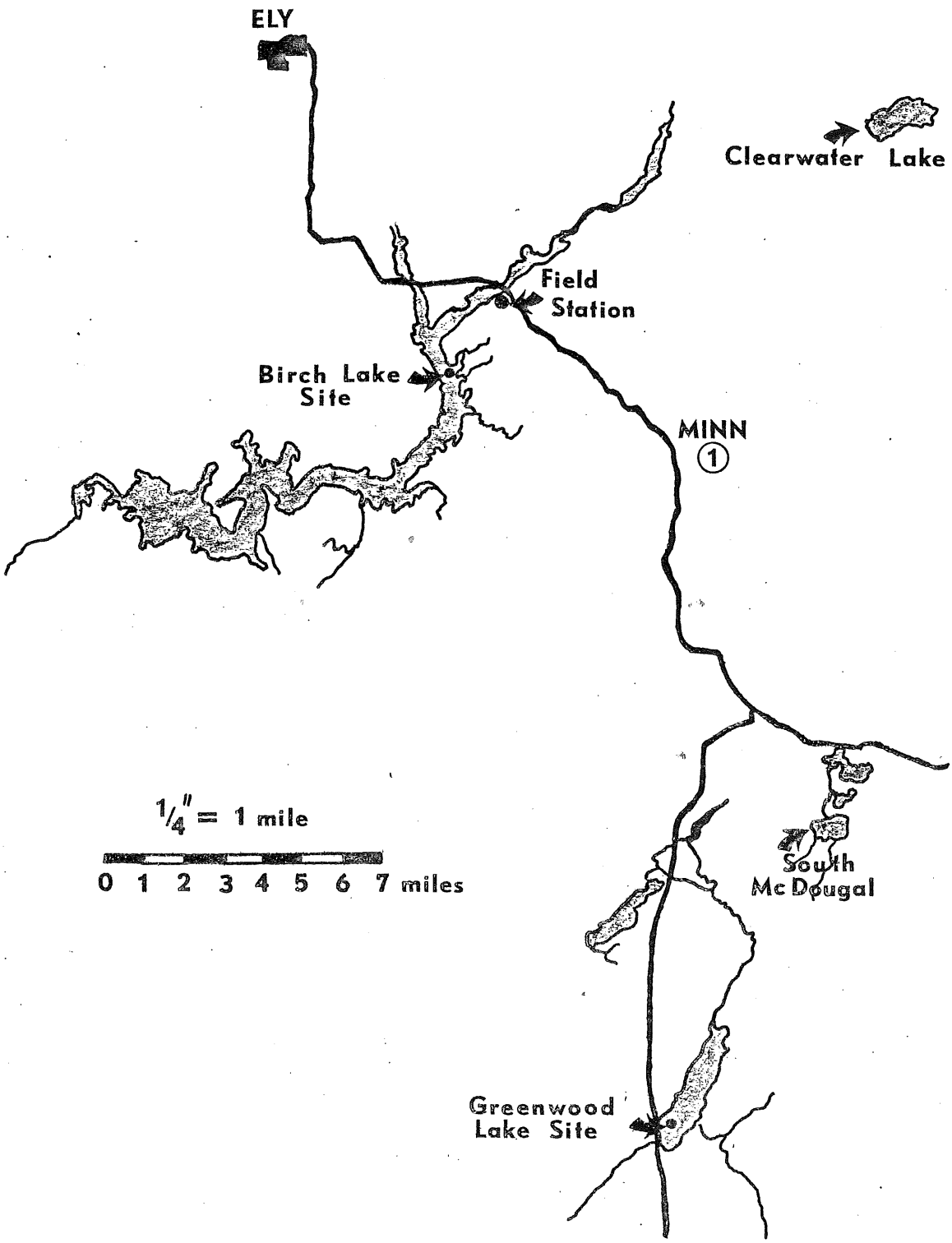

Figure 2. *In situ* Enclosure System.

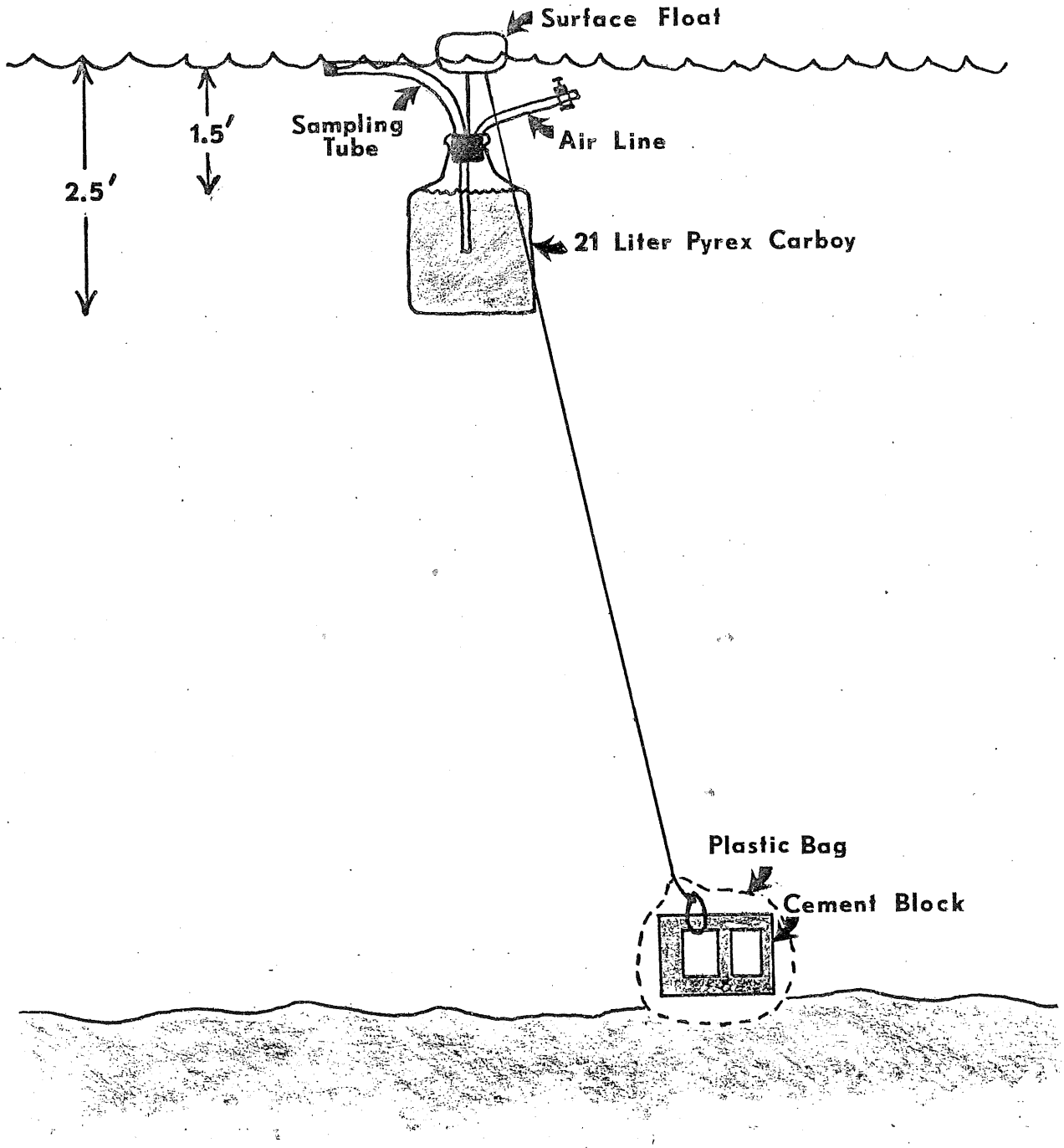

Figure 3. Detail of Enclosure Bottle.

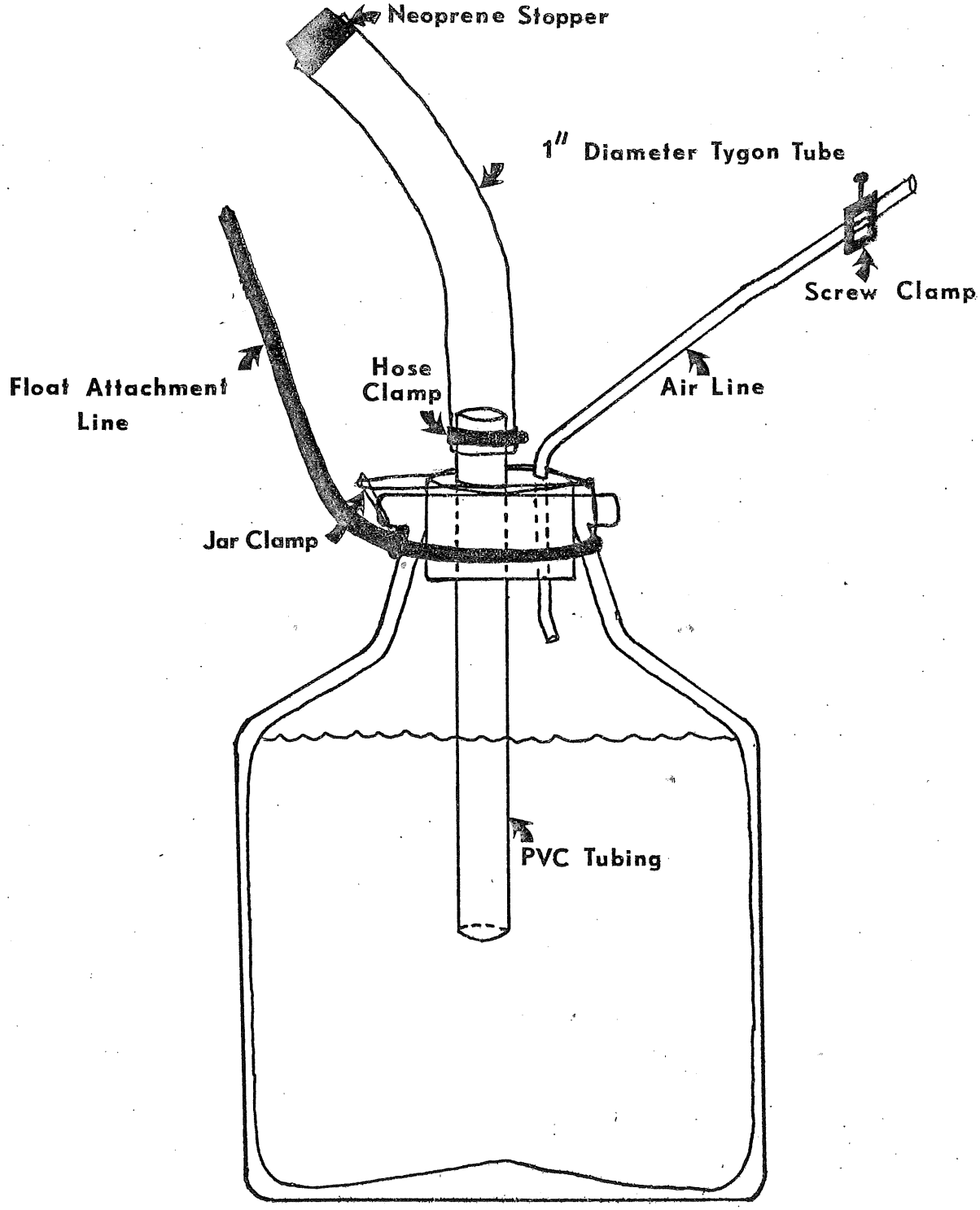

Figure 4. Chlorophyll a, Field Run 1, Birch Lake.

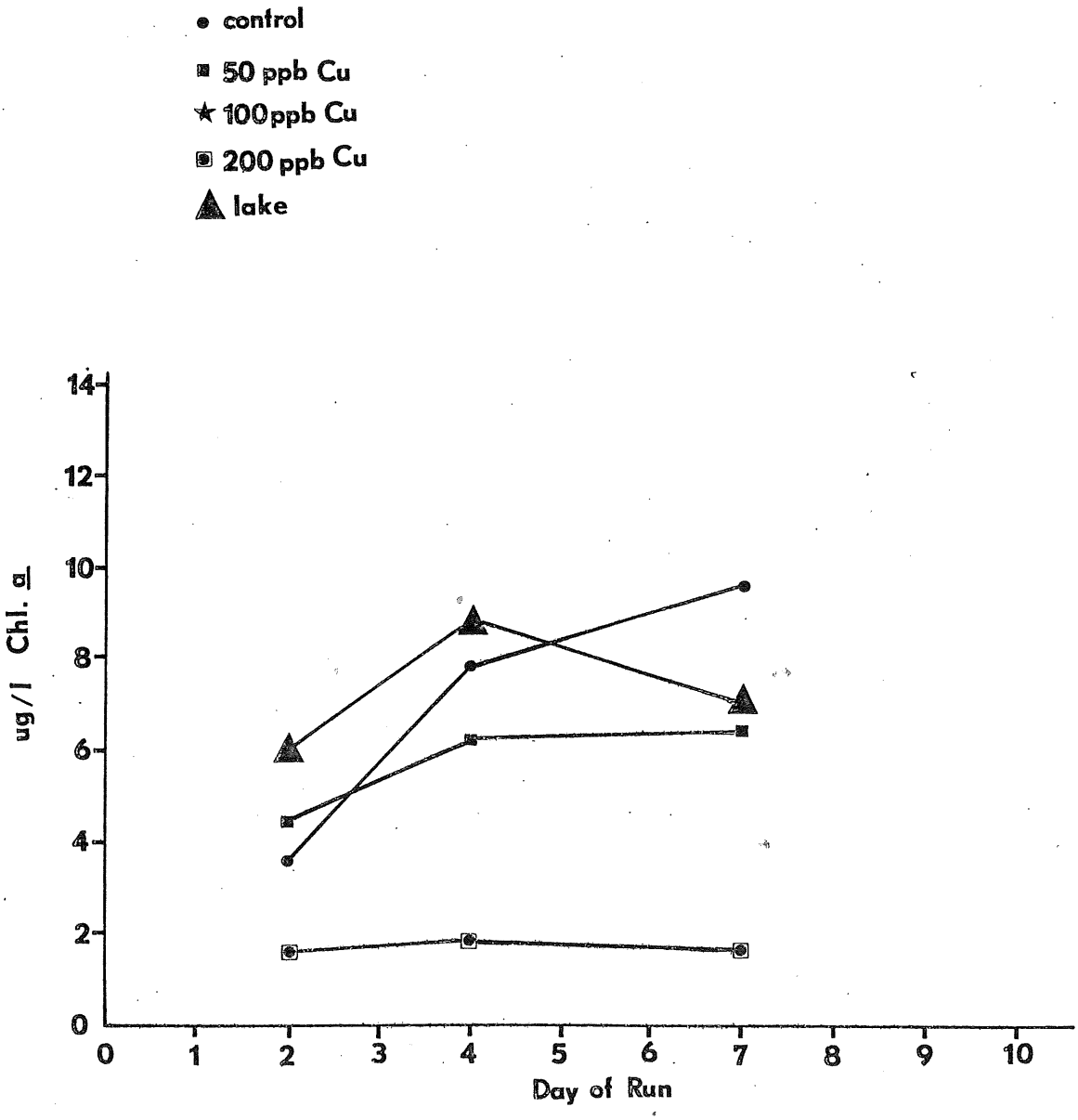

Figure 5. Chlorophyll a, Field Run 1, Greenwood Lake.

