HEAVY METAL CONCENTRATIONS, PRIMARY PRODUCTIVITY, CHLOROPHYLL-A LEVELS, AND THE AUTOTROPHIC INDICES OF STREAM PERIPHYTON SUBJECTED TO WOOD PRESERVATION WASTE

by

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I. INTRODUCTION

Culpeper Wood Preservers' Toxic Spill

On January 31, 1981, 200,000 gallons of a dilute chromium, copper, and arsenic solution spilled from a waste lagoon at Culpeper Wood Preservers (CWP) in Culpeper, Virginia. The spill occurred at the plant, located just northwest of US 15 and the US 29 Bypass, about 35 miles from Fredericksburg, Virginia. The contaminated waste flowed into an unnamed tributary of Jonas Run, which runs into Mountain Run and eventually empties into the Rappahannock River, the drinking water source for the City of Fredericksburg (1).

CWP was allegedly expanding an existing waste lagoon when the accident occurred. The Virginia State Water Control Board (SWCB) and other authorities were not notified about the spill until three days after it had occurred. According to the SWCB, CWP had no permit to dump the hazardous chemicals. Also, the Culpeper Planning Commission announced that CWP was in violation of a county zoning ordinance for having such a holding pond. The zoning permit which CWP held did not allow for storage of chemical wastes.

Tests performed by the SWCB shortly after the spill showed 34,000 parts per billion (ppb) chromium (Cr), 5,400 ppb arsenic (As), and 240 ppb copper (Cu) 100 feet downstream from the plant (2). Five to six miles downstream, the metal concentrations were 460 ppb Cr, 3 ppb As, and 10 ppb Cu (2). As time passed, the metal concentrations in the creek's water decreased.

Three days after officials learned of the toxic spill, a ban was

placed on milk from cattle grazing near or downstream from CWP. This was prompted by the discovery of an unusually high, but not harmful, chromium concentration in the milk (3). The ban was lifted on February 14th but imposed again on the 17th for a short period (4). When the milk ban was lifted the first time, a quarantine was placed on all cattle, swine, and poultry in the affected area. This remained in effect for approximately two weeks (5).

Farmers below the spill site reported an unusually high mortality rate for their cattle and calves following the accident. Some cattle were reported to be losing much of their hair, and poultry and fish kills were noted. However, it has not been proven that the CWP spill was the cause for these problems.

Design and Objectives of This Study

Benthic algae, also known as periphyton, are the primary producers in lotic systems (6,7,8). They are also a major source of food for higher organisms, such as invertebrates and fish (8). Periphyton communities are subject to many adverse conditions but are characterized by a quick recovery rate (6). Grzenda and Brehmer (6) have reported that the production of the periphyton community is very closely related to the quality of the water passing over them. Thus, it is very useful to consider periphyton as a measure of stream biodynamics. It is for these reasons that periphyton were chosen as the subject of this syudy of the streams affected by the CWP spill.

The objectives were:

1. to determine the total arsenic, chromium, and copper concentrations

in the periphyton and water column, both upstream and downstream of the CWP spill site;

2. to compute the autotrophic index of the periphyton at each station above and below the spill site;

3. to measure the primary productivity of the periphyton at all the sampling stations above and below the CWP spill; and

4. to determine from the data the extent of any ecological damage that might have occurred as a result of the spill.

II. LITERATURE REVIEW

Environmental Sources and Natural Concentrations of As, Cr, and Cu

Heavy metal have been generally defined as those elements which have a specific gravity greater than five units or an atomic number larger than 20 (9,10). Some of these metals are necessary trace nutrients; however, most exert toxic actions when present in high concentrations (9). The remainder of this discussion will focus on As, Cr, and Cu, the three heavy metals contained in the preservation waste spilled from the CWP lagoon.

Arsenic. Arsenic, the 20th most aboundant element in nature, has been used by mankind for many centuries (11). In the Orient, arsenical compounds were used in medicine 2000 to 3000 years ago (12). Today, As is used in many industries. Ninty-seven percent of present-day As is refined into white As and used in such products as herbicides, pesticides, desiccants, and preservatives. The remaining three percent is used as a metallurgical additive in special lead and copper alloys (11).

Volcanic activity was the orginal source of As in sedimentary rocks. In the earth's crust, As is usually found associated with sulfide as metal arsenides (11). Up until recent years, the weathering of As remained in balance with its deposition to the sediments. Human activities, such as the burning of fossil fuels, erosion, mining, and the smelting of nonferrous metal ores have upset this natural banance (11,12). Ferguson and Gavis (12) have shown that man's industrialization has increased the amount of As entering the oceans by threefold.

Today, besides the natural weathering of sedimentary rocks, As is entering the environment through fallout from the atmosphere, the leeching of agricultural pesticides and fertilizers, and from industrial releases (13).

Arsenic is naturally present in small qualtities in most soils, waters, plants, and animals (11). The amount present in a system or an organism depends upon its proxcimity to an As soucre. If the environment contains only natural amounts of As, then the plants and animals of that system are likely to contain low As concentrations. However, if that system is subject to high As concentrations, then the organisms are likely to contain higher than normal As levels.

Several studies have calculated the normal As levels in the inert and organic segments of systems relatively untouched by major As pollution sources. Seydel (13) studied the As distribution and circulation in Lakes Michigan and Superior and found that Lake Superior was relatively free of As contamination. His findings showed that the natural levels of As in sediment, plankton, and water were 4.10 mg/g, 3.34 mg/g, and 0.53 ug/l, respectively. In 1977, the Committee on Medical and Biologic Effects of Environmental Pollutants (11) published a report containing their recent findings on As concnetrations in the environment. Their findings showed that thermal waters contained the greatest levels of As (0.7 ppm), while most other waters contained trace amounts (0.25 - 180 ppb). Arsenic was present in all animals, with the greatest levels (0.03 - 10 ppm) found in marine organisms. As was true in the animal kingdom, As was present in nearly all specimens of the plant kingdom. Aquatic algae contained between 0.05 -

5 ppm As, dry weight.

Chromium. Chromium is an abundant element. Of the 29 elements of biological importance, Cr ranks fourth (14). It is also 17th in aboundance amoung all nongaseous elements. The metal Cr was not discovered until 1797, a "late comer" in relation to arsenic (14). Chromium can be very toxic when it is encountered in large concentrations, but it is also an essential trace element, necessary for the survival of most living organisms.

The greatest amounts of Cr are used in the metallurgical industry. There it is transformed into stainless and alloy steels. The refractory and chemical industries are also large users of chromium.

Some of the Cr found in the environment is from natural sources, such as the weathering of chromate containing rocks (14). However, much of that chromium found today did not originate from natural occurences but rather from man-made sources. Industrial discharges into the atmosphere, rivers, lakes, and oceans have increased the levels of Cr found in the world today. Galloway and Likens (15) have shown that atmospheric enrichment of Cr and other metals is not just a local phenomenon around industrialized areas. They studied lake sediments in the Adirondack State Park, N.Y. and concluded that chromium, along with silver, gold, cadmium, copper, lead, tin, vanadium, and zinc, were being atmospherically enriched in regions far from their source.

Chromium occurs naturally in rocks and soils, in small and varying amounts, usually as chromic acid. Normal concentrations range from traces to five percent of the total sample weight (14). A survey of American rivers in 1974 showed Cr levels ranging from 0.7 to 84 ppb,

with most between one and ten ppb (14). The same report surveyed Cr concentrations in the plant kingkom. All plants contained varying amounts of Cr, with plankton having the highest levels of 3.5 ppm (dry weight).

Copper. The use of copper dates back to 3000 B.C., where it was formed into vessels to hold water and food stuffs (16). Copper's trait of being both malleable and ductile probably accounted for its early use. Copper has remained a useful element and today is used in the metallurgical industry, in pigments,insecticides, and in herbicides.

Copper is a normal constituent of all living things, but, as with other heavy metals, it can be toxic when encountered at high concentrations (16). Average concentrations of 50 ppm Cu are found in the earth's crust (17). The metal is usually found in deposits associated with sulfur, such as chalcocite (Cu_2S), chalcopyrite ($CuFeS_2$), and covallite (CuS) (16).

In the aquatic environment, Cu is found in low levels, except where contamination is present. Trollope and Evans (18) tested the waters of the Lower Swansea Valley, England, for several metal ion concentrations. Their results showed a mean concentration of 0.02 ug/l Cu in the water column of this relatively unpolluted region. In another study, the amounts of Cu in uncontaminated aquatic plant and fish samples were determined to be one to 50 ug/g (dry weight) and 0.01 - 0.02 ug/g (dry weight), respectively (17).

Aqueous Chemistry of Arsenic, Chromium, and Copper

The chemistry of heavy metal in the aquatic environment is complex.

There are many interactions formed between metals and the other components of the system. The distribution and availability of most metals in aqueous solution depends upon the redox potential, pH, oxygen supply, and available particulates such as clay and hydrous oxides (19,20,21, 22,23,24,25). Whether a metal is found in solution or in the sediments depends on its solubility and adsorption properties. The following section will deal with the aquatic charateristics of As, Cr, and Cu.

Arsenic. Arsenic has four stable oxidation states; +5, +3, 0, and -3 and its compounds may either be organic or inorganic. According to Klumpp and Peterson (26), the predominant As species in marine waters is arsenate (AsO_4), while in fresh river waters is appears as arsenite (AsO_3^{-3}). Arsenic metal occurs only rarely, and As^{-3} is present only when the redox potential is extremely low (12). In systems with a high Eh (volt) value and well oxygenated waters, the arsenic acid species (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{-2}$, and AsO_4^{-3}) are stable. Under mildly reducing conditions, the arsenious acid species (H_3AsO_3, $H_2AsO_3^-$, and $HAsO_3^{-2}$) become stable (26). Under conditions were sulfide is stable, realgar, AsS, and orpiment, As_2S_3, have low solubilities and occur as stable solids at pH values below 5.5 and Eh values about 0 volts.

The aquatic chemistry of As is more complex than for most heavy metals, with oxidation-reduction, ligand exchange, precipitation, and adsorption reactions all occurring (12). Precipitation of the metal ion and adsorption onto clays are the two major mechanisms affecting As concentrations in the water column. Ferguson and Gavis (12) have demonstrated that ferrous iron oxides, aluminum hydroxide, and clays co-precipitate with, or adsorb to, As particles and remove them from

solution.

The distribution and circulation of As through Lakes Michigan and Superior was investigated by Seydel (13). Overall, it was found that Lake Michigan had become enriched in As from the pollution it had received. Patterns of As stratification were prevalent in both lakes. Concentrations were greater in the anaerobic bottom waters where high amounts of ferrous iron were found. Sediment concentrations of 26.8 - 28.8 ppm As were due to the precipitation and adsorption of As compounds. Seydel concluded that the co-precipitation of arsenite with hydrated heavy metal oxides played a significant role in the metal stratification within both lakes (13,27).

Clement and Faust (28) studied the release of As from contaminated sediments and muds. Anaerobic waters led to levels of arsenite approximately ten times greater than aerobic regions. The release of As from the sediments was highly dependent on the reduction of ferric to ferrous iron, but the phosphorus level was also found to play a significant role. pH was determined to most affect the release of As. Below pH 4 and above pH 9, extensive release of As occurred. At low pH values, the iron species were solubilized from the muds with a concurrent release of As. Displacement of As from the binding sites of the increasing hydroxide concentrations occurred at high pH values. Clement and Faust also examined tha relationship between arsenic and phosphorus (28). These two elements competed for the same binding sites in muds and the levels of either existing in aqueous solution depended upon the relative amounts of the other.

Chromium. Chromium has many valence states, the most common being

0, +2, +3, and +6 (14). Trivalent Cr is the most stable form. Besides Cr^{+3}, the only other oxidation state found naturally is hexavalent (+6) chromium.

In aquatic systems, the chromous ion Cr^{+2} oxidizes to Cr^{+3}. The oxidation potential of Cr^{+6} to Cr^{+3} is strong, and it is highly unlikely that the oxidation of the trivalent form could occur in vitro. The hexavalent states of chromate (CrO_4^{-2}) and dichromate ($Cr_2O_7^{-2}$) may be reduced to CrO_3^{-2} by heating or the addition of a reducing agent (29). The hexavalent form of chromium, almost always linked to oxygen, is a strong oxidizing agent (14). Under aerobic conditions, hexavalent Cr in natural waters is stable and will persist for long periods. However, under anaerobic conditions, Cr^{+6} will be reduced to the trivalent form, whose salts hydrolze in neutral or weakly alkaline solutions. The chromic oxide formed is insoluble and deposits in the sediments (29). Cr^{+3} is the most stable and important oxidation state of chromium. In this state, it has a strong tendency to form complexes with ligands whose rates of exchange are low (14).

A study performed by the Committee on Biologic Effects of Atmospheric Pollutants (14) found that the hexavalent form of Cr appeared to be relatively stable in water, probably because of the low concentrations of reducing materials. The trivalent form was associated mainly with particulate matter, which suggested that organic particles may reduce and bind the element, leaving the hexavalent form in solution. Other data suggested that Cr^{+3} may be absorbed on clay particles.

Shuman et al. (30) investigated the modes of chromium metal transport above and below a waste didcharge on the Haw River, N.C. At the

control stations above the discharge, the majority of Cr was transported as crystalline minerals. Also important was transport with oxide coatings and adsorbed particles. However, transport as soluble or organically bound species was not important. At the polluted stations, chromium was associated primarily with the oxides and organically bound fraction. The percent adsorbed or in solution was very small.

Popp and Laquer (24) conducted a similar study on the Rio Grande and surrounding tributaries in Central New Mexico. They found that the majority of Cr and Cu transported in those rivers was in crystalline particles. The remainder was found in metallic coatings. The high percent of crystalline-associated metals was due to the highly mineralized nature of the drainage basins.

Copper. The only stable form of copper in aqueous solution is Cu^{+2} (16). Cu^{+1} reverts to metallic copper and Cu^{+2}, while Cu^{+3} is a strong oxidant. Only at pH values below 6.5 is the free copper ion found in waters (52). Copper carbonate ($CuCO_3$) and copper hydroxides ($Cu(OH)_3$ and $Cu(OH)_4^{-2}$) are the major copper-containing species at higher pH values (31). The common copper compounds which are soluble in water are those formed with chloride, nitrate, and sulfate. Copper compounds which form with carbonate, hydroxide, oxide, and sulfide are generally insoluble in solution (16).

Pagenkopf and Cameron (21) analyzed the deposition of copper and other trace metals downstream from a lead smelter. The Cu concentration decreased with increased distance from the smelter. The level of Cu immediately downstream from the discharge was a factor of ten above the level at the control stations. The major species found were $CuCO_3$,

$Cu(OH)^{+}$, and $Cu(OH)_2$. They concluded that the two most important processes for the transfer of Cu from solution to the sediments were precipitation and adsorption to suspended particulates.

The study of the environmental chemistry of Torch Lake, Michigan, was undertaken by Lopez and Lee (22) to determine the affect of copper tailings dumped into its waters. Between 1946 and 1970, twenty percent of the lake's volume was filled with Cu mine tailings. Higher Cu concentrations were found in sediments of waters affected by the tailings than in waters not affected. In the water column, Cu was found to be stratified, with more Cu present in the deeper waters. Copper was controlled primarily by the hydrous oxides of iron and manganese and not by the solubility relationships of copper compounds.

Sanchez and Lee (23) used Lake Monana, Wisconsin, to study the mechanisms of copper removal. Lake Monana received over 1.5×10^6 pounds of copper sulfate ($CuSO_4$) during the past 50 years to control excessive algal growth. Complexation and precipitation of Cu were the two dominant removal mechanisms. The dissolved Cu generally showed an inverse relationship to the sulfide levels, with the highest concentrations found in sulfide-free waters. The controlling factor for Cu under oxic conditions was the precipitation of Cu as a basic carbonate. Under anoxic conditions, soluble Cu was limited by sulfide. The authors reported that the sediments were acting as a copper sink and that the $CuSO_4$ additions had not resulted in any Cu build-up in the water column.

Ecological Significance of Heavy Metals

In aquatic systems, heavy metal are a serious pollution problem. In large quantities, they are toxic to all living organisms (32). Heavy metals may be transported and concentrated in the food chain and, when present at elevated levels in water supplies, can be hazardous to public health.

All heavy metals are capable of combining with a wide variety of organics, although there is a large degree of individial specificity. Because heavy metals interact with ligands that are present in all proteins, they are potent enzyme inhibitors (9). Heavy-metal binding occurs at sensitive and unsensitive cellular sites, although the toxic action is only produced by a small proportion of the total metal fixed (9). The cellular structure governs the assessability of sensitive ligands and influences the time course of heavy-metal action. The cell membrane, as a diffusion barrier, protects the cell interior, but functions associated with this membrane are particularly susceptible to a metal's toxicity (32).

According to Furmanska (27), the salts of heavy metals are one of the most dangerous impurities of industrial wastewater. Biological decomposition of organics can be rendered difficult or even inhibited by the presence of toxic compounds in wastewaters. Heavy metals adversely affect the different stages of mineralization, inhibiting the activity of enzymes of the microorganisms participating in the purification process.

Algal Interactions with Arsenic, Chromium, and Copper

Most heavy metals are needed by plants a micronutrients. These same nutrients can be very harmful if they are encountered by the organism in large concentrations. Many times, when an organism is exposed to a toxic substance, it will accumulate the toxin. The toxic material might have no noticeable affect on the organism, but may be passed up the food chain to higher organisms. This is known as biomagnification. As will be shown, As, Cr, and Cu do accumulate and become concentrated in living organisms, but they have not been shown to biomagnify, as does mercury (33).

According to Hodson (34), the great sensitivity of most aquatic biota to toxins results from their high surface-to-volume ratios. Other influencing factors include the permeability of the cell membrane, accessability of organics, and sorption properties of the cells (11,17, 34).

Many aquatic organisms have shown the ability to concentrate metals within themselves. Concentration factors are defined as the ratio of the metal concentration in the organism to that in the water mass (18). Wagemann et al. (35) measured As levels in sediments, water, and aquatic biota in the lakes of Yellowknife, Northwest Territory, Canada. In As contaminated water, As concentration factors were 333 for invertebrates, 5,600 for benthos, and between 3,500 and 10,000 for the zooplankton (wet wight). The As concentration factors decreased with increasing concentrations of As in the lake's ecosystem. Klumpp and Peterson (26) examined As and other trace metal concentrations in an estuary of Southwest England. Arsenic was accumulated by the marine

plankton relative to levels in the environment with concentration factors up to 10,000. Although As was accumulated, there were no signs that it was also biomagnified.

Trollope and Evans (18) compared heavy metal concentrations of freshwater algal blooms in waters contaminated by smelting waste with those from unpolluted waterways. They showed that the algae appeared to regulate the uptake of individual metals. The mean metal concentrations in the algae were ordered Fe > Zn > Pb > Cu > Ni, whereas the mean metal levels in the water column were ordered Zn > Pb > Fe > Ni > Cu.

In a related study, Laube et al. (36) used Anabaena and Ankistrodesmus braunii to study the mobilization and accumulation of sediment-bound heavy metals by algae. Copper and cadmium were added to river water in situ, and it was removed by both the algal and sediment compartments. Both Anabaena and A. braunii accumulated more Cu than the sediments. These results suggest that in natural systems, algae may play a very important role in mobilizing sediment-bound metals.

Experimental algal studies examining the toxicity of heavy metals have included work with nitrogen fixation, photosynthesis, growth, morphology, and reproduction (37,38,39). Nitrogen fixation by the heterocysts of blue-green algae treated with copper solutions was studied by Horne and Goldman (37). Nitrogen fixation by blue-green algae accounts for a large portion of the nitrogen budget of a lake. Their study showed that five ug/l Cu reduced nitrogen fixation by 76 percent in two days and by 90 to 95 percent in eight days.

Lowmann et al. (40) tested chromium toxicity in marine algae. Hexavalent Cr at 0.03 - 64 ppm inhibited the growth of algae. Lower

concentrations stimulated growth in some cases. This was because Cr is a necessary nutrient for some plankton. Lethal Cr levels for most of the algae were between 0.034 and 6.4 ppm.

Dupree (41) treated ponds with soduim arsenite for weed control and analyzed the As content of the algae following its application. After the application, the water concentration of As was 4.2 mg/l. This amount decreased slowly to 2.0 mg/l at 30 days and 0.2 - 1.7 mg/l at 78 days. The As in the algae rose from an initial value in the range of 5.9 - 10.6 ppm to a peak of 6,955 ppm in 27 days. Species diversity decreased with increasing As levels in the algae.

The morphology of Scenedesmum quadricauda and Anabaena variabilis was studied by Khobot'yev et al. (39). The two algae were exposed to 7.8×10^{-5} M $CuCl_2$, after which the cells became enlarged and round. The chlorophyll content of the cells was found to decrease with increasing Cu concentrations. Distinct morphological changes and alterations in pigment composition were also observed.

Steeman and Kamp-Nielsen (38) worked with Chlorella pyrenoidosa and Nitzschia palea to determine the toxicity of Cu to their growth and photosynthesis rates. Ionic Cu was extremely toxic to both of these algae when no metal complexing agents were present. Copper concentrations of one to two ug/l had a noticeable effect on the rate of photosynthesis of N. palea. Suppression of photosynthesis was found to increase with increasing time of contact with the Cu. A decrease in growth was noted at five ug/l for both species of algae.

Heavy metal toxicity is affected in natural environments by chelation and metal synergism (31,42,43). According to Gachter et al.(42),

chelation is the single most important factor reducing copper's toxicity. Ethylenediaminetetracetic acid (EDTA), nitrilotriacetic acid (NTA), and several extracellular polypeptides also cause a decrease in copper toxicity. Copper's toxicity to fish has been shown to be greater in soft waters than in hard (31). This can be explained by the complexing action of metals. In hard waters, the alkalinity is usually high, and a greater portion of the Cu ions become complexed and are then unavailable to aquatic organisms.

Heavy Metals and Other Organisms

Heavy metals are also toxic to higher organisms, such as invertebrates, fish and even Man himself. However, Cr and Cu are needed as micronutrients by most organisms. The biologic importance of chromium is restricted to the trivalent state (63). Cr^{+3} forms complexes that are stable at or below pH 4, but which readily hydroloze at higher pH values; resulting in the formation of polynucleate bridge complexes. In humans, Cr must be supplied as a complex of suitable stability in order to be utilized. This is because at the normal pH of the blood, Cr^{+3} exists in large, insoluble macromolecules which precipitate and become biologically inert. Copper is also required in animal metabolism. It is important in invertebrate blood chemistry and for the synthesis of hemoglobin in humans (63).

Ayling (44) sampled oysters from 15 sites along the Tamar River, Tasmania, and analyzed them for copper, lead, and chromium. He found the widely accepted concept of enrichment factors (concentration) up to several hundred thousand for Cr and Cu in oysters to be grossly

misleading. The levels of the three metals in the oysters were only 10 to 40 times the values found in the sediments. Copper and chromium appeared to be absorbed up to a maximum weight that was limited by the size of the oyster and independent of the amount of metal in the sediments.

In another study dealing with invertebrates, Friant (45) examined mollusks, along with other ecosystem components, for chromium, copper, lead, mercury and zinc. The order of increasing metal concentration in the mollusks was Pb, Cr, Cu, and Zn. Bottom-dwelling mollusks and rooted plants accumulated more metals than were found in the sediments. The high metal levels found at some stations were cited as the probable cause of the stunted growth of some of the mollusks.

Surber and Meehan (64) found that fish-food organisms generally survived concentrations of 1.73 mg/l of arsenious trioxide (1.3 mg/l as As) in a sodium arsenic solution. They also noted that concentrations of 4 mg/l sodium arsenate (2.3 ml/l as As) in confined outdoor pools reduced the survival and growth of fish and reduced bottom fauna and plankton populations.

Raymont and Shields (65) researched Cr toxicity in several invertebrate species found in marine waters. They reported Cr threshold toxicity levels of 5 mg/l for small prawns, Leander squilla, 20 mg/l (as Na_2CrO_4) for the shore crab, Carcinas maenus, and 1 mg/l for the polychaete, Nereis virens. The mummichogs, Fundulus heterclitus, tolerated up to 200 mg/l Cr (as $K_2Cr_2O_4$) in seawater for over a week.

Toxicity of a metal to fish is highly variable due to differences in species tolerance and environmental conditions such as temperature,

pH, dissolved oxygen, and hardness (16). Trama (46) conducted early research on the acute toxicity of Cu to the bluegill sunfish, Lepomis macrochirus. At a pH between 5 and 6, a total alkalinity of 3 - 6 mg/l as $CaCO_3$, and a total hardness of 45 -47 mg/l as $CaCO_3$, a 96-hour medial tolerance limit (TLm) was determined to be 0.71 mg Cu per l for $CuCl_2$ and 0.77 mg Cu per l for $CuSO_4$. This research showed that the toxicity of the cupric ion was independent of its source. Pickering and Henderson (47) tested the acute toxicity of heavy metals in several fish species. They concluded that an average of 0.01 - 0.02 ppm Cu in soft, fresh water was the lethal dose for most species of warm-water fish.

The Federal Water Pollution Control Act Amendments of 1972 required the U.S. Environmental Protection Agency (EPA) to publish criteria for water quality. These criteria were designed to recommend the highest concentration of pollutants that could be found in waters without causing any adverse affects on human health or the aquatic environment. The EPA criteria for As and Cr are a maximum of 100 ug/l for the irrigation of crops and a maximum of 100 ug/l for freshwater fish, respectively (62). For freshwater and marine aquatic life, the EPA criteris for Cu is 0.1 times a 96-hour LC50 as determined through a nonaerated bioassay using a sensitive aquatic resident species (63).

Metal Toxicity to Man

The toxic actions of several metals, suchas arsenic, have been known for many years. However, it has only been during the past few years that our socieity has really become aware of just how dangerous

heavy metals can be. Depending on the size of the dose and the method of contamination, the toxicity of the metal can be very small (barely noticeable) or extremely grave (causing death).

Inorganic arsenic is absorbed readily to the gastrointestinal tract, the lungs, and to a lesser extent from the skin, and becomes distributed throughout the body tissues and fluids (63). It is excreted via urine, feces, and sweat. As excretion may continue as long as 70 days after the cessation of continuous exposure (63). Arsenic poisoning can produce many adverse effects in the human body. Acute poisoning, through the ingestion of contaminated food, can lead to profound gastrointestinal damage and cardiac abnormalities (11). Chronic poisoning, usually from the inhalation of As dust, can cause teratogenic, mutagenic, and carcinogenic mutations (11). The EPA criteria recommends a maximum As concentration of 50 ug/l in domestic water supplies (63).

Copper in water supplies must be in very high concentrations to cause health problems in humans. Most water supplies do not have sufficient Cu to cause any damage. However, copper in excess of one mg/l does impart some taste to water. Because of a possible undesirable taste in drinking supplies, the EPA recommends a limit of one mg/l Cu (63). A study by Nriagu (48) examined the various responses of the human body to high copper doses. Acute toxicosis can cause hemolysis, hepatic necrosis, and even gastrointestinal bleeding. Exposure to copper over long periods produces malignant tumors, acrodynia, and, in some cases, dermatitis.

No harmful effects have been observed when food or water containing

moderate amounts of trivalent chromium were administered to laboratory animals (63). From this test data the National Academy of Science predicted that Cr^{+3} is of little medical concern. Unfortunately, the same can not be said for hexavalent chromium. Cr^{+6} is absorbed into the body via ingestion, through the skin, and by inhalation. Hexavalent Cr is irritating and corrosive to the mucus membranes and is very toxic when introduced into laboratory animals (63). Inhalation of Cr contaminated dust particles most often leads to ulceration of the skin and perforation of the nasas septum (14). Respiratory cancer, ulcers, and dermatitis are also responces to chromium contamination. A maximum of 50 ug/l Cr is allowed in domestic water supplies for health reasons (63).

Periphyton

Periphyton, known as virtually the only primary producers in quickly flowing waters, have only been studied intensely in recent years (6,8,49,50). Many researchers have turned their interest to periphyton because they are a useful indicator of water quality. When subjected to adverse effects, such as scouring water velocities or high turbidity, periphyton are characterized by quick recovery rates (6). Also, periphytic production is closely related to the quality of water passing over their communities (6,7).

Russian biologists in 1924 were probably the first to use the term "periphyton" in a descriptive sense (8). They referred to periphyton as the assemblage of microorgamisms that grew on the surfaces of objects which they placed under water. By the mid-1930's the term

periphyton was used in the United States to refer to the bacteria collected on submerged glass slides (8,51). The definition of periphyton expanded by 1945 to include all microorganisms growing on any submerged substrate (8,52). Today, Standard Methods (49) defines periphyton as "all zoogleal and filamentous bacteria, attached protozoa, rotifers, algae and free-living organisms found swimming, creeping or lodged among the attached forms on a submerged substrate, either natural or artificial."

Periphyton communities are highly affected by the environmental factors around them. Their abundance, growth rates, and composition are governed by the water quality flowing past them (49). Changes in the water quality, due to natural or man-made causes, can affect periphyton species diversity (6,7,53). Healthy water systems are characterized by a balance between the microbial components (7). Stress in a system usually causes a shift in the balance by favoring the growth of the more tolerant species. Polluted waters also affect the productivity of the periphyton communities. It has been determined that productivity is a function of the water quality, substrate availability, and seasonal patterns in temperature and solar illumination (8,49,50,54,55).

Periphyton Analyses

The biomonitoring of periphyton communities is a useful technique in assessing the quality of a water ecosystem. The analysis of the productivity, autotrophic index, and metal accumulation capacity are three of the most common techniques used in periphyton studies.

Primary production. Because periphytic communities play a significant role in the production of a lotic system, they have been studied to determine what relationships exist between their productivity and the quality of the water surrounding them. Periphyton primary production is defined as the quantity of new organic material produced by photosynthesis or the energy stored by this organic matter (49,56). A more general meaning of the word is the increase in biomass of green plants over a period of time minus any losses by excretion, damage, respiration, grazing, or death (50). Primary production may be estimated from biomass analyses, the rate of oxygen production, and through carbon uptake studies.

Hornick (50) investigated the carbon fixation rates in several Appalachian Mountain trout streams. He tested for differences in primary production rates between first and second order streams in the same drainage basin. Allochthonous input and physiochemical parameters were also measured. The time of year had a significant affect on the primary production. The highest production occurred in July, while the lowest appeared in early autumn.

In a related study, Dawson (57) determined that the metals copper, iron, manganese, nickle, and zinc accounted for a significant amount of variance in periphyton productivities in an area subjected to urban runoff. Also, in a study utilizing periphyton to determine the amount of mercury contamination in the South River, Virginia, Barker (43) concluded that the mean primary production was highest at the station which had the greatest levels of Hg in the periphyton.

Autotrophic index. The autotrophic index (AI) is a means of re-

lating changes in plankton species composition to changes in the water quality. Standard Methods (49) defines the AI as the ratio of biomass to the chlorophyll-a content of the periphyton. Periphyton of unpolluted waters are characterized by a dominance of autotrophic algae, whereas, heterotrophic organisms dominate in organically enriched waters (7,49). Normal AI values occur between 50 and 200. This indicates a system predominated by autotrophic algae. An AI higher than 200 indicates organically enriched water and, most probably, a predominace of the heterotrophic population (49).

Matthews et al. (7) analyzed the changes in autotrophic and heterotrophic portions of a stream microbial community by measuring the ATP and chlorophyll-a content of the periphyton. These changes were related to the structural and functional changes in the stream macroinvertebrate community. Their results showed the balance between the autotrophs and heterotrophs had been considerably altered by a sewage outfall. Changes in the AI were inversely related to changes in structural and functional groups. It was concluded that the nontaxonomic monitoring of microbial communities could be a useful tool in the field analysis of stress effects.

Metal accumulation. Periphyton have the ability to concentrate heavy metals. Because of this, periphyton appear well suited for the assessment of trace metals since they offer low detection limits as a consequence of bioaccumulation (53).

Work by Patrick et al. (58); employing an artificial substrate under semi-laboratory, continuous-flow conditions; showed that the uptake of vanadium, chromium, selenium, and nickle was proportional

to the exposed metal concentration for natural communities of freshwater diatoms. In a study using natural periphyton in a closed lotic microcosm, Cushing and Rose (59) found zinc uptake to be directly proportional to the exposed metal concentrations. The mechanism of uptake was reported to be passive adsorption.

Dawson (57) determined the effects of urban runoff on the metals content and productivity of the periphyton communities in the Occoquan Reservoir and Bull Run, Virginia. Periphyton accumulated metals in the following order, Fe > Mn > Zn > Cu > Ni > Pb > Cr > Cd. Since the metals at the control stations were not significantly lower than those downstream, it was determined that urban runoff was not causing higher metal concentrations in the periphyton.

Mercury contamination of the periphyton in the South River, Virginia was analyzed by Barker (43). The mean Hg concentration in the periphyton increased to a point 8.5 km below the source of contamination, then decreased further downstream. Mercury content of the periphyton was significantly higher below the Hg source than upstream.

Wood Preservation

Most wood preserving techniques are aimed at preventing the growth of the wood-destroying Basidiomycetes fungi. Decay by this fungi is grouped into two major categories, white and brown rot (60). Brown rot attacks the cellulosic skeleton, but leaves the lignin intact. This renders the wood friable and brown in color. White rot causes both the cellulose and lignin to decompose. The wood becomes soft and linty, but the color remains unchanged.

