

THE UNIVERSITY OF MANITOBA

THE ROLE OF ALGAL COMPONENTS IN PHOSPHORUS-REMOVAL  
FROM SEWAGE EFFLUENT IN AN EXPERIMENTAL AQUATIC  
VEGETATION SITE AT ARBORG, MANITOBA

by

Mirth Rosser

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A dissertation submitted to the Faculty of Graduate Studies of  
the University of Manitoba in partial fulfillment of the requirements  
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To Michael

## ABSTRACT

The effectiveness of two algal components, the epiphyton and phytoplankton, in removing phosphorus from sewage effluent passing sequentially through a series of artificial ponds, was studied. Artificial substrates of clear cellulose acetate were provided for colonization by epiphytes, and weekly samples of scraped algal material, as well as suspended algal material were analyzed for particulate phosphorus, particulate organic carbon, and species composition and number. Two of the artificial ponds were planted with aquatic macrophytes, and one of these was found to be more effective than a control in stripping phosphorus from the effluent, while the other was not. The epiphytic component was judged to be not very significant in the amount of phosphorus removed, averaging approximately 1%, while the suspended algae removed mean amounts varying from 0.64-33.81% in different ponds over the season. However, the total epiphyton in these ponds was small, owing to the limited number of planted macrophytes. The amount of epiphytic carbon produced in a weekly interval varied from 0-620 grams. The greatest number of epiphytic and suspended algae belonged to the Cyanophyta, with fewer Chrysophyta and Chlorophyta.

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## INTRODUCTION

Over the past few decades there has been much concern about the pollution of lakes and rivers as we continue to dump wastes into them, rendering them unfit for many uses. Pollution results from a variety of natural or unnatural assaults on a biological system; one characteristic form is that of cultural eutrophication, which is defined as the greatly speeded-up accumulation of organic material in a body of water as a result of human activity. Characteristics of a eutrophic lake are high nutrient levels, abundant littoral vegetation, frequent summer stagnation with algal blooms, and the absence of cold-water fish such as salmon, lake trout and whitefish.

Natural eutrophication happens, too, but is generally a much more prolonged occurrence, often taking place over thousands of years, and therefore not as disruptive to organisms which are part of the system in which it is occurring. Natural eutrophication is more often considered to be a successional process, and, as such, more orderly than cultural eutrophication.

One of the most important factors involved in cultural eutrophication is phosphorus, an element necessary for the normal functioning of all organisms, and found in domestic, agricultural, and industrial wastes. It is a major plant nutrient, and in large supply may promote the growth of massive quantities of plant material.

Phosphorus is very often limiting in fresh-water systems (Turk et al 1974), which is to say that its limited availability exerts a controlling effect on the amount of plant production which can take place in a given body of water. The 'Law of the Minimum' states that under steady-state conditions the essential material available in amounts most closely approaching the critical minimum needed will tend to be the limiting one (Odum 1971).

Phosphate compounds tend to be soluble in water except when complexed with calcium, iron, aluminum, and some other ions. Depending on pH, these react with phosphate ions to form calcium phosphate (at high or neutral pH, which occurs especially in calcareous soils), or ferric phosphate or aluminum phosphate (in lime-deficient conditions). These salts, which are relatively insoluble in water, then become part of the sediment (Vallentyne 1971).

Most phosphorus enrichment of lakes and rivers comes from sewage-disposal plants (Smith 1976), which frequently are dealing with both municipal and industrial wastes. Primary sewage treatment (purely mechanical) removes about 10% of the phosphorus, and secondary treatment (biological) up to 30% (Smith 1976). During the heavy usage of phosphate detergents, many aquatic systems were seriously damaged by the rapid production of algal blooms that resulted. When these blooms die, they are decomposed by bacteria, and O<sub>2</sub> is used up, leaving

oxygen-deficient water which cannot support many forms of life.

Although the amount of phosphates in detergents is now greatly reduced, the effects of past practices have still not been entirely reversed (Banerji 1978, Welch 1976). Much phosphorus tied up in organic matter becomes part of the sediment of lakes, being gradually released through breakdown by microorganisms such as bacteria, fungi, and microzooplankton (Odum 1971). In its soluble orthophosphate form it may continue to promote algal development. Many years may be required before heavy loads of sedimented phosphorus are removed from a system, although there may be gradual improvement once influxes are minimized (Turk et al 1974).

The history of Lake Washington is a classic example of damage to and revival of a lake ecosystem. Abundance of algae in Lake Washington had increased several-fold from 1941-1963 as increasing volumes of effluent from eleven secondary sewage treatment plants were added to it. In 1963, diversion of effluent was begun, and by 1969, winter phosphate had decreased to about 28% of the 1963 value, but nitrate and CO<sub>2</sub> remained relatively high. Within two years, hypolimnetic release of phosphate had returned to the 1933 level. The abundance of phytoplankton had decreased, although not to 1950 conditions, and it was concluded that this system had been more influenced by

phosphorus than by other sewage components. Actually, by the time half the effluent had been diverted, it was apparent that the P-content had started to decrease, an indication that the lake was capable of absorbing the human-generated P, but not detergent P as well, which had doubled the amount (Edmondson 1972).

However, even with detergent-phosphate reduction, phosphates from domestic and industrial wastes, and to a lesser extent, agricultural practices, still impose a heavy load on rivers and lakes. It is estimated that each human excretes 0.7-1.8 kg of phosphorus per year (Smith 1976), and by far the greater proportion is not removed in most conventional sewage treatments.

Tertiary treatment designed to remove dissolved inorganic materials such as phosphates and nitrates is relatively expensive, costing two to three times as much as primary-secondary (Bylinsky 1971), and is therefore carried out in very few municipalities.

Alternative methods of waste treatment which are less expensive and which have a lower resource demand should therefore be considered and implemented wherever feasible. One of these is the sewage lagoon or oxidation pond which may be adequate for smaller municipalities or suburban developments (Warren 1971). In these systems, wastes are pumped into shallow (1-2 metres) bodies of water and allowed to remain for a period of time during which algae grow in



the upper zone, providing the aeration. This is really a 'conversion' rather than a complete treatment system, in which unsanitary organic matter is altered to sanitary algal material and nutrients, which are returned to the natural environment where space and food chains must be adequate to handle it. Potential uses for the effluent include aquaculture and agricultural irrigation, while the algae may be harvested for animal food or for fertilizer. Odum (1971) estimates that about one acre is required for the treatment of the household wastes of 100 people, although currently in Manitoba one acre is considered to effectively handle wastes of 200 people.

Another 'natural' approach to waste treatment is that of applying it to a marshland area where nutrients such as phosphorus and nitrogen can be removed very effectively by aquatic plants which can eventually be harvested for animal food. Various trials around the world have suggested that an area of 50 acres containing a marsh associated with a shallow pond of approximately equal area could handle domestic sewage for 10,000 people (Woodwell 1977). Ultimately, new industries and municipal treatment plants should be sited in the middle of natural areas large enough for treatment of degradable wastes and the containment of poisonous wastes, rather than on streams or lakes or in the middle of congested areas (Odum 1971).

The town of Hay River, N.W.T., releases its effluent into a small stream which flows through swampland and into Great Slave Lake. A five-year study of the effects of this

effluent showed that within 3600 metres downstream from the point of release, the chemical and biological condition of the water was virtually identical with that of a nearby control stream not receiving effluent. Thirty-five square metres per person-year of effluent released were found to be affected, and the 32 hectare area of swampland removed 13.1 kg per day of phosphate during the summer months (Hartland-Rowe and Wright 1974).

Michigan State University is operating a 500-acre treatment area which is handling wastewater generated by 43,000 people. The experimental project includes four artificial lakes with a mean depth of six feet, three marshes, and a spray irrigation area. Plants, including algae, take up large amounts of nutrients and are then harvested for animal feed, fertilizer, and soil conditioners. Water passes sequentially through the series of lakes, improving in quality at each stage, and the final lake is intended for recreational use (Michigan State University 1977).

It was this sort of system that was examined in this study. A secondary sewage lagoon was serving a sub-northern community of 1000 people, and during the warm months, effluent from this lagoon was passed sequentially through a series of artificial shallow ponds, some of which were planted with aquatic macrophytes. It was assumed that the macrophytes were making a major contribution to

nutrient removal (Mulligan et al 1976), and that the final stage effluent was considerably reduced in P and N content as a result of uptake by the vegetation. What was not known was the proportional contribution to nutrient-stripping made by the algae in the system, and this was examined.

Experiments with radioactive phosphate added to lake surface waters have shown it to be taken up by algae within minutes (Vallentyne 1971). However, some studies of the relationship between algal biomass and nutrients have yielded a lack of correlation between the two because the level of nutrient was being controlled by the decomposition of large amounts of detritus in sediments. That is, the release of nutrients from the sediment was proceeding at a greater rate than algal uptake of nutrients (Munawar and Burns 1975).

A consideration of the phosphorus cycle in water explains how this can happen. (See Appendix 1). In the upper oxygenated zone, orthophosphate is removed from solution by the algae. In the deeper aphotic zone, organic phosphorus compounds are decomposed and orthophosphate released. In this area the iron cycle regulates orthophosphate concentration.

Under aerobic conditions ferric iron combines with orthophosphate to form insoluble ferric phosphate. Under anaerobic conditions ferric phosphate is reduced at the water-sediment interface to soluble ferrous phosphate, and

enters the water. In this way, orthophosphate which may have accumulated in sediments may be gradually released to diffuse to areas where it can be utilized by algae (Levine 1975).

Generally, the uptake rate of phosphorus is more rapid than the release rate (Odum 1971), but in warm weather the rise in water temperature does increase the rate of release of P from sediments due to increased rate of organic decomposition by bacteria, especially in shallow water (Hutchinson 1957). Under anaerobic conditions, the rate of P-release from sediments can be ten times that under aerobic conditions (Welch 1976).

Any expected correlation between orthophosphate and algal biomass may be complicated by the ability of some algae to utilize organic P compounds, depending upon their ability to generate appropriate phosphatase enzymes (Healey 1975).

Knauer (1975) found that an increase in algal biomass exhibited a two-week lag period in response to fertilization by P-loading.

Relatively large amounts of orthophosphate may be stored in the ice cover of lakes, presumably from algal cells trapped during freeze-up, and potentially increased in density as a result of freeze-concentration. These cells may release stored inorganic phosphates, providing an almost instantaneous large source at spring melt (Bozniak and Kennedy 1968).

A further complication which can interfere with the correlation between dissolved phosphate and algal biomass is that many algae may store phosphate in excess of their immediate needs (Krishnamurthy 1967, Hooper and Robinson 1978a). This so-called 'luxury' uptake during high concentrations of phosphates can later be used under conditions of P-deficiency (Wuhrmann and Eichenberger 1975). This tendency can result in there being a very low P concentration prior to an algal bloom, the P being taken up by the algae and stored (Vallentyne 1971).

Furthermore, the turnover time for algae can be very short, varying among species, so that, unless harvested rather quickly, the nutrients removed by algae will be returned to the system.

Soltero et al (1975) suggest that the critical threshold below which nuisance blooms will not occur is 0.01 mg/l in lakes. Also, it is estimated that only about 30% of the particulate phosphorus measured may be available to the algae (Cowen and Lee 1976).

Nitrogen is another element which, in excess, is often considered to be an important factor in algal blooms. Large quantities of N in the forms of nitrites, nitrates, and ammonia are also found in untreated domestic, agricultural, and industrial wastes.

The form most commonly used by plants is nitrate, although ammonia may be utilized to some extent. On the death of any organism a series of decomposer bacteria

convert the organic N to ammonia, nitrites, or nitrates. Nitrates may be utilized directly by green plants or returned to the atmosphere through the action of denitrifying bacteria. A large proportion of this elemental N is then converted to organic form through the N-fixing action of certain bacteria and algae.

Requirements for N vary considerably among the algae, some cyanophytes demanding more N and P than do many other types. Experimentally, it has been shown that P is necessary to facilitate N-uptake by algae and that there is a P requirement of 10% of the available N in order for the N to be utilized (Prescott 1968).

When the ratio of N:P is reduced, certain heterocystous N-fixing blue-greens such as Anabaena, or Aphanizomenon may be favoured and become predominant (Schindler 1971 and 1977). These algae tend to be the most objectionable since they occur in filamentous mats, are covered with a gelatinous mucous, float near the surface of the water owing to the presence of gas vacuoles, impart disagreeable odours and tastes to water and are not generally used as food by larger organisms.

Because in certain areas the atmospheric input of N can be as high as 50% that coming from waste water, control of N alone is not usually effective in reducing eutrophication effects in fresh-water lakes (Welch 1976, Kallquist 1975). Algal bioassays of 49 U.S. lakes found

P to be limiting in 35; N in 8; and other constituents in 6 (Miller et al 1974). However, in marine coastal environments or saline lakes, as well as in lakes and reservoirs in central Africa, N has been found to be more limiting than P to phytoplankton (Schindler 1971, Lund 1965).

Vollenweider (1969) has suggested that, while P may be the controlling factor in the early stages of an accelerated eutrophication process, N metabolism becomes accelerated with increasing eutrophication, probably because of an increased rate of denitrification, and beyond a certain point, N becomes limiting. This might explain claims that in the summer it is inorganic N which limits phytoplankton growth, while P or  $\text{SiO}_2$  are more likely to limit spring growth (Nicholls 1976). Since spring blooms are frequently composed of large numbers of diatoms, the silicon concentration of waters at that time is critical, because of the silicon cell wall (Prescott 1968, Lund 1965, Hooper-Réid and Robinson 1978a).

Miller et al (1976) generalize that in most natural waters, the N:P ratio can be used to predict nutrient limitation: a N:P ratio of less than 10 is N-limiting, and a N:P ratio greater than 10 is P-limiting. However, Lund (1965) has observed that algae in lakes will grow when the nitrate concentration is below the minimum level established for laboratory cultures; and maximum summer phytoplankton standing crops may develop while inorganic N is lower than the lowest detectable limit of  $10 \mu\text{g l}^{-1}$  (Nicholls 1976).

It should be realized that the significance of instantaneous estimates of N:P ratios, and of any nutrient regime, must depend upon loading rates and rates of nutrient flux. According to Odum (1971), a rapid flux of phosphate is typical of highly productive systems, and flux rate is more important than concentration in maintaining high rates of organic production.

In laboratory streams, it has been found that, while addition of low levels of nitrate and phosphate (1.2 ppm  $\text{NO}_3$  and 0.2 ppm  $\text{PO}_4$ ) produce a relatively low level of production, intermediate levels (10 times these amounts) and high levels (100 times these amounts) of fertilization did not result in very different productivities (Wilhm and Long 1969). That is, an intermediate level of nutrient may result in maximum productivity, and increasing nutrients may have no further effect, possibly because of limitation by the lack of other essential nutrients. Also, at certain levels, N and P can exert toxic effects on algae. The amounts at which detrimental effects may be felt vary for different species (Prescott 1968).

Wilhm and Long (1969) found that certain genera were common at all three nutrient levels (e.g. Mougeotia, Phormidium, Anabaena, Oscillatoria, Scenedesmus); while at the very high third level, some appeared for the first time (e.g. Gloeocystis, Ankistrodesmus, Chroococcus, Closterium), and others disappeared (e.g. Nostoc, Tetraspora, Spirulina, Aphanothece, Aphanocapsa). Production actually



declined slightly at the hyper-fertilized third level.

It is generally accepted that diversity of algal species decreases with increased fertility of waters (Prescott, 1968, Moss 1972, Cooper and Wilhm 1975). Moss suggests one possible explanation for the greater diversity in oligotrophic lakes is adaptation of lower growth rates as a response to limiting amounts of essential nutrients. Because of the longer time periods before peak populations, there is greater overlap between successive species, leading to high diversity (Moss 1972, Moss 1972a).

In more fertile waters with fast-growing species, the winter buildup of nutrients can be quickly used by these algae which achieve rapid dominance. This results in low diversity (Moss 1972).

In Holland Marsh, Ontario, high loadings of N ( $13.2 \text{ g N m}^{-2} \text{ year}^{-1}$ ) and P ( $1.2 \text{ g P m}^{-2} \text{ year}^{-1}$ ) from surrounding agricultural land have produced very eutrophic waters. In spring and fall, diatoms, especially Stephanodiscus, were dominant, sometimes comprising more than 90% of the total phytoplankton biomass. In May, the biomass was as high as  $3.2 \times 10^7 \mu\text{m}^3 \text{ ml}^{-1}$ . In summer, chlorophytes, especially Scenedesmus spp. dominated, with a smaller volume of cyanophytes, especially Oscillatoria spp. Biomass in summer ranged from  $1.0 - 3.0 \times 10^7 \mu\text{m}^3 \text{ ml}^{-1}$ . There was some evidence that a shift in the limiting nutrient for spring phytoplankton from  $\text{SiO}_2$  to P was occurring from 1971 to 1972, indicating an improvement in nutrient retention since

the initiation of P-control at municipal sewage plants in southern Ontario in 1971. Other springtime dominants in Holland Marsh were Melosira spp., Synedra spp., Asterionella sp ., Fragilaria crotonensis, and Cyclotella meneghiniana. Summertime dominance by Scenedesmus quadricauda, as well as two cyanophyte blooms, Oscillatoria articulata in early June, and O. tenuis in early September, were typical of eutrophic conditions (Nicholls 1976).

In India, cyanophytes and euglenophytes were found to predominate in raw sewage, with little seasonal variation, while in stabilized sewage, marked seasonal changes occurred, with members of the Chlorococcales ultimately becoming dominant (Singh and Saxena 1968). Planktonic Chlorococcales are mostly absent from oligotrophic waters (Round 1957).

In a study by Traaen (1975), effluent subjected to three different types of sewage treatment, primary settled, secondary biologically-treated (i.e. activated sludge), and chemically-treated (i.e.  $Al SO_4$  flocculation) was added to oligotrophic waters in Norway. The addition of 5% primary settled or 5% biologically-treated effluent both caused a marked shift from chrysophytes and chlorophytes to cyanophytes and Nitzschia while 0.5% of primary or secondary effluent resulted in heavy developments of filamentous greens, plus diatoms Nitzschia, and Tabellaria. However, 5% or 0.5% chemically-treated effluent had little

effect on the species composition. Secondary treatment produced slightly less benthic biomass than did primary, but after chemical treatment, the biomass was close to that of a control.

A cycle typical of temperate eutrophic waters seems to be: spring periphyton and planktonic diatom dominance, succeeded by large populations of Eudorina and Pandorina in late spring (Golterman 1975), blue-greens and filamentous greens in summer, and a second smaller autumnal diatom peak in September (Golterman 1975, Stockner and Armstrong 1971). Ceratium may be present in late summer, and cryptomonads may be abundant at most times of the year (Golterman 1975).

In a Pennsylvania lake subjected to high nutrient loadings from domestic sewage, a similar pattern was found, and cyanophytes, although less diverse in terms of relative numbers of genera produced the largest blooms, while the chlorophytes had the greatest number of genera (Casterlin and Reynolds 1977).

The effects of increasing temperatures and light intensity must not be overlooked in considering the initiation of the spring peak. These factors may be at least as important as nutrient availability (Stockner and Armstrong 1971, Prescott 1968), but they are so interrelated that it is difficult to attribute prime importance to any one.

Mutual inhibition between algal species is often mentioned as an explanation of periodicity when other reasons are not apparent; for example, grazing by zooplankton may be a very important factor, both in ending a spring bloom and in eliminating certain algal species, but be overlooked (Lund 1965, Golterman 1975). The major herbivores are crustaceans such as Daphnia spp. (Ward and Robinson 1974), copepods and cladocerans, and the rotifers (Golterman 1975). Grazing may be somewhat preferential, depending partly on the proportional sizes of the herbivore and the alga (Uhlmann 1971), and can lead to equilibrium, as one algal species replaces the declining one, or the grazers decline; or it may lead to greatly reduced numbers of algae (Lund 1965, Golterman 1975).

It is the smaller algae which tend to disappear when the number of grazers is experimentally increased. Some of these are small, naked green algae, nanoflagellates, cryptomonads, and certain diatoms. Algae which tend to be rejected by grazers are generally large, unicellular desmids and dinoflagellates, filamentous diatoms, and colonial blue-greens. Some of the gelatinous chlorophytes may actually increase in numbers in the presence of grazers, as they pass unharmed through the gut, picking up carbon and phosphorus compounds before being excreted (Porter 1977).

Parasitism by protozoans or fungi is another factor which can decimate algal populations (Lund 1965).

In sewage lagoons and hyperfertilized ponds at high temperatures, grazing by zooplankton may at times suppress phytoplankton growth to such an extent that the large supply of nutrients remains unused (Uhlmann 1971). Furthermore, excretion by zooplankton may contribute significantly to existing levels of P. The amount of P excreted daily by some zooplankters almost equals their total P-content (Pomeroy et al 1963, Johannes 1964), and may exceed the P-requirements of the algae present. This may be of great significance in recycling of nutrients, particularly in shallow water in warm temperatures (Hargrave and Geen 1968).

In terms of productivity, the littoral zone of many waters may be very important. Allen (1971) found that in Lawrence Lake, Michigan, the littoral community accounted for 71% of the total lake's production, and that the epiphytic algae contributed 31% to seasonal production and 21% to total annual lake production. He suggests that the epiphytic algal community is among the most productive in both fresh-water and marine environments, and is probably more productive than most epibenthic communities, especially if submerged macrophytes are well developed. Wetzel (1964) found that phytoplankton, epiphyton and macrophytes in Borax Lake, California, each played a significant role in primary production. On an annual basis, phytoplankton and periphyton made similar

contributions, each outweighing that of the macrophytes.

Love and Robinson (1977) found mean productivity of submerged macrophytes (Chara, Potamogeton, and Myriophyllum) in West Blue Lake, Manitoba to be about  $8 \text{ mg C m}^{-2} \text{ day}^{-1}$  during the growing season, while phytoplanktonic production averaged  $320 \text{ mg C m}^{-2} \text{ day}^{-1}$  during summer. Here, however, submerged macrophytes were limited by steeply-sloping shorelines of the lake.

In experimental ponds artificially-fertilized with N and P, McIntyre and Bond (1962) found large quantities of planktonic organisms were the early producers, followed by the development of the benthic community. It was this benthic community which developed most rapidly and produced the greatest biomass in the pond most heavily fertilized.

It must be remembered that, in determining productivity of attached organisms, standing crop estimates may include both living and dead components and might not represent the actual photosynthesizing community. Short-term artificial substrates may therefore provide a more realistic estimate of periphyton production as colonization proceeds. There may be two linear components in the growth curve of accumulating attached material, one representing a colonization and lag phase; and the second, an acceleration phase. This 'instantaneous growth rate' which follows the first phase may be a good estimate of periphyton production (Kevern et al 1966, Tilley and Hauschild 1975).

In a study of Typha latifolia in Oklahoma, maximum productivity occurred in May and early June and declined considerably with the hot weather in July due to continuing death of individuals, death of lower leaves and insect depredation. Also, the rate of photosynthesis is often less than that of respiration during periods of hot days and nights in midsummer (Penfound 1956).

Dying and dead tissues of macrophytes may constitute an important component in the metabolism of a lake, as a large proportion of the dissolved organic matter is released on autolysis. From laboratory analysis, Otsuki and Wetzel (1974) estimated that in Lawrence Lake, Michigan, release of dissolved organic matter by autolysis of Scirpus would reach  $26-35 \text{ g C m}^{-2} \text{ year}^{-1}$  and that these values were equivalent to 63-85% of the total dissolved organic carbon input to the planktonic organic carbon budget in the lake.

In considering the P-content of macrophytes, Caines (1965) found that an artificial increase in phosphate concentration of a Scottish lake resulted in increased concentration of P in the tissues of only those submerged macrophytes having thin or finely dissected leaves, such as Potamogeton praelongus and Myriophyllum spp., thus offering great surface area for uptake of nutrient ions directly from the surrounding water. Concentration of P is found to be higher in the growing tips than in other tissues of terrestrial and aquatic plants, and may also be

four to five times higher in healthy green leaves than in unhealthy ones. In late summer, dying plants release large amounts of phosphorus into the surrounding medium, where it is most easily assimilated by the microflora.

Mickle and Wetzel (1978) feel it is unlikely that macrophytes can compete effectively with microflora for simple low molecular weight compounds. They found that axenic Najas flexilis actively assimilated simple carbohydrates and amino acids, but ceased when epiphytized with bacteria.

Several authors believe that artificial substrates are essentially accurate in colonizing representative epiphytic algae, although Tippett (1970) found glass slides incubated for two or four week intervals did not colonize representative species or numbers of epiphytes as compared with those found on natural hosts over the same time period, and he concluded that under certain conditions, the use of artificial surfaces is not a valid method. Rare species particularly failed to become 'seeded' on slides. However, one would expect that buildup on natural hosts over time would alter any selective surface properties differently from those of newly exposed artificial substrates.

Studies have been carried out using glass slides by Stockner and Armstrong (1971), Castenholz (1960), and Allen (1971), all of whom conclude that few epiphytic species are selected against by these artificial substrates. The



positioning of glass slides can make a difference to the quantity of attached material. Held horizontally, large amounts of detritus and sediment may collect, while vertically-supported substrates may have more uniform colonization but retain it less effectively (Wetzel and Westlake 1969). Scraping epiphytes off natural substrates has the inherent problem of contamination by macrophytic tissue (Allen 1971).

Productivity on colonized cellulose acetate strips, vertically suspended, was compared with that of epiphytes colonizing macrophytes in Delta Marsh, Manitoba. No significant differences were found, but reliability in estimating periphyton production from colonized artificial substrates depends largely on the interval chosen for colonization and this may vary according to the trophic conditions of the water (Hooper and Robinson 1976, Sladeckova 1962). Sladeczek and Sladeckova (1964) obtained good results from four to six week incubations of glass slides, and Hooper and Robinson (1976), and Castenholz (1960) with two week intervals. Preliminary tests are advisable to determine the appropriate period for prevailing conditions. Kevern et al (1966) have suggested that a biomass accumulation curve can be plotted from a series of increasing exposure times.

In this study, artificial substrates of cellulose acetate were used to colonize epiphyton with the assumption that attached algal communities were representative of

those occurring on the natural host. During the first season, attention was focussed on the epiphytic material, but in the second season, the same determinations were carried out on the suspended material as well. The relative production and phosphorus-uptake were determined for both algal components, with a view to assessing the potential of this relatively simple method of cleaning waste water with minimal disturbance to the surrounding environment.

## MATERIALS AND METHODS

### 1. Description of the Experimental Site

The site of the Aquatic Vegetation Project is located adjacent to the Arborg municipal sewage lagoons 2.2 km south-east of Arborg, Manitoba at  $51^{\circ}$  latitude,  $97.5^{\circ}$  longitude. (See location map, Appendix 2.) This area is a till plain about 700' above sea level, with black-grey wooded soil and calcareous glacial till with clay and silt surface material deposits. Average annual precipitation is 48-50 cm; the surrounding area is gently undulating, with mixed stands consisting mainly of spruce and aspen. The project site consists of fifteen ponds or 'cells' which were excavated in the fall of 1974. Once dug out, the cells were lined with 10 mil polyethylene so as to allow neither leaching of effluent into the subsoil nor entry of ground water and other material into them. A layer of gravel about 8 cm thick was put on top of the plastic liners to provide an anchor for root growth and to hold the plants and liners in place.

As illustrated in Figure 1, the experimental site consisted of five rows of 'A', 'B', and 'C' cells through which effluent from the secondary lagoon was sequentially passed.