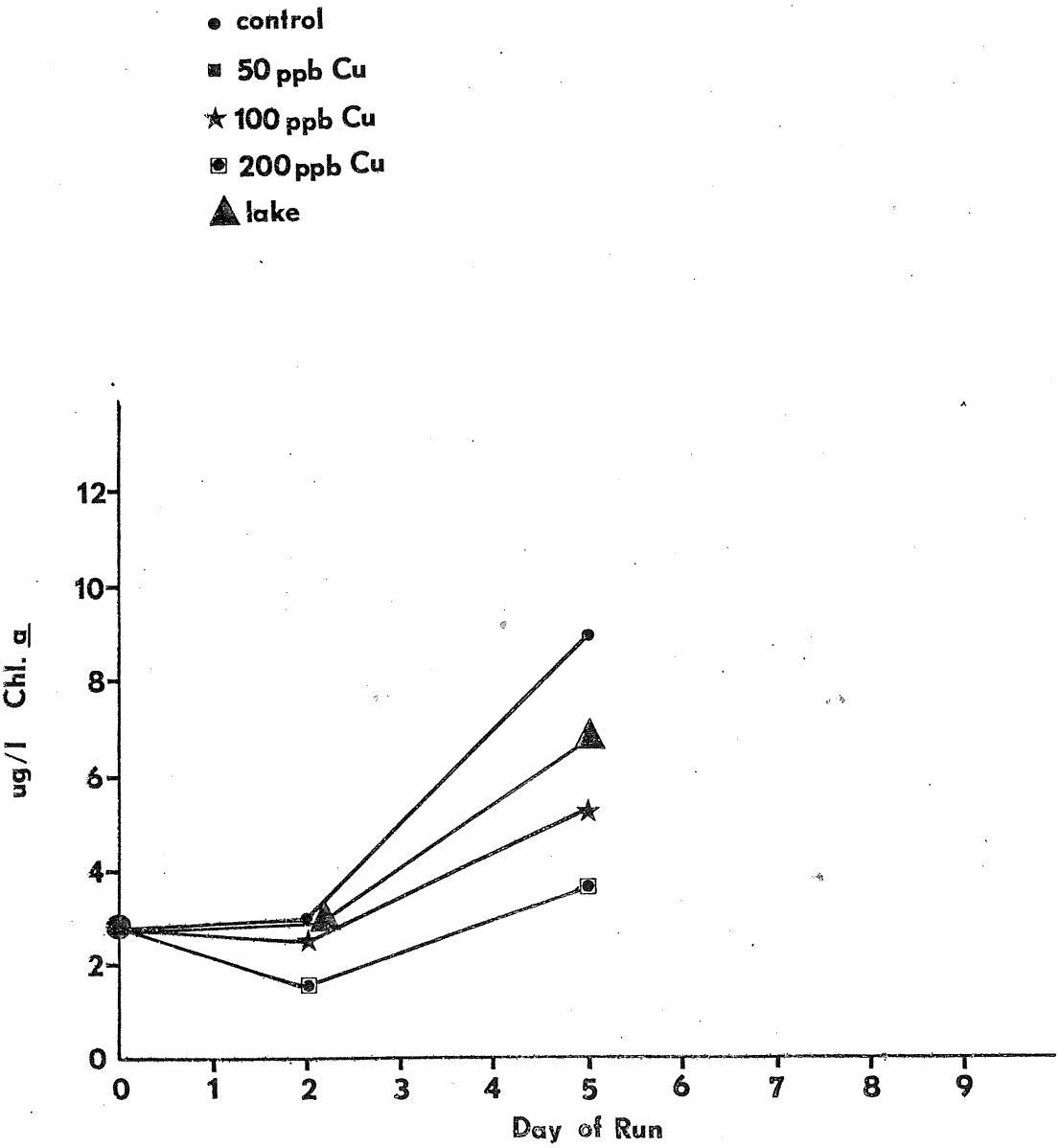

Figure 6. Chlorophyll a, Continuous culture Run 1, Birch Lake.

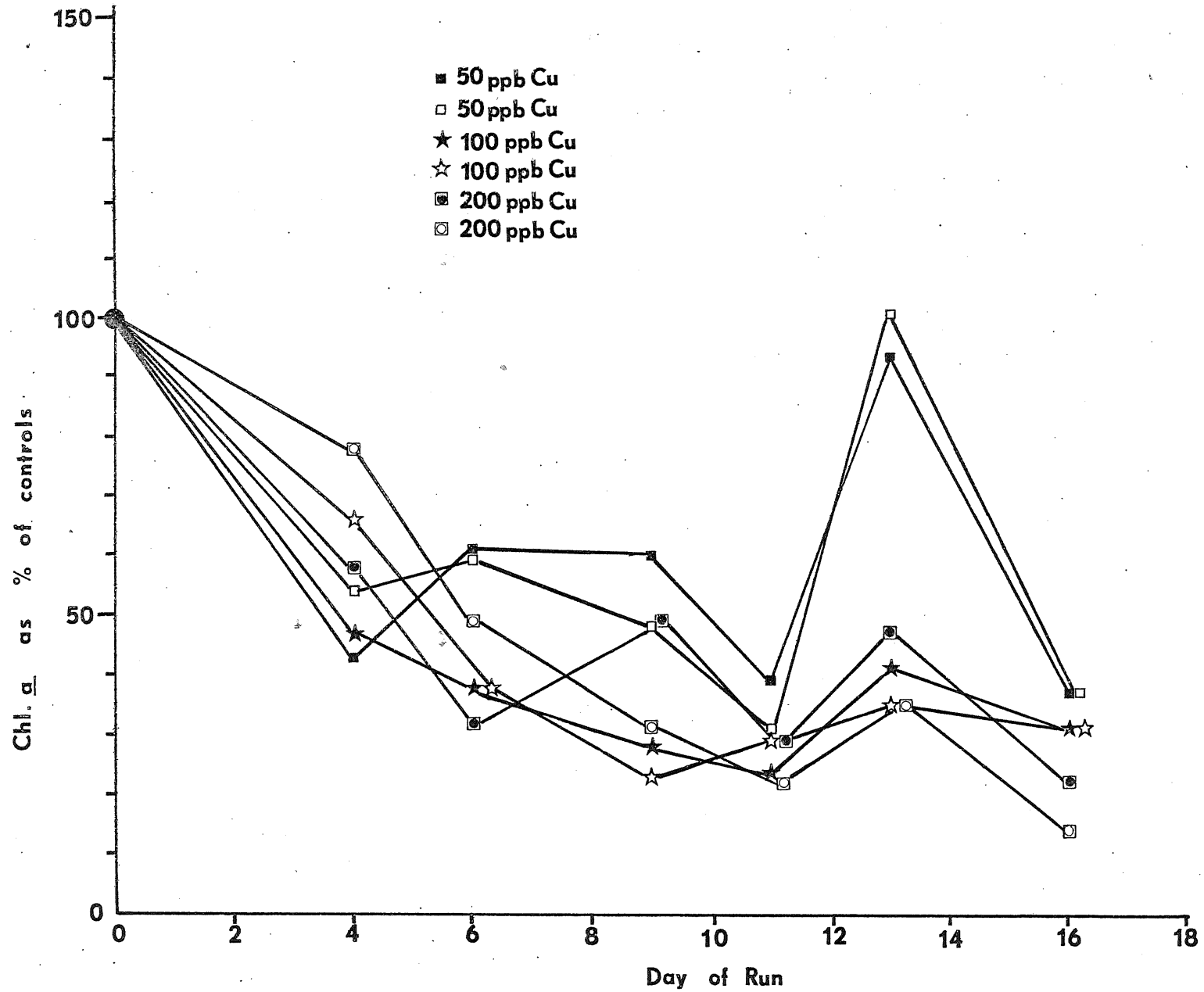

Figure 7. Chlorophyll a, Continuous culture Run 1, Greenwood Lake.

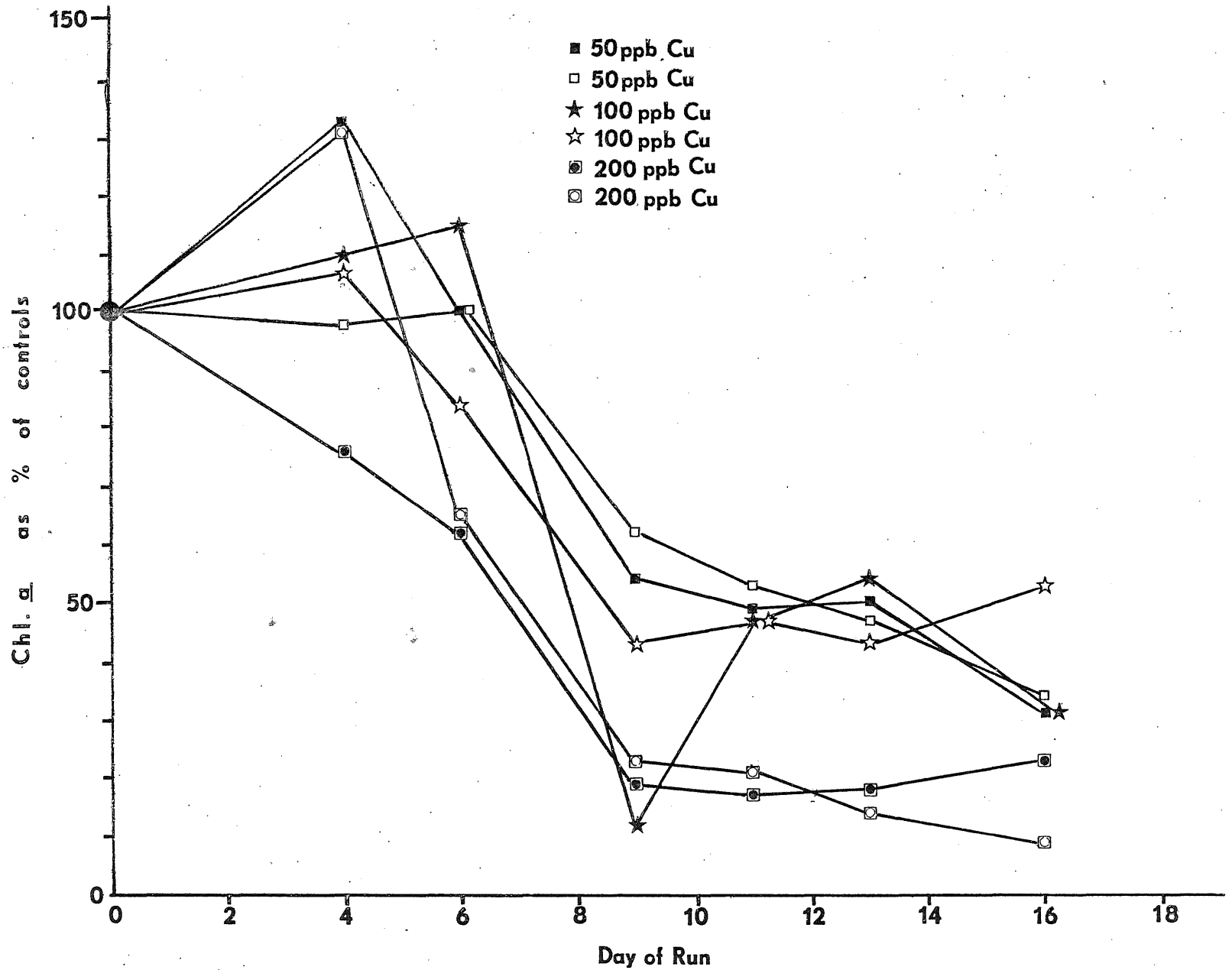

Figure 8. Chlorophyll a, Field Run 2, Birch Lake.

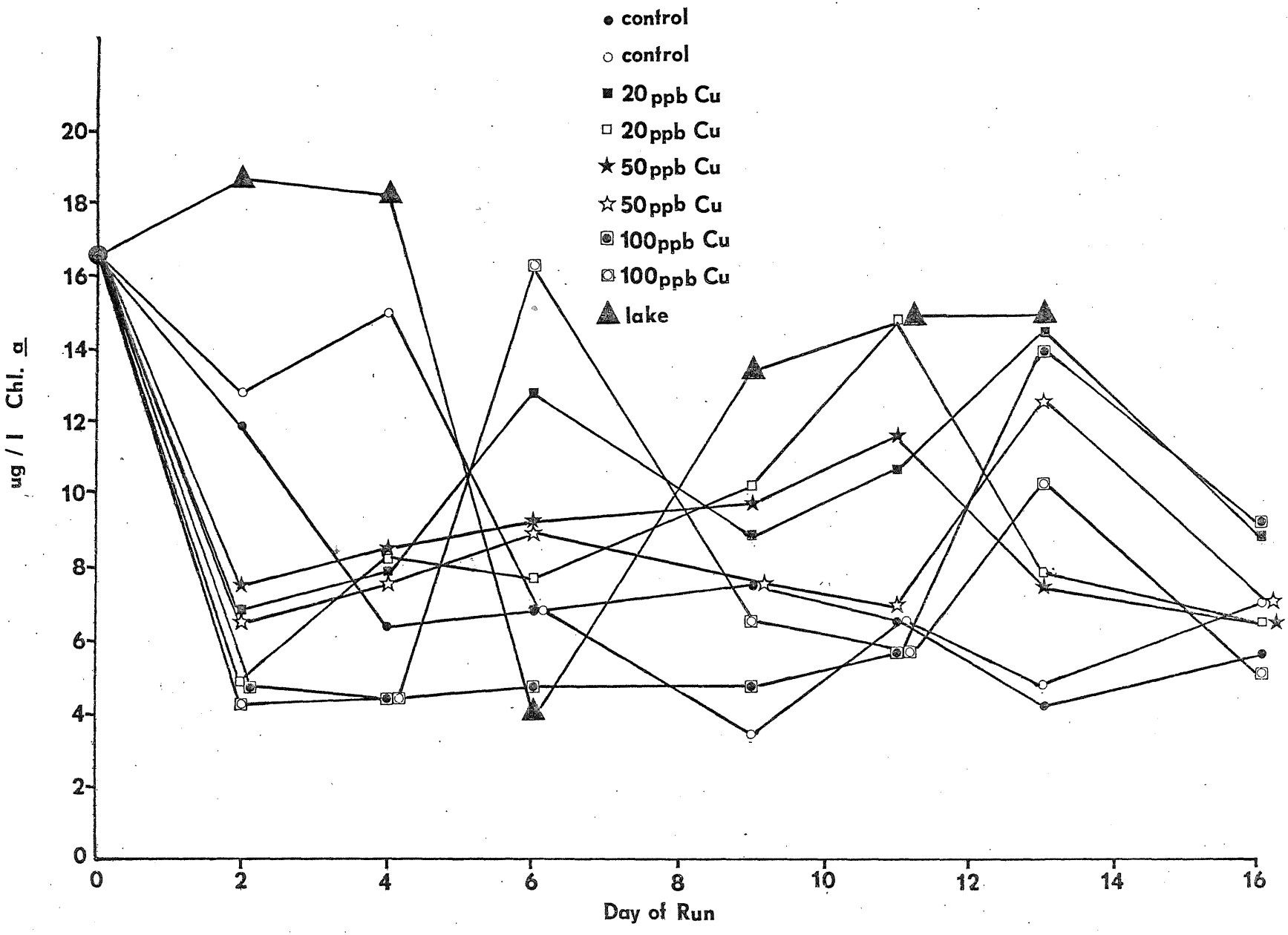

Figure 9. Chlorophyll a, Field Run 2, Greenwood Lake.