Preservation systems rely on mechanisms which either prevent the start of decay or are toxic to the decomposing fungi (60). A preservative is fungistatic if it will prevent the fungus from attacking the wood, but it does not necessarily kill the fungi. If the preservative is toxic to the attacking organism, then it is referred to as fungicidal. A greater percentage of preservatives rely on the direst toxic fungicidal mode of action (60).

The remainder of this discussion will center around chromated-copper-arsenate (CCA) preservatives. This was the type of preservative utilized by CWP when the waste was spilled from their lagoon on January 31, 1981. CCA preservatives fall into the category of fungistatic because they eliminate the source of nourishment for the fungi.

CCA, a water-borne preservative, consists of a solution of salts. The three toxicants are expressed as oxides, CuO, CrO_3, and As_2O_5 (60, 61). The arsenic component acts as an insecticidal agent as well as being fungistatic (61). CCA was developed in Scotland in 1926 and was first marketed as Celcure (60).

The American Wood Preserver's Association (AWPA) has subdivided the CCA preservatives into three varieties, Types A, B, and C. All types have approximately 19 percent copper oxide. Type A is high in CrO_3, Type B is high in As_2O_5, while Type C is intermediate (60,61). Hartford (61) discussed in detail the individual mechanisms of fixation for each of the CCA types. According to the AWPA, the CCA formula is the most reliable general purpose preservative currently available (60).

As with most other industries, the wood preservation industry produces a moderate amount of wastewater. Thompson (62) analyzed the

wastewater from a CCA preservation plant and found it to contain 1,700 mg/l COD, 300 mg/l As, 375 mg/l Cr (hexavalent), 170 mg/l Cu, and a pH of 5. He reported that the major contamination of the water occurred in the following areas; steam conditioning of the wood prior to preservation treatment, cooling pipes, wash water from cleaning operations, and from drippings of freshly treated lumber (62).

The treatment of this type of wastewater was developed by and borrowed from the electroplating industry. Of the three ions in the waste solution, hexavalent chromium is the only one which will not precipitate at a pH of 7 to 8. However, trivalent chromium will precipitate under these conditions. To remove all three metals from solution, the following must take place. Hexavalent Cr must be reduced to Cr^{+3} under acidic conditions. Next, the pH must be increased to approximately 7.5 to precipitate all three ions. Finally, the sludge is removed (62).

III. METHODS AND MATERIALS

Description of Sampling Streams

All sampling sites were located in Culpeper County, just east of Culpeper, Virginia (Figure 1). This area is composed of rolling hills dotted with corn fields and pastures. Only a small proportion of the natural woodlands remain. Three of the ten sampling stations were located on an unnamed tributary which drains into Jonas Run and which received the spill from CWP. Another four sites were in Jonas Run, which empties into Mountain Run, a tributary of the Rappahannock River. Another station was located in a second, small, unnamed tributary that flows into Jonas Run 0.7 miles above its confluence with Mountain Run. The remaining two sites were located in Mountain Run, one below and one above the confluence of Jonas and Mountain Runs.

The first unnamed tributary parallels Virginia Routes 762 and 706 and is a small intermittent stream. During the warm, dry, summer months, the flow ceases, leaving water only in the normally deeper pools. Most of the stream's reach drains agricultural and pasture lands.

Jonas Run is a slightly larger stream than the unnamed tributary that flows into it. Though it has been known to go dry during parts of the year, it usually flows year-round. Jonas Run flows mostly through corn fields and pasture land. It is used as a drinking-water supply by the local cattle.

The second unnamed tributary is very similar to the first. It is an intermittent stream that flows through an area used by Jim's Liquid Waste as a liquid chemical disposal site and empties into Jonas Run.

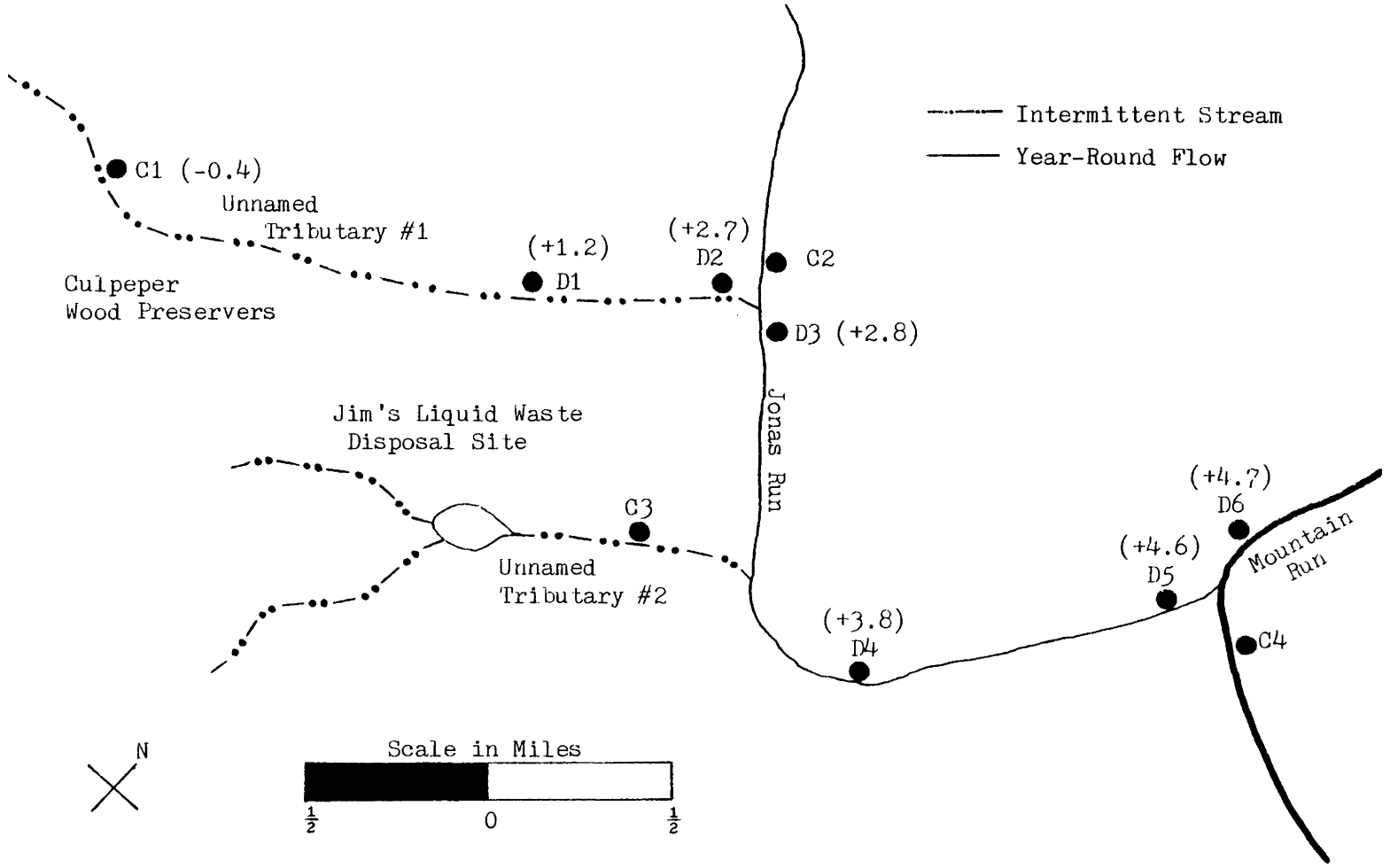

Figure 1. Location of Sampling Sites (Stream miles from CWP)

This stream drains forrested and agricultural areas.

The fourth stream involved in the study was Mountain Run. This is a moderately-sized stream which flows year-round. Similar to Jonas Run, Mountain Run flows through agricultural and pasture land and provides a drinking source for livestock.

Sampling Stations

Stations were chosen on the basis of their ecological similarity. Differing environmental conditions cause varying growths of periphyton (62). Shaded and pool regions of streams have been proven to have lower primary productivity than periphyton grown in sunny, riffle areas (62,63). The size of the streams studied in this report varied greatly, which caused difficulty in locating similar sampling locations. A total of ten sampling sites were chosen (Figure 1). One site (station C1) was used as an upstream control from the spill site and another three stations (C2, C3, and C4) were used as controls for streams entering the main drainage area below the spill area. The remaining six sites (D1, D2, D3, D4, D5, and D6) were all below the spill site at varying distances downstream.

Only one upstream control was used because of the inaccessability of the stream bed at that point. It was located beside Virginia Route 762, just above a conduit running under the route. The stream was extremely small at this point, varying from one to two feet across and from six to ten inches in depth. Water flowed past this station during the entire sampling period, even though at times it dried up downstream. The periphyton sampler, known as a diatometer, was located in a shallow, grassy area subjected to partial sunshine, as were the other samplers.

Station D1 was located 1.2 miles below the spill site along Virginia Route 706. The diatometer there was in a shallow, parially shaded location which dried up during parts of the sampling period.

The third station (D2) was approximately 2.7 miles below Culpeper Wood Preservers. It was located on the unnamed tributary, just above its confluence with Jonas Run on the Stapleton Farm. As with site D1, this site did not flow the entire sampling period. The diatometer at this site was located in a well-lit, riffle area.

Stations D3 and C2 were located in Jonas Run on the Stapleton farm approximately 2.8 miles below the spill site. It was dificult finding appropriate sites for these two stations because Jonas Run was dammed up by debris caught on bushes growing in the stream's bed just below its confluence with the unnamed tributary. Consequently, these two stations were located in slower, deeper water than would have been ecologically ideal.

Station C3 was used as a control for the unnamed tributary which flows out of Jim's Liquid Waste area. This stream flowed intermittently during the sampling period. The diatometer was located in a pool because the water was not deep enough in riffle areas to support a diatometer.

Sampling site D4 was located below the confluence of Jonas Run and the tributary which flows out of Jim's Liquid Waste area. This site was 3.8 miles below the spill site and was located beside Virginia Route 663. At this site, Jonas Run had increased in size to approximately seven to eight feet wide and six inches to one foot deep.

Apporximately 0.8 miles below station D4 was station D5, located

on property owned by T. O. Madden. This site was on Jonas Run above its confluence with Mountain Run. As with the other stations upstream, this sampler was located in a shallow, partially sunny, riffle area.

The remaining two sites were located in Mountain Run. Station C4, used as a control for Mountain Run, was located on W. E. Brown Jr.'s property, approximately 50 yards upstream from Jonas Run. Station D6, approximately 4.7 miles below the spill site, was the most distant sampling site from CWP. These two sampling stations were located in mostly sunny, riffle areas.

Periphyton Samplers

An artificial substrate, known as a diatometer, was used in this study for the collection of periphyton. The design of the diatometer was the same as that used by Barker (43) and Dawson (57) in similar studies. There are many diatometer designs available. Sladeckova (71), Cooke (52), Wetzel (51), and Patrick (58) have reviewed the merits and criticisms of several differing diatometer designs.

The main frame of the diatometer was constructed of 0.25 inch Plexiglas. This frame held twenty microscope slides (75 mm x 25 mm) vertically. There were two Styrofoam blocks, one at each end of the diatometer, to support the sampler to a depth of four inches beneath the water's surface. Two cinder blocks, attached to the diatometers with polyester cord, were used as anchors. The diatometer used for this study is depicted in Figure 2 . For this study, two diatometers were placed in tamden to obtain sufficient periphyton growth for the analyses.

The exposure periods for the diatometer's slides ranged between eight and 17 days. The length of exposure was determined by the tem-

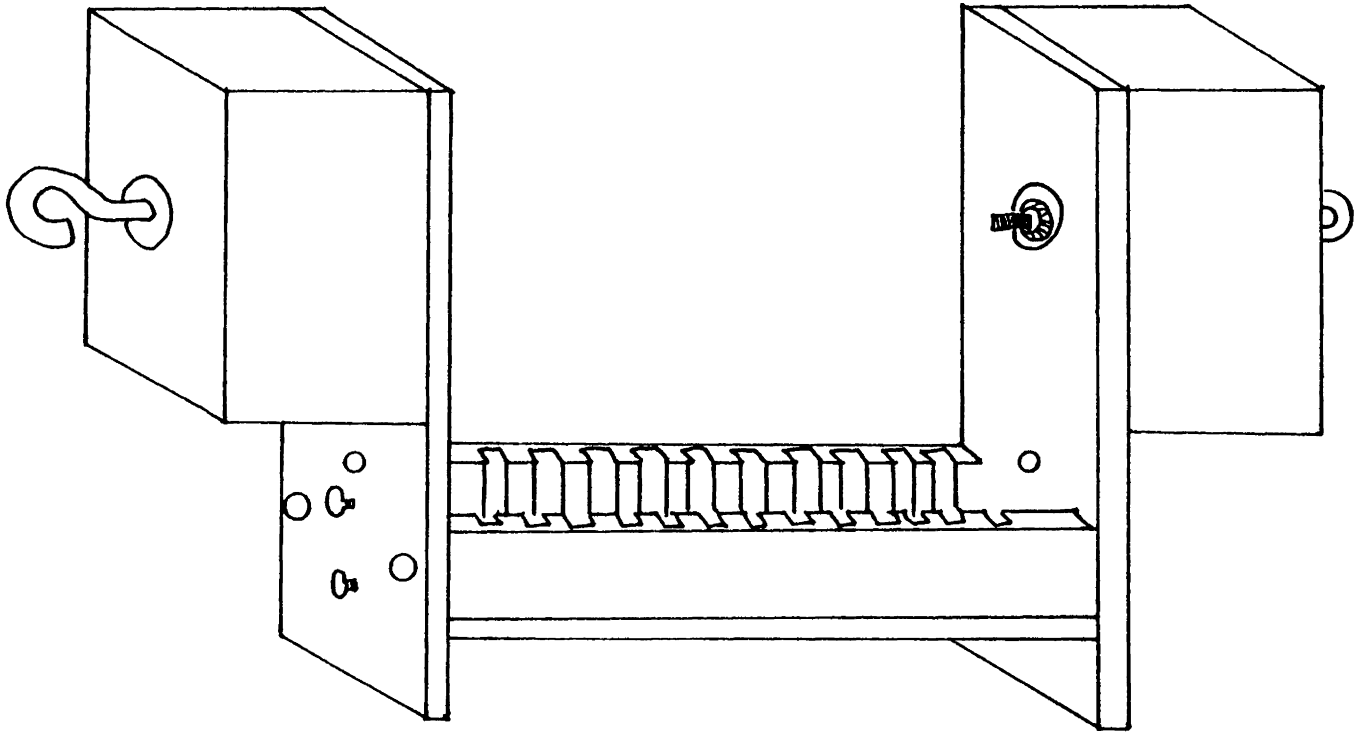

Figure 2. Diatometer Design Used in Periphyton Colonization

perature and day length. As the nights grew longer and colder, a longer exposure period was necessary for adequate periphyton growth. The eight to 17 day period was long enough for a dense mat of periphyton to accumulate but short enough so that sloughing did not occur. At the end of each exposure period, the slides were retreived and brought back to the laboratory for analysis. The dates and exposure length for each accrual period are shown in Table 1.

Sampling Procedures

At the end of each accrual period, the glass slides were removed from the diatometers in random order and transported back to Virginia Tech. Before field collection and laboratory analysis, all apparatus were acid-washed according to Section 4.1 of the EPA Manual of Methods for Chemical Analysis of Water and Wastes (72). After the growth period was complete, ten slides from each station were placed in a glass bottle containing five to ten milliliters of a 90 percent acetone and distilled water mixture. Another ten slides were placed in pre-labeled, plastic vials. Both the bottles and vials were stored in the dark on ice for the return trip to the laboratory. Once back in the lab, all samples were stored in the dark in a 4° C refrigerator until analyzed. The ten slides contained in the bottles were used for chlorophyll and gravimetric analyses. The vials contained the slides for the metals analyses. Immediately prior to the actual testing, the periphyton was scraped from the slides with a galvanized steel razor blade.

Water samples were taken three times during the sampling period. These were collected in acid-washed, pre-labeled, polyethylene bottles

Table 1. Inclusive Dates and Accrual Periods for Periphyton Sampling

Inclusive Sampling Dates, 1981	Total Days Exposure
June 22 - July 8	17
July 8 - July 16	9
July 16 - July 24	9
July 24 - Aug. 3	11
Aug. 3 - Aug. 11	9
Aug. 11 - Aug. 22	11
Aug. 22 - Sept. 3	13
Sept. 3 - Sept. 13	11
Sept. 13 - Sept. 25	13

and kept in the dark at 4° C until analyzed. Both total and soluble metals were analyzed.

Analytical Procedures

Metals analysis. All metal analyses were completed in accordance with Section 4.1.3 of the EPA Manual of Methods for Chemical Analysis of Water and Wastes (49), as modified by Dawson (57). Glassware and lab apparati were carefully acid-washed with both 50 percent nitric and 50 percent hydrochloric acids, then rinsed with tap and distilled water. Whenever possible, plastic labware was used instead of glass. Glass has been shown to adsorb metals and can also leach other metals from its surface (49).

The periphyton from the entire surface of six glass slides was scraped into a 50-ml Griffin beaker. The slides and blade were washed with 30-ml distilled water and combined with the scrapings in a previously tared beaker. The beaker was then dried in a Thelco (Model 28) oven at 105° C for 24 hours, cooled for one hour, then weighed. The beaker was then returned to the oven for another hour, cooled for an hour, and reweighed to assure constant weight. This was repeated until two similar, consecutive weighings were obtained.

After a constant weight had been determined, three-ml concentrated nitric acid was added to the beaker and evaporated without boiling to near dryness on a hotplate. Spectrograde nitric acid was utilized in the metal digestions for the first six accrual periods. The last three digestions were with Ultrex® nitric acid. The Ultrex® acid is freer of impurities than the spectrograde nitric acid. However, metal con-

centrations in the blanks were only slightly lower when Ultrex® was used. After cooling the beakers, an additional three-ml nitric acid was added. The temperature of the hot plate was increased and watch glasses were placed on top of the beakers so gentle refluxing could occur. Additional acid was added until the digestion was complete (as indicated by a light-colored residue). In general, a total of 11-ml of nitric acid was necessary in the digestion process. To keep the results comparable, the same amount of acid was used in each digestion.

After the digestion was complete, the beakers and watch glasses were washed with 0.5 percent nitric acid and the washings poured into a graduated, polystyrene centrifuge tube. The volume was adjusted to approximately 10-ml with 0.5 percent nitric acid and recorded. The samples were allowed to settle and the supernatant was analyzed with a Perkin Elmer Model 703 Atomic Absorption Spectrophotometer. Chromium and copper were analyzed by flame while arsenic was analyzed by a Perkin Elmer HGA 2100 graphite furnace because it was found in much smaller concentrations. Some samples had to be diluted with distilled water to reach the detection levels of the spectrophotometer. Blanks were prepared by carrying 30-ml of distilled water through the same procedures as were followed for digestion of the periphyton. Each time a periphyton digestion was performed, a blank was prepared also. Metal concentrations were calculated as follows:

$$[ME]p, \text{ ug/g} = \frac{([ME]d - [ME]b)\ V}{M}$$

where $[ME]_p$ = calculated metal concentration in the periphyton, ug metal/g periphyton sample
$[ME]_d$ = total concentration of metal in the digested periphyton sample, ug/l
$[ME]_d$ = concentration of metal in the digested blank, ug/l
V = volume of digested sample, l
M = mass of periphyton digested, g

Water analysis. Both total and soluble metal concentrations were analyzed at various times during the sampling period. Samples to be analyzed for total metals were acidified to a pH of 2 immediately after collection. Those to be analyzed for soluble metals were brought back to the lab without acidification and filtered through a 0.45 millipore filter as soon as possible. Both types of samples were then analyzed with a Perkin Elmer Model 703 Atomic Absorption Spectrophotometer, as were the digested periphyton samples.

Chlorophyll analysis. The spectrophotometric (Trichromatic) method described in Standard Methods (49) was used in analyzing the chlorophyll concentrations in the periphyton. The concentration of pheophyton, a degradation product of chlorophyll-a, was calculated to achieve a more exact concentration of the chlorophyll-a. Two replicates for each station were completed.

Growth on both sides of five slides were scraped into a tissue grinder, a few milliliters of 90 percent aqueous acetone were added, and the contents subsequently macerated to extract the phytopigments. The sample was then transferred into a centrifuge tube and steeped overnight in the dark at 4 C. After the sample warmed to room temperature, it was centrifuged for 20° minutes at 5000 revolutions per minute. The volume of the centrifugate was measured and the macerated periphyton pellet was set aside for the dry weight analysis. The optical

density was determined at 750, 663, 645, and 630 nm with a Perkin-Elmer Double Beam Spectrophotometer (Coleman 124). The reading at 750 nm was a correction factor for turbidity. Chlorophyll-a may be overestimated by including other pigments (pheophyton-a in particular) that absorb in the same wavelength range. To correct for this, the samples were acidified with 0.02-ml 1N HCl per milliliter of extract. The optical density was then determined at 750 and 665 nm, one to two minutes after acidification.

Chlorophyll-a concentrations were determined as follows (49):

$$\text{Chl-a, mg/l} = 11.64(OD663) - 2.16(OD645) + 0.10(OD630)$$

where OD663, OD645, and OD630 are the optical densities at the respective wavelengths.

On an areal basis, chlorophyll-a was calculated as follows (49):

$$\text{Chl-a, mg/m}^2 = \frac{\text{(Chl-a)(extract volume,l)}}{\text{surface area of slides, m}^2}$$

The calculation for the corrected chlorophyll-a and pheophyton concentrations were as follows (49):

$$C, \text{ mg/m}^2 = \frac{26.73(OD663b - OD665a) \times V}{A}$$

$$P, \text{ mg/m}^2 = \frac{26.73\,[1.7(OD665a) - OD663b] \times V}{A}$$

where C = chlorophyll-a concentration corrected for pheophyton, mg/m^2
P = pheophyton concentration, mg/m^2
OD663b = optical density before acidification
OD665a = optical density after acidification
V = volume of supernatant, l
A = total surface area of slides, m^2

Gravimetric analysis. In order to determine the dry and ash-free weight of the periphyton sample, the acetone extract from the chlorophyll analysis and the pellet from centrifuging were combined in a tared crucible and allowed to evaporate to near dryness. The sample was then transferred to a 105°C oven to dry to constant weight, then ignited for one hour at 500°C. It has been shown that temperatures as high as 500°C drive off the water of hydration of the clay and other minerals (49). To avoid this loss, the ash was re-wetted with distilled water and dried to a constant weight at 105°C. The ash-free weight (volatile solids) was determined by the following equation:

$$\begin{matrix}\text{Ash-free} \\ \text{dry weight, g}\end{matrix} = \begin{matrix}\text{Total} \\ \text{dry weight, g}\end{matrix} - \begin{matrix}\text{Dry weight} \\ \text{of ash, g}\end{matrix}$$

Autotrophic index determinations. The autotrophic index (A.I.) is a useful indicator of the water quality. It is a ratio of the chlorophyll-a to the biomass and was calculated as follows (49):

$$\text{A.I.} = \frac{\text{Biomass (ash-free dry weight), mg/m}^2}{\text{Chlorophyll-a, mg/m}^2}$$

A.I. values in the range of 50 to 200 are indicative of a healthy system. Larger values indicate a predominace of heterotrophs and most likely, an organically enriched body of water (49).

Calculation of productivity. Primary productivity can be defined as the rate of organic accumulation on a substrate. It varies with the water quality, seasonal patterns, and substrate availability (40, 49). The productivity was determined by the following equation (49):

$$\text{Productivity, g/m}^2\text{/day} = \frac{\text{ash-free dry weight/slide, g}}{\text{T x A}}$$

where T = exposure time, days
A = area of slide, m^2

Statistical Analysis of Data

Using the Statistical Analysis System (SAS) program at Virginia Polytechnic Institute and State University, a randomized complete-block analysis of variance (ANOVA) of the data was performed to determine the affects of the sampling stations and sampling dates on the values for arsenic, chromium, copper, productivity, chlorophyll-a, and autotrophic index. Duncan's Multiple Range Comparison (DMRC) test was employed to test for differences between stations and between sampling periods at the 0.05 level of significance.

IV. RESULTS

Periphyton Heavy Metal Concentrations

Arsenic. The total arsenic concentration in the periphyton at each station was determined at the end of each accrual period. These arsenic concentrations are depicted in Figure 3. Tabular data corresponding to Figure 3 are found in Table B2. The highest individual arsenic concentration of 132 ug/g was recorded at station D4, downstream from the confluence of the second unnamed trubutary and Jonas Run, during the period September 13 - 25, while the lowest concentration of approximately 1 ug/g was found during the second accrual period (July 8 - 16) at station C1, the control upstream from the CWP spill site.

An analysis of variance (ANOVA) by the SAS program showed that the As concentration in the periphyton was a function of the location of the sampling station and the time of sampling did not significantly affect those concentrations. See Table B1. A DMRC test of the mean total As concentration in the periphyton at seven stations is shown in Figure 4 and in Table B8. The lowest mean As concentration in the periphyton was found at control site C1. The highest mean arsenic concentration was at station D1, the first sampling station below the spill site. Duncan's test showed that upstream station C1 and downstream stations D3, D4, D5, and D6 were not significantly different from each other at the 0.05 level. Also, stations D1, D2, and D4 were not significantly different from one another; however, sampling sites D1 and D2 were significantly different from the upstream

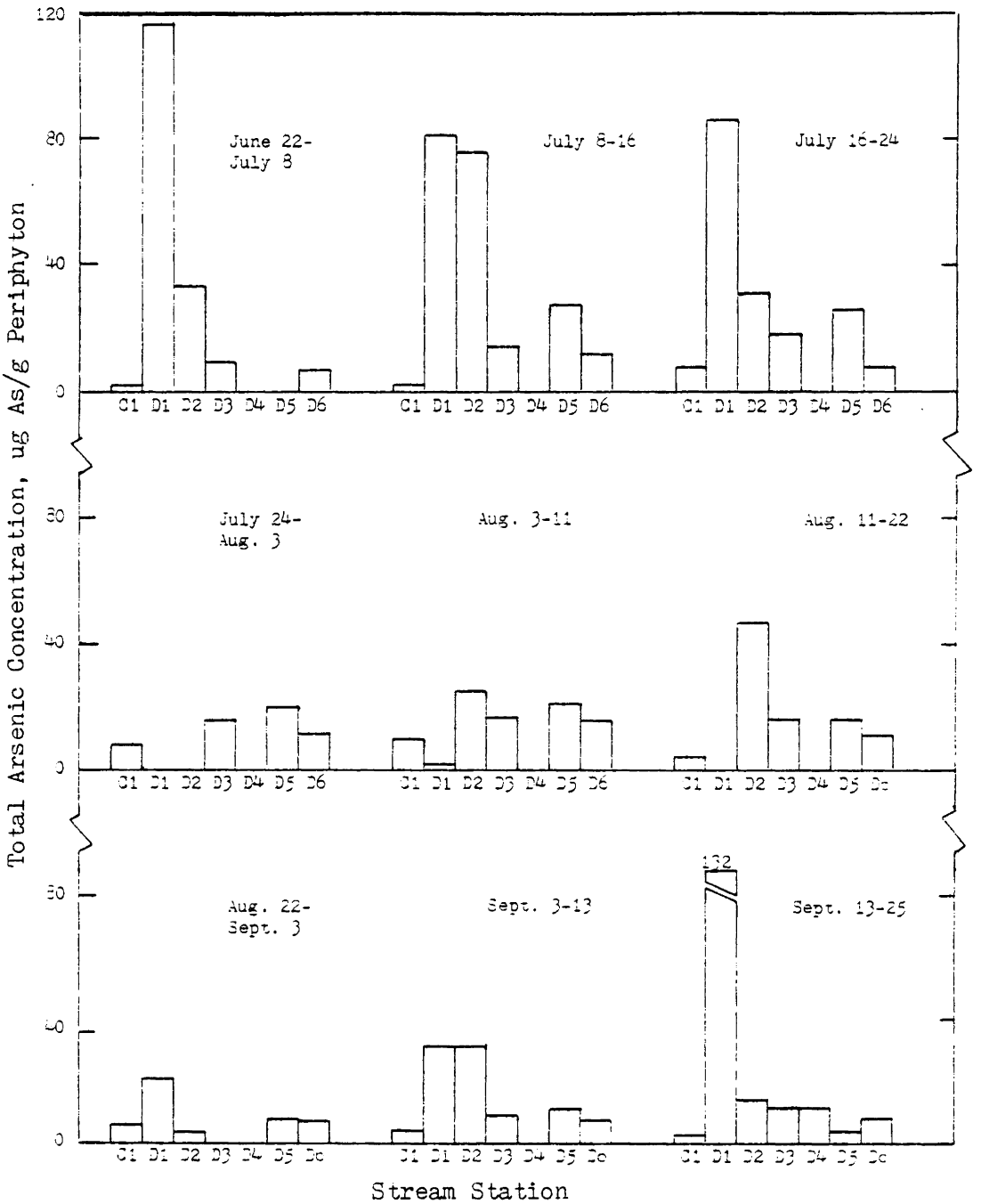

Figure 3. Total Arsenic Concentrations in Periphyton Total Solids at Seven Locations in Culpeper, Virginia, During the Period June Through September, 1981. Dates Indicate Periods of Periphyton Accumulation.

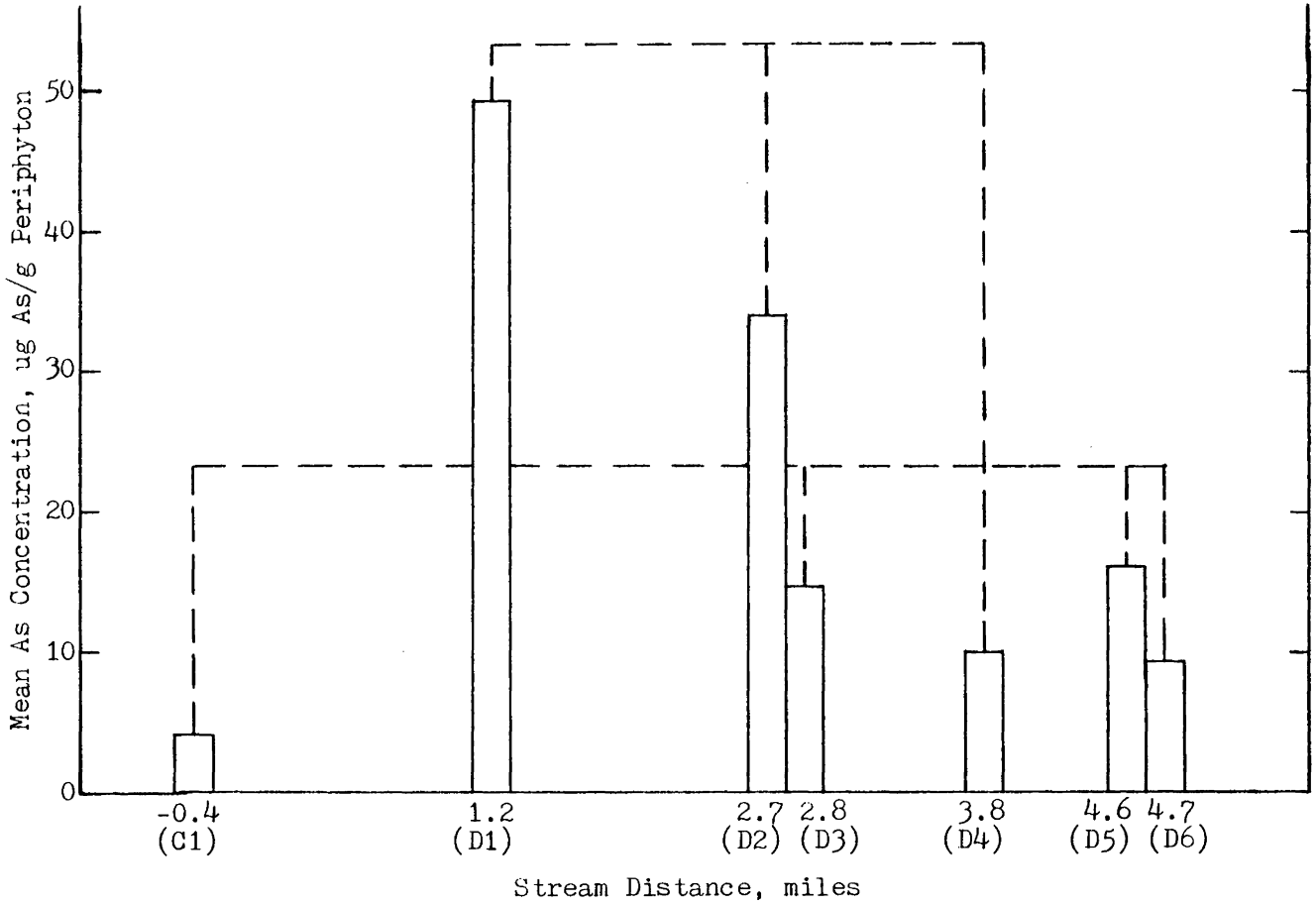

Figure 4. Comparison of the Mean Arsenic Concentration in Periphyton Total Solids at Seven Stations During the Period June Through September, 1981

(Brackets indicate no significant difference between means at the 0.05 level.)

control, C1, and from three of the downstream sites, D3, D5, and D6. The mean arsenic concentrations at the control site on Jonas Run (C2), on the second unnamed tributary (C3), and on Mountain Run (C4) were compared by the DMRC test to the mean As levels found at the stations below these control sites. This test showed no significant differences at the 0.05 level among these downstream stations and their corresponding controls.

