The effectiveness of algal biofilms in the removal of copper, zinc, and cadmium from freshwater systems

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ABSTRACT.

Experiments were carried out in 1985, to determine whether algal biofilms were efficient in the removal of heavy metals from lake water. Acrylic plates were colonized in a field laboratory for one month by organisms from the Humpback Lake Reservoir (Vancouver Island, B.C.). These colonized plates were then subjected to a mixture of copper, cadmium, and zinc at four different concentrations in pulse-dose experiments. Mixtures of metals were at concentrations which corresponded to 1x, 2x, and 5x those allowed for raw public drinking water. Depletion of metal from the solution and uptake of metal by the biofilm were measured by flame atomic absorption spectrophotometry, and the difference was determined as the daily depletion of metal from the solution. These experiments were repeated in parallel for slightly acidified conditions (0.5 pH point less).

Average daily depletion for the three mixtures ranged from 0.13, through 0.35, to 0.91 mg Cu/L and 5.26, 22.12, and 36.55 mg Zn/L under neutral conditions. Under acidified conditions, average daily depletion values were 0.21, 0.43, and 0.34 mg Cu/L and 8.16, 26.68, and 45.01 mg Zn/L for the three mixture. When these values were corrected for uptake by the exposed areas of the tanks, depletion values ranged from 0.12, 0.27, and 0.63 mg Cu/L and 6.47, 23.11 and 42.48 mg Zn/L for the three mixtures. Under acidified conditions depletion values were 0.11, 0.385, and 0.748 mg Cu/L, and 6.272, 24.670, and 36.930 mg Zn/L for the three mixtures It was not possible to determine whether there was any depletion of cadmium, because concentrations in the input solutions were below the detection levels of the methods used.

Average metal concentrations in the biomass ranged from 0.001 (control), 0.011, 0.033, and 0.053 mg Cu/mg Biomass (dry weight) and 0.003 (control), 0.018, 0.021, and 0.028 mg Zn/mg Biomass (dry weight) for the mixtures under neutral conditions. Under acidified conditions uptake values were 0.001 (control), 0.006, 0.010, and 0.014 mg Cu/mg Biomass (d.w.), and 0.003 (control), 0.005, 0.006, and 0.009 mg Zn/mg Biomass (d.w.) When compared to the exogenous concentration, copper was concentrated at factors of $2.1\text{x}10^4$ - $3.3\text{x}10^4$, and zinc at factors $1.1\text{x}10^3$ - $3.7\text{x}10^3$ under neutral conditions. Under acidified conditions these factors ranged from $5.7\text{x}10^3$ - $1.3\text{x}10^4$, and $3.5\text{x}10^2$ - $6.0\text{x}10^3$ for copper and zinc respectively. There was no measurable magnification of cadmium.

Copper depletion from solution values closely related to copper uptake by the algal biomass values under neutral conditions (0.18 vs. 1.26, 2.61 vs. 3.65 and 10.32 vs. 6.63 mg copper for depletion vs uptake respectively). This relationship held to a lesser extent for copper under acidified conditions. The relationship did not hold for zinc under neutral or acidified conditions. Total zinc depletion was much higher than total zinc uptake.

The algal species involved in the metal uptake ranged from a diverse mixture, with *Synedra ulna*, *Tabellaria fenestrata*, and *Achnanthes minutissima* being the most abundant during July - August, to a mix of *Achnanthes minutissima*, *Bulbochaeta insignis*, *Mougeotia* sp., and *Dinobryon* sp. in August - September, to an almost monoculture of *Achnanthes minutissima* during October - November.

It was concluded that although bioconcentration factors may be high, algae may not be effective in the clean-up of water bodies unless the total algal biomass is tailored to the exogenous concentration, or the contact between the metal-containing waters and the algae is prolonged via a long stream, for example. Furthermore, acidification by as little as 0.5 of a pH unit will severely decrease the ability of algae to incorporate metals.

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PREFACE

For my children, Arjen and Melody Spruit

Chapter I

INTRODUCTION AND LITERATURE REVIEW

Heavy metals in the aquatic environment and acid precipitation are among today's most pressing environmental concerns. Although physico-chemical means of control go a long way towards reducing the concentrations of these contaminants, biological control may present an alternative or supplementary method and needs more intensive study.

One such form of biological control that might offer promises, are the algae in fresh water systems. The algae's ability to concentrate heavy metals from aqueous media has been well established in the literature. However, studies of this ability to 'clean up' a stream or a pond that has been contaminated with metals are scarce. Therefore, this project attempts to measure the efficiency of a natural algal community in the removal of metals from freshwater systems under field conditions. In order to address the question fully and fit the study in the context of the existing experience a literature review was conducted related to copper, cadmium, and zinc in aqueous systems (the metals under study).

1.1 GENERAL CHEMISTRY

Metals in freshwater systems can act in a variety of ways. Under alkaline conditions many precipitate out and form less toxic compounds. Under acidic conditions most are held in the water column in some toxic form. In this case, metal toxicity varies depending on the chemical form of the metal, with the free ion generally as the most toxic form.

Peterson *et al.*, (1984) clearly showed the relationship between pH and chemical speciation for cadmium and copper. More than 90 percent of cadmium in water was in ionic form for a pH range of 5.5 - 8.5, whereas copper occurred primarily as the free copper ion over pH values ranging from 5.5 - 7.5, and as inorganic copper complexes in the range 7.5 - 8.5.

Speciation for zinc is not as clearly defined and methods to determine zinc species are still being developed (Florence, 1980; Spear, 1981). Zinc speciation is dependent on alkalinity. At low alkalinity the free (hydrated) zinc ion can comprise from almost 100% at pH 6.5 to 1% at pH 9, while zinc hydroxide ranges from <0.1% to about 0.2% over this pH range.

Metal speciation is affected by complexation with natural ligands. If there is significant competition between a given metal and other metals, complexation of that metal is decreased and the concentration of its ionic form will increase (Sunda and Hansen, 1987).

In natural waters, the controlling mechanism for metal ion concentrations includes the formation of metal-organic complexes and adsorption to the sediment. Guy and Chakrabarti (1975) found that the binding capacity of the following substances to sorb metals decreased in the order:

MnO_2 > humic acid > iron oxide > clay,

while metals were adsorbed in the order:

Pb(II) = Cu(II) > Cd(II) = Zn(II) > Ca(II) > K^+

Sorption of metals onto humic acid and hydrous oxides was found to follow Langmuir isotherms.

1.2 ORGANO-CHEMICAL INTERACTIONS

Live organisms detoxify metals in the aquatic environment in three ways:

1. through the release of soluble complexing agents into the surrounding water,
2. through the adsorption onto insoluble extracellular polymers, and
3. through incorporation into the cell and subsequent inactivation and storage in a nontoxic form.

1.2.1 Release of complexing agents

In natural waters, toxic metals can exist as complexes with both organic molecules or inorganic ions. By releasing complexing agents into the surrounding water, algae can reduce the concentration of toxic free ions available to the cells, or promote incorporation of needed metals. (Ramamoorthy and Kushner, 1975a and b; Jonas *et al.*, 1984; Starodub *et al.*, 1987; Ogiwara and Kodaira, 1989). For example in a wastewater stabilization pond half of the Zn concentration was found to be removed from the solution by an algal secretion which was responsible for Zn^{2+} complexation, and which was of a proteinaceous nature (Kaplan *et al.*, 1987). However, the chelating effects of extracellular material may not become apparent until a phase of maximal exudate production is reached (Kuwabara *et al.*, 1986).

Complexation depends on both the nature of the metal and the chelating agent. Florence (1977) showed that in lake waters with pH 6.0-6.1 copper was associated mainly with organic matter, whereas cadmium existed principally as free Cd ions or simple complexes. Very little cadmium was associated with organic chelators. Nitrotriacetic acid (NTA) and Ethylenediaminetetraacetic acid (EDTA) prevent metal sorption onto sediments, whereas glycine, citric acid, and humic acid showed little or no effect (Dehnad and Forster, 1988). Ionic forms of zinc predominated under these conditions

Complexation of metals by extracellular inorganic and organic ligands is also pH dependent (Benjamin and Leckie, 1981). In general, the degree of complexation increases with increasing pH. However, while lead and copper complexation increased steadily with an increase in pH, cadmium and zinc had almost no complexes between pH 2-6. Above pH 6 complexing increases steadily (Bolter and Butz, 1975). When the pH was held constant at 4.5, complexing of lead, copper, zinc and cadmium increased with increasing organic acid concentrations.

The ability of algae to produce soluble extracellular metal-complexing agents was found to depend on the metal tolerance of the algae. (Jardin and Pearson, 1984). A copper-tolerant strain of cyanobacteria produced a greater amount of copper complexing products than a normal strain, indicating that certain cyanobacterial strains can actively respond to toxic copper concentrations by complexing more of the metal and so reduce the toxic free ion concentration.

Other algae may ameliorate the toxic effect of a metal by binding the metal intracellularly, using a metallothionein-like protein (Hart and Scaife, 1977). Such proteins have been implicated for mussels and fish, but are not well researched for algae.

1.2.2 Metal uptake

When live organisms take up metals, the initial attachment is adsorptive to the outside of cells. Then metals are actively transported across the cell membrane into the cell (absorption), where they can be stored in the cells in less toxic forms (Mang and Tromballa, 1978)

Adsorption

Algae have a requirement for some metals for metabolic and growth processes. To obtain these essential metals, uptake mechanisms must exist.

Initial adsorption to the cell wall may facilitate diffusion, through the cell wall, of the metals necessary for growth and metabolism, or enhance the protective function of the insoluble extracellular polymer by increasing its resistance to decomposition. (Rudd *et al.*, 1984).

Adsorption of nonlethal concentrations of toxic metals onto cell surfaces could induce permeability changes that permit a freer flow of nutrients, and thereby increase cellular metabolic activity (Bollag and Duszota, 1984).

Metal affinities to cells differ. Cadmium was found to have the greatest affinity for the cells of cyanobacteria, while copper had intermediate and zinc the lowest affinity (Les and Walker, 1984). Initial binding was considered to be adsorptive rather than absorptive.

Adsorption of zinc onto the cell surface of green algae was a function of the free zinc ion concentration rather than the total zinc concentration of the growth medium (Bates *et al.*, 1982).

Absorption

Absorption follows the initial adsorption. Mang and Tromballa (1978) found that labelled cadmium was taken up by *Chlorella fusca* by adsorption, followed by diffusion and by energy dependent transport. Zinc inhibited this transport of cadmium. *Anabena floss-aquae* was found to incorporate cadmium (both in the cellular cytoplasm and the polyphosphate bodies of the cell) at concentrations three orders of magnitude higher than the incipient lethal concentration. (Rachlin, *et al.*, 1984). Rebhun and Ben-Amotz (1984) found considerable cadmium absorption by *Chlorella stigmatophora.*

Algae grown in batch cultures were found to have incorporated copper into the cell after 6 days. Copper precipitates were found in the vacuoles, the cytoplasm and the nucleus, but were absent from the cell walls and plastids (Ferstenberg *et al.*, 1975).

Zinc transported into the cells of green algae was a linear function of the free zinc ion concentration of the medium, not of the adsorbed zinc. This suggests that when zinc is transported into the cell, it is not totally derived from the adsorbed fraction (Bates *et al.*, 1982).

Desorption

Release of cadmium from loaded cells of *Chlorella fusca* is possible by diffusion (Mang and Tromballa, 1978). Hart *et al.*, (1979) reported that absorbed cadmium leached out of the cells of *Chlorella pyrenoidosa* at a slow rate, when placed in a cadmium free medium.

1.2.3 Storage of metals

Polyphosphate bodies play a role in the accumulation of heavy metals. Polyphosphate bodies are known to form during periods of nutrient imbalance and when excess inorganic phosphate is present. This accumulation is referred to as luxury consumption or phosphate overplus, and occurs in a wide variety of organisms (Hensen *et al.*, 1982; Dubois *et al.*, 1984; Manley and North, 1984).

The polyphosphate bodies are thought to be a storage compartment for metals, since metals were found to be stored in the phosphate bodies of a chlorophyte (Rosko and Rachlin, 1977: Rachlin *et al.*, 1982), bacillariophytes (Rachlin *et al.*, 1982), and a cyanophyte (Rachlin *et al.*, 1982). Cadmium increased the volume of the cell's polyphosphate bodies for *Chlorella* sp., *Navicula* sp., and *Nitzschia* sp. (Rachlin *et al.*, 1982). Compartmentalization of lead and cadmium into polyphosphate bodies and the cell wall may be a means by which some algae reduce the toxicity of these cations (Jensen *et al.*, 1982).

1.3 EFFECTS OF METALS ON ORGANISMS - TOXICITY

The toxicity to algae of metals is varied. It is dependent on the speciation of the metal, the species of alga, the tolerance of the algal species or strain, and the presence of other elements and metals. Metal toxicity results primarily in a reduction in growth and/or of chlorophyll content.

1.3.1 Metal species

Generally the ionic form of a metal is more toxic than its complexed species. The toxicity of both Cd and Cu increases with increasing pH (5.5-8.8 for cadmium, 5.0-6.5 for copper). This is due to a competition between free metal cations and H^+ ions for cellular binding sites (Peterson *et al.*, 1984). With a lower pH more H^+ ions are available for competition. At higher pH values relatively more Cd^{2+} ions are available for binding. Allen *et al.*, (1980) found that the toxicity of zinc was not related to the total metal ion concentration. They conclude that chelation of zinc reduces toxicity. Florence (1977) found that ionic copper was far more toxic to aquatic organisms than complexed copper, and that the more stable the copper complex the lower its toxicity. The same held true for zinc. Hargreaves and Whitton (1976) found that copper is less toxic than zinc at pH 3.5, and much more toxic than zinc at pH 7 to *Hormidium rivulare*. Copper was found to be toxic to *Anabena floss-aquae* even in low free ion concentrations (Wageman and Barica, 1979). Cadmium, for which organisms have no known use, exists primarily in the ionic form in aqueous media.

In a mixture, metals may interact. Copper and nickel were additive in their toxic effect, as were copper and zinc mixtures (Anderson and Weber,1975).

Cadmium was found to increase the toxicity of lead if the concentration of cadmium was greater than the concentration of lead. With a greater lead concentration there was a marked decrease in the cadmium toxicity (Pietilainen, 1975).

1.3.2 Growth

Copper affects the growth of blue-green and green algae differently depending on the species as well as the concentration of the free metal ion in the water. In general, it reduced growth in most of the species tested, but the effect was highly species dependent (Young and Lisk, 1972).

Zinc, copper, and cadmium were found to be increasingly toxic (in the order given) to growing cultures of *Chroococcus paris* (Les and Walker, 1984). All were found to reduce the growth rate at concentrations greater than 1 mg/L. With increasing cadmium concentrations growth rates were also decreased for a green alga and two diatom species (Rachlin *et al.*, 1982).

Cadmium, copper, and mercury were found to have a greater effect on cell division than on chlorophyll 'a' content in *Chlorella vulgaris*, whereas the reverse was found to be true for zinc and lead (Rosko and Rachlin, 1977). These findings contradict Rebhun and Ben-Amotz (1984), who found that cadmium did not inhibit the growth rate.

The effects of deleterious concentrations of copper in *Chlorella pyrenoidosa* was found to be due not to the copper entering into the plasma, but rather to a binding to the cytoplasmic membrane, after which the cells became unable to divide (Steemann Nielsen *et al.*,1969: Steemann Nielsen and Kamp-Nielsen, 1970). The cells remained alive, although no growth occurred. When the algae were transferred to a copper-free medium they resumed growth (Steemann Nielsen and Kamp-Nielsen, 1970).

Elevated zinc concentrations were highly detrimental to the growth of *Selenastrum capricornutum* affecting all phases (lag, exponential and stationary)

(Kuwabara,1985). Zinc inhibited growth of *Selenastrum* sp. (Kuwabara *et al.*, 1986).

1.3.3 Chlorophyll

Cadmium decreased photosynthesis and the growth rate of *Chlorella pyrenoidosa* (Hart and Scaife, 1977). Rebhun and Ben-Amotz (1984) found that cadmium did not inhibit growth of the marine alga *Chlorella stigmatophora*, but a considerable decrease of chlorophyll content was established. Loss of chlorophyll from *Anabena floss-aquae* was also recorded by Rachlin *et al.* (1984). They state that cadmium caused significant reductions in the surface area of the cell's thylakoids.

Photosynthesis was found to be more affected than growth in natural populations of phytoplankton under copper stress (Cote, 1983). Copper was found to adversely affect the rate of photosynthesis of mature periphyton communities, but the rate did not decline for periphyton on newly-colonized surfaces (Leland and Carter, 1985).

1.3.4 Other toxic effects

Several other morphological changes have been observed. For example, cadmium toxicity decreased the size and number of the cell's polyphosphate bodies of *Anabena floss-aquae* (Rachlin *et al.*, 1984). These writers also mention a marked shrinking away of the plasma membrane from the cell wall and that cadmium causes the polyphosphate bodies to lose Mg and Ca.

Bartlett and Rabe (1974) studied the effects of combined metals on *Selenastrum capricornutum* and found that combinations of copper, zinc and cadmium were similar in toxicity to equal concentrations of zinc. Combinations of copper and cadmium resulted in a greater growth rate than equal concentrations of copper, suggesting that cadmium inhibits copper toxicity.

Enzyme activity in phytoplankton cultures was inhibited by free copper ions (Rueter, 1983).

1.4 EFFECTS OF METALS ON ORGANISMS - TOLERANCE

Tolerance is dependent on the speciation of metal and the species of alga. Tolerance for one metal does not necessarily extend to another metal. For some metals tolerance may be lost when the algae are exposed to a metal-free environment, while for others, tolerances are maintained.

Stokes (1975) stated that tolerance is correlated with the presence of metals in the environment and appears to be specific for each metal. For cultures of *Scenedesmus* and *Chlorella*, copper tolerance required the presence of copper in the medium. Having been exposed to both copper and nickel, copper-depleted cultures lost their tolerance for copper but retained their tolerance for nickel.

Tolerant cells accumulated more of a metal than the non-tolerant counterparts of the same strain (Stokes, 1975). A copper tolerant alga showed increased tolerance to nickel, zinc, silver and cobalt when compared with a reference isolate (Stokes, 1981). Tolerance may be due to the ability of metal-adapted strains of algae to produce complexing compounds (Jardin and Pearson, 1984).

When isolates from a mine-contaminated river were subjected to copper, lead, zinc and cadmium, they were all found to be resistant to the levels normally present in their habitat. Furthermore copper tolerant green algae from areas with a high copper concentration tended to be also lead resistant, but algae from high lead sites were copper sensitive (Foster, 1982a).

Tolerant organisms often take up metals in greater amounts than their non-tolerant counterparts. These organisms are thought not to restrict metal uptake, so that metal exclusion is unnecessary. However, Foster (1977) found that a copper tolerant strain of *Chlorella vulgaris* excluded copper. When comparing sensitive and nonsensitive algal species Button and Hostetter (1977) found that copper remained bound to the cell wall of the nonsensitive species, whereas it readily entered the sensitive species.

Tolerant strains of a given species tend to have a lower growth rate. Growth of metal tolerant strains of Cyanobacteria was slower than that of normal strains in the presence of low copper concentrations in the medium (Jardin and Pearson, 1984). Green algae were more resistant to the effects of cadmium than were blue-greens (Hart and Scaife, 1977).

1.5 UPTAKE OF METALS - MAGNIFICATION IN THE LABORATORY

A number of metal-uptake studies have been carried out in the laboratory under controlled conditions. Most were carried out with a single algal species and a single metal.

For example, *Anabena floss-aquae* was found to incorporate cadmium at concentrations three orders of magnitude higher than the incipient lethal concentrations. Cadmium was incorporated into both the cellular cytoplasm and the cell's polyphosphate bodies (Rachlin *et al.*, 1984). Rebhun and Ben-Amotz (1984) found considerable cadmium uptake by *Chlorella stigmatophora*.

Jonas *et al.* (1984) question the validity of such studies. They state that the components of a nutrient culture medium can cause changes in the physicochemical equilibrium of the metal species compared to the equilibrium found in natural waters. Furthermore, such experiments have been restricted to the culturable component of the community, which limits the usefulness of such bioassays. They conclude that toxicity testing with biological organisms should be conducted under conditions as similar as possible to those existing in the natural environment.

1.6 UPTAKE OF METALS - FIELD EXPERIMENTS

Field studies in different environments have shown that algae accumulate and concentrate metals, regardless of the climate or whether they occur in lotic or lentic environments. In all cases uptake and concentration appears to dependent on the exogenous free metal ion concentration.

Periphyton was found to concentrate copper and zinc about 1.7×10^4 times compared to the concentrations in the water. (Hutchinson *et al.*, 1976: Patrick and Loutit, 1976).

In field studies of English lakes contaminated with zinc smelting waste, green algae concentrated copper at factors ranging from 1.8×10^3 to 8.3×10^4, and zinc at factors 5.2×10^2 to 9.0×10^3 depending on the concentrations in the surrounding waters (Trollope and Evans, 1976). Denny and Welsh (1979) studying British lakes found lead concentration factors in phytoplankton in excess of 10^5. Field samples of filamentous green algae from a Cornwall river were found to have concentrated copper at factors 5.0×10^2 to 3.0×10^4, depending on the concentrations in the surrounding waters (Foster, 1982).

Studies of plankton organisms in a heavily polluted coastal area of Sweden showed that metal concentration in the plankton was greater than the metal content in the water (Lindahl *et al.*, 1983). Iron and copper were most often accumulated, and were found to be associated with the silica frustules of diatoms.

Metal uptake by periphytic algae was dependent on the position of the periphyton in the water column, since metal concentrations vary in the water column (Friant and Koerner, 1981). In an area (South Carolina) contaminated by coal ash all metals were more concentrated in the sediment and the biota than in the water column (Cherry and Guthrie, 1977). Algae cultured in dialysis tubing in a river in Connecticut concentrated copper considerably (Klotz, 1981).

In Lake Ontario (Canada) zinc, cadmium, lead and copper were found to have concentration factors of respectively $2.9\mathrm{x}10^3$, $4.9\mathrm{x}10^4$, $1.6\mathrm{x}10^4$, and $2.2\mathrm{x}10^3$ in the alga *Cladophora glomerata* (Keeney *et al.*, 1976).

Ndiokwere (1984) reported that algae in the River Niger (Nigeria), accumulated metals from the water, although concentrations in the algae were lower than concentrations in the sediments.

These data, although interesting, do not indicate whether algae can be used efficiently as metal scavengers for the purpose of removing metal from contaminated waters. To answer such a question, studies need to be carried out that measure the metal concentration of the water before and after it has been exposed to the algae. Only one such study was found in the literature. At a mine-site in Missouri, Jennet and Wixson (1975) have shown that a one-kilometer-long stream containing primarily *Cladophora* sp. was an effective metal remover.

1.7 FACTORS AFFECTING METAL UPTAKE

All physical, chemical, and biological factors of the environment affect metal uptake. These include pH, time, concentration of the metals in the surrounding water, the presence of other elements, temperature, light, the nature of the organic and inorganic matrix in between algal cells, availability of binding sites, the algal species and algal concentration, and the leaching capacity of the cells.

1.7.1 Uptake and pH

Metal uptake is directly related to pH. Although the free metal ion concentration in water increases with decreasing pH, more metals are taken up at relatively higher pH levels. This is due to the competitive behaviour between metal ions and H^+ ions. H^+ ions are preferentially adsorbed in waters with high H^+ ion concentrations.

Gipps and Coller (1980) found that cadmium uptake in *Chlorella pyrenoidosa* was affected by both the pH and the temperature. Uptake increased with pH and temperature. Les and Walker (1984) found that the amount of metal bound increased with pH. A high pH microenvironment around photosynthesizing microbes represents a condition unfavorable for Zn desorption. (Kuwabara *et al.*, 1986).

The amount of heavy metal bound by sewage sludge is positively correlated to the pH (Tam and Wong, 1983). The stability of heavy metal complexes in sludge is pH dependent, and is decreased at lower pH values. The concentrations of metals released from sludge to the supernatant increased with decreasing pH (Adams and Sanders, 1984).

Adams (1985) found that the quantity of metal taken up by sewage sludge at a constant pH increased with added metal concentration. While in some cases there appeared to be a saturation point, the author states that his data did not fit Langmuir or Freundlich adsorption isotherms closely.

Increased pH levels promoted zinc uptake and retarded zinc loss in the marine algae *Ulva*, *Porphyra* sp. and *Laminaria* sp. A similar relationship occurred for dead cells (Gutknecht, 1961 and 1963)

1.7.2 Uptake and time

The rate of metal adsorption can be rapid. Les and Walker (1984) stated that the majority of metals (copper, zinc and cadmium) were bound to algal cells within 1 minute and nearly all of the metal that was ultimately taken up was bound within 10 minutes.

The rate of absorption is dependent on the kind of metal present. For example zinc, nickel, chromium, iron and manganese were removed continuously by periphytic algae , while copper, cadmium, cobalt and lead were found to be taken up in the first two hours after which uptake was only slight (Vymazal, 1984).

When algal cells become older, they can accumulate larger amounts of heavy metals (Bollag and Duszota, 1984). In natural systems, considerable seasonal variation may occur with metal concentrations at times far exceeding what would be expected according to the estimated free ion activity during the summer (Deniseger, 1985).

1.7.3 Uptake and the exogenous concentration

Metal uptake is dependent on the concentration of the particular metal in the surrounding water. In general with an increased exogenous metal concentration metal uptake by organisms increases. This holds true for freshwater (Trollope and Evans, 1976; Hart and Scaife, 1977; Hart *et al.*, 1979; Friant and Koerner, 1981; Foster, 1982b) estuarine (Rebhun and Ben-Amotz, 1984), and marine algae (Gutknecht, 1961), as well as for microalgae in estuarine mud (Lion *et al.*, 1982).

Other factors may play a role. For example, dependence on the concentration of cadmium ions could be limited by the presence of PO_4^{3-} ions (Gipps and Coller, 1980). Hart *et al.*, (1979) indicated that the cadmium transport system could be saturated above certain levels. Furthermore, uptake may be dependent on the metal itself. Foster (1982b) found that algal zinc concentrations were nearly constant and independent of the external zinc concentration which suggested that the uptake of zinc may be regulated by the algae.

1.7.4 Uptake and temperature

Gipps and Coller (1980) suggested that metal uptake appears to be directly related to the temperature of the surrounding water, although few studies were found in the literature to support their hypothesis.

Hart and Scaife (1977) state that *Chlorella pyrenoidosa* did not sorb cadmium at 4 °C. Uptake was higher at 31 °C than at 21 °C (Hart *et al.*, 1979).

1.7.5 Uptake and light

Light also appears to play an important role, but only few studies were found in the literature. In a freshwater laboratory system, zinc uptake was stimulated by light, suggesting that the uptake mechanism is related to photosynthesis (Cushing and Rose, 1970). Exposure to light stimulated both uptake and the loss of zinc in larger marine algae (Gutknecht, 1963).

Hart and Scaife (1977) found no uptake of cadmium by *Chlorella pyrenoidosa* in the dark. This light dependence suggests an active transport process requiring ATP derived from photophosphorylation. In a later paper (1979) Hart *et al.* state that uptake in the dark is decreased by 25 percent when compared to uptake in the light (75 w incandescent bulb).

1.7.6 Uptake and the organic matrix

When algae attach to a substrate, a great deal of insoluble organic and inorganic matter may get entangled in amongst the algae. The algae themselves may lay down a mucilagenous layer. Dead cells and diatom frustules may all be part of this matrix.

Several writers have studied the adsorptive capacities of live cells compared to dead cells. In all cases, dead cells were found to adsorb considerably higher concentrations of cadmium and other metals than live cells. This phenomenon occurred in bacterial strains (Bollag and Duszota, 1984), fungi (Tobin *et al.*, 1984) and in a chlorophyte (Mang and Tromballa, 1978).

Dead cells of *Ulva*, *Porphyra*, and *Laminaria* adsorbed more zinc than the live seaweeds (Gutknecht, 1963). On the other hand, Hart and Scaife (1977) found that there was no uptake of cadmium by dead cells of *Chlorella pyrenoidosa*. and Cushing and Rose (1970) found that dead cells took up less zinc than live cells, The sheath material of *Chroococcus paris* did not seem to cause a greater affinity for metals in the organism compared to organisms that do not produce sheaths (Les and Walker, 1984). When exposed to cadmium, copper and zinc this alga sorbed the total amount of these metals in the medium within one minute.

A study of settling particles in Lake Zurich shows that biogenic material is very important for removing metals from the water column, especially copper and zinc, but also lead, cadmium and chromium. High removal rates from the water column of copper, zinc, cadmium, and lead coincided with high sedimentation rates of organic matter (Sigg *et al.*, 1987) and uptake by the dead cells was considerable.

1.7.7 Uptake and the inorganic matrix

Inorganic material may be abundant in amongst the periphyton community especially in areas with high concentrations of hydrous ferric and manganese oxides or large amounts of particulate matter in the water.

The influence of this matrix on measurements of metal uptake by algae is dependent on the nature of the particulate matter and the kind of metal.

1.7.7.1 Inorganic particulate matter.

In cultures, Zn adsorbed on particulate matter was not as readily available to *Selenastrum* sp. as were the dissolved fractions, but Zn adsorbed on particles could become available to the algae when the exogenous concentration decreased. Inorganic particles could thus serve to increase the pool of nutrient or toxic substances and to some extent could buffer ion activities if desorption kinetics are rapid with respect to algal uptake rates (Kuwabara *et al.*, 1986).

Florence (1977) found that trace metals were not adsorbed on inorganic colloids in his experiments. Though adsorption on hydrous ferric oxides is known to occur in some cases (Newman *et al.*, 1983 and 1985), this did not occur when iron concentrations were low (Florence, 1977).

Uptake by clay at pH 5 showed a Langmuir type adsorption isotherm. In this experiment, with constant initial concentrations of copper and zinc, adsorption increased with the pH until the threshold for precipitation was exceeded (pH 7) (Slavek and Pickering, 1981).

1.7.7.2 Hydrous ferric and manganese oxides.

When present amongst the periphyton, hydrous oxides can play an important role in the uptake of metals. Indications are that this uptake is metal specific and dependent on the availability of the iron and manganese oxides.

Organic matter was more efficient in metal uptake than inorganic matter in Lake Zurich. In addition to the biogenic material iron oxides contributed in the scavenging of Cu, Zn, Pb, and Cr and settled with the biological material, while precipitated manganese oxides took up other elements (Sigg *et al.*, 1987).

When estuarine muds were cleaned of their organic components, the adsorption of cadmium was decreased by a factor 4, indicating the importance of the organic component (Lion *et al.*, 1982). These researchers also noted a possible partitioning of metals and their adsorption. Substantial fractions of extracted copper and cadmium were associated with the organic component of the mud, whereas lead was associated primarily with the Fe-Mn oxide coating.

Jones (1986) found that under acid conditions (pH 3) hydrated ferrous oxides precipitated in a Welsh river, which resulted in subsequent binding of metals with the oxides. In this river system cadmium remained in solution.

Newman *et al.* (1983) found that lead was mostly associated with an iron- and manganese-rich matrix. In another field test Newman *et al.* (1985) found similar patterns for periphyton collected in an area with a high fly ash incidence.

Davies and Leckie (1978) suggest that trace metals in natural water systems may be binding onto colloidal particles that are coated with humic compounds, rather than reacting simply with oxide surface sites.

1.7.8 Uptake in the presence of other elements

The presence of other elements and metals can lead to antagonistic or synergistic behaviour of the metals. The adsorption of a given metal by activated carbon was hindered by the presence of other metals, indicating that metals compete for adsorption sites (Netzer and Hughes, 1984). Combination experiments, with any combination of three metals acting at the same time, showed that there was synergism with regards to toxic effects between manganese and copper, antagonism between manganese and lead, and antagonism between copper and lead (Christensen *et al.*, 1979).

Hart and Scaife (1977) state that calcium, magnesium, molybdenum, copper, zinc, or cobalt did not affect the uptake of cadmium by *Chlorella pyrenoidosa*. Manganese and iron did inhibit cadmium uptake by this alga (Hart *et al.*, 1979). Copper and cadmium compete with algae for phosphate ions (Briand *et al.*, 1978).

Calcium and zinc ions have chemical properties that are similar to cadmium ion (Abel and Baerlocher, 1984) and zinc and cadmium compete for active sites.

High concentrations of zinc increase the chance of this ion being bound, which reduces the toxic effect of cadmium. Les and Walker (1984) mention several other instances of metal competition. Manganese partially relieves inhibition of photosynthesis by copper. Cadmium uptake decreases when approximately equimolar amounts of Na, Mg, C, Ca, Mn, Co, Ni, and Zn are incubated with the toxic metal. Cadmium inhibits nitrogen fixation in *Nostoc* sp..

It is possible, that these contradictory results are due to the nature of the binding between the metal and the algae. What holds true for one metal species , may well be reversed in another. In mixed metal experiments, harmful effects should not be attributed to absolute concentration of the excess metal, rather to the ratio of this metal to other metals present (Wong and Beaver, 1979). These authors cite similar patterns found in studies of diatoms, concluding that algal growth inhibition is related to the ratio of copper and zinc, not to the absolute activities of either.

1.7.9 Uptake and available binding sites

Studies on the uptake of metals by biomass are complicated not only by the nature of the metal species, but also by the nature of the adsorbent materials Metals compete for these adsorption sites (Netzer and Hughes, 1984; Tobin *et al.*, 1984).

The nature of bonding between metal ions and the algal cell walls is thought to range from covalent to ionic charge bonding, with copper more covalently bound and zinc bonding more like ionic charge bonding (Crist *et al.*, 1981). Hart *et al.* (1979) found that cadmium uptake in *Chlorella pyrenoidosa* is carrier mediated and an active process that occurs against a cadmium gradient. However, even although cadmium is bound inside the cells, slow leaching of intracellular cadmium out of the cells does occur.