The Manitoba Department of Northern Affairs was primarily interested in the relative success of a number of aquatic macrophytes within such cells and their ability to effectively alter a number of the parameters of the effluent

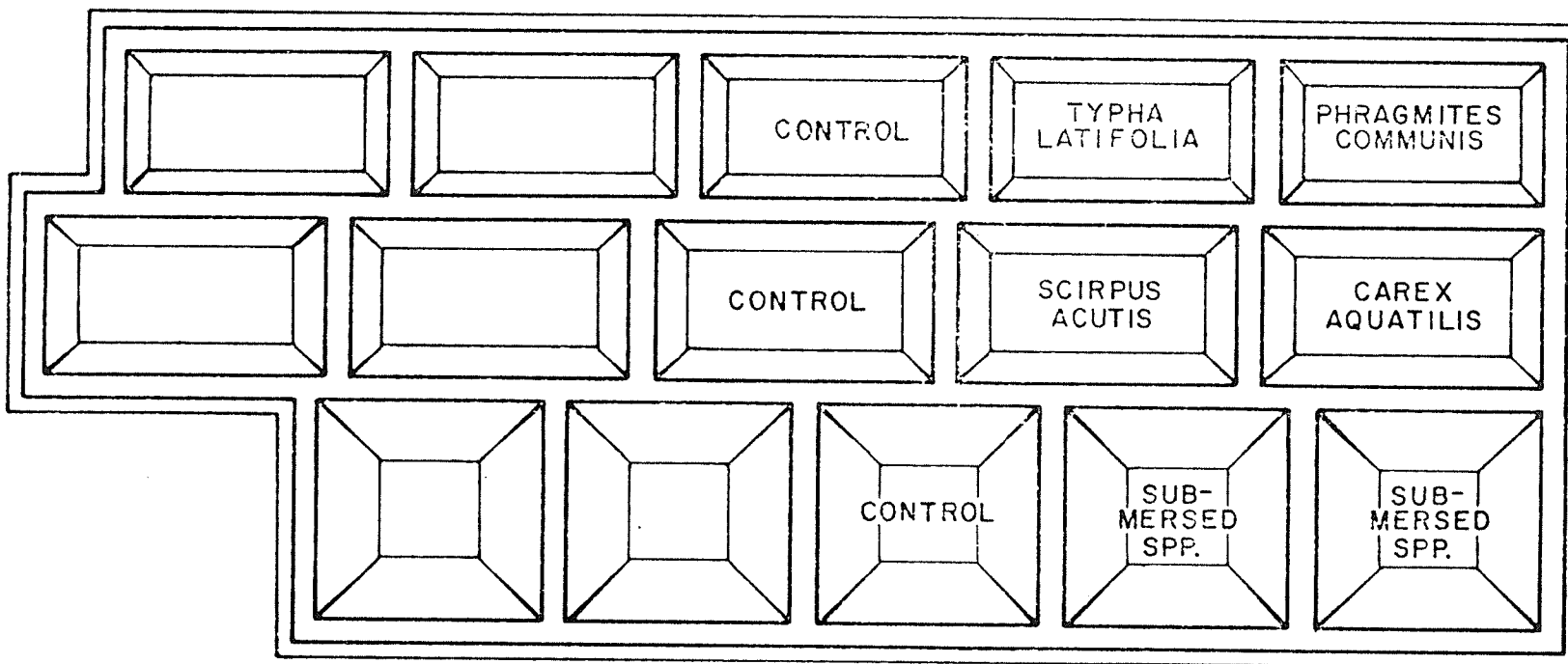
## FIGURE 1

Schematic diagram of the experimental cells  
of the Aquatic Vegetation project at Arborg,  
Manitoba.

2° LAGOON



SCALE 1:40



"A" SERIES

"B" SERIES

"C" SERIES

SUBMERSED SPP. : CHARA VULGARIS  
MYRIOPHYLLUM EXALBESCENS  
ZANNICHELLIA PALUSTRIS  
ELODEA CANADENSIS

AQUATIC VEGETATION.  
PROJECT: EXPERIMENTAL  
CELLS AT ARBORG, MAN.

passing through them. Accordingly, samples of plant tissue were analyzed as part of their study, while water samples collected at the beginning and end of each retention period of effluent in each cell were analyzed by the Water Services Board in Winnipeg for: pH, specific conductance, non-filterable residue, B.O.D., total Kjeldhal nitrogen, ammonia-nitrogen ( $\text{NH}_3$ ), nitrate-nitrogen ( $\text{NO}_3$ ), nitrite-nitrogen ( $\text{NO}_2$ ), ortho-phosphate, and total phosphorus. Standard methods of analysis were utilized (A.P.H.A. 1971).

The phycological research described herein was confined to the first vegetated series of cells ( $A_1, B_1, C_1$ ) and the third series ( $A_3, B_3, C_3$ ) which was not vegetated. An aerial photograph (Figure 2) shows the first series of cells.

The  $A_1$  cell of the first series contained Phragmites communis Trin., Typha latifolia L., and Scirpus acutus Muhl, and the second cell of that series,  $B_1$ , contained Carex spp.L., and Scirpus acutus Muhl. Some submerged macrophytes, such as Myriophyllum exalbescens Fern and Chara vulgaris L. (Wood 1967) were transplanted into the deeper  $C_1$  cell in the spring of 1976, but these did not develop significantly. The third series, not vegetated, served as a control.

A series of pumps and hoses connected the lagoon with each cell of the 'A' series so that a 'run' began with 30,000 gallons (136,380 litres) of effluent being pumped into each A cell simultaneously, where it stayed for five days with minimal disturbance. After this period, pumping

## FIGURE 2

Aerial photograph of part of the Aquatic Vegetation site at Arborg, Manitoba. The bottom three cells are, from right to left, A<sub>1</sub>, B<sub>1</sub>, and C<sub>1</sub>, referred to as the vegetated series. The secondary lagoon is off the the right.





began into the B cells, the A's being refilled with effluent from the lagoon. This was repeated from B to C, after which the effluent was transferred to a drainage ditch. Pumping took approximately two days, so the total interval between filling and emptying of a cell was approximately seven days, and a complete run was three weeks from the pumping into A to the emptying of C. Periodic equipment failure led to occasional minor deviation from this schedule. Data were collected for thirteen complete runs in 1976 and twelve complete runs in 1977, and the sampling period extended from the latter part of May until the end of September.

## 2. Algal Sampling Procedures

Since it had been decided to provide artificial substrates for the colonization of epiphyton rather than disturb the limited transplanted vegetation, a method was devised to allow vertical positioning of strips of clear cellulose acetate. An assumption made was that samples of epiphytic material removed from the strips were representative of the epiphyton attached to the macrophytes.

Support structures for the attachment of the acetate strips were built of wood and angle-iron and are illustrated in Appendix 3. Enough strips of cellulose acetate to provide substrate samples at weekly intervals from May to September were cut. These approximately 4 cm-wide strips were stapled to the diagonal wooden crossbars at the top and bottom of the frames. Frames were constructed so

that the substrate strips extended from the sediment to above the surface of the water when the cells were flooded.

Two series of acetate strips were utilized, one continuing over the season and sampled weekly, and one replacement series, with strips removed and replaced every week. The former would represent the continuing colonization expected on the macrophytes as the season progressed, while the weekly strips would represent fresh colonization of newly-exposed areas as the macrophytes grew. Since the acetate strips did not exactly mimic daily plant growth, this representation would only be approximate. Moreover, in the event of stationary or apparent negative 'growth', these representations were not applied.

One frame with its attached substrates was placed near the centre of each of the cells under investigation, and at weekly intervals strips were removed for analysis. In the vegetated series, a strip was removed from each continuing series, along with a strip which had been incubated for the preceding week. The latter was replaced with a new strip to be sampled the following week.

In the unvegetated series, because of the absence of macrophytic substrate, it was assumed that no significant buildup of epiphyton was occurring over the season, and so investigation of the epiphytic material was confined to samples after weekly colonization periods. Consequently, at each weekly sampling time two short-term strips were removed from the frames, and two new ones substituted in their place. Values obtained from analysis were averaged for the two.

Leaf blank to correct  
numbering

On removal, each strip was divided into its upper and lower halves to account for any vertical stratification which might be occurring in the epiphyton. In the first sampling season, each half was subsequently divided into a number of pieces, each one bottled and labelled for each separate analysis, and transported to the laboratory. The following season the central portion (approximately 10 cm) of upper and lower halves was cut, sealed in a jar of distilled water and transported in a cold chest to the laboratory. All analyses were carried out on aliquots of filtered material scraped from these portions.

During the first experimental season, no direct examination of the algal material other than that attached to the acetate strips was carried out, but in 1977 it was decided to make comparable determinations for both attached and suspended material. With this in mind, a 2 litre water sample was collected from near the surface of each cell and the secondary lagoon and returned to the laboratory along with the acetate strips.

All samples were held in a dark room at 4°C until the following day when each colonized artificial substrate was removed from its jar, placed in a petri dish, and the attached material carefully scraped with a razor blade into distilled water. The resultant mixture was made up to a known volume, portions of which were filtered for each determination. The area of cellulose acetate scraped was noted for future conversion of algal parameters to standard

volume. During the second season, measured portions of the water samples were also filtered and the filters were kept frozen until determinations could be carried out.

### 3. Estimation of Macrophyte Surface Area

An integral part of the 1977 investigation was the estimation of actual algal activity occurring in these ponds. This involved quantification of the real macrophyte surface area available for colonization by the epiphyton. Every two or three weeks macrophyte numbers were estimated in the  $A_1$  and  $B_1$  cells by counting plant species and numbers in nine random  $0.25 \text{ m}^2$  quadrats. Twenty plant stem diameters of each species were measured and averaged, so that with the aid of an aerial photograph showing the extent of macrophyte coverage, and the known cell area and depth, the actual submerged macrophytic surface area available for colonization was extrapolated as it changed over the growing season. A sample calculation follows:

Area of macrophyte bed	-	E
$\bar{X}$ species A/.25 $\text{m}^2$	-	$N^A$
$\bar{X}$ species B/.25 $\text{m}^2$	-	$N^B$
$\bar{X}$ species C/.25 $\text{m}^2$	-	$N^C$
$\bar{X}$ circumference species A	-	$C^A$
$\bar{X}$ circumference species B	-	$C^B$
$\bar{X}$ circumference species C	-	$C^C$
Depth of cell $\text{cm}^2$	-	D

$$\text{Sum of } \frac{N^A \times C^A \times D}{0.25} \times E = \text{surface area of macrophytes available for colonization.}$$

#### 4. Determinations of Algal Parameters

##### A. Dry Weight Determinations

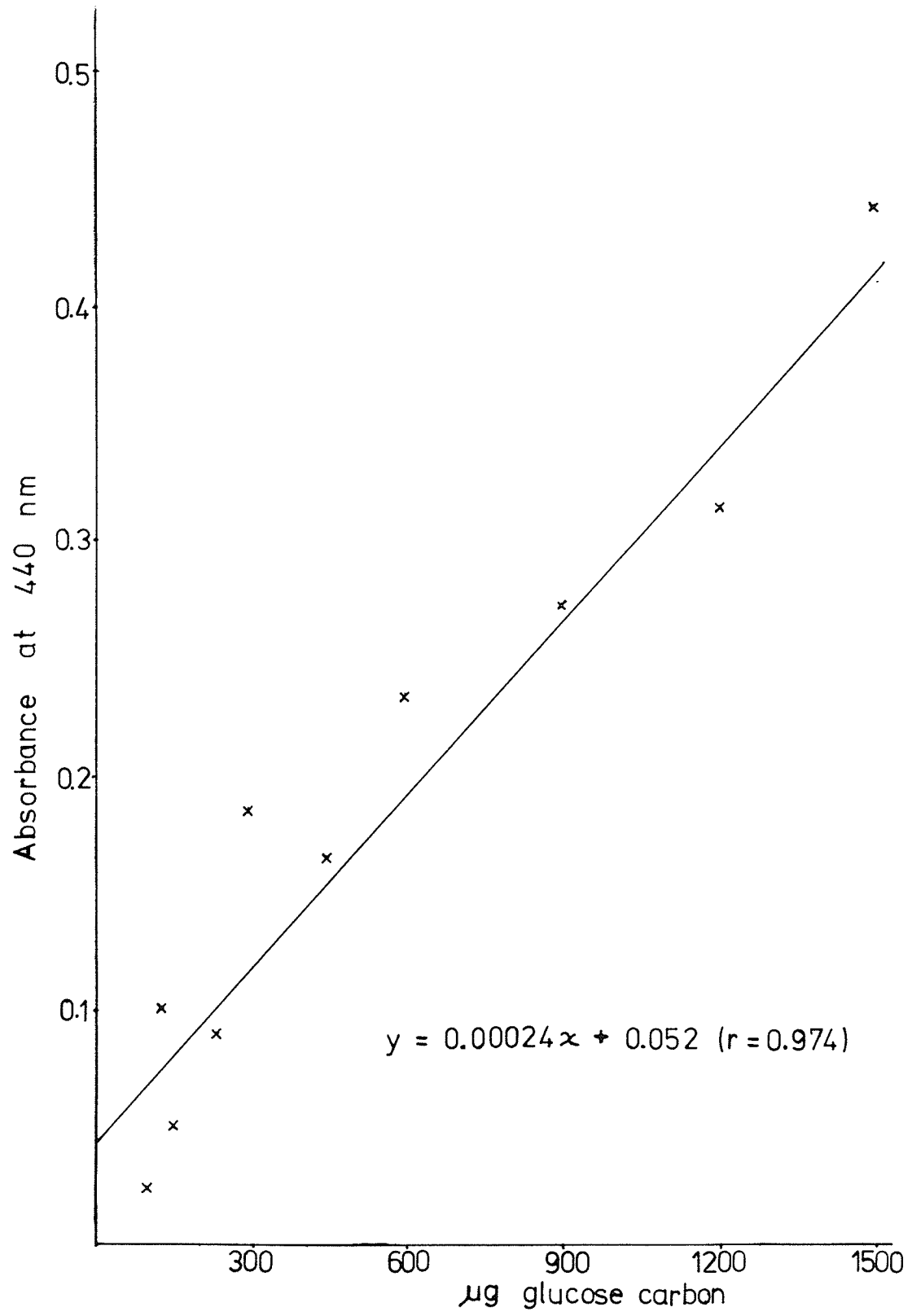
Sartorius 47 mm or Whatman (GF/C) glass fibre filters were pre-dried in an oven at 100-110°C for one hour and stored in a dessicator until required. Prior to filtering the weight of each was recorded. Once the attached material had been filtered, filters were dried to a constant weight at the same temperature and reweighed. Dry weight values obtained were expressed on a square centimeter basis. In addition, in 1977, filtered suspended particulate material was treated in the same way and results expressed per millilitre.

##### B. Particulate Organic Carbon (POC) Determinations

Sartorius 47 mm glass fibre filters were freed of organic material by placing them in a muffle furnace at 450-500°C for up to ten hours. They were then stored under dust-free conditions until required. Ashed filters containing the scraped epiphytic material were wet-oxidized in a mixture of concentrated sulphuric acid and potassium dichromate after the procedure of Strickland and Parsons (1968). Absorbance of the resultant solution was measured at 440 nm in an SP500 spectrophotometer. The procedure was calibrated using glucose-carbon standards and absolute values of organic carbon made from a calibration regression equation as shown in Figure 3. Again, values were

## FIGURE 3

Calibration regression line determined from spectrophotometric absorbance at 440 nm of increasing concentrations of glucose carbon standards.





expressed on a POC per  $\text{cm}^2$  basis. In 1977, filtered suspended material was similarly analyzed and results expressed per millilitre.

#### C. Particulate Phosphorus (PP) Determinations

In the 1976 season, scraped epiphytic material was centrifuged and the supernatant discarded. The remaining pellet was digested in a perchloric acid/concentrated nitric acid mixture (Strickland and Parsons 1968). The method included the hydrolysis of any polyphosphates. In this way, all phosphorus was converted to inorganic phosphate, which was then determined by standard molybdate blue colorimetric procedures. The procedure was calibrated using potassium dihydrogen phosphate standards and results were expressed per  $\text{cm}^2$ .

The method used the following year was essentially that of Stainton, Capel and Armstrong (1974), and is based on the extraction of phosphorus by dilute hydrochloric acid and its conversion to ortho-phosphate. Whatman (GF/C) glass fibre filters, pre-ignited at  $450-500^\circ\text{C}$  for two hours, were used to collect aliquots of scraped epiphytic material and suspended particulate matter. Sample filters plus two unused filters to serve as blanks and two to serve as standards, were placed in screwcap Pyrex vials with caps removed and put in a muffle furnace at  $550^\circ\text{C}$  for one hour to incinerate organic matter. After cooling, 2 ml of 1N HCl plus 10 ml of distilled water were added to all tubes;

2.5  $\mu\text{gP}$  in a potassium dihydrogen phosphate standard solution was added to the two blank filters serving as standards; and all tubes were capped tightly and placed in an oven at  $104^{\circ}\text{C}$  for two hours. After cooling, 2.5 ml of acid molybdate-antimony reagent was added to each vial, thirty minutes allowed for colour development, and absorbance of samples and standards read against a blank in a Spectronic 20 (Bausch & Lomb) at 885 nm. The unit extinction factor in terms of  $\mu\text{gP}$  of  $\text{PO}_4\text{-P}$  per sample is then:

$$\frac{2.5}{\text{standard absorbance}} \times \text{absorbance of sample.}$$

#### D. Qualitative Examinations

Direct examination of thoroughly mixed suspensions of both scraped epiphytic and suspended algal material was carried out using compound phase microscopy at 400x magnification and Palmer counting cells.

The number of fields counted varied with the complexity of the sample, the minimum number for a sample with one or very few species being twenty. Counts in groups of ten fields were continued until no new species had been observed for at least ten fields. They were then stopped and species numbers averaged for ten fields, after which conversion was made to the volume of the counting grid. Finally, algal cell numbers were expressed in terms of cells per square centimeter for the epiphyton and cells per litre for the phytoplankton.

## RESULTS

### 1. Dry Weight Determinations

Data representing dry weights of attached material are presented for 1976 in Appendix 4. These data appear spurious, showing neither a seasonal trend nor a consistent pattern with each nutrient run. This is likely due to the greatly varying proportions of inorganic material that must have been included in determinations. Other than providing a very rough indication of the proportion of attached organic material, these data have not been considered as contributing to the overall understanding of the topic. The 1977 dry weight data are equally spurious, and a further problem involved the use of filters for several weeks which tended to disintegrate somewhat on filtering, giving inaccurate results. These data are left out entirely.

### 2. Particulate Organic Carbon Determinations

#### A. Epiphytic Material (EM)

##### (i) Short-term Incubations

Figures 4 and 5 show the rate of POC accumulation for the 1976 season from June 1 to September 26. Values are given in  $\mu\text{g C cm}^{-2} \text{ day}^{-1}$  for the upper and lower strata of 13 consecutive runs. Figure 4 shows epiphytic POC in the vegetated series ( $A_1, B_1, C_1$ ), and Figure 5, the epiphytic POC in the control series ( $A_3, B_3, C_3$ ).

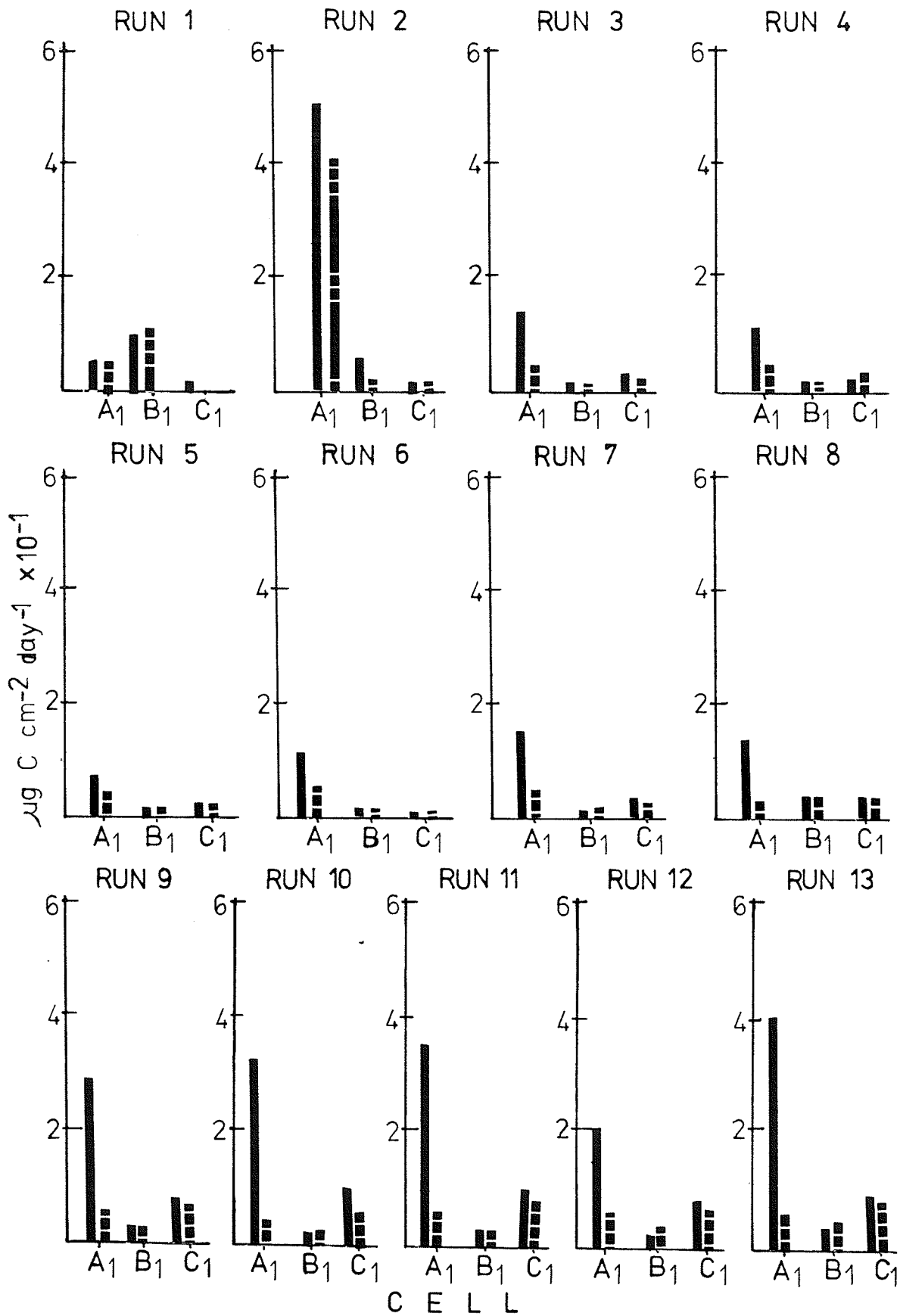
The first observation is that there was definite vertical stratification occurring in the vegetated series. Average values are given in Table 1. In most cases, and

## FIGURE 4

Rates of accumulation of particulate organic carbon in the epiphytic material on upper and lower halves of cellulose acetate substrate in the vegetated series of cells ( $A_1$ ,  $B_1$ , and  $C_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1976.

Values are derived from measurements made at the end of each nutrient stripping run.

————— upper stratum  
- - - - - lower stratum



## FIGURE 5

Rates of accumulation of particulate organic carbon in the epiphytic material on upper and lower halves of cellulose acetate substrate in the control series of cells ( $A_3$ ,  $B_3$ , and  $C_3$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1976.

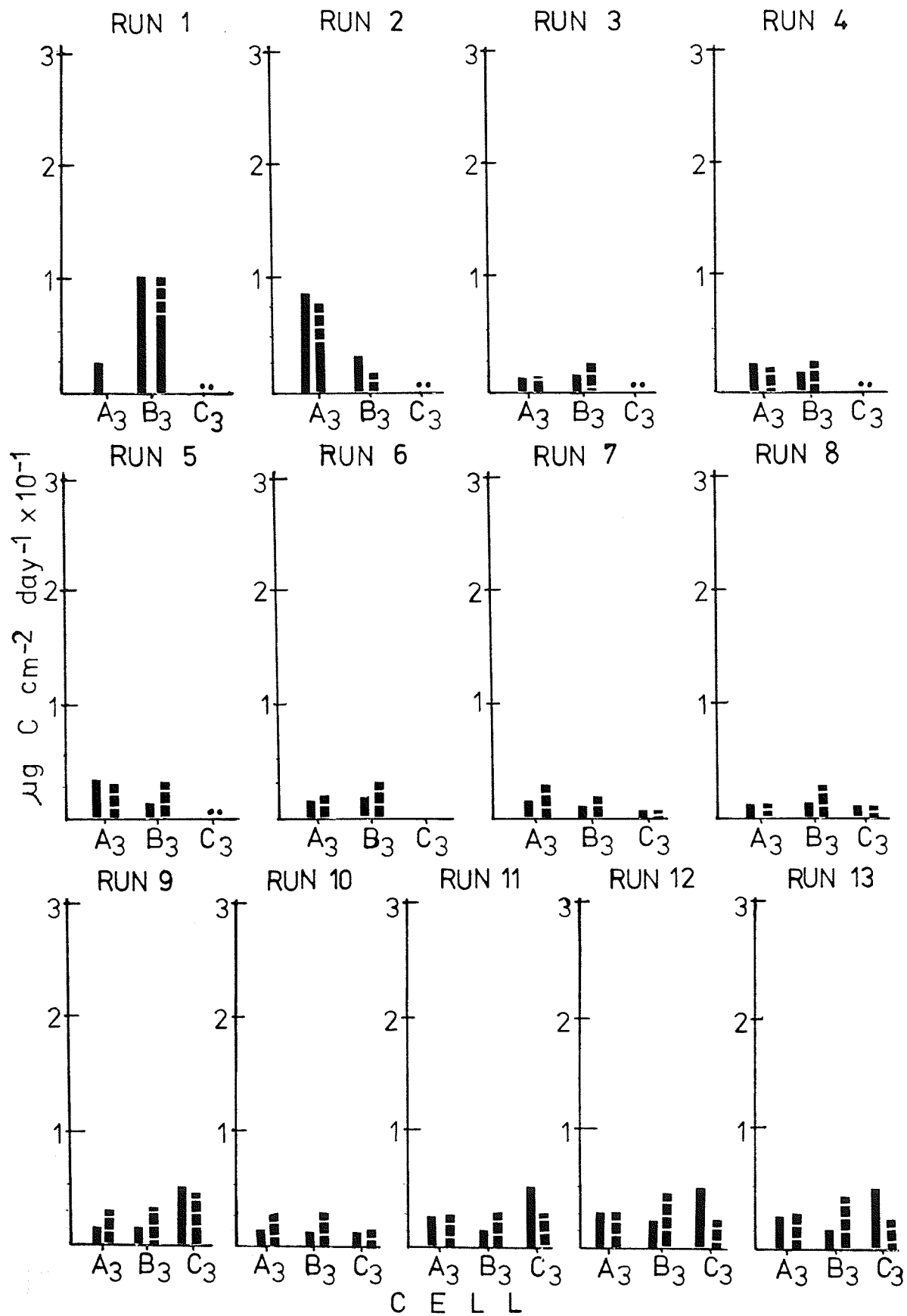
Values are derived from measurements made at the end of each nutrient stripping run.

———— upper stratum

■■■■■■■■ lower stratum

A dot indicates missing data.





particularly in the  $A_1$  cell, there was more POC accumulation in the upper stratum than in the lower (Figure 4). The reverse was true for the control series (Figure 5). In most runs in  $A_3$  and  $B_3$ , the attached material from the lower stratum was higher in POC than material from the upper.

Table 1. Mean rate of POC accumulation in the epiphytic material in 1976. (n = 13 runs)

Stratum	Vegetated Cells			Control Cells		
	$A_1$	$B_1$	$C_1$	$A_3$	$B_3$	$C_3$
Upper	22.60	1.57	4.13	2.38	2.02	3.14
Lower	5.44	1.72	3.21	2.63	3.29	1.90

In most runs of the vegetated series there was more daily POC accumulation in epiphytic material in the  $A_1$  cell than in the  $B_1$  and  $C_1$  cells, while the  $C_1$  cell was intermediate between  $A_1$  and  $B_1$ . This was not the trend in the control series, there being only 3 runs in which epiphytic POC accumulation was highest in  $A_3$ . In only one of these runs (run 7) was there a marked decrease in POC from  $A_3$  to  $B_3$  to  $C_3$ ; unexpectedly, the decrease is reversed in runs 9 and 12.

The greatest amount of epiphytic POC in  $A_1$  occurred in the second run with 48.0  $\mu\text{g}$  upper and 40.83 lower; in  $C_1$  it occurred in the eleventh run with 11.4  $\mu\text{g}$  upper and



8.03  $\mu\text{g}$  lower. EM was relatively low in POC in  $B_1$  over the entire season. Because of mechanical problems at the site, sampling did not begin as early in 1977, and runs 1 through 12 extended from June 24 to September 28. Values for daily rates of POC accumulation in this second season are presented in Figures 6 and 7. Mean values for upper and lower strata of all cells are given in Table 2.