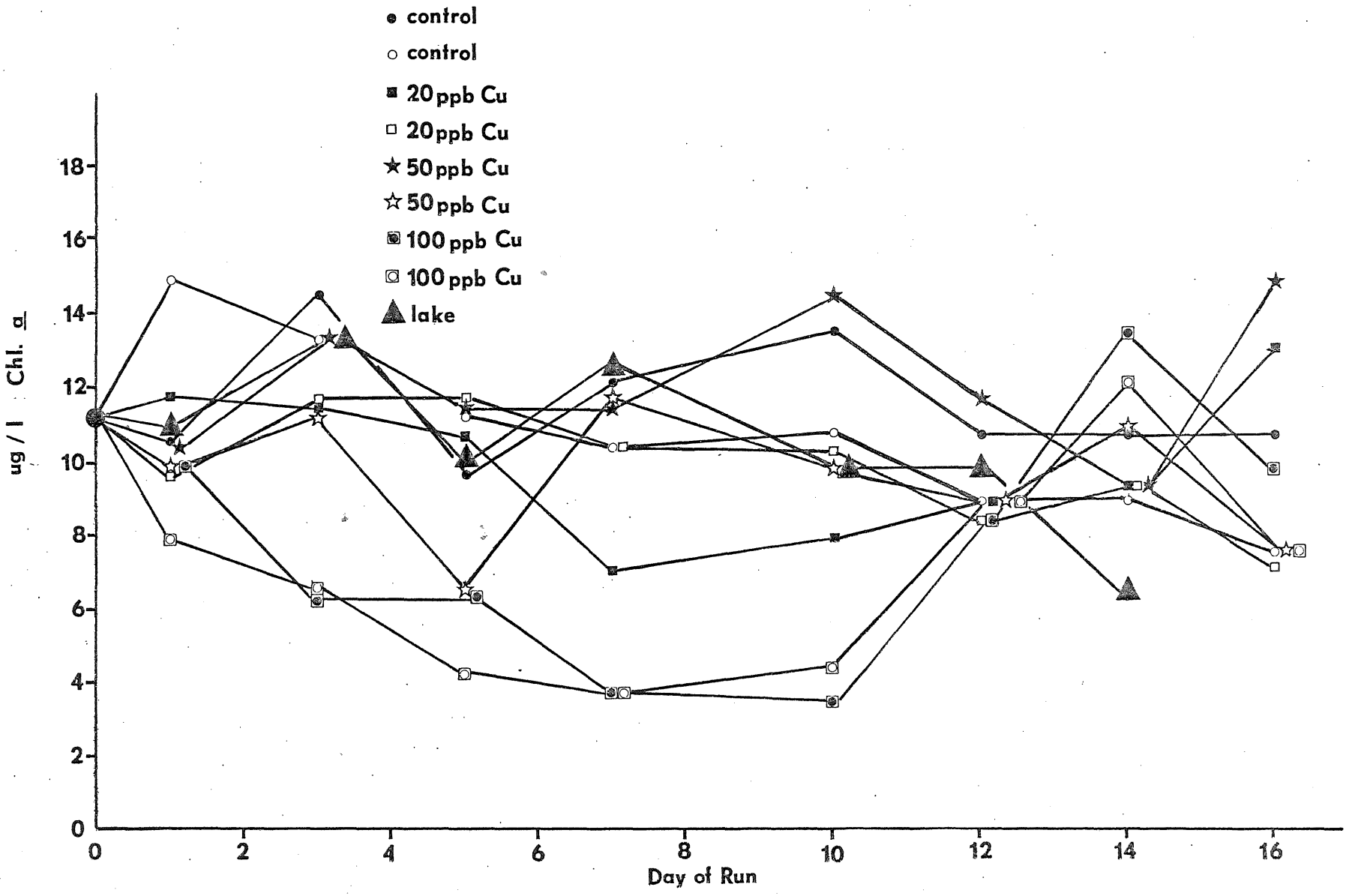

Figure 10. Chlorophyll a, Continuous culture Run 2, Birch Lake.

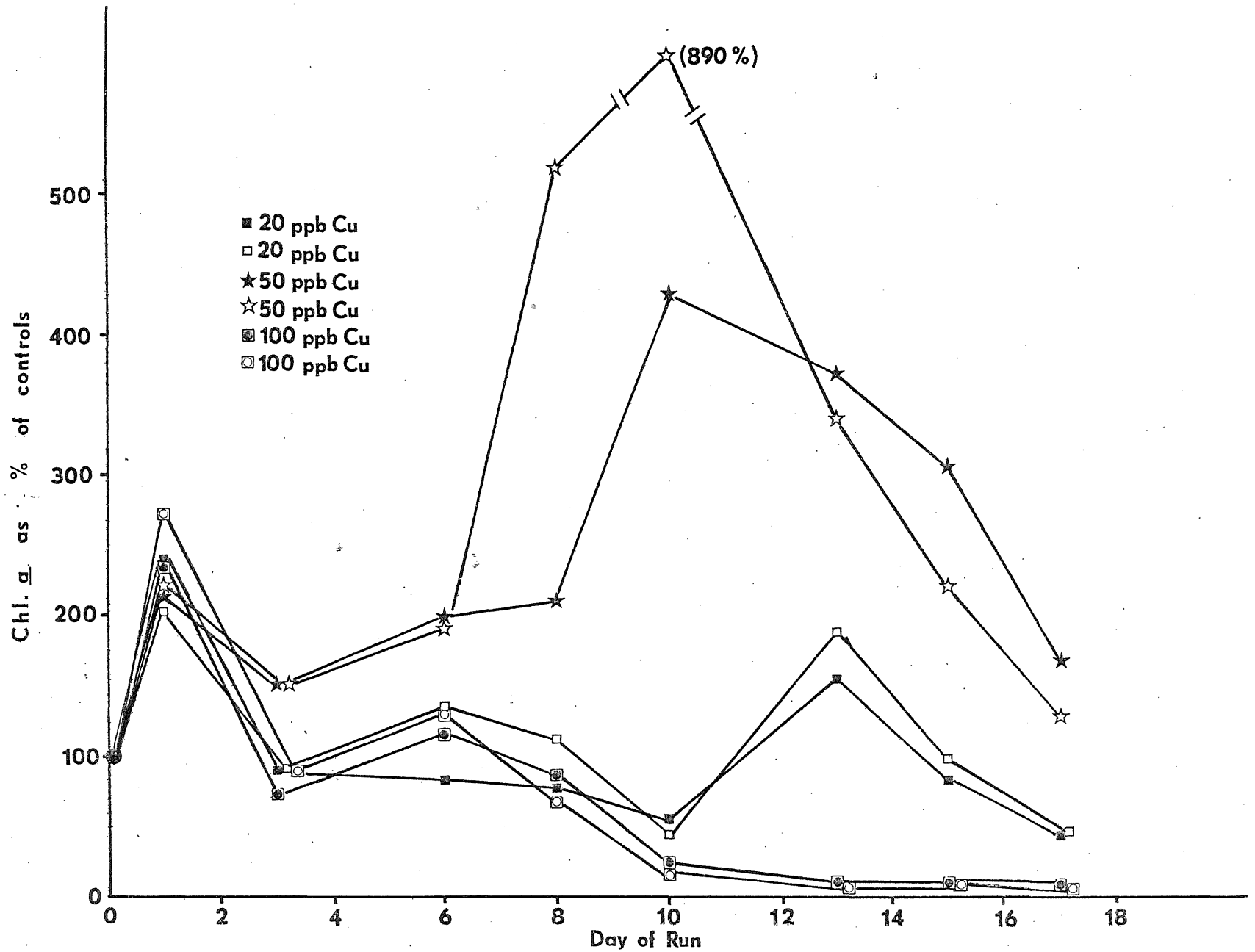

Figure 11. Chlorophyll a, Continuous culture Run 2, Greenwood Lake.

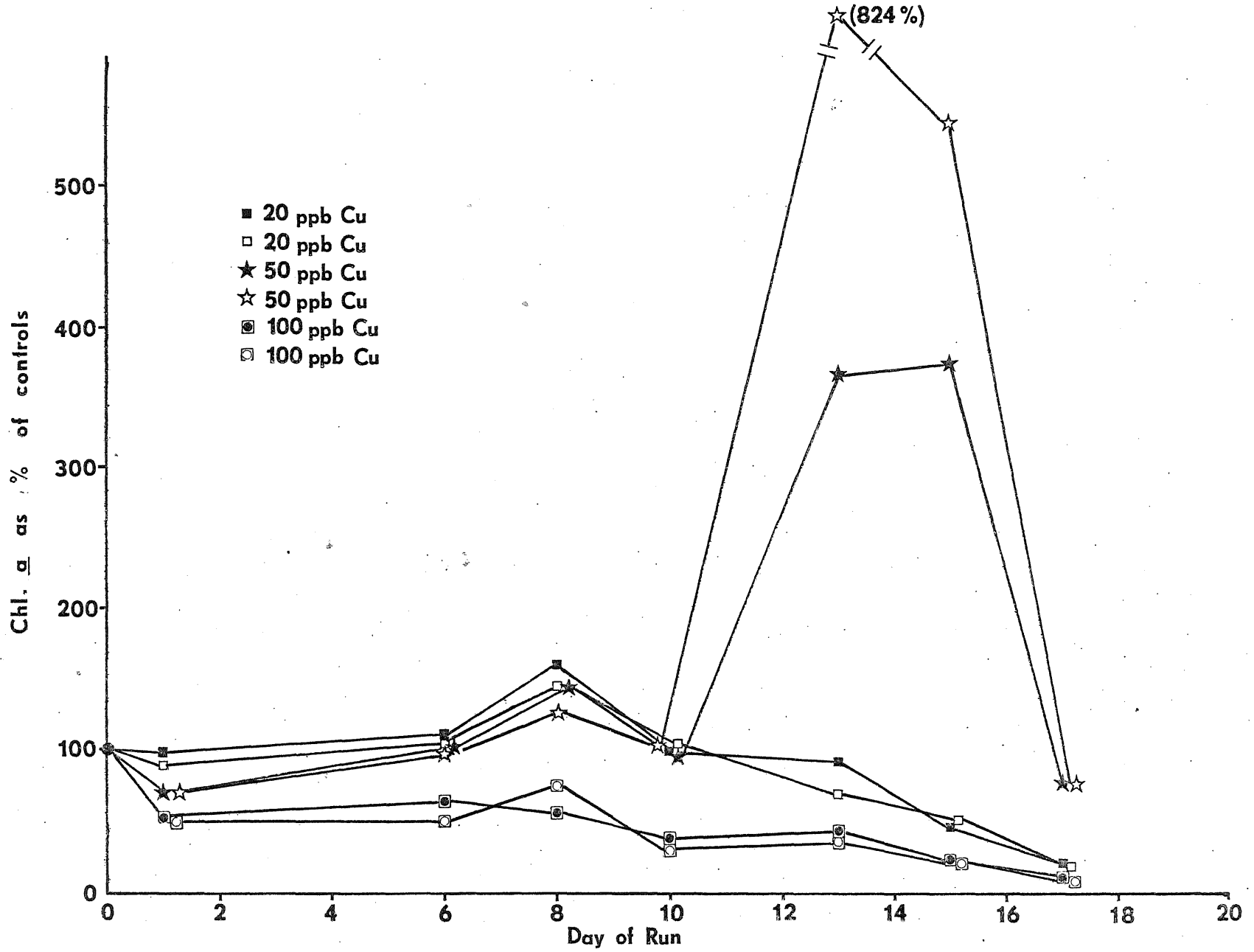

Figure 12. Chlorophyll a, Field Run 3, Birch Lake.

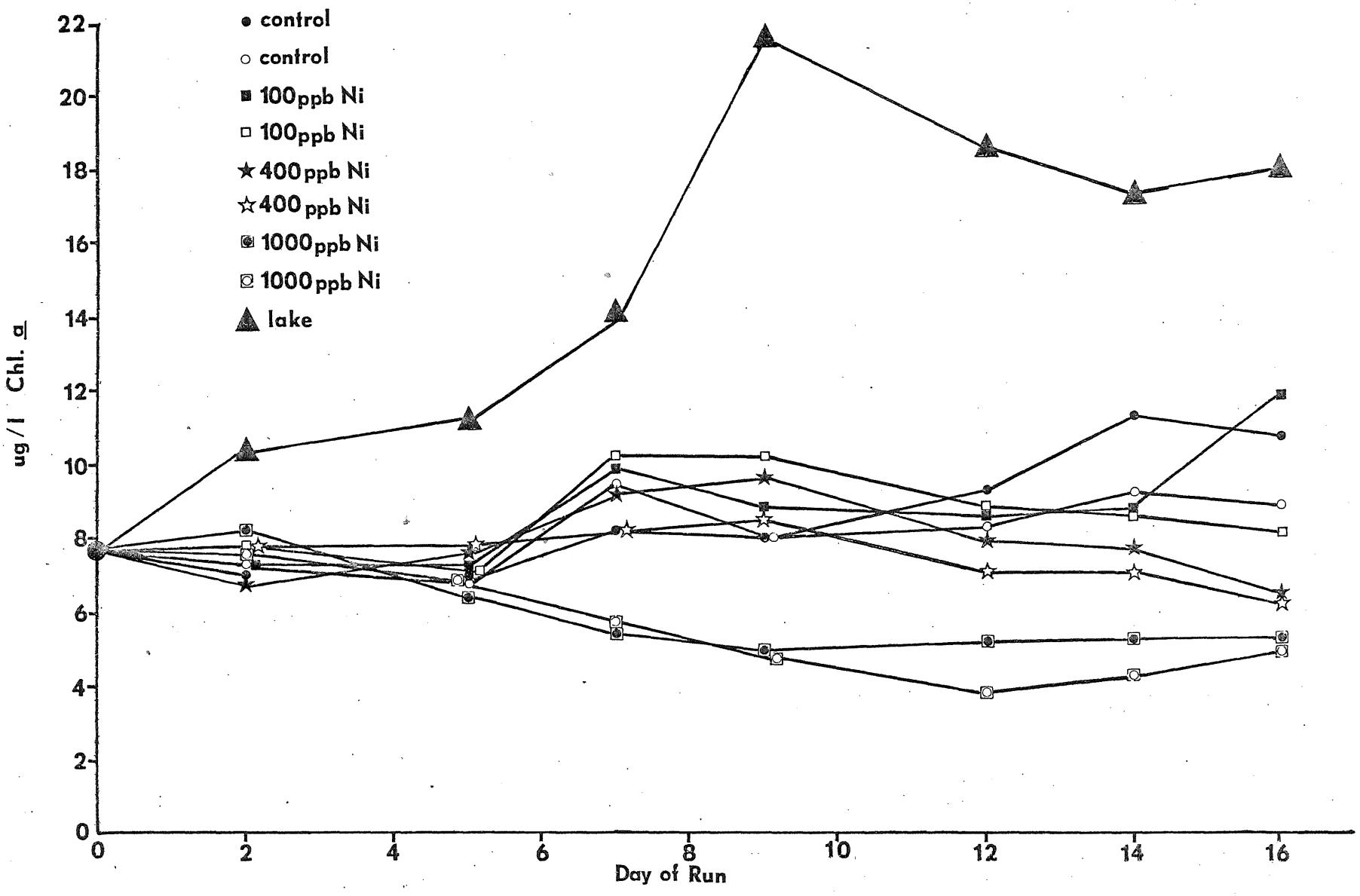

Figure 13. Chlorophyll a, Field Run 3, Greenwood Lake.