The mean arsenic concentration for each sampling period is shown in Figure 5 and Table B14. Both the ANOVA and DMRC test showed no significant differences at the 0.05 level among As concentrations in periphyton accrued during the various sampling periods. Reasons for these arsenic concentrations and trends will be discussed in the following chapter.

Chromium. At the end of each accrual period, the total chromium concentrations in the periphyton from each of the sampling stations was determined. Figure 6 and Table B3 depict the Cr levels at each station during the nine accrual periods. The highest individual Cr concentration of 377 ug/g was found at station C1 during the period June 22 - July 8. Chromium was not detected on two occassions; once at station C3 during the second accrual period and again during the fifth accrual period at station D1.

Results of the statistical analysis (ANOVA) of the Cr concentrations in the periphyton showed no significant differences at the 0.05 level caused by either the sampling location or time of sampling. However, a DMRC test of the mean Cr concentrations in the periphyton showed significant differences among the sampling sites at the 0.05

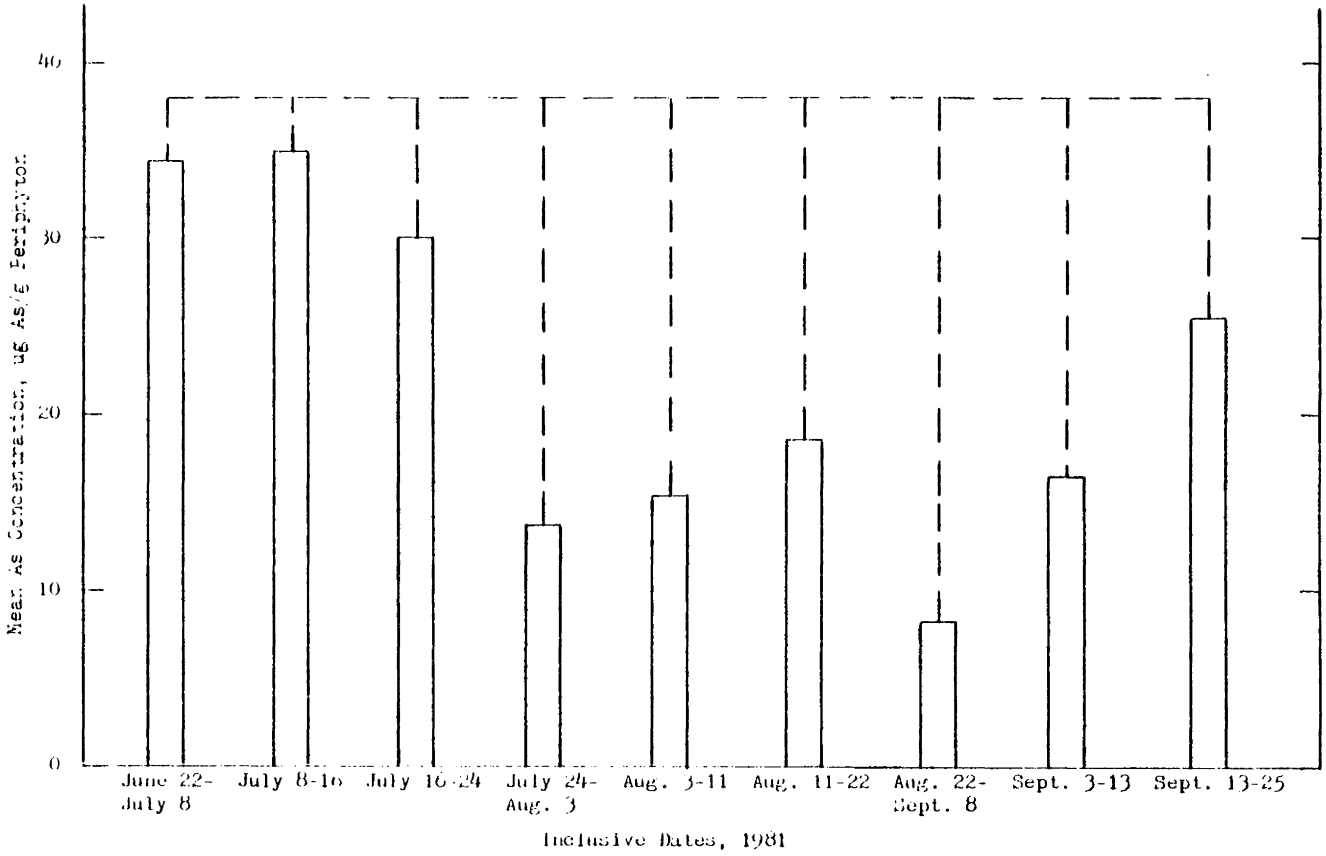

Figure 5. Comparison of the Mean Total Arsenic Concentration in Periphyton Total Solids During Each of the Nine Accrual Periods

(Brackets indicate no significant difference between means at the 0.05 level.)

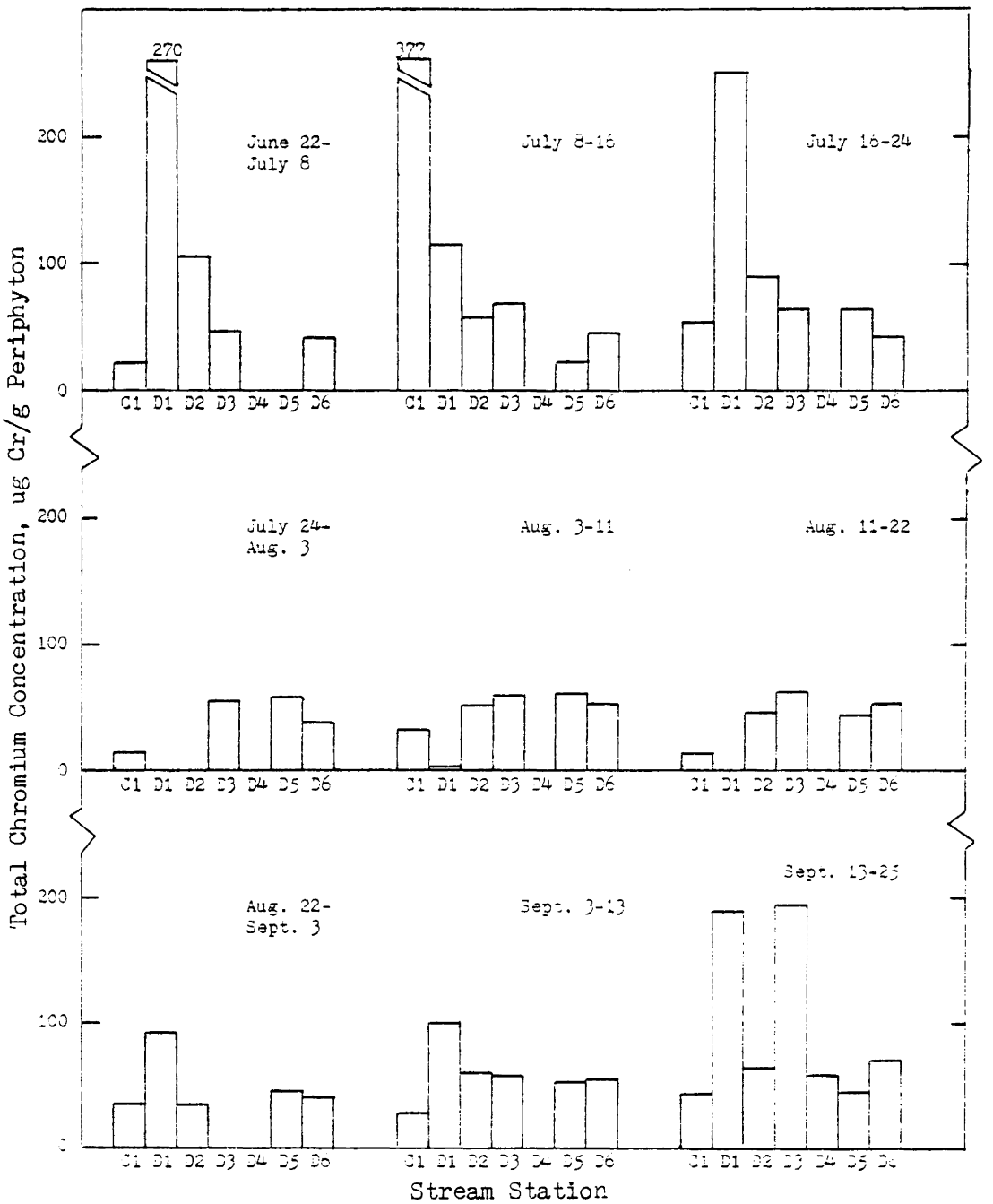

Figure 6. Total Chromium Concentrations in Periphyton Total Solids at Seven Locations in Culpeper, Virginia, During the Period June Through September, 1981. Dates Indicate Periods of Periphyton Accumulation.

level. See Figure 7 and Table B2. The lowest mean Cr concentration of 34 ug/g occurred at station C1, while the greatest mean Cr concentration of 161 ug/g was recorded at sampling site D1. The periphyton Cr levels at sites C1, D2, D3, D4, D5, and D6 were not significantly different from one another and were grouped together. Stations D2 and D4 also had periphyton Cr concentrations that were not significantly different from one another and grouped together. Only the mean Cr level in the periphyton at sampling site D1, just below the spill site, was significantly different from any other site. This station differed in Cr concentration at the 0.05 level of significance from sites C1, D2, D3, D5, and D6. Duncan's test showed there were no significant differences among the periphyton Cr concentrations at the control stations C2, C3, and C4 and their corresponding downstreams sites, D3, D4, and D6.

The mean total Cr levels at each of the sampling stations are presented in Figure 8 and Table B15. Neither the ANOVA nor the DMRC test showed any significant differences among concentrations of Cr in the periphyton caused by different accrual periods. A further discussion of the chromium data and trends will be presented in the next chapter.

Copper. The copper concentration in the periphyton total solids at each of the sampling stations for the nine accrual periods are found in Table B4. A graphical representation of these periphyton Cu levels is found in Figure 9. The highest single Cu concentration of 1225 ug/g was recorded at control station C3 during the second sampling period. A low of 20 ug Cu/g periphyton was found during

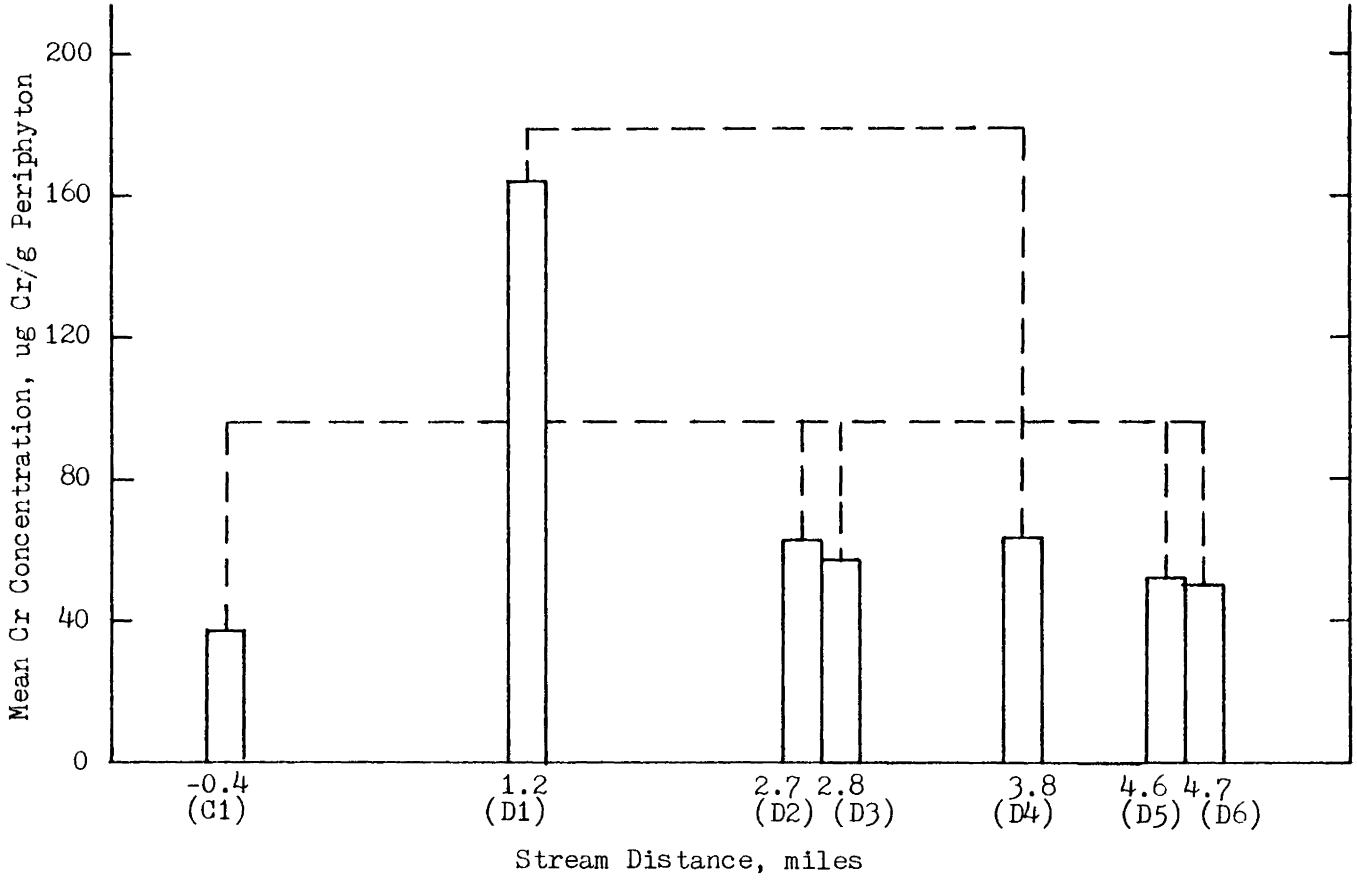

Figure 7. Comparison of the Mean Chromium Concentration in Periphyton Total Solids at Seven Stations During the Period June Through September, 1981

(Brackets indicate no significant difference between means at the 0.05 level.)

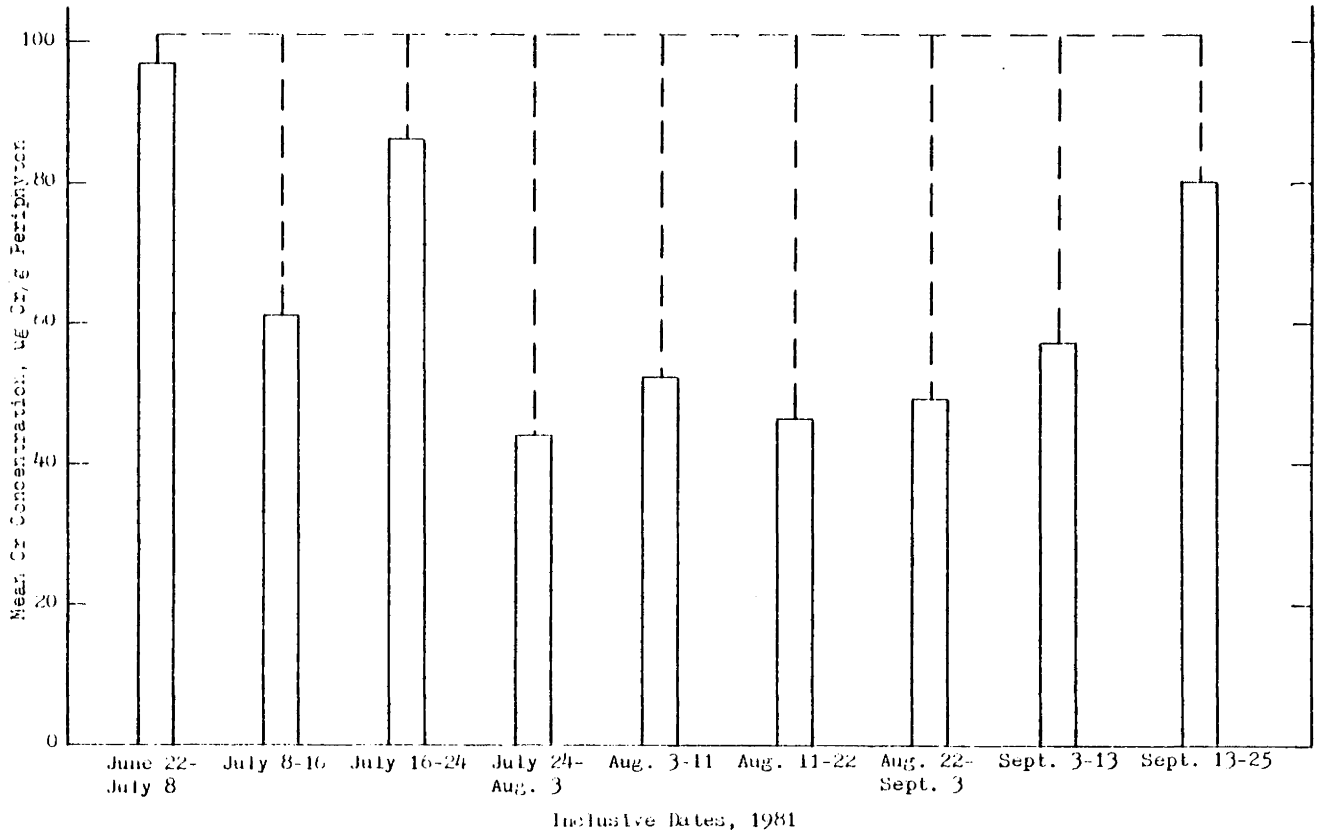

Figure 8. Comparison of the Mean Total Chromium Concentration in Periphyton Total Solids During Each of the Nine Accrual Periods

(Brackets indicate no significant difference between means at the 0.05 level.)

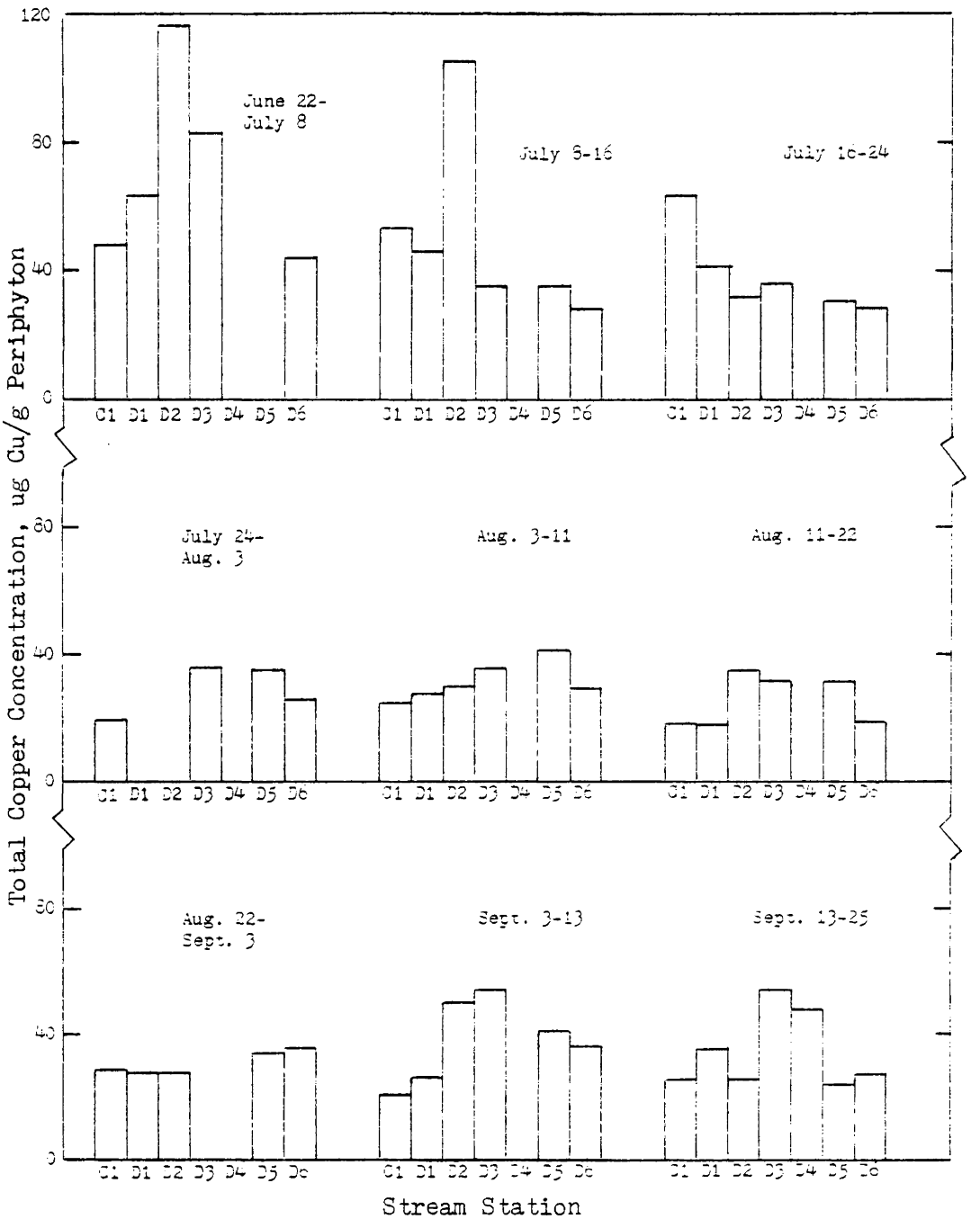

Figure 9. Total Copper Concentrations in Periphyton Total Solids at Seven Locations in Culpeper, Virginia, During the Period June Through September, 1981. Dates Indicate Periods of Periphyton Accumulation.

the fourth accrual period (July 24 - August 3) at control station C1.

The ANOVA by the SAS program showed that the copper concentrations were not a function of the location of the sampling station; however, the date of sampling did play a significant role in determining these concentrations. The results of the ANOVA for copper are found in Table B1. The outcome of the Duncan's test is displayed in Figure 10 and Table B10. The lowest mean Cu concentration of 31 ug/g was observed at station D6, while the greatest Cu concentration of 53 ug/g was found at sampling site D2. In contrast to the ANOVA's findings, the DMRC test showed the periphyton Cu concentrations were a function of the sampling location. Periphyton copper concentrations at sampling sites C1, D1, D3, D4, D5, and D6 did not differ significantly at the 0.05 level. Stations D1, D2, D3, and D4 were also similar in periphyton Cu concentration and grouped together. The only site which differed significantly in periphyton Cu level from any of the other stations was station D2, 2.7 miles below the CWP spill site on the unnamed tributary. This sampling location differed significantly from stations C1, D5, and D6 in periphyton Cu levels. The Duncan's test showed that control stations C2 and C4 were not significantly different from their corresponding downstream stations, D3 and D6, with respect to their periphyton Cu concentrations. However, control station C3, located on the second unnamed tributary, did differ significantly in copper concentration from its downstream station, site D4, on Jonas Run.

The mean copper levels in the periphyton during each accrual period are shown in Figure 11. The analysis of variance, as well as

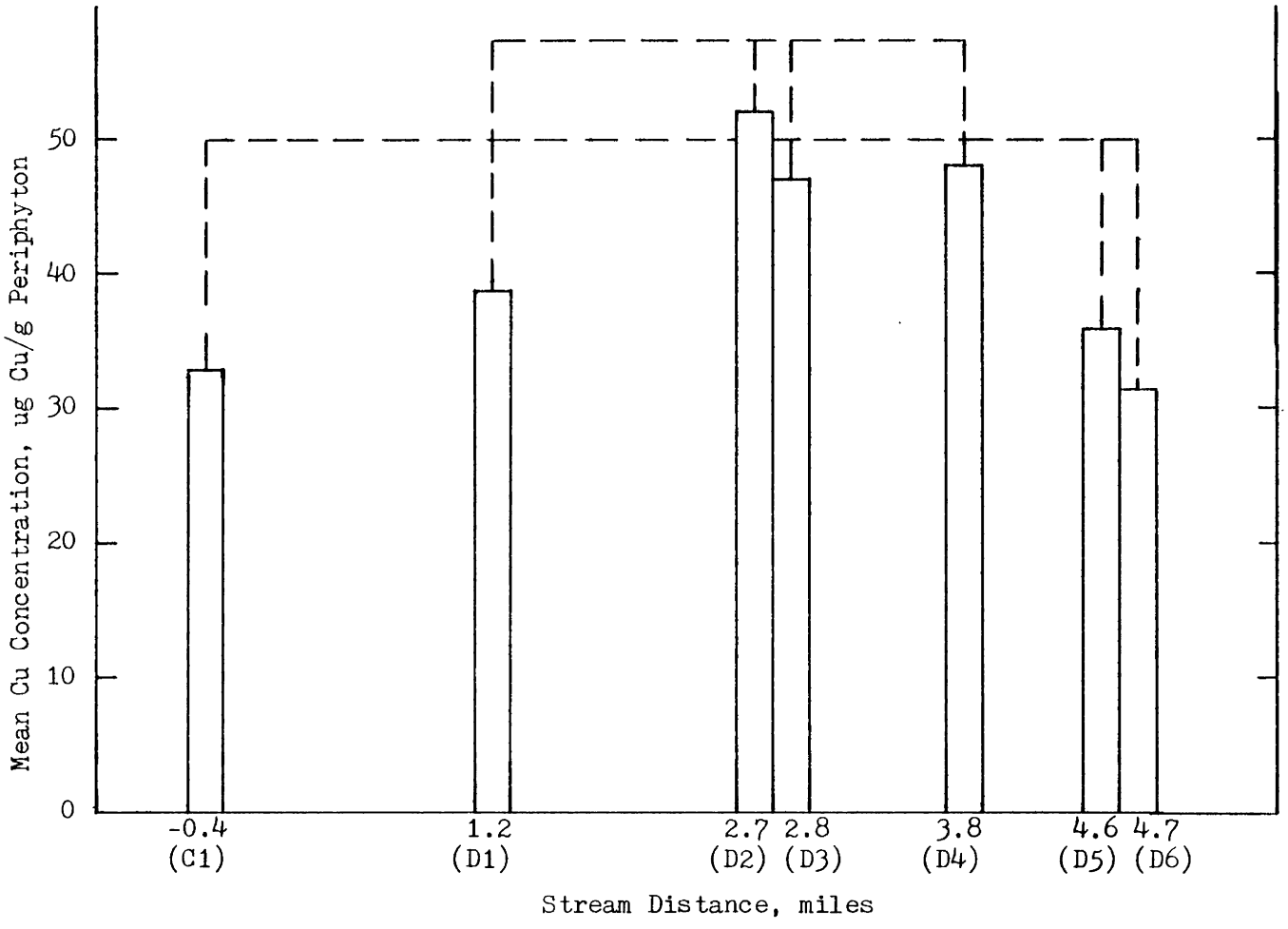

Figure 10. Comparison of the Mean Total Copper Concentration in Periphyton Total Solids at Seven Stations During the Period June Through September, 1981

(Brackets indicate no significant difference between means at the 0.05 level.)

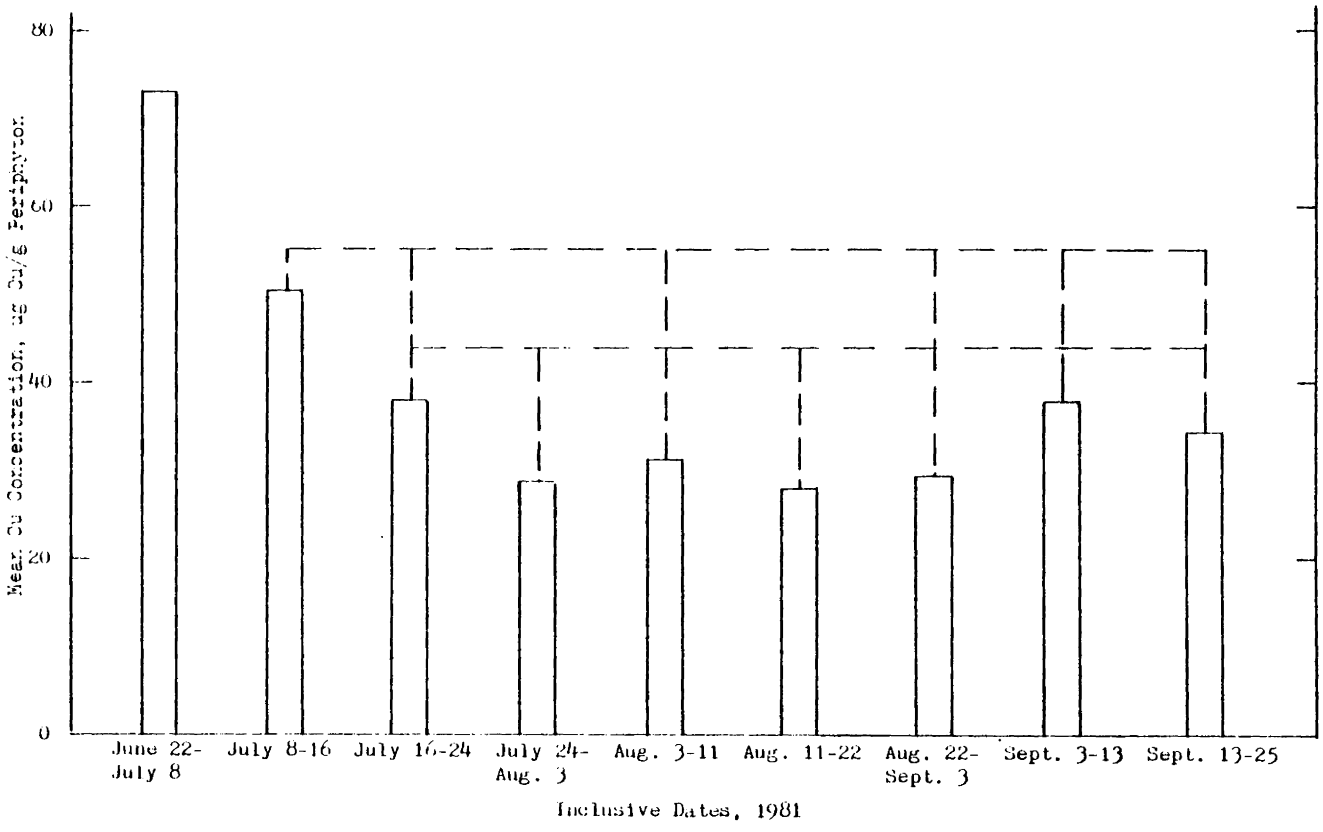

Figure 11. Comparison of the Mean Total Copper Concentration in Periphyton Total Solids During Each of the Nine Accrual Periods.

(Brackets indicate no significant difference between means at the 0.05 level.)

the DMRC test, showed the Cu concentrations were a function of the sampling location (Tables B1 and B16). According to Duncan's test, periphyton Cu concentrations appearing during accrual periods two, three, four, five, seven, eight, and nine were not different from one another at the 0.05 level of significance. Also not significantly different from one another were Cu concentrations obtained during accrual periods three, four, five, six, seven, eight, and nine. The mean periphyton Cu concentration from accrual period one (June 22 - July 8) was significantly different from the mean concentrations at all other sampling periods, while the periphyton Cu level from period six (August 11 - 22) was different only from those measured after accrual periods one and two. A further discussion of the copper data is contained in the next chapter.

Water Analysis

Results of the arsenic, chromium, and copper analysis for the water samples collected at several of the periphyton sampling stations are in Table 2. Water samples were collected three times during the testing period; once in each of the months June, July, and August. The June sample was analyzed for only total metals. Water samples collected in July were analyzed for both total and dissolved heavy metals, while only the dissolved metals were analyzed in the August sampling. Of the 36 samples analyzed for total metals, 27 were at or below the detection limits of the atomic adsorption spectrophotometer (AA). Of the dissolved metal samples, 29 of the 39 sample concentrations were at or below the detection limits.

Table 2. Total and Dissolved Arsenic, Chromium, and Copper Concentrations in Water Samples from Culpeper, Virginia

Sampling Date (1981)	Sampling Site	Arsenic, ug/l		Chromium, mg/l		Copper, mg/l	
		Total	Dissolved	Total	Dissolved	Total	Dissolved
June 22	C1	<1.0	*	<0.05	*	<0.02	*
	C2	*	*	*	*	*	*
	C3	*	*	*	*	*	*
	D1	11.0	*	<0.05	*	<0.02	*
	D2	5.0	*	<0.05	*	<0.02	*
	D3	<1.0	*	<0.05	*	<0.02	*
	D4	*	*	*	*	*	*
	D5	<1.0	*	<0.05	*	<0.02	*
	D6	<1.0	*	<0.05	*	<0.02	*
July 15	C1	<1.0	<1.0	<0.05	0.07	<0.02	<0.02
	C2	1.0	<1.0	<0.05	<0.05	0.03	0.06
	C3	1.0	<1.0	<0.05	<0.05	<0.02	<0.02
	D1	*	*	*	*	*	*
	D2	*	*	*	*	*	*
	D3	1.0	<1.0	<0.05	<0.05	0.09	<0.02
	D4	2.0	2.0	<0.05	0.14	<0.02	<0.02
	D5	*	*	*	*	*	*
	D6	1.0	<1.0	<0.05	<0.05	<0.02	<0.02
Aug. 22	C1	*	<1.0	*	<0.05	*	<0.02
	C2	*	*	*	*	*	*
	C3	*	<1.0	*	1.07	*	0.07
	D1	*	2.0	*	<0.05	*	<0.02
	D2	*	<1.0	*	<0.05	*	<0.02
	D3	*	<1.0	*	<0.05	*	<0.02
	D4	*	*	*	*	*	*
	D5	*	<1.0	*	<0.05	*	0.02
	D6	*	<1.0	*	<0.05	*	0.03

* Water samples were not collected at these sites.