Table 2. Mean rate of POC accumulation in the EM in 1977. (n = 12 runs)

Stratum	Vegetated Cells			Control Cells		
	$A_1$	$B_1$	$C_1$	$A_3$	$B_3$	$C_3$
Upper	18.39	36.33	14.50	19.42	21.09	11.74
Lower	15.76	37.68	13.56	12.54	14.88	6.96

In general, values for daily POC accumulation were higher in the 1977 EM than comparable values in 1976. Vertical stratification occurred, but in the vegetated series did not give consistently higher values in the upper stratum. In almost as many individual cases, there was considerably greater POC accumulation in the EM from the lower substrate portions as from the upper. In the control series in 1977 there was a definite trend toward more POC in the upper stratum, however.

In Figure 6, illustrating the vegetated series of 1977, the tendency was for the  $B_1$  cell to show the

## FIGURE 6

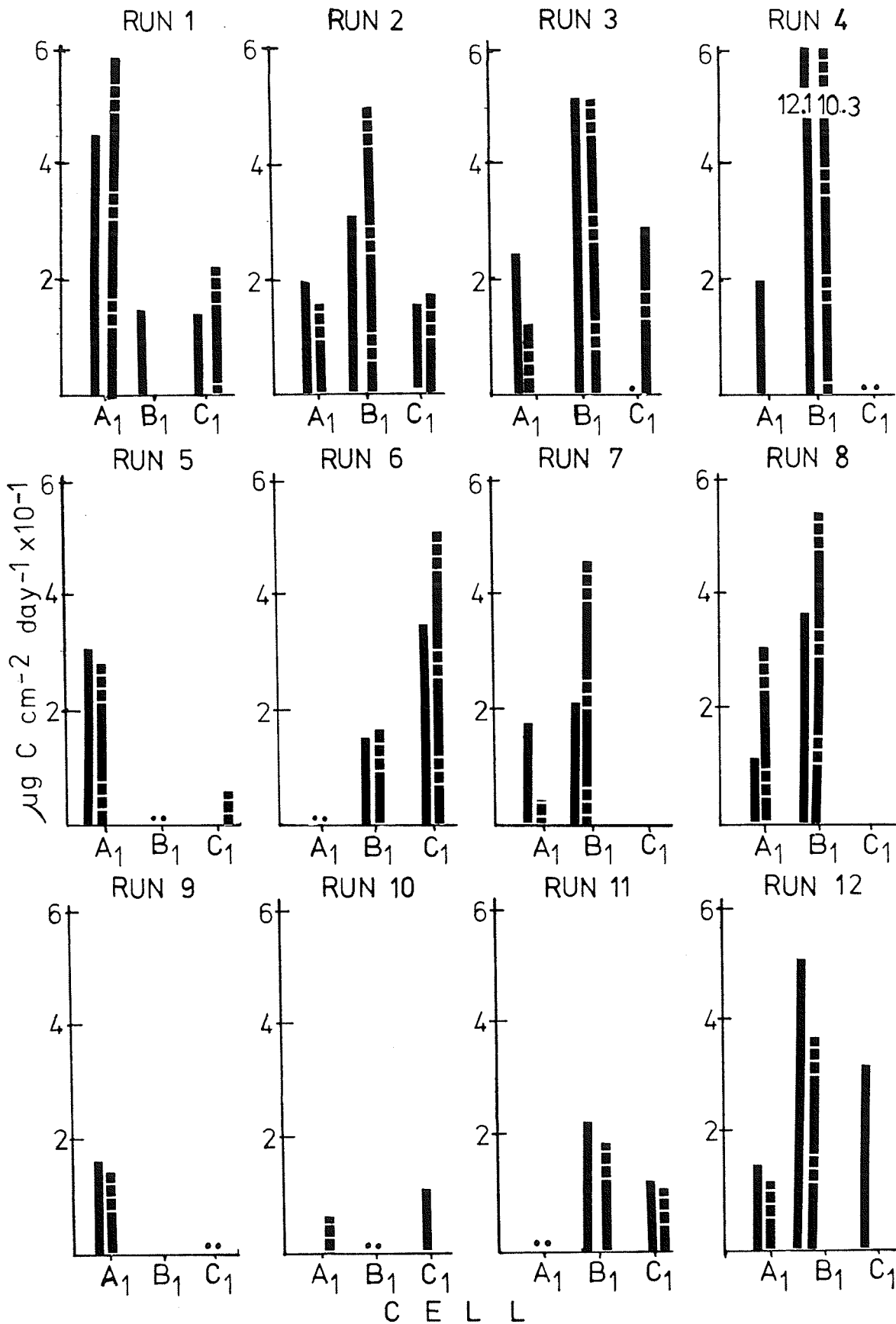
Rates of accumulation of particulate organic carbon in the epiphytic material on upper and lower halves of cellulose acetate substrate in the vegetated series of cells ( $A_1$ ,  $B_1$ , and  $C_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Values are derived from measurements made at the end of each nutrient stripping run.

———— upper stratum

— · — · — · lower stratum

( A dot indicates missing data.



## FIGURE 7

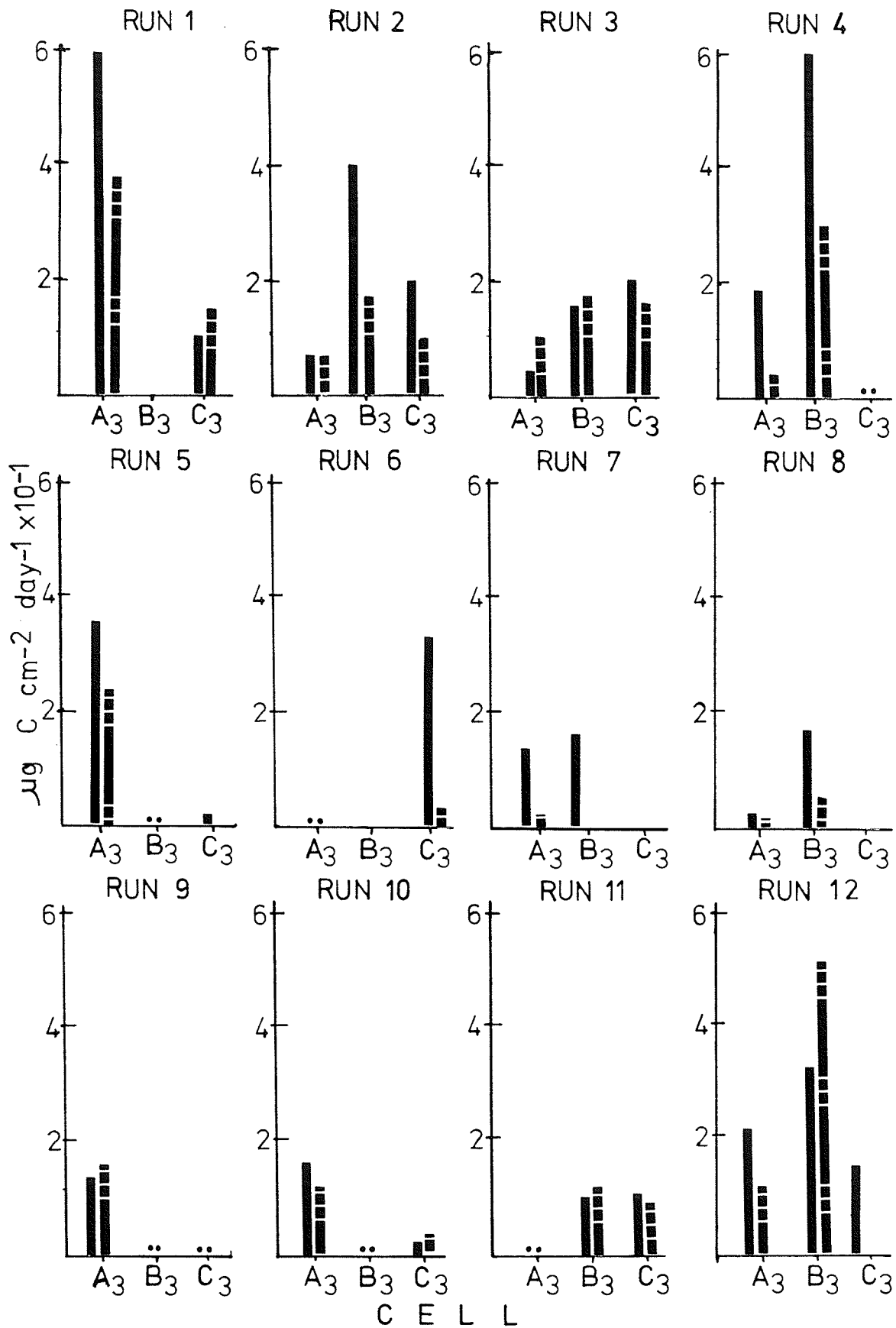
Rates of accumulation of particulate organic carbon in the epiphytic material on upper and lower halves of cellulose acetate substrate in the control series of cells ( $A_3$ ,  $B_3$ , and  $C_3$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Values are derived from measurements made at the end of each nutrient stripping run.

———— upper stratum

■■■■■■■■ lower stratum

A dot indicates missing data.



greatest rate of accumulation of epiphytic POC. This was the case for 5 of the 6 complete\* runs, and it exceeded the rate of accumulation in the A<sub>1</sub> cell in 7 runs. Similarly, in the control series, in the majority of cases, the POC content was higher in the EM in the B<sub>3</sub> than in the A<sub>3</sub> cell.

(ii) Continuing Incubations

The amounts of POC as they accumulated in the attached material on the continuing strips over the course of the 1976 season in the vegetated cells are presented in Table 3. These values are derived from periodic sampling of on-going accumulation, and, as such, should represent epiphytic development on standing macrophytes as it progressed throughout the growing season.

With the exception of the first two runs, amounts were highest in the A<sub>1</sub> cell and lowest in the B<sub>1</sub> cell, the C<sub>1</sub> cell being intermediate. (Table 3). Significant vertical stratification was apparent only in the first cell, with considerably more POC in the upper stratum than in the lower. In C<sub>1</sub>, there was slightly more POC in the upper stratum.

In A<sub>1</sub>, the quantity of POC tended to increase rapidly until the eighth run, after which it began to level off. The same trend appeared in B<sub>1</sub> and C<sub>1</sub>, although

\*In some cases over the course of the season, samples were damaged and data were not available. Here, a 'complete' run is one in which there are data available for the upper and lower strata of all 3 cells of the series.

Table 3. Continuing accumulation of particulate organic carbon in the epiphytic material of the upper and lower strata in the vegetated series in 1976. Values are in  $\mu\text{g cm}^{-2}$  for each run.

Run No.	Epiphytic POC $\mu\text{g cm}^{-2}$					
	$A_1$		$B_1$		$C_1$	
	Upper	Lower	Upper	Lower	Upper	Lower
1	18.46	22.03	45.48	43.46	* 4.90	* 18.90
2	271.50	419.40	* 29.90	* 52.60	8.31	6.50
3	* 358.70	* 285.40	8.05	5.70	9.11	7.03
4	77.37	23.09	7.32	6.16	12.20	13.60
5	44.75	28.40	7.90	8.98	14.50	12.32
6	106.30	29.80	7.54	9.87	* 16.10	* 18.52
7	123.40	28.20	* 9.20	* 10.53	19.10	22.30
8	* 213.70	* 116.70	11.03	11.42	28.70	32.79
9	222.80	151.50	14.19	13.70	99.98	77.40
10	270.90	146.11	19.00	15.50	188.95	109.80
11	279.10	166.82	19.11	19.10	162.40	157.60
12	257.10	155.11	15.89	18.50	140.90	134.50
13	227.7	151.20	17.01	22.10	89.60	102.50

\* These values represent determinations made on epiphytic material of longer colonization intervals than normal, owing to variations in the sampling schedule. All other periods between sampling times were one week ( $\pm 1$  day).

Table 4. Continuing accumulation of particulate organic carbon in the epiphytic material of the upper and lower strata in the vegetated series in 1977. Values are in  $\mu\text{g cm}^{-2}$  for each run.

Run No.	Epiphytic POC $\mu\text{g cm}^{-2}$					
	$A_1$		$B_1$		$C_1$	
	Upper	Lower	Upper	Lower	Upper	Lower
1	368.24	565.88	161.10	319.58	173.70	281.79
2	73.46	54.05	304.46	368.30	186.80	116.50
3	331.10	368.30	215.33	335.79	287.64	279.90
4	234.16	240.50	464.97	585.32	-	-
5	366.98	293.65	-	-	* 507.63	* 228.06
6	-	-	* 170.08	* 384.11	701.70	511.52
7	* 260.81	* 132.80	389.93	881.41	497.50	553.21
8	291.67	174.67	367.89	411.41	291.98	230.34
9	253.68	208.33	124.19	284.09	-	-
10	86.99	322.02	-	-	* 205.46	* 226.45
11	-	-	* 593.03	* 564.15	567.28	536.07
12	* 239.87	* 331.22	269.90	328.95	420.37	249.08

\* These values represent determinations made on epiphytic material of longer colonization intervals than normal, owing to variations in the sampling schedule. All other periods between sampling times were one week ( $\pm 1$  day).



levelling off occurred slightly later.

The comparable 1977 data for ongoing accumulation of particulate organic carbon in the vegetated series are shown in Table 4. Greatest accumulation occurred in the B<sub>1</sub> cell and least in the A<sub>1</sub> cell. Lower stratum accumulation was higher in A<sub>1</sub> and B<sub>1</sub>, and less in C<sub>1</sub>. Differences between upper and lower strata were most pronounced in B<sub>1</sub> (Table 4). In C<sub>1</sub>, more POC accumulated in the upper stratum than in the lower.

There does not appear to have been any systematic progressive buildup to one peak in any of the cells. Rather, relatively large amounts of POC occurred sporadically, interspersed with lower levels.

#### B. Suspended Particulate Material (SPM)

Weekly POC accumulation in SPM in vegetated and control cells is presented for the 1977 season in Table 5. This analysis was not carried out in 1976.

Values for POC in the SPM can be adjusted to represent upper and lower limits of material accumulating in a weekly run. The upper limit represents not only the increase in material, but also that already present in the SPM pumped into a cell at the beginning of each run, minus sedimentation and grazing losses. Therefore, the upper limit is the actual value obtained in each SPM sample. The lower limit should represent the actual weekly increase in POC in the SPM, and is obtained by

Table 5. Accumulation of particulate organic carbon in the suspended particulate material in the vegetated and control series in 1977.

These are maximum values and means, in  $\mu\text{g ml}^{-1}$ , and may represent not only net weekly increases, but also material pumped into each cell at the beginning of a run. (n = 10 runs)

Run No.	Suspended POC $\mu\text{g ml}^{-1}$					
	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	A <sub>3</sub>	B <sub>3</sub>	C <sub>3</sub>
1	21.3	32.9	16.2	21.3	0	10.0
2	37.1	37.1	7.9	37.1	28.7	0
3	66.2	45.6	24.6	66.2	1.7	20.4
4	32.9	45.4	-	68.3	22.5	-
5	62.1	-	0	28.7	-	0
6	-	14.2	18.3	-	41.2	7.9
7	51.7	24.6	0	66.2	26.7	10.0
8	32.9	10.0	0	43.3	51.7	0
9	22.5	0	-	28.7	10.0	-
10	20.4	-	0	10.0	-	0
11	-	10.0	28.7	-	9.0	10.0
12	41.2	18.3	7.9	26.7	28.7	1.7
$\bar{X}$	38.8	23.8	10.4	39.6	22.0	6.0

subtracting the inflow value at the start of a run from the value obtained at the end of that run.

Upper means for each cell are included. The means indicate very little difference between the vegetated and control series, being highest in both A cells at approximately  $40 \mu\text{g ml}^{-1}$ , and intermediate in both B cells. The C cell means were lowest at or less than  $10 \mu\text{g ml}^{-1}$ . Lower limit values for most runs were very low, in many cases negative, so minimum values and means are not shown here.

### 3. Particulate Phosphorus Determinations

#### A. Epiphytic Material

##### (i) Short-term Incubations

Figures 8 and 9 illustrate the rates of epiphytic PP accumulation in the vegetated and control series in 1976. Within the vegetated series, 10 of the 13 runs showed progressively decreasing amounts of PP from the  $A_1$  through  $B_1$  to  $C_1$  cells (Figure 8), while this trend occurred in 6 of the 8 complete runs in the control series. (Figure 9). Table 6 presents average daily uptake values in all cells in both strata.

Vertical stratification was marked in the vegetated cells, with more PP in the upper stratum than in the lower, although this was less dramatic in  $C_1$ , than in  $A_1$  or  $B_1$ .

## FIGURE 8

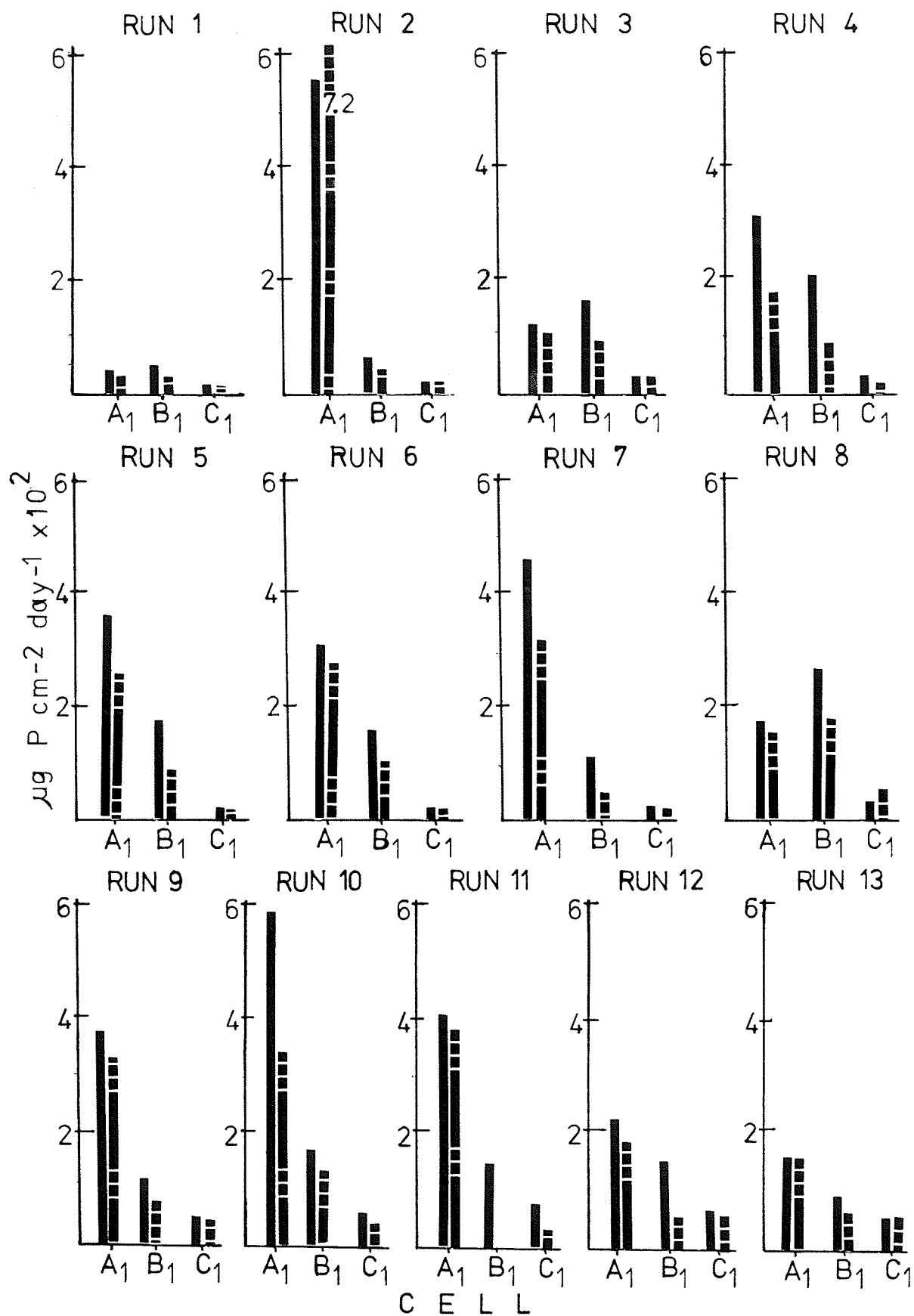
Rates of accumulation of particulate phosphorus in the epiphytic material on upper and lower halves of cellulose acetate substrate in the vegetated series of cells ( $A_1$ ,  $B_1$ , and  $C_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1976.

Values are derived from measurements made at the end of each nutrient stripping run.

———— upper stratum

— ■ ■ ■ — lower stratum

A dot indicates missing data.



## FIGURE 9

Rates of accumulation of particulate phosphorus in the epiphytic material on upper and lower halves of cellulose acetate substrate in the control series of cells ( $A_3$ ,  $B_3$ , and  $C_3$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1976.

Values are derived from measurements made at the end of each nutrient stripping run.

————— upper stratum

■■■■■■■■■■ lower stratum

A dot indicates missing data.

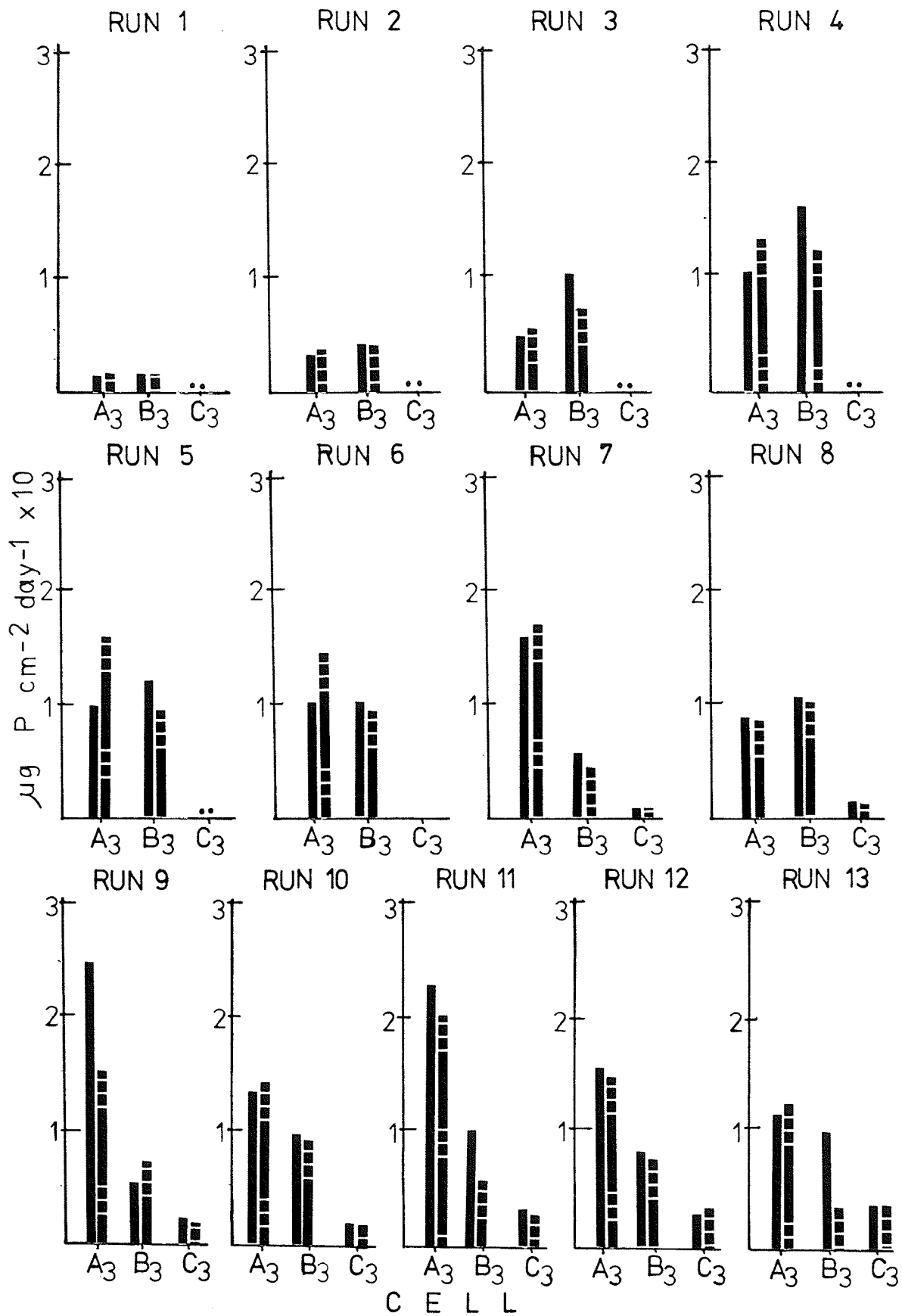


Table 6. Mean rate of PP accumulation in the EM in 1976. (n = 13 runs)

		$\mu\text{g P cm}^{-2} \text{ day}^{-1}$					
		Vegetated Cells			Control Cells		
Stratum		A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	A <sub>3</sub>	B <sub>3</sub>	C <sub>3</sub>
Upper		0.031	0.013	0.004	0.011	0.087	0.002
Lower		0.025	0.006	0.003	0.012	0.007	0.002

This trend was not apparent in the unvegetated series, with some exception in the B<sub>3</sub> cell (Figure 9).

The greatest rate of PP accumulation in the A<sub>1</sub> cell occurred in the second run with  $0.055 \mu\text{g cm}^{-2} \text{ day}^{-1}$  in the upper stratum and  $0.072 \mu\text{g cm}^{-2} \text{ day}^{-1}$  in the lower. In the B<sub>1</sub> cell, most PP accumulation occurred during run 8 with  $0.024 \mu\text{g cm}^{-2} \text{ day}^{-1}$  upper and  $0.015 \mu\text{g cm}^{-2} \text{ day}^{-1}$  lower. The 3 final runs in C<sub>1</sub> showed somewhat more PP accumulation in the EM than did earlier runs.

The trend in the control series was for epiphytic PP accumulation to increase in all cells toward the end of the season.

As Table 6 illustrates, overall rates of accumulation of epiphytic PP were higher in the vegetated than in the unvegetated series. This was especially noticeable in the first cell of each.

Equivalent 1977 phosphorus data are shown in Figures 10 and 11, and mean values of daily rates of PP accumulation in upper and lower strata are given in Table 7. These mean values are considerably higher than in 1976.



Table 7. Mean rate of PP accumulation in the EM in 1977. (n = 12 runs)

Stratum	$\mu\text{g P cm}^{-2} \text{ day}^{-1}$ Vegetated Cells			Control Cells		
	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	A <sub>3</sub>	B <sub>3</sub>	C <sub>3</sub>
Upper	1.51	0.70	0.61	1.23	0.47	0.40
Lower	0.90	0.88	0.36	1.10	0.41	0.27

Distinct vertical stratification occurred in the A<sub>1</sub> and C<sub>1</sub> cells (Figure 10) with considerably more PP accumulation in the upper stratum than lower, while in the B<sub>1</sub> cell there was somewhat more epiphytic PP in the lower stratum.

The mean daily PP accumulation values (Table 7) illustrate a decrease from A<sub>1</sub> through B<sub>1</sub> to C<sub>1</sub> in both series, and slightly higher overall values for the vegetated series than the control series, although this discrepancy is not as pronounced as in the 1976 season.

In the planted series, the highest individual values in A<sub>1</sub> occurred in the first run (3.92  $\mu\text{g cm}^{-2} \text{ day}^{-1}$  upper and 3.29  $\mu\text{g cm}^{-2} \text{ day}^{-1}$  lower); and in the 11th run in B<sub>1</sub> (2.87  $\mu\text{g cm}^{-2} \text{ day}^{-1}$  upper and 3.88  $\mu\text{g cm}^{-2} \text{ day}^{-1}$  lower). In C<sub>1</sub>, greatest amounts of epiphytic PP occurred in run 10 (2.58  $\mu\text{g cm}^{-2} \text{ day}^{-1}$  upper and 1.50  $\mu\text{g cm}^{-2} \text{ day}^{-1}$  lower).