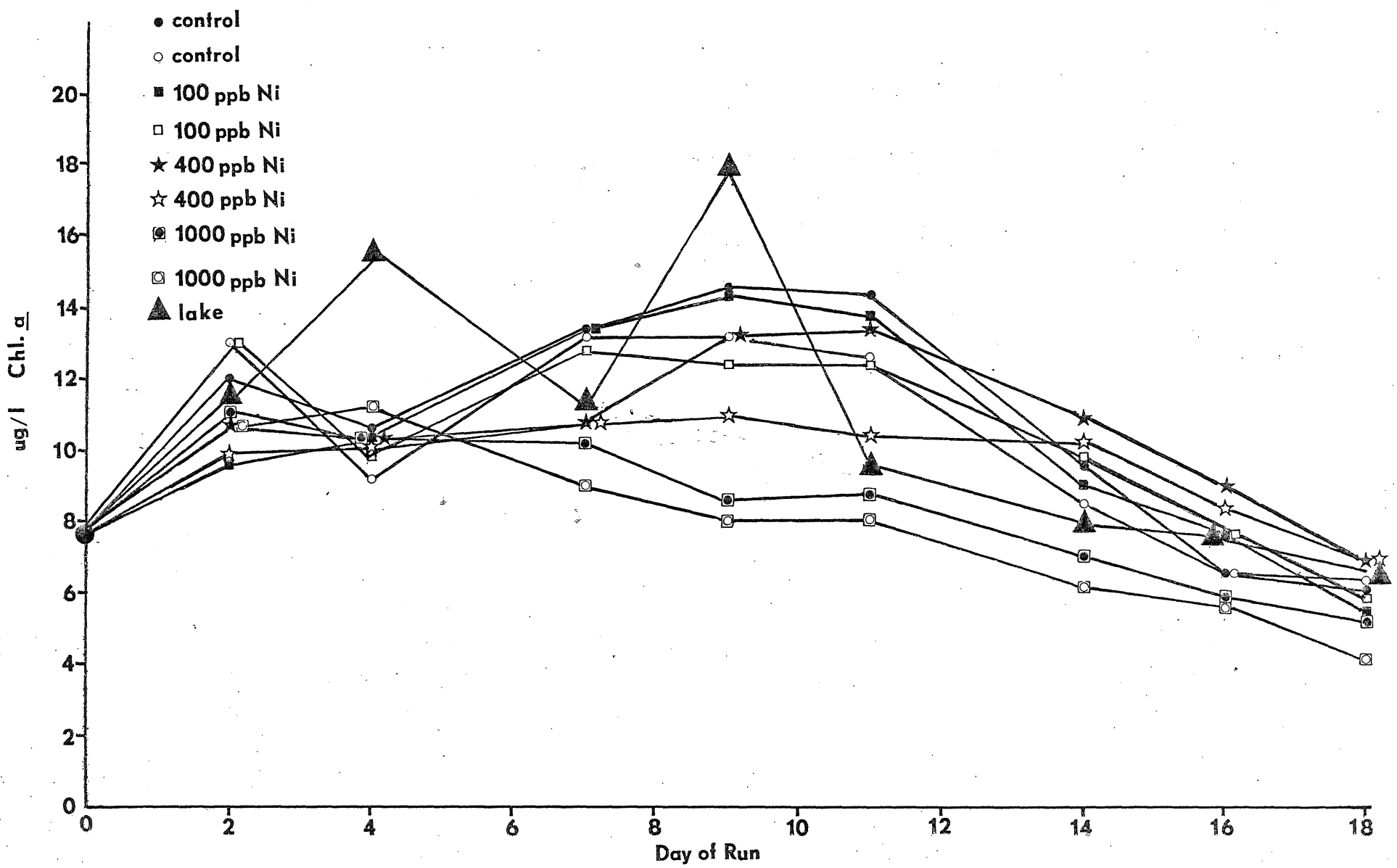

Figure 14. Chlorophyll a, Continuous culture Run 3, Birch Lake.

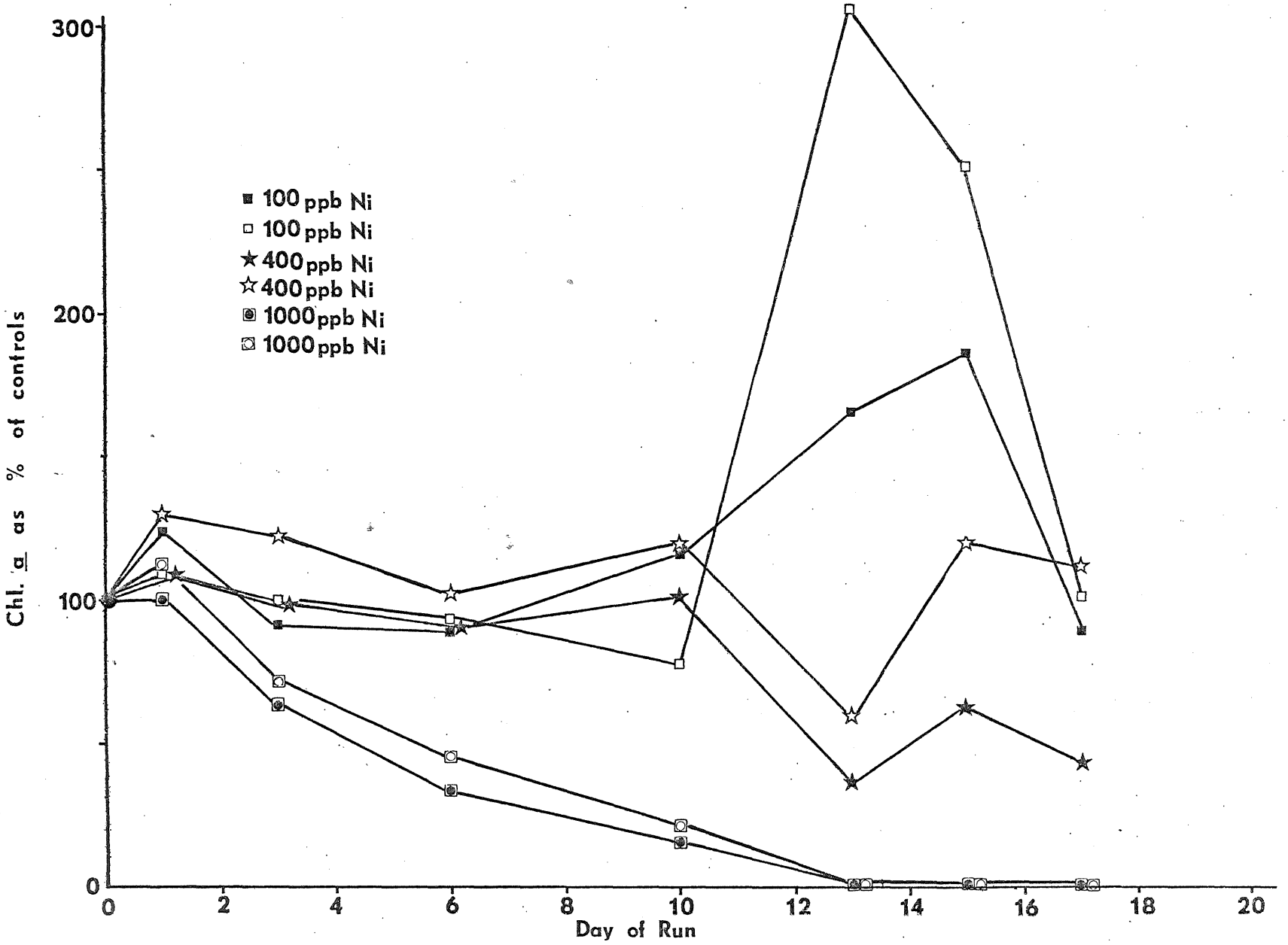

Figure 15. Chlorophyll a, Continuous culture Run 3, Greenwood Lake.

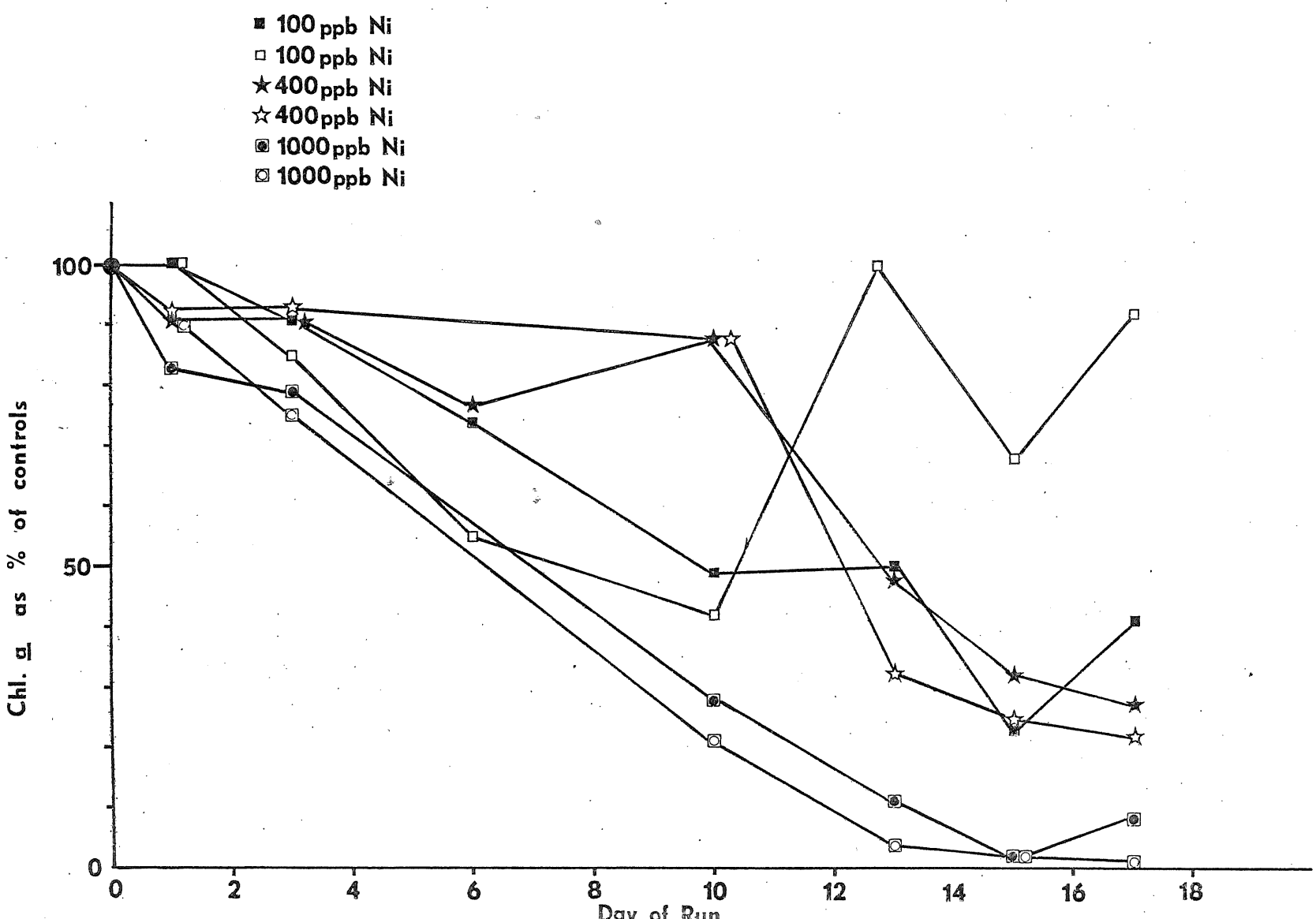

Figure 16. Chlorophyll a, Field Run 4, Birch Lake.