Primary Productivity

The primary productivities of the periphyton at each station during the nine sampling periods are shown in Figure 12. Table B5 contains the data used in the preparation of Figure 12. The analysis of variance by the SAS program showed both the location and time of sampling played a significant role in the determination of the primary productivity. Figure 13 contains the DMRC test of the mean productivities at each station for the period June through September, 1981. The highest mean productivity of 5 g/m^2/day was at station C1. The lowest mean productivity of slightly less than one g/m^2/day was found at control station C4 on Mountain Run. The mean primary productivity levels of the periphyton at stations D1, D2, D3, D4, D5, and D6 were significantly similar at the 0.05 level and thus, grouped together. Also significantly similar to one another were the productivities at stations C1, D4, and D6. The periphyton production at upstream control site C1 was significantly different from those recorded at downstream stations D1, D2, D3, and D5. Control stations C2, C3, and C4 were found to be significantly similar in their preiphyton productivity levels to their downstream stations, sites D3, D4, and D6, respectively.

The mean primary productivity of the periphyton during each accrual period is shown in Figure 14. The periphyton productivity varied considerably from one sampling period to the next. Both the ANOVA and the DMRC test showed significant differences between the productivities during the different accrual periods. (Tables B1 and B17, respectively). The productivities during the first, third,

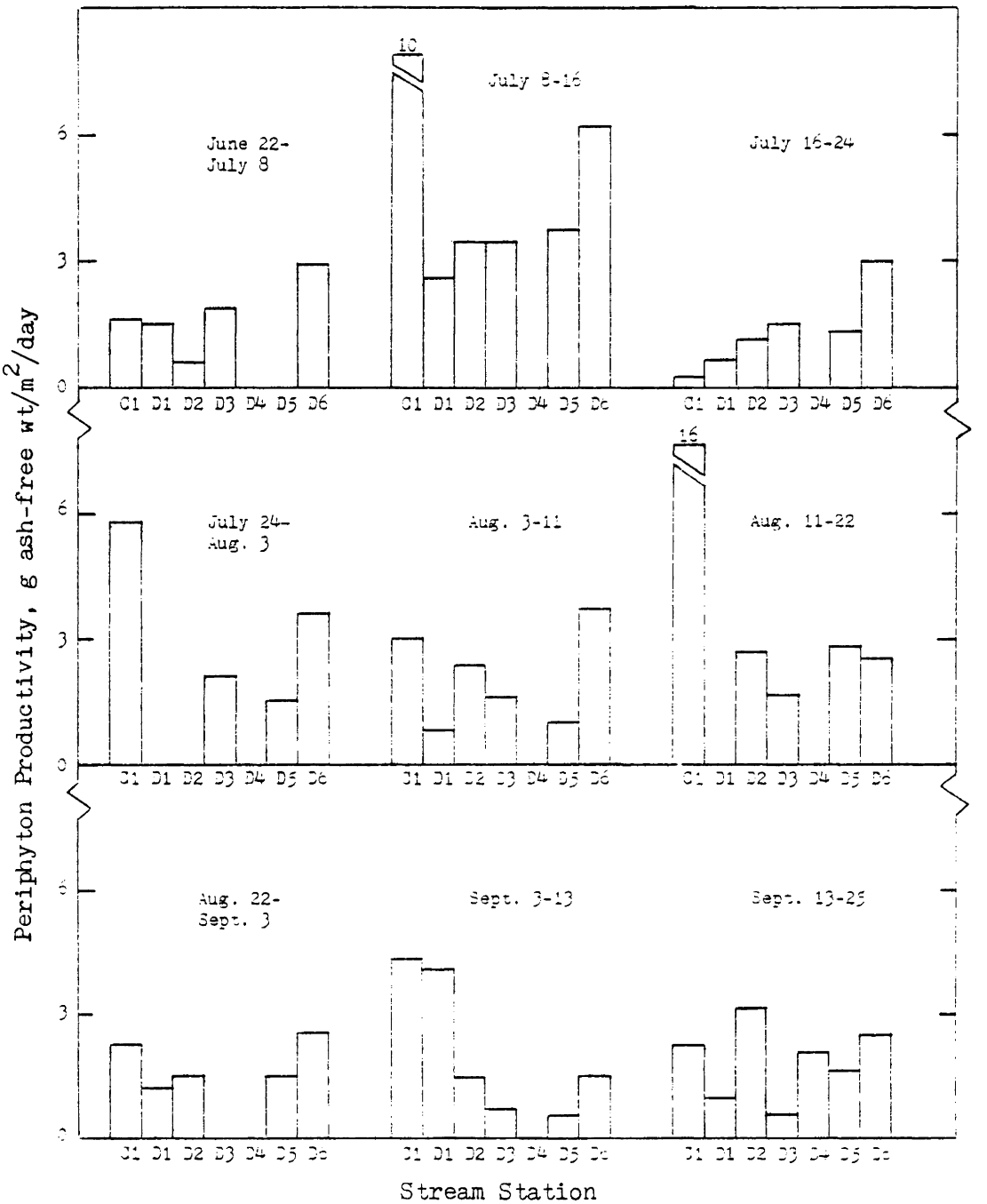

Figure 12. Periphyton Productivity at Seven Locations in Culpeper, Virginia, During the Period June Through September, 1981. Dates Indicate Periods of Periphyton Accumulation.

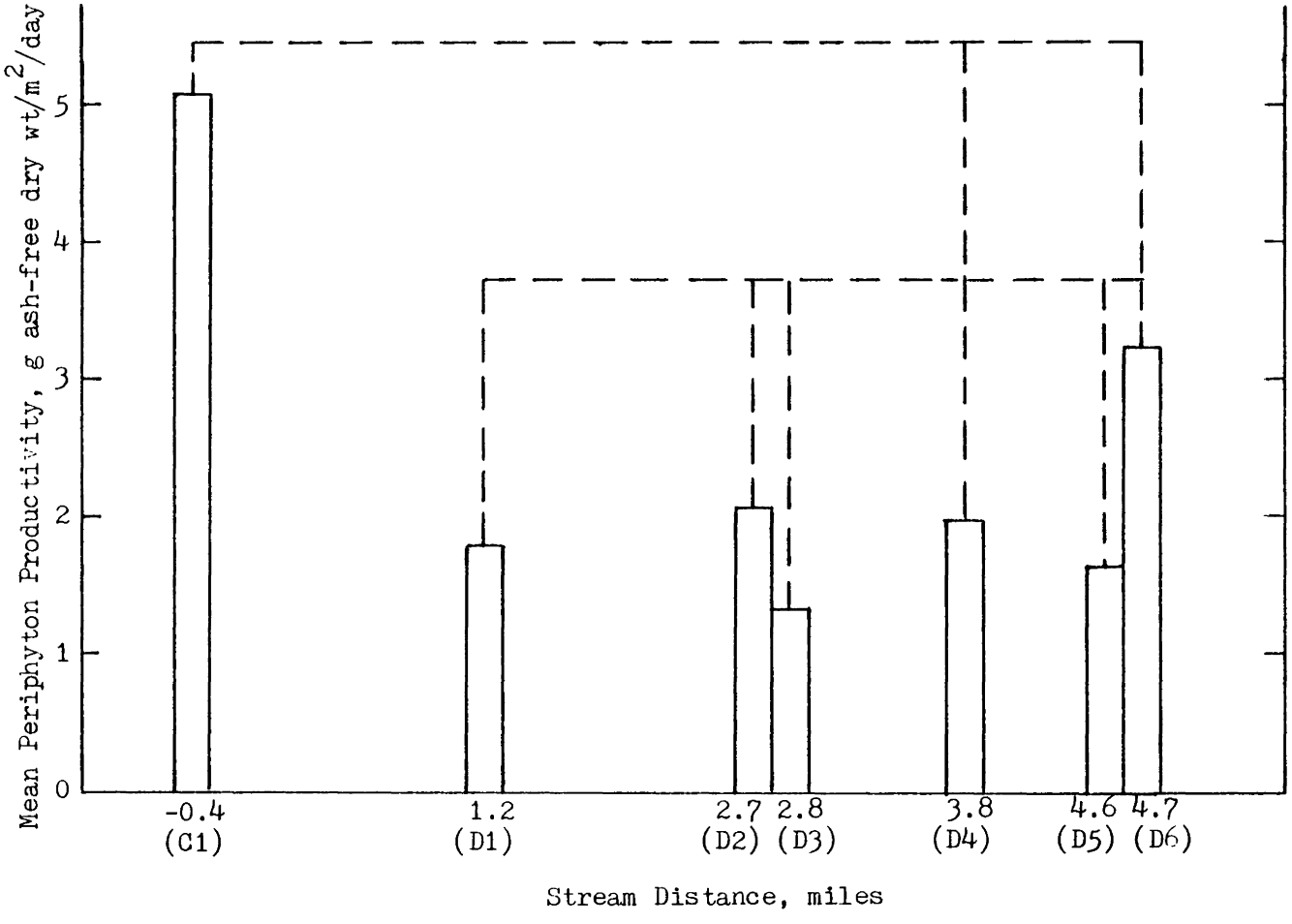

Figure 13. Comparison of the Mean Primary Productivity at Seven Stations During the Period June Through September, 1981

(Brackets indicate no significant difference between means at the 0.05 level.)

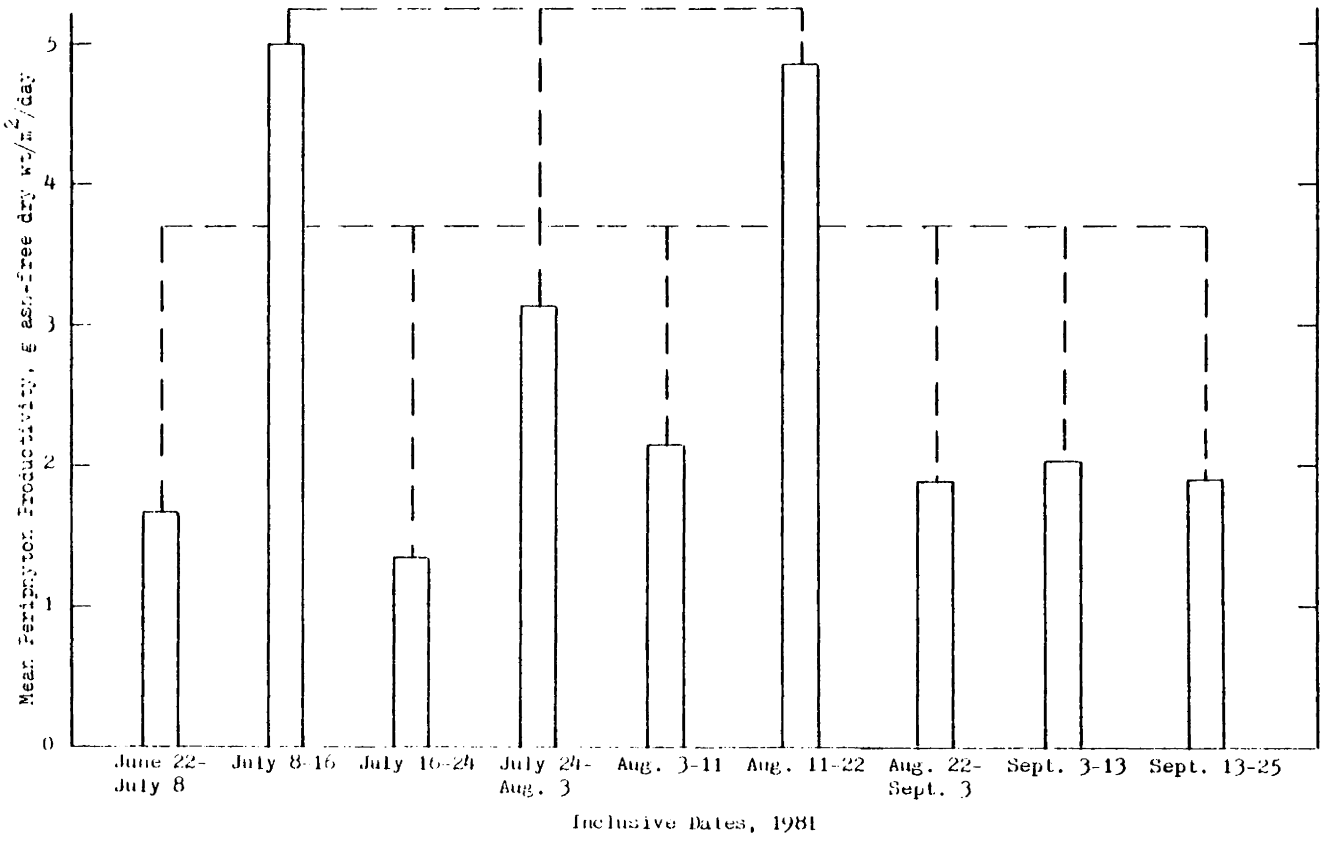

Figure 14. Comparison of the Mean Periphyton Primary Production During Each of the Nine Accrual Periods. (Brackets indicate no significant difference between means at the 0.05 level.)

fourth, fifth, seventh, eighth, and ninth sampling periods were grouped together because of their similarity. Also not significantly different form one another were the periphyton productivities during accrual periods two, four, and six. Those production rates during accrual periods two and six were different from those during sampling periods one, three, five, seven, eight, and nine at the 0.05 level of significance. Differences in productivity due to sampling location and trends caused by the time of sampling will be discussed in the following chapter.

Chlorophyll-a

At the end of each accrual period, the chlorophyll-a concentration in the periphyton from each of the sampling sites was determined. These data are contained in Figure 15 and Table B6. Results of the analysis of variance are in Table B1. The ANOVA showed there were no significant differences in chlorophyll-a concentration caused by either the location or date of sampling. The DMRC test gave the same results for the location of sampling. Figure 16 contains the comparison of the mean chlorophyll-a concentrations at seven stations. These data are also presented in Table B12. Control C2 was the station where the lowest mean chlorophyll-a concentration of 0.14 mg/m^2 appeared. The greatest chlorophyll-a level of 1.65 mg/m^2 was recorded at station D4. The mean chlorophyll-a concentrations in periphyton at all sampling stations were similar and no significant differences were noted. Controls C2, C3, and C4 were all significantly similar in periphyton chlorophyll-a levels to their corresponding downstream

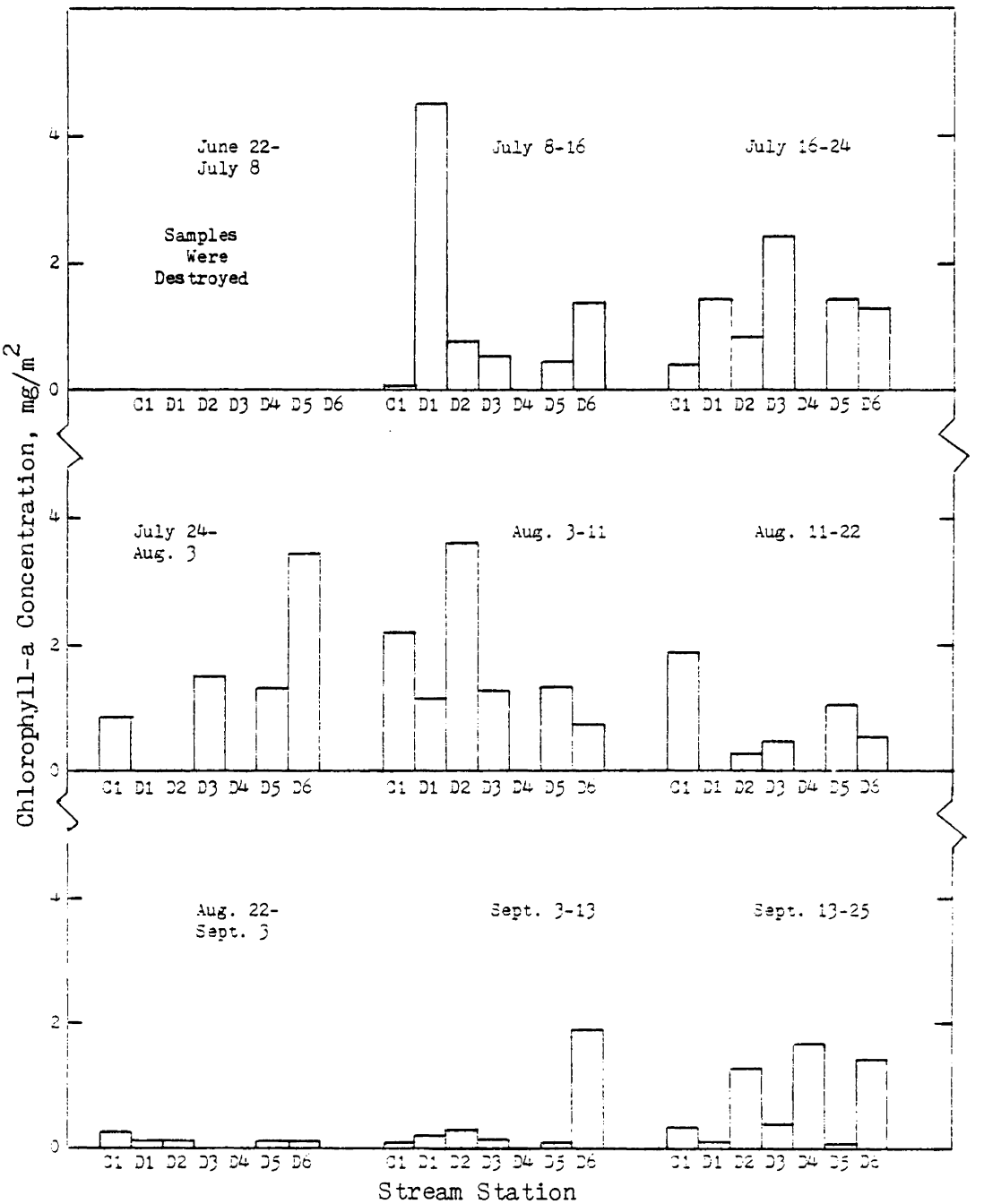

Figure 15. Chlorophyll-a Concentrations of Periphyton at Seven Locations in Culpeper, Virginia, During the Period June Through September, 1981. Dates Indicate Periods of Periphyton Accumulation.

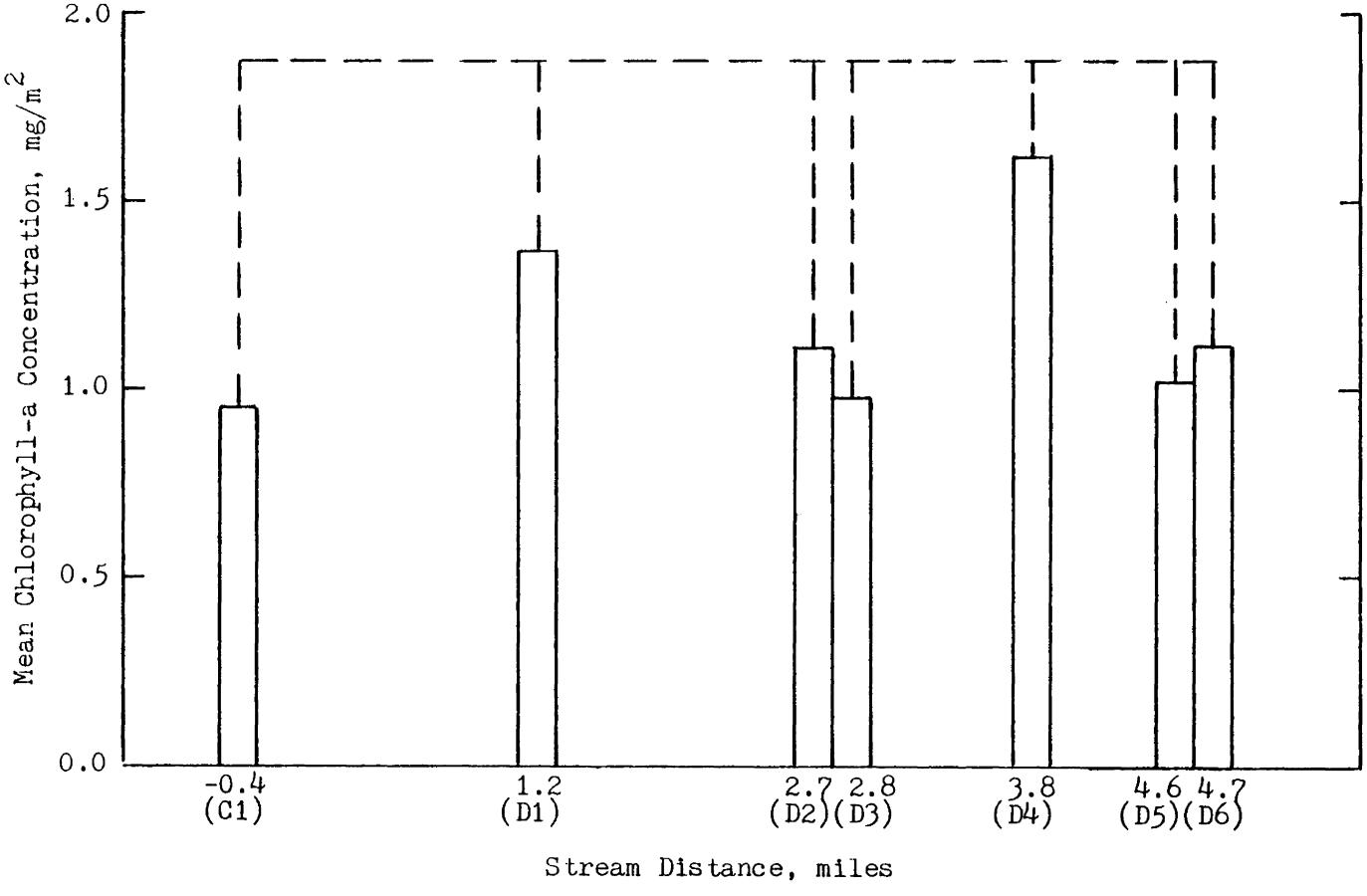

Figure 16. Comparison of the Mean Chlorophyll-a Concentration in Periphyton Total Solids at Seven Stations During the Period June Through Sept., 1981

(Brackets indicate no significant difference between means at the 0.05 level.)

stations, sampling sites D3, D4, and D6.

The mean chlorophyll-a concentrations in the periphyton during each sampling period are shown in Figure 17. The chlorophyll-a concentration in the periphyton was not determined during accrual period one. The ANOVA showed there were no significant differences in chlorophyll-a levels among the accrual periods. However, the Duncan's test showed that the time of sampling did have an influence. Table B18 contains the mean chlorophyll-a concentrations in the periphyton during each of the nine sampling periods. Chlorophyll-a levels during accrual periods two, three, four, five, and nine were similar and thus, grouped together. The periphyton chlorophyll-a concentrations during sampling periods two, three, four, six, eight, and nine were significantly simialr to one another also. The sixth, seventh, eighth, and nineth accrual periods were also grouped together because of similar periphyton chlorophyll-a concentrations. The Chlorophyll-a level after the fifth sampling period was significantly different from those after sampling periods six, seven, and eight. The concentration of chlorophyll-a in periphyton harvested after the seventh accrual period was significantly lower than those harvested after accrual periods two, three, four, and five. Further analysis of the chlorophyll-a data will be presented in the next chapter.

Autotrophic Index

The autotrophic indices derived from analyses of the periphyton from each station are depicted in Figure 18 and in Table B7. The highest individual AI of 22,582 was recorded at station D1 during

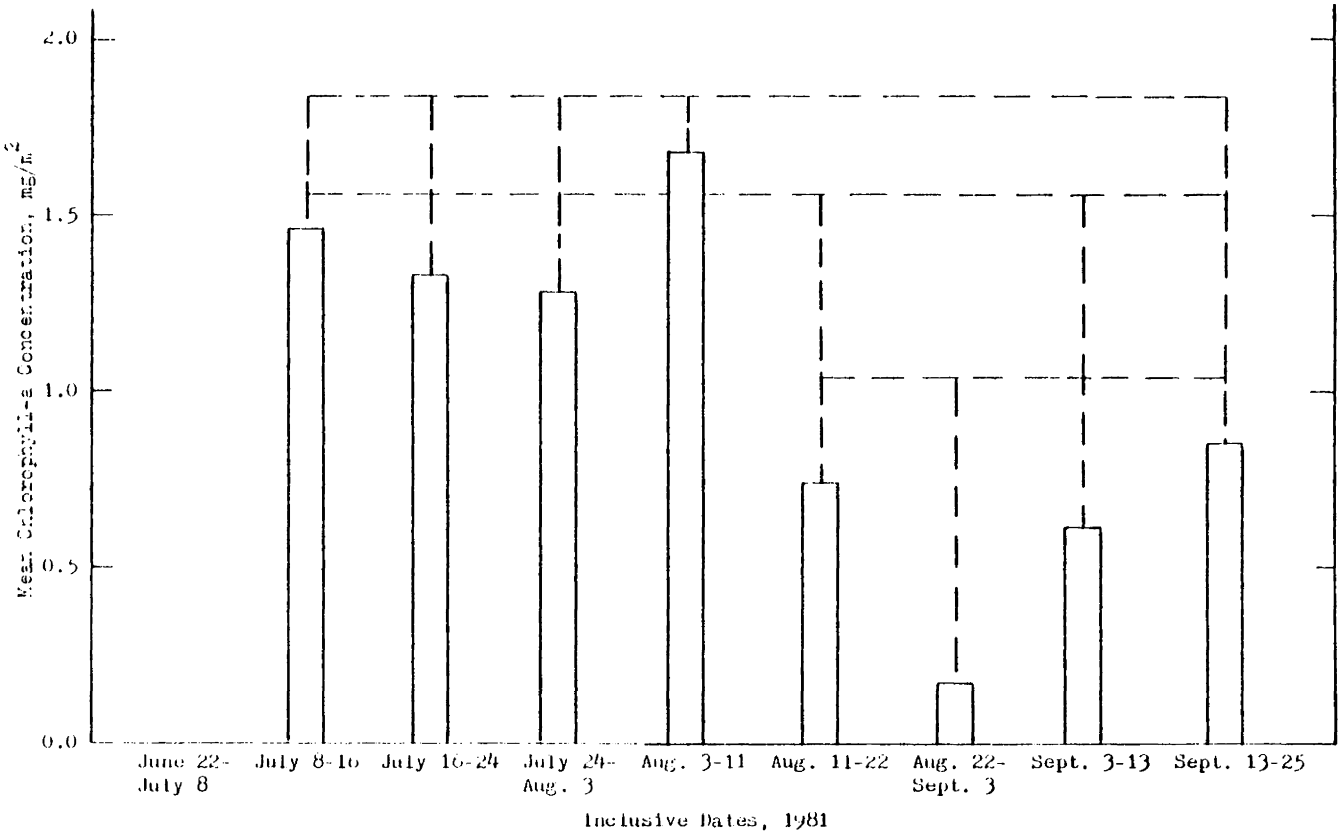

Figure 17. Comparison of the Mean Chlorophyll-a Concentration in the Periphyton During Each of the Nine Accrual Periods

(Brackets indicate no significant difference between means at the 0.05 level.)

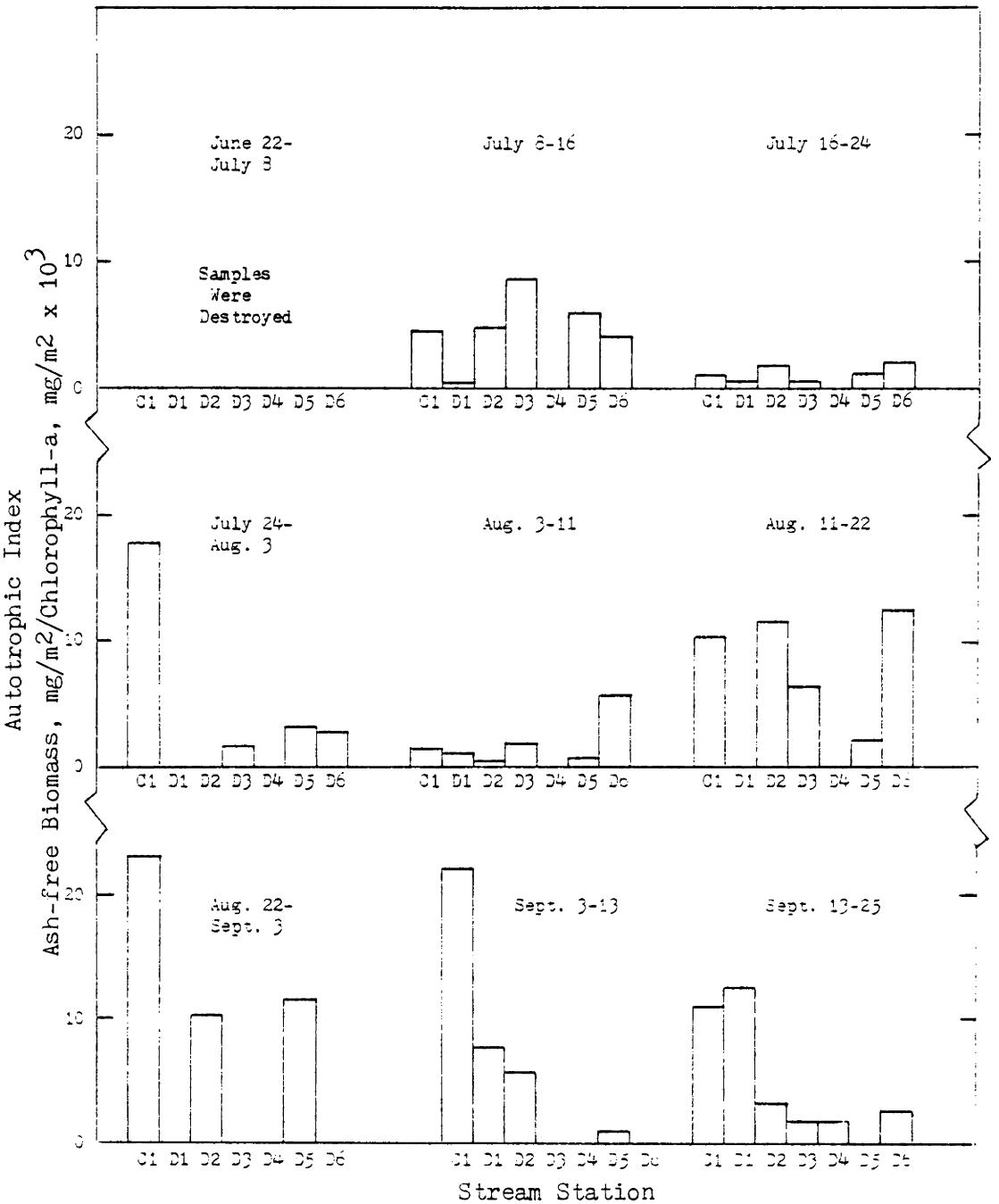

Figure 18. Autotrophic Index of Periphyton at Seven Locations in Culpeper, Virginia, During the Period June Through September, 1981. Dates Indicate Periods of Periphyton Accumulation.

the eighth accrual period. Station D1 also had the lowest AI. During the third accrual period, that station had an AI of only 462. Results of the ANOVA for the AI data are located in Table B1. According to the ANOVA, the location of the sampling site played no role in the determination of the AI; however, the time of sampling did have a significant role in this determination.

The Duncan's test for the mean autotrophic indices from seven stations is located in Figure 19. The greatest mean AI of 10,371 was observed at control site C2 on Jonas Run, while the lowest mean AI of 1490 was found at station D4. The DMRC test revealed significant differences among the mean AI values from different sampling stations. Sites C1, D1, D2, and D4 were found to be significantly similar in their AI values at the 0.05 level. The same was found to be true for stations D1, D3, D4, D5, and D6. The two highest mean autotrophic indices were from samples collected at sites C1 and D2 and these were significantly different from those collected at stations D3, D5, and D6. Control stations C2, C3, and C4 were not significantly different from their corresponding downstream stations, D3, D4, and D6 with respect to their mean autotrophic indices.