Individual values in the control series were highest in the last 3 runs than in earlier ones, the A<sub>3</sub> cell having

## FIGURE 10

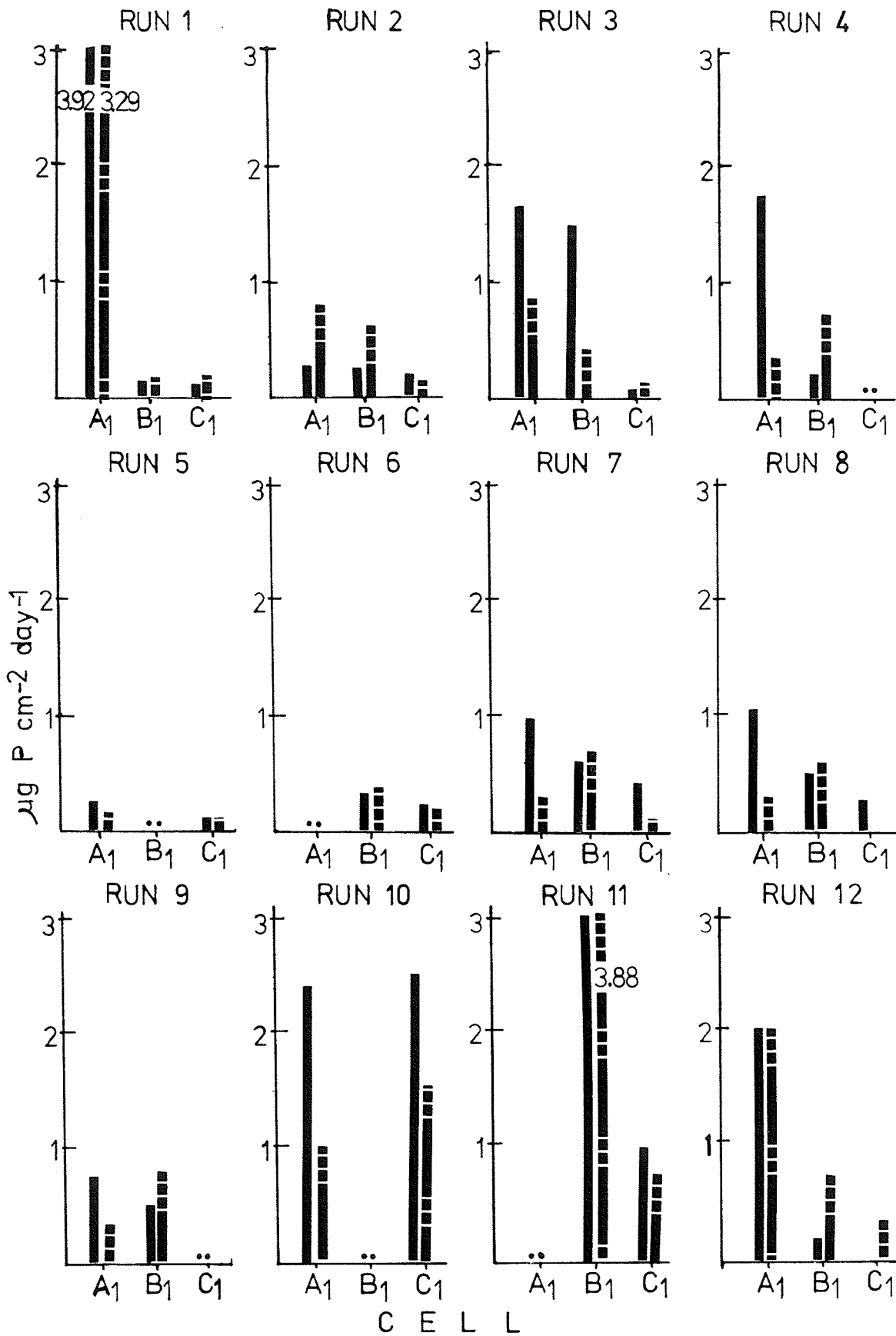
Rates of accumulation of particulate phosphorus in the epiphytic material on upper and lower halves of cellulose acetate substrate in the vegetated series of cells ( $A_1$ ,  $B_1$ , and  $C_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Values are derived from measurements made at the end of each nutrient stripping run.

————— upper stratum

■■■■■■■■■■ lower stratum

A dot indicates missing data.



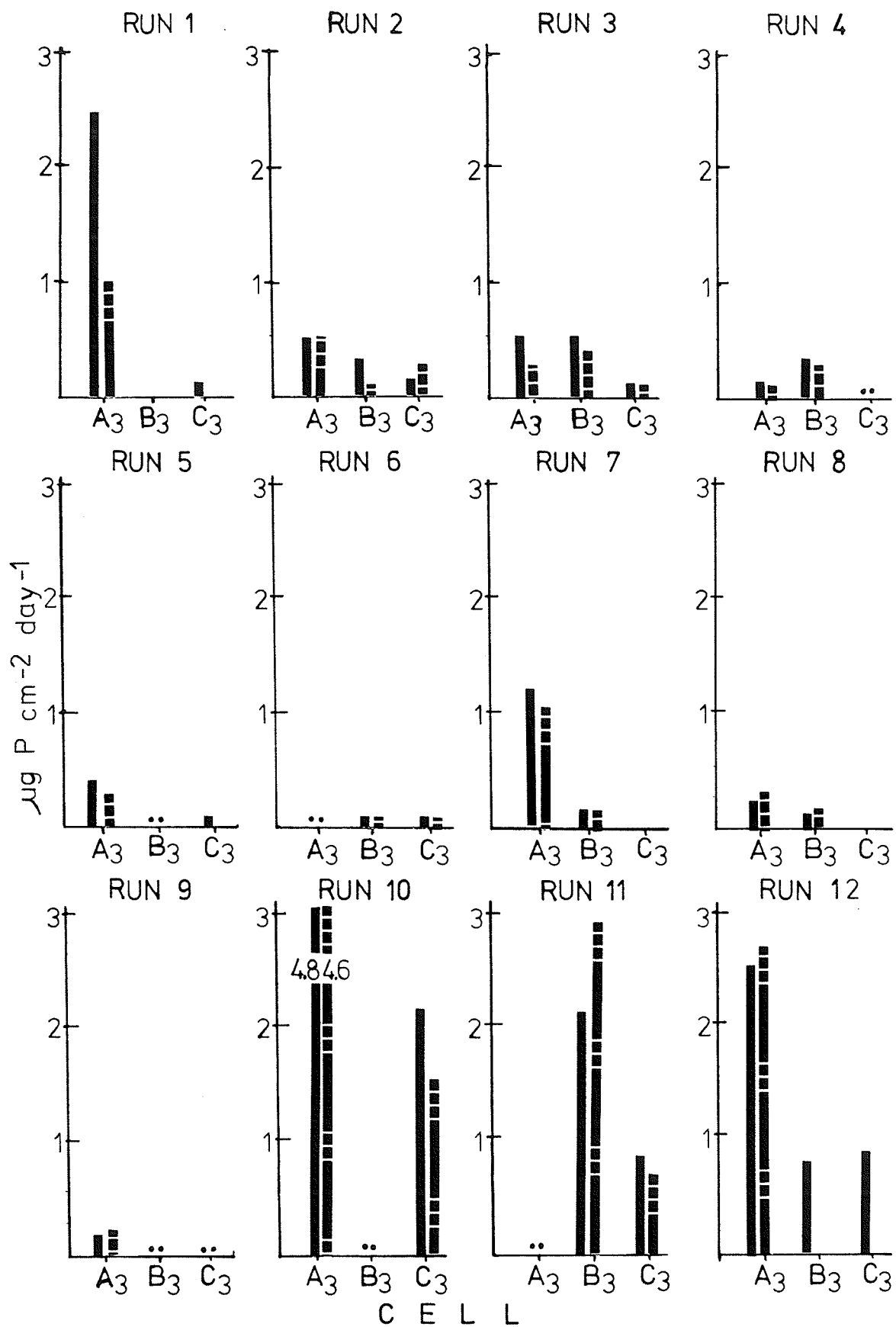
## FIGURE 11

Rates of accumulation of particulate phosphorus in the epiphytic material on upper and lower halves of cellulose acetate substrate in the control series of cells ( $A_3$ ,  $B_3$ , and  $C_3$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Values are derived from measurements made at the end of each nutrient stripping run.

————— upper stratum  
- - - - - lower stratum

A dot indicates missing data.



the greatest epiphytic PP accumulation in run 10 ( $4.8 \mu\text{g cm}^{-2} \text{ day}^{-1}$  upper and  $4.6 \mu\text{g cm}^{-2} \text{ day}^{-1}$  lower), while in  $B_3$ , run 11 had peak values of  $2.16 \mu\text{g cm}^{-2} \text{ day}^{-1}$  in the upper stratum and  $2.79 \mu\text{g cm}^{-2} \text{ day}^{-1}$  in the lower stratum. Individual values peaked in  $C_3$  in the tenth run with  $2.15 \mu\text{g cm}^{-2} \text{ day}^{-1}$  upper and  $1.54 \mu\text{g cm}^{-2} \text{ day}^{-1}$  lower.

(ii) Continuing Incubations

Table 8 gives the weekly values for the epiphytic PP as it accumulated over the course of the 1976 season on the continuing acetate strips in the vegetated series.

The tendency was for values to decrease from  $A_1$  through  $B_1$  to  $C_1$ , most often with amounts slightly higher in the upper than in the lower strata. The discrepancy between strata is small in the  $A_1$  cell. (Table 8)

In both strata of  $A_1$ , levels of PP increased gradually until the ninth run and then levelled off around  $1.0 \mu\text{g cm}^{-2} \text{ day}^{-1}$ . In both strata of  $C_1$ , and in the upper stratum of  $B_1$ , amounts increased slowly but did not level off. The lower stratum of  $B_1$  was rather erratic.

The 1977 ongoing epiphytic PP results are given in Table 9. Accumulation of PP tended to be greatest in  $A_1$ , intermediate in  $B_1$ , and least in  $C_1$ . Vertical stratification was not apparent. Increases in epiphytic PP accumulation were, for the most part, erratic over the season, except for the upper stratum in  $A_1$ , which increased fairly steadily.

Table 8. Continuing accumulation of particulate phosphorus in the epiphytic material of the upper and lower strata in the vegetated series in 1976. Values are in  $\mu\text{g cm}^{-2}$  for each run.

Run No.	Epiphytic P $\mu\text{g cm}^{-2}$					
	A <sub>1</sub>		B <sub>1</sub>		C <sub>1</sub>	
	Upper	Lower	Upper	Lower	Upper	Lower
1	0.027	0.044	0.043	0.019	*0.014	*0.012
2	0.425	0.464	*0.123	*0.088	0.016	0.017
3	*0.391	*0.303	0.122	0.121	0.014	0.017
4	0.460	0.312	0.134	0.100	0.012	0.015
5	0.471	0.413	0.120	0.086	0.014	0.014
6	0.603	0.488	0.132	0.085	*0.018	*0.014
7	0.705	0.395	*0.147	*0.077	0.017	0.013
8	*0.786	*0.583	0.132	0.082	0.038	0.038
9	0.937	0.857	0.149	0.104	0.052	0.039
10	0.977	1.040	0.160	0.120	0.073	0.041
11	0.697	0.837	0.242	0.090	0.107	0.047
12	0.819	1.030	0.305	0.153	0.303	0.102
13	0.818	0.819	0.306	0.195	0.434	0.209

\* These values represent determinations made on epiphytic material of longer colonization intervals than normal, owing to variations in the sampling schedule. All other periods between sampling times were one week ( $\pm 1$  day).

Table 9. Continuing accumulation of particulate phosphorus in the epiphytic material of the upper and lower strata in the vegetated series in 1977. Values are in  $\mu\text{g cm}^{-2}$  for each run.

Run No.	Epiphytic P $\mu\text{g cm}^{-2}$					
	A <sub>1</sub>		B <sub>1</sub>		C <sub>1</sub>	
	Upper	Lower	Upper	Lower	Upper	Lower
1	28.16	7.32	17.40	12.23	1.57	1.57
2	15.33	25.59	12.74	16.16	2.45	2.25
3	20.01	22.93	23.44	12.30	3.29	0.99
4	28.33	24.73	8.65	15.07	-	-
5	50.81	14.60	-	-	*10.96	*5.62
6	-	-	*13.36	*13.02	18.05	13.54
7	*25.15	*28.25	42.85	42.27	2.05	13.82
8	44.88	54.96	10.29	17.51	16.57	11.87
9	42.28	20.79	23.99	32.71	-	-
10	64.95	93.94	-	-	*36.31	*36.31
11	-	-	*58.15	*58.18	18.61	22.85
12	*55.95	*68.02	17.21	20.08	25.23	10.63

\* These values represent determinations made on epiphytic material of longer colonization intervals than normal, owing to variations in the sampling schedule. All other periods between sampling times were one week ( $\pm 1$  day).



## B. Suspended Particulate Material

The particulate phosphorus in the SPM was analyzed from weekly water samples of the vegetated and control cells during 1977 only. These data were calculated for upper and lower limits in the same way as for POC; however, as with the suspended POC, minimum values tended to be low or negative, and are not shown in this section. Maximum values and means are given in Table 10.

As with the suspended POC, there was little difference between the two series. Also, the high mean values occurred in the A cells, being close to  $2 \mu\text{g ml}^{-1}$ . Intermediate values just over  $1 \mu\text{g ml}^{-1}$  occurred in the B cells; and the smallest mean amounts of PP in the SPM were found in the C cells, being less than  $1 \mu\text{g ml}^{-1}$ .

## 4. Algal Cell Numbers

### A. Epiphytic Algae

Figures 12 and 13 show the number of attached algal cells per square centimeter of substrate in each run of the 1977 season in the vegetated and control series. Only material from the upper stratum was examined. The substrates examined in this context were those positioned at the beginning, and removed at the end, of each run. Values, therefore, give an indication of algal colonization of new substrate, and as such, are considered to be equivalent to colonization on newly produced macrophyte surface.

Table 10. Accumulation of particulate phosphorus in the suspended particulate material in the vegetated and control series in 1977.

These are maximum values and means, in  $\mu\text{g ml}^{-1}$ , and may represent not only net weekly increases, but also material pumped into each cell at the beginning of a run. (n = 10 runs)





Suspended P  $\mu\text{g ml}^{-1}$

Run No.	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	A <sub>3</sub>	B <sub>3</sub>	C <sub>3</sub>
1	1.33	0.75	0.48	1.19	1.67	0.53
2	1.77	1.38	0.45	0.89	0.71	0.11
3	3.10	1.31	0.50	3.45	0.94	0.43
4	1.32	2.17	-	2.90	1.10	-
5	2.25	-	0.16	1.33	-	0.20
6	-	0.77	0.38	-	0.93	0.13
7	1.29	0.65	0.67	1.11	0.65	0.20
8	1.81	0.20	0.43	1.67	1.22	0.16
9	1.22	0.70	-	1.24	1.23	-
10	1.64	-	3.28	1.11	-	2.13
11	-	3.28	0.73	-	3.28	0.33
12	4.59	1.53	0.64	5.25	1.03	1.14
$\bar{X}$	2.03	1.27	0.77	2.01	1.28	0.54

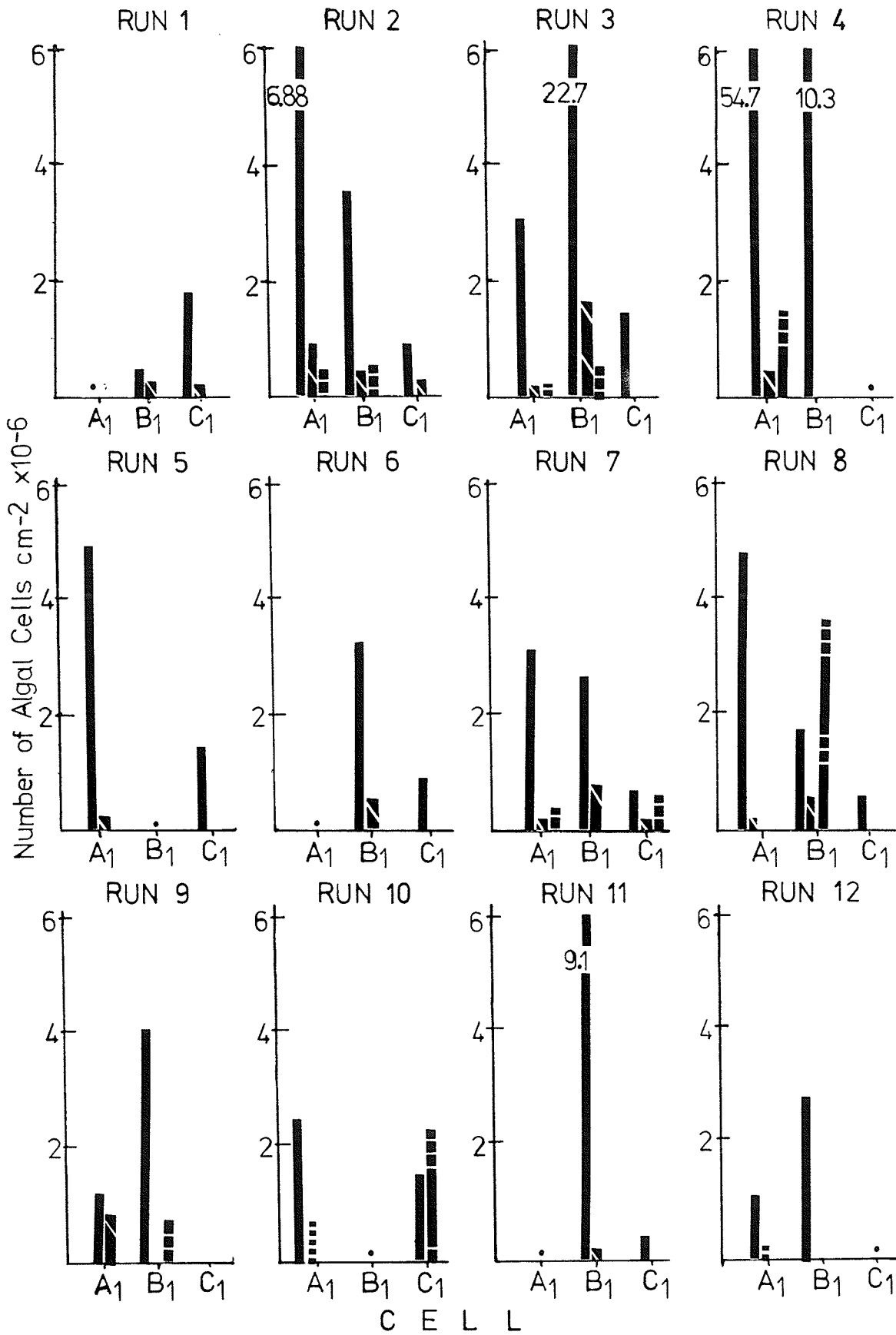
## FIGURE 12

Numbers of algal epiphytes colonizing cellulose acetate substrates in the vegetated series of cells ( $A_1$ ,  $B_1$ , and  $C_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Values are derived from counts made of material scraped from upper stratum portions of short-term strips.

	Cyanophyta
	Chrysophyta
	Chlorophyta
	Euglenophyta

A dot indicates missing data.



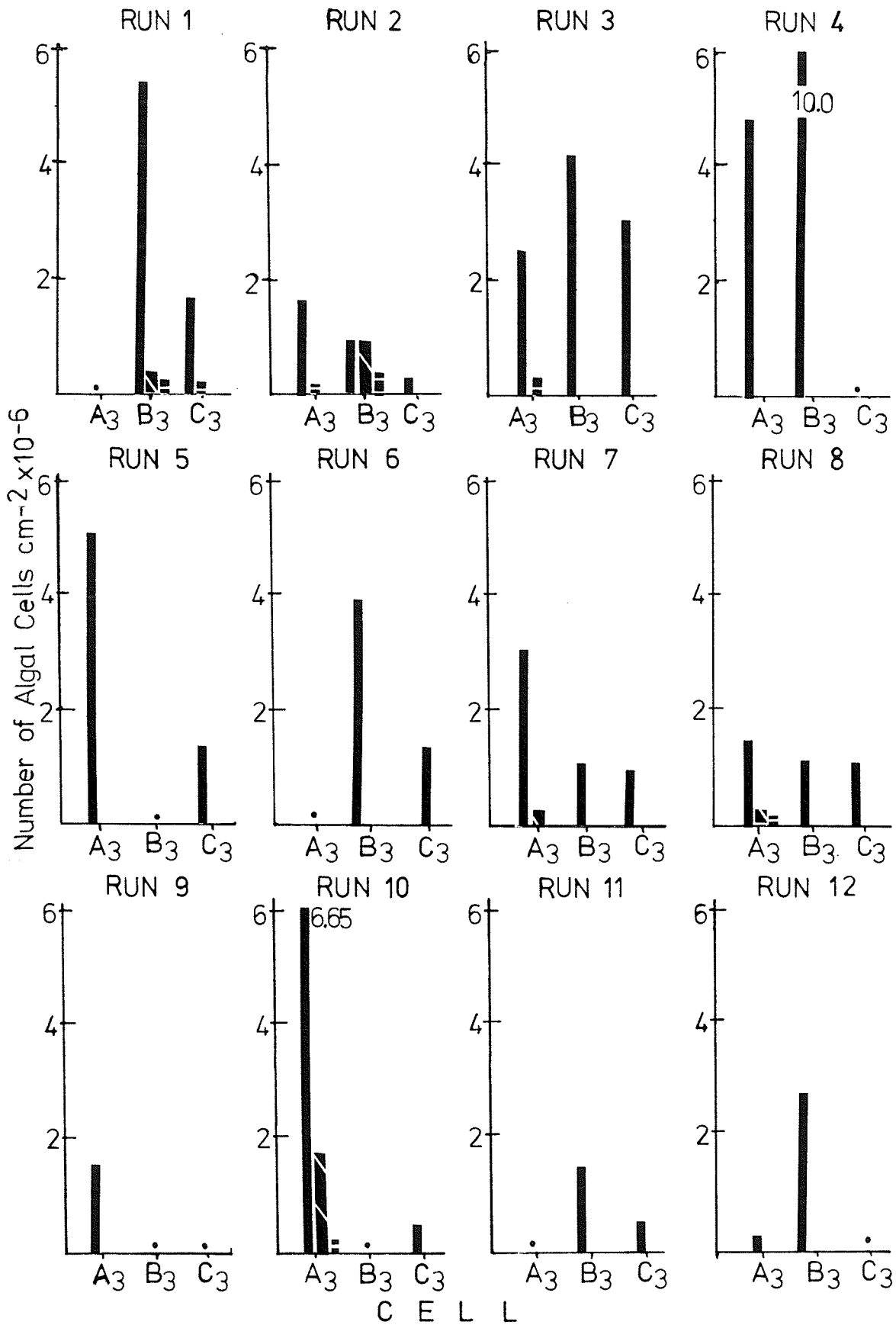
## FIGURE 13

Numbers of algal epiphytes colonizing cellulose acetate substrates in the control series of cells ( $A_3$ ,  $B_3$ , and  $C_3$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Values are derived from counts made of material scraped from upper stratum portions of short-term strips.

————— Cyanophyta  
// // // // Chrysophyta  
- - - - - Chlorophyta  
..... Euglenophyta

A dot indicates missing data.



They do not provide information on the dynamics of long-term colonization and succession, but may indicate algal responses to immediate chemical and physical parameters, rather than to combinations of these parameters with biological factors.

Algae were identified to genus, and were finally grouped into four major phyla: Cyanophyta, Chrysophyta, Chlorophyta, and Euglenophyta.

The majority of epiphytic cyanophytes belonged to the genera Anacystis Meneghini, Dactylococcopsis (Reinsch) Hansging, and Oscillatoria Vaucher. Chlorophytes consisted mainly of Ankistrodesmus Corda emend. Ralfs, Coleochaete de Brébisson, Scenedesmus Meyer, and Chlorococcum Fries. The chrysophytes were mostly pennate diatoms such as Nitzschia Hassall, Navicula Bory, Synedra Ehr., and Achnanthes Bory. The centric diatom Cyclotella Kützing was also quite common. The only euglenid found with the attached material was Phacus Dujardin, and that near the end of the season. From Figures 12 and 13, it is quite apparent that in both series cyanophytes were dominant.

Table 11 gives the mean number of attached cells per square centimeter of substrate for the vegetated and control series. The values for the cyanophytes in A<sub>1</sub>, B<sub>1</sub>, and C<sub>1</sub> showed progressively decreasing amounts from the first through the third cell with the average high in A<sub>1</sub> being  $9.021 \times 10^6 \text{ cm}^{-2}$ , and the average low occurring in

Table 11. Mean number of algal cells attached to the upper stratum of short-term cellulose acetate strips in the vegetated and control cells in 1977.

Phylum	Number of cells $\text{cm}^{-2}$ x 1000					
	Vegetated Cells			Control Cells		
	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	A <sub>3</sub>	B <sub>3</sub>	C <sub>3</sub>
Cyanophyta	9021	6590	1000	2927	3245	1088
Chrysophyta	226	374	16	214	126	0
Chlorophyta	198	455	421	25	22	35
Euglenophyta	51	0	0	31	0	0

C<sub>1</sub> with  $1.0 \times 10^6 \text{ cm}^{-2}$ . Highest mean values of chrysophytes and chlorophytes were in B<sub>1</sub>, being  $0.374 \times 10^6 \text{ cm}^{-2}$  and  $0.455 \times 10^6 \text{ cm}^{-2}$  respectively.

Within the vegetated series, the highest individual number of cyanophytes was found in run 4 in the A<sub>1</sub> cell, with  $54.695 \times 10^6 \text{ cell cm}^{-2}$ . The chrysophyte peak occurred in run 3 in B<sub>1</sub> with  $1.591 \times 10^6 \text{ cells cm}^{-2}$ , and the largest number of chlorophytes in run 8 in B<sub>1</sub> with  $2.719 \times 10^6 \text{ cm}^{-2}$ .

In the control series, cyanophytes peaked in run 4 in B<sub>3</sub> with  $10.024 \times 10^6 \text{ cells cm}^{-2}$ , chrysophytes in run 10 in A<sub>3</sub> with  $1.784 \times 10^6 \text{ cells cm}^{-2}$ , and chlorophytes were nonesistant in most runs with a high of  $0.265 \times 10^6 \text{ cm}^{-2}$  in run 1 in C<sub>3</sub>.

Within the vegetated series, the total number of



epiphytes was highest in runs 2, 3, and 4 covering the period from July 6 to August 3, 1977. No such pattern existed in the control series.

#### B. Suspended Algae

Figures 14 and 15 illustrate the numbers of phytoplanktonic algae in the water samples taken throughout the 1977 season from the vegetated and control cells. Values are expressed in millions of algal cells per millilitre.

As with the epiphyton, identification was made to genus; these were finally grouped into three phyla: Cyanophyta, Chrysophyta, and Chlorophyta.

Major cyanophytes were Anacystis, Dactylococcopsis, Chroococcus Naegeli, Anabaena Bory, and Oscillatoria Vaucher. Chrysophytes consisted mainly of Chromulina Cienkowski, Cyclotella, Navicula, and Nitzschia. Chlorophytes were mainly Ankistrodesmus Corda, emend, Ralfs, Scenedesmus Meyer, and Pandorina Bory.

The total number of genera observed in the phytoplankton was slightly higher than in the epiphyton, although many were sporadic and few in number. It is, however, apparent that the dominant genera in the suspended material are much the same as those recorded in the periphyton. It does seem, in fact, that these genera might be most expected in some form of herpo- or haptobenthic situation. Accordingly, organisms recorded here as being planktonic are likely to have been thychoplanktonic.