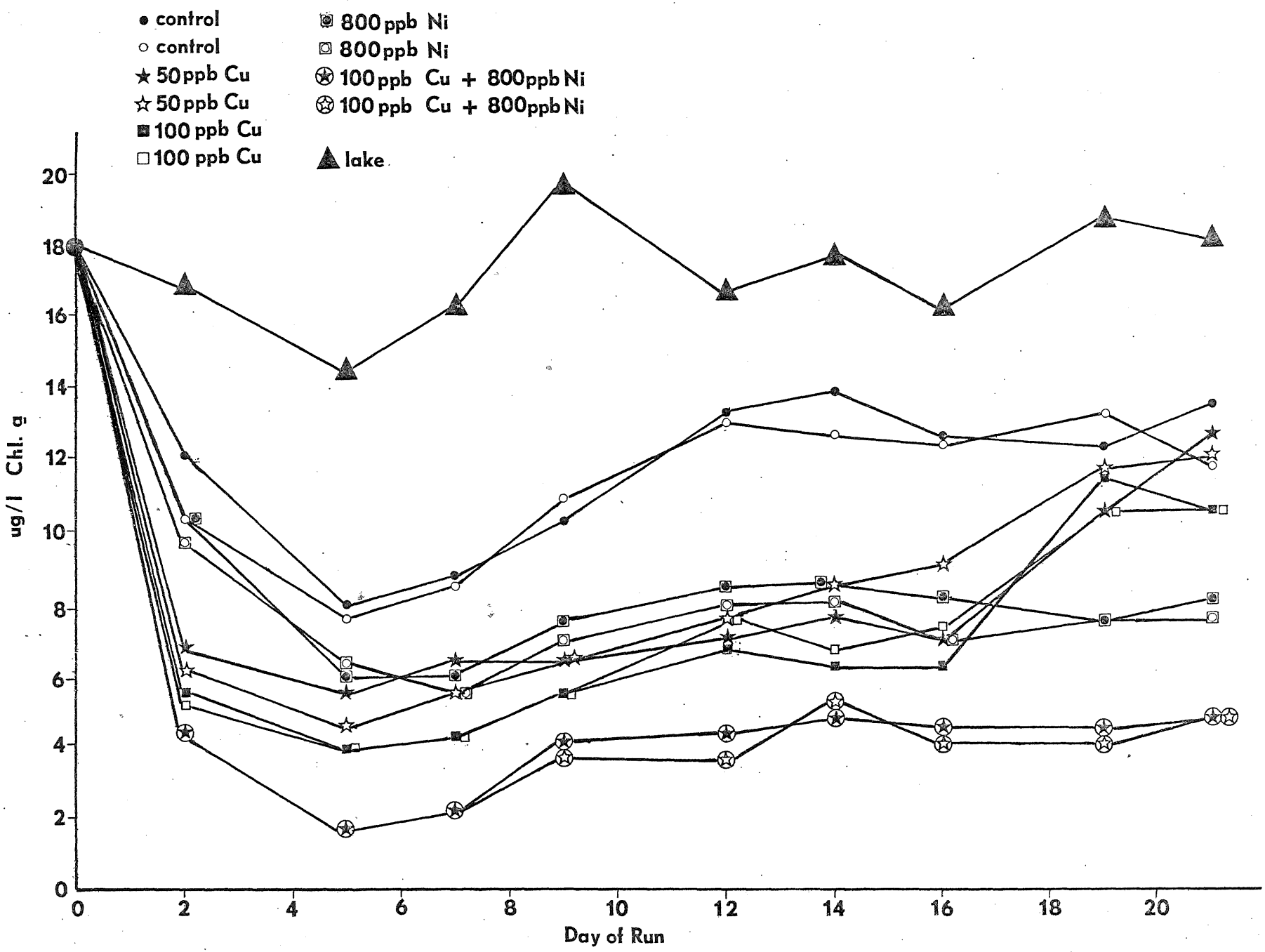

Figure 17. Chlorophyll a, Field Run 4, Greenwood Lake.

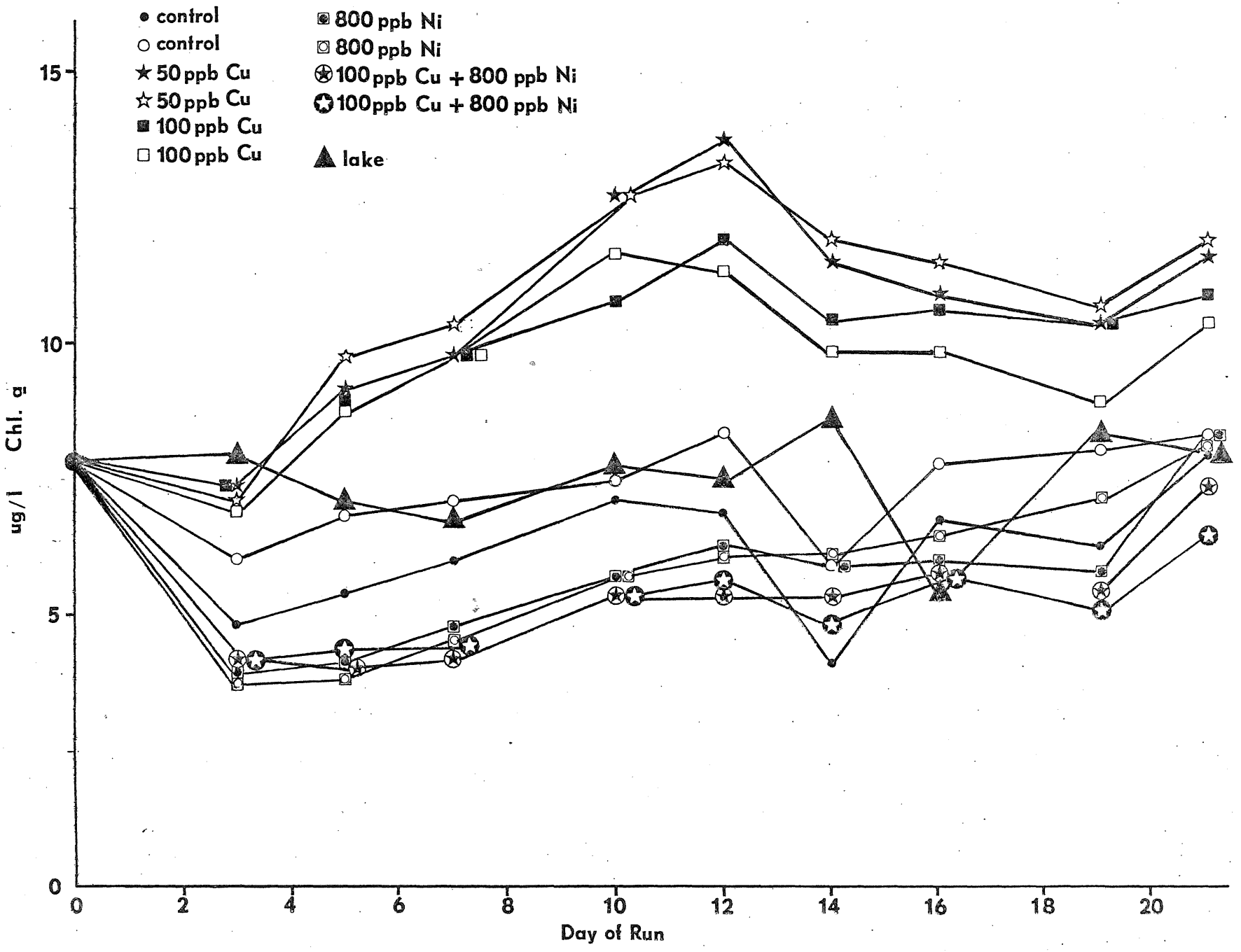

Figure 18. Chlorophyll a, Continuous culture Run 4, Birch Lake.

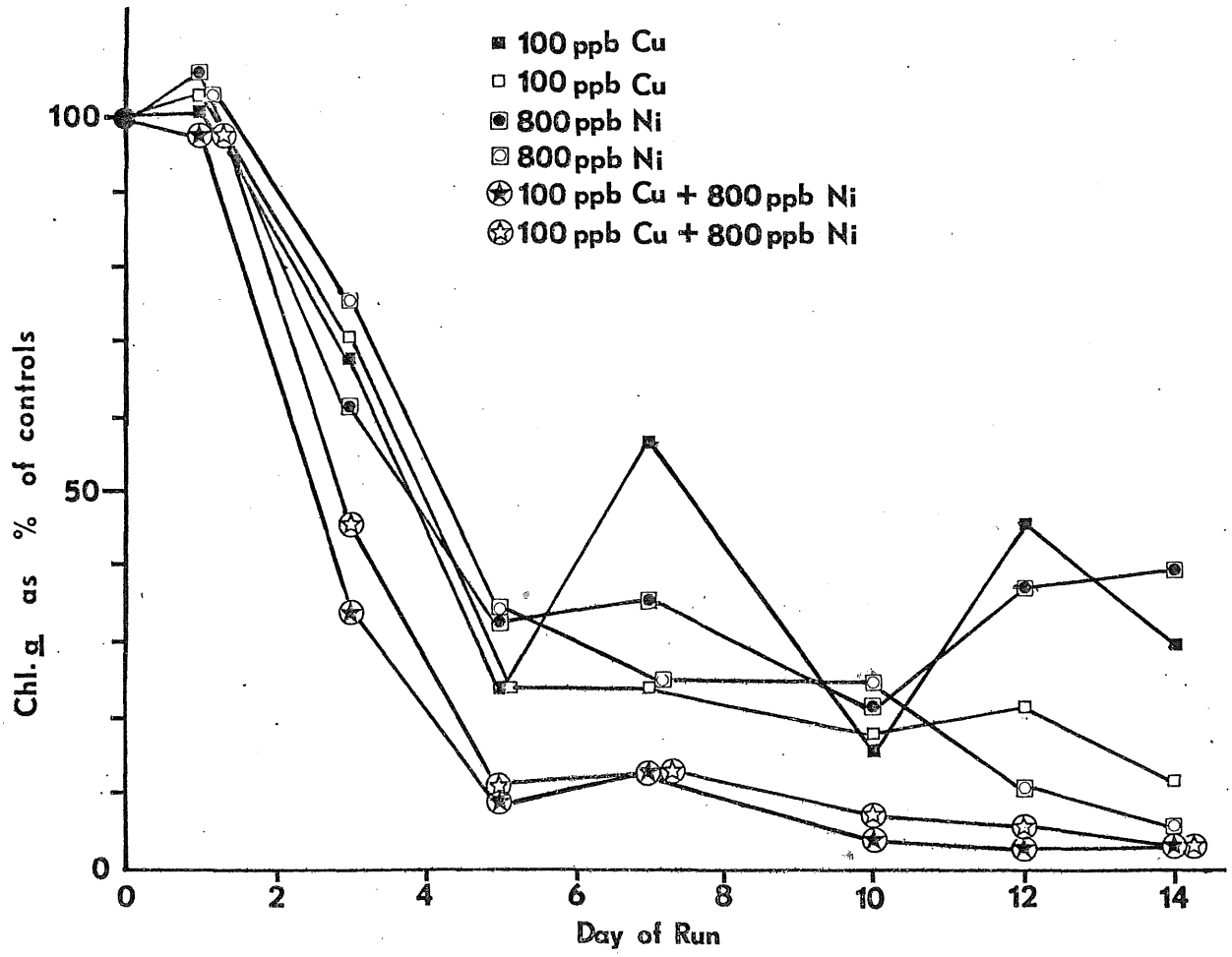

Figure 19. Chlorophyll a, Continuous culture Run 4, Greenwood Lake.

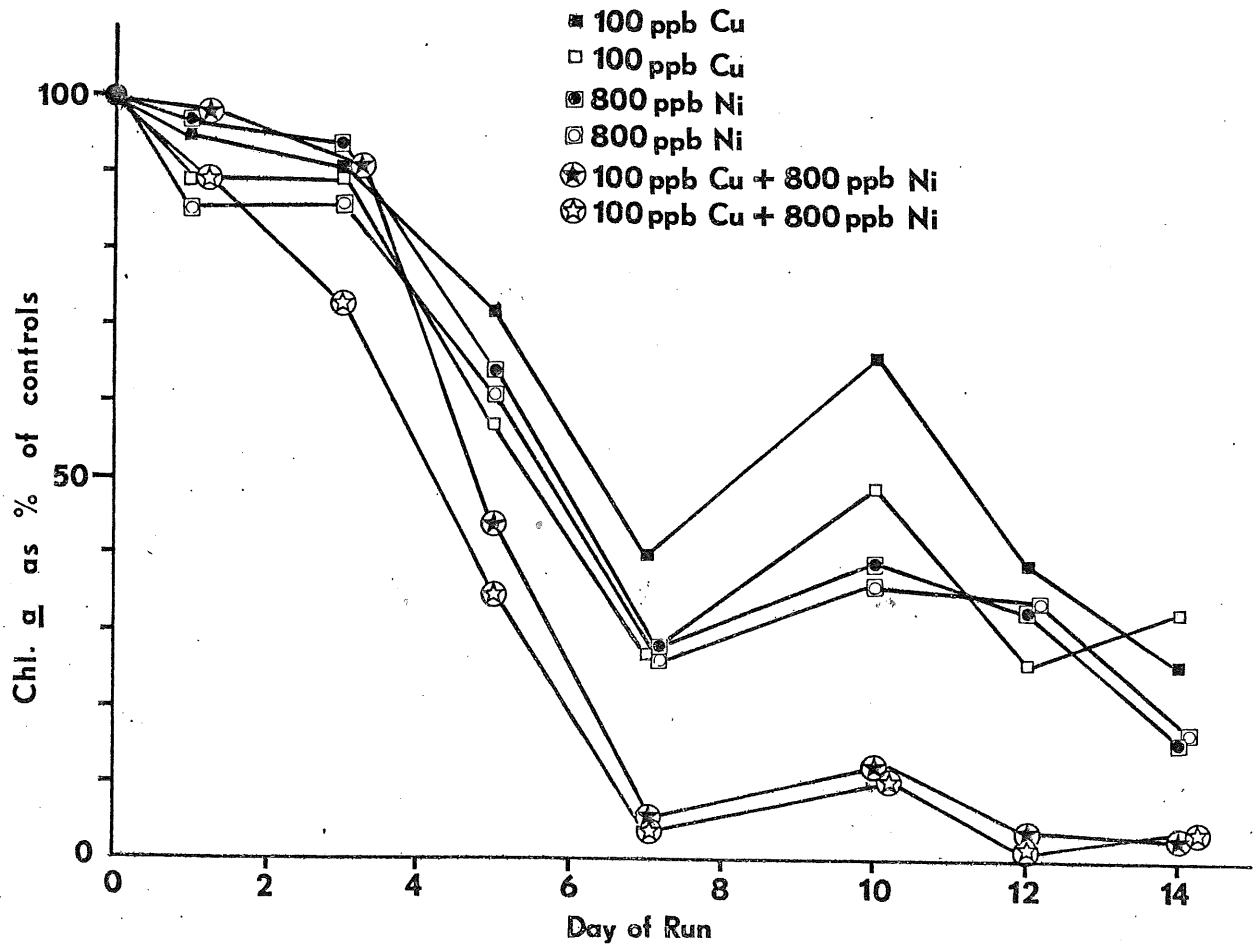

Figure 20. Chlorophyll a, Field Run 5, Greenwood Lake.