The mean autotrophic index of the periphyton collected during each accrual period is shown in Figure 20. The two lowest mean autotrophic indices of 1203 and 1958 were recorded during accrual period three and five, respectively. The mean AI was highest during sampling period seven when it increased to 14,030. The results of the DMRC test are in Table B19. Three groupings of significant similarity were found in the station autotrophic indices. They were: accrual

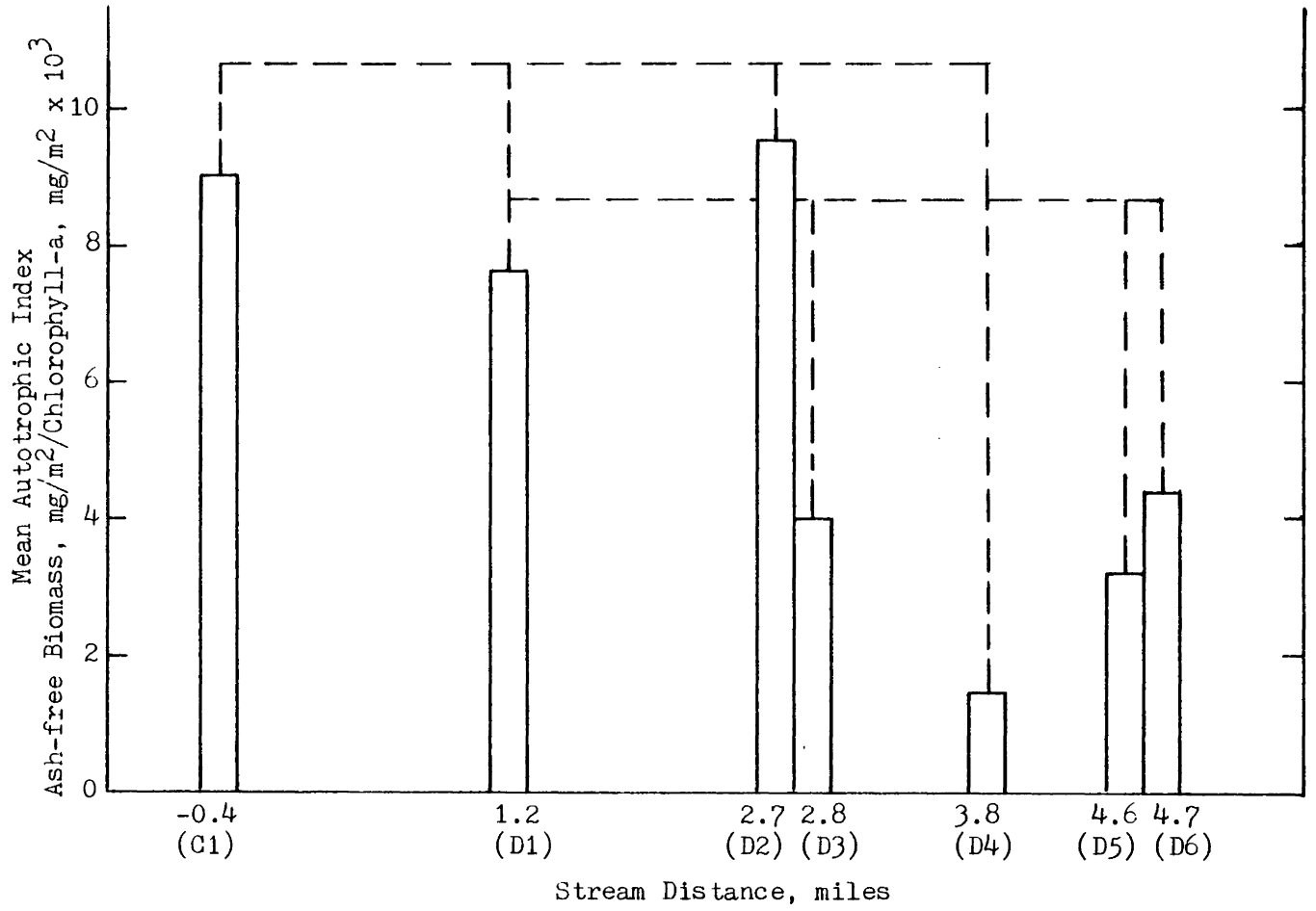

Figure 19. Comparison of the Mean Autotrophic Index at Seven Stations During the Period June Through September, 1981

(Brackets indicate no significant difference between means at the 0.05 level.)

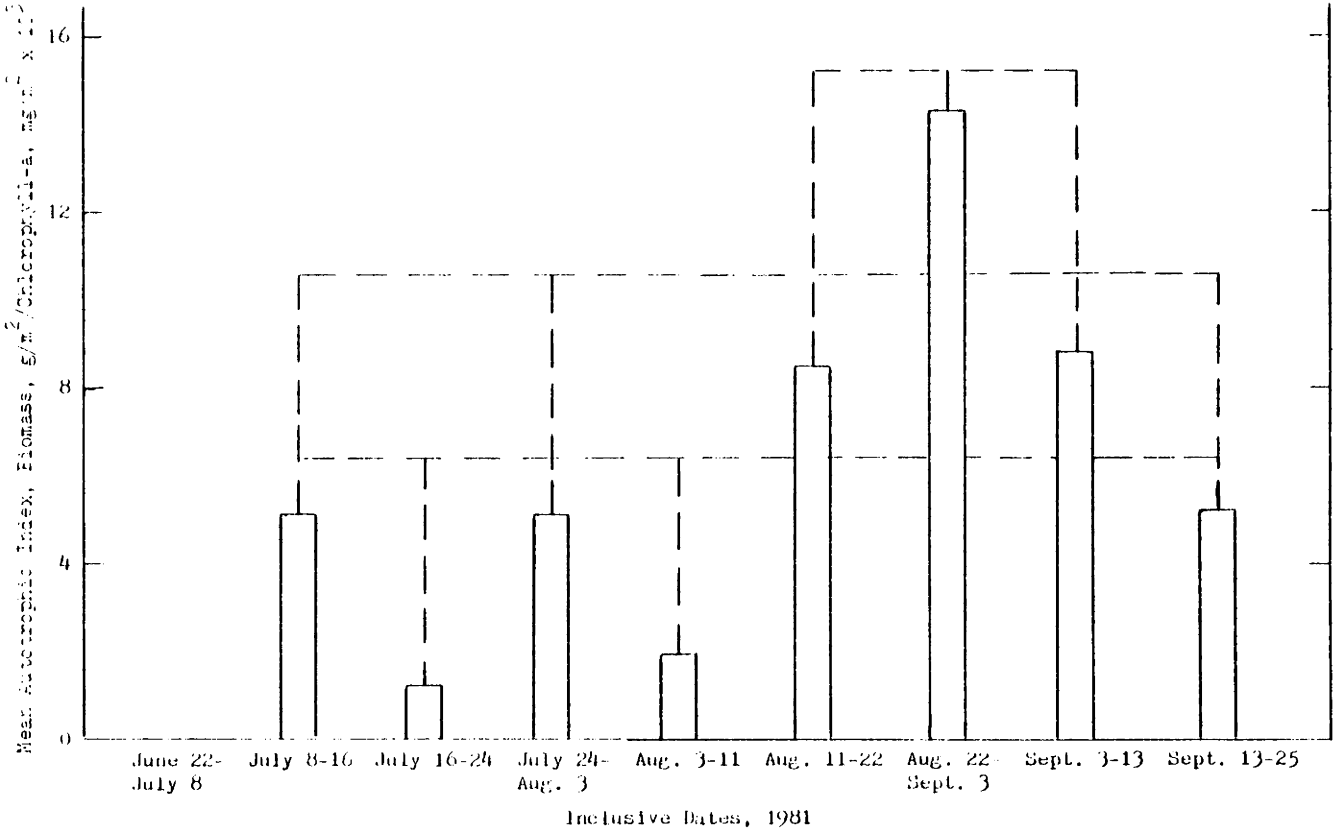

Figure 20. Comparison of the Mean Autotrophic Index of the Periphyton During Each of the Nine Accrual Periods

(Brackets indicate no significant difference between means at the 0.05 level.)

periods two, three, four, five, and nine; periods two, four, six, eight, and nine; and periods six, seven, and eight. The mean autotrophic indices during sampling periods three and five were significantly different from those determined during periods six, seven, and eight. The AI during the seventh accrual period, besides differing significantly from those during periods three and five, also differed from the autotrophic indices from periods two, four, and nine. Explanations of these AI values and trends caused by time of sampling will be disscussed in the next chapter.

Gravimetric Analyses and Weather Data

A weight analysis, consisting of dry and ash-free weight determinations, was conducted on the periphyton samples collected after each accrual period. The total dry weight, ash-free dry weight, and percentages of organics and inorganics in the periphyton samples are presented in Table 3. Periphyton from control site C3 consistently contained the greatest amounts of volatile solids.

Weather data collected during the sampling period are summorized in Table 4. Of the nine accrual periods, four were generally clear with dry weather, while stormy and wetter conditions prevailed during the remaining five. During accrual periods four, five, and six, both of the unnamed streams partially dried-up and ceased to flow, leaving only the deeper pools of water. A discussion of all the data collected in Culpeper, Virginia, is presented in the following chapter.

Table 3. Dry Weight and Volatile Solids Analyses for all Samples Collected in Culpeper, Virginia, During the Period June Through September, 1981

Accrual Period, Inclusive Dates, (1981)	Station	Dry Weight, (g)	Ash-free Weight, (g)	Percent Organic	Mean Percent Organic	Percent Inorganic	Mean Percent Inorganic
June 22-July 3	C1	0.2221	0.0259	13		87	
	D1	0.1873	0.0239	14		86	
	D2	0.0327	0.0099	31	17	69	83
	D3	0.2081	0.0283	14		86	
	D6	0.3942	0.0470	12		88	
July 8-16	C1	0.2887	0.0832	29		71	
	D1	0.0466	0.0223	48		52	
	D2	0.0494	0.0360	72		28	
	D3	0.1132	0.0362	32	51	68	49
	D5	0.0769	0.0412	54		46	
	D6	0.0689	0.0527	31		69	
	C3	0.0227	0.0209	92		8	
July 16-24	C1	0.0274	0.0040	15		85	
	D1	0.0353	0.0062	18		82	
	D2	0.0478	0.0106	23		77	
	D3	0.0633	0.0122	19	20	81	80
	D5	0.0535	0.0113	22		78	
	D6	0.0665	0.0267	16		84	
	C3	0.0035	0.0010	28		72	

(continued)

Table 3. (continued)

Accrual Period, Inclusive Dates, (1981)	Station	Dry Weight, (g)	Ash-free Weight, (g)	Percent Organic	Mean Percent Organic	Percent Inorganic	Mean Percent Inorganic
July 24-Aug. 3	C1	0.5220	0.0718	14		86	
	D3	0.1081	0.0209	19		81	
	D5	0.0893	0.0163	19	26	81	74
	D6	0.2271	0.0454	20		80	
	C3	0.0079	0.0046	58		42	
Aug. 3-11	C1	0.2133	0.0316	15		85	
	D1	0.0174	0.0075	43		57	
	D2	0.0560	0.0197	35		65	
	D3	0.0650	0.0130	20	32	80	68
	D5	0.0449	0.0101	23		77	
	D6	0.3005	0.0377	13		87	
	C3	0.0049	0.0038	78		22	
Aug. 11-22	C1	1.2247	0.1215	14		86	
	D2	0.0916	0.0243	27		73	
	D3	0.0889	0.0170	19	19	79	81
	D5	0.0929	0.0200	21		79	
	D6	0.1665	0.0249	15		85	
Aug. 22-Sept. 3	C1	0.3852	0.0333	9		91	
	D1	0.2573	0.0152	6		94	
	D2	0.1166	0.0195	17	12	83	88

(continued)

Table 3. (continued)

Accrual Period, Inclusive Dates, (1981)	Station	Dry Weight, (g)	Ash-free Weight, (g)	Percent Organic	Mean Percent Organic	Percent Inorganic	Mean Percent Inorganic
Aug. 22-Sept. 3	D5	0.1876	0.0192	11		89	
	D6	0.1942	0.0362	19		81	
	C4	0.1790	0.0154	9		91	
Sept. 3-13	C1	0.4107	0.0478	12		88	
	D1	0.6395	0.0432	10		90	
	D2	0.0330	0.0193	62		38	
	D3	0.0282	0.0070	25		75	
	D5	0.0222	0.0050	23	28	77	72
	D6	0.0650	0.0154	24		76	
	C2	0.0315	0.0072	25		75	
	C3	0.0170	0.0068	56		44	
	C4	0.0391	0.0055	14		86	
Sept. 13-25	C1	0.2070	0.0284	14		86	
	D1	0.2030	0.0393	13		87	
	D2	0.1051	0.0140	19		81	
	D3	0.1037	0.0231	22		78	
	D4	0.1037	0.0231	24		76	
	D5	0.1497	0.0215	14	25	86	75
	D6	0.1385	0.0317	23		77	
	C2	0.0654	0.0226	35		65	
	C3	0.0163	0.0101	62		38	
	C4	0.0321	0.0099	31		69	

Table 4. Weather Data From Culpeper, Virginia, During the Nine Accrual Periods.

Accrual Period, Inclusive Dates, (1981)	General Weather Conditions During Accrual Period
June 22-July 8	Mostly Cloudy with Many Thunderstorms
July 8-16	Fair
July 16-24	Mostly Cloudy with Many Thunderstorms
July 24-Aug. 3*	Mostly Fair
Aug. 3-11*	Fair
Aug. 11-22*	Partly Fair with Occasional showers
Aug. 22-Sept. 3	Very Stormy with Large Amounts of Precipitation
Sept. 3-13	Fair
Sept. 13-25	Partly Fair with a Few Showers

* The two unnamed tributaries partially dried-up during these accrual periods, leaving only the deeper pools of water. The diatometers were in stagnant water.

V. DISCUSSION

Concentrations of the three heavy metals (arsenic, chromium, and copper) were determined in the periphyton from ten sampling locations in Culpeper, Virginia, during the period June through September, 1981. Water samples were taken three times during the sampling period and analyzed for the same three metals. The productivity and chlorophyll-a content of the periphyton were analyzed because periphyton are important primary producers in lotic systems. And finally, the autotrophic index was calculated at each periphyton sampling station to indicate the relative health of that location, in terms of its organic enrichment.

Downstream Distributions of Periphyton Heavy Metals

Arsenic concentrations in periphyton total solids. The periphyton As concentrations at the control station C1, above the spill site, are shown in Figure 3. This station was approximately 0.4 miles above the location where the CWP spill occurred. Low levels of As in the periphyton at this station were recorded at the end of each accrual period. The analysis of variance on the arsenic data showed that the time of sampling did not have any effect on the periphyton As concentrations. A mean of 4 ug As/g periphyton was recorded at station C1. This station was used to determine the background As concentrations in the periphyton of Culpeper, Virgina. The periphyton As concentrations at this site were similar to As concentrations found in periphyton or aquatic algae from uncontaminated waters. Seydel (13) reported the natural As level in the plankton of Lake Superior was 3.34 ug/g. Another report found the algae in arsenic-free waters to contain between 0.05 and 5 ug/g As

(11). It was assumed that the As concentrations found in the periphyton at station C1 were good indicators of the natural background As levels.

The arsenic concentrations found in the periphyton at sampling sites D1, D2, D3, D4, D5, and D6 are located in Figure 3. Station D1, 1.2 miles below the CWP spill site, recorded the highest mean periphyton As concentration of 49 ug/g. Arsenic concentrations in the periphyton at station D1 could not be determined after two of the nine accrual periods because the diatometers were destroyed during these periods. The low As concentration recorded after the fifth accrual period (August 3-11) was significantly lower than other concentrations at this station. This low could have possibly been the result of the very small amount of material collected on the diatometer's slides during this period or because of the stagnation in the water column produced by the low level of water in the stream bed. The DMRC test (Figure 4) showed the mean periphyton As concentration was significantly higher at station D1 than at the control, station C1. This increase is most probably due to the CWP spill on January 31, 1981. The mean periphyton As concentration at station D1 was approximately ten times greater than the concentration at the control station. This periphyton As level was greater than the levels cited in the literature for uncontaminated waters.

The As concentrations in the periphyton at station D2, 2.7 miles below the spill site on the unnamed tributary, are found in Figure 3. Becuase of a damaged diatometer, the periphyton As level was not determined at this station after the fourth accrual period (July 24 August 3). Except for during the seventh accrual period (August 22 - September 3), the periphyton As concentration at this station was consistently lower

than the concentration found at station D1, but still higher than the concentration found at the control, C1. This decrease in arsenic level was probably due to either the increased distance downstream from the CWP spill site or by dilution of the arsenic in a greater flow of water. Under most conditions As does not remain in solution, but either precipitates or adsorbs onto clays, and is removed from the water column (12,13,27). The DMRC test showed the mean periphyton As concentrations at stations D1 and D2 were significantly similar to one another. Also, the Duncan's test showed the As level in the periphyton at station D2 was still significantly higher than the concentration found at control station C1.

The periphyton As levels found at stations D3, D4, D5, and D6 were similar to one another. Their mean As concentrations ranged from a low of 9 ug/g at station D6 to a high of 16 ug/g at station D5. All four of these stations had mean periphyton As concentrations below those found at stations D1 and D2, and from two to four times higher than that found at the control station C1. Results of the DMRC test showed the As concentrations in the periphyton from these four stations were significantly similar to one another and to that of the upstream control as well. The Duncan's test also showed the periphyton As concentrations found at stations D3, D5, and D6 were significantly lower than those found at sampling sites D1 and D2. Station D4 was not significantly different in periphyton As concentration from either stations D2 or D2. This unusual findings was caused by the fact that only one sampling was completed at this station, and this caused the Duncan's test to be inconclusive because of a lack of data. From these analyses, it can be shown

that the periphyton As concentration increased significantly directly below the CWP spill site and then decreased to near background concentrations at a point 2.8 miles below the spill site. All five downstream stations had periphyton with higher than normal As concentrations. This tends to indicate that there was an abnormally high amount of arsenic present in the environment below the CWP spill site.

Chromium concentration in periphyton total solids. The periphyton Cr concentrations at seven stations in Culpeper, Virginia, are found in Figure 6. The Cr level in the periphyton at station C1 was used to determine background concentrations of chromium in the Culpeper area. Except for the second accrual period (July 8 - 16), periphyton Cr concentrations at this station were low. During the second accrual period, a concentration of over 377 ug Cr/g periphyton was found. This value was significantly higher than other Cr concentrations found at this site and could have resulted from either a chromium spill along VA Route 762 (next to the station) or from laboratory contamination of the sample. However, since the blank sample analyzed at the same time did not produce unusually high Cr concentrations, it is questionable whether the sample from site C1 could have become as contaminated as it was through laboratory contamination. Results of the ANOVA showed the time of sampling had no significant impact on the amount of Cr found in the periphyton total solids.

Chromium occurs naturally in the environment and is found in most plant species. In the plant kingdom, plankton contains the greatest amounts of Cr, with concentrations usually averaging 3.5 ppm (14). The mean periphyton Cr concentration for control station C1 was 34 ug/g, ten

times greater than the normal amount. Being located next to VA Route 762, station C1 might have been receiving small amounts of chromium in the runoff from the road.

The Cr concentrations found in the periphyton at sampling site D1, downstream from the CWP spill site, are located in Figure 6. A mean periphyton Cr concentration of 161 ug/g was observed at site D1. The periphyton Cr level for the period July 24 - August 3 is missing because the diatometer at that station was destroyed during the accrual period. During the fifth accrual period no chromium was found in the periphyton at this station. This concentration was significantly lower than the mean of 161 ug/g. It was during this same accrual period and at this same station that the abnormally low As concentration mentioned earlier, was noted. As before, this low Cr reading was probably caused by either the low water level or the small amount of periphyton collected on the diatometer's slides during the accrual period. The DMRC test showed the mean Cr concentration in the periphyton at station D1 was significantly greater than the concentration found at the upstream control. The concentration was over 45 times greater than concentrations recorded in periphyton from uncontaminated waters. This increase in periphyton Cr concentration was probably caused by the CWP spill on January 31, 1981.

The Cr levels found in the periphyton samples from downstream sampling sites D2 through D6 were relatively constant. These concentrations ranged from 48 - 65 ug Cr/g periphyton. Missing periphyton Cr concentations at stations D2, D3, D4, and D5 were due to damaged diatometers.

The mean Cr concentrations in the periphyton total solids from stations D2, D3, D4, D5, and D6 are represented in Figure 7. All five

of these mean Cr concentrations were proven significantly similar to each other by the DMRC test. Duncan's test showed that these five Cr concentrations were significantly similar to the concentrations obtained at upstream control station C1. The mean periphyton Cr concentrations at sampling sites D2, D3, D5, and D6, but not that at D4, were significantly lower than the concentration recorded at station D1. This decrease in the periphyton Cr concentration was probably caused by the increased distance from the CWP spill site. Many forms of chromium will precipitate to the sediments quickly and do not remain in the water column for long periods. This makes the chromium unavailable to the periphyton (14,29,30). The mean Cr level in the periphyton at site D4 was found to be significantly similar to the concentration obtained at station D1. Because only one measurement was obtained at station D4, the result of the Duncan's test was inconclusive.

From the above data analyses, it can be stated that the periphyton Cr concentration increased significantly immediately below the CWP spill site, then decreased significantly at a point 2.7 miles below the spill. The concentration then remained at background levels to a point 4.7 miles below the spill area. All sampling stations had periphyton with higher than normal amounts of chromium. However, the periphyton directly below the area of contamination (site D2) recorded much higher Cr levels than the other five sampling sites.

Copper concentration in periphyton total solids. The periphyton Cu concentrations from seven of the sampling stations in Culpeper, Virginia, are found in Figure 9. The concentration of copper in the

periphyton at station C1 was used to establish background levels of copper in the Culpeper area. The Cu concentrations recorded at this site, 0.4 miles above the CWP spill site, ranged from 19 - 64 ug Cu/g periphyton, with a mean concentration of 33 ug/g. This concentration fell in the range considered normal for aquatic plants in uncontamianted waters. Normal Cu levels for aquatic plants are 1 - 50 ug/g (dry weight) (17). From these data, it was assumed that the stream at control site C1 adequately represented an uncontaminated body of water and that the periphyton Cu concentrations recorded were natural background amounts.

The analysis of variance showed the periphyton Cu concentrations were dependent upon the time of sampling. The mean Cu concentration in the periphyton total solids was highest during the first three accrual periods. During the first accrual period (June 22 - July 8), the highest mean periphyton Cu concentration of 72 ug/g was observed. This mean concentration was significantly greater than any of the other mean concentrations found during the sampling period. Accrual period two (July 8 -16) had the second greatest mean periphyton Cu concentration (51 ug/g). Overall, the Cu concentration in the periphyton total solids decreased through the fourth accrual period, then remained fairly constant until the end of the study.

The Cu concentrations in the periphyton at stations D1, D5, and D6 were very similar to those at control station C1. Missing data were due to damaged diatometers. All Cu concentrations recorded in the periphyton from sites D1, D5, and D6 were within the range considered normal for nonpolluted waters. The DMRC test showed that these three stations were significantly similar in periphyton Cu levels to that at

the upstream control, C1.

The periphyton Cu concentrations at stations D2 and D3 were the only ones to exceed the limit of 50 ug Cu/g periphyton considered normal for a copper-free environment. The copper concentration in the periphyton at station D2 exceeded 50 ug/g once, while the concentration at station D3 exceeded that limit four separate times. The DMRC test showed the mean concentration of Cu in the periphyton at sampling site D2 was significantly greater than the concentrations in the periphyton at stations C1, D5, and D6. This increase in the periphyton copper content at site D2 might have been caused by the CWP spill. It could be that the copper in the waste solution travelled further downstream than either the arsenic or chromium by the time this study was conducted. This would explain why there is a greater concentration of Cu in the periphyton 2.7 miles below the spill than at 1.2 miles below it. The decrease in the periphyton Cu content below station D2 might have been caused by either the precipitation of copper to the sediments or by the dilution of the Cu in a greater flow of water (21,23).

In summary, using the data analyzed above, it can be stated that the copper concentration in the periphyton total solids increased significantly at a point 2.7 miles below the site of contamination, then began to decrease a tenth of a mile further downstream. This decrease in the periphyton Cu level continued to a point 4.7 miles below the spill site. At this point, the Cu content of the periphyton was significantly lower than it had been only 0.8 miles upstream, and was significantly similar to the Cu concentrations recorded in the periphyton at the control site, C1. The mean periphyton Cu content at

sampling sites C1, D1, D3, D4, D5, and D6 all remained within the bounds considered normal for copper-free waters. However, the periphyton at station D2 had a mean concentration higher than the 50 ug Cu/g periphyton considered normal for unpolluted systems. From this data, it can be assumed that this stream, at a point 2.7 miles below the CWP spill site, contained periphyton with abnormally high Cu concentrations.

Arsenic, chromium, and copper in periphyton organic solids. All metals previously measured were expressed in terms of the total dry weight of the periphyton. Dry weight determinations include both the organic and inorganic components of the periphyton, and these fractions vary (49,66,68). Table 3 contains the percentages of organics and inorganics found in the periphyton collected at the ten sampling stations in Culpeper, Virginia. A graphical representation of these data is presented in Figure A1. Siltation in the water column increases the amount of inorganics found in the periphyton total solids. Standard Methods (49) recommends the diatometer slides be placed vertically in the water column to reduce the accumulation of silt and debris.

The amount of silt embedded in the periphyton community is not the only factor determining the percentage of inorganics found in the periphyton sample. Species of algae differ from one another in their inorganic (ash) content. Vollenweider (66) found the ash content of most planktonic algae to be approximately five percent of the total dry weight. He also reported the ash content of some algae (especially those with massive inorganic skeletal structures, such as the diatoms) to be as high as 50 percent or more of the total dry weight. According to Wetzel (67), diatoms predominate in periphyton communities and the

inorganic fraction of their dry weight can be as high as 40 to 60 percent of their total weight. Newcombe (68) found the organic fraction of attached organisms in Sodon Lake, Michigan, to range between 21 and 97 percent. The inorganic content of the periphyton collected in Culpeper during this study was found to range between eight and 94 percent of the total dry weight, with a mean inorganic content of 74 percent.

The total arsenic, chromium, and copper concentrations in the periphyton organic solids from Culpeper are located in Tables A1, A2, and A3, respectiviely. Although the As and Cr concentrations in the periphyton organic solids were much higher than those found in the periphyton total solids, it is important to note that similar trends in the metal concentrations were still evident. Both the As and Cr concentrations were low at control station C1, rose to a higher level at the first downstream D1, then proceeded to decrease further downstream.

The Cu concentrations in the periphyton organic solids were higher that those recorded in the periphyton total solids. However, unlike the As and Cr concentrations, the Cu concentrations did not follow the same trend as was produced when the data were expressed in terms of total solids. The Cu level remained relatively constant over the entire stretch of sampling area, instead of increasing below the source of contamination, then decreasing below station D2. This would tend to indicate that the peaks in periphyton Cu concentrations were actually produced by increases in the inorganic fraction of the samples. This subject will be discussed in greater detail later in this report.

Arsenic, Chromium, and Copper Concentrations in Water

Water samples were collected three times during the period of this study. Table 2 contains the total and dissolved As, Cr, and Cu concentrations found in the water samples collected in Culpeper, Virginia. Most of the metal concentrations in the water were very low, with a majority of their concentrations being at or below the detection limits of the atomic absorption spectrophotometer. Total As, Cr, and Cu concentrations were analyzed in the first and second collections, while dissolved metals were analyzed in the second and third collections. For the most part, the concentrations of As, Cr, and Cu were greater in the total metals analysis than they were in the dissolved metals analysis, which was expected. There did not appear to be any upstream or downstream increase in any of the three metals' concentrations, nor did the date of sampling appear to have any effect in their determination.

Arsenic levels in the water ranged from less than 1.0 - 11.0 ug/l and from less than 1.0 - 2.0 ug/l for the total and dissolved metal samples, respectively. Arsenic concentrations in the range of 0.25 - 180 ug/l are considered normal in most waters (11). The samples collected in Culpeper all fell within this range. These water samples also had concentrations of As which were lower than those required by the EPA for both the irrigation of crops and human consumption (63)

The majority of the water samples analyzed for chromium contained less than 0.05 mg/l, which was below the detection limits of the AA used. Only three of the 25 samples registered Cr concentrations above 0.05 mg/l. A high concentration of 1.07 mg/l Cr was recorded at station

C3, the control for the second unnamed tributary, on August 22, 1981. This value was significantly higher than all others and might have been caused by laboratory contamination; however, blanks analyzed at the same time did not have high concentrations of chromium. It is always possible that there was some type of chromium contamination in this stream above station C3. A survey of American rivers showed normal Cr concentrations ranging from 0.7 - 84 ppb (14). Since these concentrations were below the detection limits of the AA used in this study, it was impossible to determine whether the Culpeper samples were representative of normal water Cr levels, but they were probably within the "normal range". Unfortunately, the EPA criteria limits were also too low to be compared with the results of this study for the same reasons as given above.

The copper concentrations in the water samples collected in Culpeper ranged from less than 0.02 to 0.09 mg/l. Most of the samples' concentrations were below the AA detection limit of 0.02 mg/l Cu, so an exact value could not be determined. Normal Cu levels in uncontaminated waters have been reported to be approximately 0.02 ug/l (18). A few of the Culpeper water samples contained Cu levels greater than 0.02 ug/l. However, all water samples met the EPA drinking water criteria of 1.0 mg/l Cu (63).

Periphyton Concentration Factors

Concentration factors are defined as the ratio of the metal concentration in the organism to that found in the water mass (18). Periphyton have shown the ability to concentrate heavy metals within them-

selves many times the concentration found in the water column (18,26, 35). Concentration factors may be reported higher than they truly are because of the metal-ladened inorganic silt which becomes lodged in their biomass. Because it is difficult to separate the silt from the periphyton, the concentration of a metal in the silt is recorded as if it were actually contained in the periphyton. This increases the metal's concentration in the periphyton sample and subsequently increases the concentration factor.

Periphyton concentration factors were calculated for the three metals involved in this study. Arsenic concentration factors as high as 10,000 have been reported in some plankton (26,35). The As concentration factors recorded for the Culpeper periphyton were much lower. Arsenic was concentrated in the periphyton a maximum of 8,800 times over the concentration found in the water during this study. Most of the As concentration factors were between 1,000 and 1,500. Chromium did not concentrate within the periphyton and the concentration factors for copper were very low, ranging from zero to two.

Primary Productivity of Culpeper Sampling Stations

Periphyton communities are the major primary producers in streams and small rivers. Most other components of a stream ecosystem depend upon the periphyton for their continued survival. Periphyton primary productivity is defined as the quantity of new organic material produced by the photosynthesis of these organisms (49,56). The primary production rates determined at seven sampling station in Culpeper, Virginia are shown in Figure 12. The ANOVA showed both the time and

location of sampling had a significant effect on the periphyton productivity.

As mentioned above, the date of the periphyton sampling played a significant role in determining the primary productivity. Figure 12 shows the comparison of the mean periphyton primary productivity during each of the nine accrual periods. The productivity varied considerably from one accrual period to the next. The DMRC test showed the productivity increased significantly from the first accrual period to the second, decreased significantly from the second to the third, and continued to follow this pattern of alternately increasing and decreasing until the seventh accrual period (August 22 - September 3). There was no significant variation in the productivity after this date.

The erratic periphyton productivity was probably a result of a number of different factors. It is likely that the weather during each accrual period had a noticeable effect on the periphyton production. Table 4 contains the weather data collected during this study. Accrual periods during which heavy rains occurred also produced the lowest mean productivities. Large amounts of agricultural runoff caused greater flow velocities, more silt, and larger quantities of debris to be present in the streams. These conditions are not conducive to prolific periphyton growth. Another contributing factor to the erratic productivity could have been caused by the cattle grazing near the streams. On warm summer days many cattle were found in the sampling streams. The organic loads these cattle added to the streams could have increased or decreased the rate of production during certain periods, depending on the quantity of the organics.

A comparison of the mean primary production rates at seven of the sampling locations in Culpeper, Virginia, is shown in Figure 11. The location of the sampling station produced differences in the periphyton production rates, as shown by the ANOVA. Productivity was consistently lower at stations D1, D2, D3, D4, and D5. These sampling sites were in the midst of cow pastures. Their low productivities could have resulted from the high organic loading these streams received from the cattle. Increased organic loading has been shown to stimulate bacterial growth (67). The filamentous bacterium, Sphaerotilus, and the heterotrophic component of the periphyton compete with the autotrophs for nutrients in the organic waste and systematically "squeeze out" the photosynthetic component of the periphyton, causing a decrease in primary production (67,69). Also, the increased turbidity caused by disturbances of the stream bed by the cattle in the stream could have limited the amount of light which reached the periphyton and, thus, decreased their production. Another reason for the decrease in primary productivity at these stations could have been the toxicity of the heavy metals in the CWP spill. Heavy metals can exert toxic actions upon periphyton and limit their growth (36,52,57). The very low mean production rate found at station D3 was probably caused by a combination of the above reasons and by the slower stream current found at this site. Because Jonas Run was partially dammed by debris just below sampling site D3, the water at this station was much deeper and the current much slower than at other stations. Streams with slow flow velocities have been shown to exhibit lower primary production rates (49).

Stations C1 and D6 exhibited higher mean production rates than

the other five sampling stations. The high primary productivity at control site C1 was probably due to the fact that this location was in a sunny region, free from the effects of the grazing cattle. The primary productivity at station D6, on Mountain Run, was lower than the productivity at station C1, but higher that at the other five sites. The diatometer at this station was located in a mostly sunny, riffle area. These conditions are more conducive to the growth of the periphyton and, thus, the result can be a higher rate of primary productivity (46,49).

Primary production rates have been used as an indicator of the quality of a water system. In general, high production is indicative of a more organically enriched ecosystem. In some cases, such as in an area contaminated by a toxic agent, the productivity is actually lowered (69). Wetzel (67) calculated the general range of primary productivity rates in the phytoplankton associated with lake systems. These production rates are shown in Table 5. Based on the values for lake systems, the streams of Culpeper, Virginia, would be classified as mesotrophic to eutrophic. These classifications reflect the high degree of organic enrichment in these streams. Agricultural runoff contains many nutrients and organics which tent to cause eutrophication (67,69). The results obtained in this study indicate that a toxic substance, if present, did not affect the rate of periphyton primary production.