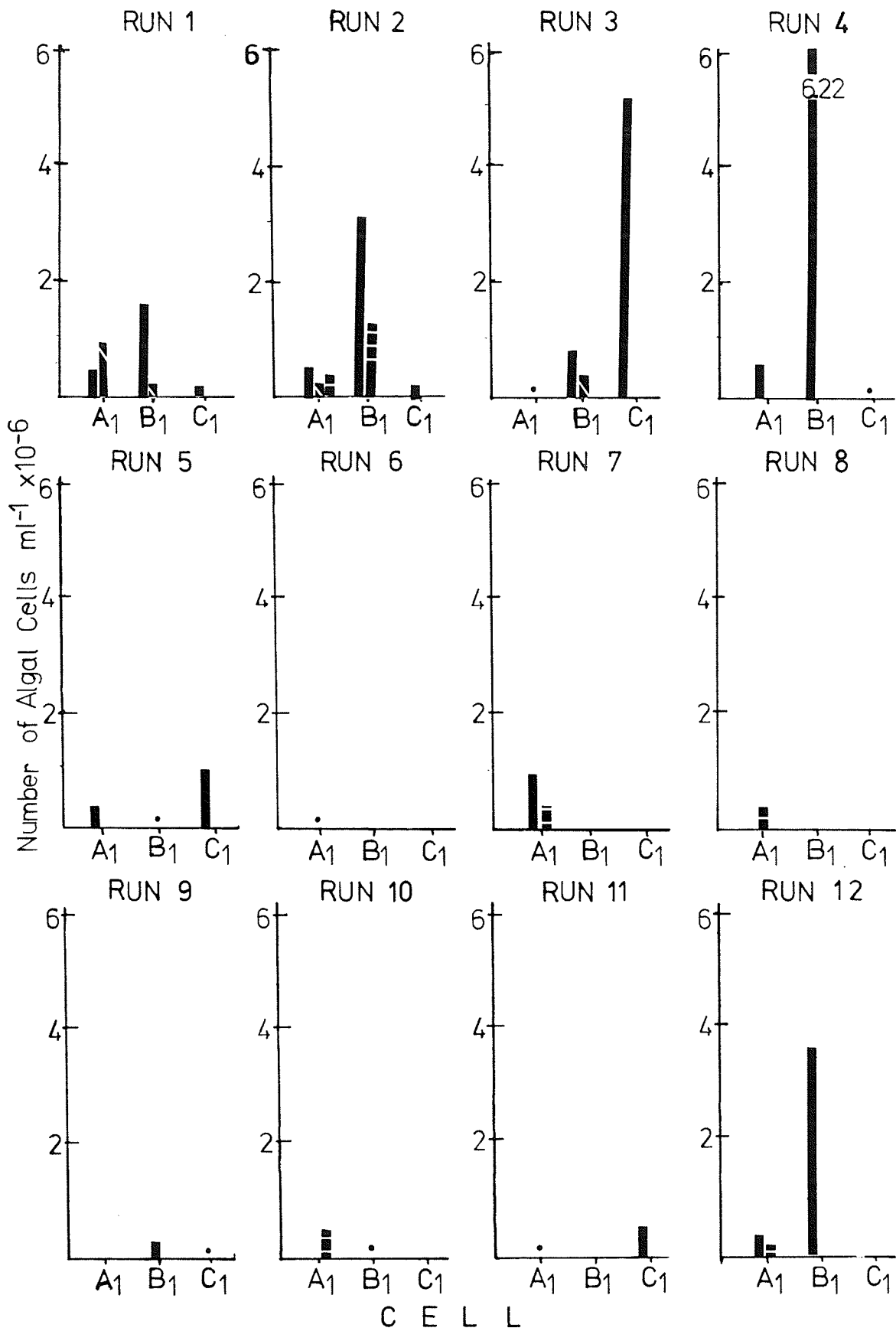
## FIGURE 14

Numbers of algal cells in suspended material in the vegetated series of cells ( $A_1$ ,  $B_1$ , and  $C_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Values are derived from counts made from weekly water samples.

———— Cyanophyta  
———/—— Chrysophyta  
- - - - - Chlorophyta

A dot indicates missing data.



## FIGURE 15

Numbers of algal cells in suspended material in the control series of cells ( $A_3$ ,  $B_3$ , and  $C_3$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Values are derived from counts made of weekly water samples.

———— Cyanophyta

——— Chrysophyta

■■■■ Chlorophyta

A dot indicates missing data.

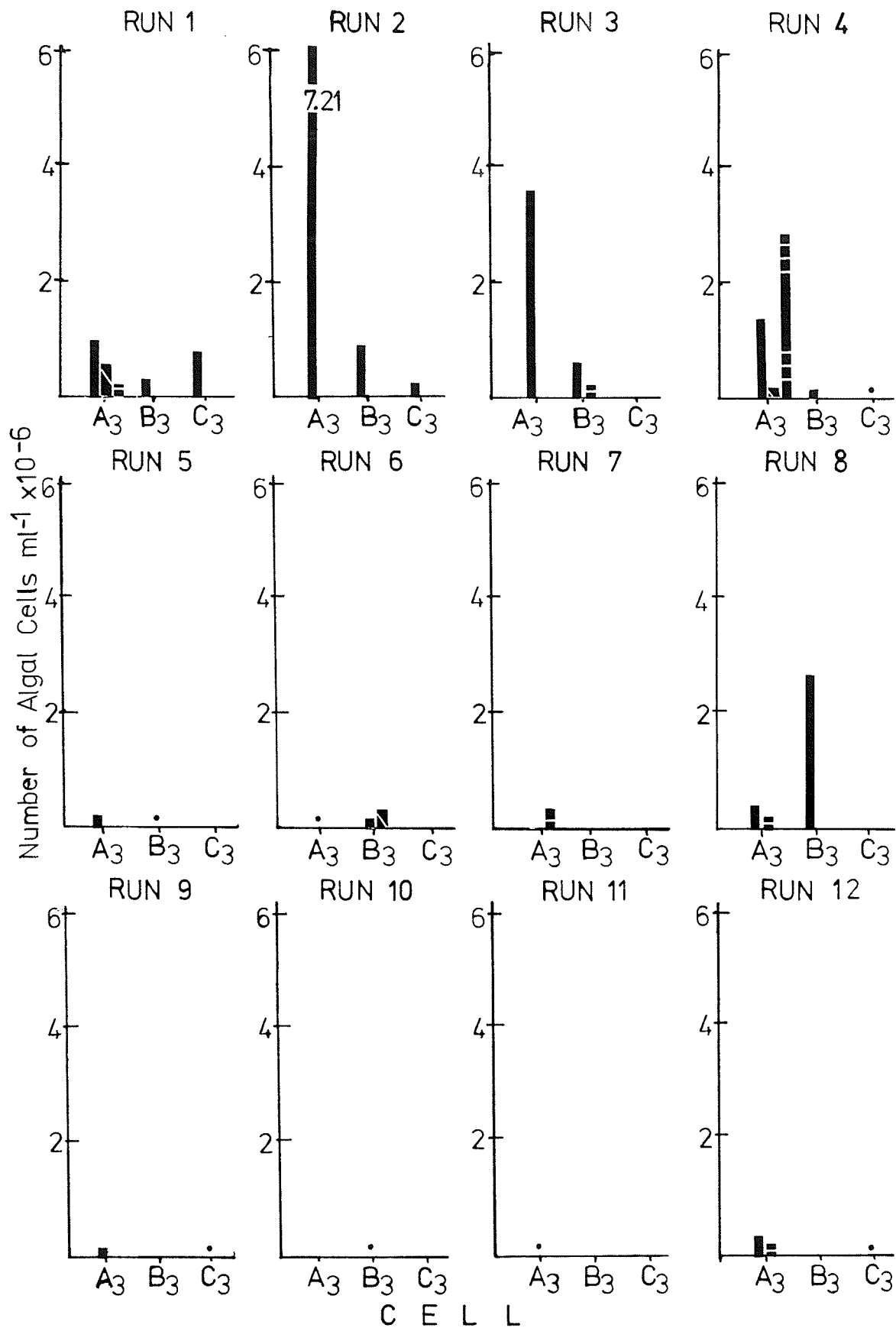


Table 12. Mean number of suspended algal cells in the weekly effluent samples from the vegetated and control cells in 1977.

Phylum	Number of cells ml <sup>-1</sup> x 1000					
	Vegetated Cells			Control Cells		
	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	A <sub>3</sub>	B <sub>3</sub>	C <sub>3</sub>
Cyanophyta	278	1453	691	1383	398	86
Chrysophyta	83	25	0	65	4	0
Chlorophyta	87	118	0	395	22	0

As can be seen in Table 12, in both vegetated and control cells the number of cyanophytes greatly exceeded the other groups. In the former, the greatest number of blue-green algae occurred in the B<sub>1</sub> cell with  $1.454 \times 10^6$  ml<sup>-1</sup>, while in the control series, average values decreased from A<sub>3</sub> through B<sub>3</sub> to C<sub>3</sub>. Numbers of chrysophytes and chlorophytes were relatively low, being zero in both C cells.

Also in both series, as illustrated in Figures 14 and 15, most algal development occurred in the first four runs, from June 28 to August 3, 1977, with very little apparent growth thereafter. In the vegetated series, cyanophytes peaked in B<sub>1</sub> in run 4 with  $6.219 \times 10^6$  cells ml<sup>-1</sup>, chrysophytes in A<sub>1</sub> in run 1 with  $6.653 \times 10^6$  ml<sup>-1</sup>, and chlorophytes in B<sub>1</sub> in run 2 with  $1.129 \times 10^6$  cells ml<sup>-1</sup>.

In the controls, the greatest number of blue-green algae occurred in A<sub>3</sub> in run 2, with  $7.207 \times 10^6$  cells ml<sup>-1</sup>.

Chrysophytes again peaked in run 1 in  $A_3$  with  $0.573 \times 10^6$  cells  $\text{ml}^{-1}$ , and the number of green algae was greatest in run 4 in  $A_3$  at  $2,658 \times 10^6 \text{ ml}^{-1}$ .

## 5. Macrophyte Surface Area

Figure 16 illustrates the estimated area in square centimeters of plant surface area per  $0.25 \text{ m}^2$  quadrat available for colonization by epiphyton as it developed over the 1977 season in cell  $A_1$ . Relatively little growth occurred for a considerable portion of the season, but there was a marked increase in the last few runs.

The macrophytic surface area per  $0.25 \text{ m}^2$  quadrat in  $B_1$  in 1977, as depicted in Figure 17, suggests a similar tendency for slow growth early in the season followed by a plateau (or slight decline) and then a marked increase around run 9.

## 6. Total Particulate Organic Carbon in the Vegetated Cells

### A. Epiphytic Material

Extrapolating from the amount of macrophyte surface area per quadrat to the total amount of macrophyte surface in cells  $A_1$  and  $B_1$ , and combining the ongoing and weekly values of epiphytic POC per square centimeter, one can estimate the total POC in all attached material in the two vegetated cells during the 1977 season.

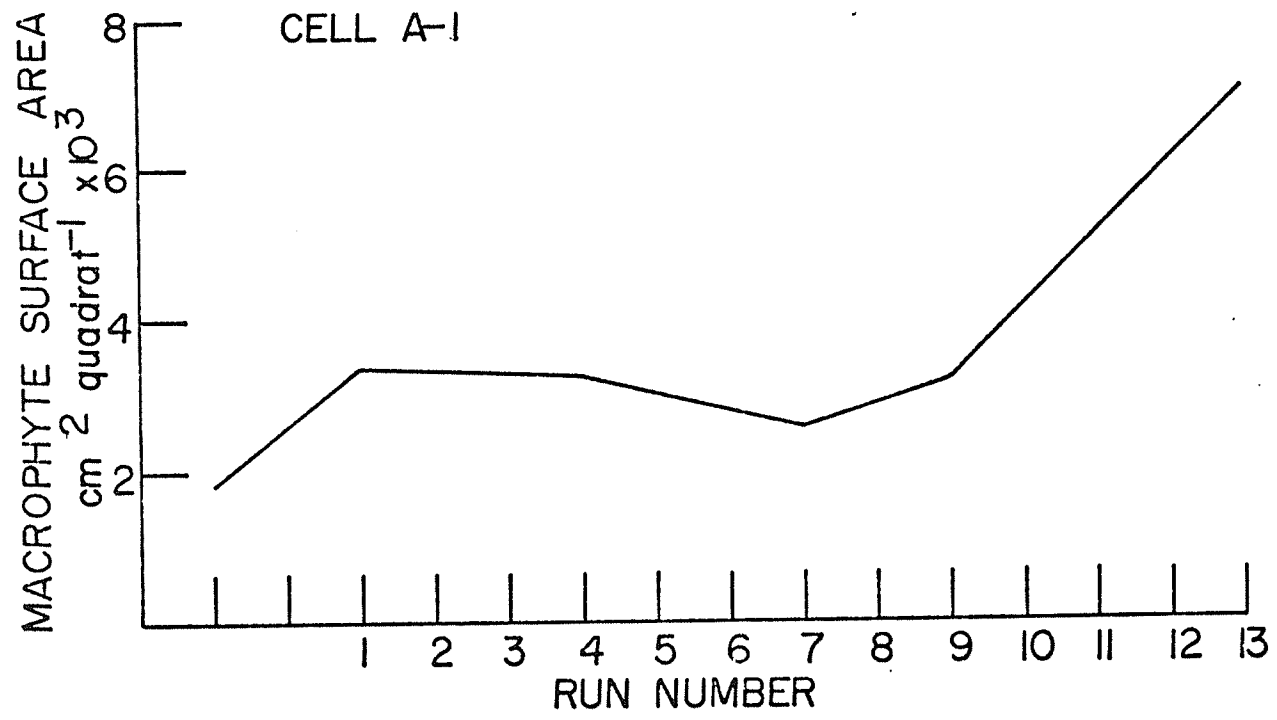
This was accomplished by subtracting the previous

## FIGURE 16

Surface area per  $0.25 \text{ m}^2$  quadrat of macrophytes in the first vegetated cell ( $A_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba.

Values are derived from regular measurements made in 1977 of 9 quadrats (averaged) and 20 stem diameters (averaged).

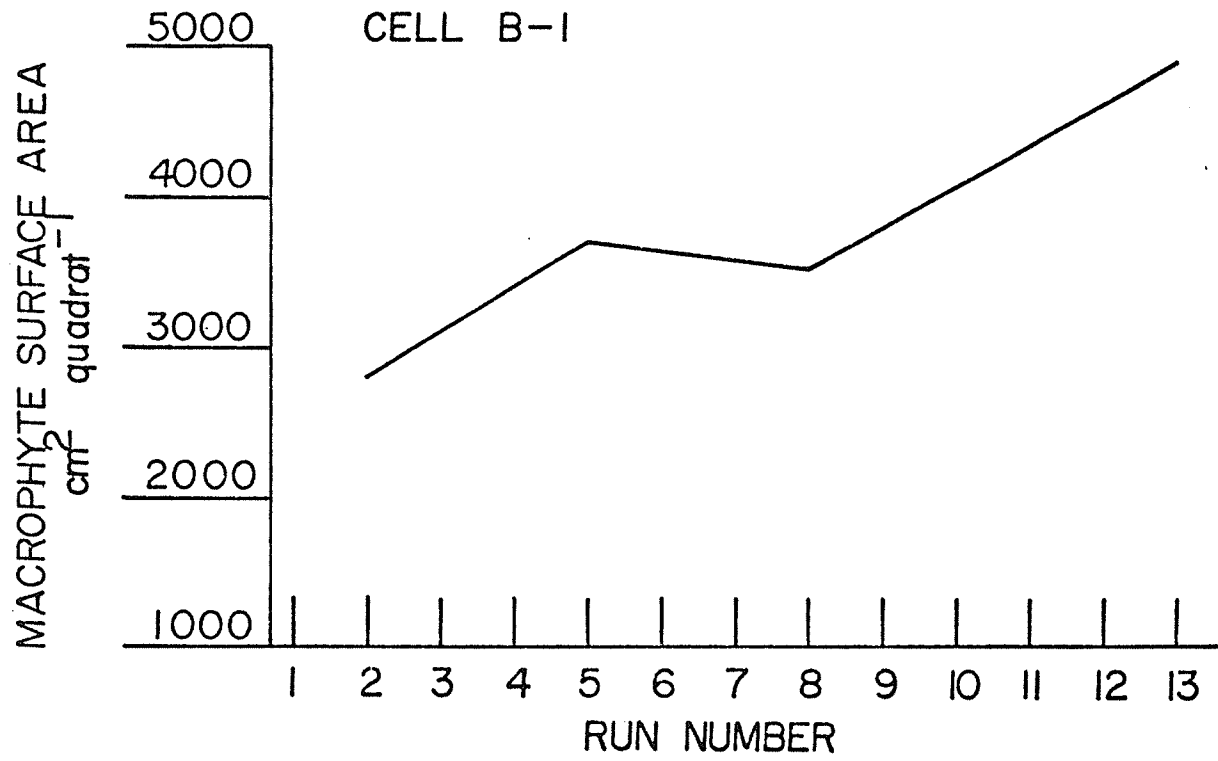




## FIGURE 17

Surface area per  $0.25 \text{ m}^2$  quadrat of macrophytes in the second vegetated cell ( $B_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba.

Values are derived from regular measurements made in 1977 of 9 quadrats (averaged) and 20 stem diameters (averaged).



week's value for the ongoing strips from each sample to provide an estimate of the net amount of POC which had accumulated during the week on already existing macrophyte surfaces and adding it to the amount of POC on the weekly short-term strips. This weekly value represented colonization of newly exposed plant surfaces.

The net ongoing value was then multiplied by the total macrophytic surface area in the two vegetated cells at each weekly interval. The weekly value was multiplied by the difference in macrophytic surface area that had occurred during the preceding week. These two figures were then combined, thus representing the total accumulation of POC in  $A_1$  and  $B_1$  during each run.\* Sample calculations are shown in Table 13. POC values estimated in this way for  $A_1$  and  $B_1$  are appended (Appendix 5).

Figure 18 represents the total amount of POC in EM in the first vegetated cell,  $A_1$ , and Figure 19, the total POC in EM in the second,  $B_1$ .

With respect to  $A_1$  (Figure 18), the trend was for higher amounts of epiphytic POC accumulation in the early runs, with a decline in the middle of the season. The highest value of 620 grams occurred in run 2, while the

\*Run numbers are different in this section, since data are displayed chronologically for each vegetated cell, rather than sequentially from A to B to C. Each 'run' here refers only to the weekly incubation period in a cell, and not to a complete 3-week effluent treatment through three cells.

Table 13. Method of calculating total Particulate Organic Carbon in Epiphyton in runs 1 and 2 in cell A<sub>1</sub> in 1977.

Run	Date	Tot. Macr. s.a. per quadrat	Stratum	Ongoing POC (µg)	Weekly POC (µg)	Estimated Total POC accumulation in Epiphyton in cell A <sub>1</sub> (mg)
1	21/6	2600 cm <sup>2</sup>	upper	181.58	-	$\frac{181.58 \times 2600 \times 565/2}{1000} = 133,370.5$ <sup>(3)</sup>
1	"	"	lower	173.24	-	$\frac{173.24 \times 2600 \times 565/2}{1000} = 127,244.8$
1	21/6	715 <sup>(1)</sup> cm <sup>2</sup>	upper	-	32.66 x 3.5 <sup>(2)</sup> = 114.31	$\frac{114.31 \times 715 \times 565/2}{1000} = 23,089.2$
1	"	"	lower	-	22.1 x 3.5 = 77.35	$\frac{77.35 \times 715 \times 565/2}{1000} = 15,623.7$
TOTAL (mg)						299,328.2

(1) Run value minus previous run value: gives new surface area (s.a.) available; if the net value appeared to be negative, zero value was used.

(2) Half weekly value attributed to upper stratum & half to lower stratum.

(3) Total number of macrophyte-covered quadrats in A<sub>1</sub>. Half value attributed to upper stratum and half to lower stratum.

(4) Conversion from µg to mg.

continued . . .

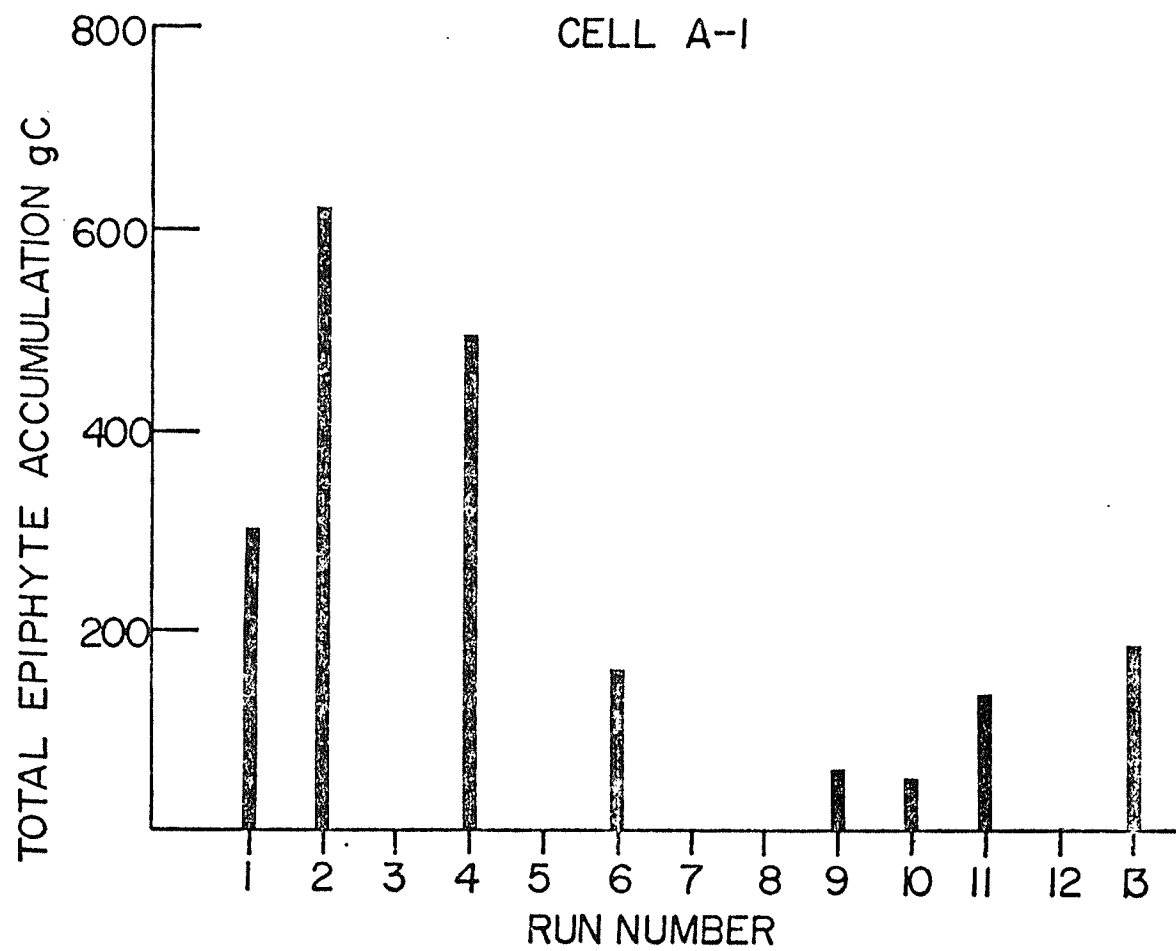
Table 13 (cont.)

Run	Date	Tot. Macr. s. a. per quadrat	Stratum	Ongoing POC ( $\mu\text{g}$ )	Weekly POC ( $\mu\text{g}$ )	Estimated Total POC accumulation in Epiphyton in cell A <sub>1</sub> (mg)
2	29/6	3328 cm <sup>2</sup>	upper	186.66 <sup>(5)</sup>	-	$\frac{186.66 \times 3328 \times 565/2}{1000} = 175,477.1$
2	"	"	lower	392.64	-	$\frac{392.64 \times 3328 \times 565/2}{1000} = 369,116.7$
2	29/6	728 cm <sup>2</sup>	upper	-	$46.52 \times 3.5 = 162.82$	$\frac{162.82 \times 728 \times 565/2}{1000} = 33,476.4$
2	"	"	lower	-	$58.27 \times 3.5 = 203.95$	$\frac{203.95 \times 728 \times 565/2}{1000} = 41,931.8$
TOTAL (mg)						620,002.0

(5) Run value minus previous run value;  
gives net weekly POC accumulation.

## FIGURE 18

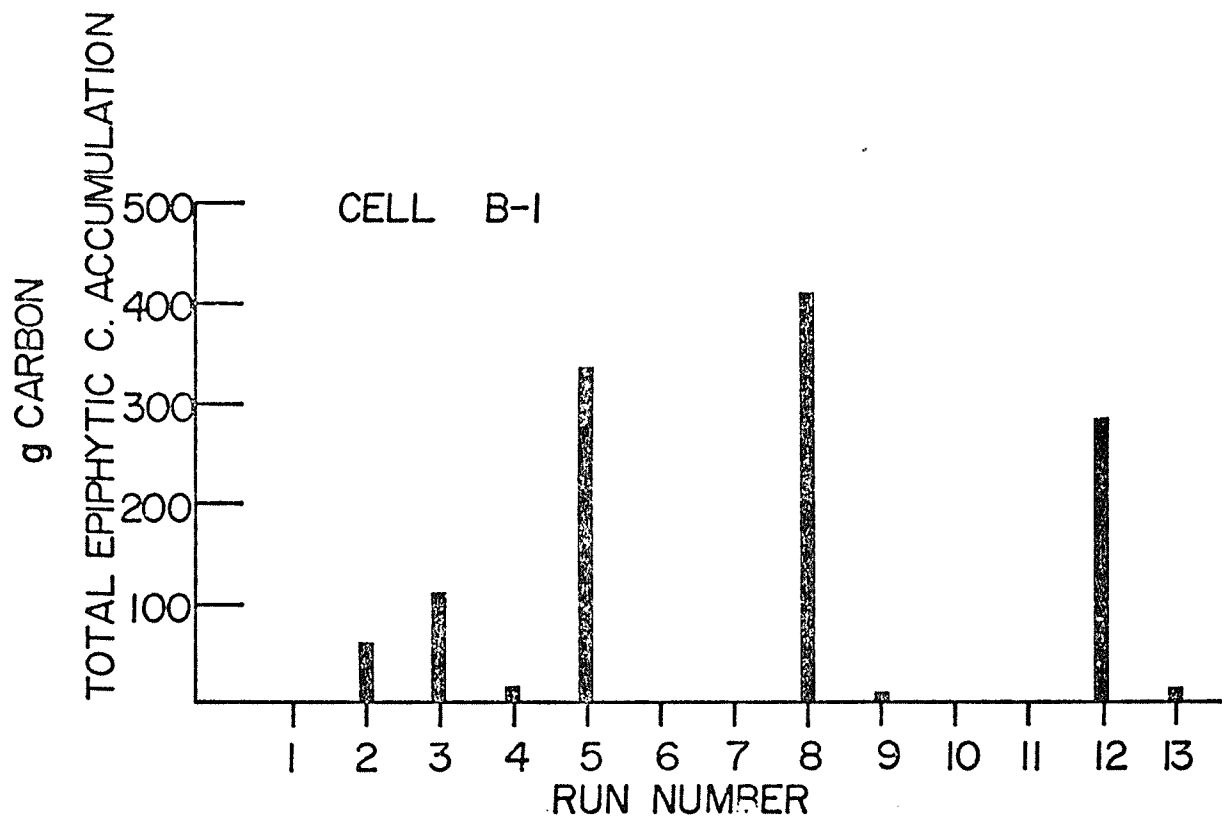
Total accumulation of particulate organic carbon in the epiphytic material attached to all submerged macrophyte surfaces in the first vegetated cell ( $A_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.





## FIGURE 19

Total accumulation of particulate organic carbon in the epiphytic material attached to all submerged macrophyte surfaces in the second vegetated cell (B<sub>1</sub>) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.



mean was 182.7 grams.

In  $B_1$  (Figure 19), colonization was very uneven, with sporadic peaks occurring throughout the 1977 season. Overall, the amount of epiphytic POC was less in  $B_1$  than in  $A_1$ , the maximum being 411.2 g in the eighth run, and the mean being 125.0 g.

#### B. Suspended Particulate Material

The total amounts of POC present during each run in the SPM in cells  $A_1$  and  $B_1$  were estimated for 1977 by converting the values obtained per millilitre to litres, and multiplying by the total number of litres in the cell during incubation periods. (30,000 gallons = 136,380 litres)

Upper and lower limits were determined for these figures, maximum values being the straightforward extrapolation from the measured value (in ml) to cell volume. Minimum values, which should represent net increase, were obtained as described earlier, and extrapolated to cell volume. Where the net value appeared to be negative (because of a higher inflow value than the value at the end of a run) the net lower limit was considered to be zero. Disturbance of sediment during pumping may have partially accounted for net negative or zero values in suspended POC during a run, so that actual values probably fall somewhere between the upper and lower limits.