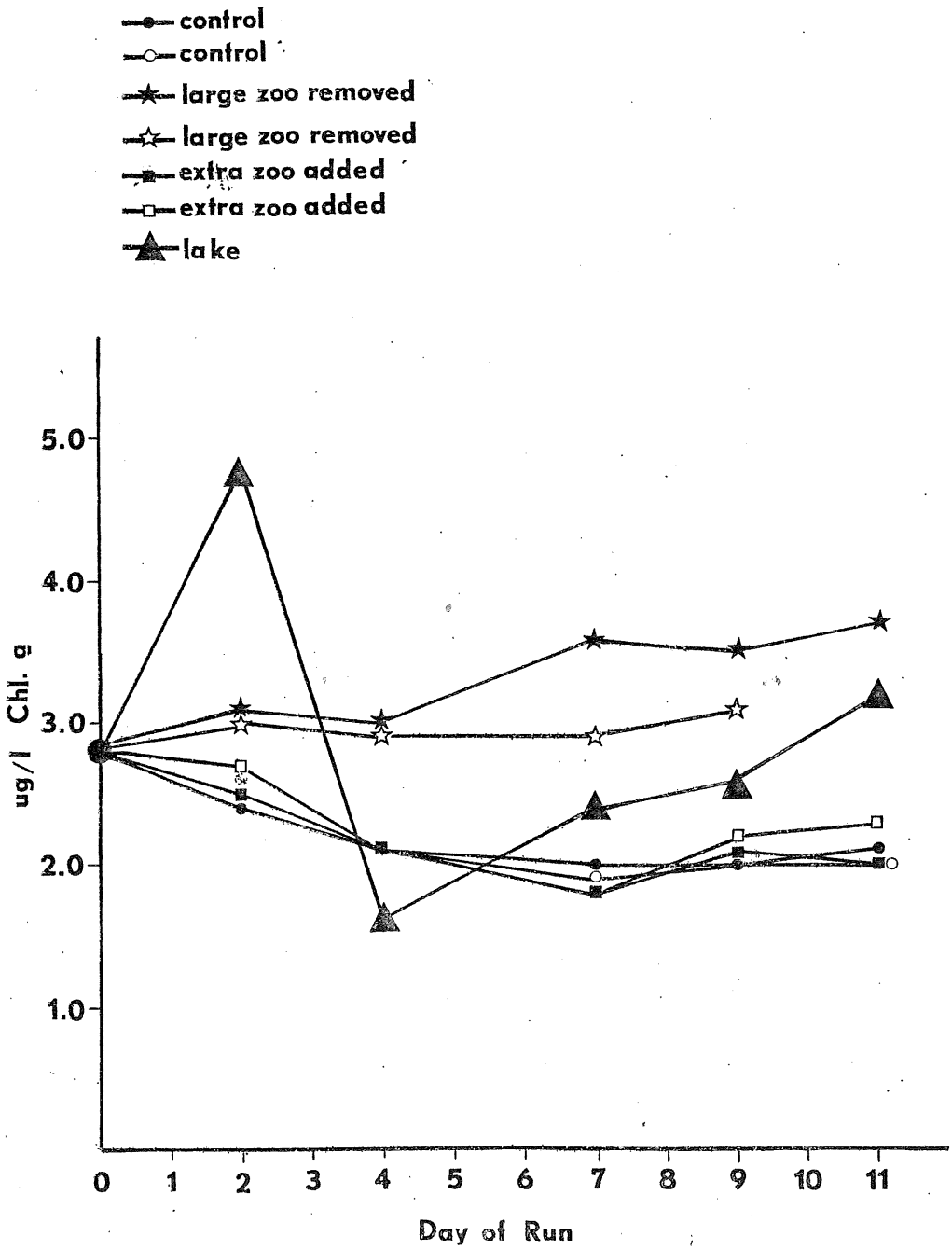

Figure 21. Chlorophyll a, Continuous culture Run 5, Clearwater Lake.

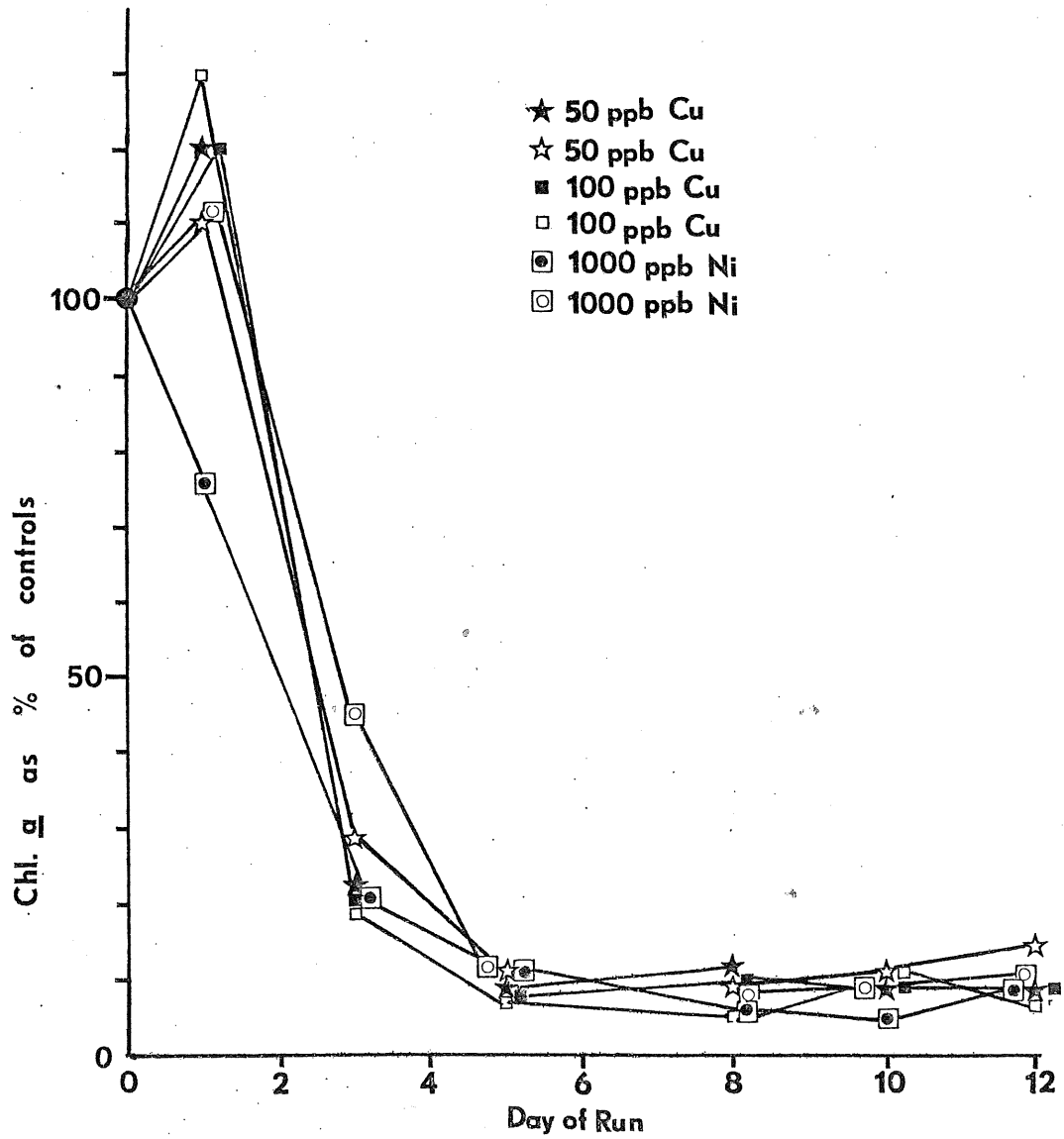

Figure 22. Chlorophyll a, Continuous culture Run 5, South McDougal Lake.

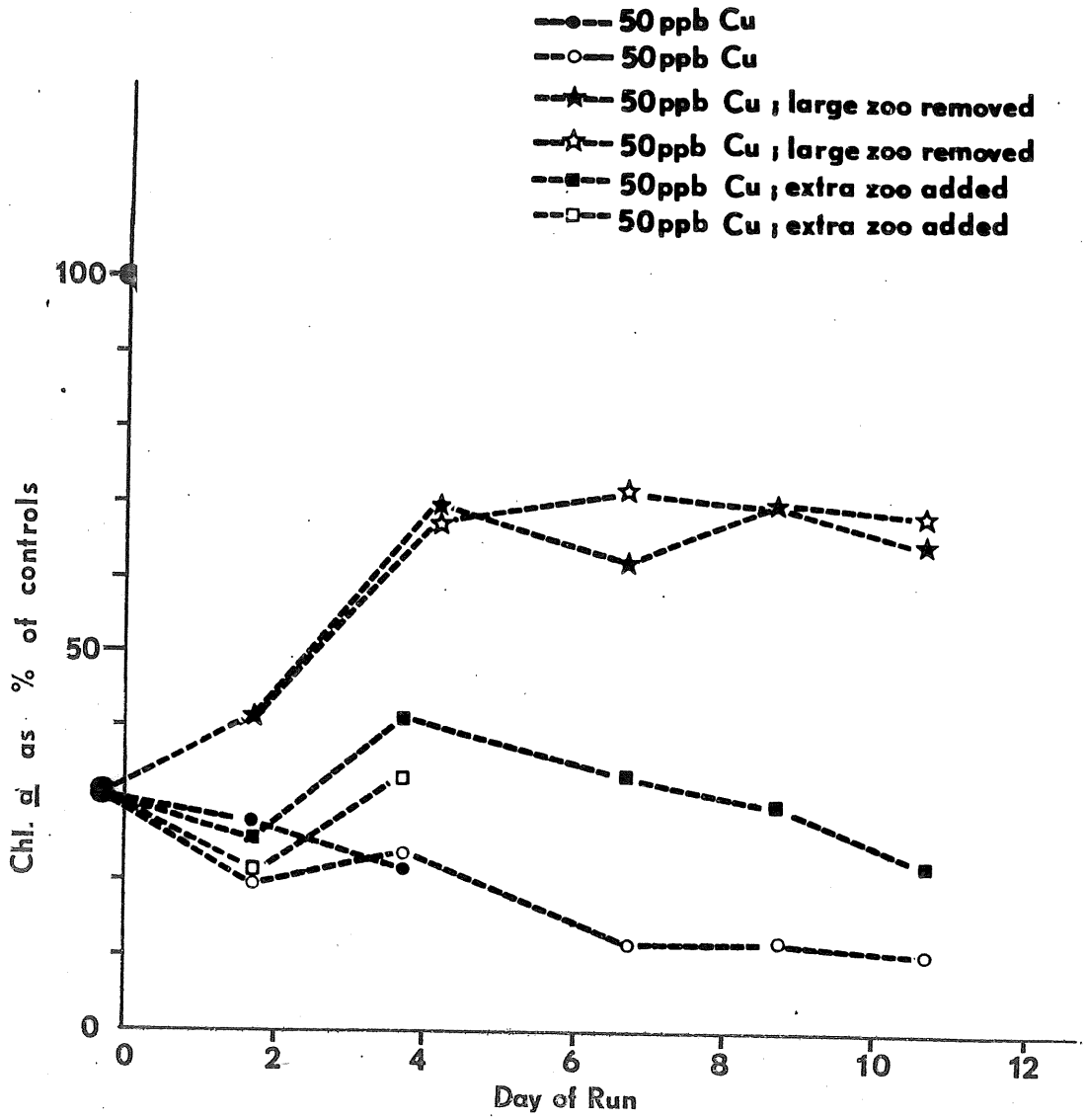

Figure 22. Chlorophyll a, Continuous culture Run 5, South McDougal Lake.

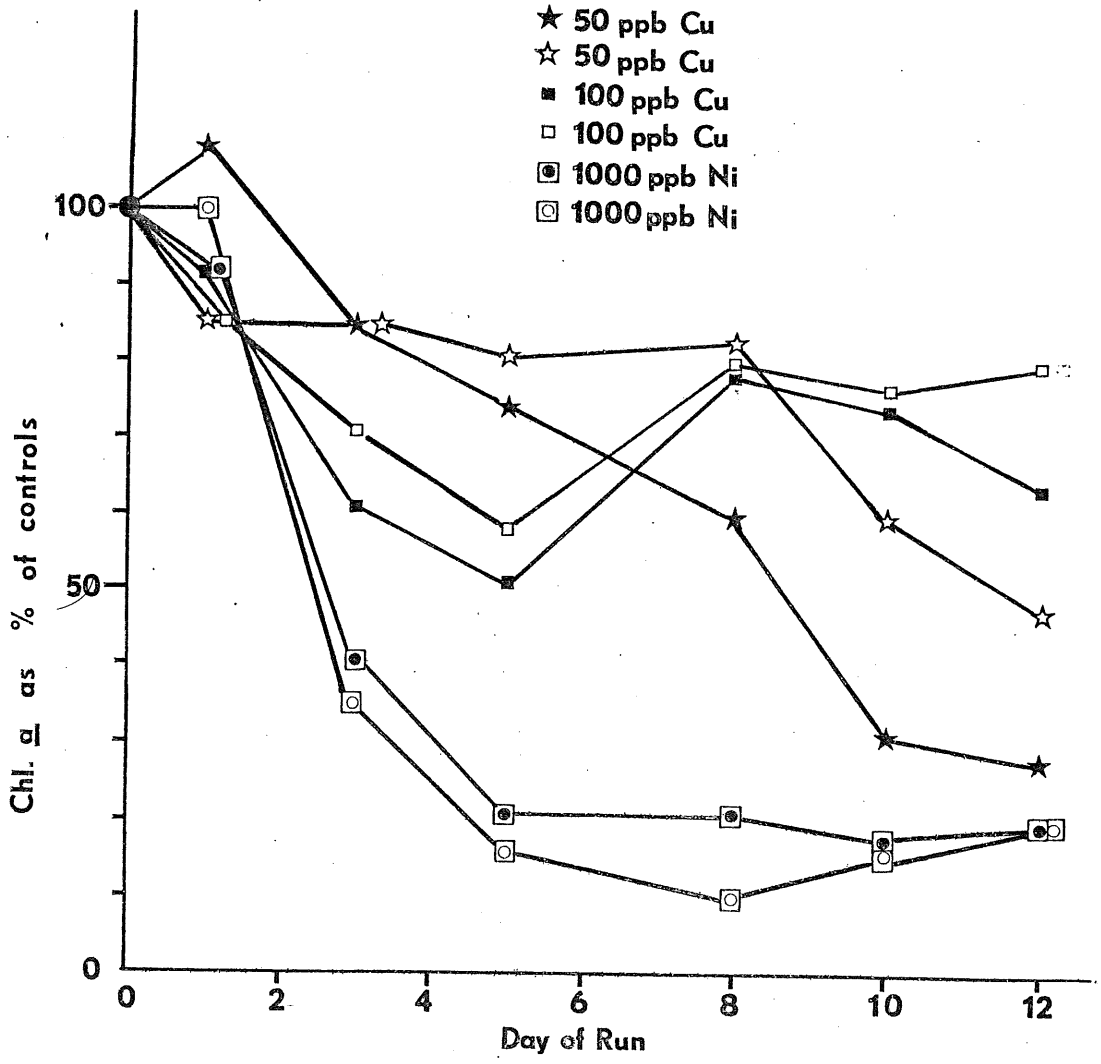

Figure 23. Chlorophyll a, Continuous culture Run 6, Birch Lake.