The chemical make-up of the extracellular materials surrounding a cell affects the kinds and concentration of metal bound. Rudd *et al.* (1984) found that metal (copper and cadmium) uptake occurred after the initial complexation capacity had been exceeded, which suggests the presence of more than one binding site, but nickel adsorption ceased when the complexation capacity was reached.

1.7.10 Uptake, algal species and algal concentration

Changes in algal cell numbers can affect solute partitioning and chemical speciation in aqueous and suspended particulate phases (Kuwabara *et al.*, 1986). Briand *et al.* (1978) found that the binding capacity for copper, cadmium and other metal ions was related to the species composition rather than to the total biomass. Most of the binding in their experiment was accounted for by certain species of greens, diatoms and chrysomonads that constituted a minor fraction of the total algal volume. On the community level, the major site of sorption of zinc in natural matlike periphyton was the upper surface of the community (Rose and Cushing, 1970).

1.7.11 Uptake and sampling devices

Materials used in sampling, such as sampling plates or slides, storage containers, and sample handling equipment can be an important source of possible contamination (Erickson, 1977). Soda-lime glass has a high ion-exchange capacity and high levels of trace metal impurities. High purity plastics (polyfluorocarbons, polyethylene, and polymethylacrylates) are low in trace impurities, although there can be considerable variation in metal impurities depending on the manufacturing process. Karin *et al.* (1975) reported that metals were present on or just below the surface of polyethylene. Polymer stabilizers could react with metals in solution and interfere with metal analysis (Heiden and Aikens, 1977). Robertson (1968) found that Plexiglass contained <10 ppb copper and zinc compared to 4 and 90 ppb respectively for polyethylene and 22 and 9.3 ppb respectively for Teflon. Borosilicate glass contained 730 ppb zinc.

1.8 PRESENT EXPERIMENT

The complexity of metal-organism, metal-organics, as well as metal-metal interactions is apparent. It is clear that realistic measurements of metal sorption by algae cannot be derived from the study of a single biologically active element and a single species under laboratory conditions. All such elements and species follow characteristic spatial and temporal variations in the environment and their effects are tightly interrelated. The following experiment therefore proposed to test the uptake of metals by an algal film using a mixture of three metals (zinc, copper, and cadmium) in natural water under field conditions. Zinc, copper and cadmium were chosen because zinc and copper are known to be essential to plant life, while cadmium has no known function. In addition, they are most commonly mentioned as contaminants in freshwater environments. Finally, their concentrations are among the highest found in the Buttle Lake system. This is the only freshwater body on Vancouver Island that is affected by active mining operations at this time.

Several baseline mixtures were considered:

1. Metal levels allowable for aquatic life. These were rejected as experimental concentrations because levels were too low to be easily measured directly by flame atomic absorption spectrophotometry.

2. Average metal concentrations found yearly in Buttle Lake. These were rejected because they were too variable to make a representative choice.

3. Spring concentrations in Buttle Lake were considered too variable and too high to work with safely in a field laboratory, where contamination could mean contamination of the public drinking water

4. Metal concentrations allowed in untreated (raw) public drinking water. These were chosen, because of their mid-level quality, which might allow larger plants and animals to survive but would kill off sensitive or smaller species.

Chapter II

MATERIALS AND METHODS.

2.1 General outline of the experiments.

The objective of the experiment was to test the uptake of metals by attached biofilm organisms. To this end several experiments were proposed (Appendix 1), which led to a prototype field design in 1984. Since this design was drastically altered in 1985, only a short description will follow here, and a more extensive description is given in Appendix I.

In 1984, acrylic plates were suspended in Myra Creek (Vancouver Island, B.C.) to be colonized by organisms known to have metal tolerant strains downstream from a copper mine. During this time, a field laboratory was built at the Humpback Lake Reservoir (Vancouver Island, B.C.). A drip-flow system was set up consisting of 2 wetbenches (to be used as cooling systems) in which glass tanks were placed to receive the colonized plates and to subject them to metal and acid stress. After one month the plates were transported in coolers from Myra Creek to the field laboratory, where they were subjected to metal solutions.

Initially these metal solutions were from the original tailings obtained from the tailing ponds of the mine. The high pH (9.75) of the tailings (due to alkalinity of the tailing ponds) however, made it impossible to extract the metals from the tailing slurry. Therefore, stock solutions were prepared from metal acetates at pH levels ranging from 2.5-3. At this pH all organisms died within a short time.

The pH was therefore increased to 6 in the final experiments, since the experiment was designed to measure metal uptake by live organisms. It was also thought that nutrients might be a limiting factor, and a hydroponic fertilizer was added to the stock solutions.

After an experimental run of one month, the daily metal concentrations of the stock solutions as well as the outgoing solutions were measured and were found to contain insignificant amounts of metals, by the time they reached the colonized plates. It was thought that the addition of nutrients precipitated the metals out of the stock solution. In addition to this, the opaque plastic buckets that contained the stock solutions were found to have a thin layer of green or blue green algae growing on the inner surface, and these were thought to have contributed to the metal loss from the stock solution. Furthermore, the colonized plates had been set in parallel with the flow of the water, thereby reducing contact of the organism with the metal containing water.

As a result, the design of the experiment was changed to include

- the sterilization of lake water, through filtration followed by UV irradiation to prevent algal growth in the stock solution,
- a decrease in the amount of stock solution in the field, i.e. instead of a large bucketful, 1 liter plastic bags were used. These bags were covered with black plastic to block the light. The smaller bags reduced the exposure time and prevented possible build-ups of algae in the stock solutions,
- an altered tank design, (described later) made of acrylic, that ensured greater contact of the metal solution with the organisms,

- and after one experimental run with the new system, the addition of a 'blank' experiment where clean plates were subjected to the same treatment as the colonized plates.

With this new system two sets of three experiments were carried out in 1985, each lasting about one month. The first experiment (Experiment 1) ran from July 20 to halfway August 11, the second from August 23 to September 27 (Experiment 2) and the third from October 8 to November 12 (Experiment 3). Each set of three experiments consisted of the colonization of acrylic plates for one month. These colonized plates were then placed into tanks and subjected to four different metal treatments. The metal content of the water going into and coming out of the tanks was measured and the difference was calculated to give a measure of metal depletion.

At the end of each experiment the acrylic plates were removed from the tanks and the biofilm was scraped off and collected for biomass, metal content, and species analysis. This process was run under both neutral (Experimental set 1) and slightly acidified conditions (Experimental set 2).

2.2 Location

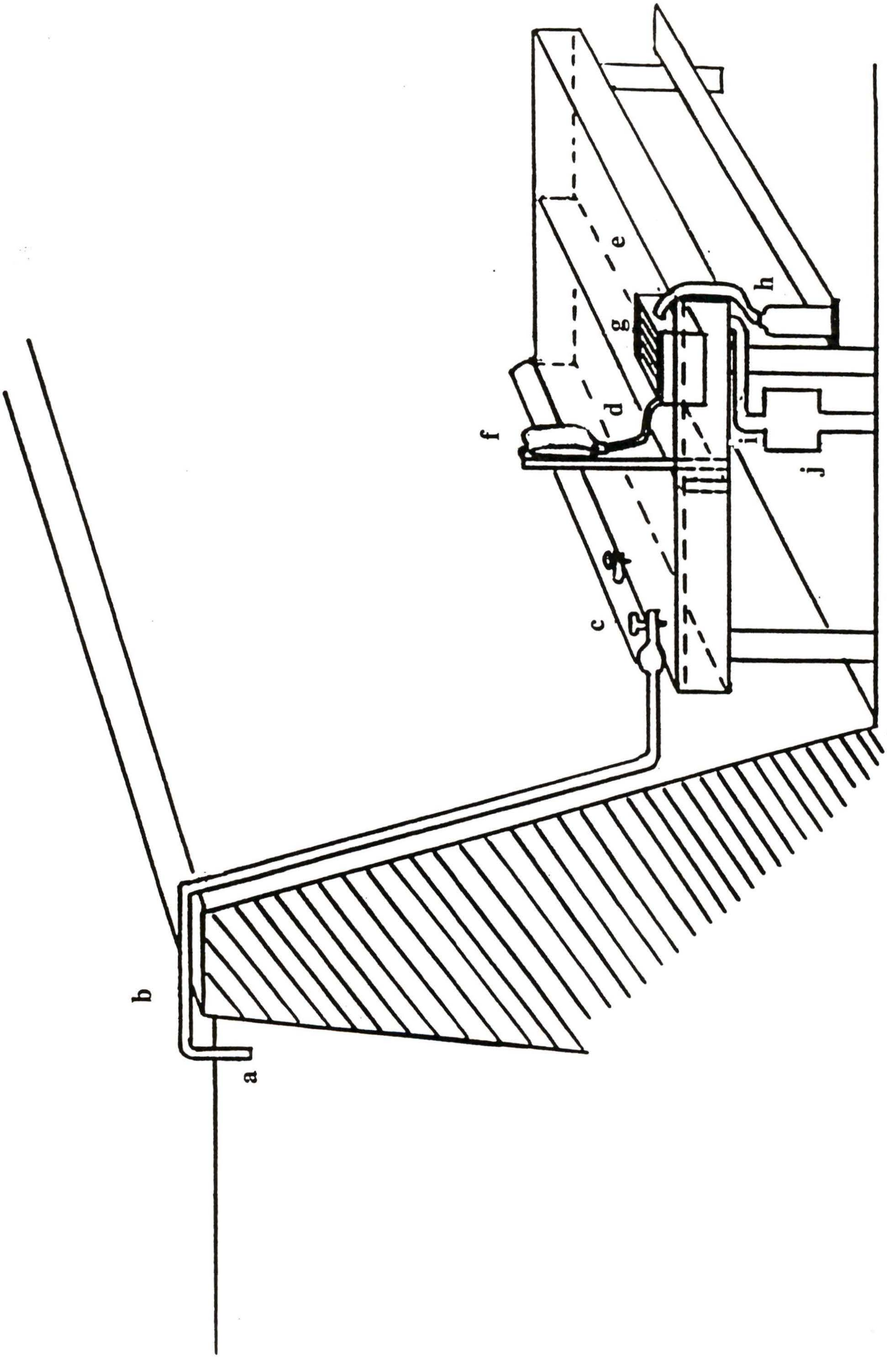
The experiments were carried out in a field laboratory at the Humpback Reservoir, Vancouver Island B.C. This site was chosen primarily for the purity of its water, since the Humpback Reservoir supplies the drinking water for the Greater Victoria region.

2.3 Waterpath

Water was taken from one meter below the surface of the reservoir. It was syphoned over the dam through PVC piping to a manifold, which emptied into a plastic covered wooden wetbench (1.3 x 2.6 m by 22.5 cm) (Figure 1).

Figure 1: Experimental field laboratory.

. Water was taken 1 m below the surface (a) and siphoned over the dam through PVC tubing (b), it then entered a manifold (c), which entered into the colonization compartments (d), From there the water passed over a low divider into the cooling compartment (e), where FEN containers (f) dripped into experimental slotted tanks(g) and outflowing water was collected in PVC bottles (h). The cooling water was carried out of the wetbench via a drain (i), and passed through an ion exchange column (j) before being drained into the environment


a
b
c
d
e
f
g
h
i
j

The wetbench was divided into 5 sections, 4 on the north side of the bench were used for colonizing plates to be used in the experiments, and the fifth was used as a cooling system for the experimental tanks. Wastewater from the experiment was passed through two ion exchange columns, to prevent metals from entering the surrounding environment, before being discharged into the stream below the dam. Tests of the wastewater discharged to the stream showed no measurable copper, cadmium or zinc.

2.4 Colonization

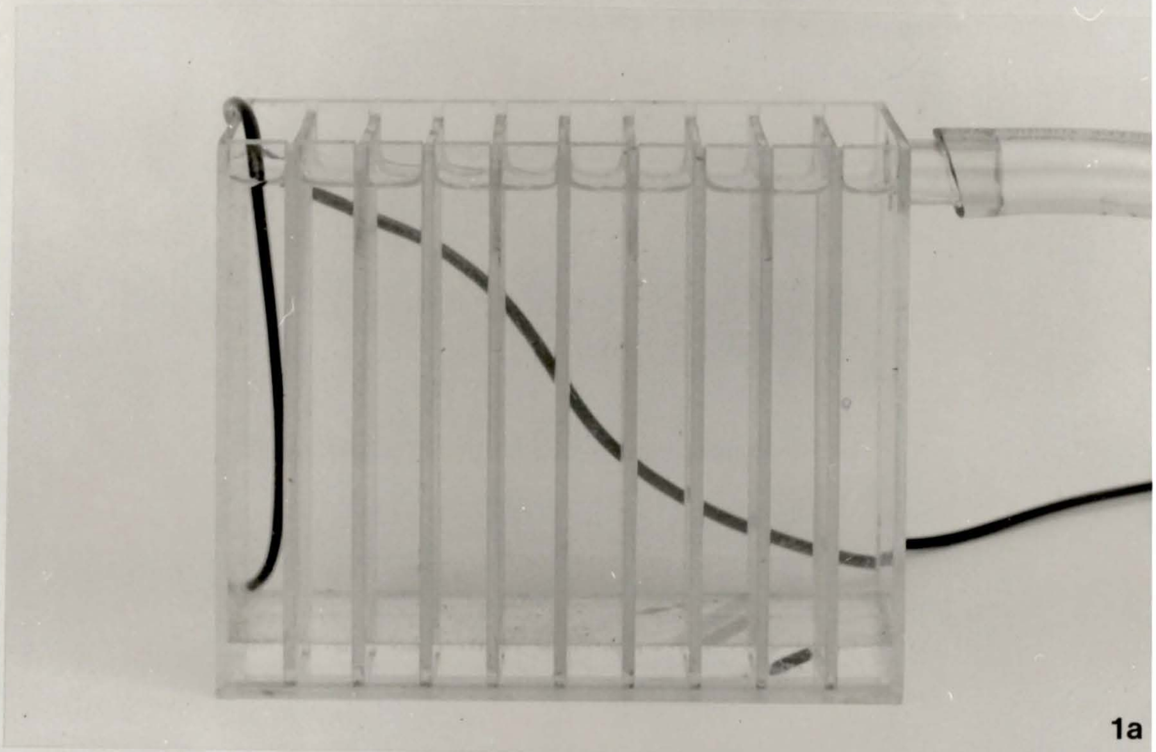
Clear acrylic, plates (11.3 x 14.2 cm) were evenly sanded to roughen the surface, and facilitate attachment of the periphyton biofilm. Acrylic was chosen, because it is generally low in metal impurities (depending on the manufacturing process). Regular glass has a high ion exchange capacity and high levels of trace metal impurities (Erickson, 1977). Acrylic can also be shaped more easily and does not break as easily as glass. The number of plates made available at the start of the first experimental interval was insufficient to collect samples for species analysis, and only samples for metal content could be taken.

The plates were placed upright in slotted strips of PVC and submerged in the colonization partitions where they remained for a month. The plates were aligned with the direction of the water flow to ensure maximum exposure. During the first week the plates were exposed to a high flow rate (turnover time 3 hours). After the first week flow was reduced to a turnover time of once every 24 hours. Colonization was allowed to continue for another three weeks, after which the plates were transferred to slotted tanks where the metal depletion experiment took place.

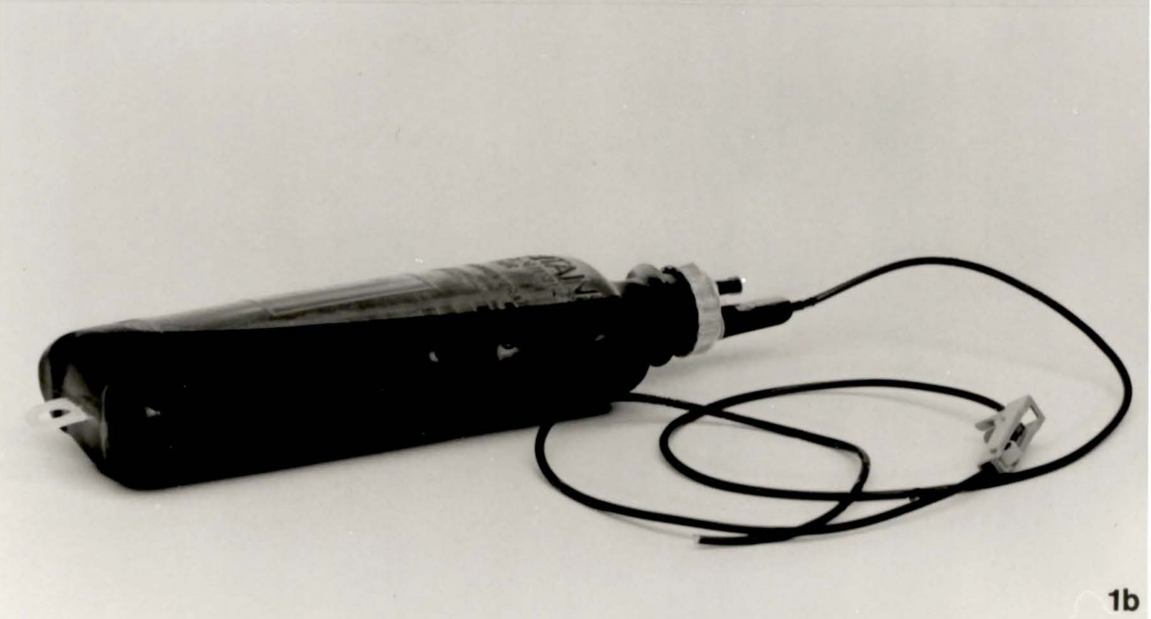
An effort was made to colonize under conditions of metal stress, but an accurate dosage rate could not be maintained, and the procedure was abandoned. After one month the plates were transferred to clear acrylic tanks (15.4 x 12.5 x 18.4 cm) (Fig. 2a). The tanks were designed to hold 9 plates snugly in slotted grooves. The plates were inserted in such a way that the water described a vertically meandering path (Fig. 3) over the attached biofilm community and the tanks were covered with acrylic lids held down with an elastic band.

Figure 2: a. Slotted acrylic tank with inserted sanded plates and b. FEN container used to dispense metal solutions.

. Photograph a. shows the slotted tanks, with the plates inserted. The metal solution traveled from the middle of the first compartment to the left bottom corner, from where it flowed up to the right top corner, and down to the bottom left corner, continuing in this pattern until it reached the outlet. Photograph b. shows the FEN bag with the dispenser system attached which allowed for a slow drip-rate.



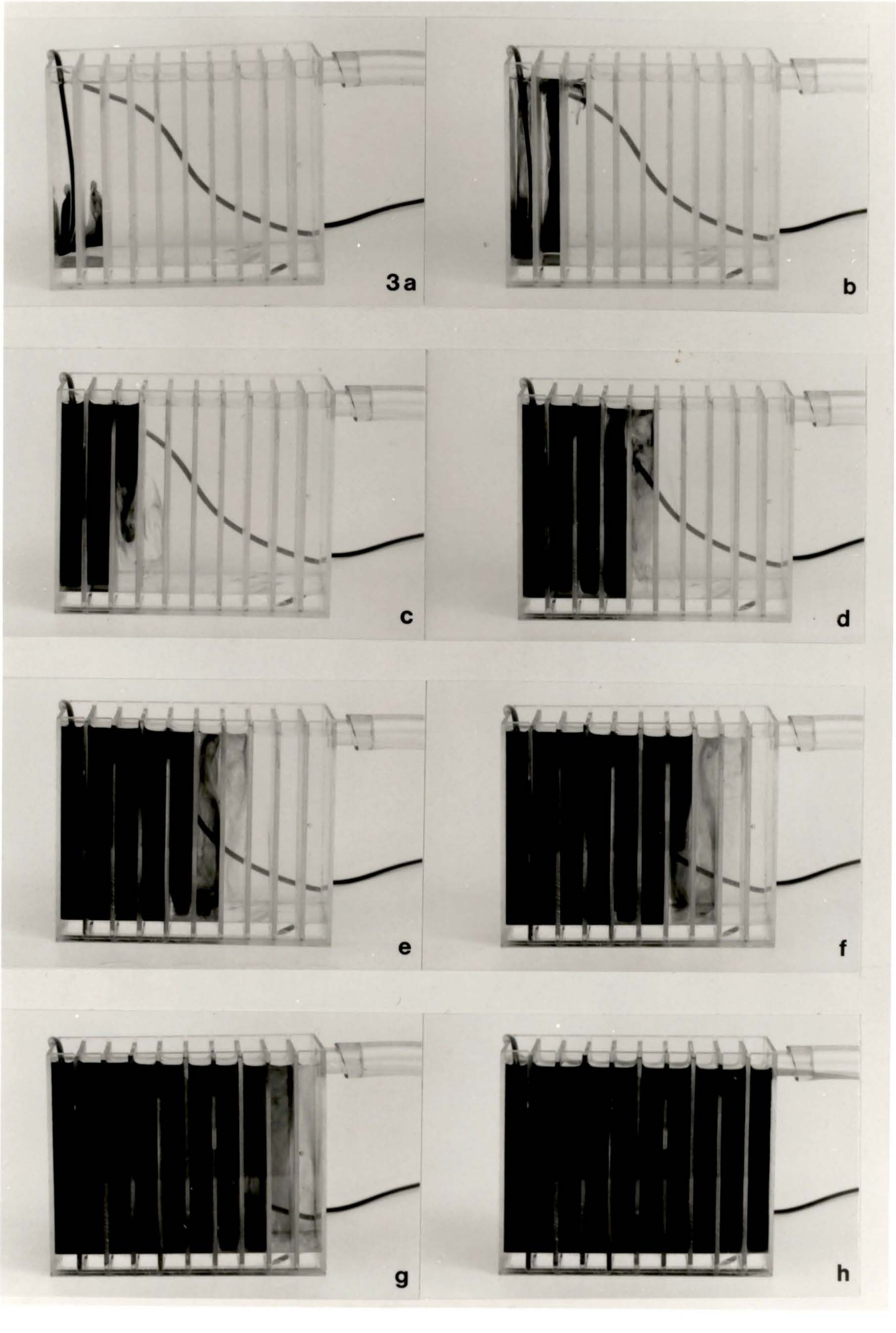
1a



1b

Figure 3: Water path in the tanks.

. Dye test showing the vertical meandering path taken by the water solution through the tank (moving from 3a through to h).


3a
b
c
d
e
f
g
h

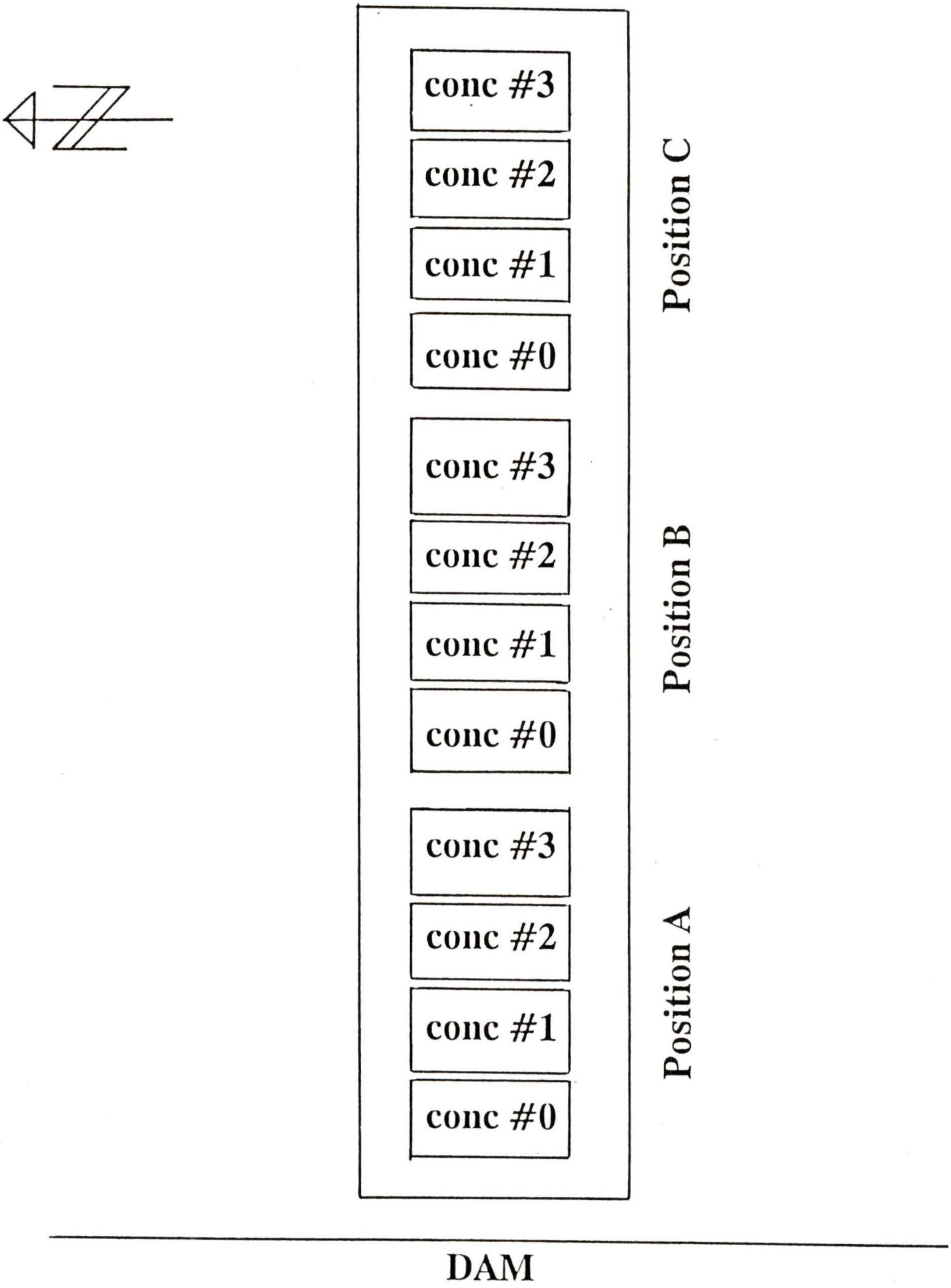
2.5 Experimental design

Humpback lake water was transported to the laboratory at the University of Victoria, Victoria, B.C., and sterilized for at least 24 hours with an Angstrom 2537 UV sterilizer (model AN-8). Copper, zinc and cadmium were added from metal acetate stock solutions to make up 20 liters of metal mix stock solutions and for both the neutral and the acidified treatments; the pH of the stock solutions was adjusted to about 6 for the acidified treatment, and remained unchanged for the neutral treatment.

Concentrations for a mix of three metals (Cd, Cu and Zn), were made up at values of 1, 2, and 5 times the levels allowed for raw public drinking water (Reeder *et al.*, 1979; Demayo and Taylor, 1981; Taylor and Demayo, 1980), i.e drinking water before it has received chlorine and alkaline treatment. For the metals involved the allowable concentrations are; 5 mg/L Zn, 0.5 mg/L Cu and 0.01 mg/L Cd, which were the concentrations used for tank 1. These solutions were used to fill 1 liter Flexiflo Enteral Nutrient (FEN) containers (plastic bags holding nutrient solutions and used in forced feeding) with Flexiflo dispenser systems obtained from the Victoria General Hospital in Victoria (Fig. 2a). The containers were covered with black plastic garbage bags to prevent air bubbles from forming and creating airlocks. Three sets (A, B, and C) of tanks were set up with 4 tanks per set as is shown in Figure 4. This set-up was repeated for an otherwise identical acidified set of experiments.

Figure 4: Tank position.

. Orientation of the tanks and their ingoing concentrations with respect to the dam, where Concentration #3 is the highest, and Concentration #0 the lowest. Position A was subjected to the same treatments as B and C, except that there were no organism and the experiments were kept in the dark.


conc #3
conc #2
conc #1
conc #0
Position C
conc #3
conc #2
conc #1
conc #0
Position B
conc #3
conc #2
conc #1
conc #0
Position A
DAM

Tank 0 received U.V. sterilized lake water from the FEN bags and was used as the control (Concentration #0).

Tank 1 received approximately 5mg/L Zn, 0.5 mg/L Cu and 0.01 mg/L Cd. (Concentration #1).

Tank 2 received approximately 10 mg/L Zn, 1.0 mg/L Cu and 0.02 mg/L Cd. (Concentration #2).

Tank 3 received approximately 25 mg/L Zn, 2.5 mg/L Cu and 0.05 mg/L Cd. (Concentration #3).

In order to determine whether the position in the wetbench had any effect on metal depletion, three sets of 4 colonized tanks, sets B, C, and D were tested during the first one-month period. Preliminary statistical analysis showed no difference in uptake due to position, and set D was dropped from the experiment. Position A was used to determine the effect of the acrylic plates by themselves. To this end the tanks were filled with blank plates, subjected to the same concentrations as the colonized plates, and all four tanks in this set were kept in the dark (Fig. 4).

After one month, the plates were removed from the tanks, and the biofilm was harvested by scraping it off with acrylic scrapers. During this experimental period new plates had been colonized for a month, in preparation for the subsequent experimental period.

This procedure was carried out both for neutral metal solutions (pH 7) and for acidified metal mixes that had a slightly reduced pH of about 6. The pH was reduced by the addition of H_2SO_4.

2.6 Metal depletion of the input solution.

Every second morning, the FEN containers were taken to the field, and flow rates were set in such a way that the 1 liter container emptied in approximately 24 hours. Empty containers were carried to the laboratory and filled for the following day, each bag being filled with the same concentration from the same stock solution.

At the downstream end of each experimental tank, the outflowing solution was collected in plastic bottles. These bottles were taken into the laboratory, where 7 ml of the collected water was fixed with 1 drop of Baker's Instra Analyzed Nitric Acid (Erickson, 1977) for metal analysis, while another part was stabilized to 20 ^{o}C, for pH determination according to the methods described in Standard Methods for the Examination of Water and Wastewater (1966). The empty plastic bottles were acid-cleaned overnight with 30 % Baker's Instra Analyzed Nitric Acid, thoroughly rinsed with glass distilled water, and returned the next day to collect from the same tank.

Light intensities at the surfaces of the plates in the tanks could not be measured, due to the narrow distance between plates. Measurements would have involved the removal of one or more plates, which could have resulted in contamination and losses of periphyton.

pH values were measured with digital field pH meter (CANLAB Portable digital pH meter H5503-1, model #607). Temperature (+ 0.5 ^{o}C) of the water was measured with an alcohol thermometer.

2.7 Clean up

In preparation for the next run, tanks, plates, and plastic bottles were scrubbed free of any residual organic matter with soap and water, and acid cleaned overnight in 30 % Baker's Instra Analyzed Nitric Acid, after which they were rinsed in double glass distilled water and left to dry.

2.8 Sample analysis

At the end of each one month period, the plates were removed from the tanks and put into Ziplock Freezer bags for transport to the University where the biofilm was completely scraped off the plates with acrylic scrapers into the same Ziplock bags. Any residual biofilm was rinsed off into the bags with glass distilled water. Each set of 9 plates was divided into subsets for different analyses. Three plates were used for biomass and metal analysis, and another three for species analysis.

2.9 Analysis of sorbed metal

Millipore membrane filters (pore size 0.47 um) were rinsed 3 times with glass distilled water and were oven dried (at 60 ^{o}C overnight) in a desiccator and weighed. Harvested biofilm samples were filtered through these filters and dried and weighed again. Filters and organisms were digested with 2 ml concentrated Baker's Instra Analyzed Nitric Acid which was diluted to 10 ml with glass distilled water and kept in a waterbath at 95 ^{o}C overnight. Samples were stored in acid-cleaned scintillation vials. Vials were stored until analysis by atomic absorption flame spectrophotometry.

Atomic absorption measurements for copper were carried out in the Chemistry Department of The University of Victoria (Varian Model AA475 series). Zinc and biomass metal-concentration measurements were carried out with the help of a multisampling Atomic Absorption Spectrophotometer (Varian Model 1475, fitted with an automatic sampler) at the Institute of Ocean Sciences in Sidney, Vancouver Island, B.C.. Zinc concentrations were too high to be measured a standard nebulizer head, so measurements were made on a reduced flow (with a variable nebulizer head), with the flame turned at an angle of 45 ^{o}. This increased the error of the readings to some extent.

2.10 Species Analysis

Plates were scraped with acrylic scrapers and the organisms were stored in 3 % formalin. Prior to the species analysis and counts, diatom species were identified by ashing subsamples to obtain the clean frustules for identification. Wet subsamples were subsequently analyzed for species identification and abundance. 15 random fields per wet subsample were checked and counted at 40x magnification. Duplicate samples for each metal concentration were analysed and the results were totalled.

2.11 Statistical analysis

Daily metal depletion data were analyzed with a Friedman rank-order correlation test (Zar, 1974). In addition to this, the data were pooled for acidified and neutral treatments and an analysis of variance was carried out as exploratory analysis. The data were also subjected to regression analysis to determine the percentage uptake. Metals-in-biomass data were analyzed with Pearson's correlation tests (Zar, 1974).