Figures 20 and 21 show the total amounts of suspended POC in A<sub>1</sub> and B<sub>1</sub> respectively. In A<sub>1</sub>, the greatest accumulation occurred during the first half of the season, the largest amount being 9035 g in run 4, with a mean of 5297 g. With the exception of run 6, all lower limit values were 0.0 g.

In B<sub>1</sub> also, most POC accumulation took place in the first 5 runs, but overall there was less activity than in A<sub>1</sub>, the average amount being 3318 g.

#### C. Combined Epiphytic and Suspended Particulate Material

Combined data representing the total POC of EM and SPM in each run through cells A<sub>1</sub> and B<sub>1</sub> are presented in Figures 22 and 23. The profiles are essentially the same as in the previous two figures; more activity took place in A<sub>1</sub> than in B<sub>1</sub>, and accumulation is more pronounced during the early part of the season.

### 7. Total Particulate Phosphorus in the Vegetated Cells

#### A. Epiphytic Material

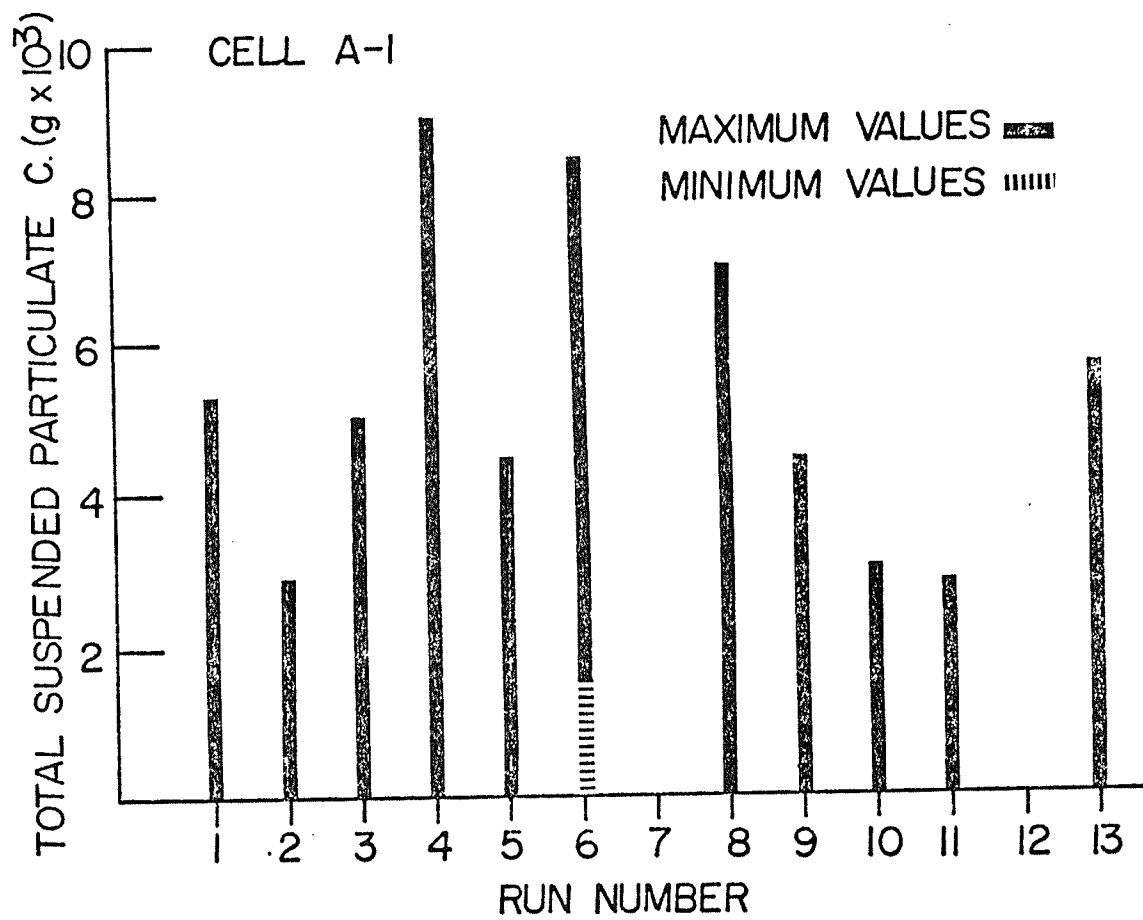
In the same way that the previous total figures for POC were generated, total epiphytic accumulation of PP in 1977 was extrapolated in the two cells A<sub>1</sub> and B<sub>1</sub>. Figures 24 and 25 depict this information. Values for each run are presented in Appendix 6.

## FIGURE 20

Total accumulation of particulate organic carbon in the suspended particulate matter in the first vegetated cell ( $A_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Maximum values are derived from measurements made at the end of each nutrient stripping run and extrapolated to the total volume of effluent in the cell.

Minimum values are obtained by subtracting the total inflow value at the start of a run from the value representing the amount of carbon in the total volume of effluent at the end of that run.

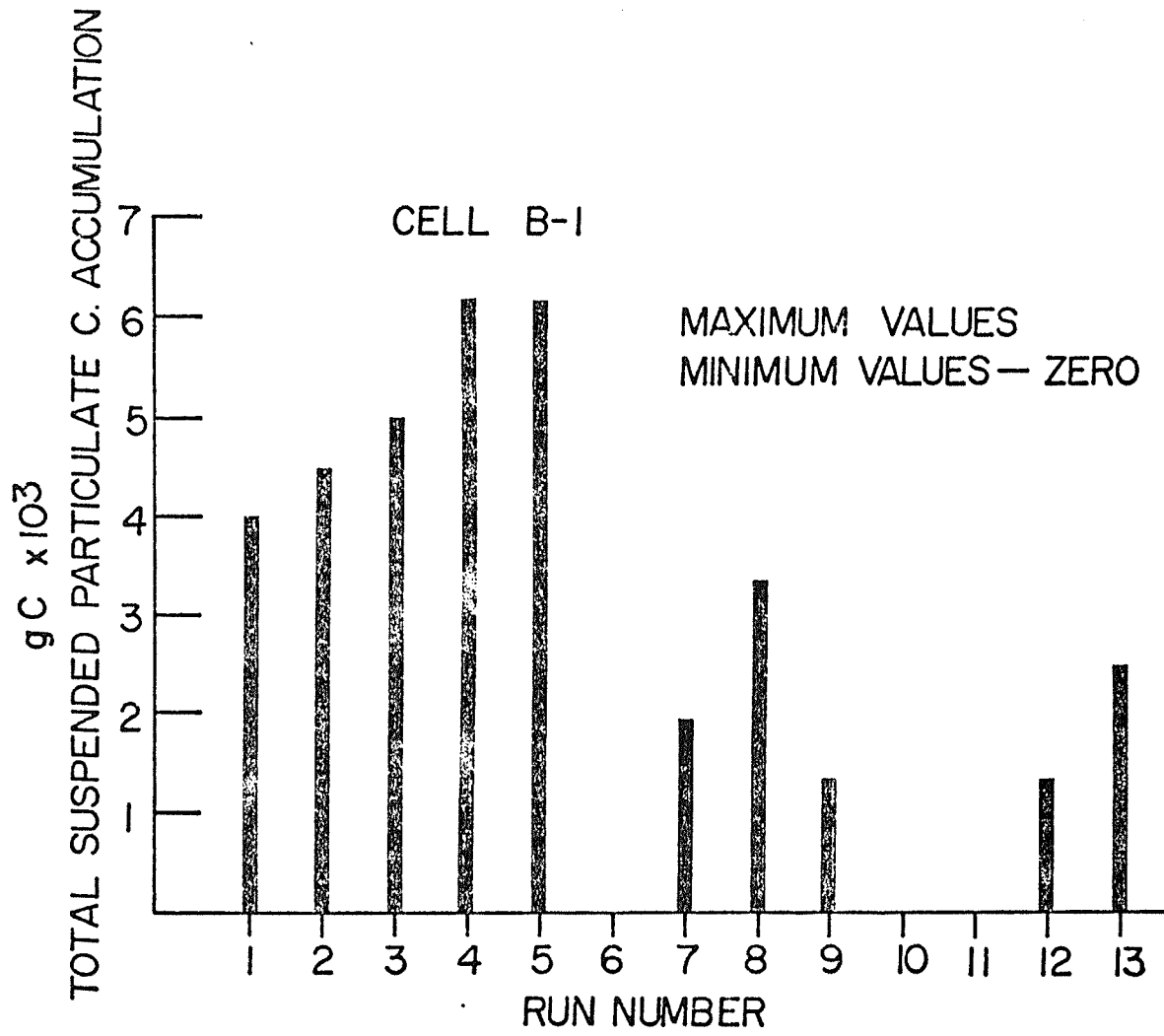


## FIGURE 21

Total accumulation of particulate organic carbon in the suspended particulate material in the second vegetated cell ( $B_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Maximum values are derived from measurements made at the end of each nutrient stripping run and extrapolated to the total volume of effluent in the cell.

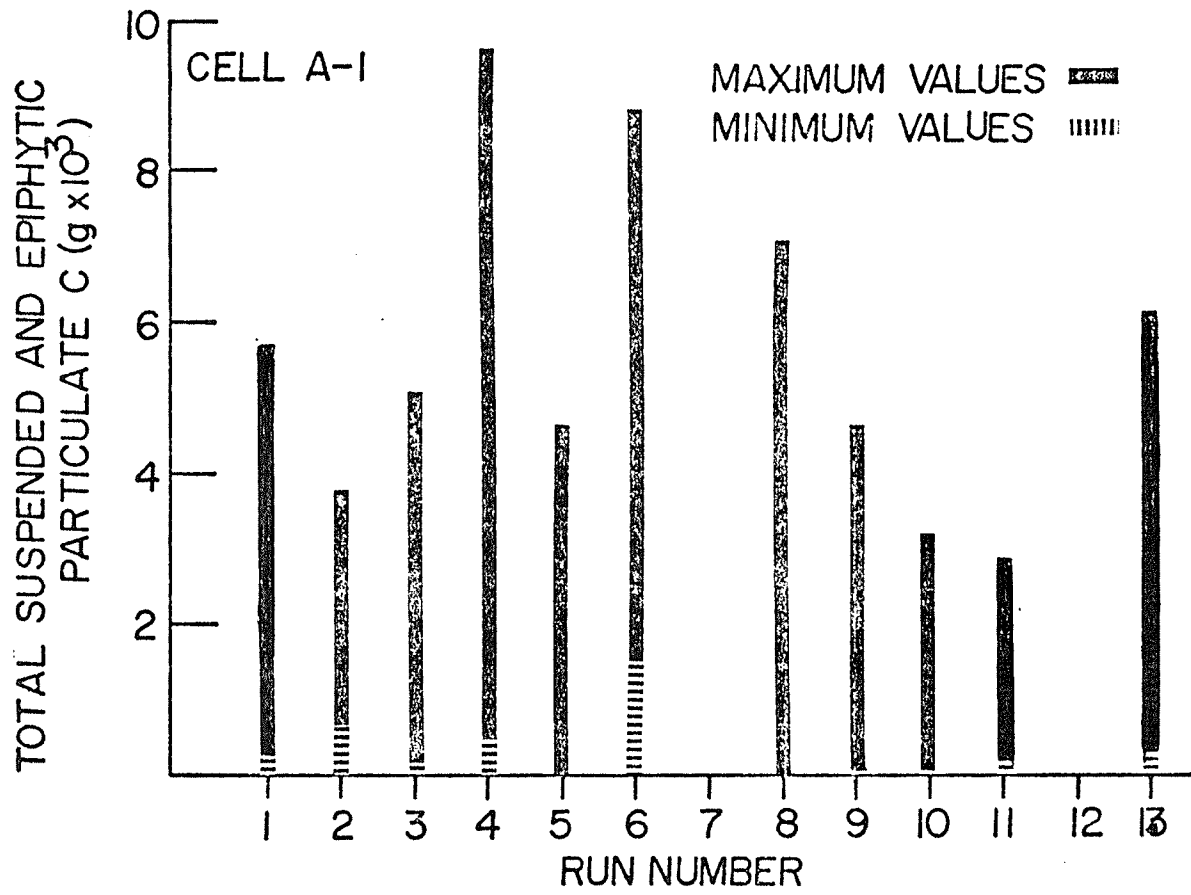
Minimum values are obtained by subtracting the total inflow value at the start of a run from the value representing the amount of carbon in the total volume of effluent at the end of that run.





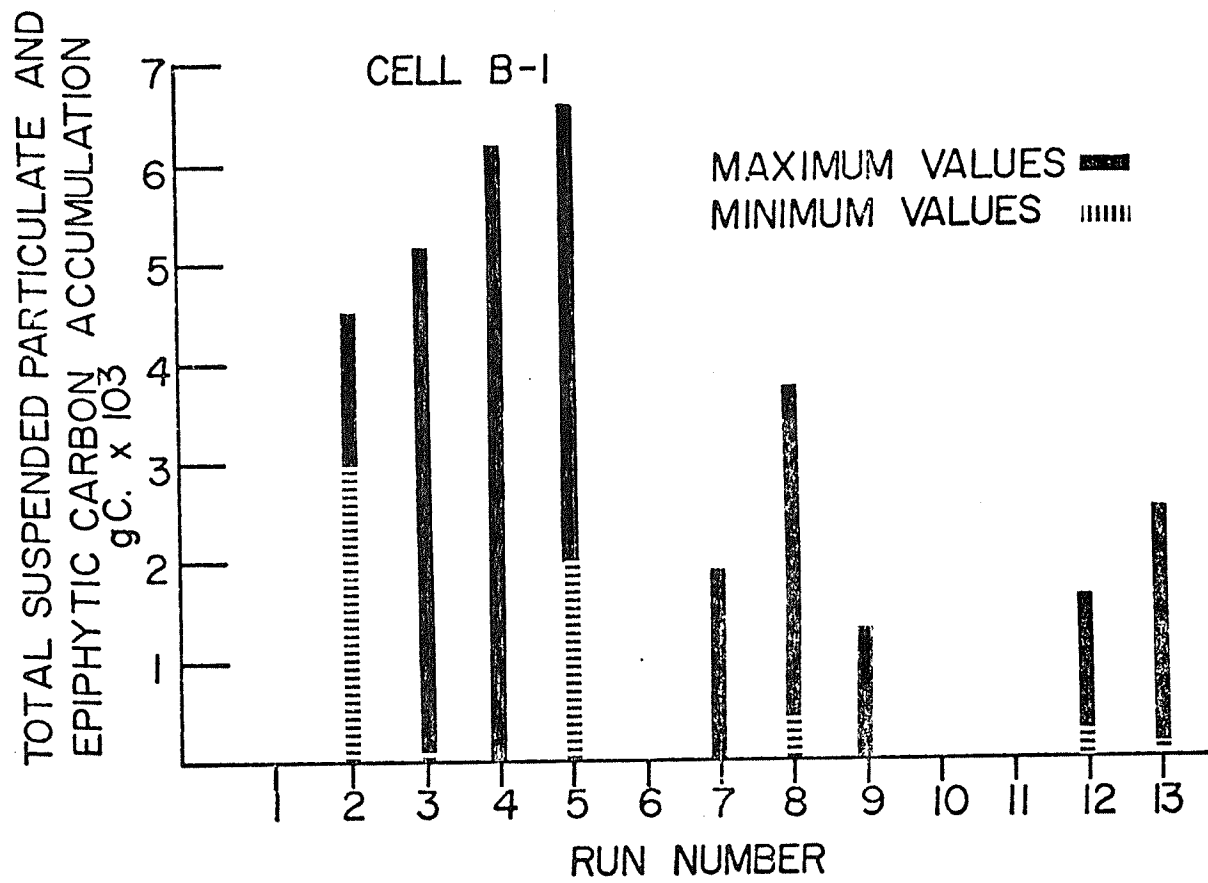
## FIGURE 22

Total combined accumulation of particulate organic carbon in the suspended particulate matter and the epiphytic material attached to all submerged macrophyte surfaces in the first vegetated cell ( $A_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.



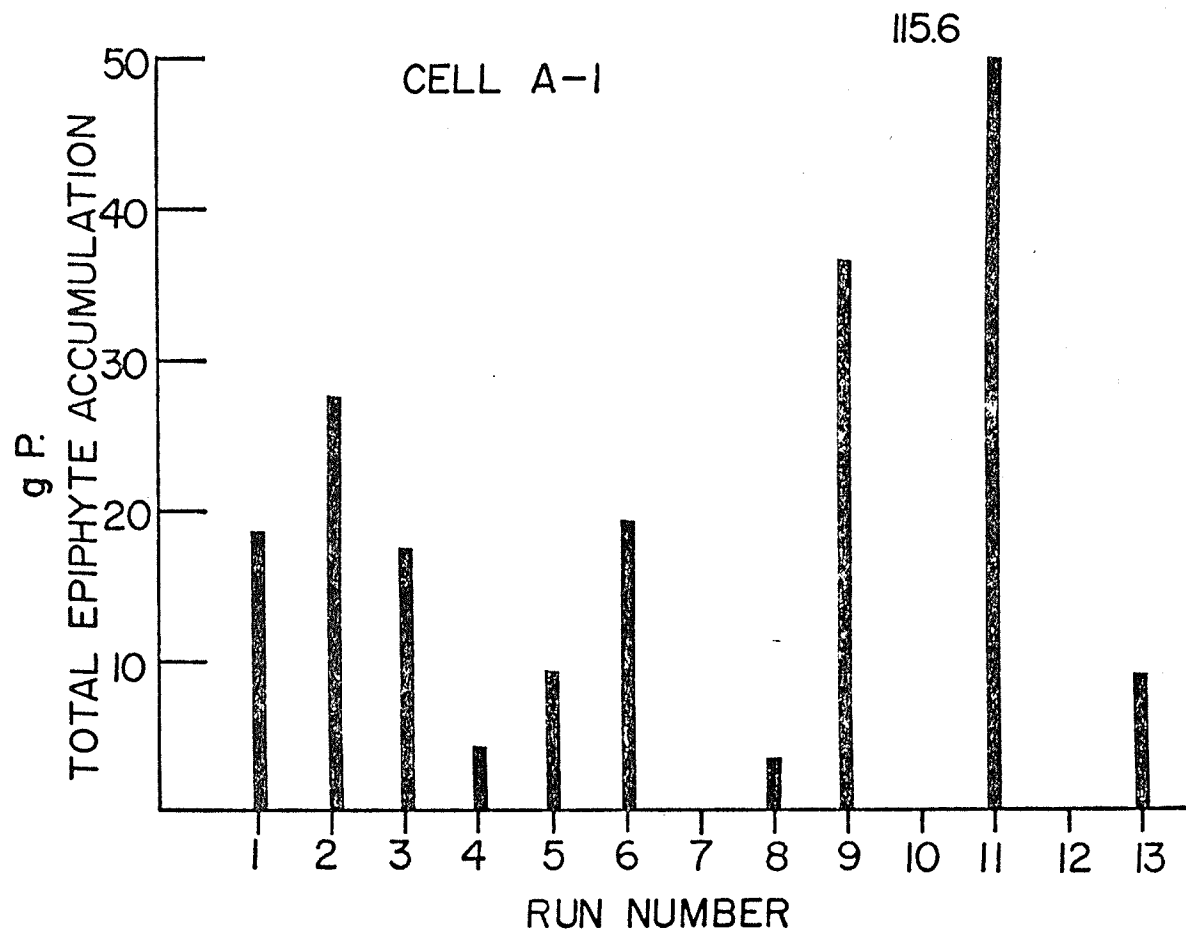
## FIGURE 23

Total combined accumulation of particulate organic carbon in the suspended particulate matter and the epiphytic material attached to all submerged macrophyte surfaces in the second vegetated cell ( $B_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.



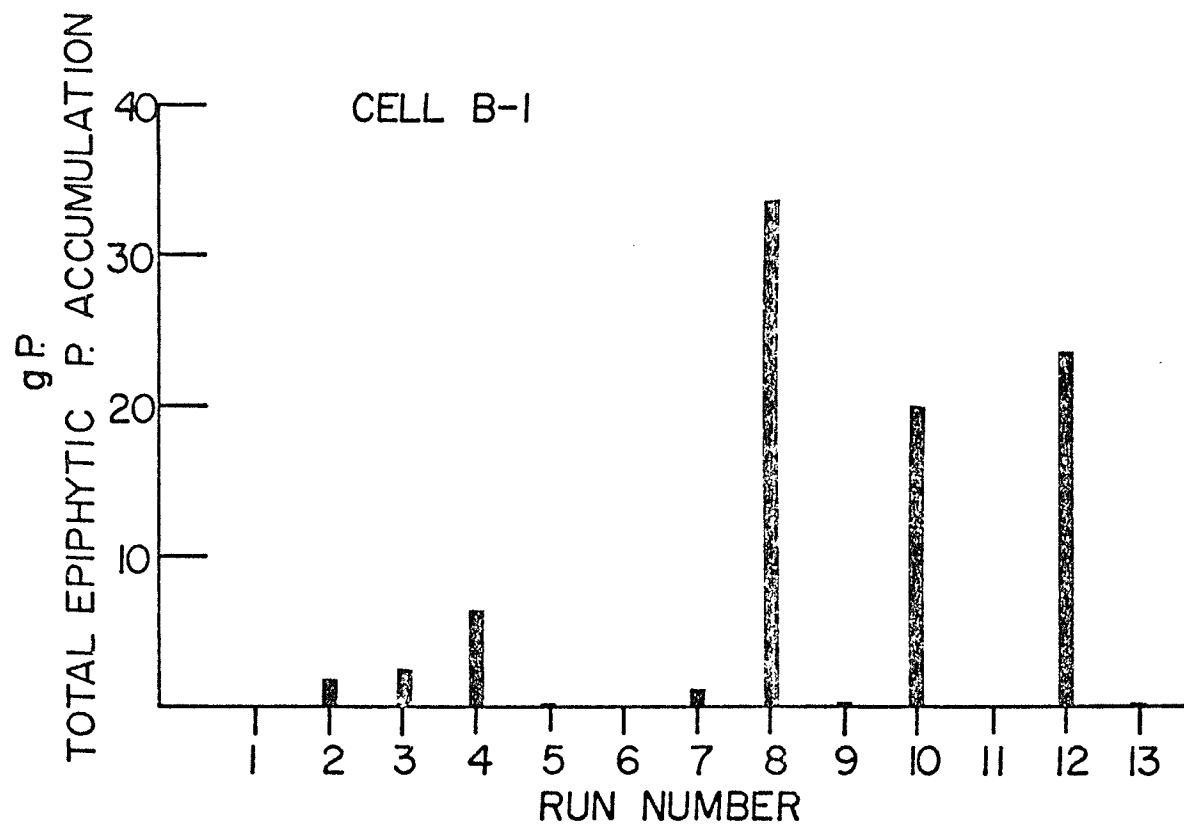
## FIGURE 24

Total accumulation of particulate phosphorus in the epiphytic material attached to all submerged macrophyte surfaces in the first vegetated cell ( $A_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.



## FIGURE 25

Total accumulation of particulate phosphorus in the epiphytic material attached to all submerged macrophyte surfaces in the second vegetated cell ( $B_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.





In  $A_1$ , epiphytic PP accumulation was higher during the first part of the season, with a few high peaks in the latter half (Figure 24). The highest value was 115.6 g in run 11 and the mean was 23.9 g.

In  $B_1$  (Figure 25) values were considerably lower, the maximum of 34.1 g occurring in run 8, the mean being 9.0 g.

#### B. Suspended Particulate Material

Figures 26 and 27 illustrate the total amounts of PP in each run in the SPM in the vegetated cells  $A_1$  and  $B_1$ . These values were obtained in the same way as for suspended POC. In both cases, suspended PP was somewhat higher during the early part of the season, with a peak toward the end. There was distinctly more suspended PP in  $A_1$ , the upper mean being 271.5 g, compared with 172.8 g in  $B_1$ . Table 14 shows this information for  $C_1$ , the amounts being considerably less, with an upper mean of 99.2 g. These data for  $C_1$  are included to permit eventual discussion of total P-removal in all six cells.

#### C. Combined Epiphytic and Suspended Particulate Material

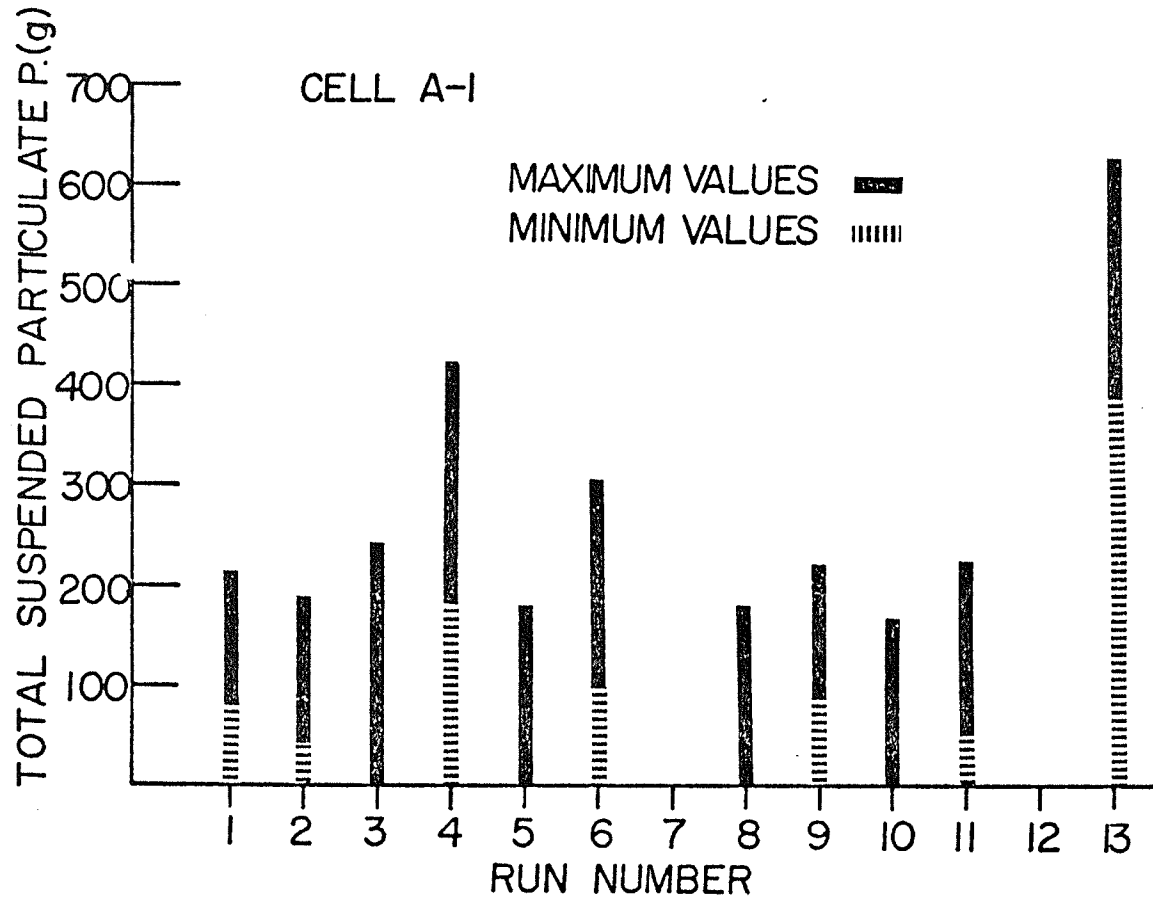
As with the POC data, the total combined PP in the EM and SPM, shown in Figures 28 and 29, are very similar to the SPM results alone. This reflects the greater biological activity of the SPM in these cells, and the relatively small contribution by the EM. The maximum mean accumulation for  $A_1$  was 295.5 g, and the upper limit mean for  $B_1$  was 166.1 g.

## FIGURE 26

Total accumulation of particulate phosphorus in the suspended particulate material in the first vegetated cell ( $A_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Maximum values are derived from measurements made at the end of each nutrient stripping run and extrapolated to the total volume of effluent in the cell.

Minimum values are obtained by subtracting the total inflow value at the start of a run from the value representing the amount of phosphorus in the total volume of effluent at the end of that run.