Periphyton Chlorophyll-a Content

The concentrations of chorophyll-a in the periphyton at seven of

Table 5. General Range of Primary Productivity of Phytoplankton of Lakes of Different Trophic Categories*

Trophic Type	Mean Primary Productivity, $g/m^2/day$
Ultra-oligotrophic	<0.01 (<0.005 g $C/m^2/day$)
Oligotrophic	0.01 - 0.06 (0.005 - 0.03 g $C/m^2/day$)
Mesotrophic	0.05 - 2.0 (0.025 - 1.0 g $C/m^2/day$)
Eutrophic	>2.0 (>1.0 g $C/m^2/day$)

* Modified from Wetzel (67). Measured by the ^{14}C uptake method (g $C/m^2/day$) and converted to organic matter ($g/m^2/day$). Assumption is make that carbon is equal to 50 percent of total organic biomass (67).

the Culpeper sampling stations are shown in Figure 15. Results of the ANOVA showed there were no station effects on these concentrations. Mean chlorophyll-a contents of the periphyton at these sampling sites ranged between 0.86 mg/m^2 and 1.64 mg/m^2. (See Figure 16).

A comparison of the mean chlorophyll-a content of the periphyton during each of the accrual periods is shown in Figure 17. The periphyton samples from the first accrual period (June 22 - July 8) were destroyed during the analysis. The time of sampling proved to be significant in the determination of the periphyton chlorophyll-a content. Starting with the second accrual period (July 8 - 16) and continuing until the end of the fifth (August 3 - 11), the periphyton chlorophyll-a concentration remained high. Most likely, a good ecological balance existed between the autotrophic and heterotrophic segments of the periphyton population during this period. However, the heterotrophic component of the population must have become dominant during the period of August 11 - September 25 because of the extremely small amounts of chlorophyll-a present in the periphyton during that period.

To support the above hypothesis, the primary productivity was low during three of these four last accrual periods. During the sixth period (August 11 -22), when the periphyton chlorophyll-a content was low and the productivity was high, a large heterotrophic population most likely accounted for the majority of the production produced on the substrates. Wührmann (69) explained that areas directly below organically enriched pollution outfalls become dominated by the heterotrophs. In such areas, the production of these heterotrophs may be wrongly re-

ported as primary productivity. Most of the sampling stations in Culpeper were subject to high organic loadings.

Autotrophic Indices of Culpeper Sampling Stations

To relate changes in the periphyton species diversity to changes in the water quality, the autotrophic index can be employed. The AI is the ratio of periphyton biomass to the chlorophyll-a content of that periphyton (49). An analysis of variance showed the time of sampling played a significant role in calculating the AI. The location of that sampling station also proved to be a determining factor. The autotrophic indices of the periphyton at seven of the ten Culpeper sampling stations are presented in Figure 18. The AI could not be determined during the first accrual period (June 22 - July 8) because the samples were destroyed during analysis.

Overall, the mean autotrophic indices at all sampling stations were greater than 200, values which are typical of organically polluted waters. Stations C1, D1, and D2 had the three highest mean autotrophic indices. The mean AI at each of these stations was over 7000, reflecting the great abundance of heterotrophs in the periphyton population. Factors which affect the concentration of the phytopigments in the periphyton can also affect the AI. Shading and turbidity are two such controlling factors. Stations D3, D4, D5, and D6 had mean autotrophic indices which were slightly lower than the other three stations. High autotrophic indices were expected at all of the stations downstream from the CWP spill site because of their high organic load. Even though control site C1 was not exposed to the high organic load experienced

by the downstream station, the AI reported at this station was characteristic of an organically polluted waterway. Obviously, the periphyton community at this station was heavily dominated by a heterotrophic population.

The comparison of the mean autotrophic indices during each accrual period is shown in Figure 20. The date effect could be attributed to the changes in periphyton biomass and community structure caused by climatic factors (49). The lower mean autotrophic indices experienced during the second through fifth accrual periods reflected a periphyton population with a lesser dominance by the heterotrophs. The high autotrophic indices found during the last four accrual periods coincide with the low mean periphyton chlorophyll-a concentrations recorded during this same period. The mean AI values of 5,300, 8,500, 9,100, and 14,000 recorded during the last four accrual periods reflected the near total dominance by the heterotrophic portion of the periphyton community.

Relationships Among Periphyton Heavy Metal Concentrations

Figures A2, A3, and A4 are graphical comparisons of the Cr and As, Cu and As, and Cu and Cr periphyton concentrations, respectively, at all sampling stations. Correlation coefficients for these comparisons are shown in Table A4. A highly significant correlation of 0.60 was obtained from the comparison of the Cr to the As periphyton concentrations. This correlation meant that as the As concentration in the periphyton increased, a similar increase was noted in the periphyton Cr levels. However, this correlation did not necessarily mean that the

change in one variable caused the change in the other variable, but that a mutual dependence existed between the two. It is possible, though, that a complex was formed between arsenic and chromium which truly made each metal dependent upon the ohter's concentration. If this were the case, metal synergism could play an important role. This could not be proven, however. No correlations were found between the Cu and As or Cu and Cr periphyton concentrations. The concentrations of these metals were independant of each other.

Periphyton Metal Concentrations and Primary Productivity

The three heavy metals involved in this study have been shown to affect algae and periphyton in many ways. Some of these effects have included: decreased nitrogen fixation, growth inhibition, reduced production rates, adverse morphological changes, and decreased photosynthesis. Lowman (40) discovered that low concentration of chromium in water could inhibit the growth of most species of algae. Steeman and Kamp-Nielsen (38) found that ionic copper reduced the photosynthesis and growth rates in two types of plankton. Following heavy metal contamination, periphyton have shown an amazing ability to recover to their normal growth and productivity rates (6,43,57).

The graphical comparisons between the productivities and the periphyton As, Cr, and Cu concentrations are located in Figures A5, A6, and A7. Neither the substrates at stations with high arsenic concentrations nor those with high copper concentrations had lower periphyton productivities. However, there was a significant negative correlation

of -0.27 between the periphyton chromium concentrations and the primary productivity. This meant the stations with high periphyton chromium concentrations had low primary productivities. Station D1, which had the highest periphyton Cr concentration, had one of the lowest primary production rates. The station with the greatest productivity (control site C1) had the fourth lowest periphyton Cr concentration.

It is impossible to determine whether this decrease in periphyton production was caused solely by the increased concentrations of chromium in the periphyton. There were many contaminating substances present in the streams sampled. Without elaborate testing, it is difficult to determine which substance or condition produced this decrease in periphyton primary productivity.

Periphyton Metal Concentrations and Chlorophyll-a

Graphical comparisons of the periphyton arsenic, chromium, and copper concentrations and the chlorophyll-a content of the periphyton are located in Figure A8, A9, and A10. No negative or positive correlations of any significance were reported. See Table A4. There were no relationships between any of the metals and the chlorophyll-a concentrations.

Sampling stations D1 and D2, which had the highest mean periphyton As concentrations, were the sites where two of the highest mean periphyton chlorophyll-a levels occurred. These high concentrations of arsenic did not seem to cause lower levels of chlorophyll-a in the algae. On the other hand, the periphyton at station D4 had one of the lowest mean As concentrations, but also had the highest chlorophyll-a content.

Also, the high concentrations of chromium and copper did not produce periphyton with lower amounts of chlorophyll-a.

As mentioned earlier, heavy metal contamination has been shown to decrease algal photosynthesis and reduce the amount of chlorophyll present in aquatic algae (38,39,40). Such effects could not be demonstrated from the results of this study. However, this study did not begin until five months after the CWP spill.

Periphyton Metal Concentrations and Autotrophic Indices

Graphical comparisons of the periphyton As, Cr, and Cu concentrations and the station autotrophic indices are in Figures A11, A12, and A13. There were no correlations between high periphyton metal concentrations and poor water quality (indicated by AI values over 200). It is highly unlikely that the As, Cr, or Cu concentrations in the periphyton caused the heterotrophic periphyton population to dominate at all sampling stations.

Station C1, which exhibited the lowest concentration of arsenic in the periphyton, had the second greatest AI. The poor water quality at this station was probably produced by some type of contamination other than arsenic, because station C1 was located above the site of As contamination. Stations D1 and D2, whose periphyton recorded the greatest levels of As, also had very high autotrophic indices. The high AI values at these two stations were most probably caused by the high organic load entering the streams at these two points. The remaining four downstream sampling stations, D3, D4, D5, and D6, had lower periphyton As concentrations along with lower AI values.

The trends were much the same with the relationships between the chromium and copper levels in the periphyton and the station autotrophic indices. The station AI was not dependent upon the concentration of either of these two metals. The poor water quality, as indicated by the high AI values, was most probably caused by the high organic load these streams received from the surrounding agricultural area.

Periphyton Metal Concentrations, Inorganic Weight, and Organic Weight Percentages

Periphyton dry weight and ash-free dry weight were used in the calculation of the percentages of organics and inorganics found in the periphyton at each sampling station. It was hoped that these data could show which portion of the samples really were collecting the metals As, Cr, and Cu. Because of the turbidity in the streams' water at nearly all stations, a large amount of silt was collected on the diatometer's slides along with the periphyton. It was impossible to separate the silt from the periphyton before digestion, so the combination was analyzed for these three metals.

Organic Weight. Figures A14, A15, and A16 contain the graphical comparisons of the periphyton As, Cr, and Cu concentrations and the percentages of organic matter found in the periphyton samples. The correlation coefficients corresponding to these figures are in Table A4. No correlation was recorded between either the arsenic or chromium concentrations in the periphyton and the sample's percentage of organic material. This reveals that the As and Cr concentrations in the periphyton were not dependent upon the organic component of the sample.

The amount of organics in the periphyton sample did not affect these metals' concentrations.

A highly significant correlation of 0.60 was obtained in the comparison of the periphyton copper level to the sample's organic content. This showed that as the sample's organic content increased, a greater concentration of copper was found in the periphyton. This leads one to believe that the copper was associated with the periphyton rather than adsorbed onto the silt in the sample.

Inorganic Weight. The comparisons of the periphyton metal concentrations to the inorganic fractions in these samples are found in Figures A17, A18, and A19. The results of these comparisons were very similar to the results in the preceding section. This is so because the inorganic percent is the complement of the organic percent. (% inorganic = 100% - % organic). The arsenic and chromium levels in the periphyton were not associated with the percent of inorganic sediment in the sample, as expected. As in the section above, a highly sigfinicant correlation coefficient of -0.60 was recorded between the percentage of inorganic silt and the amount of copper in the periphyton samples. This negative correlation meant that as the periphyton copper concentration increased, the percent of inorganic sediment in the sample decreased. This indicated that the copper was not associated with the inorganic or silt fraction of the sample.

VI. SUMMARY AND CONCLUSIONS

A summary of the major findings of this study are as follows:

1. Regarding arsenic concentrations in periphyton total solids:
   a. Mean periphyton arsenic concentrations were greatest (49 ug/g) at station D1, 1.2 miles below the CWP spill site.
   b. Mean arsenic concentrations in periphyton total solids increased downstream to 1.2 miles and then decreased continually to station D6, 4.7 miles downstream. Arsenic concentrations in the periphyton were significantly higher at stations D1 and D2 than at the control, C1, or downstream sampling sites, D3, D5, and D6.
   c. Mean periphyton arsenic concentrations at all downstream stations were higher than the concentrations cited in the literature as normal for uncontaminated waters.
   d. Arsenic concentrations in the periphyton did not appear to be time dependent. That is, the quantity of arsenic appearing in the periphyton was not related to the time of year the accrual took place.
   e. There were no significant relationships between the periphyton arsenic levels and either the percent of organics or inorganics in the periphyton samples.
2. Regarding chromium concentrations in periphyton total solids:
   a. Mean chromium concentrations in periphyton total solids were highest (161 ug/g) at downstream station D1.

b. The periphyton chromium concentration increased at site D1, 1.2 miles below the CWP spill site and then decreased to near background concentrations at site D2, 2.7 miles downstream. The periphyton at station D1 had a mean chromium concentration which was significantly greater than all other mean chromium concentrations.

c. All sampling sites had periphyton with above normal concentrations of chromium (i.e. 3.5 ppm), including the upstream control, station C1.

d. Chromium concentrations in the periphyton did not appear to be related to the time of year the sample was collected.

e. There were no correlations between the chromium concentrations in the periphyton and either the percentage of organics or inorganics in the periphyton sample.

3. Regarding copper concentrations in periphyton total solids:

a. Mean periphyton copper concentrations were greatest (53 ug/g) at the second downstream sampling site, D2, 2.7 miles below the site of contamination.

b. Mean copper levels in periphyton total solids increased downstream to station D2, then decreased to station D6, 4.7 miles downstream. The mean periphyton copper concentration was significantly higher at site D2 than at control site C1 or downstream stations D5 and D6.

c. All sampling stations, except site D2, had mean periphyton copper concentrations which were normal for uncon-

taminated waters (i.e. 50 ppm).

d. According to Duncan's Multiple Range test, time of sampling did affect the periphyton copper level. Periphyton collected during the first three accrual periods contained the greatest concentrations of copper.

e. A significant relationship was noted between the periphyton copper concentrations and both the percent of organics and inorganics in the periphyton samples. The copper appeared to be associated with the organic segment in the periphyton samples.

4. Regarding periphyton primary productivity:

a. Mean periphyton productivity was greatest at control station C1, 0.4 miles above the site of contamination.

b. Periphyton productivity was significantly affected by both the location and time of sampling.

c. A negative correlation was observed between the periphyton chromium concentration and the periphyton productivity. No significant relationships were observed between periphyton arsenic or copper concentrations and periphyton productivity.

5. Regarding periphyton chlorophyll-a concentrations:

a. Location of the sampling station did not affect the periphyton chlorophyll-a level.

b. The chlorophyll-a concentration in the periphyton was dependent upon the date of sampling. Significantly

higher chlorophyll-a levels were observed in the periphyton during the first half of this study.

c. No correlations were found between the periphyton arsenic, chromium, or copper concentrations and the periphyton chlorophyll-a level.

6. Regarding autotrophic index values:

a. The location and time of sampling significantly affected the periphyton autotrophic index.

b. All sampling stations recorded autotrophic indices over 200, indicating organically enriched waters.

c. No significant relationships were observed between station autotrophic indices and periphyton arsenic, chromium, or copper concentrations.

The significant conclusions derived from this study are:

1. The major effect of the CWP toxic spill, which occurred on January 31, 1981, on the periphyton communities downstream was noted most at sampling site D1, 1.2 miles below the discharge point. The periphyton at this site did collect significant amounts of the three metals; arsenic, chromium, and copper.

2. Many of the high periphyton metal concentrations were above the concentrations considered normal for unpolluted waters. However, for the most part, the metal concentrations in the periphyton did not significantly affect the periphyton productivity (chromium being an exception), chlorophyll-a concen-

trations, or the station autotrophic index.

3. Considering the above facts, the Culpeper Wood Preserver spill of January 31, 1981, probably did not cause any long-term, irreversible damage to the primary producer organisms and other components of the periphyton community in the streams into which the waste flowed.

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APPENDIX A

Tables and Figures Noted in

Discussion Section

Table A1. Total Arsenic, Chromium, and Copper Concentrations in Periphyton Organic Solids from Culpeper, Virginia, During the Period June Through September, 1981

Station	Accrual Period, Inclusive Dates, (1981)	Total As Concentration, (ug/g)	Mean Total As Concentration, (ug/g)	Total Cr Concentration, (ug/g)	Mean Total Cr Concentration, (ug/g)	Total Cu Concentration, (ug/g)	Mean Total Cu Concentration, (ug/g)
C1	June 22-July 8	10.89		33.41		399.92	
	July 8-16	3.03		1,301.55		184.03	
	July 16-24	46.46		363.40		424.00	
	July 24-Aug. 3	48.71		190.93		142.21	
	Aug. 3-11	46.93	33.08	214.00	358.75	162.67	236.91
	Aug. 11-22	27.57		176.86		135.57	
	Aug. 22-Sept. 3	55.67		383.22		314.33	
	Sept. 3-13	33.83		229.67		174.42	
	Sept. 13-25	24.69		335.69		195.00	
D1	June 22-July 8	877.95		313.84		449.43	
	July 8-16	108.50		239.67		95.87	
	July 16-24	480.44		1,111.94		230.78	
	July 24-Aug. 3	*		*		*	
	Aug. 3-11	5.12	458.81	0.00	809.36	63.88	260.21
	Aug. 11-22	*		*		*	
	Aug. 22-Sept. 3	349.00		1,538.17		442.83	
	Sept. 3-13	313.50		998.70		256.00	
	Sept. 13-25	1,017.15		1,463.38		282.69	
D2	June 22-July 3	110.00		148.86		396.97	
	July 8-16	105.00		79.75		146.19	
	July 16-24	137.95		410.36		230.78	
	July 24-Aug. 3	*		*		*	

(continued)

Table A1 (continued)

Station	Accrual Period, Inclusive Dates, (1981)	Total As Concentration, (ug/g)	Mean Total As Concentration, (ug/g)	Total Cr Concentration, (ug/g)	Mean Total Cr Concentration, (ug/g)	Total Cu Concentration, (ug/g)	Mean Total Cu Concentration, (ug/g)
D2	Aug. 3-11	73.08	82.28	146.20	202.07	85.97	172.72
	Aug. 11-22	83.89		200.30		150.22	
	Aug. 22-Sept. 3	21.65		194.35		159.88	
	Sept. 3-13	51.66		97.60		80.37	
	Sept. 13-25	75.05		339.16		131.49	
D3	June 22-July 8	60.37		54.03		642.77	
	July 8-16	39.54		204.91		106.76	
	July 16-24	97.47		341.58		185.00	
	July 24-Aug. 3	82.21		289.89		186.05	
	Aug. 3-11	85.85	64.68	303.35	327.50	183.15	241.99
	Aug. 11-22	83.89		331.58		165.79	
	Aug. 22-Sept. 3	*		*		*	
	Sept. 3-13	33.72		202.28		219.16	
	Sept. 13-25	34.41		892.36		247.27	
D4	June 22-July 8	*		*		*	
	July 8-16	*		*		*	
	July 16-24	*		*		*	
	July 24-Aug. 3	*		*		*	
	Aug. 3-11	*	49.59	*	295.14	*	220.36
	Aug. 11-22	*		*		*	
	Aug. 22-Sept. 3	*		*		*	
	Sept. 3-13	*		*		*	
	Sept. 13-25	49.59		295.14		220.36	

(continued)

Table A1. (continued)

Station	Accrual Period Inclusive Dates, (1981)	Total As Concentration, (ug/g)	Mean Total As Concentration, (ug/g)	Total Cr Concentration, (ug/g)	Mean Total Cr Concentration, (ug/g)	Total Cu Concentration, (ug/g)	Mean Total Cr Concentration, (ug/g)
D5	June 22-July 8	*		*		*	
	July 8-16	49.19		38.91		66.70	
	July 16-24	115.73		288.91		138.18	
	July 24-Aug. 3	110.17		315.22		190.50	
	Aug. 3-11	90.56	73.68	270.69	267.61	181.09	177.98
	Aug. 11-22	80.67		216.71		147.90	
	Aug. 22-Sept. 3	68.40		442.80		341.90	
	Sept. 3-13	44.73		242.23		186.36	
	Sept. 13-25	30.00		325.43		171.28	
D6	June 22-July 8	52.25		47.40		372.00	
	July 8-16	31.42		141.68		90.81	
	July 16-24	45.62		261.24		174.19	
	July 24-Aug. 3	56.15		187.25		125.95	
	Aug. 3-11	133.25	53.12	445.00	241.88	249.83	184.48
	Aug. 11-22	60.07		333.60		186.93	
	Aug. 22-Sept. 3	32.16		218.58		183.21	
	Sept. 3-13	31.33		225.21		151.79	
	Sept. 13-25	35.86		317.00		125.59	
G2	June 22-July 8	*		*		*	
	July 8-16	*		*		*	
	July 16-24	*		*		*	
	July 24-Aug. 3	*		*		*	
	Aug. 3-11	*	39.98	*	241.97	*	203.70
	Aug. 11-22	*		*		*	

(continued)

Table A1. (continued)

Station	Accrual Period, Inclusive Dates, (1981)	Total As Concentration, (ug/g)	Mean Total As Concentration, (ug/g)	Total Cr Concentration, (ug/g)	Mean Total Cr Concentration, (ug/g)	Total Cu Concentration, (ug/g)	Mean Total Cu Concentration, (ug/g)
C2	Aug. 22 Sept. 3	*		*		*	
	Sept. 3-13	36.04		162.80		186.04	
	Sept. 13-25	43.93		321.14		221.36	
C3	June 22-July 8	*		*		*	
	July 8-16	10.87		0.00		1,331.52	
	July 16-24	35.41		265.59		177.04	
	July 24-Aug. 3	20.93		153.49		237.20	
	Aug. 3-11	16.54	21.00	330.87	144.96	202.19	389.44
	Aug. 11-22	*		*		*	
	Aug. 22-Sept. 3	*		*		*	
	Sept. 3-13	13.45		57.61		326.48	
	Sept. 13-25	28.78		62.21		62.21	
C4	June 22 July 8	*		*		*	
	July 8-16	*		*		*	
	July 16-24	*		*		*	
	July 24 Aug. 3	*		*		*	
	Aug. 3-11	*	39.28	*	408.92	*	273.61
	Aug. 11-22	*		*		*	
	Aug. 22-Sept. 3	57.25		521.25		368.00	
	Sept. 3-13	51.43		410.36		342.93	
	Sept. 13-25	9.17		295.14		109.90	

* Diatometers at these stations were damaged during the accrual period.

Table A2. Correlation Coefficients Between the Sampling Parameters From Culpeper, Virginia

Sampling Parmeter / Sampling Parameter	Sampling Parameter		
	Arsenic	Chromium	Copper
Chromium	0.6054**		
Copper	-0.0264	-0.1006	
Primary Production	-0.1150	-0.2670*	-0.0513
Chlorophyll-a	0.1751	0.1286	-0.0762
Autotrophic Index	0.0193	-0.1704	-0.0211
Percent Organic	0.1148	0.0089	0.6001**
Percent Inorganic	-0.1128	-0.0074	-0.6022**

* Correlation significant

** Correlation highly significant

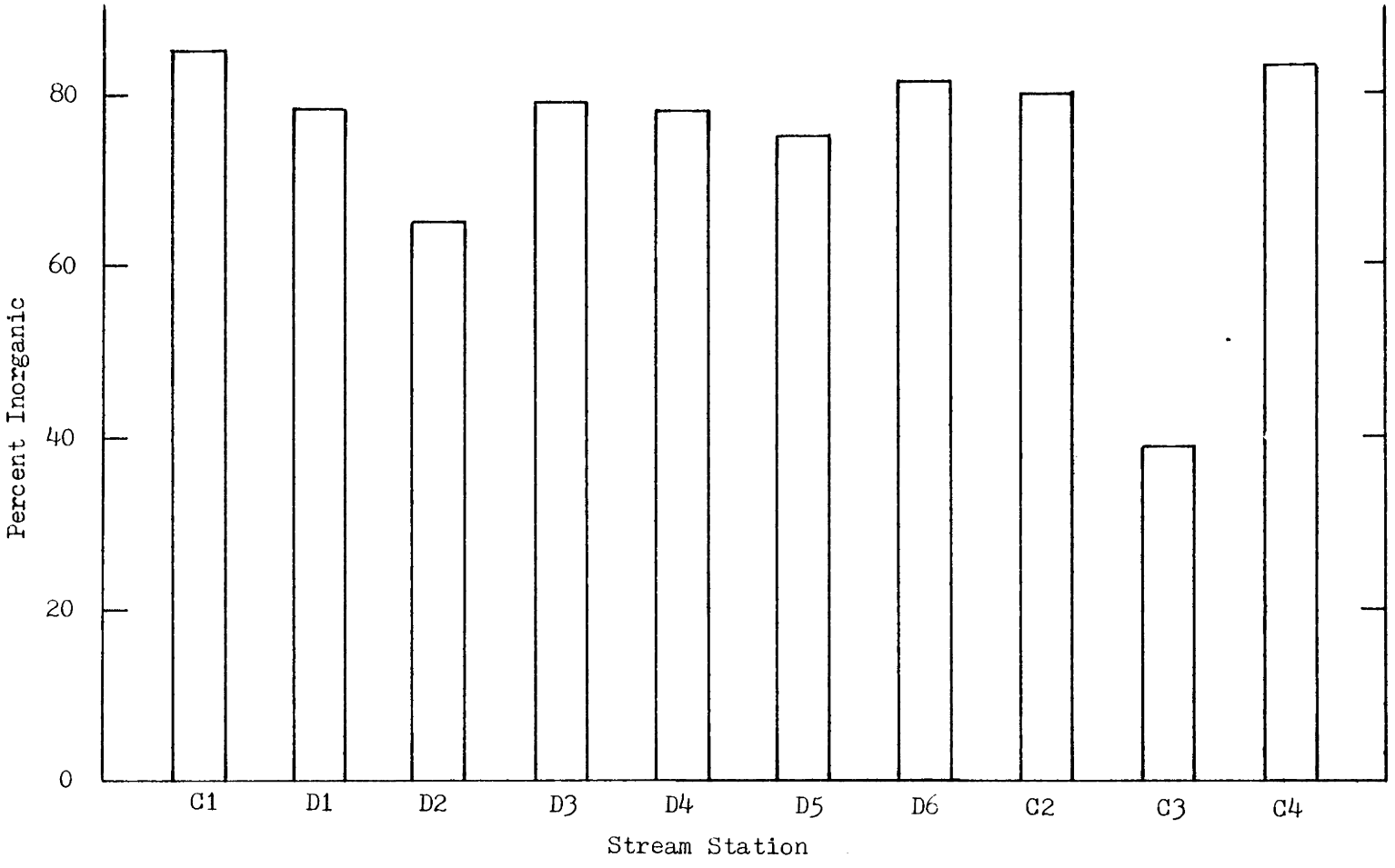

Figure A1. Inorganic Fraction of Periphyton Total Solids at Ten Stations in Culpeper, Virginia, During the Period June Through September, 1981.

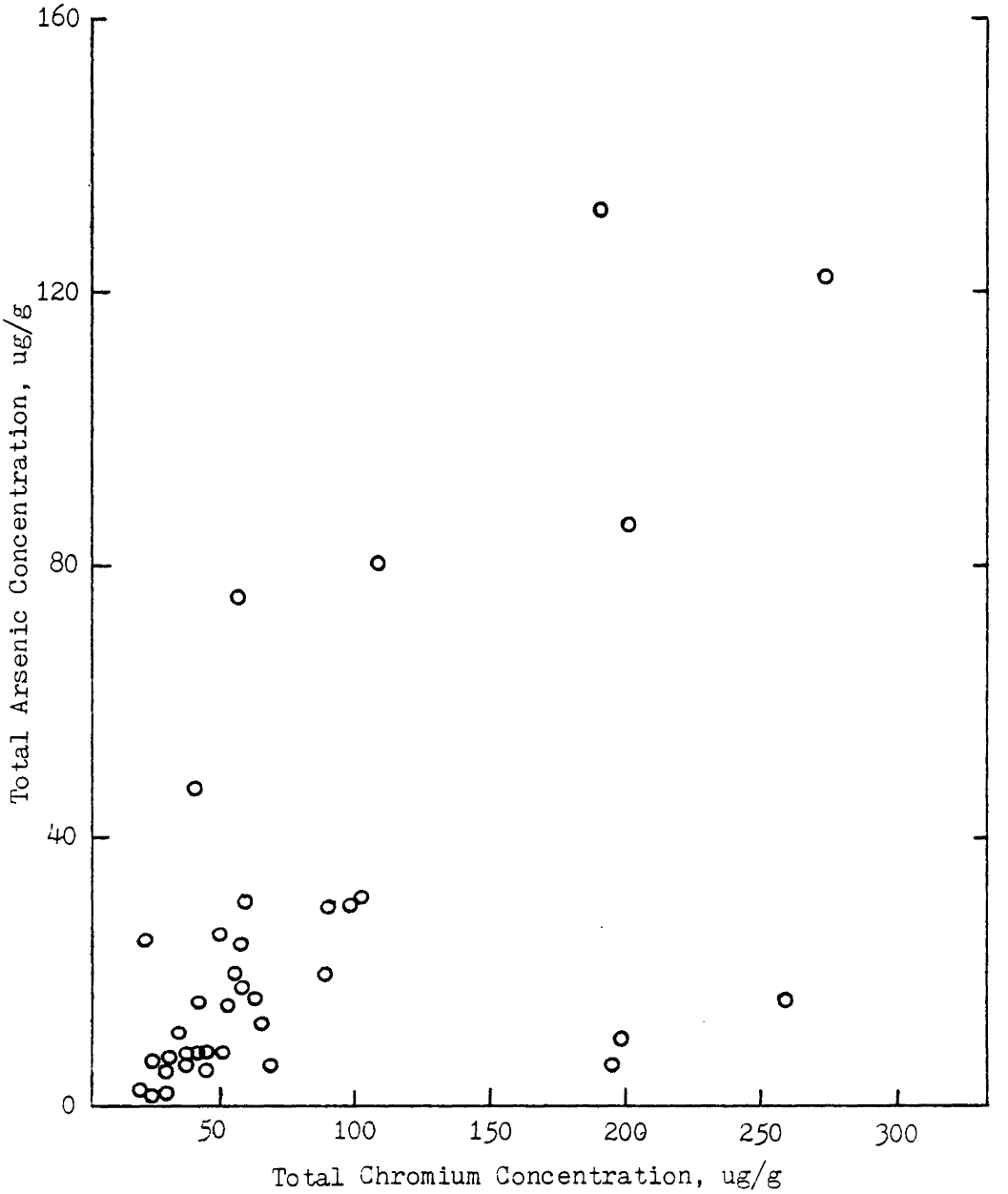

Figure A2. Relationship Between Arsenic and Chromium Concentrations in Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

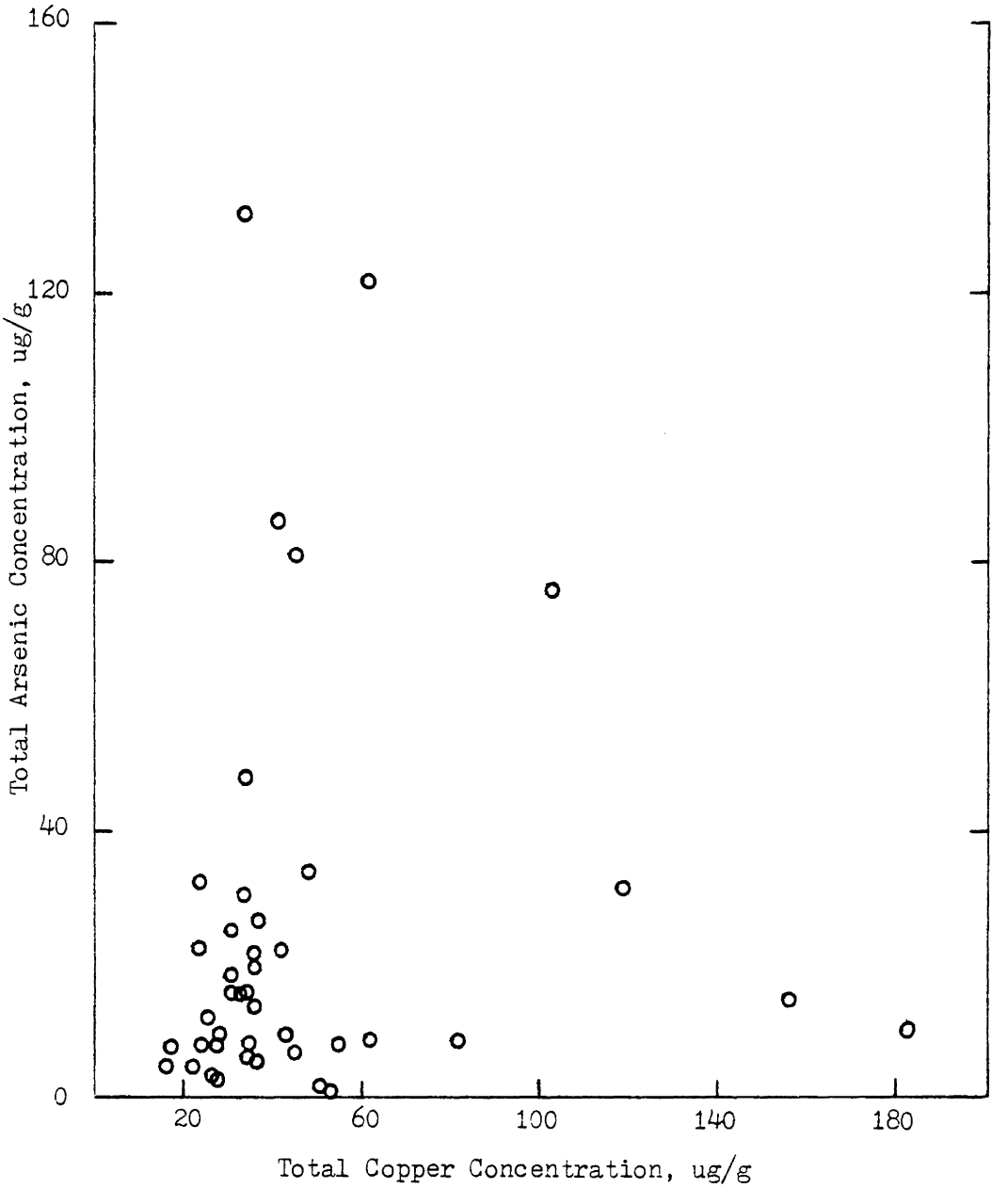

Figure A3. Relationship Between Arsenic and Copper Concentrations in Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