Chapter III

RESULTS

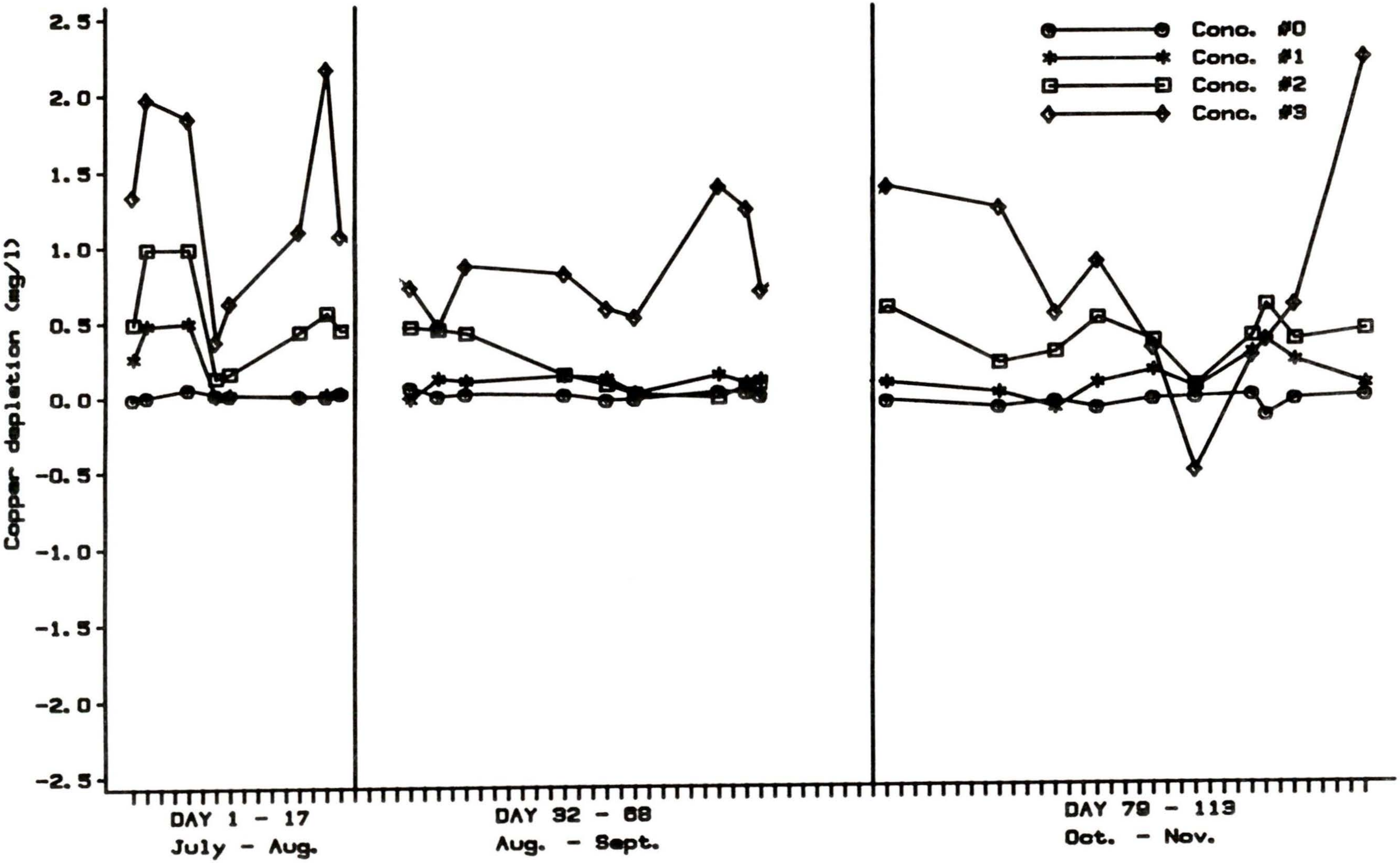
3.1 Average daily metal depletion

The average daily metal depletion, expressed as the difference between input and output, is shown in Figures 5-8. An example of the measurements and calculations, as well as standard deviations is given in Appendix II. Initially metal depletion from the ingoing water was high, then it decreased, sometimes to the point of releasing the metals back into the environment. Daily copper depletion was generally higher for higher concentrations (Fig. 5), with Concentration #1 (average depletion 0.13 mg Cu/L) showing little difference from the control. Concentration #2 (average depletion 0.35 mg Cu/L) shows some difference from the control, and Concentration #3 (average depletion 0.91 mg Cu/L) showing the greatest difference. Standard deviations for these values ranged from 0.00 to 0.77 (see Appendix II). Daily zinc metal depletion is given in Figure 6. (average depletions 5.26 mg Zn/L for Concentration #1, 22.12 mg Zn/L for Concentration #2, and 36.55 mg Zn/L for Concentration #3). The relation between zinc depletion and external concentration was not as clear as for copper.

Acidification of the ingoing metal solution affected the metal depletion of copper (Fig. 7) and zinc (Fig. 8) by increasing the variability of the daily metal depletion (average values; 0.21, 0.43, and 0.34 mg Cu/L, and 8.16, 26.68, and 45.01 mg Zn/L for Concentrations #1, #2, and #3 respectively). Metal depletion patterns were similar per metal, under neutral conditions. The greater variation due to acidification obscured this pattern. Daily depletion levels for cadmium could not be measured as they were below the detection limits of the system.

Figure 5: Copper depletion from the water under neutral conditions.

. The difference between input and output concentration (mg/L) of copper in solution is shown for the three sets of experiments.



Cono. #0
Cono. #1
Cono. #2
Cono. #3
Copper depletion (mg/l)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 6: Zinc depletion from the water under neutral conditions.

. The difference between input and output concentration (mg/L) of zinc in solution is shown for the three sets of experiments.

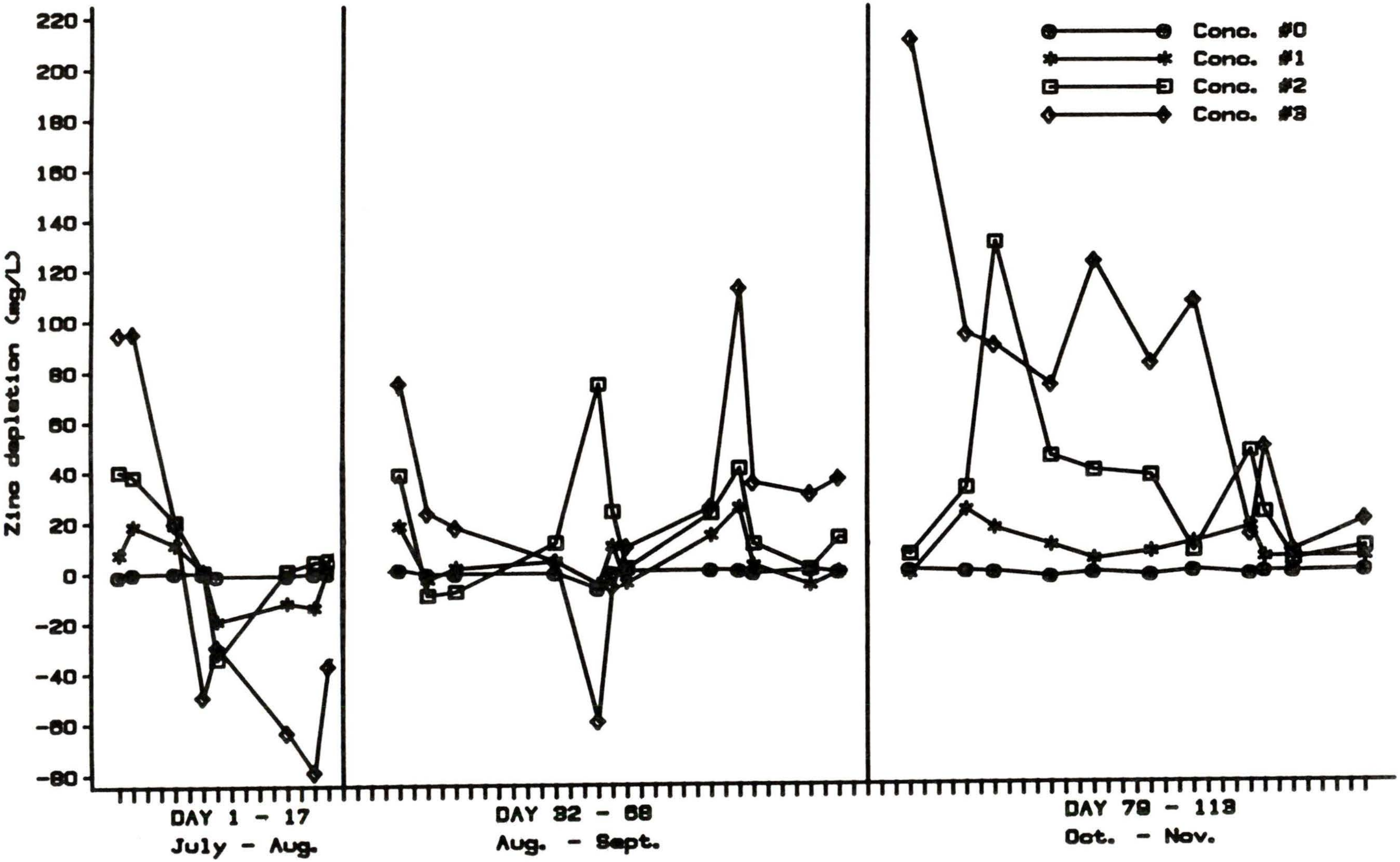

Zinc depletion (mg/L)
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
Conc. #0
Conc. #1
Conc. #2
Conc. #3
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 7: Copper depletion from the water under acidified conditions.

. The difference between input and output concentration (mg/L) of copper solution is shown for the three sets of experiments.

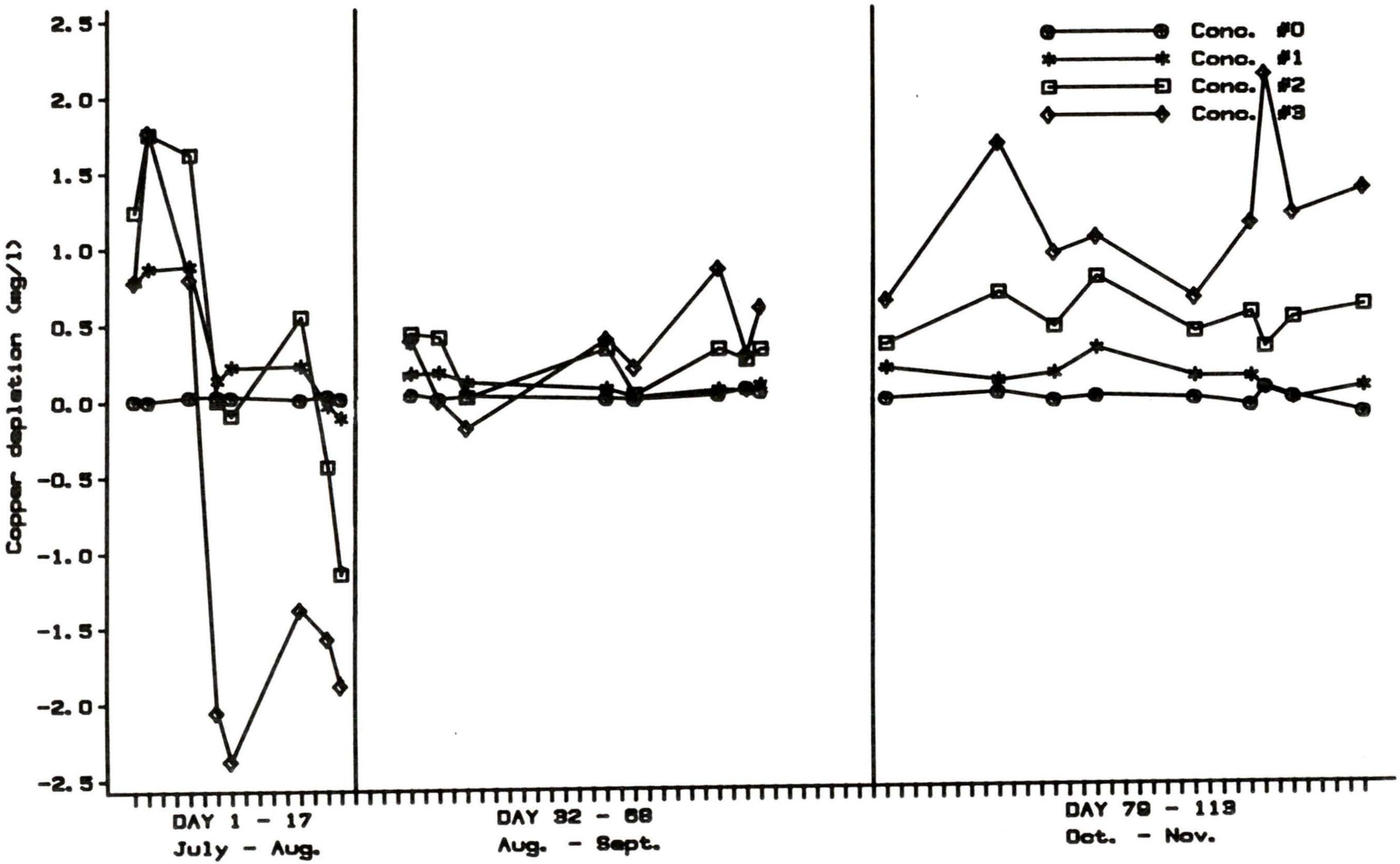
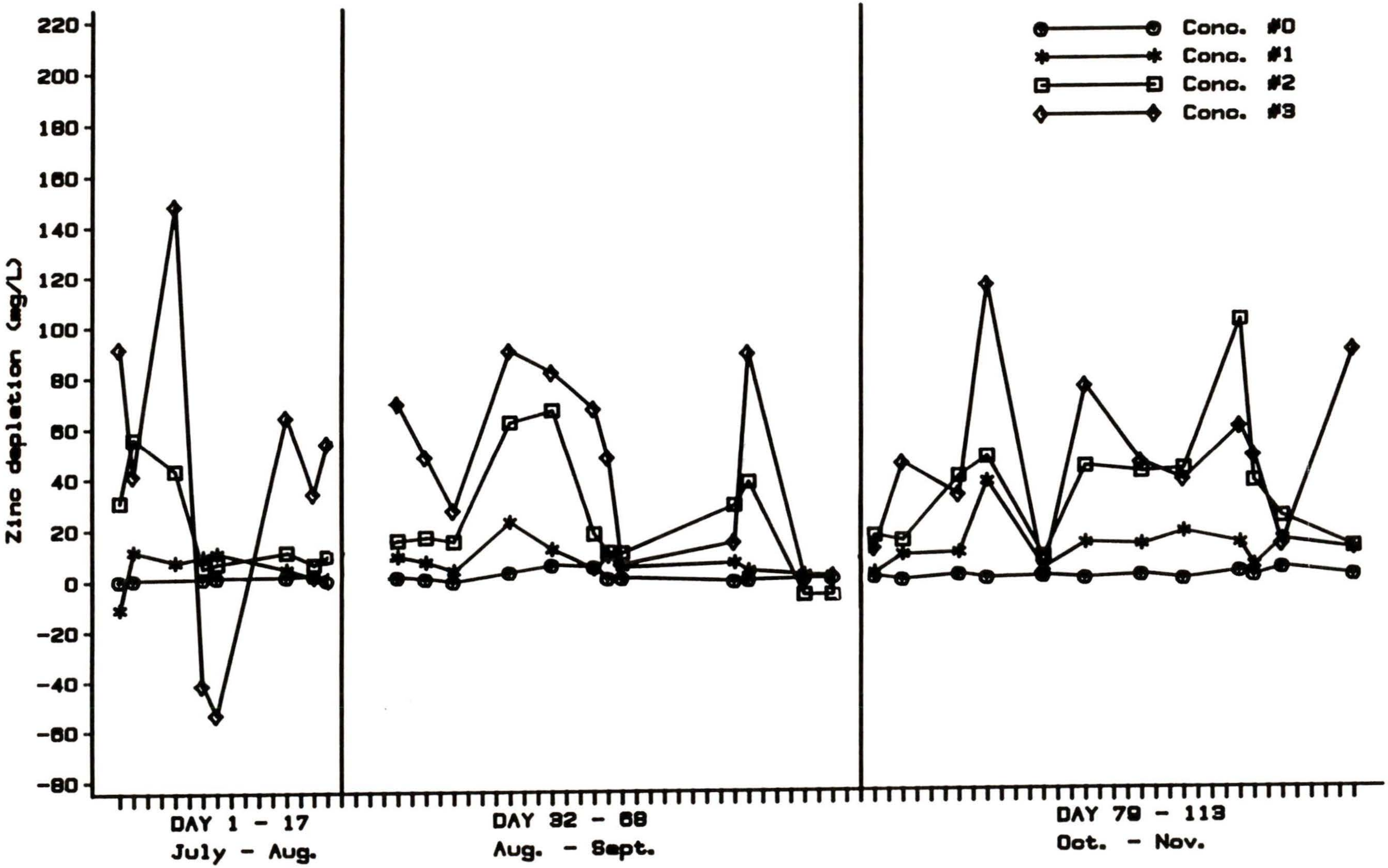

Copper depletion (mg/l)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Conc. #0
Conc. #1
Conc. #2
Conc. #3
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 118
Oct. - Nov.

Figure 8: Zinc depletion from the water under acidified conditions.

. The difference between input and output concentration (mg/L) of zinc solution is shown for the three sets of experiments.


Zinc depletion (mg/L)
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
Conc. #0
Conc. #1
Conc. #2
Conc. #3
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

3.2 Metal depletion by acrylic tanks, blank tests

The experiment measuring the metal depletion by the acrylic tanks (blank) in the dark was carried out during the periods of August-September and October-November and showed patterns of metal depletion that were almost identical to those due to the organism, although depletion by the blank was generally lower. Metal depletion by acrylic under neutral conditions is shown in Figures 9 and 10 for copper and zinc respectively. Figures 11 and 12 show the metal depletion under acidified conditions for copper and zinc respectively. An example of the similarity in patterns between blank and organism metal depletion is given for copper in Figure 13-15, for the three concentrations #1, #2, and #3 respectively.

Figure 9: Copper depletion in the acrylic tank (blank) under neutral conditions.

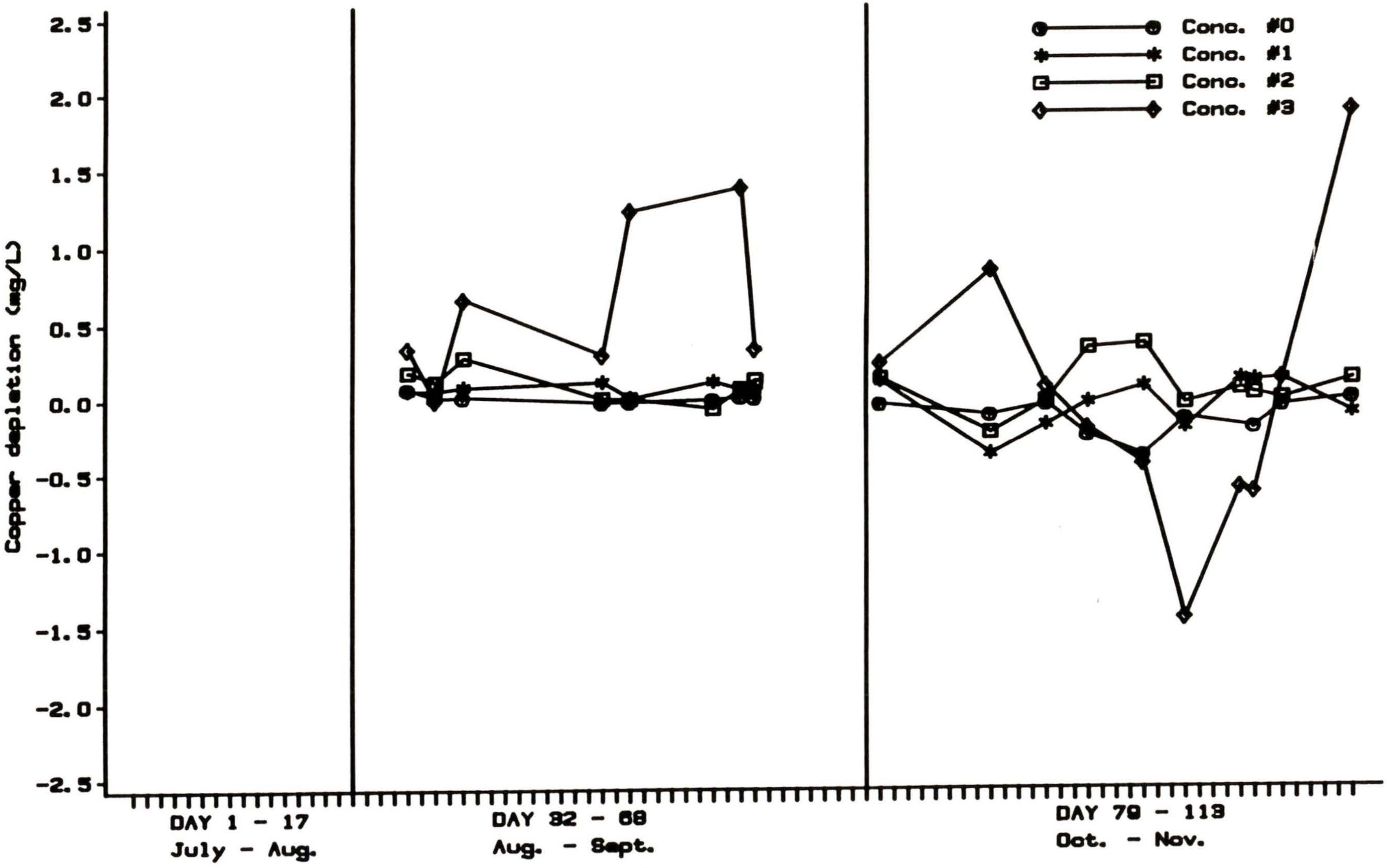

Copper depletion (mg/L)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.
Conc. #0
Conc. #1
Conc. #2
Conc. #3

Figure 10: Zinc depletion in the acrylic tank (blank) under neutral conditions.

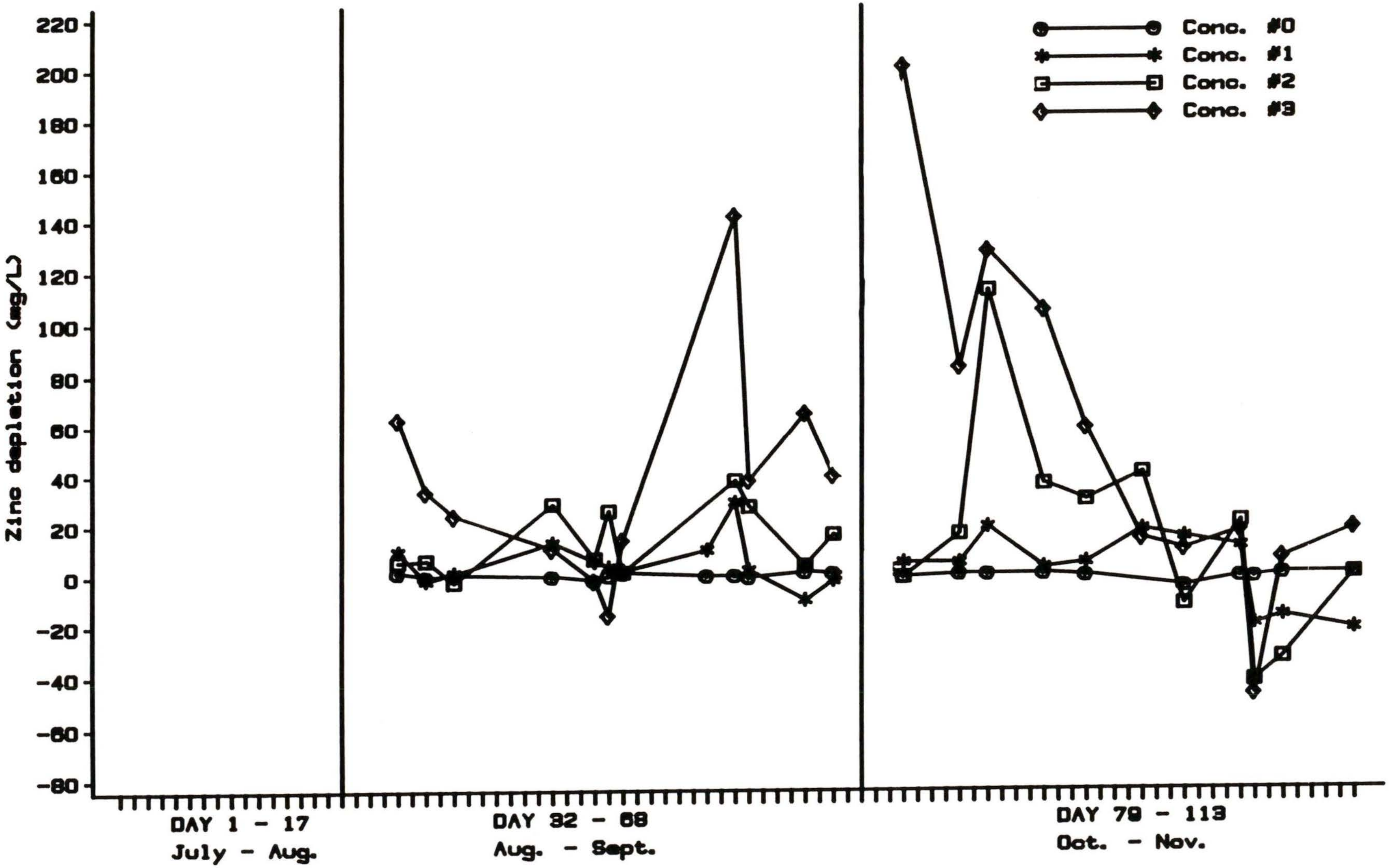

Zinc depletion (mg/L)
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.
Conc. #0
Conc. #1
Conc. #2
Conc. #3

Figure 11: Copper depletion in the acrylic tank (blank) under acidified conditions.

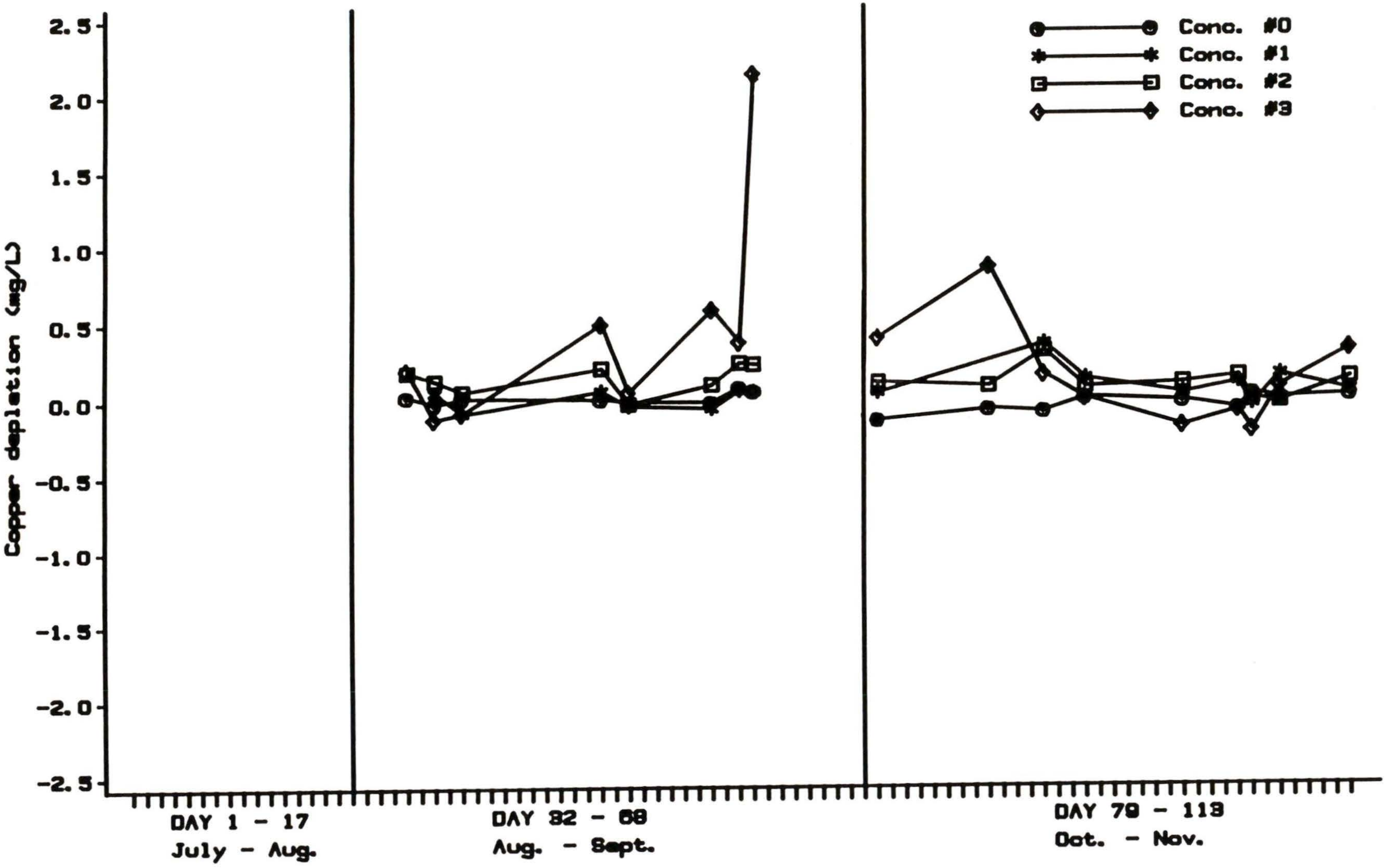

Cono. #0
Cono. #1
Cono. #2
Cono. #3
Copper depletion (mg/L)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 12: Zinc depletion in the acrylic tank (blank) under acidified conditions.

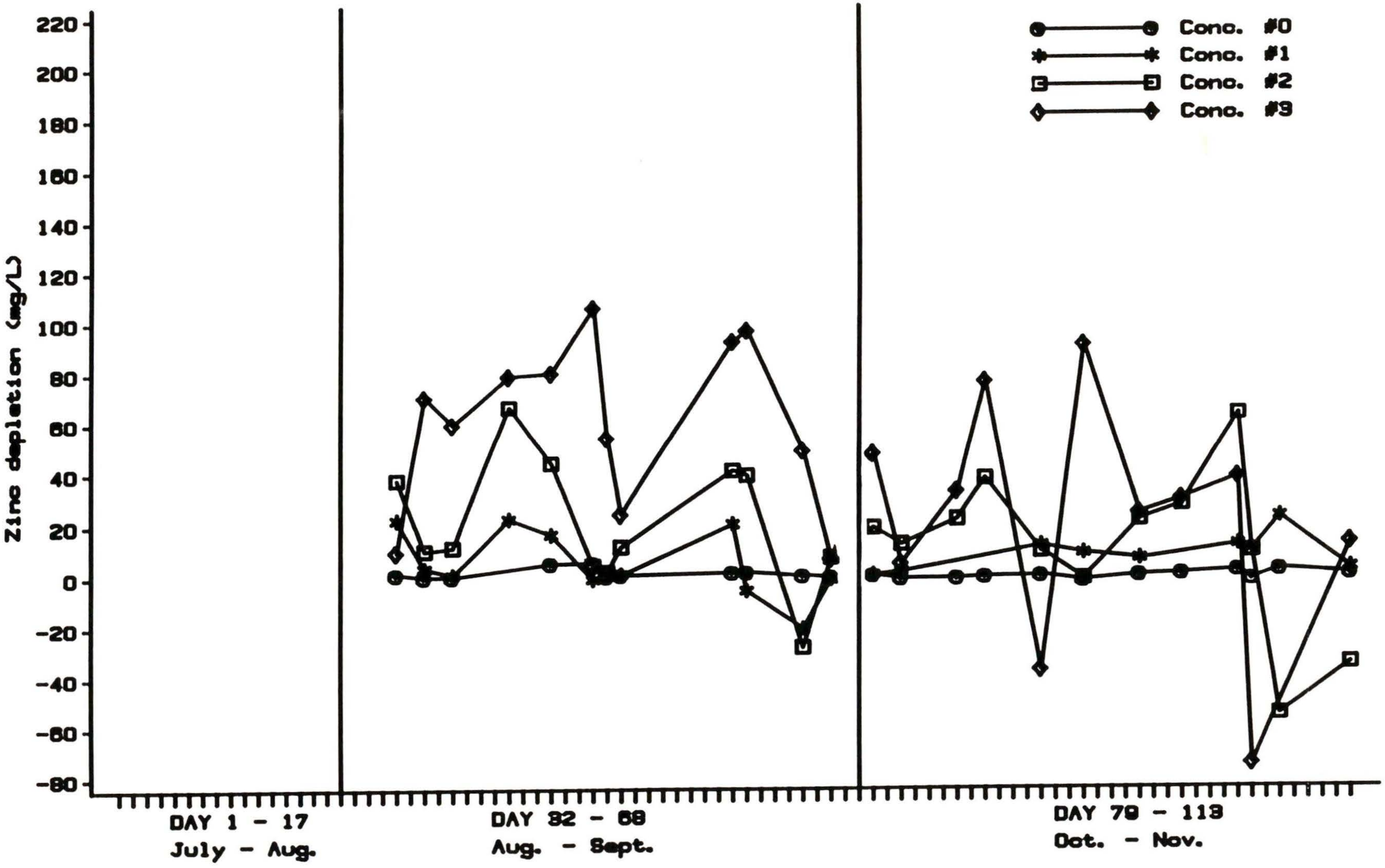

Conc. #0
Conc. #1
Conc. #2
Conc. #3
Zinc depletion (mg/L)
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 13: Copper depletion by organisms vs. blank (Concentration #3).