## FIGURE 27

Total accumulation of particulate phosphorus in the suspended particulate material in the second vegetated cell ( $B_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

Maximum values are derived from measurements made at the end of each nutrient stripping run and extrapolated to the total volume of effluent in the cell.

Minimum values are obtained by subtracting the total inflow value at the start of a run from the value representing the amount of phosphorus in the total volume of effluent at the end of that run.

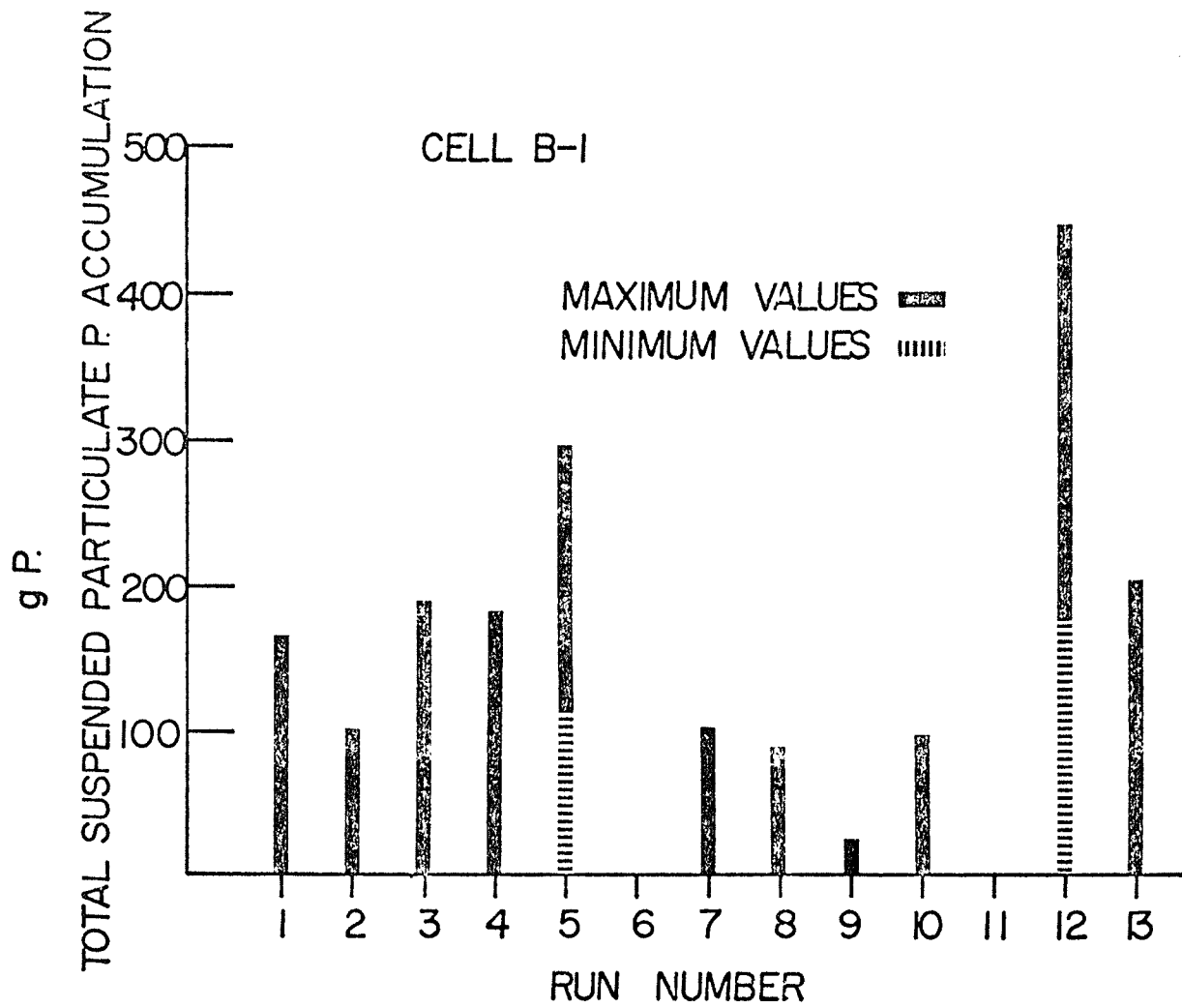
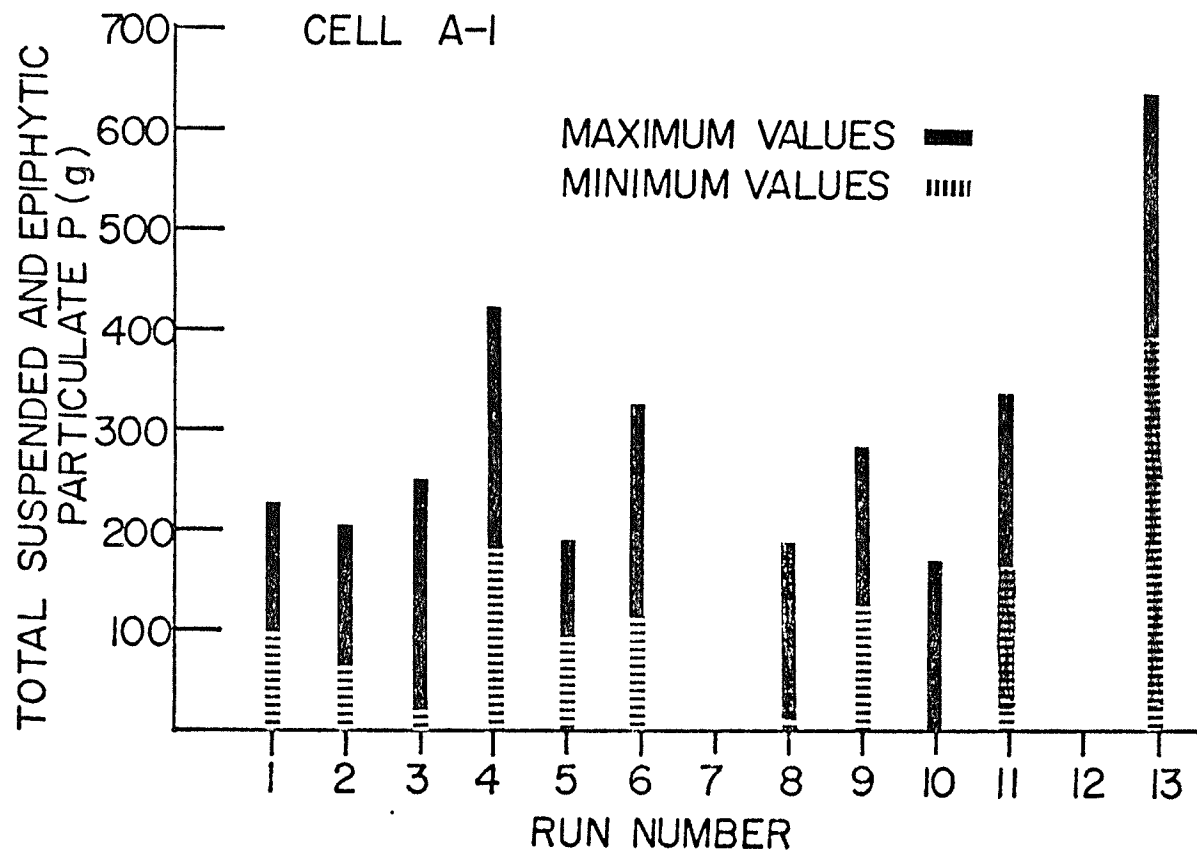


Table 14. Total accumulation of particulate phosphorus in the suspended particulate material in cell C<sub>1</sub> in 1977. Values have been extrapolated from weekly samples to cell volume.

Run No.	Upper Limit (g)	Lower Limit (g)
1	38.2	0
2	65.5	0
3	61.4	0
4	68.2	0
5	-	-
6	21.8	0
7	51.8	0
8	91.4	2.7
9	58.6	31.4
10	-	-
11	447.3	256.4
12	99.6	0
13	87.3	0
$\bar{X}$	99.2	26.4

## FIGURE 28

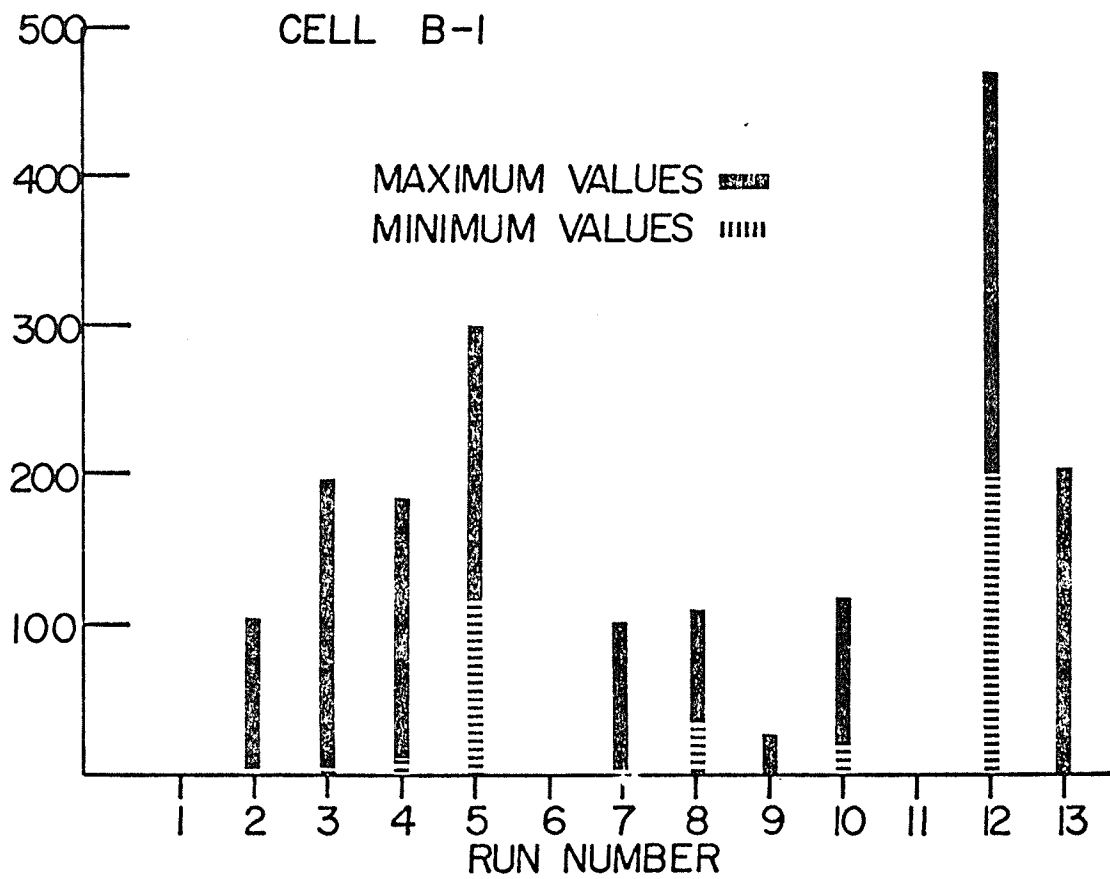
Total combined accumulation of particulate phosphorus in the suspended particulate matter and the epiphytic material attached to all submerged macrophyte surfaces in the first vegetated cell ( $A_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.





## FIGURE 29

Total combined accumulation of particulate phosphorus in the suspended particulate matter and the epiphytic material attached to all submerged macrophyte surfaces in the second vegetated cell ( $B_1$ ) at the Aquatic Vegetation site at Arborg, Manitoba in 1977.

TOTAL SUSPENDED AND EPIPHYTIC  
PARTICULATE P. ACCUMULATION  
g P.

## 8. Percentage of Ortho-Phosphate Removed in the Vegetated and Control Series

### A. Vegetated Series

The following three tables (Tables 15, 16, and 17) give the percentages of orthophosphate removed in the vegetated series. The value representing the total orthophosphate removed from a run was calculated from the water analysis carried out for the overall vegetation project, and represented the difference between the amount of orthophosphate in the effluent in the cell at the beginning of a run (the 'in' value), and at the end of a run.

In cells A<sub>1</sub> and B<sub>1</sub>, proportions of phosphate-removal are assigned to epiphytic and suspended matter; while in C<sub>1</sub> (Table 17), where there was insignificant development of submerged macrophytes, only the percent removal by the total SPM is included.

With respect to the the SPM, maximum and minimum values are given, these having been obtained as previously explained.

By far the greater amount of algal uptake of orthophosphate in A<sub>1</sub> occurred in the SPM, the maximum mean being 10.9% and the minimum mean being 3.1%, with 1.19% removal taking place in the epiphyton.

This is also the case in B<sub>1</sub> (Table 16), with a maximum average of 23.9% in the SPM, while the EM contribution is again less than 1%.

The maximum and minimum averages for the SPM in C<sub>1</sub>

Table 15. Percent of Orthophosphate Removed from  $A_1$  by Epiphyton and Suspended Particulate Material.

Run No.	Total Ortho $PO_4$ Removed (g)	% Removal by Epiphyton	Maximum % Removal by SPM	Minimum % Removal by SPM
1	2045.7	0.93	10.53	3.80
2	3000.4	0.91	6.05	1.32
3	2216.2	0.78	10.89	0
4	* -	-	-	-
5	0	-	-	-
6	1404.7	1.40	21.84	6.70
7	327.3	-	-	-
8	2182.1	0.23	8.06	0
9	2625.3	1.39	9.4	3.32
10	0	-	-	-
11	3102.6	3.73	7.21	1.58
12	-	-	-	-
13	4773.3	0.17	13.11	8.08
$\bar{X}$	1970.7 n = 11	1.19 n = 8	10.9 n = 8	3.10 n = 8

\* A dash indicates data which were incomplete or not applicable because of apparent zero values of ortho  $PO_4$  removed from a cell.

Table 16. Percent of Orthophosphate Removed from B<sub>1</sub> by Epiphyton and Suspended Particulate Material.

Run No.	Total Ortho PO <sub>4</sub> Removed (g)	% Removal by Epiphyton	Maximum % Removal by SPM	Minimum % Removal by SPM
1	2386.7	* -	-	-
2	613.7	0.32	16.67	0
3	-	-	-	-
4	0	-	-	-
5	1588.8	0.01	18.63	7.30
6	0	-	-	-
7	170.5	0.75	61.6	0
8	2625.3	1.30	3.38	0
9	0	-	-	-
10	1329.7	1.50	7.20	0
11	0	-	-	-
12	1295.6	1.81	34.53	13.47
13	1363.8	0.01	15.30	0
$\bar{X}$	947.8 n = 12	0.81 n = 7	22.47 n = 7	2.97 n = 7

\* incomplete or non-applicable data

Table 17. Percent of Orthophosphate Removed in  $C_1$  by Suspended Particulate Material.

Run No.	Total Ortho $PO_4$ Removed (g)	Maximum % Removal by SPM	Minimum % Removal by SPM
1	0	* -	-
2	-	-	-
3	0	-	-
4	88.6	76.97	0
5	-	-	-
6	313.7	6.97	0
7	0	-	-
8	0	-	-
9	340.9	17.2	9.2
10	1363.8	-	-
11	2182.1	20.50	13.43
12	7773.7	1.28	0
13	3409.5	2.56	0
$\bar{X}$	1289.4 n = 12	20.91 n = 6	3.77 n = 6

\* incomplete or non-applicable data

(Table 17), are very similar to that of  $B_1$ , at 20.91% and 3.77% respectively.

#### B. Control Series

The following three tables, Tables 18, 19, and 20 show the maximum and minimum percentage removal of phosphorus by the SPM in the control cells  $A_3$ ,  $B_3$ , and  $C_3$ . Both the  $B_3$  and  $C_3$  cells had mean upper limit removal values of just over 14%, while the average removal in  $A_3$  ranged from 11.81 - 2.03%.

Table 18. Percent of Orthophosphate Removed in A<sub>3</sub> by Suspended Particulate Material

Run No.	Total Ortho PO <sub>4</sub> Removed (g)	Maximum % Removal by SPM	Minimum % Removal by SPM
1	4057.3	* -	-
2	3068.6	5.29	0.67
3	2863.9	4.24	0
4	-	-	-
5	1568.4	25.22	0
6	681.9	26.60	0
7	0	-	-
8	3443.6	4.40	0
9	3239.0	7.03	2.10
10	340.9	4.96	0
11	2898.1	5.22	0
12	0	-	-
13	3068.6	23.33	15.51
$\bar{X}$	2102.5 n = 12	11.81 n = 9	2.03 n = 9

\* incomplete or non-applicable data



Table 19. Percent of Orthophosphate Removed in B<sub>3</sub> by Suspended Particulate Material

Run No.	Total Ortho PO <sub>4</sub> Removed (g)	Maximum % Removal by SPM	Minimum % Removal by SPM
1	340.9	3.20	3.2
2	0	* -	-
3	-	-	-
4	68.2	100.00	0
5	0	-	-
6	954.7	-	-
7	1091.0	11.63	0
8	3239.0	2.74	0
9	0	-	-
10	0	-	-
11	0	-	-
12	0	-	-
13	272.8	51.5	0
$\bar{X}$	497.2 n = 12	33.81 n = 5	0.64 n = 5

\* incomplete or non-applicable data

Table 20. Percent of Orthophosphate Removed in C<sub>3</sub> by Suspended Particulate Material

Run No.	Total Ortho PO <sub>4</sub> Removed (g)	Maximum % Removal by SPM	Minimum % Removal by SPM
1	0	* -	-
2	-	-	-
3	54.6	27.5	0
4	0	-	-
5	0	-	-
6	136.4	20.0	0
7	0	-	-
8	566.0	4.82	0
9	0	-	-
10	0	-	-
11	1022.8	28.40	12.80
12	8319.2	0.54	0
13	3750.5	4.15	0.40
$\bar{X}$	1154.1 n = 12	14.20 n = 6	2.20 n = 6

\* incomplete or non-applicable data

## DISCUSSION

In observing the preceding data, some definite trends can be seen. Tables 15-20 illustrate the relative lack of effectiveness of the epiphyton in removing orthophosphate from the effluent when compared with the phytoplankton in cells A<sub>1</sub> and B<sub>1</sub> in 1977.\* This partially reflects the limited development of the macrophytes in these cells, providing little surface area for attachment. Thus, the suspended algae appear to have been much more active. It is also worth noting that in the A and C cells, there was very little difference in the total amount of phosphate removed, whether vegetated or not. In both A<sub>1</sub> and A<sub>3</sub>, (Tables 15 and 18), an average of approximately 2000 g of orthophosphate was removed per run. This suggests that the macrophytes in the first vegetated cell were not contributing significantly to nutrient removal, since there was roughly the same proportion of orthophosphate removal unaccounted for in both A<sub>1</sub> and A<sub>3</sub>. Using the maximum suspended material removal figure in both, and adding the epiphytic removal value in A<sub>1</sub>, there was a mean percentage removal of approximately 12%, leaving 88% unaccounted for. Since there were no macrophytes in the control series,

\* Total phosphorus removal values would have been more desirable, but the water analysis yielded incomplete total-P results for the first five runs, so orthophosphate removal results were used instead.

this 88% can not be attributed to the effectiveness of the macrophytes in  $A_1$  on the basis of this study.

Possibly, sedimentation was a major factor, operating in both these cells as a nutrient sink. Or, turbulence at the start of a run may have caused an artificially-inflated initial orthophosphate value. 'In' values of orthophosphate were relatively high from midseason on, which may have corresponded with warm weather increases in release-rate of P from sediments (Hutchinson 1957). It is interesting to note that in two of the five runs (6 and 11) in which  $A_1$  appears to have been superior to  $A_3$  in phosphate-removal, ( $A_1$  minus  $A_3$ , Figure 30A), epiphytic orthophosphate was quite high. This would suggest that, without the EM, the vegetated cell would have been decidedly less effective than the unvegetated cell on average.

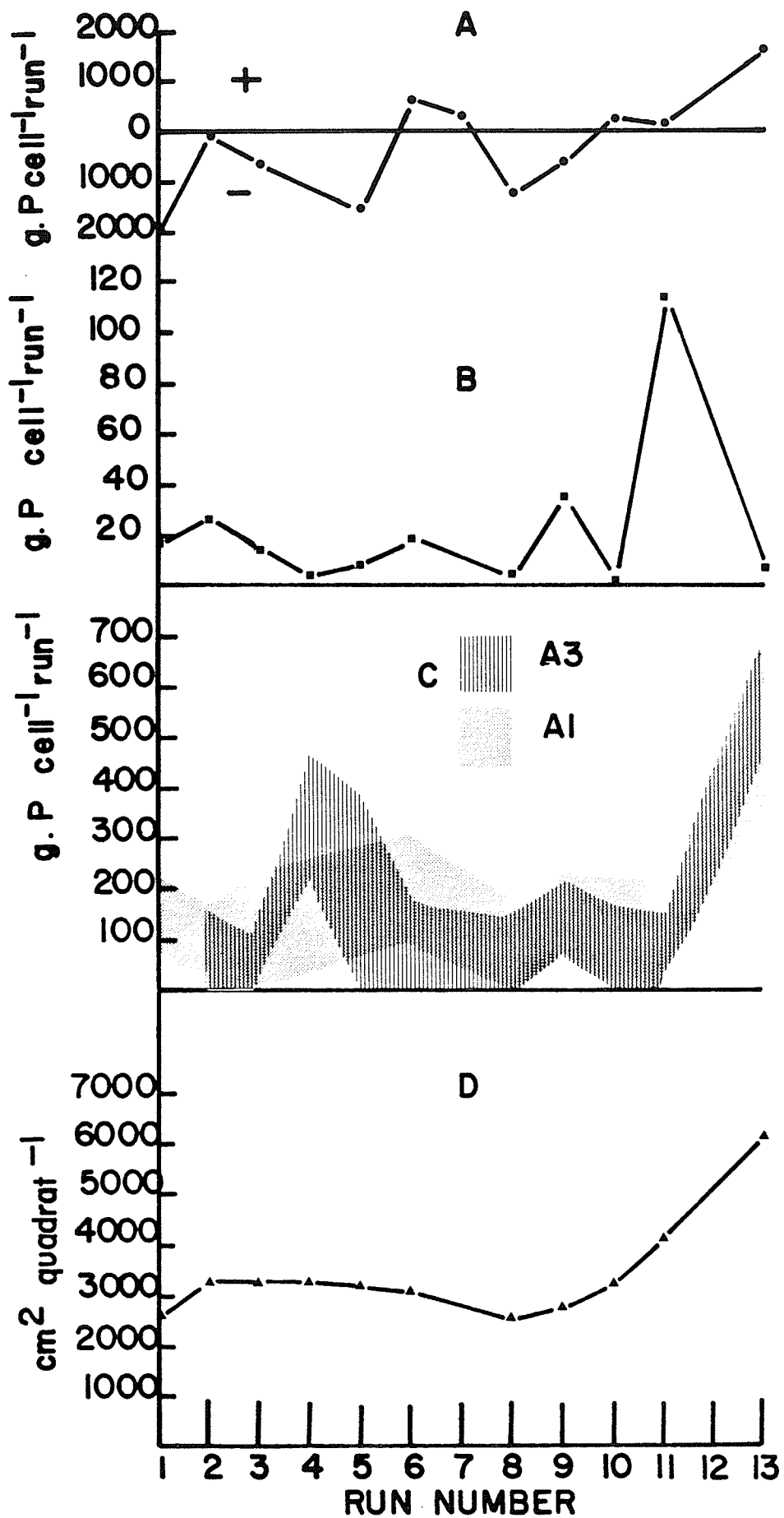
The macrophytes in  $A_1$  may have been planted with abundant soil which could have met their nutrient demands, or which may even have contributed considerable P to the system, which, along with increased release-rate, may have masked the macrophytic contribution to nutrient-stripping. This, of course, is purely speculative, since no sediment-analysis was carried out.

The B cells (Tables 16 and 19) were quite different, with very small amounts removed by the epiphytic algae ( $\bar{X} = 0.81\%$ ) in  $B_1$ , and upper limit mean values of 22.47% and 33.81% by the phytoplanktonic algae of  $B_1$  and  $B_3$

## FIGURE 30

A summary of run to run trends within the A cells during the 1977 season.

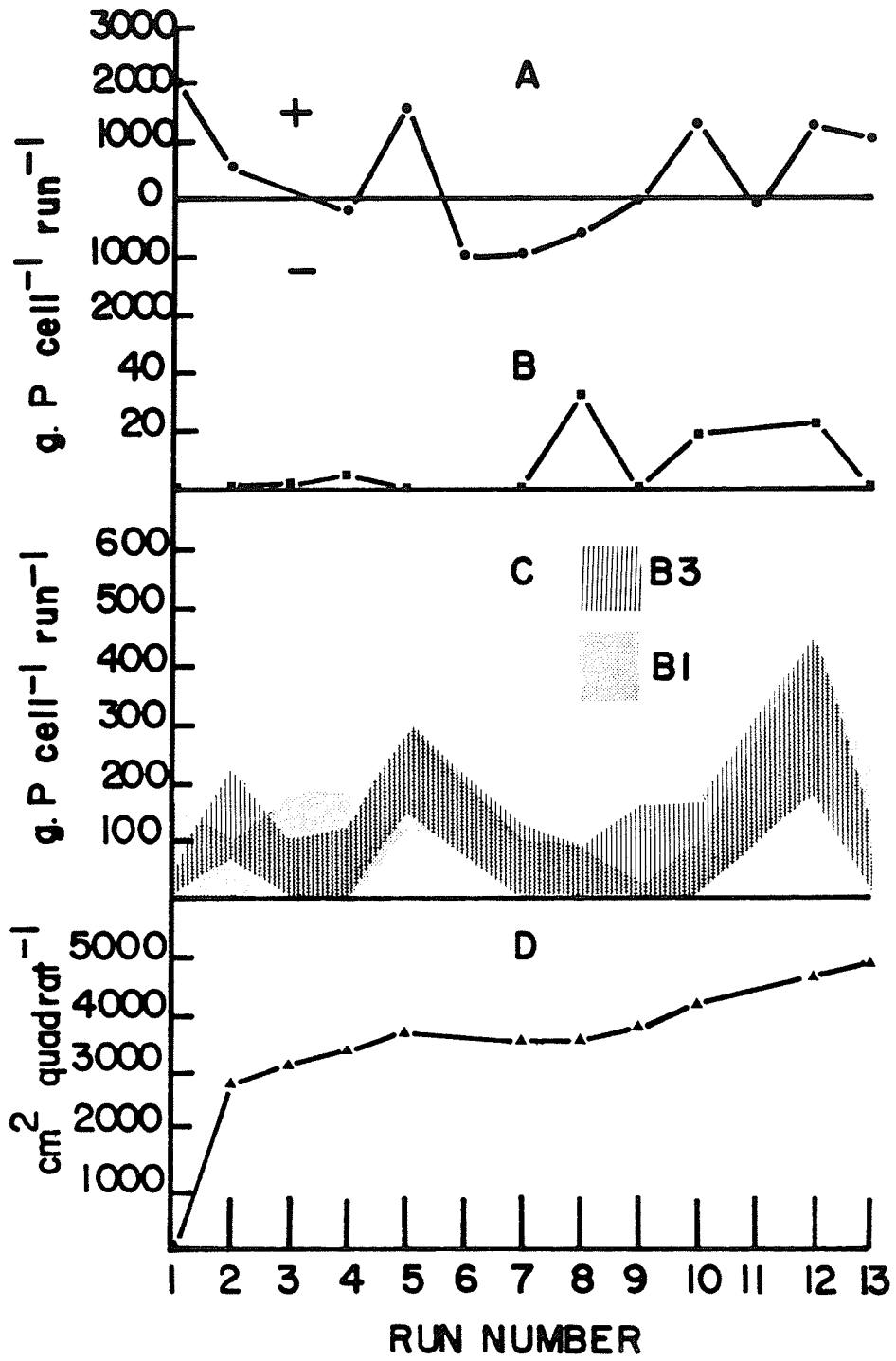
- A. The effectiveness of the planted cell relative to the control. Data are derived by the subtraction of the total orthophosphate removed per run in the control cell ( $A_3$ ) from the equivalent values in the vegetated cell ( $A_1$ ). Points in the positive (+) area indicate greater effectiveness of the  $A_1$  cell, and points in the negative (-) area indicate greater effectiveness of the  $A_3$  cell.
- B. The total per run accumulation of phosphorus in the epiphytic material in the  $A_1$  cell.
- C. The total maximum and minimum amounts of phosphorus accumulated in the suspended particulate material in  $A_1$  and  $A_3$ .
- D. Macrophyte surface area  $0.25 \text{ m}^{-2}$  quadrat in  $A_1$ .