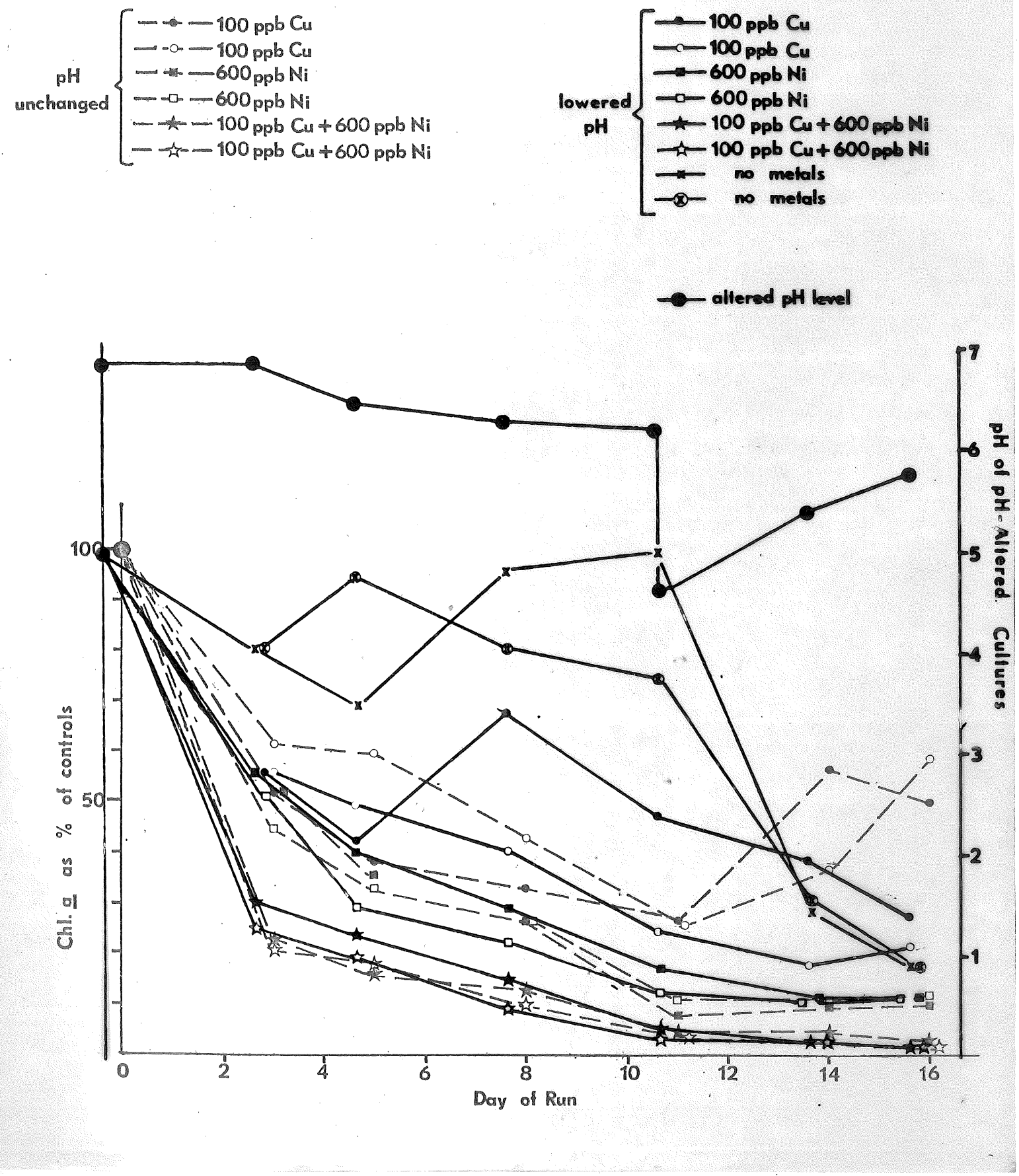

Figure 23. Chlorophyll a, Continuous culture Run 6, Birch Lake.

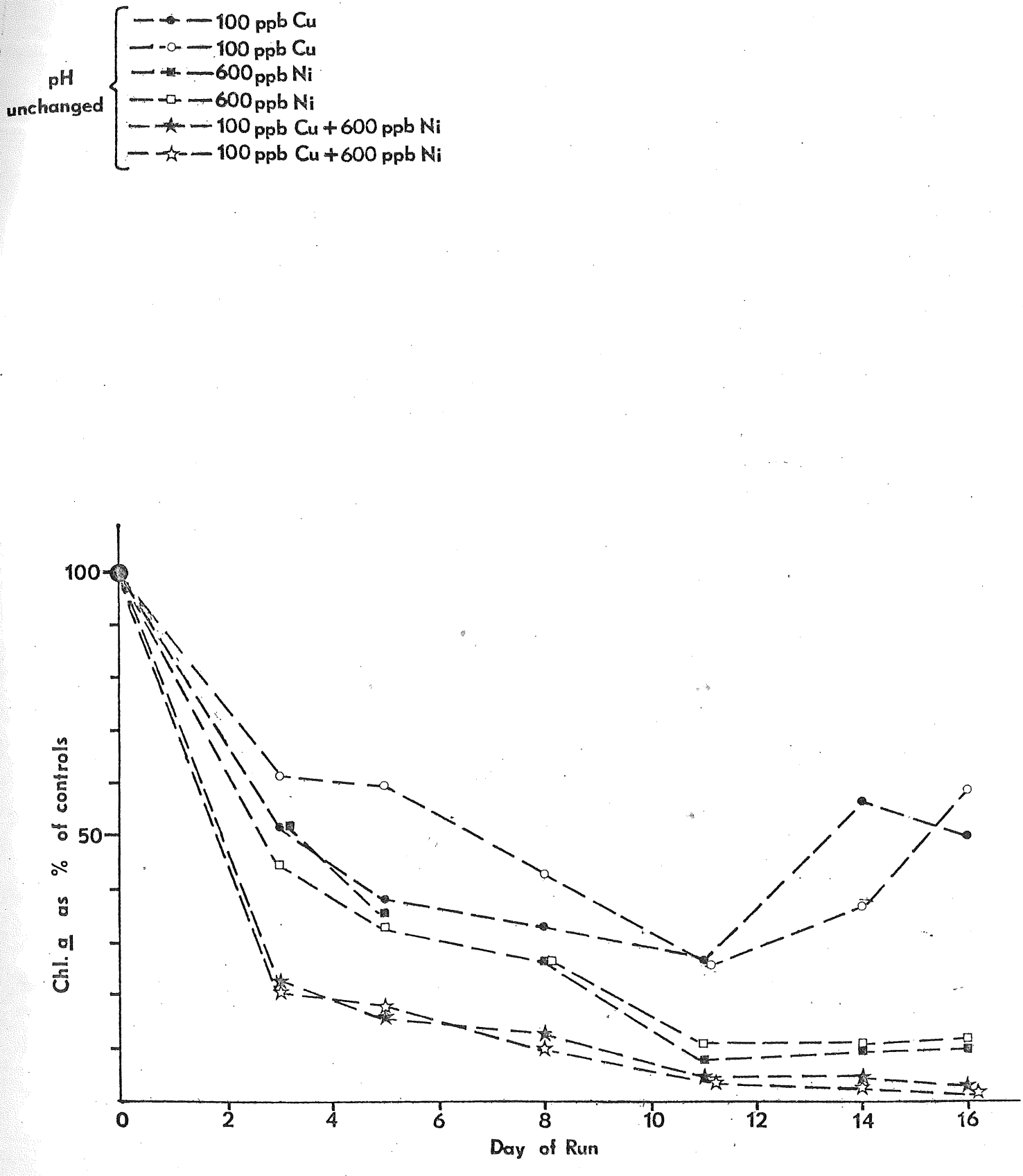

Figure 24. Chlorophyll a, Continuous culture Run 7, Birch Lake.

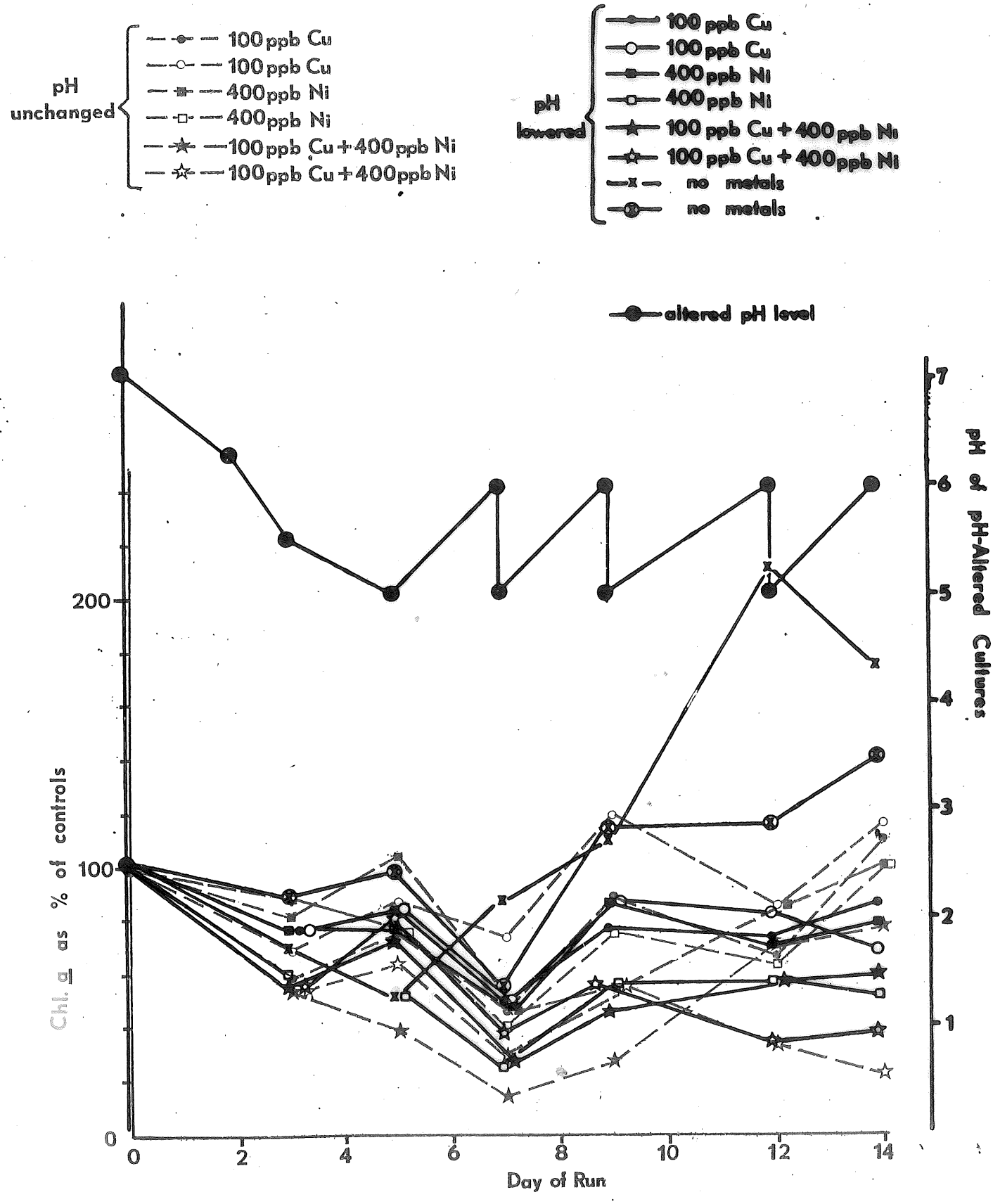

Figure 24. Chlorophyll a, Continuous culture Run 7, Birch Lake.

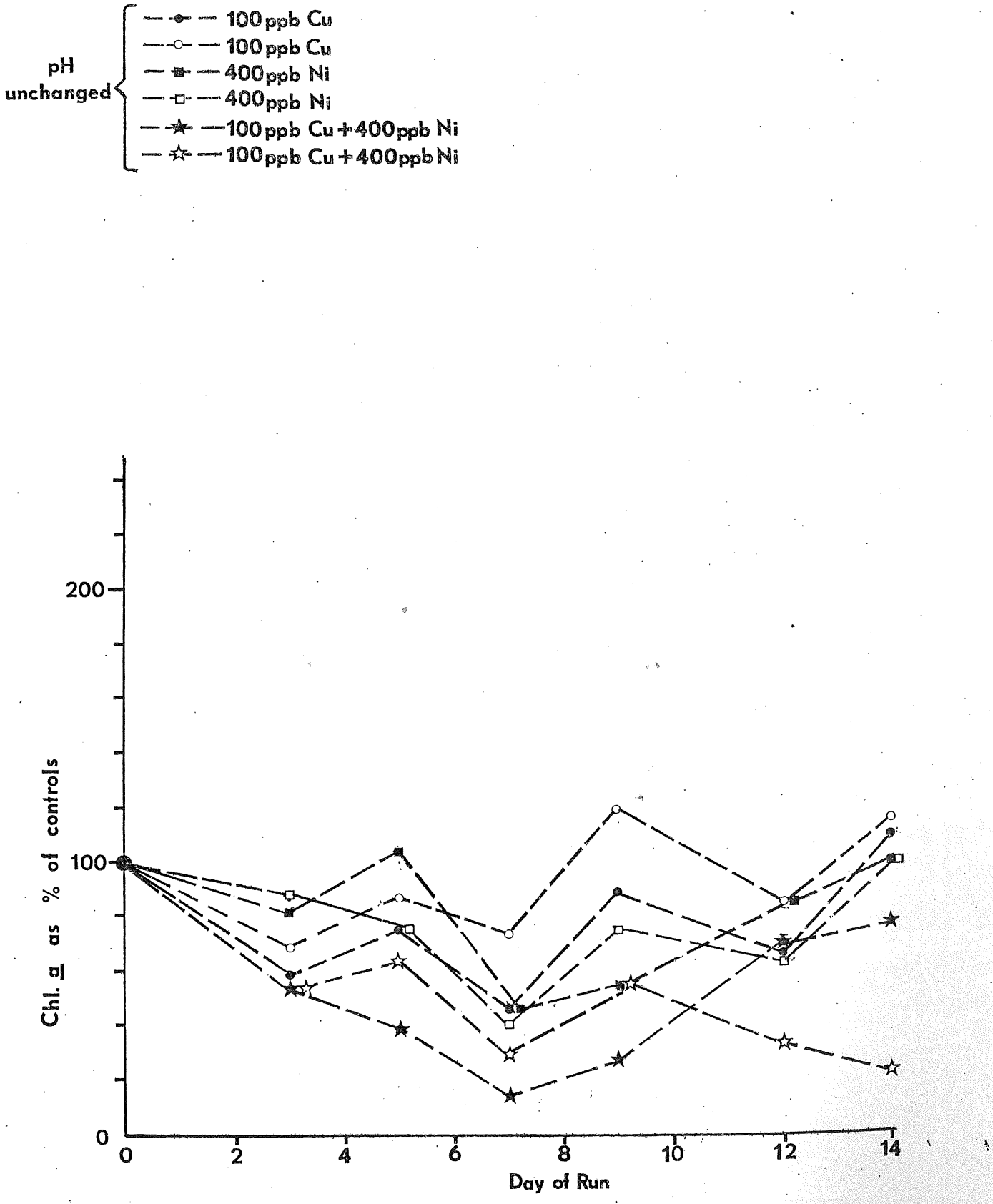

Table 1. Physical and chemical characteristics of study lakes.