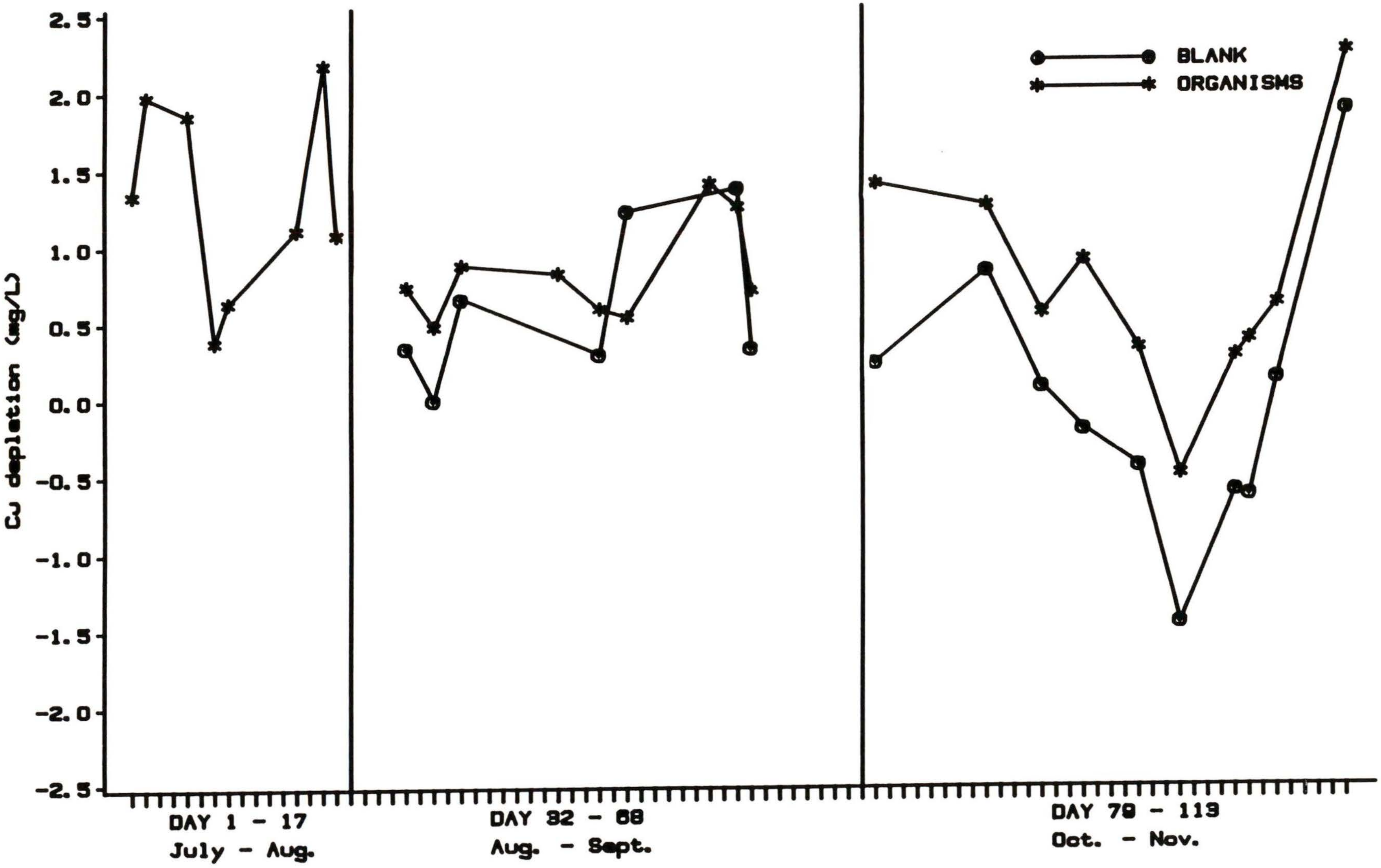

2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Cu depletion (mg/L)
BLANK
ORGANISMS
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 14: Copper depletion by organisms vs. blank (Concentration #2).

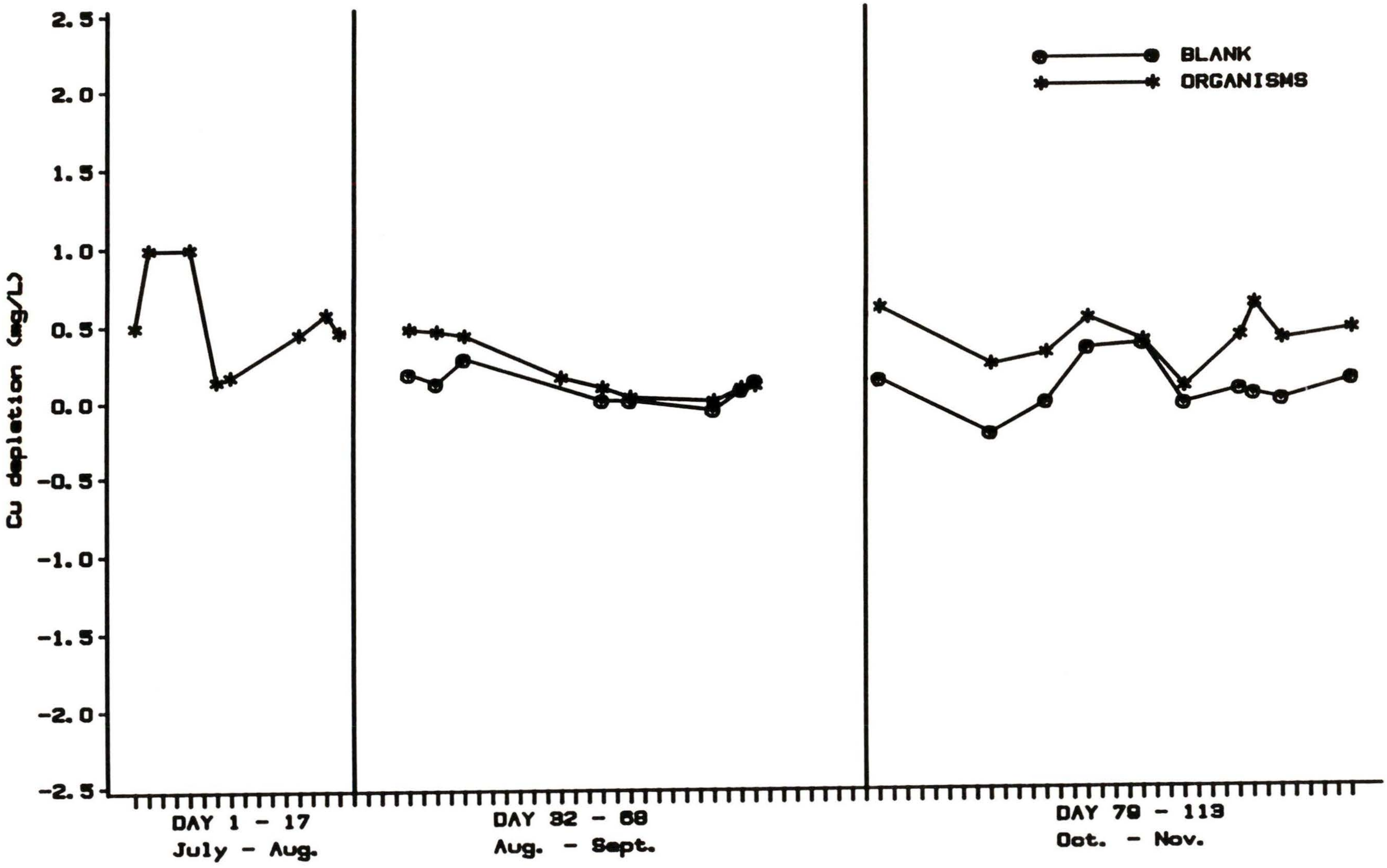
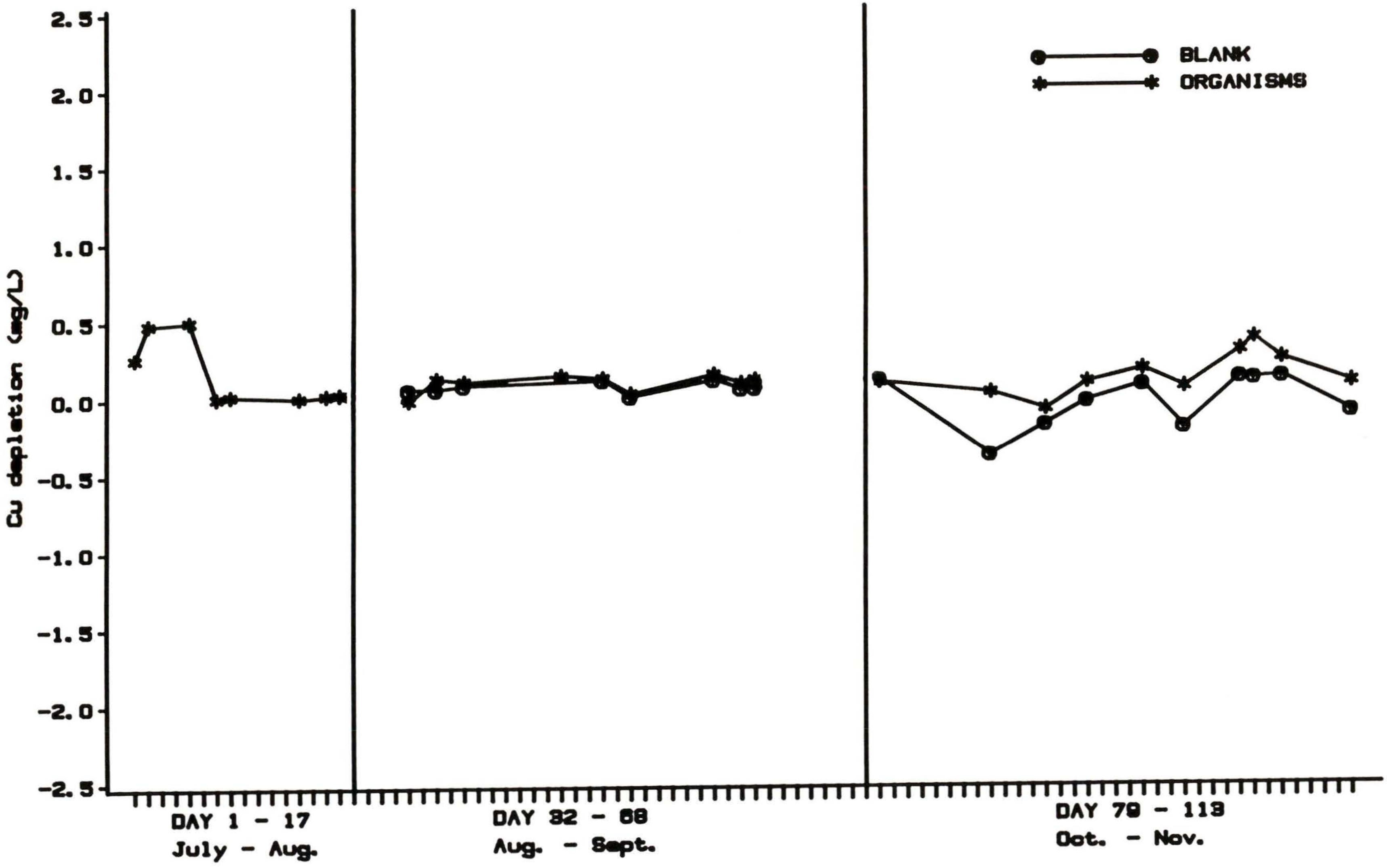

BLANK
ORGANISMS
Cu depletion (mg/L)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 15: Copper depletion by organisms vs. blank (Concentration #1).


BLANK
ORGANISMS
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Cu depletion (mg/L)
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Friedman's non-parametric rank order analysis (Zar, 1974) is given in Table 1 and shows that metal depletion per concentration was significantly ($P<0.05$) different for copper, and depletion from the ingoing solutions was higher for higher concentrations. This relationship is much reduced for zinc and for the acidified conditions.

There are also fewer significant differences for the blank experiment for both metals and both treatments (neutral and acidified).

Table 1: Friedman's rank order analysis

Significant ($P<0.05$) differences between metal depletions by organisms per input concentration.

Treatment	*Expt #*	*Copper* Conc.#	*Zinc* Conc#
Neutral	1	#3>#2>#1>#0	#2>#0
	2	#3>#2>#1>#0	#3=#2>#1=#0
	3	#2>#1>#0, #3>#0	#3>#2>#1>#0
Acidified	1	#1=#2>#3	#2>#0
	2	#2>#1>#0	#3>#2>#1>#0
	3	#3>#2>#1=#0	#3=#2>#1>#0

Significant ($P<0.05$) differences between metal depletions by acrylic tanks.

Treatment	*Expt. #*	*Copper* Conc. #	*Zinc* Conc. #
Neutral	2	#2=#1>#0	#3=#2=#1>#0
	3	#2>#0	#3>#0
Acidified	2	#2>#1=#0	#3>#2>#1=#0
	3	#1>#0	#3=#2=#1>#0

Copper concentrations #1, #2, and #3 were equivalent to input solutions with 0.5, 1.0, and 2.5 mg/L respectively and 0.09, 0.29, and 0.78 mg/L average copper depletion respectively under neutral conditions. Zinc concentrations #1, #2, and #3 were equivalent to input solutions of 5, 10, and 25 mg/L respectively and to zinc depletion concentrations of 3.32, 16.90, and 30.45 mg/L respectively. Under acidified conditions average depletion concentrations amount to 0.15, 0.32 and 0.11 mg Cu/L, and 6.82, 21.69 and 41.38 mg Zn/L.

3.3 Neutral vs. Acidified

Comparison of neutral and slightly acidified treatments also showed the similarity in patterns of depletion (Figures 16 and 17). When this was expressed in percentages, the patterns merged for copper, and to a lesser degree for zinc. Analysis of variance of the averaged values showed no significant ($P<0.5$) difference in metal depletion between neutral and acidified treatments.

Figure 16: Comparison of copper depletion under neutral vs. acidified conditions for Concentration #3.

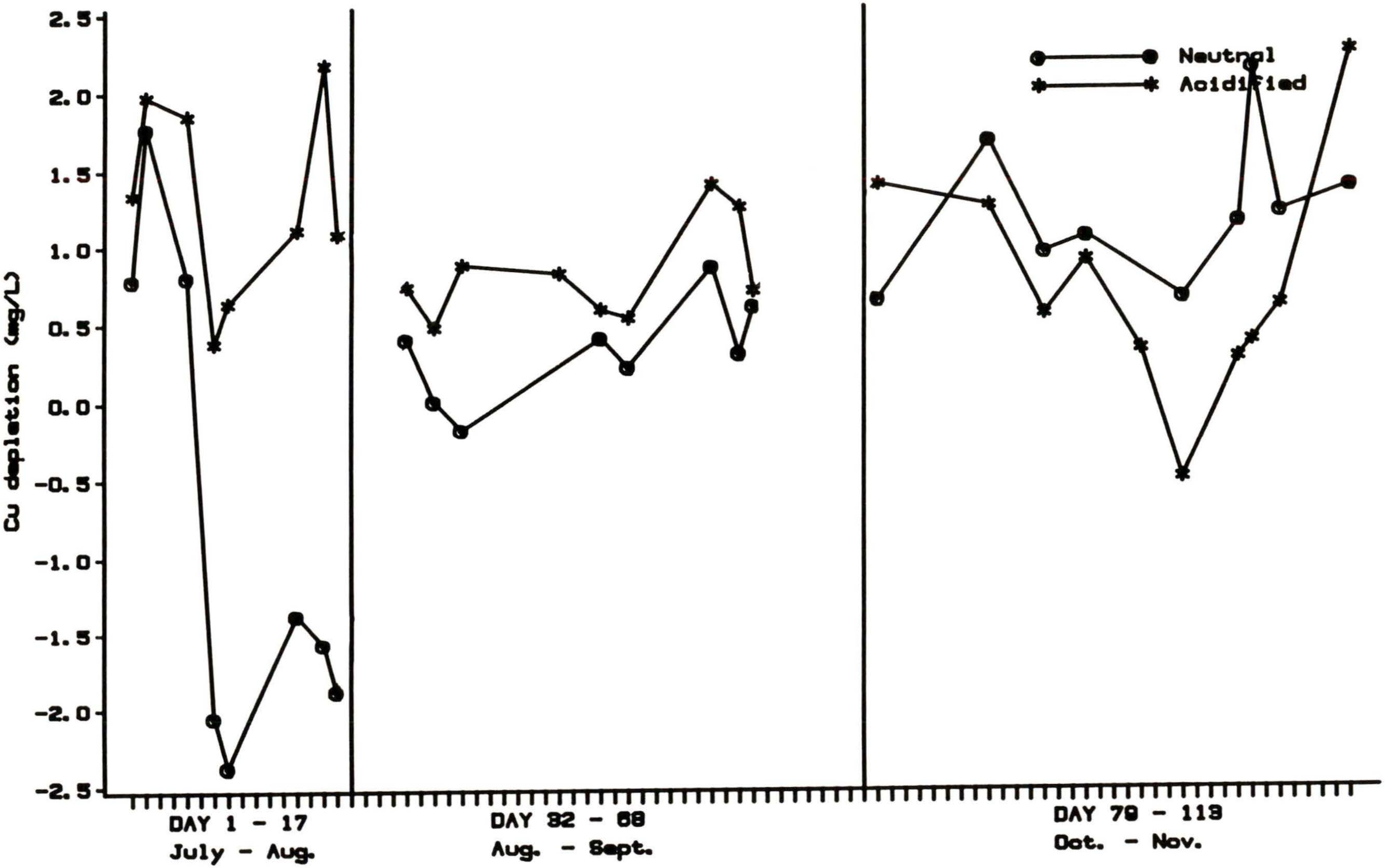
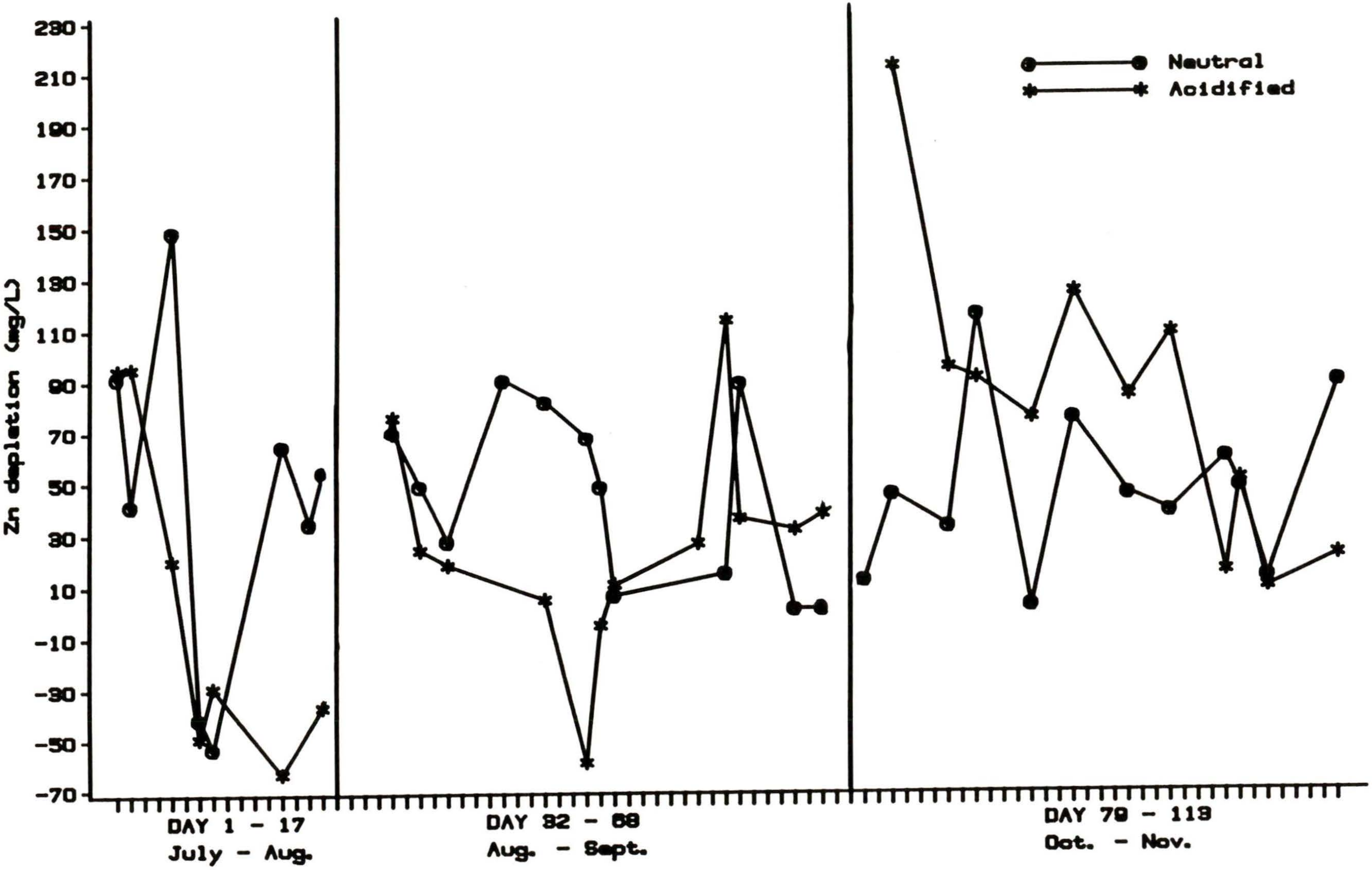

2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Cu depletion (mg/L)
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.
Neutral
Acidified

Figure 17: Comparison of zinc depletion under neutral vs. acidified conditions for Concentration #3.


230
210
190
170
150
130
110
90
70
50
30
10
-10
-30
-50
-70
Zn depletion (mg/L)
Neutral
Acidified
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

3.4 Changes in biomass

Changes in the biomass were estimated by sampling before and after the periods of study. The algal film was scraped off the plates, filtered dried and weighed. The periods of study lasted about one month each. Changes in biomass are given in Table 2. In most instances there was an estimated loss of biomass over the periods of study.

Table 2: Changes in biomass per experiment (g/plate)

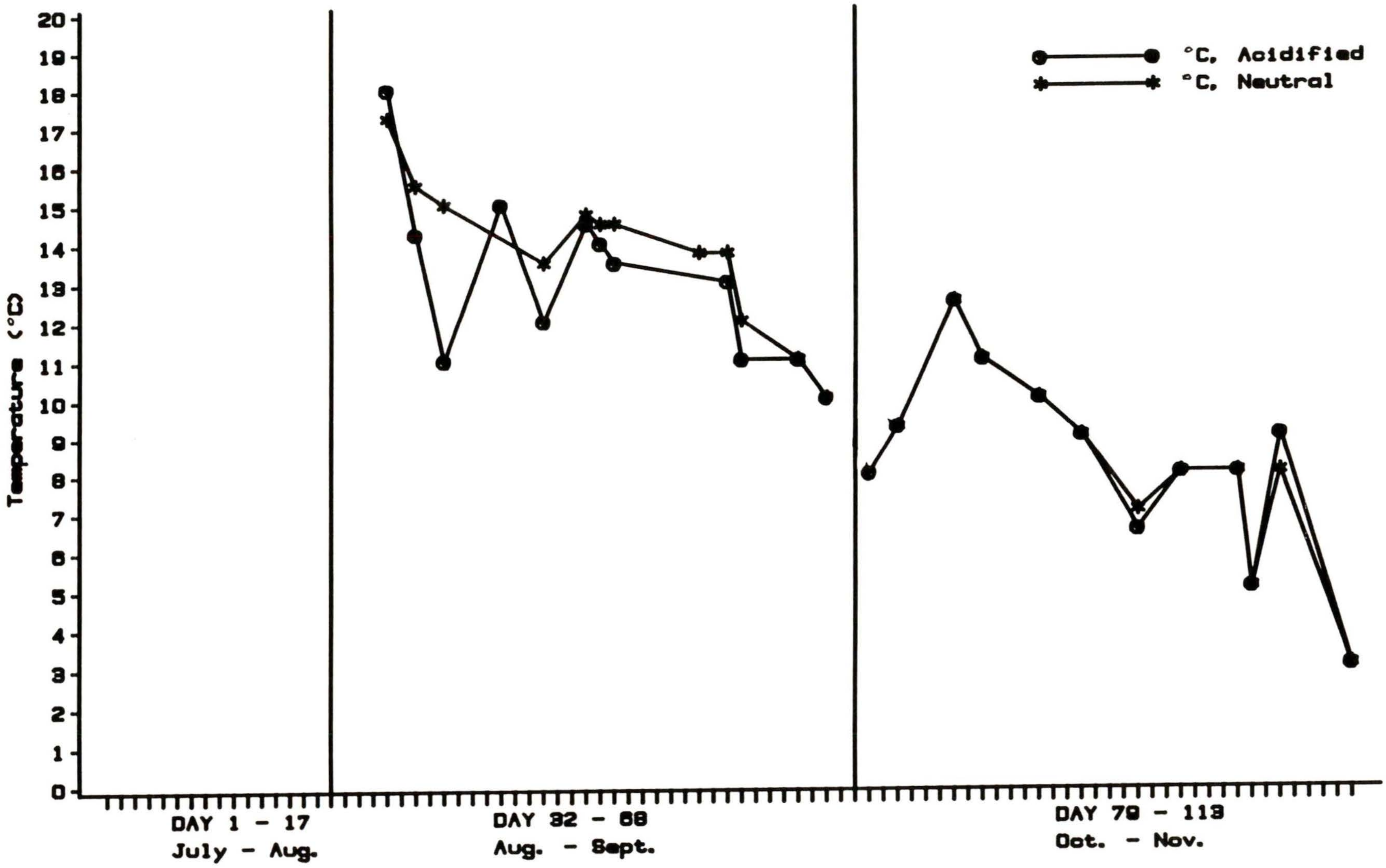
Conc.	Experiment 1			Experiment 2			Experiment 3		
				Neutral					
	Before	After	Change	Before	After	Change	Before	After	Change
0	0.0153	0.0078	-0.0075	0.0189	0.0034	-0.0155	0.0210	0.0076	-0.0134
1	0.0215	0.0193	-0.0022	0.0064	0.0015	-0.0049	0.0251	0.0173	-0.0078
2	0.0254	0.0173	-0.0082	0.0178	0.0017	-0.0161	0.0177	0.0180	+0.0003
3	0.0317	0.0278	-0.0052	0.0072	0.0046	-0.0026	0.0171	0.0094	-0.0077
				Acidified					
	Before	After	Change	Before	After	Change	Before	After	Change
0	0.0115	0.0038	-0.0077	0.0129	0.0091	-0.0038	0.0148	0.0079	-0.0069
1	0.0218	0.0141	-0.0076	0.0087	0.0072	-0.0015	0.0179	0.0098	-0.0082
2	0.0239	0.0162	-0.0077	0.0068	0.0084	+0.0016	0.0232	0.0149	-0.0083
3	0.0298	0.0223	-0.0075	0.0044	0.0045	+0.0001	0.0138	0.0058	-0.0080

Biomass values ranged from 6 mg to 32 mg at the start of the experiments and from 1 mg to 28 mg at the end.

3.5 Environmental parameters

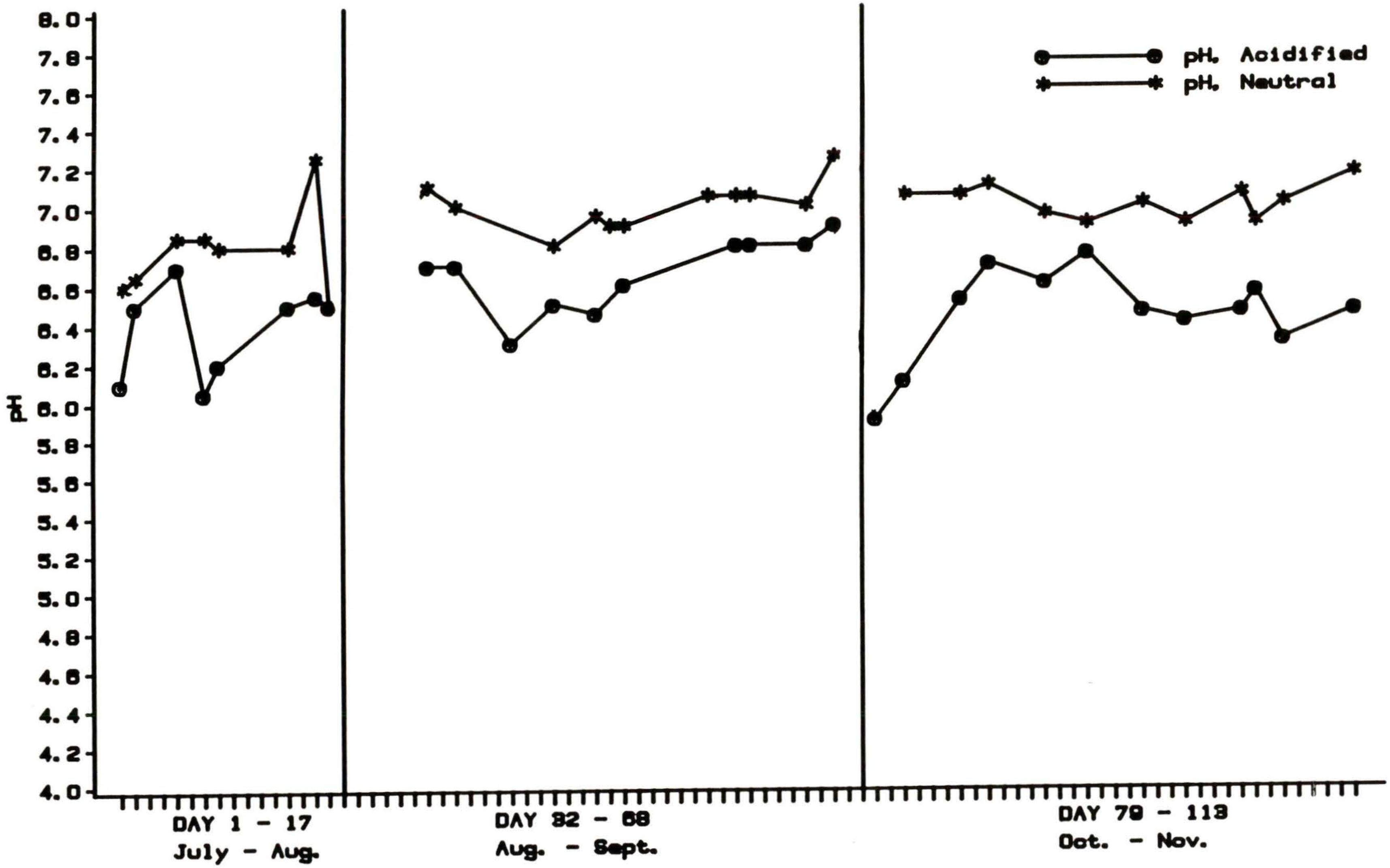
Temperatures for August-September and October-November are given in Figure 18, showing that the temperatures were similar for both the neutral and the acidic treatment. Temperatures ranged from 18°C late in August to 3°C in November, and decreased at a steady rate. pH values are given in Figure 19, indicating that the reduced pH treatment was consistently lower by about 0.5 pH units than the neutral experiments during all periods. The pH was essentially constant during all three experiments, both for the neutral and the slightly reduced pH.

Figure 18: Mean temperatures for Experiments 2 and 3.



°C, Acidified
°C, Neutral
Temperature (°C)
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 19: Mean pH values for Experiments 1, 2 and 3.


8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
pH
pH, Acidified
pH, Neutral
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

3.6 Corrected values

Since 25 % of the interior surface of the experimental tank was not covered by organisms, metal depletion measurements were corrected by subtracting 25% of the daily metal depletion of the blank from the total metal depletion . These "corrected" values under neutral conditions are shown in Figures 16 and 17 for copper and zinc respectively, and for acidified conditions in Figures 18 and 19 respectively. Average depletion values ranged from 0.12, 0.26, and 0.68 mg Cu/L and 6.55, 23.22, and 40.55 mg Zn/L for Concentrations #1, #2, and #3 respectively under neutral conditions. Under acidified conditions values ranged from 0.11, 0.39, and 0.75 mg Cu/L and 6.27, 24.67, and 36.93 mg Zn/L for concentrations #1, #2, and #3 respectively. Corrected metal depletion showed some leveling out of the pattern for copper, and a lowering of the uptake values for zinc.

Figure 20: Corrected metal depletion values for copper under neutral conditions.