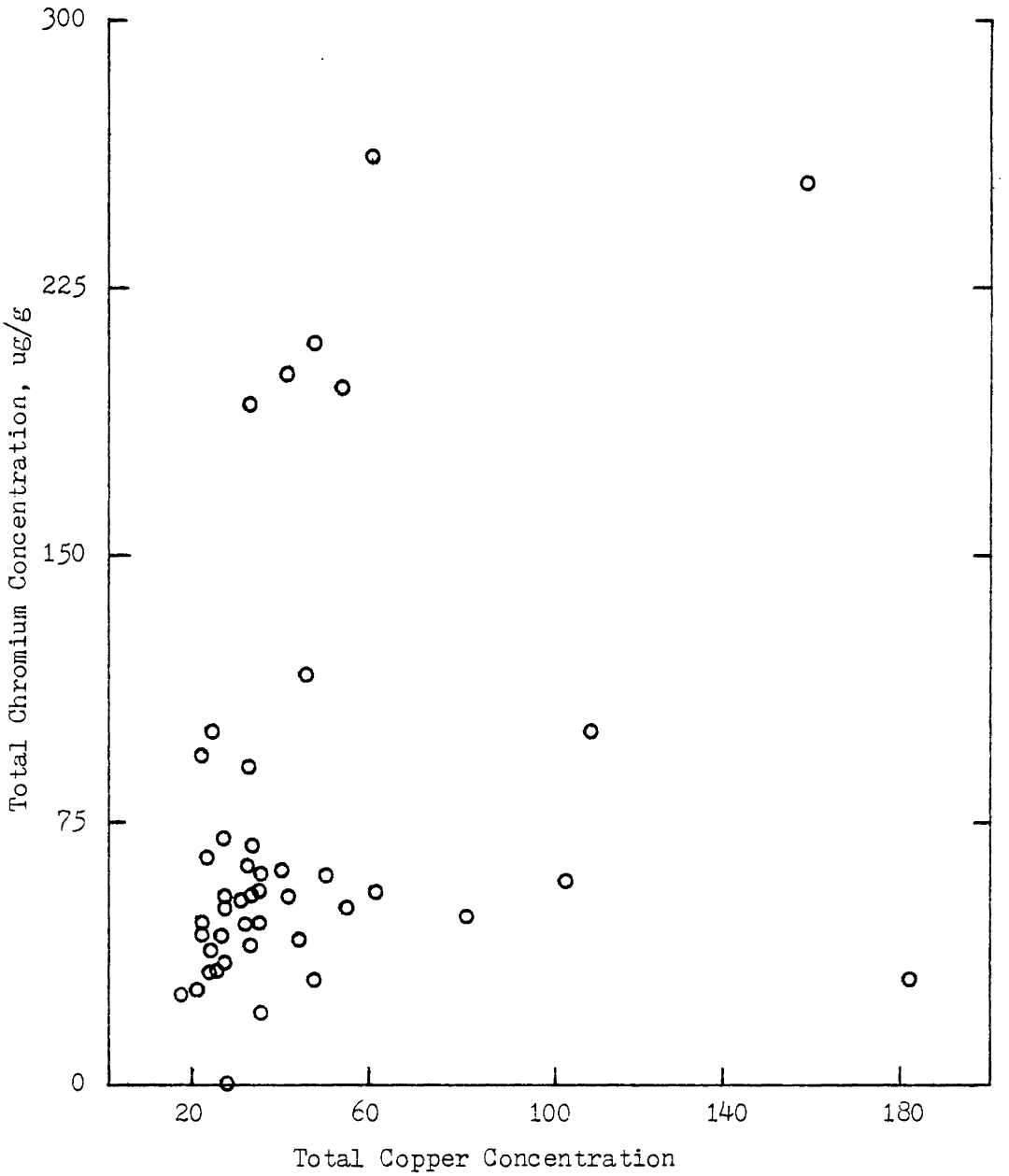

Figure A4. Relationship Between Chromium and Copper Concentrations in Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

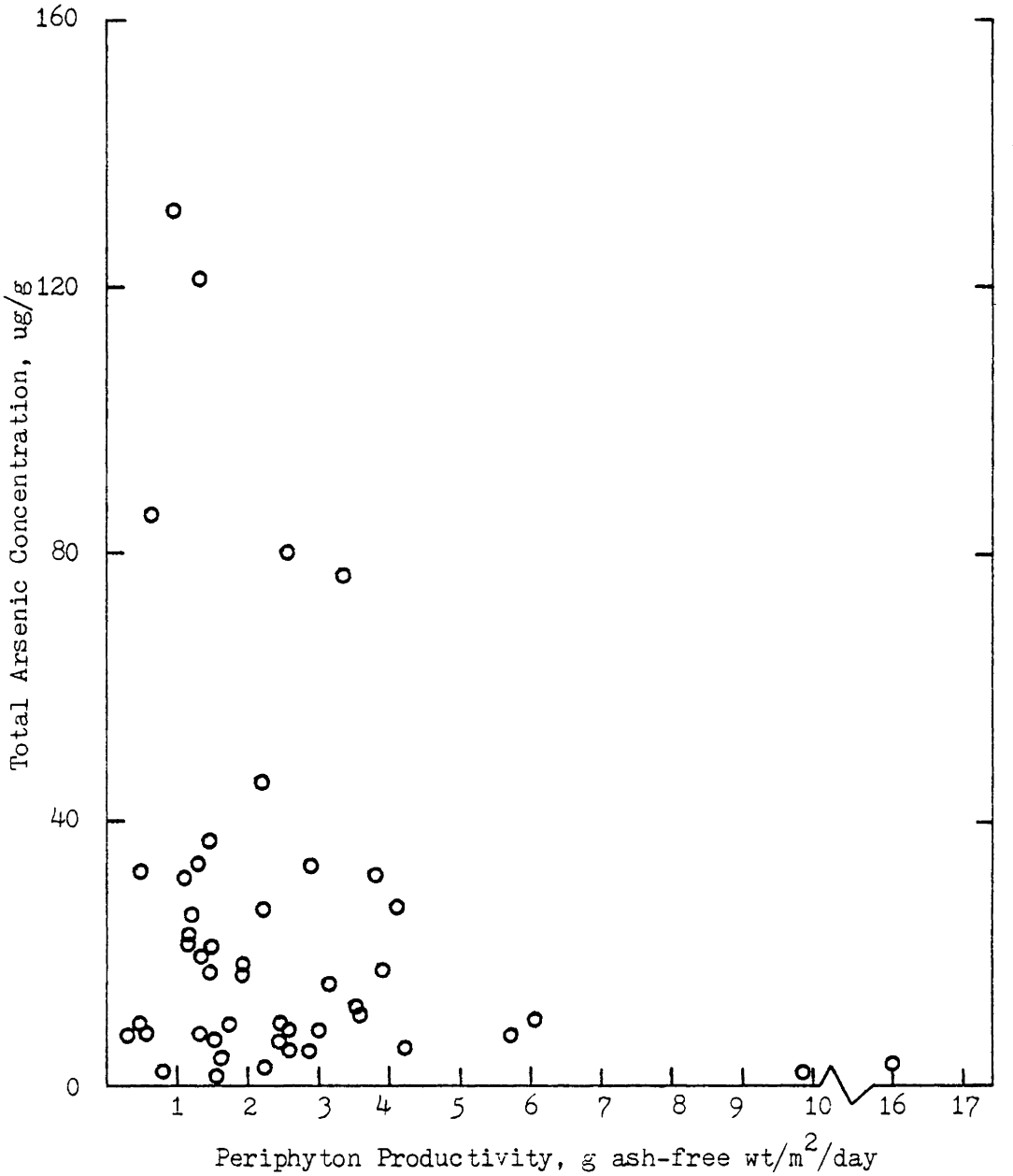

Figure A5. Relationship Between Periphyton Productivity and Total Arsenic Concentration in Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

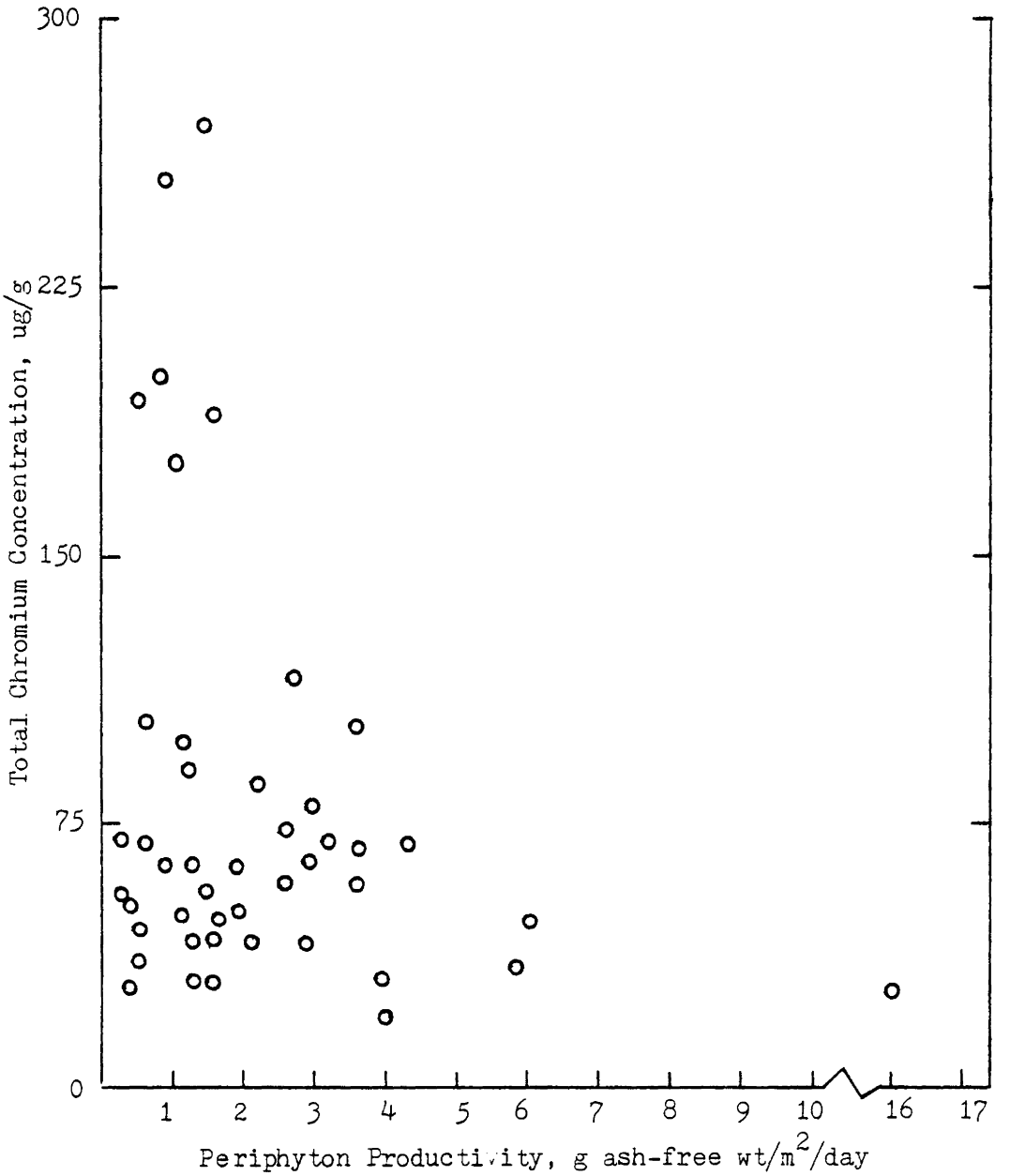

Figure A6. Relationship Between Periphyton Productivity and Total Chromium Concentration in Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

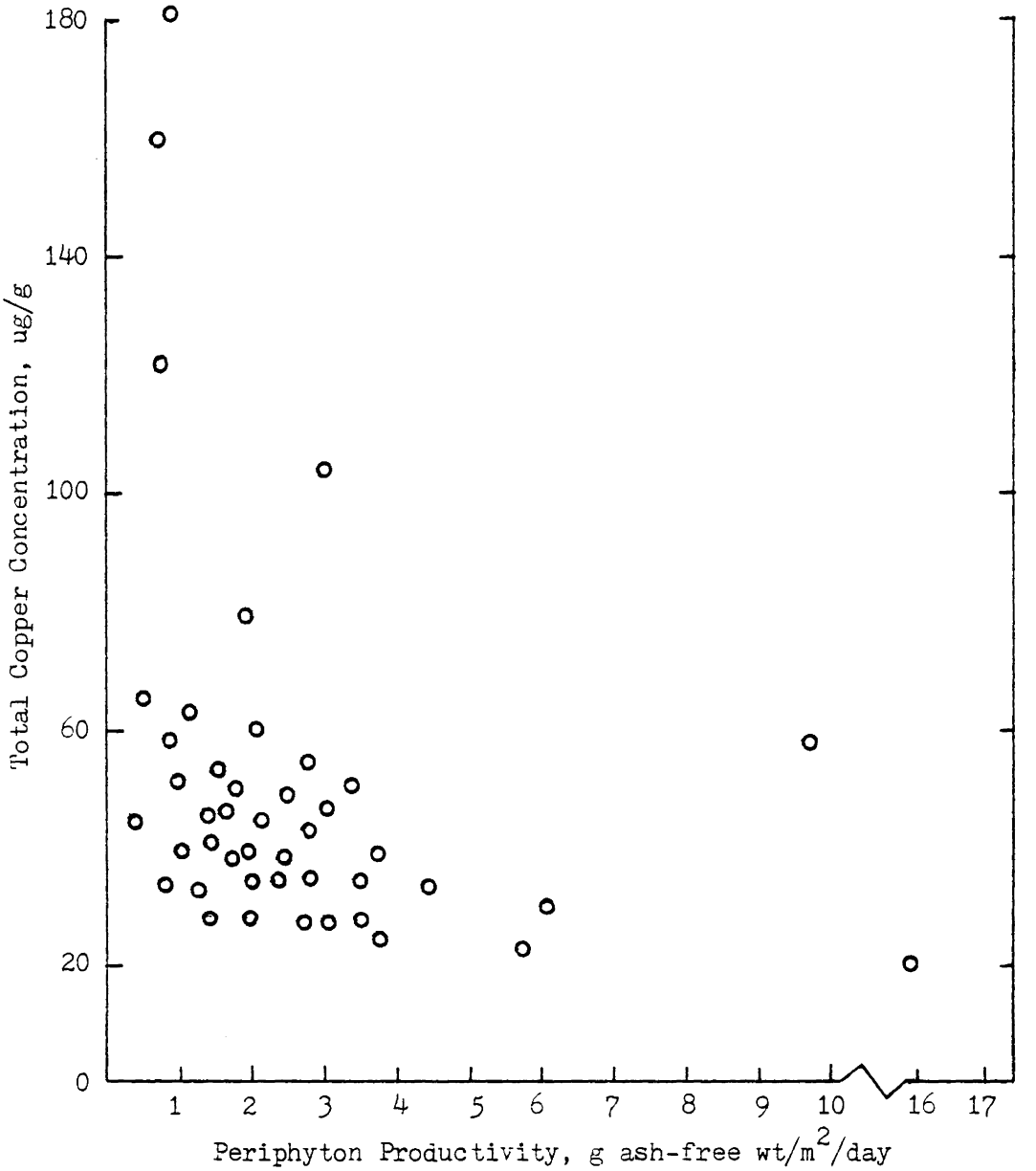

Figure A7. Relationship Between Periphyton Productivity and Total Copper Concentration in Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981

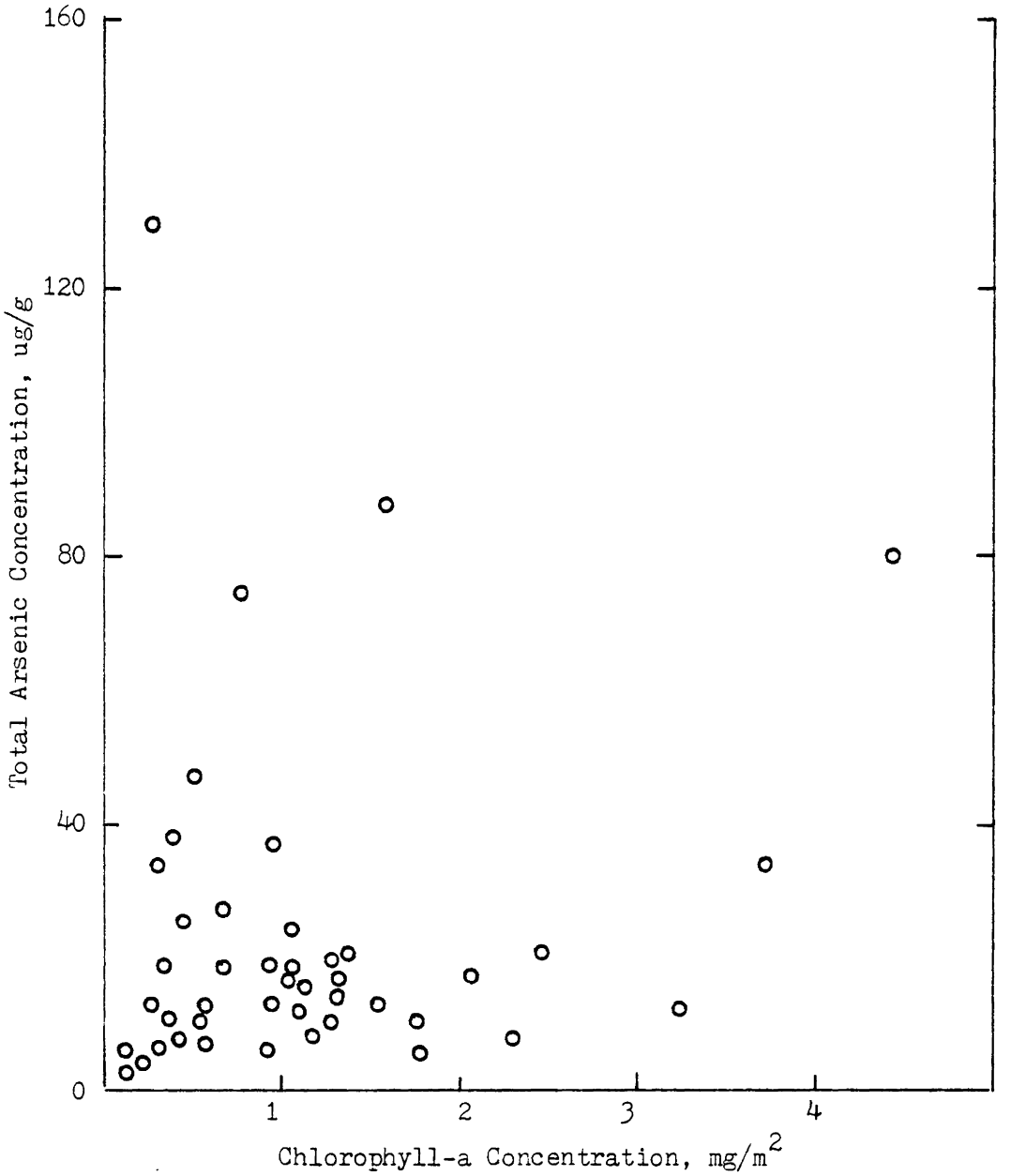

Figure A8. Relationship Between Total Arsenic Concentration and the Chlorophyll-a Concentration in Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

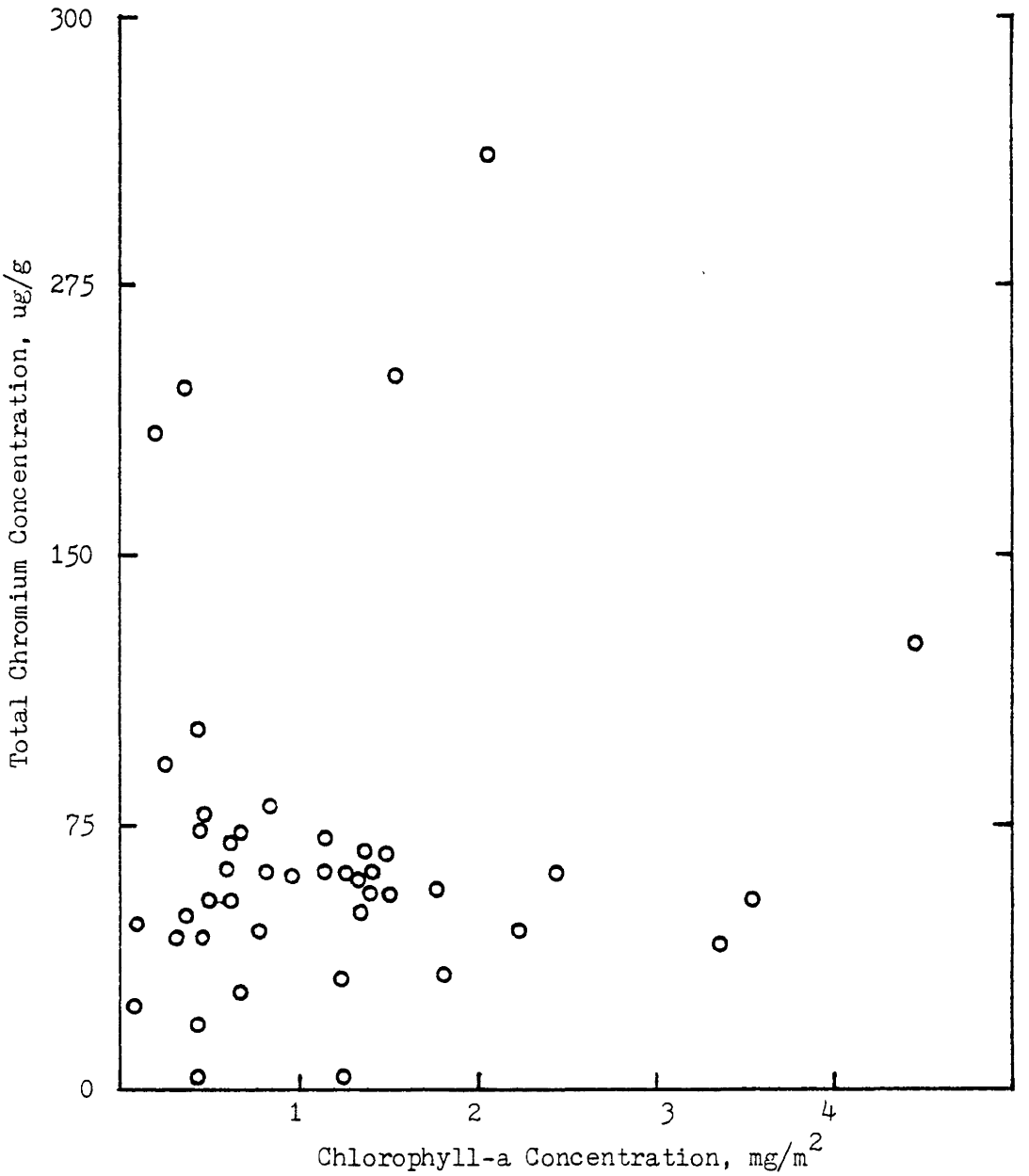

Figure A9. Relationship Between Total Chromium Concentration and the Chlorophyll-a Concentration in Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

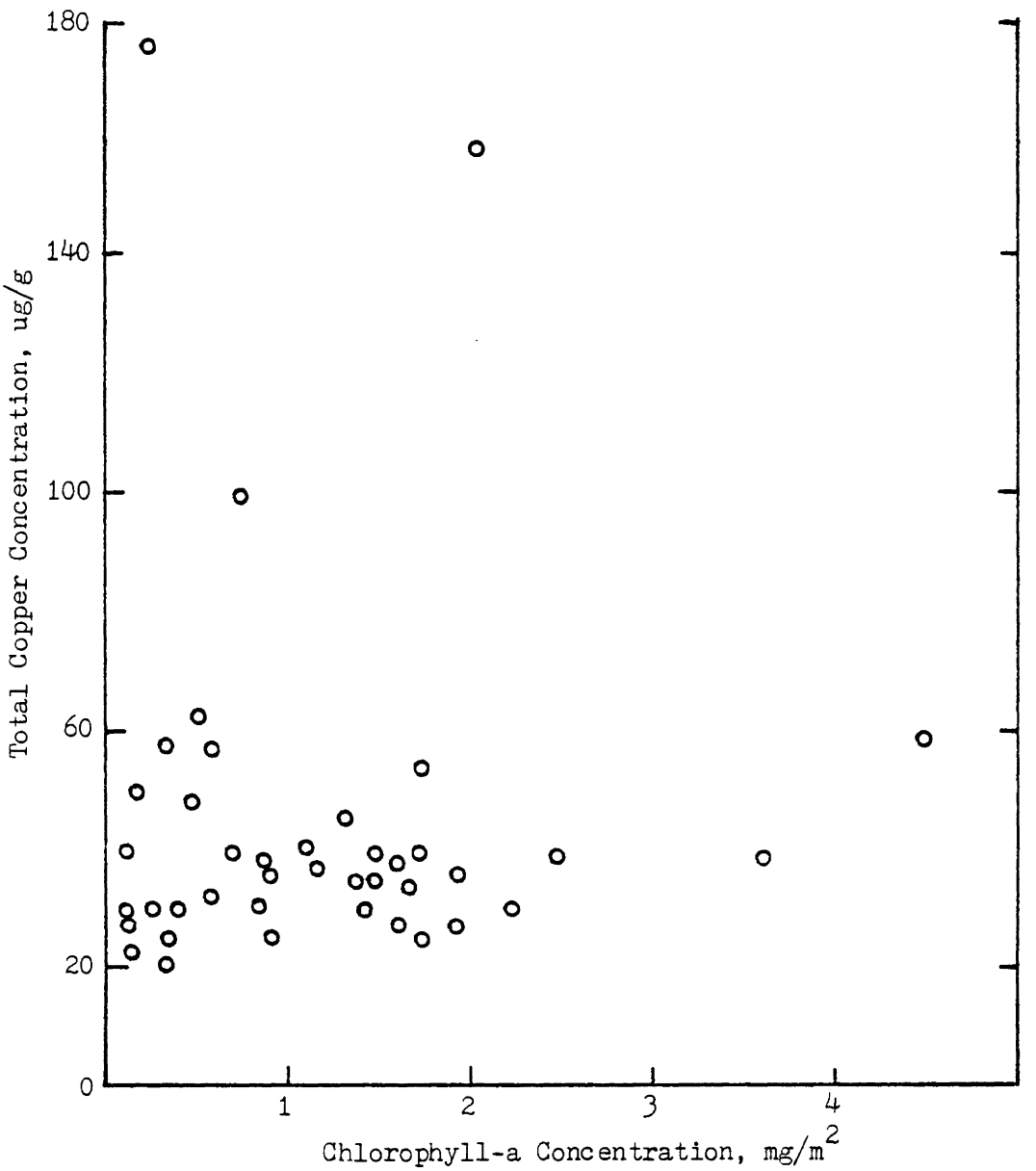

Figure A10. Relationship Between Total Copper Concentration and the Chlorophyll-a Concentration in Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

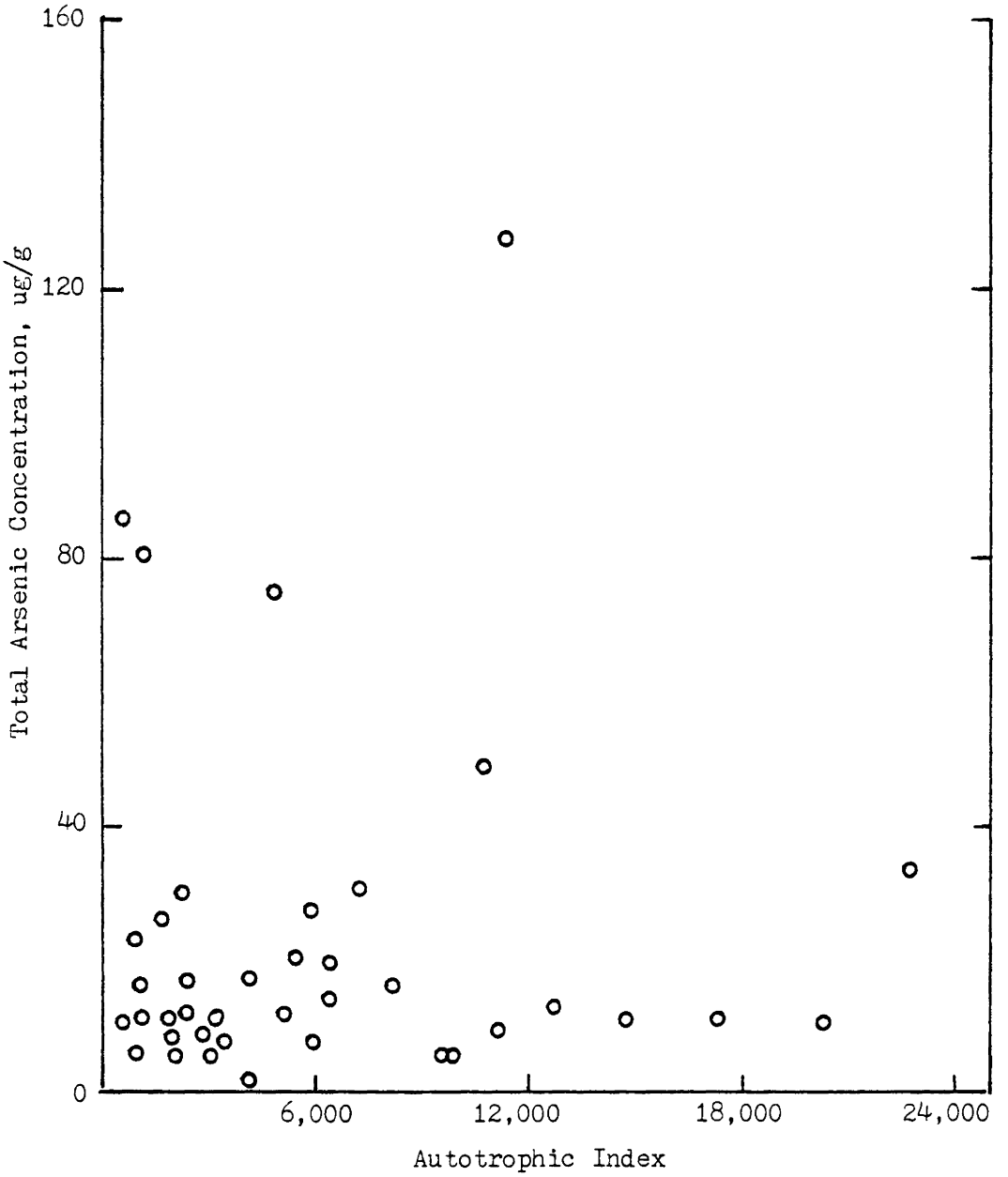

Figure A11. Relationship Between the Total Arsenic Concentration in the Periphyton Total Solids and the Autotrophic Index at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

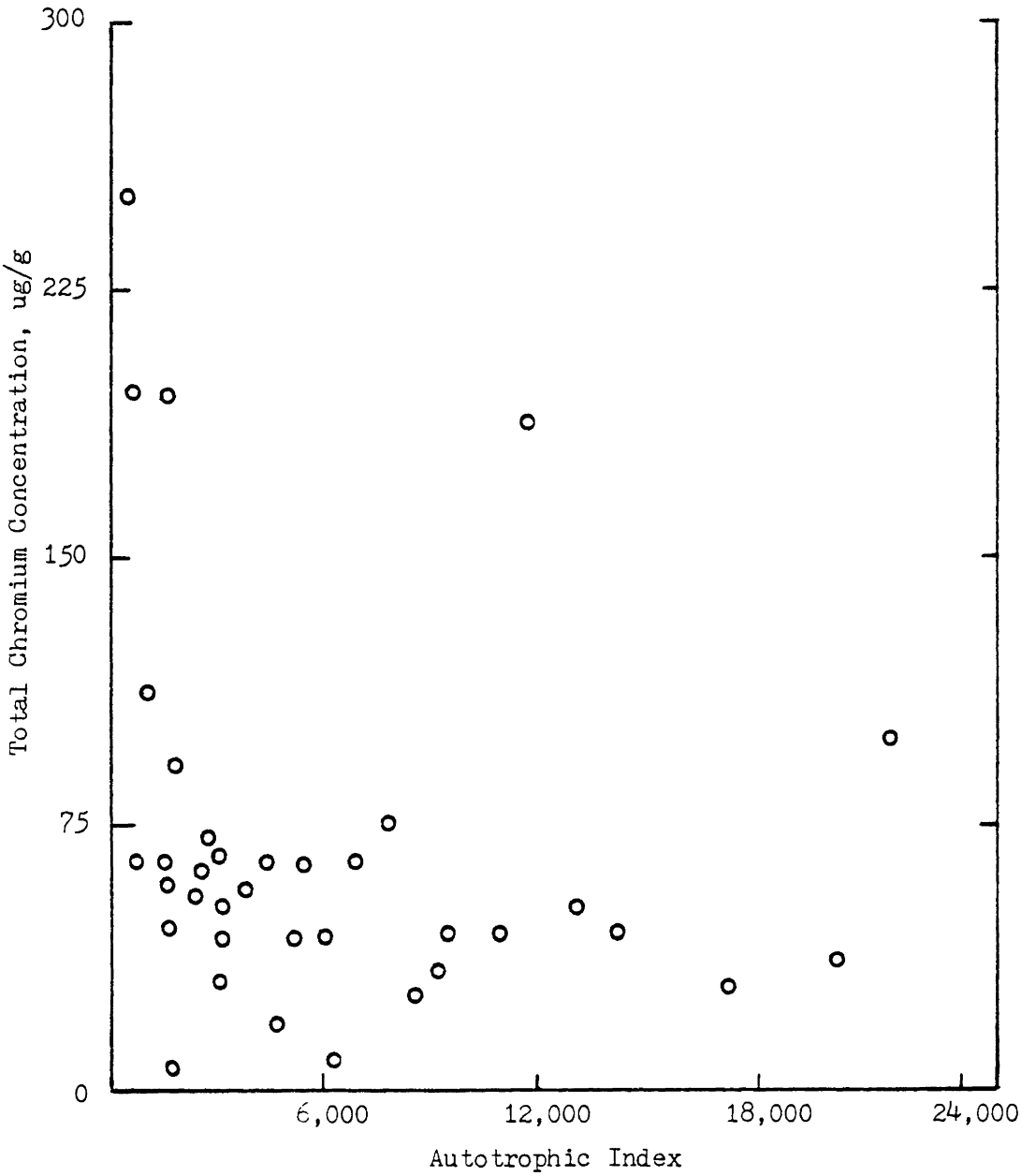

Figure A12. Relationship Between the Total Chromium Concentration in the Periphyton Total Solids and the Autotrophic Index at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