Lake	Maximum Depth (m)	Secchi Disk (m)	pH	Conductivity (μmhos/cm)	Total Alkalinity (mg/l $CaCO_3$)	Color (Pt-Co units)	TOC (mg/l)	Complexing Capacity (μM Cu)
Birch (site 1)	5	2	6.8	85	22	54	16	1.6 - 2.6
Greenwood	2	1	6.3	60	6	190	25	2.6
Clearwater	14	4	6.7	39	16	2	7	0.3 - 2.2
South McDougal	1.5	1.5	6.4	36	11	180	38	2.8 - 3.2

Table 2. Summary of bioassays.

Bioassay Type	Treatments[1]				Lakes	Dates (1977)		Duration
Continuous Cultures	Copper (µg/l)	Nickel (µg/l)	pH	Combinations		Start	End	(days)
Run 1	50, 100, 200	-	-	-	Birch, Greenwood	June 11	June 27	17
2	20, 50, 100	-	-	-	Birch, Greenwood	July 6	July 23	18
3	-	100, 400, 1000	-	-	Birch, Greenwood	July 27	Aug. 13	18
4	100	800	-	100 Cu + 800 Ni	Birch, Greenwood	Aug. 20	Sept. 3	15
5	50, 100	1000	-	none	Clearwater, South McDougal	Oct. 14	Oct. 26	13
6	100	600	4.5	all	Birch	Nov. 5	Nov. 21	17
7	100	400	5.0	all	Birch	Dec. 1	Dec. 15	15
Field Tests Run 1[2]	50, 100, 200	-	-	-	Birch, Greenwood	June 15	June 20	6
2	20, 50, 100	-	-	-	Birch, Greenwood	July 2	July 18	17
3	-	100, 400, 1000	-	-	Birch, Greenwood	July 26	Aug. 13	19
4	50, 100	800	-	100 Cu + 800 Ni	Birch, Greenwood	Aug. 18	Sept. 8	22
5	50	Increased and decreased large zooplankter density		all	Greenwood	Sept. 26	Oct. 7	12

[1]Metal concentrations are nominal values. See Tables 4a and 4b for measured values.

[2]Polyethylene bag enclosures.

Table 3. Lake temperature and water chemistry at 0.5 m depth during bioassays.

Run	Lake	Lake Temperature (°C)	Total Alkalinity (mg/l $CaCO_3$)	pH	Metals (µg/l)		Organic Carbon (mg/l)	
					Total Cu	Total Ni	TOC	DOC
1 (Field and Lab)	Birch	16-22	24	6.7	1.5	-	15	14
	Greenwood	18-20	7	6.1	< 2	-	25	24
2 (Field and Lab)	Birch	22-23	22	6.7	1.4	-	16	17
	Greenwood	17-22	6	6.3	1.8	-	26	26
3 (Field and Lab)	Birch	19-23	21	6.8	-	< 2	17	17
	Greenwood	18-20	6	6.3	-	< 2	25	25
4 (Field and Lab)	Birch	18	20	6.7	2.0	12	18	20
	Greenwood	15-18	5	6.3	2.3	5	25	27
5 (Field)	Greenwood	11	3	6.2	-	-	34	33
5 (Lab)	Clearwater	8	11	6.5	0.9	< 1	6	7
	S. McDougal	5	9	6.9	1.6	< 1	35	34
6 (Lab)	Birch	7	16	6.8	-	-	22	-
7 (Lab)	Birch	3	16	6.8	-	-	23	-

Table 4a. Summary of nominal and measured metal concentrations (µg/l) in continuous culture bioassays.

Run	Lake	TREATMENT					
		Copper		Nickel		Copper/Nickel	
		Nominal	Measured	Nominal	Measured	Nominal	Measured
1	Birch	50	44	-		-	
		100	91				
		200	200				
	Greenwood	50	46	-		-	
		100	85				
		200	170				
2	Birch	20	18	-		-	
		50	43				
		100	103				
	Greenwood	20	20	-		-	
		50	45				
		100	94				
3	Birch	-		100	88	-	
				400	350		
				1000	900		
	Greenwood	-		100	93	-	
				400	360		
				1000	925		
4	Birch	100	80	800	725	100/800	71/730
	Greenwood	100	77	800	745	100/800	77/740
5	Clearwater	50	43	1000	990	-	
		100	85				
	South McDougal	50	43	1000	1000	-	
		100	90				
6	Birch	100	98	600	640	100/600	98/630
	Birch (low pH)	100	101	600	570	100/600	91/610
7	Birch	100	100	400	370	100/400	99/400
	Birch (low pH)	100	101	400	400	100/400	99/400

Table 4b. Summary of nominal and measured metal concentrations (ug/l) in field bioassays.

Run	Lake	TREATMENT					
		Copper		Nickel		Copper/Nickel	
		Nominal	Measured (Mean)	Nominal	Measured (Mean)	Nominal	Measured (Mean)
1	Birch	50	44	-		-	
		100	-				
		200	160				
	Greenwood	50	-	-		-	
		100	86				
		200	165				
2	Birch	20	20	-		-	
		50	37				
		100	81				
	Greenwood	20	19	-		-	
		50	40				
		100	80				
3	Birch	-		100	97	-	
				400	360		
				1000	895		
	Greenwood	-		100	93	-	
				400	350		
				1000	900		
4	Birch	50	40	800	783	100/800	83/810
		100	85				
	Greenwood	50	38	800	763	100/800	78/790
		100	77				
5	Birch	50	44	-		-	
	Birch (hi zoo)	50	50				
	Birch (low zoo)	50	48				

Table 5. Summary of bioassay results.

Run	Lake	Treatment Cu (µg/l)	Ni (µg/l)	pH	Effects Lab	Field
1	Birch	50	-	-	toxic, peak day 13	toxic
		100			toxic	-
		200			toxic	toxic
	Greenwood	50	-	-	toxic	-
		100			toxic	toxic
		200			toxic	toxic
2	Birch	20	-	-	slightly toxic? peak day 13	No conclusions due to poor replication of treatments
		50			stimulatory, peak day 10	
		100			toxic	
	Greenwood	20	-	-	slightly toxic?	no effect
		50			stimulatory, peak day 14	no effect
		100			toxic	toxic, then recovers
3	Birch	-	100	-	stimulatory, peak day 14	no effect
			400		slightly toxic?	slightly toxic?
			1000		toxic	toxic
	Greenwood	-	100	-	toxic, one culture peaks	no effect
			400		toxic	no effect
			1000		toxic	slightly toxic
4	Birch	50	800	-	-	toxic, then recovers
		100			toxic	toxic, then recovers
		100 +	800		toxic	toxic
					toxic	toxic
	Greenwood	50	800	-	-	stimulatory
		100			toxic	stimulatory
		100 +	800		toxic	toxic, then recovers
					toxic	toxic, partially recovers
5	Greenwood	50			-	no effect
		50 - zooplankton removed				stimulatory
		50 - zooplankton added				stimulatory, then declines
		zooplankton removed				stimulatory
		zooplankton added				no effect
5	Clear-water	50	1000	-	toxic	-
		100			toxic	
					toxic	
	South McDougal	50	1000	-	toxic	-
		100			toxic, partially recovers	
					toxic	
6	Birch	100	600	6.2	toxic, partially recovers	-
		100 +	600		toxic	
					toxic	
		100			no effect until pH = 4.5	
			600		toxic	
		100 +	600		toxic	
					toxic	
7	Birch	100	400	5.0	slightly toxic, recovers	-
		100 +	400		slightly toxic, recovers	
					toxic	
		100			stimulatory	
			400		slightly toxic	
		100 +	400		toxic	
					toxic	

Table 6. Relative production/biomass ratios, field Run 4, Greenwood Lake. Values from different dates are not comparable.

Date	Treatment	Relative P/B
Sept. 9 (Day 22)	Control	179 206
	50 µg/l Cu	152
	100 µg/l Cu	186 195
Sept. 4 (Day 17)	Control	112
	50 µg/l Cu	104 113
	100 µg/l Cu	127 124

Table 7. Relative production/biomass ratios, field Run 5, Greenwood Lake. Values from different dates are not comparable.

Date	Treatment	Relative P/B
Oct. 5 (Day 10)	Control	715 639
	Large zooplankton removed	738
	Large zooplankton added	722
	50 µg/l Cu	704
	50 µg/l Cu, large zooplankton removed	473
	50 µg/l Cu, large zooplankton added	765 572
Sept. 30 (Day 5)	Control	865 800
	Large zooplankton removed	386
	Large zooplankton added	987
	50 µg/l Cu	855
	50 µg/l Cu, large zooplankton removed	487
	50 µg/l Cu, large zooplankton added	994 928

Table 8. Bacteria population counts, field Run 5, Greenwood Lake. Samples collected and plated Oct. 7, 1977; plates counted Oct. 9, 1977.

Treatment	Colonies/ml	Standard Deviation
Control	3350 345	141 120
Large zooplankton removed	1080	156
Large zooplankton added	590 668	127 263
50 µg/l Cu	1535	149
50 µg/l Cu, large zooplankton removed	3360 3605	481 403
50 µg/l Cu, large zooplankton added	720 1460	42 57
Lake water, 0.5 m depth	159	31

Table 9. Cell counts, continuous culture Run 2, day 14. Numbers are units/ml. NS = no sample. P = present.

Lake	Species	Treatment		
		Control	50 µg/l Cu	100 µg/l Cu
Birch	Fragilaria crotonensis	130	499	P
		NS	1,019	NS
	Synedra incisa	332	2,589	5
		NS	309	NS
	Synedra rumpens	1,443	8,203	0
		NS	10,436	NS
Greenwood	Synedra rumpens	1,622	4,586	0
		0	NS	0

Table 10a. Cell counts, field Run 2, day 16 (Birch Lake).
Numbers are units/ml. P = present.

Lake	Species	Treatment			
		Control	20 µg/l Cu	50 µg/l Cu	100 µg/l Cu
Birch (day 16)	Achnanthes minutissima	26	145	444	5
		67	170	398	5
	Fragilaria crotonensis	1,694	4,969	1,952	98
		2,753	2,960	3,698	253
	Nitzschia spp.	77	279	398	36
		46	145	274	5
	Tabellaria fenestrata	243	300	P	26
		274	72	41	21
	Crucigenia quadrata	36	10	0	P
		88	0	P	P
	Total blue-green algae (except Agmenellum)	98	30	5	5
		294	10	0	25
	Dinobryon bavaricum	5	10	5	103
		P	0	10	129
	Dinobryon divergens	21	129	31	656
		160	5	119	408
	Dinobryon sociale & subsp.	0	31	5	310
		0	0	72	449
	Hyalobryon spp.	0	0	P	310
		0	0	21	516

Table 10b. Cell counts, field Run 2, day 14 (Greenwood Lake). Numbers are units/ml.

Lake	Species	Treatment			
		Control	20 µg/l Cu	50 µg/l Cu	100 µg/l Cu
Greenwood (day 14)	Asterionella formosa	21	62	114	181
		10	93	196	268
	Fragilaria crotonensis	10	46	57	1,441
		72	93	227	836
	Fragilaria vaucheriae	0	0	108	0
		0	41	114	1,426
	Nitzschia spp.	98	129	62	1,839
		186	160	532	124
	Tabellaria fenestrata	36	124	129	93
		36	222	222	72
	Spondylosium planum	52	145	145	287
		46	83	196	434
	Coelosphaerium naegelianum	1,188	744	0	114
		780	0	150	0

Table 11. Cell counts, field Run 3, day 16 (Birch) and day 18 (Greenwood). Numbers are units/ml.

Lake	Species	Treatment			
		Control	100 µg/l Ni	400 µg/l Ni	1000 µg/l Ni
Birch (day 16)	*Synedra* spp.	420 306	275 46	6 62	13 9
	Micractinium pusillum	36 2	166 253	22 0	5 12
	Anabaena circinalis	104 80	42 0	0 24	13 0
	Anabaena planctonica	213 150	166 31	21 69	0 19
	Aphanocapsa delicatissima	99 73	36 67	6 28	12 1
	Total blue-green algae (except *Agmenellum*)	706 542	358 273	98 209	135 114
Greenwood (day 18)	*Oscillatoria pseudo-geminata*	77 62	98 0	8 10	0 0
	Unidentified flagellates	62 26	10 0	5 0	0 0

Table 12a. Cell counts, field Run 4, day 21 (Birch Lake).
Numbers are units/ml. P = present.

Lake	Species	Treatment				
		Control	50 µg/l Cu	100 µg/l Cu	800 µg/l Ni	100 µg/l Cu + 800 µg/l Ni
Birch (day 21)	Asterionella formosa	337	654	186	196	126
		305	374	238	176	119
	Fragilaria crotonensis	130	218	170	41	70
		341	254	155	55	71
	Nitzschia spp.	202	913	77	23	6
		62	726	150	3	14
	Rhizosolenia eriensis	0	21	176	28	13
		10	16	289	14	17
	Synedra spp.	16	343	P	10	13
		15	737	15	17	12
	Anabaena planctonica	145	0	0	52	0
		114	0	0	19	0
	Aphanocapsa delicatissima	192	15	5	2	6
		57	0	0	3	10
	Aphanizomenon flos-aquae	1,157	5	0	111	1
		744	0	0	158	0
	Coelosphaerium naegelianum	171	26	0	31	P
		67	16	P	14	0
	Oscillatoria geminata	36	0	0	13	0
		41	0	0	10	0
	Phormidium spp.	73	0	10	10	5
		186	0	P	7	7
	Hyalobryon spp.	0	0	1,751	0	0
		5	10	1,178	0	0
	Dinobryon divergens	0	99	72	10	9
		5	42	83	7	5

Table 12b. Cell counts, field Run 4, day 21 (Greenwood Lake).
Numbers are units/ml. P = present.

Lake	Species	Treatment				
		Control	50 µg/l Cu	100 µg/l Cu	800 µg/l Ni	100 µg/l Cu + 800 µg/l Ni
Greenwood (day 21)	Asterionella formosa	0	1,131	296	86	325
		100	882	309	127	168
	Tabellaria fenestrata	225	300	5	65	28
		168	145	39	65	99
	Aphanocapsa delicatissima	29	P	0	13	0
		38	10	16	26	0
	Chroomonas acuta	42	0	10	2	0
		74	62	78	0	0
	Cryptomonas erosa	24	0	5	0	0
		42	62	28	5	0
	Cryptomonas reflexa	28	0	8	0	0
		45	36	8	0	0

Table 13. Cell counts, field Run 5, day 11 (Greenwood Lake).
Numbers are units/ml. NS = no sample. P = present.

Lake	Species	Treatment		
		Control	Large zooplankters removed	Large zooplankters removed + 50 µg/l Cu
Greenwood (day 11)	Asterionella formosa	82	86	238
		NS	NS	97
	Chroomonas acuta	0	67	55
		NS	NS	58
	Cryptomonas spp.	0	P	67
		NS	NS	31