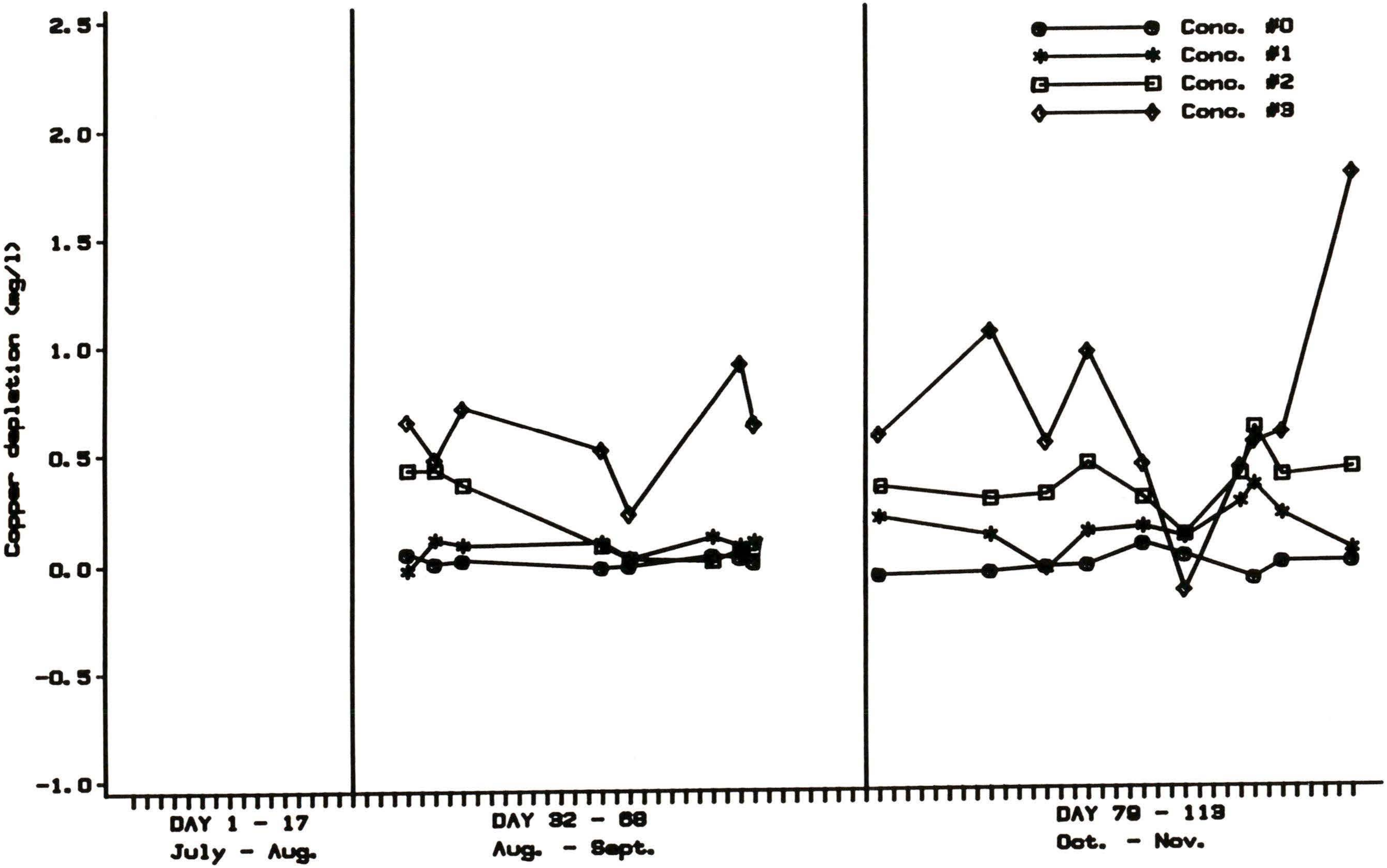

2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
Copper depletion (mg/l)
Cono. #0
Cono. #1
Cono. #2
Cono. #3
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 21: Corrected metal depletion values for zinc under neutral conditions.

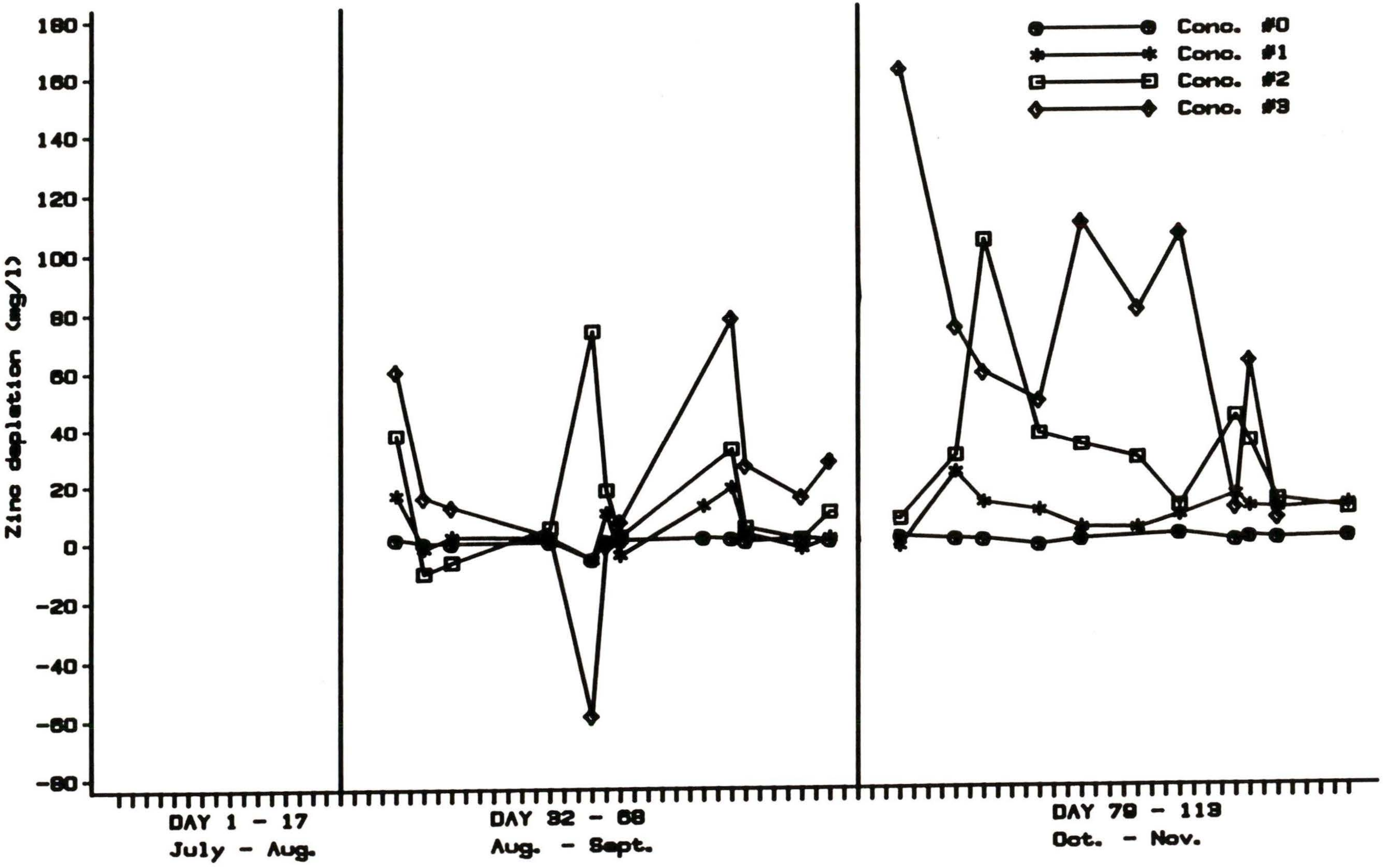

180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
Zinc depletion (mg/l)
Conc. #0
Conc. #1
Conc. #2
Conc. #3
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 22: Corrected metal depletion values for copper under acidified conditions.

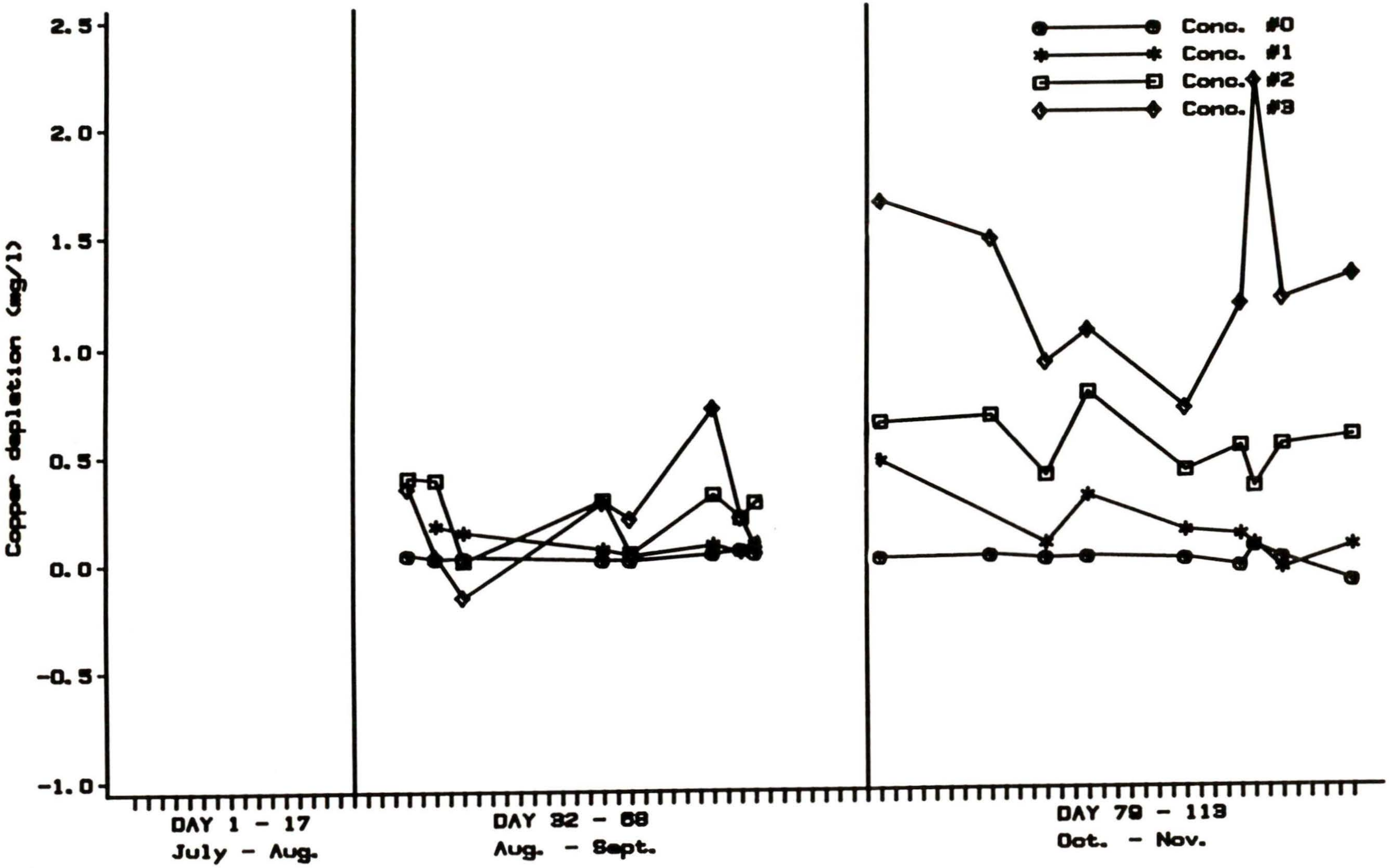
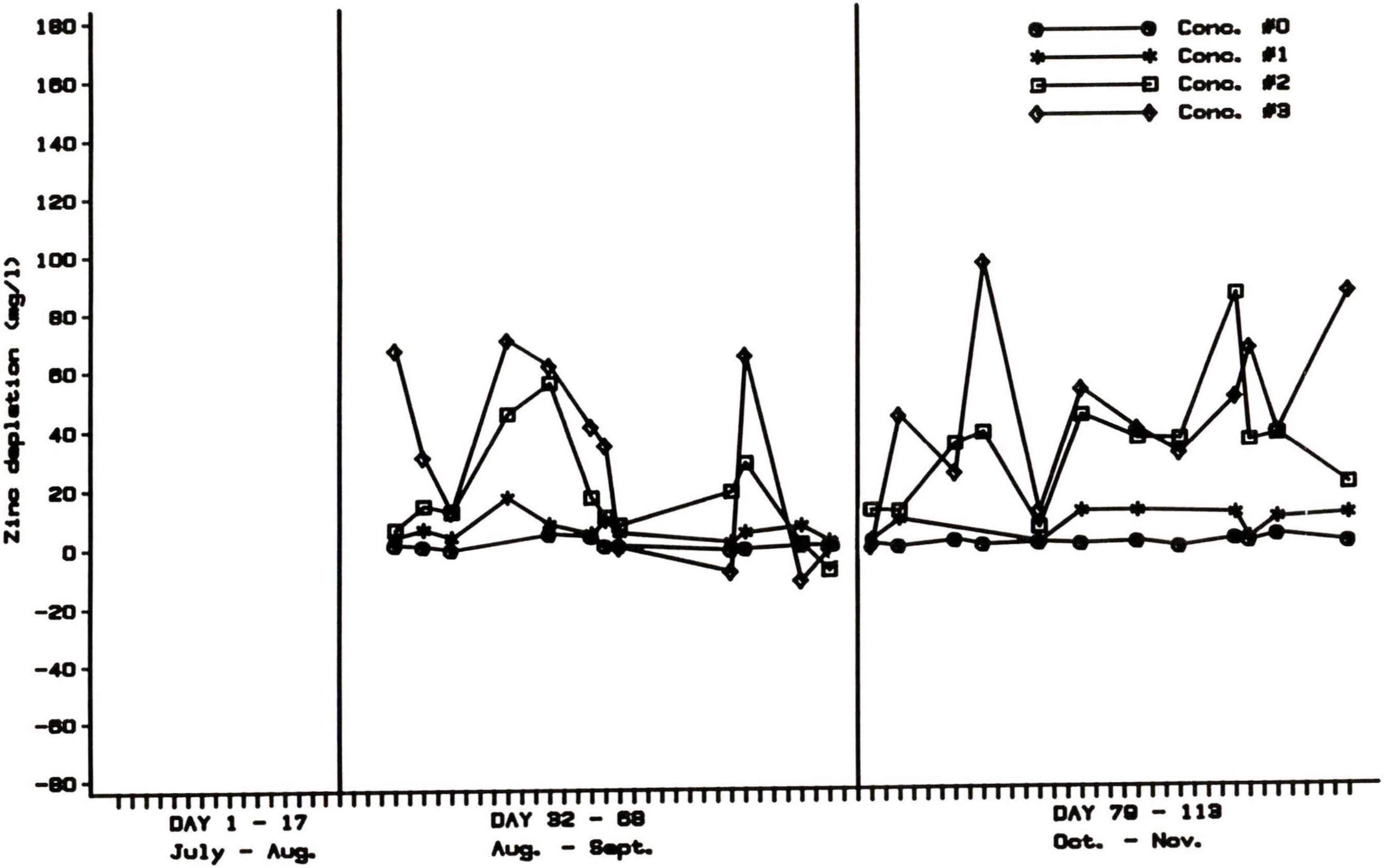

Conc. #0
Conc. #1
Conc. #2
Conc. #3
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
Copper depletion (mg/l)
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 23: Corrected metal depletion values for zinc under acidified conditions.


180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
Zinc depletion (mg/l)
Conc. #0
Conc. #1
Conc. #2
Conc. #3
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

A Friedman rank order analysis for the corrected values is given in Table 3.

Table 3: Friedman's rank order analysis

Significant (P<0.05) differences between metal depletions by organisms per concentration.

Treatment	*Expt.#*	*Copper* Conc. #	*Zinc* Conc. #
Neutral	2+3	#3>#2>#1>#0	#3>#2>#1>#0
	2	#3>#2=#1>#0	#3=#2>#1,#3>30
	3	#3=#2>#1>#0	#2>#1>#0,#3>#0
Acid.	2+3	#2>#1>#0	#3=#2>#1>#0
	2	#3=#2=#1>#0	#2>#1>#0
	3	#3>#2>#1>#0	#3=#2>#1>#0

When the experiments are combined, depletions per concentration are significantly different (P<0.05), with the higher concentrations showing greater metal depletion. On a monthly basis the differences are not as clear, although all except one (zinc, neutral, 3rd experiment) were significantly different from the control.

A simple regression analysis was carried out on both raw and the corrected data to test whether metal depletion by algae is a constant fraction of the input concentration. Metal depletion was tested against the input concentration. The results are given in Table 4.

Table 4: Metal depletion as a function of the input concentration

		NEUTRAL		ACIDIFIED	
Metal	Expt #	R^2	Slope	R^2	Slope
		"raw ratios"			
Cu	1	0.7161	0.4380	0.7016	-0.2118
Cu	2	0.8477	0.3656	0.3288	0.1213
Cu	3	0.6762	0.3934	0.7618	0.3872
Zn	1	0.0647	0.0203	0.3877	0.3295
Zn	2	0.3829	0.3371	0.5639	0.3644
Zn	3	0.7914	0.5273	0.6950	0.3852
		corrected ratios			
Cu	2	0.7574	0.2604	0.1693	0.0716
Cu	3	0.7786	0.3410	0.7610	0.3776
Zn	2	0.2860	0.2467	0.3876	0.2411
Zn	3	0.7479	0.4168	0.6397	0.3303

For the raw copper data under neutral conditions R^2 values range from 0.6762 to 0.8477 with slopes ranging from 36.6 to 43.8 %. Zinc showed no consistent value per season, nor did it show consistent metal depletion .

For the corrected metal depletion values, copper shows R^2 values of 0.7574 to 0.7786, consistent with the raw metal depletion values, whereas zinc metal depletion maintains the inconsistent pattern. Slopes for the corrected values are somewhat less for copper, ranging from 26 to 34 %. Acidification upsets the relationship, though it affects copper metal depletion percentages more than zinc metal depletion percentages.

Metal depletion was tested against the environmental parameters temperature and pH, and the changes in biomass. Biomass was assumed to change at a constant rate. The Pearson's correlation coefficients (Zar, 1974) are given in Table 5 for these parameters

Table 5: Metal depletion , temperature, pH and biomass.

Pearson's correlation coefficients.

Metal Treatment	*Conc*	*pH*	*Temp*	*Average Biomass per plate*
Cu Neutral	#1	0.1518	-0.4413	0.3570
	#2	-0.3753	-0.5839	0.8127
	#3	-0.1135	0.4466	-0.0729
Cu Acidified	#1	-0.0776	-0.0536	0.2382
	#2	-0.2026	-0.4998	0.7577
	#3	0.1713	-0.4163	0.6841
Zn Neutral	#1	0.2489	-0.5224	0.3437
	#2	0.0820	0.4067	0.2374
	#3	-0.0550	-0.0150	0.6924
Zn Acidified	#1	-0.1423	0.2640	0.0196
	#2	-0.0532	-0.3580	0.2582
	#3	0.1101	0.0000	0.0522

Daily metal depletion values do not correlate with the daily pH and temperature. There was some correlation between metal depletion and the changes in biomass but it was not consistent.

3.7 Copper–zinc competition

To determine the interactions between copper and zinc in the presence of organisms, the percent uptake per concentration is shown in Figures 24, 25, and 26 for Conc. #1, Conc. #2, and Conc. #3 respectively. These graphs show no clear difference between the % zinc and % copper metal depletion. Similarly, copper and zinc interactions compared in the blank experiments (Figs. 27-29, for Conc. #1, Conc. #2 and Conc. #3 respectively) showed no distinct differences, although zinc depletion was generally somewhat higher.

Figure 24: Copper-zinc competition (Concentration #1) on the colonized plates.

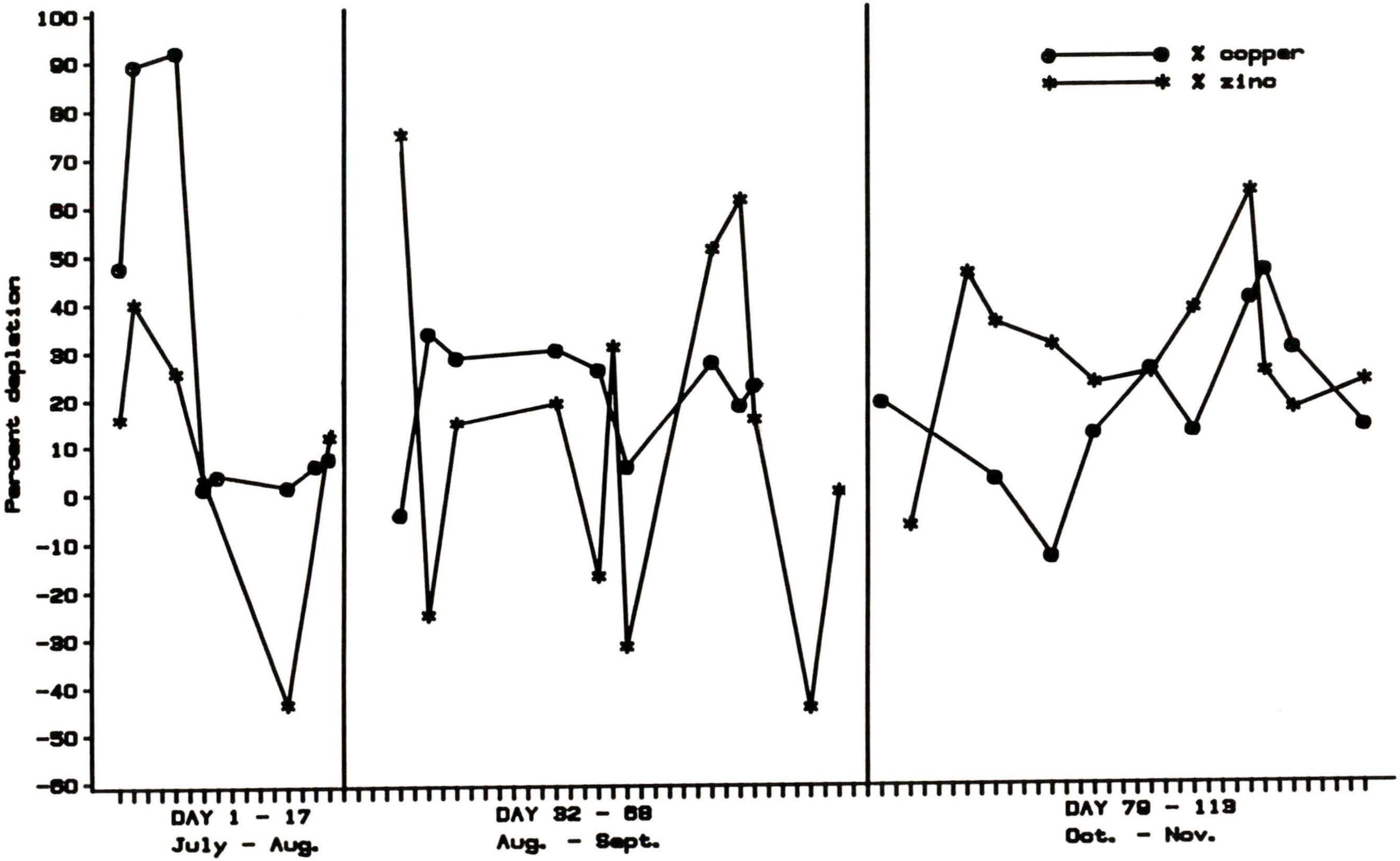

100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
Percent depletion
% copper
% zinc
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 25: Copper-zinc competition (Concentration #2) on the colonized plates.

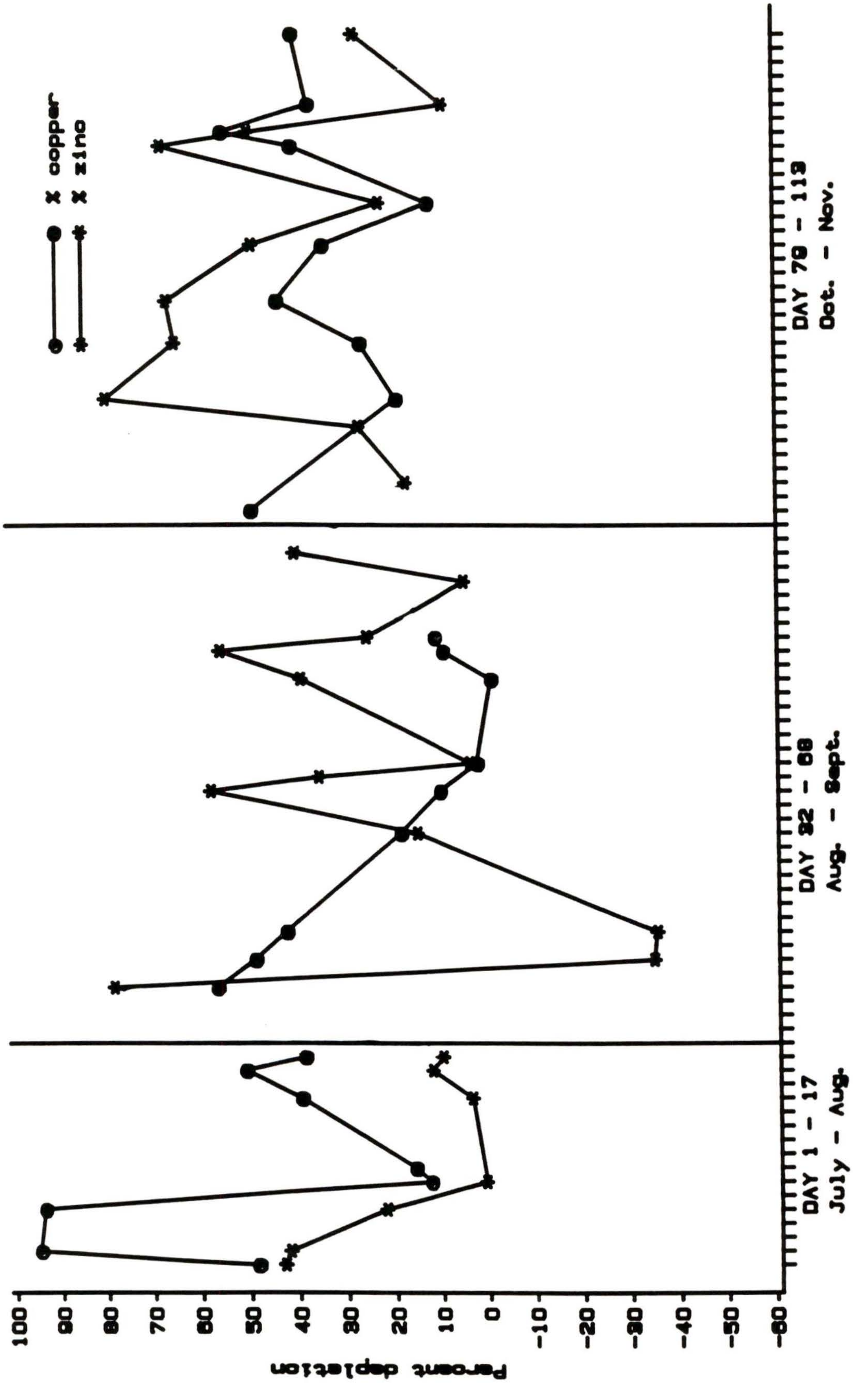

Percent depletion
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.
% copper
% zinc

Figure 26: Copper-zinc competition (Concentration #3) on the colonized plates.

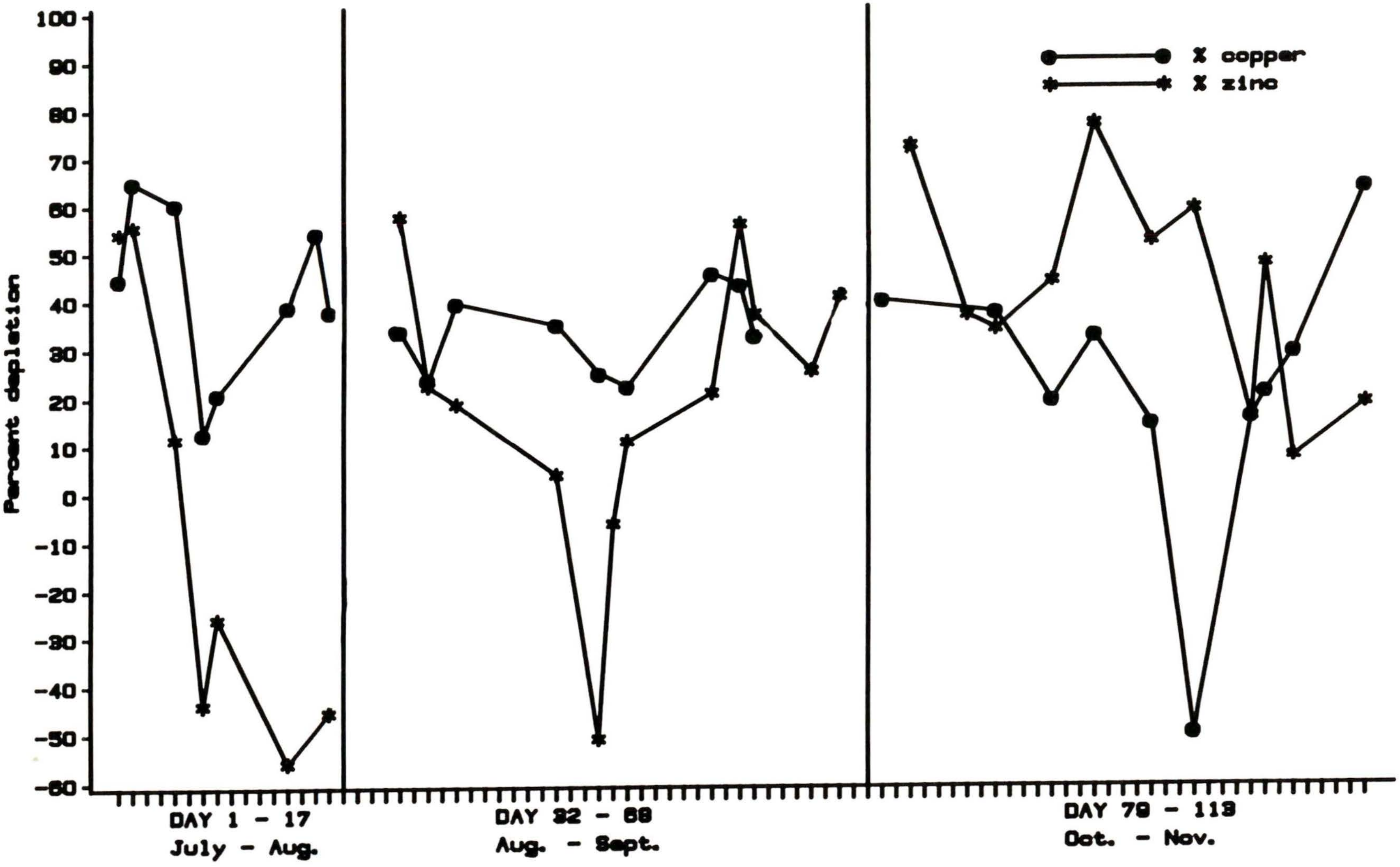

% copper
% zinc
Percent depletion
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 27: Copper-zinc competition (Concentration #1) on the blank plates.