## FIGURE 31

A summary of run to run trends within the B cells during the 1977 season.

- A. The effectiveness of the planted cell relative to the control. Data are derived by the subtraction of the total orthophosphate removed per run in the control cell ( $B_3$ ) from the equivalent values in the vegetated cell ( $B_1$ ). Points in the positive (+) area indicate greater effectiveness of the  $B_1$  cell, and points in the negative (-) area indicate greater effectiveness of the  $B_3$  cell.
- B. The total per run accumulation of phosphorus in the epiphytic material in the  $B_1$  cell.
- C. The total maximum and minimum amounts of phosphorus accumulated in the suspended particulate material in  $B_1$  and  $B_3$ .
- D. Macrophyte surface area  $0.25 \text{ m}^{-2}$  quadrat in  $B_1$ .





respectively. However, the B<sub>1</sub> cell, which was planted largely with Carex sp., was nearly twice as effective as the B<sub>3</sub> cell in the average amount of orthophosphate removed. Possibly, these species contributed more to nutrient removal through greater effectiveness than did the Typha, Scirpus, and Phragmites of the A<sub>1</sub> cell, and may account for some of the difference.

The C cells, (Tables 17 and 20), both of which were unvegetated, were approximately equal in terms of mean amounts of orthophosphate removed per run (about 1200 g), the phytoplankton accounting for a maximum removal of 20.91% in C<sub>1</sub> and 14.2% maximum removal in C<sub>3</sub>.

Figures 30 and 31 illustrate some trends on a per run basis in 1977, in the A and B cells respectively. With respect to Figure 30, in general, the curve of the macrophyte surface area is closely paralleled by that of the epiphytic P, as would be expected, with some exceptions near the end of the season. Comparison may also be made between the macrophyte surface area and the curve which represents the difference between A<sub>1</sub> and A<sub>3</sub>, with major discrepancies occurring near the middle of the summer. The phytoplankton, too, increases dramatically in P-content in both A cells early in the season (run 4) and at the end (run 13). This midseason decrease in activity of macrophytes, epiphyton, and phytoplankton, coinciding with maximum yearly water temperatures, is typical of aquatic

production in stable environments (Penfound 1965, Nicholls 1976, Casterlin and Reynolds 1977), and is interesting in such a continuously disturbed system, appearing to suggest that the important factors operating in a stable system were effective here as well.

In Figure 31, it can be seen that some trends in  $B_1$  and  $B_3$  were more interrupted, although the macrophyte surface area and phytoplanktonic P in both cells followed the same basic pattern of rapid increases in the early part of the season, levelling off or declining midway, and increasing again near the end. The epiphytic P had its highest point in run 8, although this may be a spurious datum point. Consistency is lacking in the P-removal effectiveness ( $B_1$  minus  $B_3$ ) with several peaks and valleys. This may have involved differential sediment disturbances resulting in misleading P-readings. The  $B_1$  cell particularly was seen to contain large numbers of Daphnia during midseason, which may have accounted in part for the lower effectiveness of that cell at that time. Suspended algal numbers (Figure 14), as well as suspended P-values (Table 10), were low in  $B_1$  in runs 6-9. This study did not include quantification of grazers, but it is felt that their effect may have been quite important.

The 1977 POC values in the EM were generally higher than in 1976 (Figures 4-7 and Tables 1 and 2). This may reflect greater biological activity by these algae in 1977, as the ponds were better established. The  $B_1$  cell, which

had been replanted early in the second season, likely provided more plant surface for epiphyton; it is in this cell that the greatest discrepancy exists between the two seasons. Unfortunately, comparisons with amount of epiphytic production or P-uptake found by other authors in different situations are difficult to make, since units are often different, many authors stating values in terms of square metres of water surface area (Hartland-Rowe and Wright 1975, Hooper and Robinson 1976).

The epiphytic phosphorus data in both years show a decline from A to B to C in both vegetated and control series. This suggests that the very high POC values in the B<sub>1</sub> cell in 1977 do not represent just the living algal component but included other organic material adhering to the macrophyte stems (Hooper and Robinson 1976).

One of the inherent problems in the 1977 study existed in the low level of effluent in the B cells on the days that sampling was done. As it happened, the B cells were frequently almost emptied at that time each week, possibly resulting in increased concentration of debris near the sediment, and this is reflected in many of the POC data. The fact that the average amount of PP in the EM was so much lower in 1976 than in 1977 (Tables 6 and 7) does lend support to the suggestion that the cells were functioning more efficiently as time went by. (However, the method used to determine PP during the first season

(Strickland and Parsons 1968), which uses heat-digestion of polyphosphates, may not be as sensitive as that used the following year (Stainton, Capel, and Armstrong 1974), which functions through acid-digestion; so there may have been some underestimation in 1976.)

The continuing incubations of epiphytic POC (Tables 3 and 4) and PP (Tables 8 and 9) illustrated one of the difficulties in attempting to measure components of algal growth which has built up over time. So much of the material that has been allowed to accumulate over weeks or months may be non-living, or might slough off periodically, that a smooth curve showing continuous growth is not likely to be obtained (Wetzel and Westlake 1969). This was the case in the ongoing POC of 1976 and 1977 (Table 3 and 4 respectively) and in the ongoing PP of 1977 (Table 9), where periodic large decreases occurred. The ongoing epiphytic P of 1976 (Table 8) was very low and generally followed a fairly smooth upward curve.

The suspended material showed a great deal of flux on a weekly basis, in terms of POC and PP (Tables 5 and 10). This may reflect the unstable nature of the suspended algal component, specifically its rapid turnover time, but it may also involve disturbances of sediment, dilution from rainfall, the influence of grazers, changes in light intensity, and numerous other factors. Only the means show consistency, the trend to less POC and PP with passage

through the system, being very evident.

The generally low values of POC (Table 5) and PP (Table 10) in the two C cells in midseason (runs 5-9) may have occurred because of the presence of luxurious mats of a green filamentous alga, Stigeoclonium sp. Kuetzing, the effluent itself appearing very clear for the latter part of the season. This alga may have been especially effective at using the nutrients entering the cell, thus limiting the growth of phytoplankton. Unfortunately, this component of the system was not analyzed, procedures for quantifying mats being difficult, but it is strongly felt that it was the major contributor to the very large quantities of orthophosphate removed from the C cells during the last few runs.

Normally, one would expect vertical stratification to occur in epiphyton, with more development nearer the surface (upper stratum) in response to greater light intensity. Sixty percent of light may be lost in the first 2-5 metres (Prescott 1968), but this of course depends on turbidity and colour of the water. In the C cells, which were deeper than the A and B cells, vertical stratification seems to be in evidence quite consistently. Considering the amount of suspended material in the A and B cells, light extinction most likely occurred within centimetres rather than metres, and probably accounted for the considerable vertical stratification that occurred in them most of the time. The unexpected larger mean amounts of POC and PP

which occurred in the lower stratum of the B<sub>1</sub> cell in 1977 (Tables 2 and 7), are again attributed to the greater concentration of debris that accompanied the extreme shallowness in that cell on sampling days. However, Hooper-Reid and Robinson (1978) found no obvious differences in cell volumes and compositions between upper and lower depths of epiphytes colonizing cellulose acetate substrates. The maximum depth range in their study was 0-70 cm.

The reliability of the artificial substrates in terms of accurate representation of naturally-occurring epiphyton communities was an essential part of this research. Allen (1971) and Hooper and Robinson (1976) have shown that epiphytic production on artificial substrates is similar to that on natural hosts, although algal species may differ (Tippett 1970). Hence, the epiphytic POC and PP values obtained in this study are considered to be accurate, but there may be some doubt about the algal community composition.

It is unlikely that there was ever any significant nutrient limitation in any of the cells (with the possible exception of the previously-mentioned inhibition of phytoplankton in the C cells once the extensive chlorophyte mats had developed), since levels of ingoing phosphorus were quite high in all cells virtually all the time. Yet, mean amounts of algal POC and PP decreased with each sequential residence period (i.e., from A to B to C).

This probably reflects the rapid senescence and sedimentation of algae within a one-week incubation period, so that fewer cells were transported from one cell to the next with each transfer. This may also explain the problematic 'upper' and 'lower' limits determined for the suspended material (Figures 20-29 and Tables 14-20). Where the lower limit (representing net production) was zero or negative, the major factor producing this result was, of course, subtraction of the previous cell's value (the 'in' value), which no doubt overestimated the proportion of that component which would survive the second incubation period. On the other hand, taking no account of incoming material would be misleading. It is for this reason that actual 'net' production is assumed to be somewhere between the upper and lower limit values.

With respect to the algal species, the relative lack of diversity, and particularly the preponderance of cyanophytes, indicates that these cells are in extreme stages of eutrophy (Prescott 1968, Traaen 1975). There was somewhat higher diversity among the epiphyton in the first few runs (Figures 12 and 13); chrysophytes, which tend to peak in spring, were most in evidence at that time, and chlorophytes, being typical summer species (Golterman 1975), occurred in midsummer in the vegetated cells (Figure 12). These occurrences appear to reflect the interaction of seasonal events with inter-run events which were independent of seasonal parameters. The only

euglenids were found in runs 10 and 12 in A<sub>1</sub>, and in runs 8 and 10 in A<sub>3</sub>. Euglenids such as Euglena and Phacus are typical of waters with high organic content (Round 1957). The phytoplankton, too (Figures 14 and 15), was somewhat more diverse and abundant early in the season, with very low numbers from the fifth run on. A problem with preserved samples is that many of the more delicate organisms, such as the cryptomonads with their very fine cell coverings may not have survived the period of time between collection and counting; so some species may have been underestimated. This could have been avoided only by determining algal species and numbers in fresh material, which was not practically possible.

One of the essential requirements of such a system would include harvesting of all biological material at the end of the growing season, and possibly at certain points throughout the season. Otherwise, senescence of algal and other plant material would return large quantities of nutrients to the system (Otsuki and Wetzel 1974). This could be done quite readily in the case of large aquatic plants and filamentous mats such as those which occurred in the C cells. These could then be used for animal feed or agricultural fertilizer, thus constituting a recycling of nutrients and energy, rather than a waste, as is our present custom. The single-celled algae, which constituted the majority in both A and B cells, are potentially difficult



to remove. However, if this system is representative of vegetated retention ponds, nutrient status may typically be at such a stage by about the third week of residence, that harvestable floating mats of algae, such as the Stigeoclonium in this study site, may be expected to occur.

A possible method of dealing with single-celled microalgae would be to introduce into the system a higher trophic level, such as filter-feeders. Odum (1971) suggests that the role of invertebrates, although not fully studied, may be quite important in sewage systems. In the Arborg system, perhaps, this group of organisms could be used to eliminate many of the microalgae in the first and second stages, and contained in the A and B cells through some sort of filtering system, while the easily harvestable material is removed from the final cell. Final effluent could be used to irrigate what should ideally be adjacent agricultural land.

Rohlich and Uttormark (1972) have pointed to the major disadvantage of nutrient-stripping by algae in shallow ponds, as the requirement for substantial land areas, but in small or relatively-isolated communities, this need not be impractical. It is quite effective, particularly when combined with aquatic macrophytes (Hartland-Rowe and Wright 1974); it is cheaper than chemical precipitation (Rohlich and Uttormark 1972); and it results in an end product which is a valuable resource.

At the same time, it provides a nesting area for many birds and leaves intact the integrity of lakes and rivers which might otherwise be spoiled.

## SUMMARY AND CONCLUSIONS

The epiphytic community was less effective in the removal of phosphorus from the effluent in this system than was the suspended community; however, there was not a well-developed epiphytic community owing to the limited planted vegetation in the cells chosen for this study. The planktonic material appeared to be somewhat more effective overall, but because of rapid turnover rates, may have returned nutrients to the system upon degradation almost as quickly as they were removed. For this reason, any real nutrient-removal by algae from such a system would entail harvesting of the algae at regular intervals. This applies to any plant material which may have been contributing to nutrient-removal from the cells.

Many variables which may have been important were not measured, among them, sedimentation, which may have accounted for the major nutrient loss in the A and B series.

According to the mean data, in the first series of cells ( $A_1$  vs  $A_3$ ), there was no difference in nutrient-removal accounted for by the presence of macrophytes. However, in the second series ( $B_1$  vs  $B_3$ ), the vegetated cell was more effective. A strictly controlled study of nutrient-uptake by different aquatic macrophytes would therefore seem warranted.

It is intuitively felt that extensive green algal mats in the third series of cells ( $C_1$  and  $C_3$ ) were

responsible for the large quantities of phosphorus-removal occurring in those cells, and a useful study would be to examine what conditions may promote the development of such inoffensive, harvestable algae such as these.

The artificial substrates used for the colonization of epiphyton are assumed to have provided accurate representations of epiphytic organic carbon and phosphorus, although they may not have accurately represented the relative algal species attached to submerged macrophyte surfaces.

There was an interaction of inter-run events with intra-run events. That is, seasonal effects were apparent in many of the results, and predictable sequential changes during a three-week residence period occurred within each characteristic seasonal situation.

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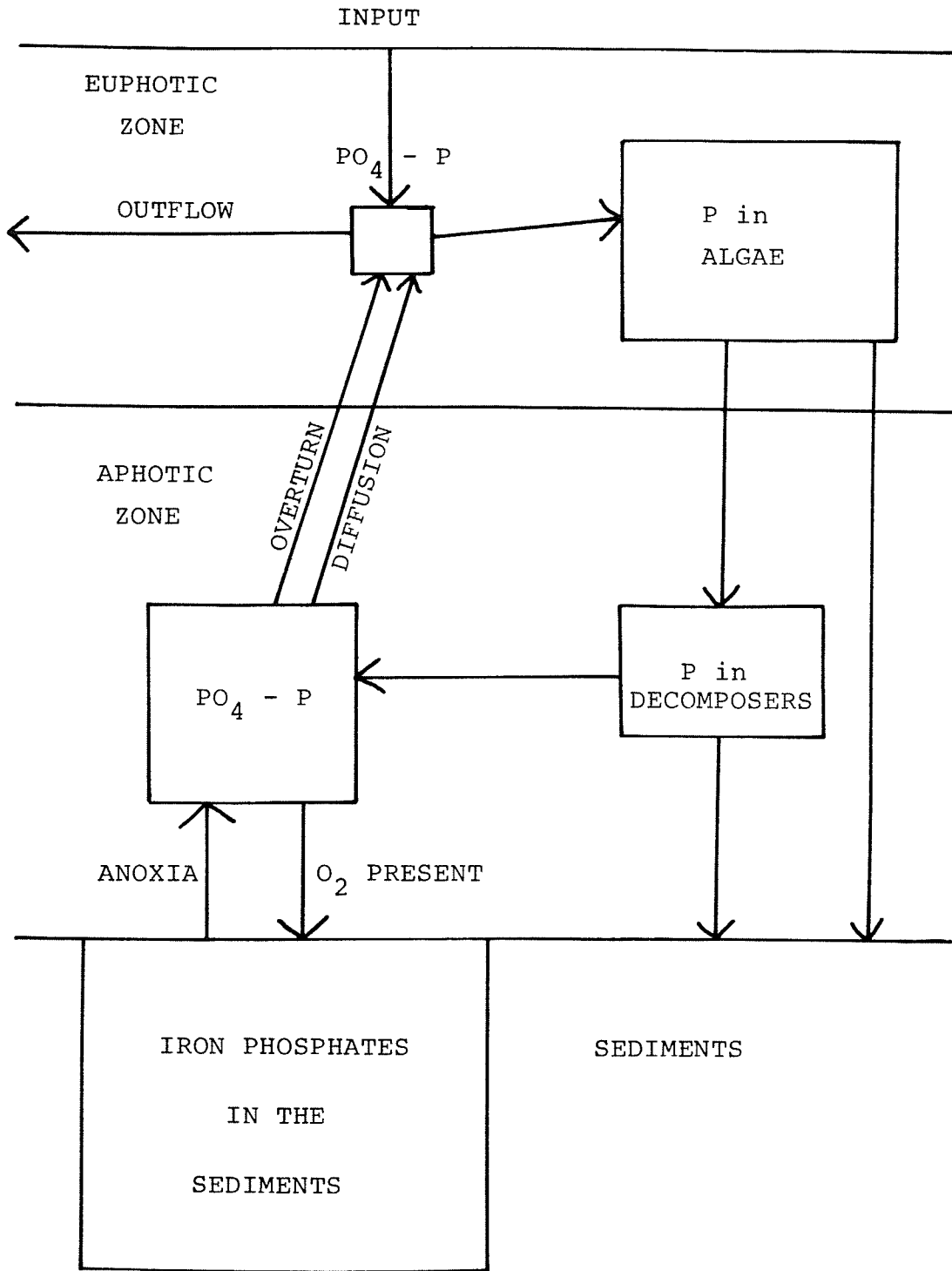
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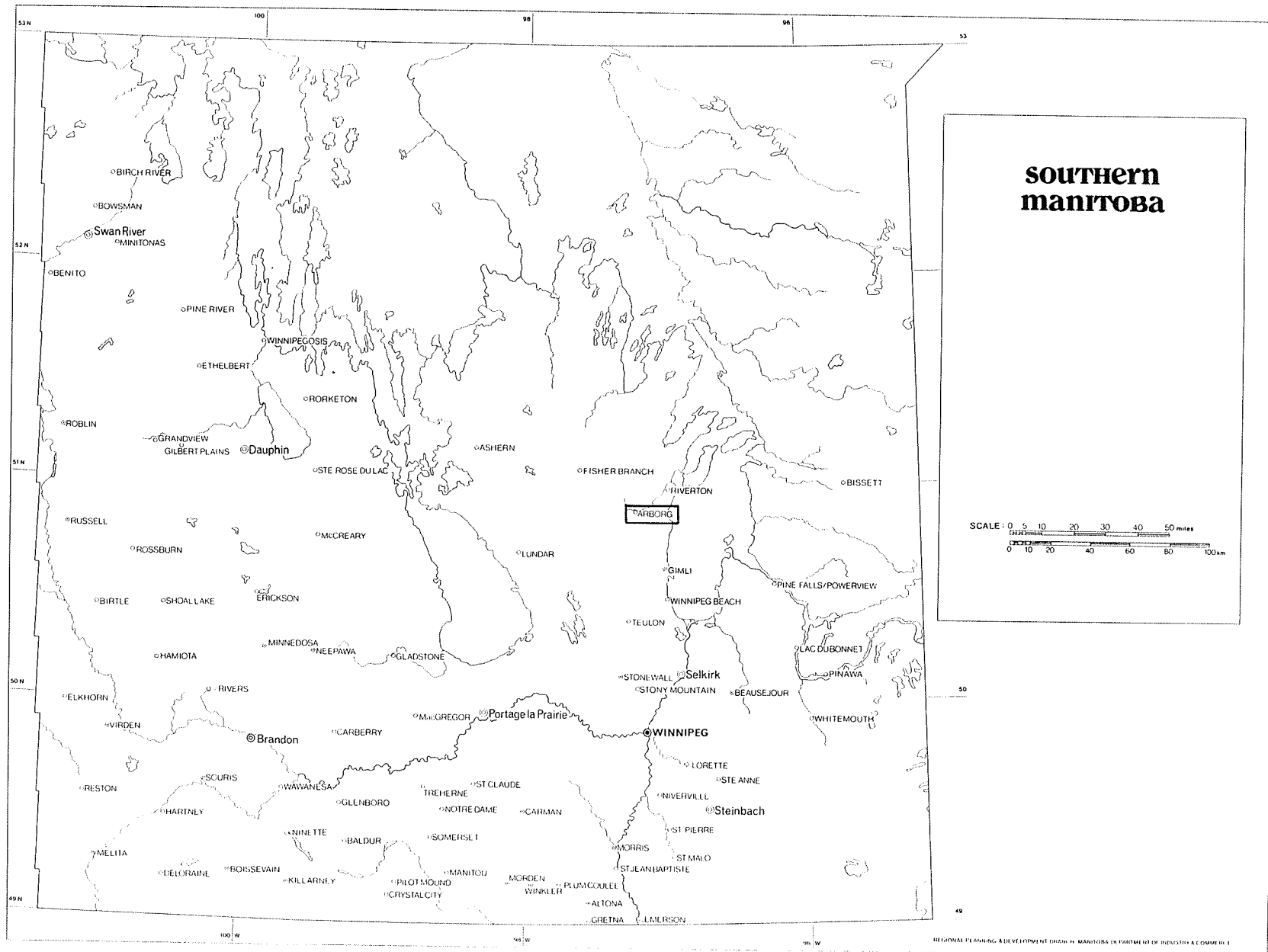
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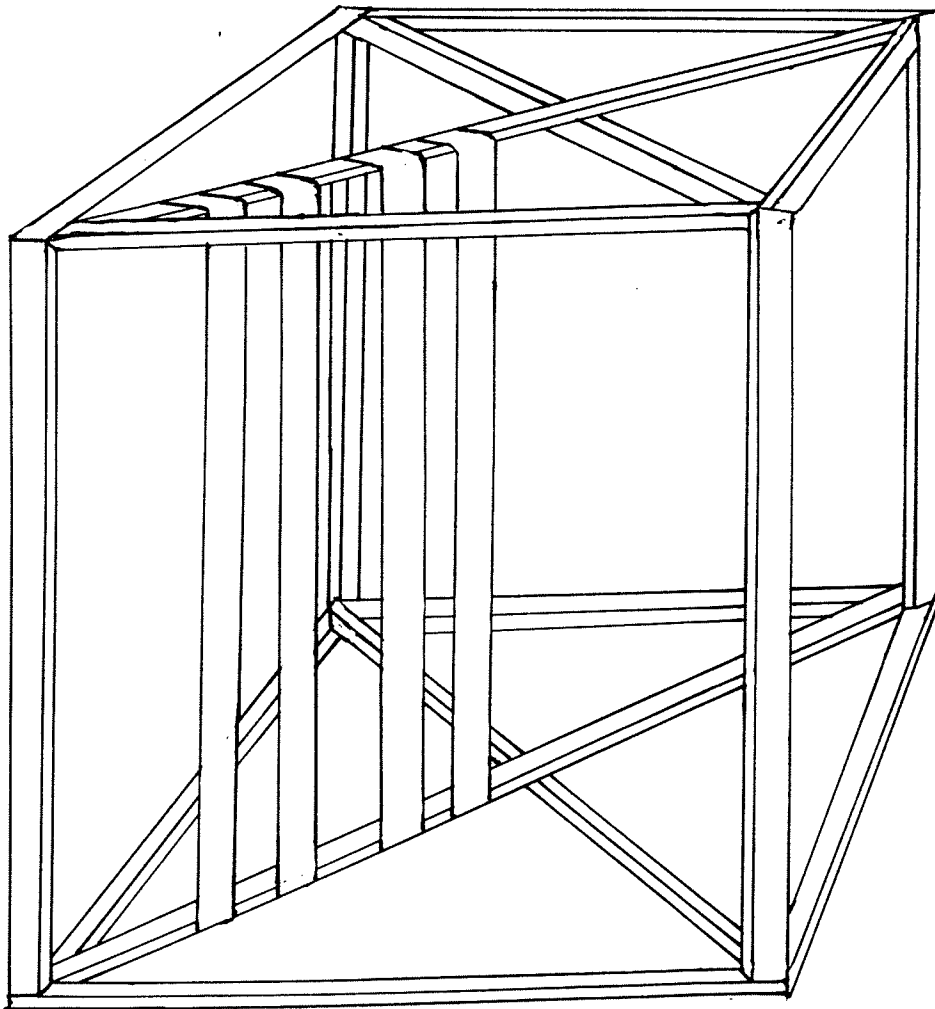
## Appendix 1. The Phosphorus Cycle in Water



Drawn from Levine (1975)



Appendix 3. Schematic diagram of the support structures used for the attachment of cellulose acetate strips. The strips were attached vertically to wooden cross bars.



## Appendix 4

Rate of Dry Weight Accumulation in the Epiphytic Material  
in the Vegetated Series in 1976.

Run	Stratum	$\mu\text{g cm}^{-2} \text{ day}^{-1}$		
		A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>
1	Upper	21.5	107.7	10.1
	Lower	20.1	169.3	11.3
2	Upper	435.5	4.2	51.6
	Lower	594.4	2.8	6.5
3	Upper	32.1	2.0	8.0
	Lower	25.1	5.4	12.2
4	Upper	229.4	11.7	12.9
	Lower	203.8	5.8	7.7
5	Upper	296.8	8.6	9.9
	Lower	406.7	14.9	14.1
6	Upper	83.3	14.7	49.9
	Lower	145.7	12.7	5.8
7	Upper	30.0	3.6	27.1
	Lower	44.9	30.2	6.6
8	Upper	77.9	20.7	147.1
	Lower	11.5	- *	115.9
9	Upper	14.3	19.6	39.9
	Lower	12.1	3.9	23.3
10	Upper	11.3	7.6	97.4
	Lower	12.5	14.3	13.9
11	Upper	13.3	18.0	95.3
	Lower	19.7	17.9	6.6
12	Upper	76.0	22.2	29.7
	Lower	23.6	13.6	4.7
13	Upper	16.7	20.2	6.9
	Lower	38.3	49.7	1.9

\* incomplete data

cont.

Rate of Dry Weight Accumulation in the Epiphytic Material in the Control Series in 1976.

Run	Stratum	$\mu\text{g cm}^{-2} \text{ day}^{-1}$		
		A <sub>3</sub>	B <sub>3</sub>	C <sub>3</sub>
1	Upper	11.7	39.7	-*
	Lower	6.5	21.3	-
2	Upper	48.4	9.0	-
	Lower	108.2	43.5	-
3	Upper	16.4	158.8	-
	Lower	23.1	167.5	-
4	Upper	28.1	117.7	-
	Lower	41.4	-	-
5	Upper	18.2	10.9	-
	Lower	43.2	12.9	-
6	Upper	15.4	14.0	18.1
	Lower	20.6	26.0	4.1
7	Upper	20.4	39.3	-
	Lower	207.1	7.4	-
8	Upper	3.2	-	20.7
	Lower	7.0	-	15.0
9	Upper	3.7	9.8	20.3
	Lower	0.9	21.0	66.1
10	Upper	32.1	56.9	66.4
	Lower	9.7	16.1	20.6
11	Upper	21.0	123.9	98.3
	Lower	32.4	17.8	29.0
12	Upper	35.4	22.2	22.7
	Lower	30.6	24.8	7.5
13	Upper	32.0	11.3	6.1
	Lower	76.2	10.3	61.0

\* incomplete data

## Appendix 5

Total Particulate Organic Carbon in Epiphyton in cells  
A<sub>1</sub> and B<sub>1</sub> by run in 1977.

Run	Total POC mg run <sup>-1</sup>	
	mg C in A <sub>1</sub>	mg C in B <sub>1</sub>
1	299,328.2	- *
2	620,003.0	62,384.2
3	170.2	110,976.5
4	495,854.5	16,111.2
5	0	336,560.1
6	162,863.2	-
7	-	0
8	0	411,203.0
9	60,698.3	12,548.4
10	48,146.8	0
11	138,302.7	-
12	-	286,123.3
13	184,670.6	13,948.7
$\bar{X}$	182,730.7 (n = 11)	124,985.5 (n = 10)

\* incomplete data



## Appendix 6

Total Particulate Phosphorus in Epiphyton in cells  
A<sub>1</sub> and B<sub>1</sub> by run in 1977.

Run	Total PP mg run <sup>-1</sup>	
	mg P in A <sub>1</sub>	mg run <sup>-1</sup>
1	18,984.4	- *
2	27,412.8	1,946.2
3	17,210.2	2,170.0
4	4,363.0	6,151.8
5	9,339.1	141.3
6	19,687.0	-
7	-	1,275.1
8	5,093.1	34,100.7
9	36,587.8	122.6
10	453.1	19,979.8
11	115,588.0	-
12	-	23,488.3
13	8,177.2	148.5

\* incomplete data