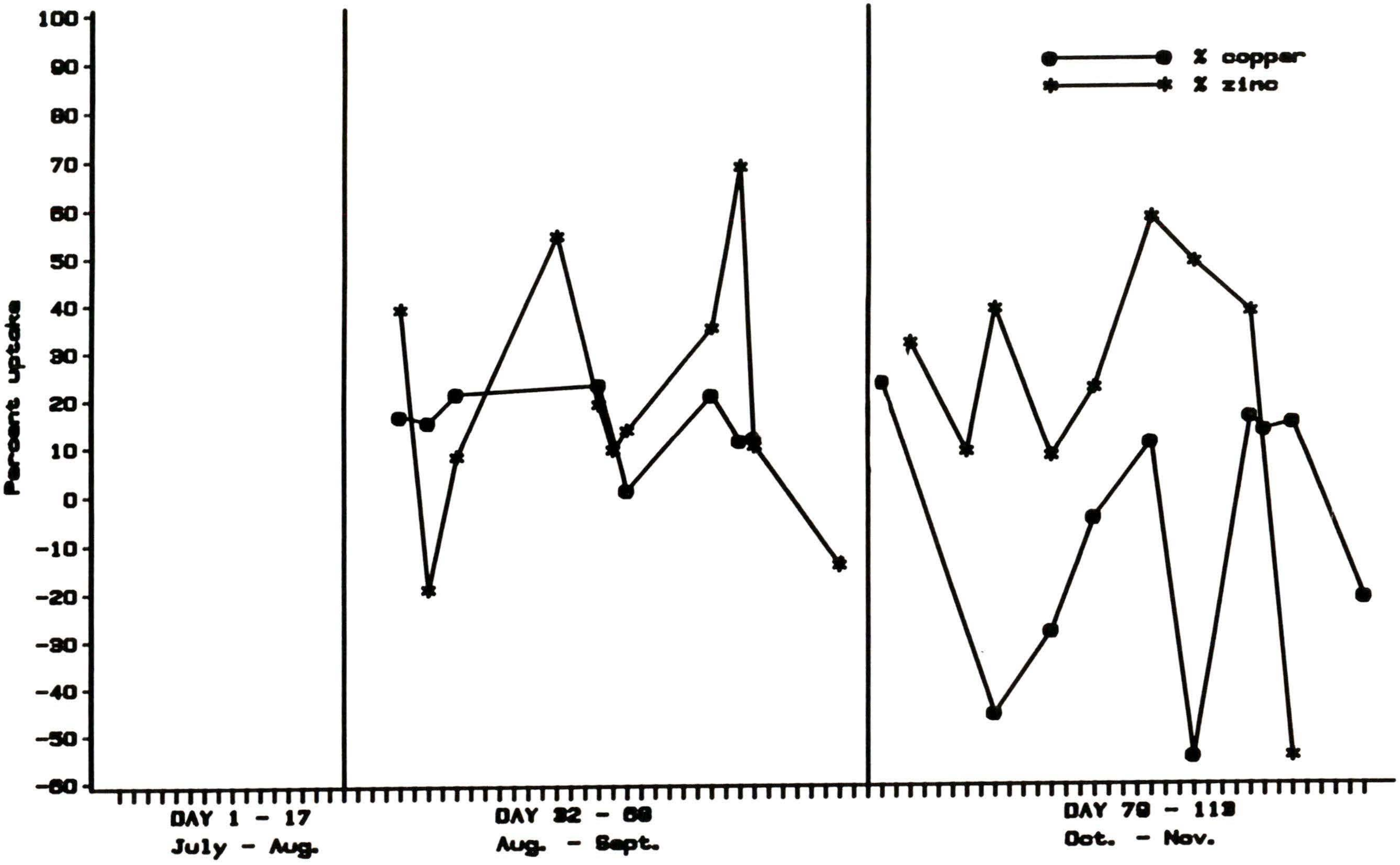
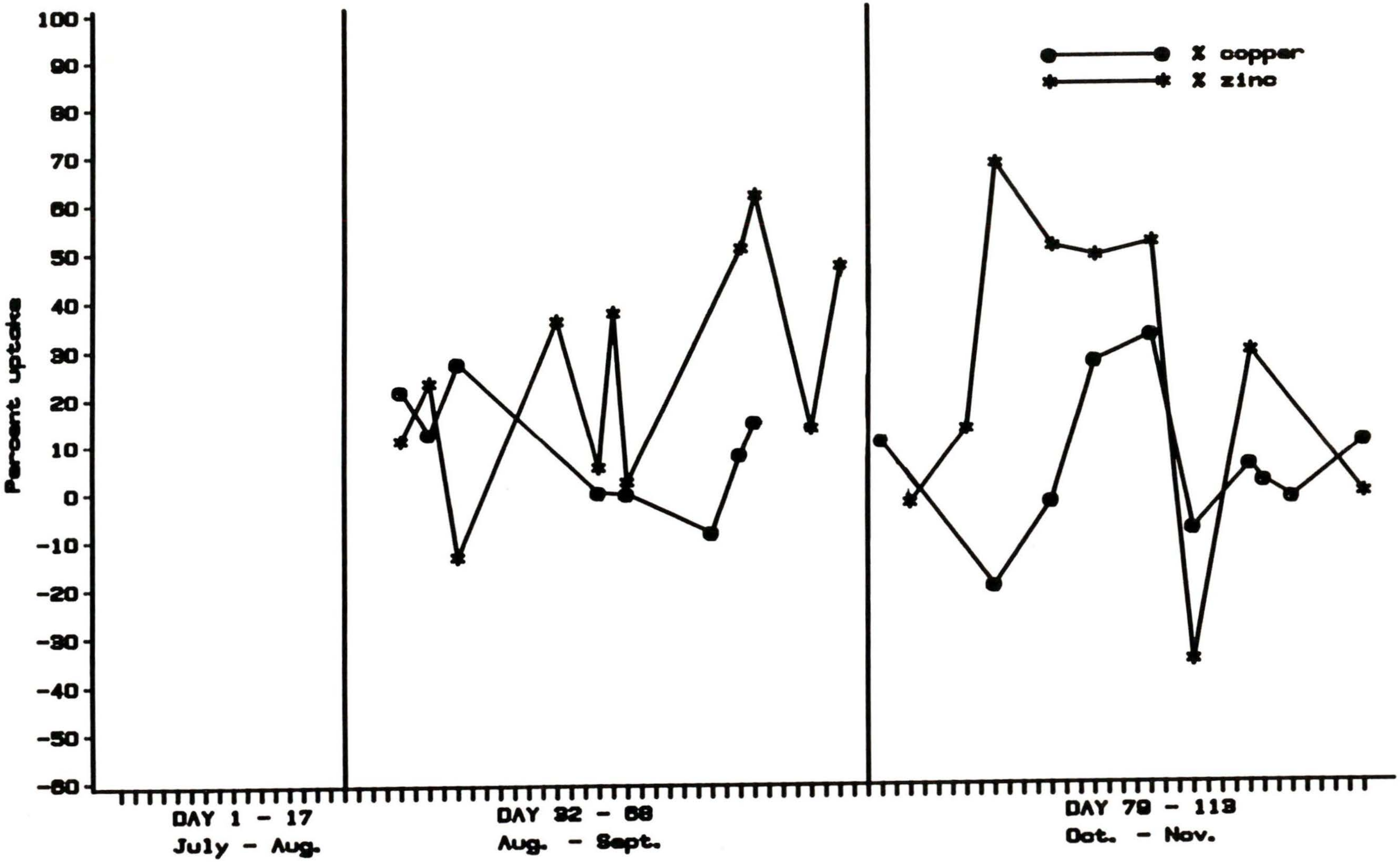
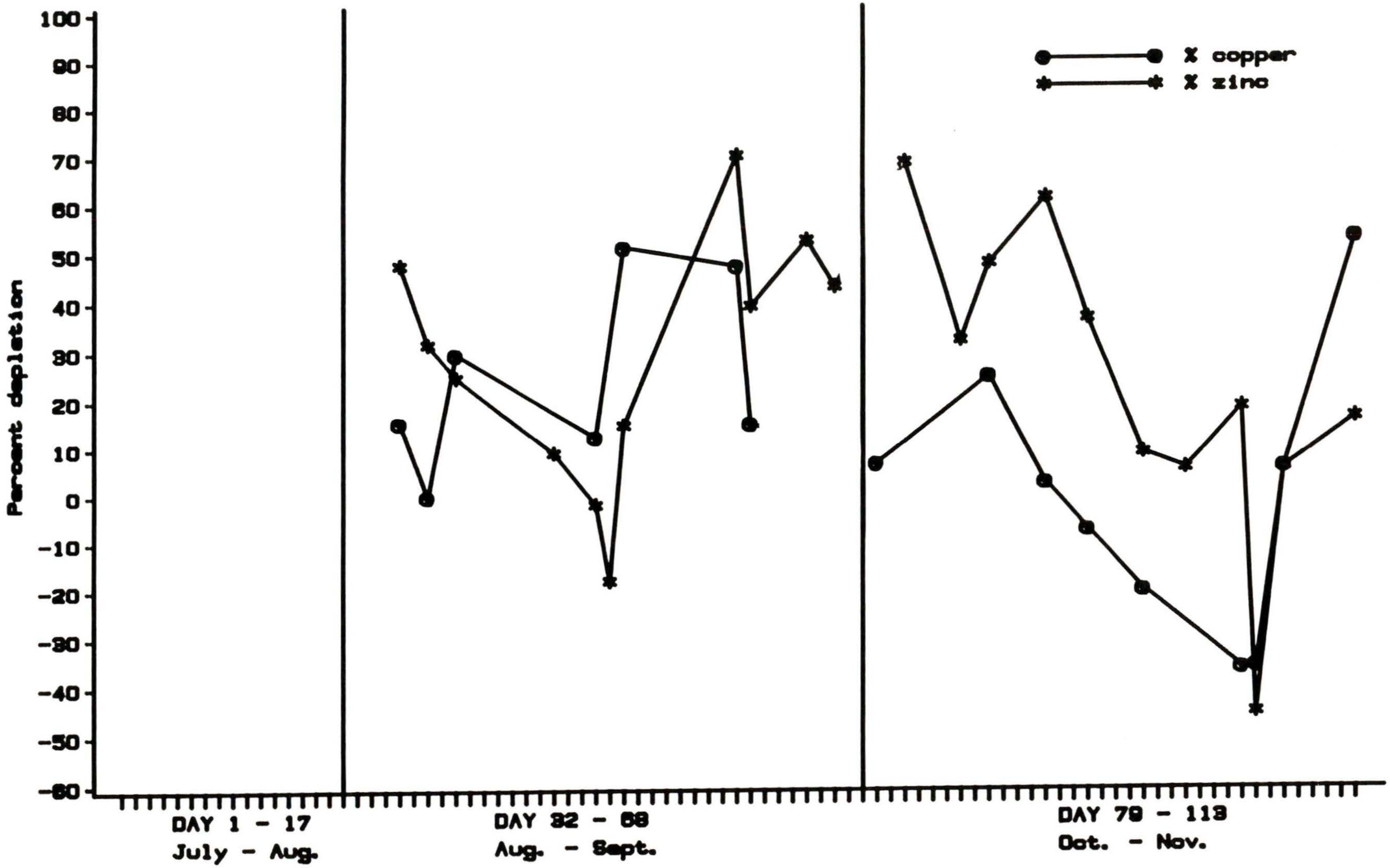

Percent uptake
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
% copper
% zinc
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 28: Copper-zinc competition (Concentration #2) on the blank plates.



100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
Percent uptake
% copper
% zinc
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

Figure 29: Copper-zinc competition (Concentration #3) on the blank plates.


% copper
% zinc
Percent depletion
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
DAY 1 - 17
July - Aug.
DAY 32 - 68
Aug. - Sept.
DAY 79 - 113
Oct. - Nov.

3.8 Metal concentrations of the biomass

When metal concentrations in the biofilms were measured, copper under neutral conditions was found to be taken up in proportion to the exogenous concentration (Fig. 30). Zinc uptake (Fig. 31) was more variable. Under acidified conditions both copper and zinc uptake were distinctly lower (Figs. 32 and 33 resp.) and the uptake pattern for copper was lost. There was no measurable cadmium in any of the samples.

Figure 30: Copper uptake by the biofilm under neutral conditions.

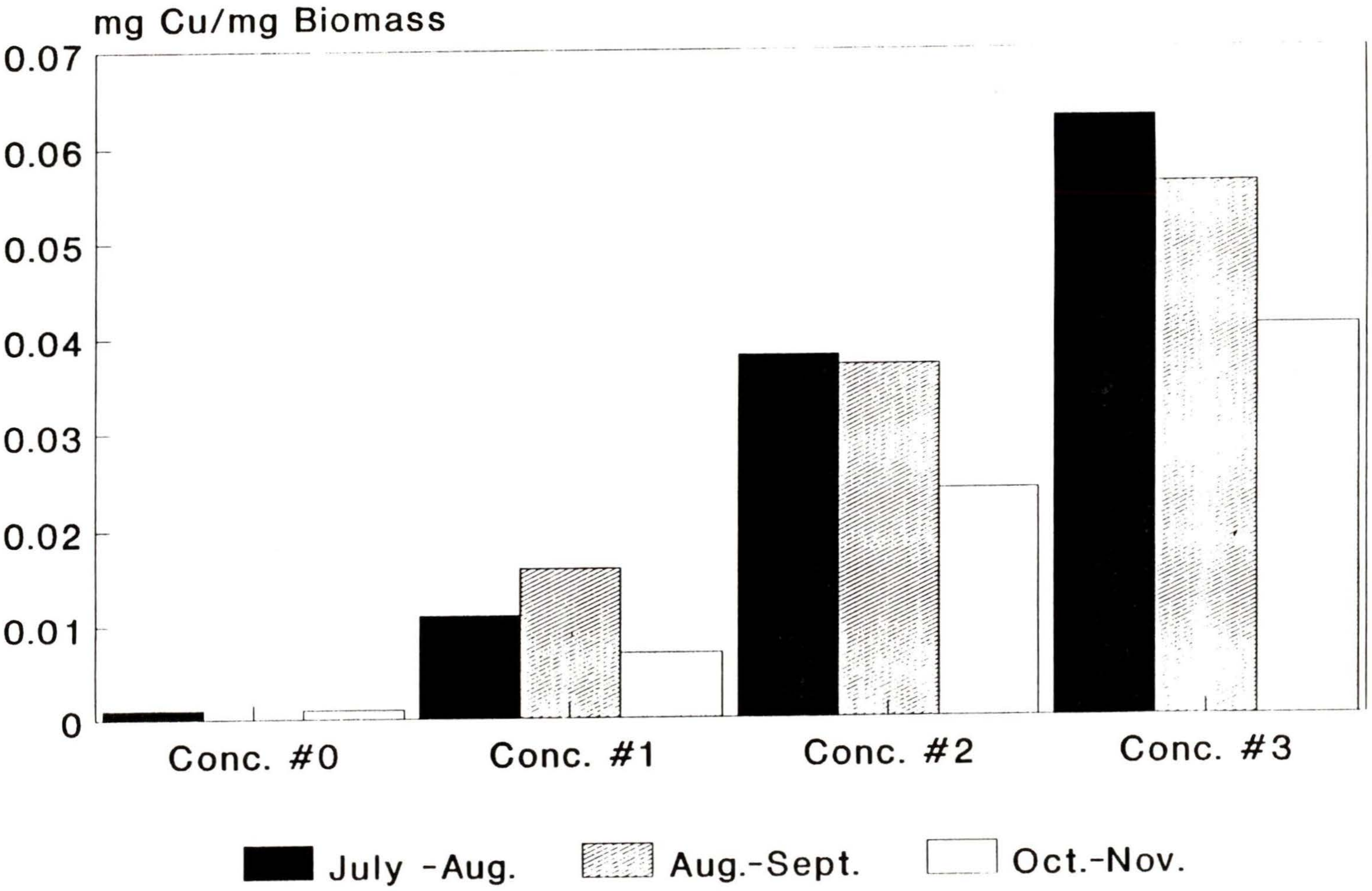

mg Cu/mg Biomass
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Conc. #0
Conc. #1
Conc. #2
Conc. #3
July -Aug.
Aug.-Sept.
Oct.-Nov.

Figure 31: Zinc uptake by the biofilm under neutral conditions.

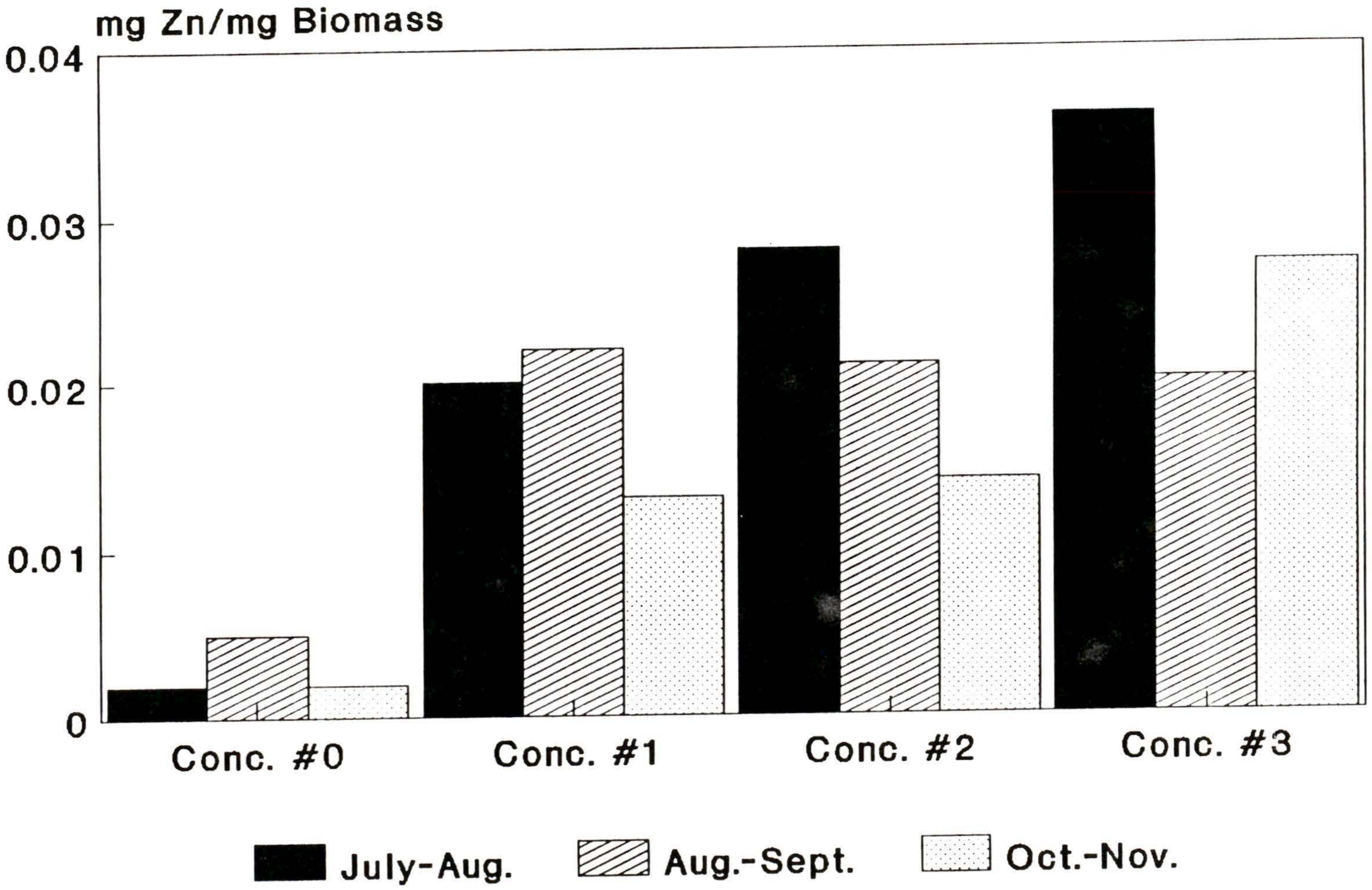

mg Zn/mg Biomass
0.04
0.03
0.02
0.01
0
Conc. #0
Conc. #1
Conc. #2
Conc. #3
July-Aug.
Aug.-Sept.
Oct.-Nov.

Figure 32: Copper uptake by the biofilm under acidified conditions.

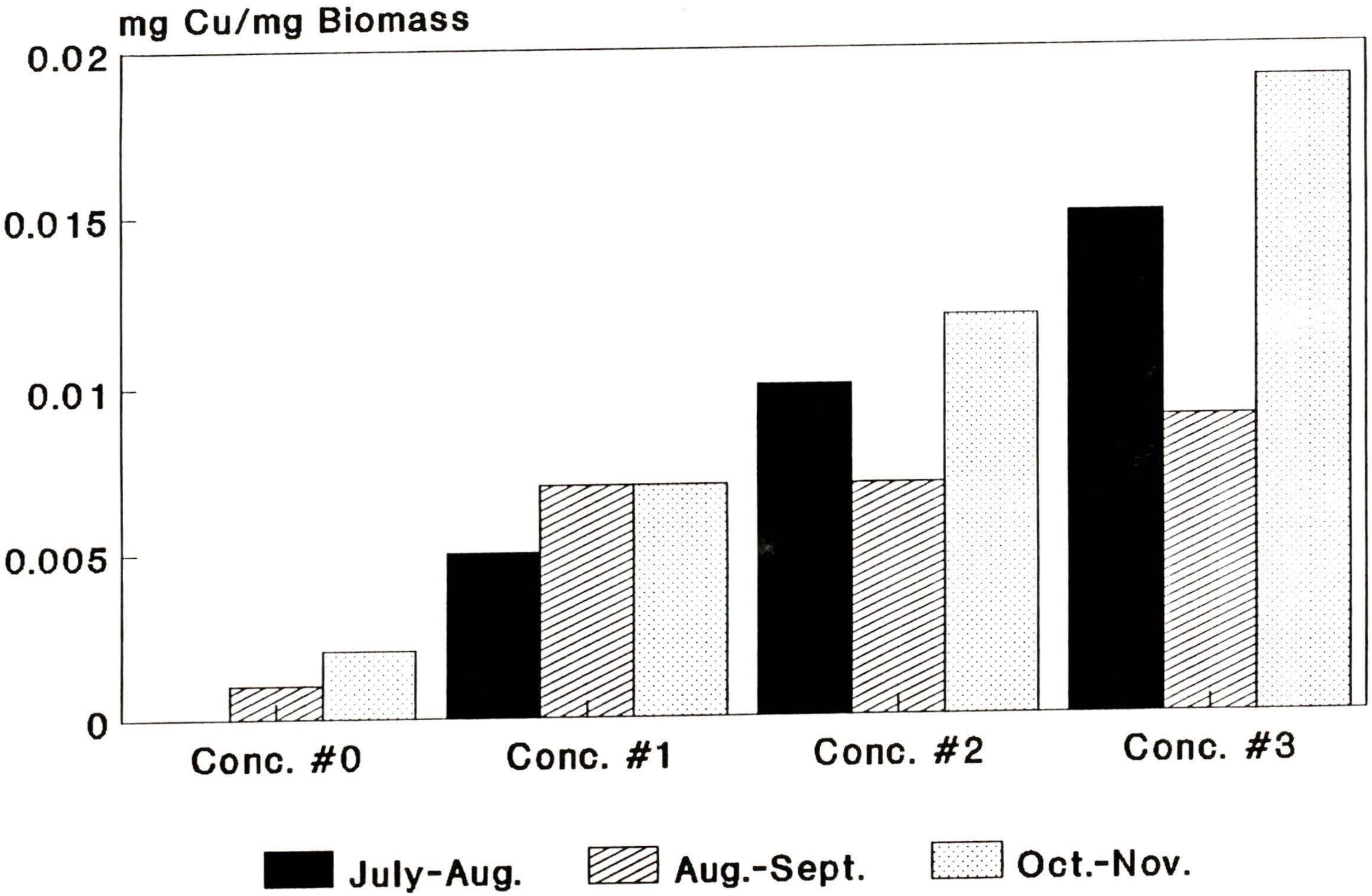
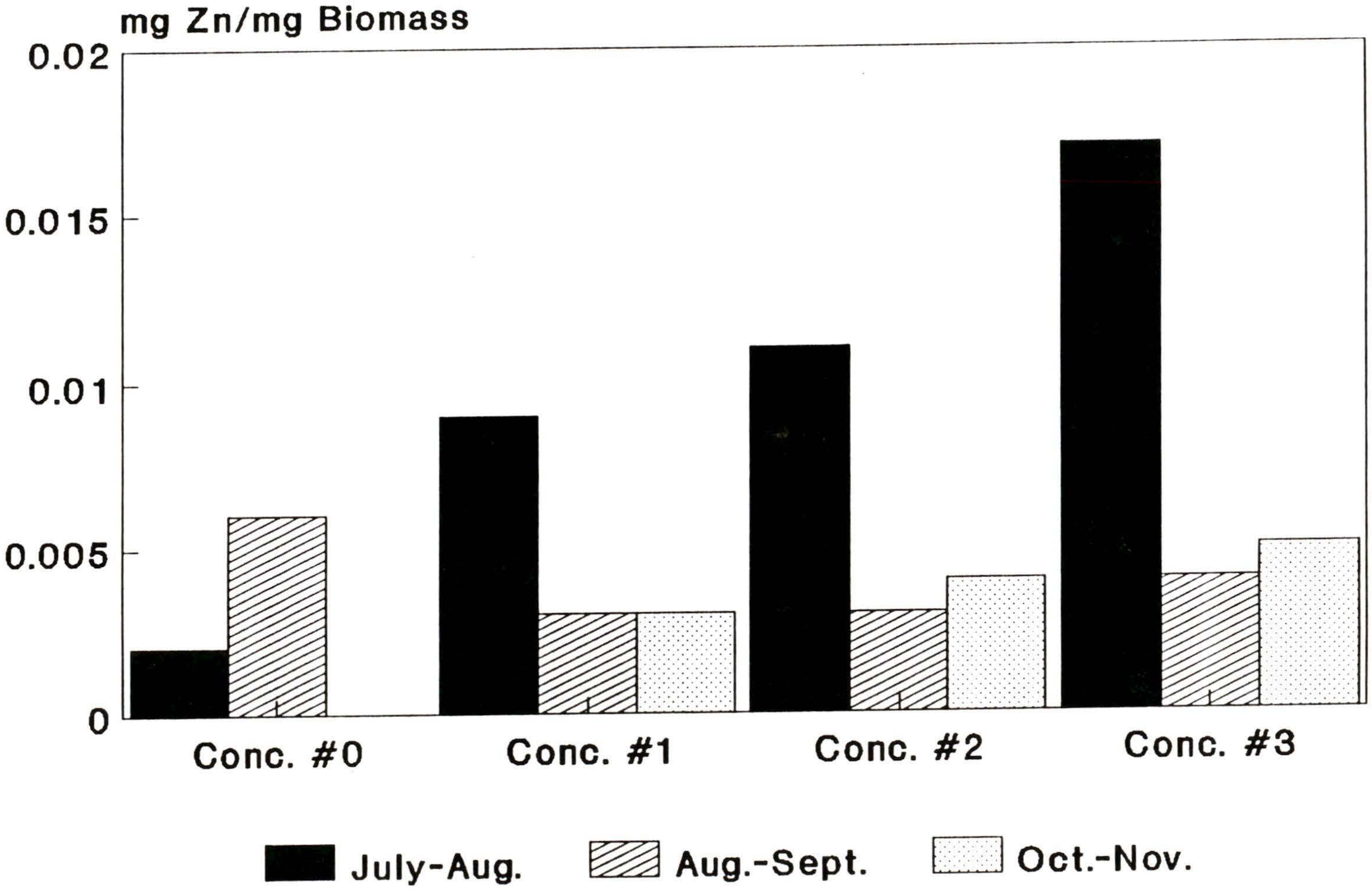

mg Cu/mg Biomass
0.02
0.015
0.01
0.005
0
Conc. #0
Conc. #1
Conc. #2
Conc. #3
July-Aug.
Aug.-Sept.
Oct.-Nov.

Figure 33: Zinc uptake by the biofilm under acidified conditions.


mg Zn/mg Biomass
0.02
0.015
0.01
0.005
0
Conc. #0
Conc. #1
Conc. #2
Conc. #3
July-Aug.
Aug.-Sept.
Oct.-Nov.

Analysis of variance for the different uptake values is given in Table 6.

Table 6: Metal concentrations in the biomass.

Significant ($P<0.05$) differences.

Test	*Cu uptake* mg/mg biomass dry weight	*Zinc uptake* mg/mg biomass dry weight
Expt #1,2,3	#1=#2=#3	#1>#2=#3
Treatment	Neutral>Acidic	Neutral>Acidic
Conc	#3>#2>#1>#0	#3>#2=#1>#0

From experiment to experiment there was no significant ($P<0.05$) difference between copper uptake per concentration, while for zinc, uptake in the first experiment was significantly higher than in the second and the third. Uptake under neutral conditions was significantly higher than uptake under acidified conditions both for copper and zinc. For copper, the uptake per concentration increased with increasing concentration, while for zinc Conc. #2 and Conc. #1 were not different from each other, but Conc. #3, Conc. #2, and Conc. #1 were all significantly greater than the control. It was not possible to measure cadmium uptake.

Concentration factors were calculated and are given in Table 7.

Table 7: Biomagnification factors.

Metal	*Conc* *mg/L*	*mg metal/ mg biomass dry weight*	*magnification factors*
		Neutral	
Cu	0.0(average 0.0129	0.001	$7.8x10^{-2}$
	0.5	0.011	$2.2x10^{4}$
	1.5	0.033	$3.3x10^{4}$
	2.5	0.053	$2.1x10^{4}$
Zn	0.0(average -0.5417)	0.004	$2.1x10^{-3}$
	5.0	0.018	$3.7x10^{3}$
	10.0	0.021	$2.1x10^{3}$
	25.0	0.028	$1.1x10^{3}$
		Acidified	
Cu	0.0(average 0.0074)	0.001	$1.3x10^{-1}$
	0.5	0.006	$1.3x10^{4}$
	1.5	0.010	$9.7x10^{3}$
	2.5	0.014	$5.7x10^{3}$
Zn	0.0(average 0.7383)	0.004	$5.4x10^{-3}$
	5.0	0.005	$1.0x10^{3}$
	10.0	0.006	$6.0x10^{3}$
	25.0	0.009	$3.5x10^{2}$

Copper was concentrated from the water by a factor of about 10 greater than zinc, for both neutral and acidified conditions. Furthermore, copper concentrations in the biomass are consistently higher than the zinc concentrations.

3.9 Total metal depletion vs. total metal uptake by the biomass

To estimate whether there is a correlation between the daily metal depletion and the quantity of metal taken up by the biomass, the total depletion value was calculated, from the 'corrected' data and averaged for the two experimental periods. The average uptake by the biomass per concentration was multiplied by the average biomass per concentration (before and after), and then multiplied by 9 (the number of plates) to give a total amount of metals taken up per tank. The results are given in Table 8.

Table 8: Total depletion vs. total biomass uptake.

Conc. #	Total metal depletion (mg)	Total metal uptake by the Biomass (mg) Before	After
	Copper Neutral		
1	0.18	1.75	1.26
2	2.61	6.03	3.65
3	10.32	8.92	6.63
	Zinc Neutral		
1	56.52	2.88	2.06
2	222.60	3.84	2.32
3	245.25	4.72	3.50
	Copper Acidified		
1	1.92	.87	.56
2	4.54	1.62	1.19
3	8.65	2.02	1.37
	Zinc acidified		
1	121.43	.72	.47
2	407.81	.97	.71
3	841.75	1.15	.79

Copper values in the biomass are within the range of those calculated from the corrected daily depletion values, both for the neutral and the acidified treatments. Total zinc values do not correlate at all. Zinc in the biomass is very much lower than the values calculated for the 'corrected' depletion values.

3.10 Species list

An all-species analysis for the three different months is given in Appendix 1. A list of the ten most common species and their ranks relative to one another are given in Table 9. Due to a lack of colonizing plates, there was no collection at the start of July-August period.

Table 9: Species before and after each experiment

Species	Before treatment	After treatment
	July - August (Neutral)	
	Averaged rank orders	
Synedra ulna	-	1
Melosira distans	-	2.75
Navicula perigrina	-	3.5
Tabellaria fenestrata	-	4
Achnanthes minutissima	-	4.75
Ceratium hirundinella	-	6
Asterionella formosa	-	7
Diatoma hiemale	-	7
Fragillaria sp.	-	7
Mougeotia sp.	-	7
Diatoma elongata	-	7.5
Melosira ambigua	-	8
Zygnema sp.	-	8.3
Tabellaria flocculosa	-	8.5
Cosmarium ralfsii	-	8.7
Spirogyra sp.	-	9
Achnanthes sp.	-	10
Frustula rhomboides	-	10
Gomphonema lanceolatum	-	10
Cosmarium sp.1	-	10
Dinobryon sp.	-	10
	August-September (Neutral)	
Achnanthes minutissima	1.25	1
Synedra ulna	3.25	2.25
Mougeotia sp.	4	4
Fragillaria construens	4	-
Gomphonema lanceolatum	8	-
Tabellaria fenestrata	3.25	4
Bulbochaeta insignis	6	4
Melosira distans	1.7	5.5
Dinobryon sp.	-	5.7
Tabellaria flocculosa	8.5	6
Coccoid greens (5u)	-	6
Gomphonema, girdle view	8.5	7
Cymbella sp.1	-	7
Cymbella sp.2	8	7
Nitzschia denticulata	-	7
Navicula perigrina	5.25	7.25
Ceratium hirundinella	5	7.5
Cosmarium ralfsii	7	8
Scenedesmus sp.	8	-
Spirogyra sp.	-	8

October-November (Neutral)

Species	Before treatment Average rank orders	After treatment
Achnanthes minutissima	1	1
Synedra ulna	2	2.75
Tabellaria fenestrata	2.7	3
Tabellaria flocculosa	6	3
Bulbochaeta insignis	3.7	3.5
Ulothrix sp.	-	4
Dinobryon sp.	5.7	4.3
Cosmarium ralfsii	7	6
Oedogonium sp.	-	6.7
Asterionella formosa	-	7
Gomphonema, girdle view	6.3	7
Melosira distans	6	7
Scenedesmus sp.	9	7
Zygnema sp.	-	7
Navicula perigrina	6.75	7.5
Nitzschia denticulata	-	8.7
Gomphonema lanceolatum	10	8
Coccoid greens	7	10
Gomphonema acuminatum	-	10
Ceratium hirundinella	8	10

July - August (Acidified)

Species	Before treatment	After treatment
Achnanthes minutissima	-	1
Diatoma vulgare	-	2
Melosira distans	-	3.75
Gomphonema,girdle view	-	4
Bulbochaeta insignis	-	4.3
Tabellaria fenestrata	-	4.5
Scenedesmus sp.	-	4.5
Fragillaria sp.	-	5
Navicula perigrina	-	5
Synedra ulna	-	5.25
Diatoma hiemale	-	6
Eunotia tibia	-	7
Melosira ambigua	-	8
Coccoid greens (5u)	-	8
Cosmarium ralfsii	-	8
Cosmarium euastrum	-	8
Spondylonium planum	-	8
Tabellaria flocculosa	-	9
Cosmarium sp.2	-	9

August-September (Acidified)

Species	Before treatment	After treatment
	Average rank order	
Achnanthes minutissima	*1*	*1*
Coccoid green (lu)	-	2
Asterionella formosa	-	*3*
Bulbochaeta insignis	*4.3*	*3*
Synedra ulna	*5.5*	*3.25*
Gomphonema, girdle view	*4*	*4*
Tabellaria fenestrata	*4.5*	*4*
Dinobryon sp.	-	*5*
Ceratium hirundinella	-	*6*
Navicula perigrina	*5*	*7*
Scenedesmus sp.	*4.5*	*7*
Mougeotia sp.	-	*7.3*
Zygnema sp.	-	*7.5*
Melosira distans	*3.75*	*7.5*
Nitzschia denticulata	-	*9*
Tabellaria flocculosa	*8.5*	*9*
Spirogyra sp.	-	*9*
Gomphonema lanceolatum	-	*10*
October-November (Acidified)		
Achnanthes minutissima	*1*	*1*
Bulbochaeta insignis	-	*2*
Coccoid greens (5u)	-	*3*
Synedra ulna	*2.25*	*3.5*
Ulothrix sp.	-	*4*
Tabellaria fenestrata	*3.5*	*4.25*
Dinobryon sp.	*4*	*4.5*
Gomphonema, girdle view	*4*	*5.5*
Mougeotia sp.	*6.3*	*6*
Spondylonium planum	-	*6*
Zygnema sp.	*4*	*6.25*
Tabellaria flocculosa	*9*	*7*
Gomphonema lanceolatum	*9.5*	*8*
Cosmarium ralfsii	-	*8*
Scenedesmus sp.	*5.5*	*8*
Ceratium hirundinella	*6*	*8.5*
Navicula perigrina	*6.25*	*8.7*
Eunotia tibia	-	*10*
Gomphonema acuminatum	-	*10*
Spirogyra sp.	*8*	*10*

In the first study period, diversity was relatively high and the community was dominated by *Synedra ulna*, *Tabellaria fenestrata*, and *Achnanthes minutissima*. During August-September *Achnanthes minutissima* became more abundant as did *Bulbochaeta insignis*, *Mougeotia* sp., and *Dinobryon* sp.. In the last study period, October-November, *Achnanthes minutissima* became almost completely dominant. There were no clear changes in the species distribution or relative numbers before and after each experiment.

Visual observation of the fixed samples showed few dead cells. Most of the diatoms were intact and still filled with cytoplasm. Chloroplasts in such species as *Zygnema* and *Spirogyra* were well preserved and clearly recognizable.

3.11 Metal concentrations in the water of Humpback Lake

Water quality data for July and August 1985 were obtained from Austin, Clark, and Lucy, (1989), and are shown in Table 10.

Table 10: Water analysis of Humpback Lake

Total metals (mg/l).

Metals	*July*	*August*
Aluminium	<0.02	<0.02
Arsenic	<0.25	<0.25
Cadmium	<0.0005	<0.0005
Chromium	<0.005	<0.05
Cobalt	<0.1	<0.01
Copper	0.003	<0.001
Iron	<0.01	0.14
Lead	0.011	<0.001
Manganese	<.01	0.015
Molybdenum	<0.01	<0.01
Nickel	<0.05	<0.05
Vanadium	<0.01	<0.005
Zinc	<0.005	<0.005

This table shows that iron and manganese concentrations are low.

Chapter IV

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

4.1 In review

The relationship between metal uptake by live organisms or nonliving matter and the factors that influence it are as follows. Metal uptake is directly related to:

1. the mass of the adsorbent (Daniels *et al.*, 1956), and by inference,
2. the total available surface area,
3. the exogenous metal concentration (Hart and Scaife, 1977; Hart *et al.*, 1979; Trollope and Evans, 1976; Friant and Koerner, 1981; and others),
4. the presence of light (Hart and Scaife, 1977; Hart *et al.*, 1979; Cushing and Rose, 1970; Gutknecht, 1963),
5. the pH (for some metals) (Gipps and Coller, 1980; Les and Walker, 1984; Adams, 1984; Tam and Wong, 1983), either by causing chelation or through protein competition,
6. the length of exposure time, although for the majority of metals binding is essentially saturated within the first 2 hours (Les and Walker, 1984; Vymazal, 1984),
7. the number of available binding sites, which is metal dependent (Crist *et al.*, 1981; Netzer and Hughes, 1984; Tobin *et al.*, 1984), and the algal species (Briand *et al.*,1978).

Uptake is inversely related to:

1. the temperature in the case of simple adsorption onto nonliving matter (Daniels *et al.*, 1956), although 3 studies show that uptake by live algae increases with increasing temperatures (Gipps and Coller, 1980; Hart and Scaife, 1977; Hart *et al.*, 1979), which may indicate other processes were operating,
2. (possibly) the amount already adsorbed, although strains made tolerant by exposure took up more metals than their nontolerant counterparts (Stokes, 1975),
3. the amounts and kinds of released chelators that change the metal speciation (Jonas *et al.*, 1984: Ramamoorthy and Kushner, 1975 a and b),
4. the amount of metal released by the cells either from the cell surface or by leaching (Mang and Tromballa, 1978; Hart *et al.*, 1979).

Furthermore, uptake is influenced (either positively or negatively) by:

1. metal competition (Netzer and Hughes, 1984; Christensen and Scherfig, 1979, Briand *et al.*, 1978),
2. the amount of detritus and insoluble extracellular material amongst algae (Bollag and Duszota, 1984; Tobin *et al.*, 1984; Mang and Tromballa, 1978; Gutknecht, 1963),
3. the presence of high concentrations of inorganic particles, hydrous ferrous oxides and manganese oxides (Sigg *et al.*, 1987; Kuwabara, 1986; Newman, 1983 and 1985; Florence, 1977).

4.2 Average daily depletion of metals from water

Comparison of the average daily metal depletion under neutral conditions showed (Fig. 1, Table 1) that copper depletion is directly related to the exogenous concentrations. All metal depletion values were significantly different ($P<0.05$) from each other as well as from the control (Concentration #0). Therefore higher copper concentrations in the surrounding waters led to higher metal depletion values. This relationship was not as clear for zinc under neutral conditions (Fig. 2, Table 1), where Concentration #2 was the only concentration that was consistently significantly different. Although some of this variability may have been partly due to an increased variability in the atomic absorption concentration measurements (caused by the 45° angle of the flame) it also indicates metal competition, where higher exogenous concentrations do not necessarily mean higher uptakes. These contradictions are reflected in the variable results obtained in these experiments, where, depending on the month and possibly the type of algal community, uptake could be either related to the exogenous concentration (Experiment 3) or to competition (Experiments 1 and 2). Differences between experiments could be a result of the changed composition of the algal community during that period. Briand *et al.* (1978) in biweekly sampling, found that metal uptake was more closely related to the species composition of the community than to the total biomass.

4.3 Neutral versus acidified

Decreasing the pH of the input concentration to 6.5 increased the variability in the metal metal depletion patterns both for copper and zinc (Figs 3 and 4, respectively). pH reduction was expected to reduce the daily uptake, due to competition for binding sites between H^+ and metal ions. However, the small total amount of biomass on the plates may not have been sufficient to show the effect of acidification. In addition, an average lowering of the pH from 7.0 to 6.5 may not have been sufficient to create measurable proton-metal competition. Since no other studies of this kind (i.e. measurements of the effects of acidification on metal uptake) were found in the literature, it is difficult to state the cause of this variability with any certainty.

Although the pH was consistently lower for the acidified treatment, there was no significant difference ($P<0.5$) in metal depletion between the two treatments (Figs. 12, 13, Table 1). At the levels of pH studied, the effects of protons competing with metals for binding sites may not have been pronounced enough to show a difference. Small biomass values (ranging from 4 to 28 mg) could also be an influencing factor, since the effect of a reduced pH showed up clearly when the metals were measured in the biofilm itself (Figs. 26-29, Table 6). If much larger amounts of biomass had been present, the effect of a slightly reduced pH might have become more apparent.

4.4 Metal depletion by acrylic tanks

A blank experiment was carried out in parallel with the colonized plates, in order to determine the interactions between the metals and acrylic (Figs 5-8). Due to the inert nature of all components, low variability and a clear distinction in metal depletion for the different concentrations between blank acrylic and colonized plates (Figs. 5 - 8) was expected. Metal depletion patterns were expected to follow Langmuir or Freundlich equations.

However, Concentration #3, the highest concentration was not significantly different from Concentration #2 for copper, while for zinc, the highest concentration was the only concentration that was consistently significantly different. This may have been the result of specific interactions between metals and acrylics.

Figures 9-11 show that metal depletion by the blank was generally lower than the colonized uptake. For copper, depletion was therefore not only a function of the surrounding metal concentration, but also of the nature of the adsorber, in this case the algae. It is possible that the increased surface area available for metal adsorption is the cause for this difference between depletion by the blank and the colonized plates. Rose and Cushing (1970) showed (through TEM photography) that metal adsorption occurred first on the outer layer of an attached algal community and then slowly penetrated between the cells. The affinity of metals for organic ligands could be another factor. Hart and Scaife (1979) mentioned that the surface of *Chlorella pyrenoidosa* is a mosaic of binding sites for different metals. An area covered with different algae would likely have a greater abundance and variety of binding sites on its surface than the same acrylic area.

Depletion patterns from day to day were similar for both the blank and the biofilm experiments. This similarity in patterns between colonized plates in the light and blank plates in the dark indicated that light was not a determining factor in the changes in the daily depletion pattern. Since metal depletion from the water by acrylics can be assumed to be adsorptive, and since the depletion patterns are the same, it is likely that uptake was rather more adsorptive than absorptive and the difference in metal depletion was due the biofilm.

4.5 Cadmium concentrations

The low (0.01 mg/L) allowable concentrations of cadmium in raw public drinking water are a reflection of its toxic and carcinogenic nature. Concentrations had to be held low to avoid contamination of the environment. As a result concentrations (even the highest) were below the limits of detection of the Flame Atomic Absorption Spectrophotometer, and could not be measured with accuracy.

4.6 Corrected values

When metal depletion values for the colonized plates were corrected for the depletion by the exposed part of the tank, the depletion pattern flattened out to some extent. This indicates that the pattern was determined by factors other than environmental or biological events.

Friedman's rank correlation analysis showed (Table 3) that when the experiments were pooled, metal depletion values were significantly larger for larger input concentrations ($P<0.05$), which indicates that metal depletion is dependent on the exogenous concentrations, both for copper and zinc. The distinction between concentrations was not always clear per experiment, although copper values were significantly different from the control. Zinc metal depletion at low concentrations was not significantly different from the control in the second experiment, indicating that at low concentrations, copper-zinc competition existed, or that the algae controlled the uptake of zinc through, for example, the release of chelators.

High correlation coefficients (Table 4) (ranging from 70 to 90 %) for copper showed that there was a direct relationship between input and metal depletion, i.e. metal depletion was dependent on the concentration. The value of the slope (Table 4) was equivalent to the percent metal depletion (about 40 %). The relationship did not fit Langmuir or Freundlich adsorption isotherm equations In standard metal adsorption-on-resin experiments, resins are exposed to different concentrations over the same period of time at the same temperature under laboratory conditions (Daniels *et al.*, 1956).

The resulting relationships are expressed as

$$c/(x/m)=(b/a)c+(1/a) \quad \text{Langmuir}$$

and

$$(x/m)=kc^{n} \quad \text{Freundlich}$$

where:
x= adsorbed weight,
m= mass of adsorber,
c= concentration in solution,
n, k, a, and b= constants

In the present experiment, neither the temperature nor the exposure time were constant. Furthermore, apart from day 1, the biofilm had already taken up metals in the preceding days. Adsorption of metals to inert material is inversely related to temperature (Daniels *et al.*, 1956). In these experiments, the temperature decreased and metal depletion should have increased. Instead of this, metal depletion decreased and then increased slightly. Depending on the biomass, variations in adsorption should be directly related to the exposure times (1 to 4 days). The longer exposure should lead to greater adsorption, especially for zinc which was taken up continuously by periphytic algae (Vymazal, 1984), while copper is taken up in the first two hours after which metal depletion is slight. Over 4 days however, greater amounts of copper and zinc should have been taken up than over 1 or 2 days. However, the metals already present should reduce the availability of binding sites. Unless these factors balance out, copper depletion must have been actively controlled at least in part, and copper depletion is likely to be a combination of adsorption and absorption. No consistent percent metal depletion was found for zinc, nor were correlation coefficients consistent from experiment to experiment. Competition with copper for available binding sites, or algal control may have been the cause of this. Depletion was higher for higher concentrations, but the relationship is not straightforward and no set fraction is taken up continuously.

4.7 The effect of environmental parameters and changes in biomass

No consistent correlation (direct or inverse) could be found for the metal depletion from the ingoing water of copper and zinc as a function of the pH or the temperature (Table 5), which was contrary to findings in the literature (Les and Walker, 1984; Kuwabara *et al.*, 1986; Adams, 1985; Gipps and Coller, 1980; Hart and Scaife, 1977; Hart *et al.*, 1979). Copper depletion appeared to be somewhat (inversely) related to the temperature (Pearson's correlation coefficients of 44 to 58 %), but this relationship did not hold under acidified conditions, nor did it apply to zinc uptake under both neutral and acidified conditions. If anything, the depletion was more closely (directly) related to the changes in biomass, although the pattern was not consistent. It should be borne in mind however, that the estimated changes in biomass were established from different samples taken before and after each study season. Furthermore, the assumption of a steady loss of biomass over the periods of study may be an oversimplification of the actual pattern of biomass losses. Metal uptake, adsorptive or absorptive, would be closely related to the available biomass. A loss in biomass should result in a reduction in metal uptake.

4.8 Copper-zinc competition

Several writers mentioned that metals compete for binding sites (Briand *et al.*,1978; Christensen *et al.*, 1979; Les and Walker, 1984) and that copper outcompetes zinc.

In these experiments, no clear pattern could be found (Figs 20-23) when percent depletion of copper and zinc were compared. This was similar in the blank experiment. If anything, % zinc metal depletion was somewhat higher than % copper uptake. The effect of an exogenous zinc concentration that was a factor 10 greater than the copper concentration, obviously outweighed the effects of competition, if any.

4.9 The influence of hydrous oxides

Hydrous metal oxides are known to bind metals such as lead (Lion *et al.*,1982; Newman *et al.*, 1983 and 1985; Sigg *et al.*, 1987). If they are present in the waters to be tested, they can significantly alter the results of metal uptake studies by algae. However, iron and manganese concentrations were low in the Humpback Lake water (Table 10), and were not likely to have played a role in these experiments. Furthermore, the literature indicates (Florence, 1977; Kuwabara, 1986; Sigg *et al.*, 1987) that copper and zinc are primarily associated with the organic components of the biofilm including algae and detritus.

4.10 Metal concentrations of the biomass

The resulting copper concentrations measured in the biomass, followed the findings of other studies, i.e. the total metal uptake was dependent on the surrounding concentration and the pH (Hart and Scaife, 1977; Hart *et al.* 1979; Friant and Koerner, 1981; Les and Walker, 1984; Adams, 1985). Analysis of variance (Table 6) showed that there was no significant ($P<0.5$) difference in copper uptake from month to month. The algae in Tank 1 (Concentration 1) took up about 1.00×10^{-2} mg Cu/mg Biomass (dry weight), in Tank 2 (Concentration #2) the algae took up 3.29×10^{-2} mg Cu/mg Biomass (dry weight), and in Tank 3 (concentration 3) they took up 6.01×10^{-2} mg Cu/mg Biomass (dry weight), regardless of the season. This showed that neither temperature nor light were important factors in the uptake patterns. If the copper uptake was solely adsorptive, or was transported into the cell by passive diffusion, uptake would not be light energy dependent. The analysis also showed that uptake under neutral conditions was significantly ($P<0.5$) higher than uptake under acidified conditions. This was oddly contrasting with the daily metal depletion analysis, where no clear difference in depletion was apparent (Figs. 12 and 13). between the two treatments. It is likely that the low biomass was insufficient to show a difference on the daily level. Furthermore, the analysis showed that the metal depletion values per exogenous concentration of metals were significantly different from each other, reinforcing the idea that copper uptake was concentration dependent. The small differences for each exogenous metal concentration for the different experiments could have been a result of the changes in species dominance in the community.

Zinc concentrations in the biomass did not follow the copper patterns (Fig. 27). Uptake concentrations in the first experiment were significantly higher than in the second and third experiment. This indicates that zinc uptake may have been light, temperature, or species dependent, or a combination of any of these. Experiments have shown that zinc uptake is stimulated by light (Gutknecht 1963; Cushing and Rose, 1970). Concentrations taken up under acidified conditions were significantly lower than those under neutral conditions, and the small amount of biomass was the likely cause of the apparent discrepancy between these values and the daily metal depletion values for zinc. There was a significant difference in metal uptake between algae exposed to Concentration #3 and those exposed to Concentration #2, as well as between uptake at Concentration #1 and at Concentration #0 but no significant difference between uptake at Conc. #2 and at Conc. #1. This showed that zinc uptake was also dependent on the exogenous concentration and that uptake was mostly adsorptive. However, in spite of the fact that the zinc input was 10 times higher than the copper input, the biofilm did not take up zinc in greater amounts than copper. In fact, the average zinc concentration in the biomass was consistently lower than the copper concentration or all input concentrations (Table 7). Therefore, as previously discussed, metal competition or the algal control may have played an important part in the uptake of zinc. There was no measurable cadmium uptake. This was probably due to the low input concentrations as well as to an observed competition with zinc (Abel and Baerlocher, 1984; Les and Walker, 1984).

Trollope and Evans (1976) found that algae concentrated copper by factors ranging from 1.8×10^3 to 8.3×10^4, while zinc magnification ranged from 5.2×10^2 to 9.0×10^3, a factor of 10 lower than copper. This coincided with the findings of these experiments, where zinc is consistently a factor 10 lower than copper (Table 7). Furthermore zinc uptake is consistently lower than copper uptake.

4.11 Total metal depletion vs. total metal uptake

When the estimated total metal depletion was compared to the total metal uptake by the biomass, depletion values for copper were closely related to the total metal uptake, especially for those calculations based on the biomass at the end of the experiments. This was true to a lesser extent for the acidified conditions. Total zinc depletion did not relate at all to the total zinc taken up by the biomass. Therefore if the dosage of a metal is too high compared to the biomass uptake, measurements will be masked by other processes.

Several researchers (Hutchinson *et al.*, 1976: Trollope and Evans, 1976: Denny and Welsh, 1979:) have stated that, since algae bioconcentrate metals, they can be used to clean up waterbodies that have been contaminated with metals. To extrapolate from the ability of algae to bioconcentrate metals to their ability to remove metals from waterbodies is an oversimplification. The mass of living algae required would have to be large. For copper, for example, only about 30 % is taken up in a given waterbody (Table 4), and depending on the concentration 0.01 to 0.05 mg of copper can be held by 1 mg of biomass (Fig. 30). To clean up 1 Liter of water containing 2.5 mg/L copper would need approximately 150 mg of algal biomass (dry weight). Furthermore, at very low concentrations metal depletion may not be measurable, while the metal could still be present in concentrations that are toxic to some aquatic life.

4.12 Species analysis

During the first season (July-August) species diversity was high, with diatoms dominating. *Synedra ulna* and *Tabellaria fenestrata* were the two most frequently encountered diatioms. During August-September some green algae became more abundant, both in numbers and in size. Of these the most common were a *Bulbochaeta* sp. and a *Mougeotia* sp.. Of the diatoms during this period *Achnanthes minutissima* was present in the greatest numbers. During the October - November experiment plates were almost entirely covered with *Achnanthes minutissima*. This species is known to be metal tolerant when found in metal contaminated waters (Deniseger, 1985) Whether it was metal tolerant in these experiments cannot be answered, since tolerance can vary between strains of the same species depending on the environment in which they grow (Foster, 1982b).

In spite of the seasonal changes, species distributions and abundances (for a given experiment) were similar before and after each experiment. No invertebrate species were found.

The abundance of diatoms may have been an important factor in the relatively high uptake of copper. Lindahl *et al.*, (1983) found that copper, taken up from the marine environment, was associated with the silica frustules of diatoms. Green algae are also found to be copper concentrators (Trollope and Evans, 1976; Foster, 1982; Klotz, 1981; Keeney *et al.*, 1976; Jennet and Wixson, 1975), and their abundance in these experiments has probably contributed to the copper uptake.

Zinc uptake may have been primarily species dependent. If diatoms took up more zinc than green algae, this could explain the greater daily metal depletion and concentration in the biomass in the first season, when few green algae were present. Briand *et al.*, (1978) found that the relative uptake of copper and cadmium was species dependent. No specific studies have been found in the literature for zinc. However, in Trollope and Evans' (1976) study green algae concentrated zinc at a factor 10 less than copper, which appears to lend some support to this idea. Hutchinson *et al.*, (1976) and Patrick and Loutit (1976) stated that periphyton concentrates copper and zinc equally, but Hutchinson *et al.* (1976) did not mention the species distribution and Patrick and Loutit (1976) studied periphytic bacteria.

4.13 Conclusion and Recommendations

Under field conditions (i.e. where some variables are not under the experimenter's control) copper uptake is directly related to the biomass (to some degree), to the exogenous concentration, and to the pH. Light, exposure time to the metal, and temperature appear to have little or no influence on the uptake. Furthermore, copper is depleted as a constant fraction of the metal concentration in the surrounding water.

Zinc uptake is also dependent on the exogenous metal concentration, and is decreased by acidification. However, metal depletion of the exogenous concentration is not a constant fraction. Zinc depletion patterns were likely the result of high dosage - low biomass ratio.

Cadmium does not appear to have been concentrated. However, levels may have been too low to be measurable and competition with zinc could have reduced uptake and magnification.

Therefore, although algae are bioconcentrators of metals, their use for clean-up, or removal of metals from water bodies has certain restrictions. In a situation where several metals are present at the same time, competition may prevent one or more of the metals from being taken up. Each metal must be considered separately.

The algal mass would have to be large, although it is remarkable that about 20 mg of algae per liter showed measurable differences in copper uptake, which depended on the external copper concentration. In future experiments the metal input must be tailored to the available biomass (i.e. <2.5 mg of metal/L for 20 mg of algal mass) to give a clearer picture of daily uptake. Zinc concentrations were likely too high in this experiment to clearly show daily metal depletion patterns, although this could not have been foreseen at the outset, since no studies of this type had been carried out before.

A slight acidification (for example from acid rain) severely reduces the ability of algae to bind copper and zinc. This could be of substantial importance in areas of high acid precipitation.

The set fraction of copper depletion shows that the length of the water path may be very important. The algal mass will take up a set fraction and no more. Consecutive batches of algal mass, each taking up this fraction, or a very long stream, would be most effective for copper. Zinc uptake cannot be as clearly defined and may be much more difficult to remove. Although zinc concentrations are generally higher in the environment than copper, nevertheless copper outcompetes zinc when adsorbed, and may have to be removed first. An example of such a system would be a long meandering stream followed by a pond. The trends towards increased metal depletion at the ends of the seasons indicate that longer experiment durations might allow one or more species in the community to develop as an efficient absorber of metals. The final restriction would be winters in colder areas when ponds and streams freeze over.

A great many questions remain unanswered. For example:

1. Metal depletion appears to be cyclic, and this experiment was not designed to test that.
2. Why and how does the season affect zinc uptake?
3. Is zinc depletion primarily species related?
4. High zinc depletion values during July-August and no differences during the other two periods indicate that light, or species composition, or both, play an important role.
5. How does an algal community change under long-term metal stress?
6. Do some species develop more tolerant strains?
7. How long will it take to develop a tolerant strain?

This study was designed to answer the question, "Are biofilm organisms efficient in the removal of metals from a metal stressed environment?" It has answered that question in part, and generated many more. More field studies of this kind are needed to answer these.

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APPENDIX I. PREVIOUS PROPOSALS AND EXPERIMENTAL DESIGNS

Several experiments where proposed that led to the final design. What these experiments had in common is that they were all to be carried out under field conditions. The main restriction for each of them, was the possible threat to the environment.

Proposed experiments.

Proposal #1.

To find and culture periphyton species that are metal resistant and can be used as a biological metal clean-up system. To accomplish this, slides were to be placed at set distances from a metal source. The metal source first considered was a plate painted with shipfouling paint. However this was thought to leach metals at concentrations that were too low to give significant results. Another possible metal source was a permeable clay cup filled with a metal solution. Metals would have leached out of the cup into the surroundings. Concentrations necessary for this experiment were too high to be used outside of a laboratory.

Proposal #2. To take periphyton communities that had been exposed to metal waste, and to determine the rate of recovery when the communities were exposed to different environmental conditions.

Periphyton communities from sites exposed to metals would be subjected to; clean water, clean water with lime, with phosphorus, with concentrated periphyton to increase colonization, and clean water with a combination of all of the above. The experiment was to be carried out at Buttle Lake under field conditions, but the logistics of setting up a semi-permanent laboratory there proved to be too complicated and expensive and the experiment was altered to:

Proposal #3.

To collect periphyton communities that had not been exposed to metals and expose them to metal concentrations found in Buttle Lake, either by exposing them to Buttle Lake water in the laboratory, or by suspended seeded slides or both. Cost of analysis was the primary deterrent in this case.

Proposal #4.

To subject periphyton communities from the Buttle Lake area and subject them to metal stress by exposing them to water run over mine tailings. This experiment was to be carried out at the field laboratory at the Humpback Lake reservoir. An effort was made to extract metals from mine tailings. These tailings were obtained from the mine tailing ponds with an Eckman dredge. The pH value of these tailings was close to 10 and it proved impossible to extract the metals without strongly acidifying the water.

In the spring of 1984, acrylic plates (9.5x9.5 cm) were submerged for 6 weeks in Myra Creek to be colonized. During this time wetbenches were built at the Humpback Reservoir to receive the experimental tanks and plates. The colonized plates were transported from Myra Creek to the Humpback Reservoir in polystyrene coolers (for a period of 5 hours). Upon arrival, the plates were submerged in (acid-cleaned) experimental tanks and left to acclimatize to field laboratory conditions.

During July 1984, these periphytic organisms were exposed to solutions o different metal concentrations at pH levels ranging from 3.5 to 2.5. Samples of the ingoing and outflowing solutions were taken daily as were the pH values for each of the metal concentrations, and the flow rates of the water running through each tank.

The metal concentrations were calculated to be from 1 to 4x those concentrations found in Buttle Lake. Water samples were preserved with Baker's Instra Analyzed Nitric Acid awaiting analysis.

After that month , the organisms found on the plates had either gone into the encysting stage or had died, except for the concentration that was equal to the concentrations found in Buttle Lake. In this case the organisms looked like they might still be alive, but all inner structures looked deformed. All plates were scraped clean and an effort was made to bring the organisms into culture, to see if they would recover. The cultures all crashed.

Metal analysis of the water showed no appreciable concentrations in the ingoing solutions. This was thought to have been caused by green and blue green algae growing in the stock solution containers. In addition, the hydroponic fertilizer was thought to have caused the metals to precipitate out. This led to a modified experiment, where plates were to be colonized in the Field laboratory instead of in Myra Creek. These colonized plates were then exposed to three different metal concentrations at neutral pH and at a pH of about 5.5 - 6.0. These experiments were to be run at one month intervals or three month as is described in Chapter 2, Materials and Metals.

APPENDIX II. EXAMPLE OF CALCULATIONS AND STANDARD DEVIATIONS

An example of the measurements and calculations of copper depletion under neutral conditions for Experiment 1 (July-August) is given here. Standard deviations (s) were calculated according to

$$s= (x_i-x)/(n-1)$$

where x=average of 3 atomic absorption spectrophotometry readings, n=3 the number of readings, and i the number of the reading.

The table shows concentrations going in (3-, 2-, 1-, and 0in), and the solutions leaving the tanks (C3-, C2-, C1-, C0-, B3-, B2-, B1-, and B0-out) that correspond with the positions and concentrations given in Figure 4 of the text.

Date	Conc#	Atomic absorption reading (mg/L)			x	s
July 23	3in	2.98	3.04	3.02	3.01	0.03
	2in	1.02	1.05	0.97	1.01	0.04
	1in	0.57	0.55	0.54	0.55	0.02
	0in	0.00	0.00	0.00	0.00	0.00
	C3out	1.53	1.50	1.58	1.54	0.04
	C2out	0.63	0.62	0.60	0.62	0.02
	C1out	0.32	0.37	0.33	0.34	0.03
	C0out	0.05	0.00	0.00	0.02	0.03
	B3out	1.81	1.83	1.81	1.82	0.01
	B2out	0.43	0.42	0.43	0.43	0.01
	B1out	0.27	0.29	0.25	0.27	0.02
	B0out	0.00	0.00	0.00	0.00	0.00
July 24	3in	3.07	3.05	3.00	3.04	0.04
	2in	1.03	1.06	1.03	1.04	0.02
	1in	0.53	0.55	0.54	0.54	0.01
	0in	0.02	0.03	0.03	0.03	0.01
	C3out	0.94	0.93	0.89	0.92	0.03
	C2out	0.05	0.04	0.05	0.05	0.01
	C1out	0.06	0.05	0.08	0.06	0.02
	C0out	0.01	0.03	0.01	0.02	0.01
	B3out	1.21	1.21	1.21	1.21	0.00
	B2out	0.05	0.05	0.08	0.06	0.02
	B1out	0.05	0.06	0.05	0.05	0.01
	B0out	0.00	0.03	0.05	0.03	0.03
July 27	3in	3.07	3.05	3.08	3.06	0.02
	2in	1.03	1.07	1.06	1.05	0.01
	1in	0.55	0.54	0.54	0.54	0.01
	0in	0.07	0.10	0.08	0.08	0.02
	C3out	1.18	1.17	1.18	1.18	0.01
	C2out	0.06	0.05	0.05	0.05	0.01
	C1out	0.05	0.04	0.04	0.04	0.01
	C0out	0.03	0.02	0.03	0.03	0.01
	B3out	1.28	1.24	1.25	1.26	0.02
	B2out	0.08	0.07	0.08	0.08	0.01
	B1out	0.04	0.05	0.04	0.04	0.01
	B0out	0.03	0.02	0.03	0.03	0.01

Date	Conc#	Atomic absorption reading (mg/L)			x	s
July 29	3in	3.01	3.01	2.98	3.00	0.02
	2in	1.06	1.05	1.08	1.06	0.02
	1in	0.54	0.53	0.53	0.53	0.01
	0in	0.02	0.02	0.06	0.03	0.02
	C3out	2.51	2.55	2.48	2.51	0.04
	C2out	0.92	0.96	0.97	0.95	0.03
	C1out	0.52	0.54	0.52	0.53	0.01
	C0out	0.01	0.01	0.03	0.02	0.01
	B3out	2.86	2.74	2.79	2.76	0.03
	B2out	0.92	0.91	0.90	0.91	0.01
	B1out	0.54	0.54	0.50	0.53	0.02
	B0out	0.00	0.02	0.00	0.01	0.01
July 30	3in	3.01	3.02	3.08	3.04	0.04
	2in	1.03	1.03	1.02	1.03	0.01
	1in	0.55	0.53	0.53	0.54	0.01
	0in	0.00	0.08	0.00	0.03	0.05
	C3out	2.51	2.53	2.47	2.50	0.03
	C2out	0.91	0.88	0.93	0.91	0.03
	C1out	0.51	0.51	0.53	0.52	0.02
	C0out	0.02	0.01	0.01	0.01	0.01
	B3out	2.31	2.32	2.29	2.31	0.02
	B2out	0.83	0.81	0.82	0.82	0.01
	B1out	0.52	0.54	0.50	0.52	0.02
	B0out	0.01	0.01	0.02	0.01	0.01
Aug.4	3in	2.86	2.85	2.88	2.86	0.02
	2in	1.11	1.11	1.11	1.11	0.01
	1in	0.37	0.35	0.35	0.35	0.04
	0in	0.01	0.01	0.03	0.03	0.40
	C3out	1.51	1.51	1.50	1.51	0.02
	C2out	0.64	0.63	0.63	0.63	0.01
	C1out	0.37	0.37	0.37	0.37	0.00
	C0out	0.03	0.01	0.02	0.02	0.01
	B3out	2.02	1.97	2.01	2.01	0.04
	B2out	0.72	0.72	0.72	0.72	0.00
	B1out	0.33	0.33	0.33	0.33	0.00
	B0out	0.02	0.04	0.03	0.03	0.01

Date	Conc#	Atomic absorption reading (mg/L)			x	s
Aug. 6	3in	3.97	4.06	4.02	4.02	0.05
	2in	1.11	1.15	1.08	1.11	0.04
	1in	0.34	0.34	0.34	0.34	0.00
	0in	0.01	0.03	0.01	0.02	0.01
	C3out	1.57	1.56	1.57	1.57	0.01
	C2out	0.50	0.50	0.46	0.49	0.02
	C1out	0.32	0.31	0.33	0.32	0.01
	C0out	0.00	0.00	0.05	0.02	0.03
	B3out	2.17	2.03	2.17	2.12	0.08
	B2out	0.62	0.61	0.60	0.61	0.01
	B1out	0.31	0.32	0.33	0.32	0.01
	B0out	0.00	0.00	0.02	0.01	0.01
Aug. 7	3in	2.89	2.84	2.86	2.86	0.03
	2in	1.14	1.19	1.16	1.16	0.03
	1in	0.36	0.37	0.36	0.36	0.01
	0in	0.00	0.00	0.09	0.03	0.05
	C3out	1.74	1.70	1.82	1.75	0.06
	C2out	0.68	0.67	0.65	0.67	0.02
	C1out	0.33	0.32	0.33	0.33	0.01
	C0out	0.00	0.00	0.00	0.00	0.00
	B3out	1.83	1.84	1.79	1.82	0.03
	B2out	0.72	0.77	0.80	0.76	0.04
	B1out	0.34	0.35	0.35	0.35	0.01
	B0out	0.00	0.00	0.00	0.00	0.00

From these data depletion values were determined by calculating the average of the B and C concentrations leaving the tanks, and subtracting them from the ingoing concentrations. The standard deviation for the average of B and C (x) was determined according to:

$$s = (x_i - x)^2/(n-1)$$

while the standard deviation of the depletion was determined by a pooled variance measure according to:

$$s_x - s(B,C) = s_p^2/n_1 + s_p^2/n_2$$

with

$$s_p^2 = (s_x + s(B,C)) / (v_1 + v_2)$$

where v_1 and v_2 are the degrees of freedom for each sample, and s_p^2 the pooled variance. The following table shows these values for Experiment 1.

Date	Conc#	x	s_x	(B+C)/2	$s_{B,C}$	Depletion	$s_{x-(B,C)}$
July 23	3	3.01	0.03	1.68	0.20	1.33	0.25
	2	1.01	0.04	0.53	0.13	0.48	0.24
	1	0.55	0.02	0.31	0.05	0.24	0.13
	0	0.00	0.00	0.01	0.01	-0.01	0.05
July 24	3	3.04	0.04	1.07	0.21	1.97	0.77
	2	1.04	0.02	0.06	0.01	0.98	0.09
	1	0.54	0.01	0.06	0.01	0.48	0.07
	0	0.03	0.01	0.03	0.01	0.00	0.07
July 27	3	3.06	0.02	1.22	0.06	1.84	0.14
	2	1.05	0.02	0.07	0.02	0.98	0.11
	1	0.54	0.01	0.04	0.00	0.50	0.04
	0	0.08	0.02	0.03	0.00	0.05	0.06
July 29	3	3.00	0.02	2.64	0.18	0.36	0.23
	2	1.06	0.02	0.93	0.03	0.13	0.11
	1	0.53	0.01	0.53	0.00	0.00	0.04
	0	0.03	0.02	0.02	0.01	0.01	0.10
July 30	3	3.04	0.04	2.41	0.13	0.63	0.22
	2	1.03	0.01	0.87	0.06	0.16	0.14
	1	0.54	0.01	0.52	0.00	0.02	0.06
	0	0.03	0.05	0.01	0.00	0.02	0.11
Aug. 4	3	2.86	0.02	1.76	0.36	1.10	0.32
	2	1.11	0.01	0.68	0.06	0.43	0.14
	1	0.35	0.02	0.35	0.03	0.00	0.11
	0	0.03	0.04	0.03	0.01	0.00	0.12
Aug. 6	3	4.02	0.05	1.85	0.39	2.17	0.35
	2	1.11	0.04	0.55	0.08	0.56	0.18
	1	0.34	0.00	0.32	0.00	0.02	0.00
	0	0.02	0.01	0.02	0.01	0.00	0.08
Aug. 7	3	2.86	0.03	1.79	0.05	1.07	0.14
	2	1.16	0.03	0.72	0.06	0.44	0.15
	1	0.36	0.01	0.34	0.01	0.02	0.07
	0	0.03	0.05	0.00	0.00	0.03	0.12

APPENDIX III. SPECIES ANALYSIS AND COUNTS.

Species before and after each experiment were identified and counted, taking 15 fields at random and counting at 40x magnification. The results per species were added for each plate. Due to the lack of colonizing plates there was no collection at the start of the July-August period.

Diatom species were initially identified from ashed specimens. Species counts were carried out using wet fixed samples. Some of the species found in the ashed samples did not appear in the species counts, and have therefore been recorded without numbers. Species lists are given here.

July - August

Species	Before treatment 0N	1N	2N	3N	After treatment 0N	1N	2N	3N
Achnanthes laterostata	-	-	-	-	-	-	-	-
Achnanthes minutissima	-	-	-	-	*51*	*33*	*16*	*56*
Achnanthes sp.	-	-	-	-	*9*	-	*1*	-
Amphora ovalis	-	-	-	-	-	-	*2*	-
Asterionella formosa	-	-	-	-	*2*	*4*	*7*	*7*
Caloneis sp.	-	-	-	-	-	-	-	-
Cocconeis placentula	-	-	-	-	-	-	-	-
Cyclotella sp.	-	-	-	-	-	*1*	-	*1*
Cymbella sp1.	-	-	-	-	*1*	*2*	-	-
Cymbella sp2.	-	-	-	-	-	-	-	-
Diatoma hiemale	-	-	-	-	*24*	*41*	*3*	*4*
Diatoma elongata	-	-	-	-	*16*	-	*6*	-
Diploneis sp.	-	-	-	-	-	-	-	-
Epithemia argus	-	-	-	-	*3*	*2*	-	-
Epithemia turgida	-	-	-	-	-	-	-	-
Eunotia denticulata	-	-	-	-	-	*2*	*1*	*1*
Fragillaria construens	-	-	-	-	-	*6*	-	-
Fragillaria sp.	-	-	-	-	-	*49*	*5*	-
Frustula rhomboides	-	-	-	-	-	*1*	*3*	*2*
Gomphonema acuminatum	-	-	-	-	*2*	-	-	-
Gomphonema lanceolatum	-	-	-	-	-	*10*	*1*	-
Gomphonema sp.	-	-	-	-	-	-	-	-
Gomphonema, girdle view	-	-	-	-	*9*	-	*2*	*4*
Mastogloia smithii	-	-	-	-	-	-	-	-
Melosira distans	-	-	-	-	*25*	*79*	*90*	*67*
Melosira ambigua	-	-	-	-	*10*	-	-	*23*
Navicula perigrina	-	-	-	-	*53*	*72*	*52*	*44*
Navicula subtilissima	-	-	-	-	-	-	-	-
Navicula vulpina	-	-	-	-	-	-	-	-
Navicula sp.1	-	-	-	-	-	-	-	-
Navicula sp.2	-	-	-	-	-	-	-	-
Naviula sp.3	-	-	-	-	-	-	-	-
Nitzschia denticulata	-	-	-	-	*1*	-	-	*1*
Nitzschia ignorata	-	-	-	-	-	-	*1*	*3*
Nitzschia amphibea	-	-	-	-	-	-	-	*2*
Nitzschia sp.1	-	-	-	-	-	-	-	*2*
Nitzschia sp.2	-	-	-	-	-	-	-	-
Rhopalodia gibba	-	-	-	-	*1*	-	-	*2*
Tabellaria fenestrata	-	-	-	-	*19*	*66*	*41*	*116*
Tabellaria flocculosa	-	-	-	-	*13*	*2*	*2*	*13*
Stauroneis phoenicenteron	-	-	-	-	-	-	-	-
Suririella biseriata	-	-	-	-	-	-	-	-
Suririella elegans	-	-	-	-	-	-	-	-
Suririella laris	-	-	-	-	-	-	-	-
Suririella sp.	-	-	-	-	*1*	-	-	-
Synedra ulna	-	-	-	-	*73*	*367*	*244*	*235*

Arthrodesmus	-	-	-	-	-	-	-	-
Bulbochaeta insignis	-	-	-	-	*2*	-	-	-
Coccoid greens (5u)	-	-	-	-	-	-	-	-
Coccoid greens (1u)	-	-	-	-	-	-	-	-
Cosmarium ralfsii	-	-	-	-	*9*	*3*	*11*	*10*
Cosmarium euastrum	-	-	-	-	-	*4*	-	-
Cosmarium sp.1	-	-	-	-	*9*	*4*	-	-
Cosmarium sp.2	-	-	-	-	-	-	-	-
Cosmarium sp.3	-	-	-	-	-	-	-	-
Cosmarium sp.4	-	-	-	-	-	-	-	-
Cosmarium sp.5	-	-	-	-	*2*	-	-	-
Docidium undulatum	-	-	-	-	-	-	-	-
Mougeotia sp.	-	-	-	-	*19*	*6*	*3*	*46*
Oedogonium sp.	-	-	-	-	-	-	-	-
Scenedesmus sp.	-	-	-	-	*4*	*4*	-	-
Schizomeisis leibnii	-	-	-	-	-	-	-	-
Spirogyra sp.	-	-	-	-	-	*12*	-	-
Spondylonium planum	-	-	-	-	-	-	*1*	*1*
Staurastrum curutum	-	-	-	-	-	-	-	-
Staurastrum sp.	-	-	-	-	-	-	-	-
Ulothrix sp.	-	-	-	-	-	-	-	-
Zygnema sp.	-	-	-	-	*1*	*13*	*5*	*16*
Dinobryon	-	-	-	-	-	-	*3*	-
Spirulina princeps	-	-	-	-	-	-	-	-
Oscillatoria sp.	-	-	-	-	-	v	-	-
Blue-green filament 1	-	-	-	-	-	-	-	-
Blue-green filament 2	-	-	-	-	-	-	-	v
Ceratium hirundinella	-	-	-	-	-	*6*	*11*	*1*
Pediastrum sp.	-	-	-	-	-	-	-	-
Fungal filaments Possibly;*Scytalidium* sp.	-	-	-	-	v	v	v	-

August-September

	Before treatment				After treatment			
Species	0N	1N	2N	3N	0N	1N	2N	3N
Achnanthes laterostata	-	-	-	-	-	-	-	-
Achnanthes minutissima	*158*	*637*	*272*	*45*	*2060*	*1654*	*3071*	*1273*
Achnanthes sp.	-	-	-	-	-	-	-	-
Amphora ovalis	-	*1*	*1*	-	-	-	-	-
Asterionella formosa	-	*2*	*1*	-	-	-	-	-
Caloneis sp.	-	-	-	-	-	-	-	-
Cocconeis placentula	-	-	*1*	*1*	-	-	-	*1*

Cyclotella sp	-	*1*	*1*	*2*	-	-	-	*2*
Cymbella sp.1	-	-	-	-	*1*	*8*	-	-
Cymbella sp.2	-	-	*2*	*3*	-	-	-	-
Diatoma hiemale	-	-	-	-	-	-	-	-
Diatoma elongata	-	-	-	-	-	-	-	-
Diploneis sp.	-	-	-	-	*1*	-	-	*1*
Epithemia argus	-	-	-	-	-	-	-	-
Epithemia turgida	-	-	-	*2*	-	-	*1*	-
Eunotia denticulata	-	-	*1*	*1*	-	-	-	-
Fragillaria construens	*28*	-	*1*	*2*	-	-	-	-
Fragillaria sp.	-	-	-	-	-	-	-	-
Frustula rhomboides	*1*	-	*1*	*1*	*1*	-	-	*1*
Gomphonema acuminatum	-	*1*	*1*	-	-	-	-	-
Gomphonema lanceolatum	*6*	-	-	*1*	*3*	*1*	*1*	*4*
Gomphonema sp.	-	-	-	-	*1*	-	-	-
Gomphonema, girdle view	*5*	*2*	*2*	*3*	*42*	*3*	-	*7*
Mastogloia smithii	-	-	-	-	-	-	-	-
Melosira distans	-	*53*	*66*	*120*	-	*2*	*10*	*10*
Melosira ambigua	-	-	-	-	-	-	-	-
Navicula perigrina	*15*	*14*	*4*	*13*	*28*	*3*	*9*	*5*
Navicula subtilissima	-	-	-	-	-	-	-	-
Navicula vulpina	-	-	-	-	-	-	-	-
Navicula sp.1	-	-	-	-	-	-	-	-
Navicula sp.2	-	-	-	-	-	-	-	-
Navicula sp.3	-	-	-	-	-	-	-	-
Nitzschia denticulata	*1*	-	*1*	-	*5*	*1*	-	-
Nitzschia ignorata	-	-	-	-	-	*2*	-	-
Nitzschia amphibea	-	-	-	-	-	-	-	-
Nitzschia sp.1	-	-	-	-	-	-	-	-
Nitzschia sp.2	-	-	-	-	-	-	-	-
Rhopalodia gibba	-	-	-	*1*	*1*	-	-	-
Tabellaria fenestrata	*42*	*26*	*42*	*38*	*1*	*65*	*3*	*65*
Tabellaria flocculosa	*4*	*1*	*1*	*7*	*2*	*2*	-	*13*
Stauroneis phoenicenteron	-	-	-	-	-	-	-	-
Suririella biseriata	-	-	-	-	-	-	-	-
Suririella elegans	-	-	*1*	-	-	-	-	-
Suririella laris	-	-	-	-	-	-	-	-
Suririella sp.	-	-	-	-	*1*	-	-	-
Synedra ulna	*58*	*17*	*12*	*44*	*138*	*83*	*39*	*30*
Arthrodesmus	-	-	-	-	-	-	-	-
Bulbochaeta insignis	*14*	-	-	-	*69*	*15*	*1*	*24*
Coccoid greens (5u)	-	-	-	4	-	-	6	-
Coccoid greens (1u)	-	-	-	-	-	-	-	-
Cosmarium ralfsii	*9*	*1*	-	-	*4*	-	-	-
Cosmarium euastrum	-	-	-	-	*1*	-	-	-
Cosmarium sp.1	-	-	-	-	-	-	-	-
Cosmarium sp.2	*14*	*1*	*1*	*1*	*4*	-	-	-
Cosmarium sp.3	-	-	-	-	-	-	-	-
Cosmarium sp.4	-	-	-	-	-	-	-	-

Cosmarium sp.5	1-	-	-	-	2	-	-	-
Docidium undulatum	-	-	-	-	-	-	-	-
Mougeotia sp.	2	-	-	-	45	19	15	15
Oedogonium sp.	-	-	-	-	-	-	-	-
Scenedesmus sp.	-	-	-	-	4	-	-	-
Schizomeisis leibnii	-	-	-	-	-	-	-	-
Spirogyra sp.	-	-	-	-	-	6	-	-
Spondylonium planum	-	-	-	-	-	-	1	1
Staurastrum curutum	-	-	-	-	-	-	-	-
Staurastrum sp.	2	-	-	-	-	-	-	-
Ulothrix sp.	-	-	-	-	-	-	-	-
Zygnema sp.	-	-	-	-	-	-	2	-
Dinobryon sp.	-	-	-	-	-	14	10	10
Spirulina princeps	-	-	-	-	-	-	-	-
Oscillatoria sp.	-	-	-	-	-	-	-	-
Blue-green filament 1	-	-	-	-	-	-	-	-
Blue-green filament 2	-	-	-	-	v	v	v	v
Ceratium hirundinella	-	1	1	22	-	2	3	7
Pediastrum sp.	-	-	-	-	-	1	-	-
Fungal filaments Possibly;*Scytalidium* sp.	v	v	v	v	-	-	-	-

October-November

	Before treatment				After treatment			
Species	0N	1N	2N	3N	0N	1N	2N	3N
Achnanthes laterostata	-	-	-	-	-	-	-	-
Achnanthes minutissima	2060	1654	3071	1273	1612	1508	5373	4218
Achnanthes sp.	-	-	-	-	-	-	-	-
Amphora ovalis	-	-	-	-	-	-	-	-
Asterionella formosa	-	-	8	-	-	-	4	1
Caloneis sp.	-	-	-	-	-	-	-	-
Cocconeis placentula	-	-	-	1	-	-	-	-
Cyclotella sp	-	-	-	-	-	-	-	-
Cymbella sp.1	-	1	-	2	-	1	-	-
Cymbella sp.2	-	-	-	-	-	-	-	-
Diatoma hiemale	-	-	-	-	-	-	-	-
Diatoma elongata	-	-	-	-	-	-	-	-
Diploneis sp.	1	-	-	1	-	-	-	-
Epithemia argus	-	-	-	-	-	-	-	-
Epithemia turgida	-	-	1	-	-	-	-	-
Eunotia denticulata	-	-	-	-	-	-	-	-
Fragillaria construens	-	-	-	-	-	-	-	-
Fragillaria sp.	-	-	-	-	-	-	-	-

Frustula rhomboides	1	-	-	1	1	-	-	1
Gomphonema acuminatum	-	-	-	-	12	-	-	1
Gomphonema lanceolatum	3	1	1	4	-	4	-	1
Gomphonema sp.	1	-	-	-	-	-	-	-
Gomphonema, girdle view	42	3	-	7	18	2	5	3
Mastogloia smithii	-	-	-	-	-	-	-	-
Melosira distans	-	2	10	10	-	1	-	12
Melosira ambigua	-	-	-	-	-	-	-	-
Navicula perigrina	28	3	9	5	20	3	-	4
Navicula subtilissima	-	-	-	-	-	-	-	-
Navicula vulpina	-	-	-	-	-	-	-	-
Navicula sp.1	-	-	-	-	-	-	-	-
Navicula sp.2	-	-	-	-	-	-	-	-
Navicula sp.3	-	-	-	-	-	-	-	-
Nitzschia denticulata	5	1	-	-	13	3	3	1
Nitzschia ignorata	-	2	-	-	-	-	-	-
Nitzschia amphibea	-	-	-	-	-	1	-	-
Nitzschia sp.1	-	-	-	-	-	-	-	-
Nitzschia sp.2	-	-	-	-	-	-	-	-
Rhopalodia gibba	1	-	-	-	-	1	-	-
Tabellaria fenestrata	1	65	18	65	7	45	48	78
Tabellaria flocculosa	2	2	-	13	-	2	-	34
Stauroneis phoenicenteron	-	-	-	-	-	-	-	-
Suririella biseriata	-	-	-	-	-	-	-	-
Suririella elegans	-	-	-	-	-	-	-	1
Suririella laris	-	-	-	-	-	-	-	1
Suririella sp.	-	-	-	-	-	-	-	-
Synedra ulna	138	83	39	30	116	108	94	31
Arthrodesmus	-	-	-	-	-	-	-	-
Bulbochaeta insignis	69	15	1	24	32	1	53	-
Coccoid greens	-	-	6	-	12	-	-	-
Coccoid greens (lu)	-	-	-	-	-	-	-	-
Cosmarium ralfsii	8	-	-	-	10	11	-	16
Cosmarium euastrum	2	-	-	-	6	-	-	-
Cosmarium sp.1	1	-	-	-	-	-	-	-
Cosmarium sp.2	4	-	-	-	-	-	-	-
Cosmarium sp.3	-	-	-	-	-	-	-	-
Cosmarium sp.4	-	-	-	-	-	-	-	-
Cosmarium sp.5	-	-	-	-	-	-	-	-
Docidium undulatum	-	-	-	-	-	-	-	-
Mougeotia sp.	45	15	15	15	-	-	-	-
Oedogonium sp.	-	-	-	-	26	8	-	10
Scenedesmus sp.	4	-	-	-	-	4	-	16
Schizomeisis leibnii	-	-	-	-	-	-	-	-
Spirogyra sp.	6	-	-	-	-	-	-	2
Spondylonium planum	-	-	-	-	-	-	-	-
Staurastrum curutum	-	-	-	-	-	-	-	-

Staurastrum sp.	-	-	-	-	-	-	-	-
Ulothrix sp.	-	-	-	-	*45*	*16*	-	-
Zygnema sp.	-	-	*2*	-	*19*	*16*	-	*6*
Dinobryon sp.	-	*14*	*10*	*10*	-	*28*	*33*	*33*
Spirulina princeps	-	-	-	-	-	-	-	-
Oscillatoria sp.	-	-	-	-	-	-	-	-
Blue-green filament 1	v	v	v	v	-	-	-	-
Blue-green filament 2	-	-	-	-	-	v	v	v
Ceratium hirundinella	-	*2*	*3*	*7*	*2*	*5*	-	*5*
Pediastrum sp.	-	*1*	-	-	-	-	-	-
Fungal filaments Possibly;*Scytalidium* sp.	-	-	-	-	-	-	-	-

July - August

	Before treatment				After treatment			
Species	05	15	25	35	05	15	25	35
Achnanthes laterostata	-	-	-	-	-	-	-	-
Achnanthes minutissima	-	-	-	-	*1274*	*189*	*848*	*359*
Achnanthes sp.	-	-	-	-	-	-	-	-
Amphora ovalis	-	-	-	-	-	-	-	-
Asterionella formosa	-	-	-	-	-	-	-	-
Caloneis sp.	-	-	-	-	-	-	-	-
Cocconeis placentula	-	-	-	-	-	-	*1*	*1*
Cyclotella sp	-	-	-	-	-	*1*	-	*1*
Cymbella sp.1	-	-	-	-	-	*1*	-	-
Cymbella sp.2	-	-	-	-	-	-	-	*1*
Diatoma hiemale	-	-	-	-	-	-	-	*13*
Diatoma elongata	-	-	-	-	-	-	-	-
Diatoma vulgare	-	-	-	-	-	-	-	*43*
Diploneis sp.	-	-	-	-	-	-	-	-
Epithemia argus	-	-	-	-	-	-	-	-
Epithemia turgida	-	-	-	-	-	-	-	-
Eunotia denticulata	-	-	-	-	-	-	-	-
Eunotia tibia	-	-	-	-	*2*	*1*	*5*	-
Fragillaria construens	-	-	-	-	-	-	-	-
Fragillaria sp.	-	-	-	-	-	-	*8*	-
Frustula rhomboides	-	-	-	-	-	*1*	*1*	*1*
Gomphonema acuminatum	-	-	-	-	-	-	-	-
Gomphonema lanceolatum	-	-	-	-	*2*	-	-	-
Gomphonema sp.	-	-	-	-	-	-	-	-
Gomphonema, girdle view	-	-	-	-	*26*	-	*1*	*1*
Mastogloia smithii	-	-	-	-	-	-	-	-
Melosira distans	-	-	-	-	*13*	*28*	*28*	*28*
Melosira ambigua	-	-	-	-	-	*4*	-	-
Navicula perigrina	-	-	-	-	*22*	*15*	*7*	*21*

Navicula subtilissima	-	-	-	-	-	-	-	-
Navicula vulpina	-	-	-	-	-	-	-	-
Navicula sp.1	-	-	-	-	-	-	-	-
Navicula sp.2	-	-	-	-	-	-	-	-
Naviula sp3	-	-	-	-	-	-	-	-
Nitzschia denticulata	-	-	-	-	*2*	-	*1*	*3*
Nitzschia ignorata	-	-	-	-	-	-	-	-
Nitzschia amphibea	-	-	-	-	-	-	*1*	*1*
Nitzschia sp.1	-	-	-	-	-	-	*1*	-
Nitzschia sp.2	-	-	-	-	-	-	-	-
Rhopalodia gibba	-	-	-	-	-	-	-	*2*
Tabellaria fenestrata	-	-	-	-	*16*	*19*	*38*	*12*
Tabellaria flocculosa	-	-	-	-	*3*	-	-	*1*
Stauroneis phoenicenteron	-	-	-	-	-	-	-	-
Suririella biseriata	-	-	-	-	-	-	-	-
Suririella elegans	-	-	-	-	-	-	-	-
Suririella laris	-	-	-	-	-	-	-	-
Suririella sp.	-	-	-	-	-	-	-	-
Synedra ulna	-	-	-	-	*34*	*13*	*9*	*6*
Arthrodesmus	-	-	-	-	-	-	-	-
Bulbochaeta insignis	-	-	-	-	*153*	*7*	-	*21*
Coccoid greens (5u)	-	-	-	-	4	-	-	-
Coccoid greens (1u)	-	-	-	-	-	-	-	-
Cosmarium ralfsii	-	-	-	-	*4*	-	*1*	-
Cosmarium euastrum	-	-	-	-	*4*	-	-	-
Cosmarium sp.1	-	-	-	-	-	-	-	-
Cosmarium sp.2	-	-	-	-	*3*	-	-	-
Cosmarium sp.3	-	-	-	-	-	-	-	-
Cosmarium sp.4	-	-	-	-	-	-	-	-
Cosmarium sp.5	-	-	-	-	*2*	-	*1*	*2*
Docidium undulatum	-	-	-	-	-	-	-	-
Mougeotia sp.	-	-	-	-	-	-	-	-
Oedogonium sp.	-	-	-	-	-	-	-	-
Scenedesmus sp.	-	-	-	-	-	*16*	*2*	*18*
Spirogyra sp.	-	-	-	-	-	-	-	-
Spondylonium planum	-	-	-	-	-	*4*	-	-
Staurastrum curutum	-	-	-	-	-	*1*	-	-
Staurastrum sp.	-	-	-	-	-	-	-	-
Ulothrix sp.	-	-	-	-	-	-	-	-
Zygnema sp.	-	-	-	-	-	-	-	-
Dinobryon sp.	-	-	-	-	-	-	-	-
Spirulina princeps	-	-	-	-	-	-	-	-
Oscillatoria sp.	-	-	-	-	-	-	-	-
Blue-green filament 1	-	-	-	-	-	-	-	-
Blue-green filament 2	-	-	-	-	-	-	-	-

Ceratium hirundinella	-	-	-	-	-	2	-	1
Pediastrum sp.	-	-	-	-	-	-	1	-
Fungal filaments Possibly;*Scytalidium* sp.	-	-	-	-	v	v	v	v

August-September

	Before treatment				After treatment			
Species	05	15	25	35	05	15	25	35
Achnanthes laterostata	-	-	-	-	-	-	-	-
Achnanthes minutissima	1274	189	848	359	374	757	2146	424
Achnanthes sp.	-	-	-	-	-	-	-	-
Amphora ovalis	-	-	-	-	-	-	-	-
Asterionella formosa	-	-	-	-	-	3	-	99
Caloneis sp.	-	-	-	-	-	-	-	-
Cocconeis placentula	-	-	1	1	-	1	-	-
Cyclotella sp	-	1	-	1	-	-	-	1
Cymbella sp.1	-	1	-	-	-	-	-	3
Cymbella sp.2	-	-	-	-	-	-	-	-
Diatoma elongata	-	-	-	-	-	-	-	-
Diatoma hiemale	-	-	-	13	-	-	-	-
Diatoma vulgare	-	-	-	43	-	-	-	-
Diploneis sp.	-	-	-	-	-	1	-	-
Epithemia argus	-	-	-	-	-	-	-	-
Epithemia turgida	-	-	-	-	-	-	-	-
Eunotia denticulata	1	-	-	-	-	-	-	-
Eunotia tibia	1	1	5	-	2	-	-	-
Fragillaria construens	-	-	16	-	-	-	-	-
Fragillaria sp.	-	-	-	-	-	-	-	-
Frustula rhomboides	-	1	2	1	-	-	-	1
Gomphonema acuminatum	-	-	-	-	3	-	-	-
Gomphonema lanceolatum	2	-	-	-	6	5	1	-
Gomphonema sp.	-	-	-	-	-	1	-	-
Gomphonema, girdle view	26	-	1	1	77	19	-	-
Mastogloia smithii	-	-	-	-	-	-	-	-
Melosira distans	13	28	28	28	2	7	4	2
Melosira ambigua	-	-	-	-	-	-	-	-
Navicula perigrina	22	15	7	21	27	13	6	10
Navicula subtilissima	-	-	-	-	-	-	-	-
Navicula vulpina	-	-	-	-	-	-	-	-
Navicula sp.1	-	-	-	-	-	-	-	-
Navicula sp.2	-	-	-	-	-	-	-	-
Naviula sp.3	-	-	-	-	-	-	-	-
Nitzschia denticulata	1	-	1	3	5	6	1	-
Nitzschia ignorata	-	-	-	-	-	-	-	-
Nitzschia amphibea	-	-	-	-	-	-	-	-
Nitzschia sp.1	-	-	1	1	1	-	-	-
Nitzschia sp.2	-	-	1	-	-	-	-	-

Rhopalodia gibba	-	-	-	*1*	*1*	*1*	-	-
Tabellaria fenestrata	*16*	*19*	*38*	*12*	*33*	*72*	*45*	*85*
Tabellaria flocculosa	*3*	-	*3*	*1*	*1*	*6*	-	-
Stauroneis phoenicenteron	-	-	-	-	-	-	-	-
Suririella biseriata	-	-	-	-	-	-	-	-
Suririella elegans	-	-	-	-	-	-	-	-
Suririella laris	-	-	-	-	-	-	-	-
Suririella sp.	-	-	-	-	-	-	-	-
Synedra ulna	*34*	*13*	*9*	*6*	*53*	*151*	*82*	*71*
Arthrodesmus	-	-	-	-	-	-	-	-
Bulbochaeta insignis	*153*	*7*	-	*21*	*114*	*36*	-	-
Coccoid greens (5u)	4	-	-	-	-	-	-	-
Coccoid greens (1u)	-	-	-	-	-	-	-	131
Cosmarium ralfsii	*4*	-	*1*	-	*2*	-	-	-
Cosmarium euastrum	*2*	-	-	-	*2*	-	-	-
Cosmarium sp.1	-	-	-	-	*1*	-	-	-
Cosmarium sp.2	-	-	-	-	-	-	*2*	-
Cosmarium sp.3	-	-	-	-	-	-	-	-
Cosmarium sp.4	-	-	*1*	*2*	-	-	-	-
Cosmarium sp.5	-	-	-	-	*2*	-	-	-
Docidium undulatum	-	-	-	-	-	-	-	-
Mougeotia sp.	-	-	-	-	*28*	*7*	*1*	*15*
Oedogonium sp.	-	-	-	-	-	-	-	-
Scenedesmus sp.	-	*16*	*2*	*18*	-	*14*	-	*13*
Schizomeisis leibnii	-	-	-	-	-	-	-	-
Spirogyra sp.	-	-	-	-	*12*	-	-	-
Spondylonium planum	-	*4*	-	-	-	-	-	-
Staurastrum corutum	*1*	-	-	-	-	*1*	-	-
Staurastrum sp.	-	-	-	-	-	-	-	-
Ulothrix sp.	-	-	-	-	-	-	-	-
Zygnema sp.	-	-	-	-	*39*	-	-	*6*
Dinobryon sp.	-	-	-	-	-	*3*	*9*	*35*
Spirulina princeps	-	-	-	-	-	-	-	-
Oscillatoria sp.	-	-	-	-	-	-	-	-
Blue-green filament 1	-	-	-	-	-	-	-	-
Blue-green filament 2	-	-	-	-	-	-	v	v
Ceratium hirundinella	*1*	*2*	-	*1*	-	*3*	*5*	*1*
Pediastrum	-	-	-	-	-	-	-	-
Fungal filaments Possibly;*Scytalidium* sp.	v	v	v	v	-	-	-	-

October-November

Before treatment After treatment

Species	05	15	25	35	05	15	25	35
Achnanthes laterostata	-	-	-	-	-	-	-	-
Achnanthes minutissima	*757*	*374*	*2146*	*424*	*685*	*438*	*2362*	*254*
Achnanthes sp.	-	-	-	-	-	-	-	-
Amphora ovalis	-	-	-	-	-	-	-	-
Asterionella formosa	*3*	-	-	*9*	-	*5*	*2*	-
Caloneis sp.	-	-	-	-	-	-	-	-
Cocconeis placentula	*1*	-	-	-	-	-	-	-
Cyclotella sp	-	-	-	*1*	-	-	-	-
Cymbella sp.1	-	-	-	-	-	-	-	*1*
Cymbella sp.2	-	-	-	-	-	-	-	-
Diatoma hiemale	-	-	-	-	-	-	-	-
Diatoma elongata	-	-	-	-	-	-	-	-
Diatoma vulgare	-	-	-	-	-	-	-	-
Diploneis sp.	*1*	-	-	-	-	*1*	-	-
Epithemia argus	-	-	-	-	-	-	-	-
Epithemia turgida	-	-	-	-	-	-	-	-
Eunotia denticulata	-	-	-	-	-	-	-	-
Eunotia tibia	-	*2*	-	-	*6*	-	*1*	-
Fragillaria construens	-	-	-	-	-	-	-	-
Fragillaria sp.	-	-	-	-	-	-	-	-
Frustula rhomboides	-	-	-	*1*	-	*1*	-	*1*
Gomphonema acuminatum	*3*	-	-	-	*6*	-	-	-
Gomphonema lanceolatum	*5*	*6*	*1*	-	*12*	-	*5*	*5*
Gomphonema sp.	*1*	-	-	-	*2*	-	-	-
Gomphonema, girdle view	*19*	*77*	*5*	*2*	*60*	*19*	*10*	*23*
Mastogloia smithii	-	-	-	-	-	-	-	-
Melosira distans	*12*	*2*	*4*	*2*	-	*2*	*1*	*6*
Melosira ambigua	-	-	-	-	-	-	-	-
Navicula perigrina	*13*	*27*	*6*	*10*	*11*	*10*	*1*	*10*
Navicula subtilissima	-	-	-	-	-	-	-	-
Navicula vulpina	-	-	-	-	-	-	-	-
Navicula sp.1	-	-	-	-	-	-	-	-
Navicula sp.2	-	-	-	-	-	-	-	-
Naviula sp.3	-	-	-	-	-	-	-	-
Nitzschia denticulata	*6*	*5*	*1*	-	*2*	*2*	*1*	*1*
Nitzschia ignorata	-	-	-	-	-	-	-	-
Nitzschia amphibea	-	-	-	-	-	-	-	*1*
Nitzschia sp.1	-	*1*	-	-	-	-	-	-
Nitzschia sp.2	-	-	-	-	-	-	-	-
Rhopalodia gibba	*1*	*1*	-	-	-	-	*1*	-
Tabellaria fenestrata	*72*	*33*	*45*	*85*	*10*	*82*	*78*	*136*
Tabellaria flocculosa	*6*	*1*	-	-	-	*4*	*12*	*20*
Stauroneis phoenicenteron	-	-	-	-	-	-	-	-
Suririella biseriata	-	-	-	-	-	-	-	-
Suririella elegans	-	-	-	-	-	-	-	-
Suririella laris	-	-	-	-	-	-	-	-
Suririella sp.	-	-	-	-	-	-	-	-
Synedra ulna	*151*	*53*	*82*	*71*	*23*	*111*	*79*	*98*

Arthrodesmus	-	-	-	-	-	-	-	-
Bulbochaeta insignis	-	-	-	-	86	5	-	-
Coccoid greens (5u)	-	-	-	-	2	-	-	104
Coccoid greens (1u)	-	-	-	-	-	-	-	-
Cosmarium ralfsii	2	-	-	-	3	6	17	12
Cosmarium euastrum	2	1	-	-	2	-	-	-
Cosmarium sp.1	1	-	2	-	-	-	-	-
Cosmarium sp.2	-	-	-	-	2	-	-	-
Cosmarium sp.3	-	-	-	-	-	2	-	-
Cosmarium sp.4	-	-	-	-	-	-	-	-
Cosmarium sp.5	1	-	-	-	-	-	-	-
Docidium undulatum	-	-	-	-	-	-	-	-
Mougeotia sp.	7	28	1	15	35	11	-	-
Oedogonium sp.	-	-	-	-	4	-	-	-
Scenedesmus sp.	14	-	-	13	-	10	-	-
Schizomeisis leibnii	-	-	-	-	-	-	-	-
Spirogyra sp.	-	12	-	-	-	6	-	-
Spondylonium planum	-	-	-	-	-	15	-	-
Staurastrum corutum	-	-	-	-	-	-	-	-
Staurastrum sp.	-	-	-	-	-	-	-	-
Ulothrix sp.	-	-	-	-	-	28	-	-
Zygnema sp.	-	39	-	-	55	10	10	27
Dinobryon sp.	3	-	9	35	-	3	38	43
Spirulina princeps	-	-	-	-	-	-	-	-
Oscillatoria sp.	-	-	-	-	v	-	-	-
Blue-green filament 1	-	-	v	v	-	-	-	-
Blue-green filament 2	-	-	-	-	-	v	v	v
Merismopedia	-	-	-	-	-	-	-	v
Ceratium hirundinella	3	-	5	1	-	9	7	4
Pediastrum sp.	-	-	-	-	-	-	-	-
Fungal filaments Possibly;*Scytalidium* sp.	-	-	-	-	v	-	-	-

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