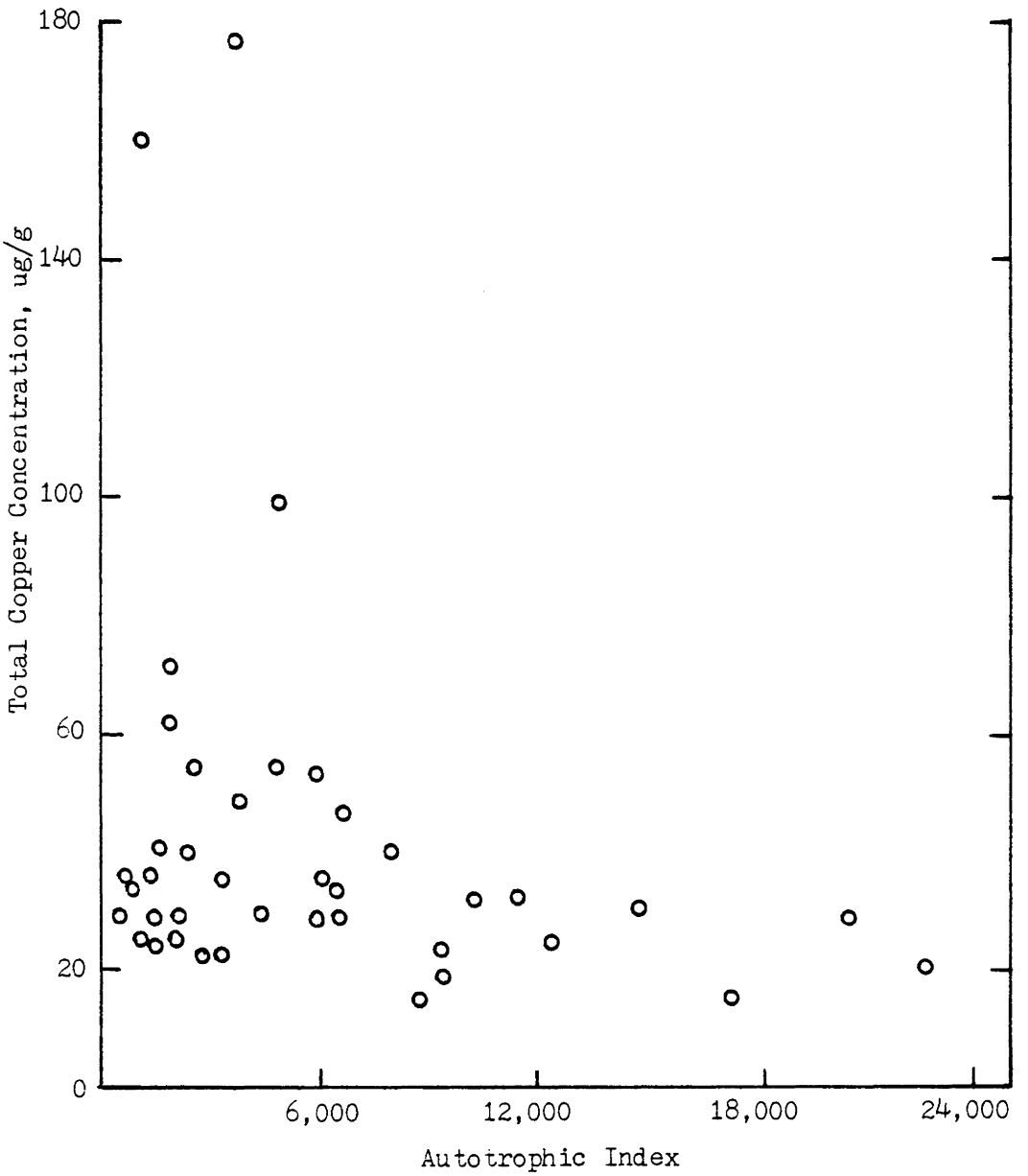

Figure A13. Relationship Between the Total Copper Concentration in the Periphyton Total Solids and the Autotrophic Index at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

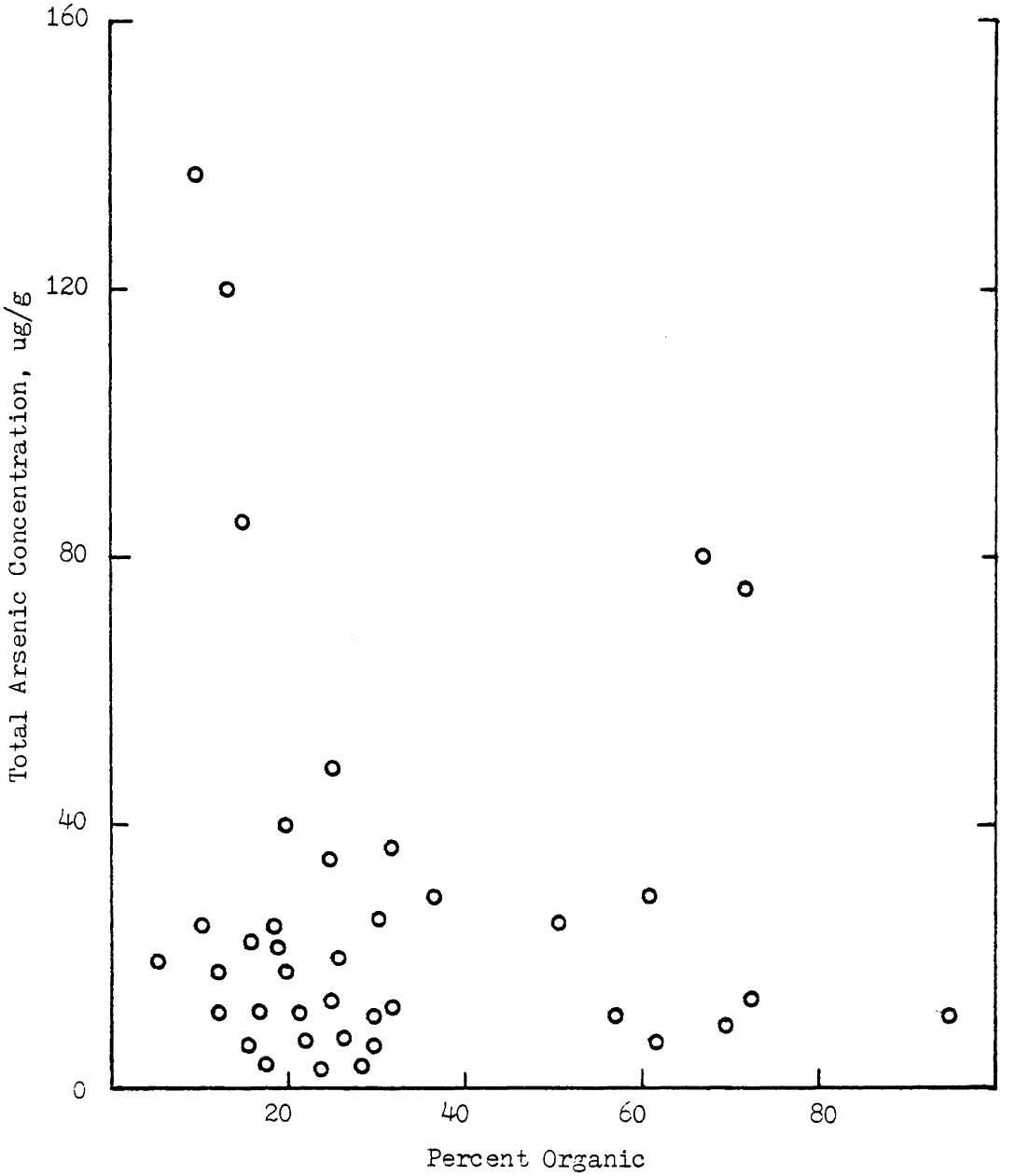

Figure A14. Relationship Between Periphyton Total Arsenic Concentration and the Percent of Organics in the Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

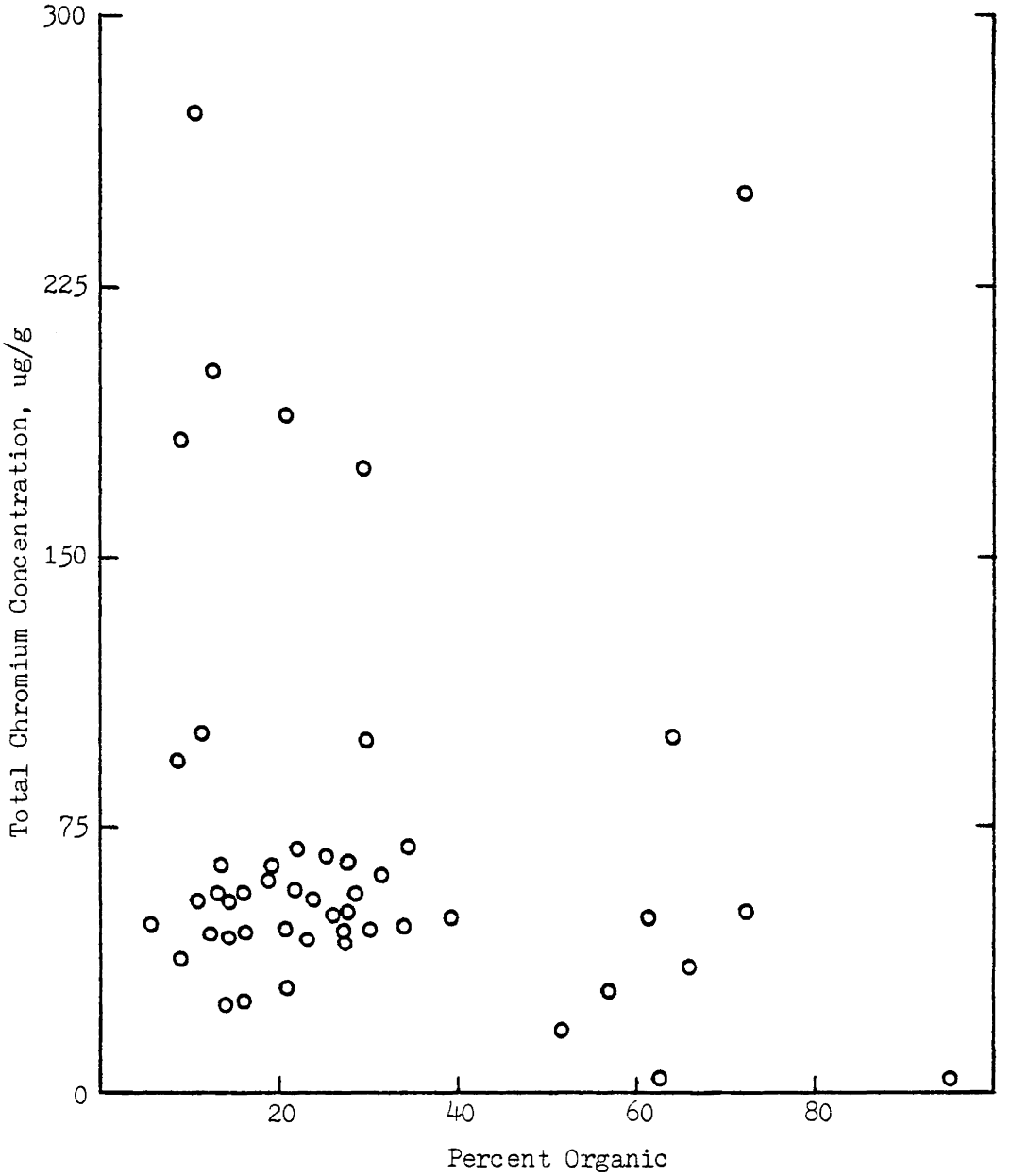

Figure A15. Relationship Between Periphyton Total Chromium Concentration and the Percent of Organics in the Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

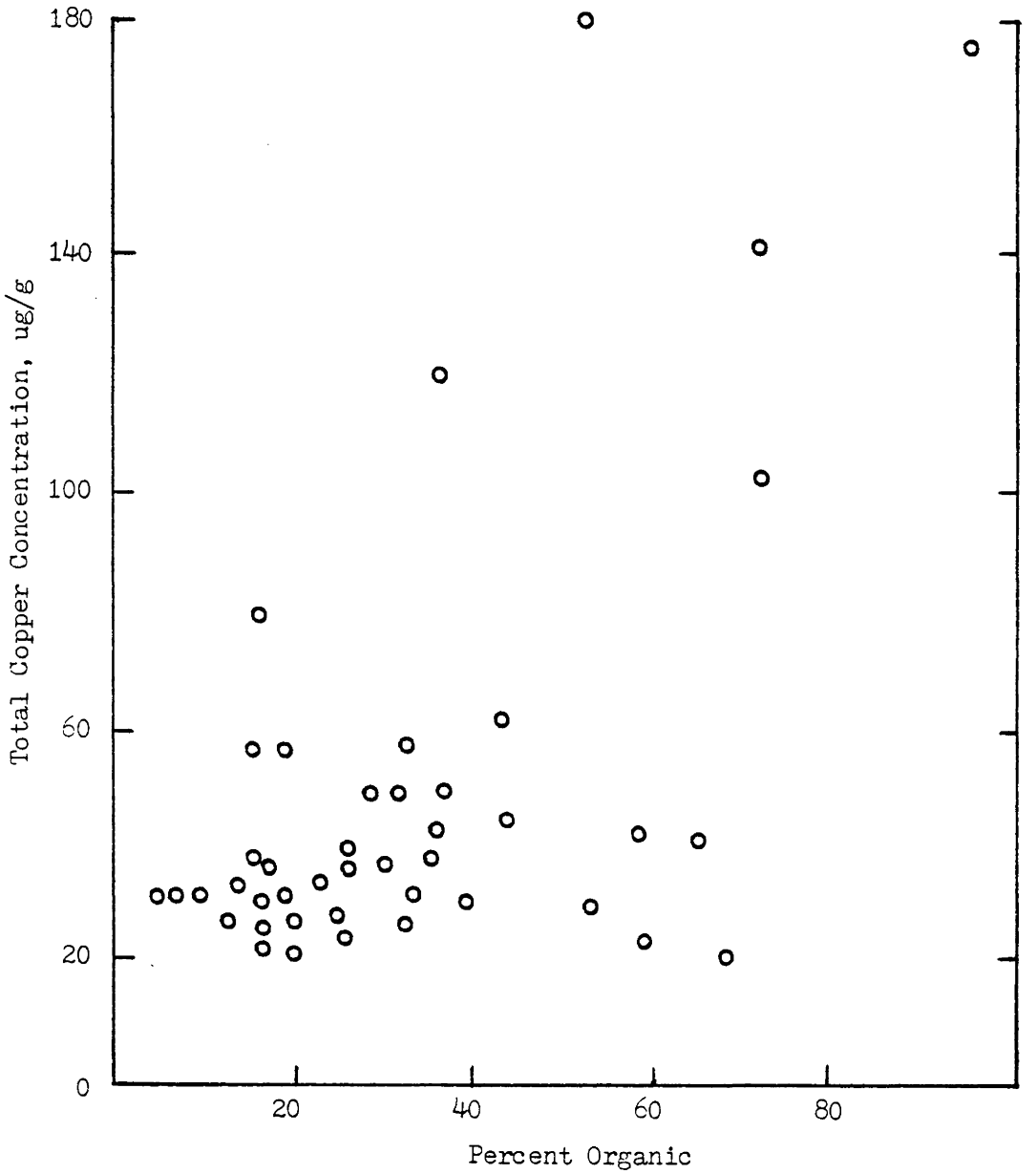

Figure A16. Relationship Between Periphyton Total Copper Concentration and the Percent of Organics in the Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

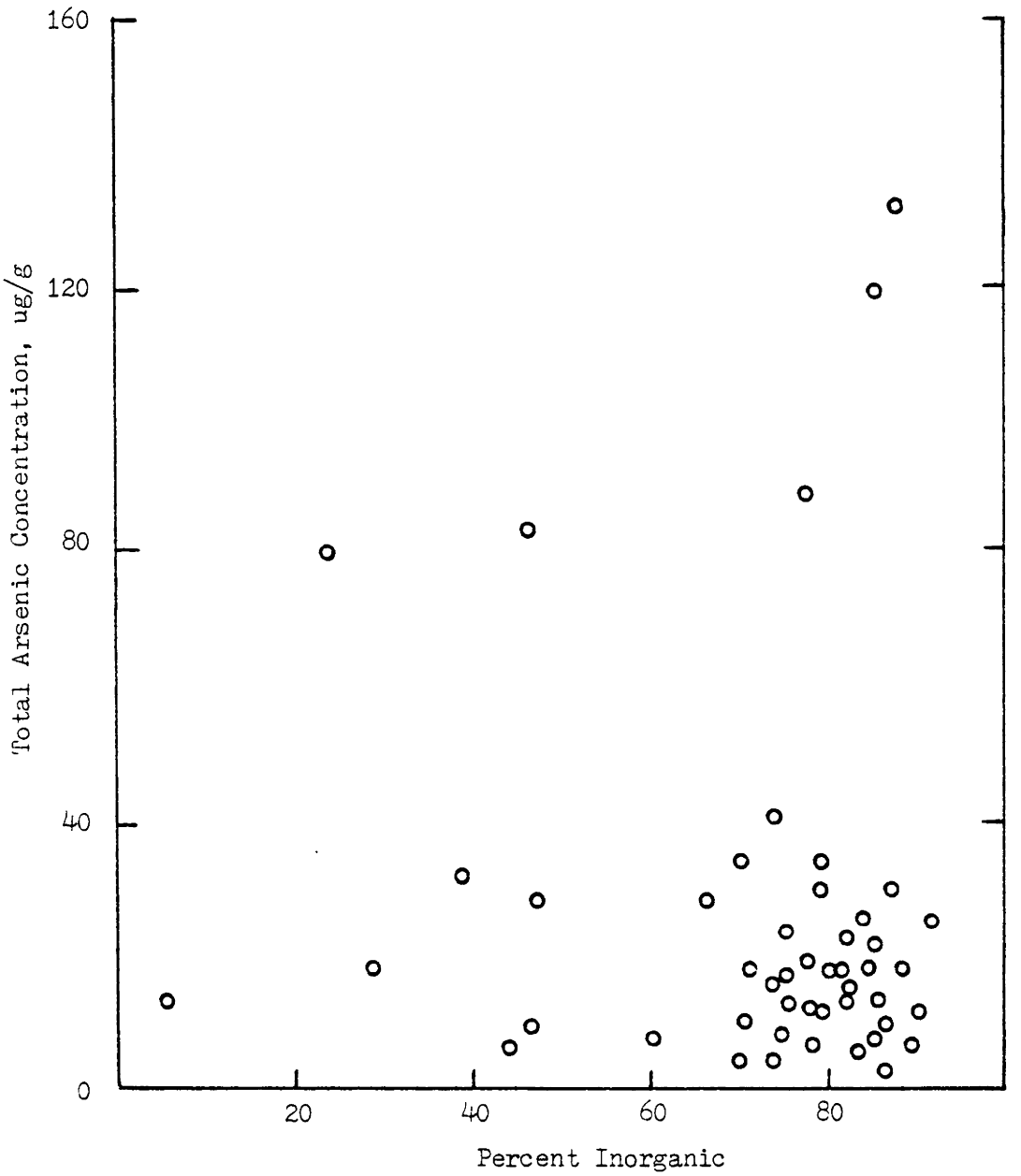

Figure A17. Relationship Between Periphyton Total Arsenic Concentration and the Percent of Inorganics in the Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

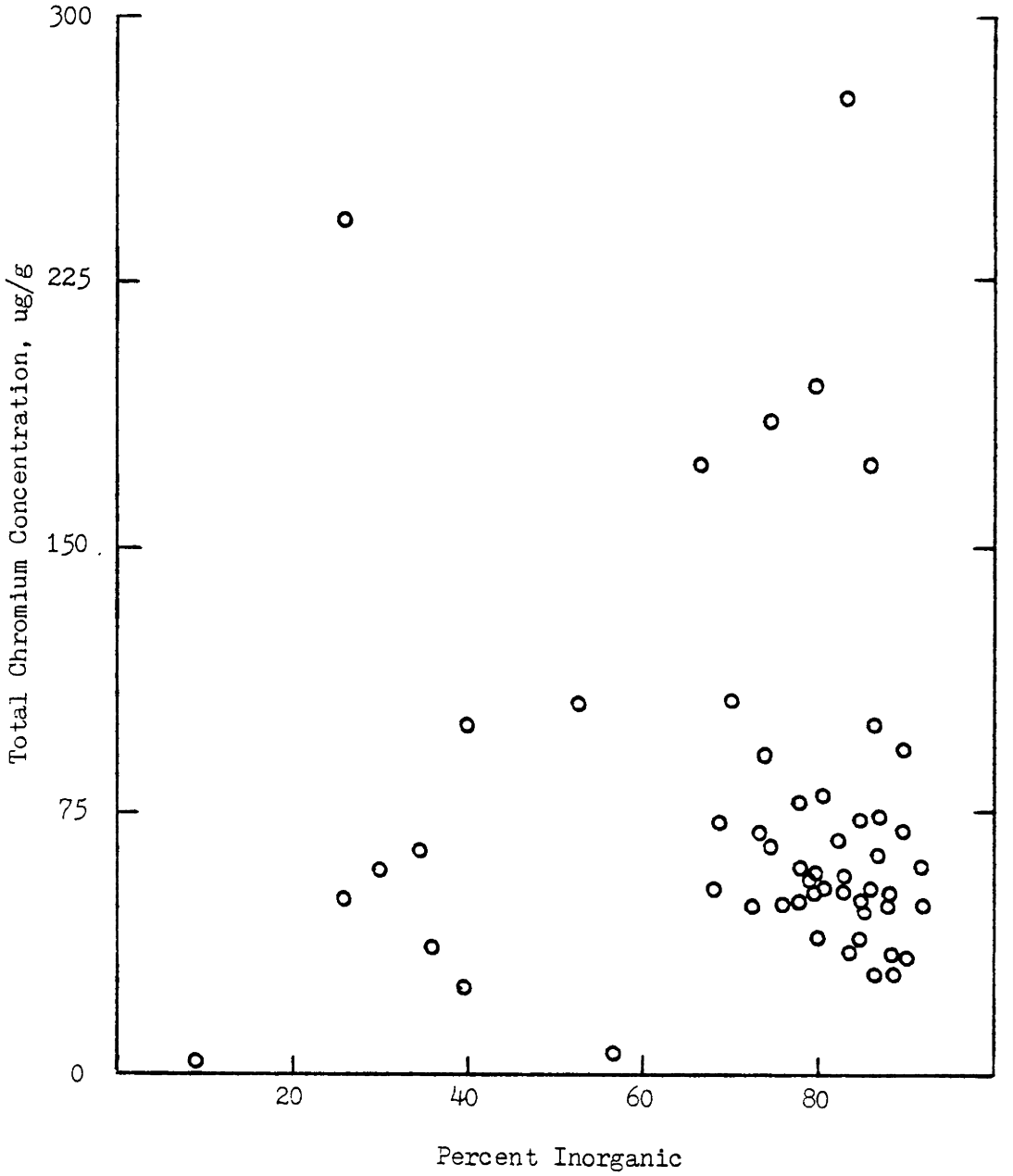

Figure A18. Relationship Between Periphyton Total Chromium Concentration and the Percent of Inorganics in the Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

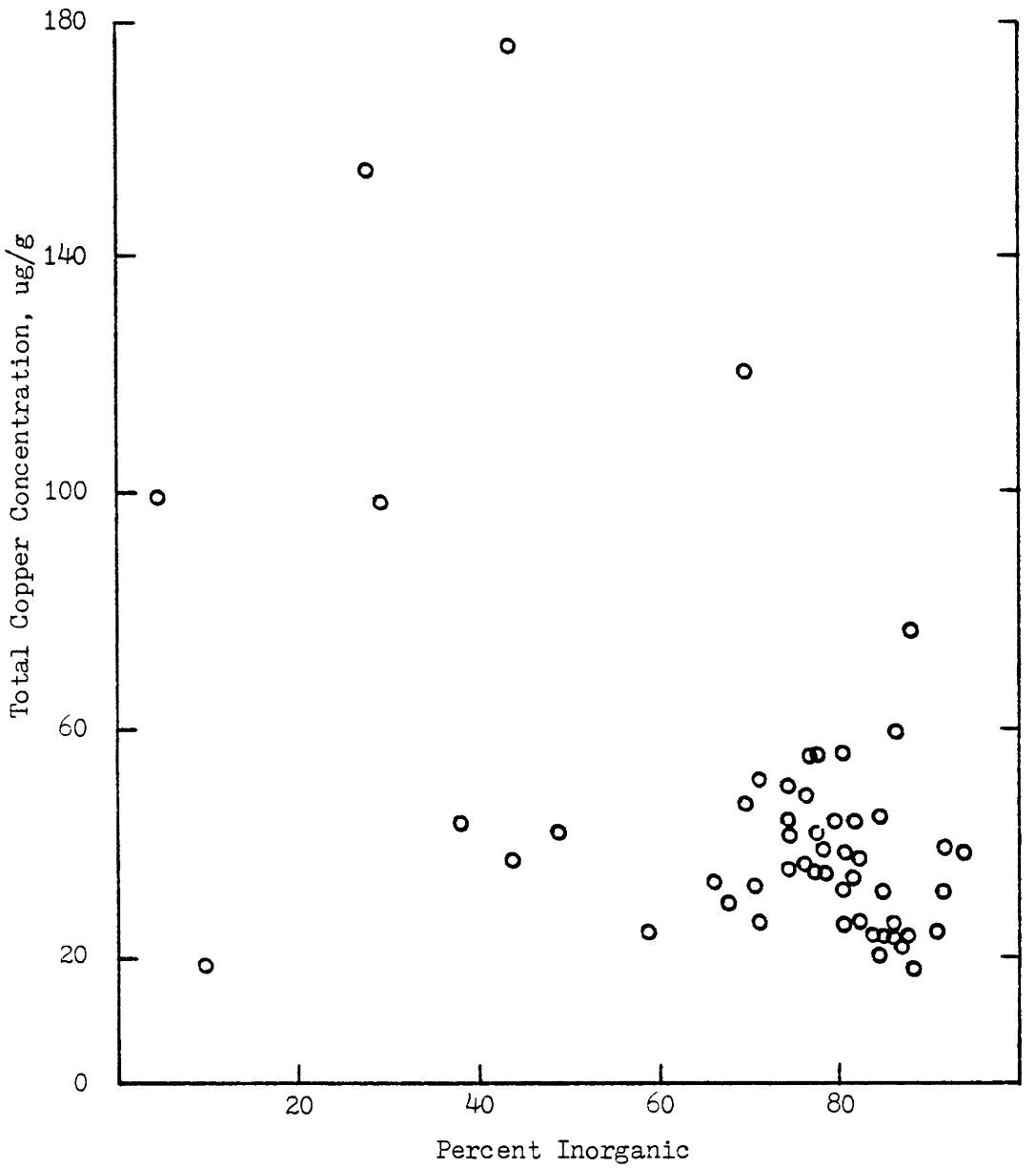

Figure A19. Relationship Between Periphyton Total Copper Concentration and the Percent of Inorganics in the Periphyton Total Solids at Ten Locations in Culpeper, Virginia, During the Period June Through September, 1981.

APPENDIX B

Tabular Presentation of Data Presented in Figures in Results Section

Table B1. Results of Analysis of Variance by the General Linear Models Procedure, (Statistical Analysis Systems SAS)

Variable	Source	Degrees of Freedom	Sum of Squares	F Value	PR F	Significance*
Total As Conc.	Station	5	2.09×10^{4}	9.38	0.0001	HS
	Time	8	4.02×10^{3}	1.38	0.3693	NS
Total Cr Conc.	Station	5	3.46×10^{4}	1.66	0.1696	NS
	Time	8	3.57×10^{4}	1.07	0.4057	NS
Total Cu Conc.	Station	5	2.46×10^{3}	2.07	0.0925	NS
	Time	8	8.06×10^{3}	4.25	0.0012	HS
Product.	Station	5	8.10×10^{1}	3.62	0.0096	S
	Time	8	8.32×10^{1}	2.33	0.0406	S
AI	Station	5	1.72×10^{8}	1.61	0.1919	NS
	Time	7	5.10×10^{8}	3.42	0.0101	S
Chlor.-a	Station	5	3.36×10^{0}	0.74	0.6000	NS
	Time	7	1.34×10^{1}	2.10	0.0735	NS

* HS: Highly significant
S: Significant
NS: Not Significant

Table B2. Total Arsenic Concentration in Periphyton Total Solids in Culpeper, Virginia, During the Period June Through September, 1981

Inclusive Dates, 1981	Stream Station and Corresponding Arsenic Concentration (ug/g)									
	C1	D1	D2	D3	D4	D5	D6	C2	C3	C4
June 22-July 8	1.36	122.43	32.75	8.15	-	*	6.27	-	*	-
July 8-16	0.88	80.88	75.60	13.05	-	26.07	9.74	-	10.00	-
July 16-24	6.96	86.48	30.35	18.52	-	25.46	7.30	-	9.56	
July 24-Aug. 3	6.82	*	*	15.62	-	19.83	11.23	-	11.51	-
Aug. 3-11	7.04	2.20	25.58	17.17	-	20.83	15.83	-	12.74	-
Aug. 11-22	3.86	*	47.75	15.94	-	16.47	9.01	-	*	-
Aug. 22-Sept. 3	5.01	20.94	3.68	*	*	6.84	6.11	*	*	4.58
Sept. 3-13	4.06	31.35	32.03	8.43	*	9.84	7.52	9.01	7.53	7.20
Sept. 13-25	3.21	132.23	14.26	7.57	10.91	4.20	7.89	6.15	18.13	2.75

* Samplers at these sites were damaged during the accrual period.
- Samplers at these sites were not installed until August 22.

Table B3. Total Chromium Concentration in Periphyton Total Solids in Culpeper, Virginia, During the Period June Through September, 1981

Inclusive Dates, 1981	Stream Station and Corresponding Chromium Concentration (ug/g)									
	C1	D1	D2	D3	D4	D5	D6	C2	C3	C4
June 22-July 8	29.40	269.90	104.20	47.01	-	*	41.71	-	*	-
July 8-16	377.43	115.04	57.42	67.62	-	20.62	43.92	-	0.00	-
July 16-24	54.51	200.15	90.28	64.90	-	63.56	41.80	-	71.71	-
July 24-Aug. 3	26.73	*	*	55.08	-	56.74	37.45	-	69.07	-
Aug. 3-11	32.10	0.00	51.17	60.67	-	62.26	53.40	-	254.77	-
Aug. 11-22	24.76	*	46.07	63.00	-	45.51	50.04	-	*	-
Aug. 22-Sept. 3	34.49	92.29	33.04	*	*	44.29	41.53	*	*	41.70
Sept. 3-13	27.56	99.87	60.51	50.57	*	53.29	54.05	40.70	32.26	57.45
Sept. 13-25	43.64	190.24	64.44	196.32	64.93	45.56	69.74	44.96	39.19	178.60

* Samplers at these sites were damaged during the accrual period.

- Samplers at these sites were not installed until August 22.

Table B4. Total Copper Concentration in Periphyton Total Solids in Culpeper, Virginia, During the Period June Through September, 1981

Inclusive Dates, 1981	Stream Station and Corresponding Copper Concentration (ug/g)									
	C1	D1	D2	D3	D4	D5	D6	C2	C3	C4
June 22-July 8	47.99	62.92	119.09	83.56	-	*	44.64	-	*	-
July 8-16	53.37	46.02	105.26	35.23	-	35.35	28.15	-	1,225.00	-
July 16-24	63.60	41.54	32.65	35.15	-	30.40	27.87	-	47.80	-
July 24-Aug. 3	19.91	*	*	35.35	-	34.29	25.19	-	130.46	-
Aug. 3-11	24.40	27.47	30.09	36.63	-	41.65	29.98	-	155.69	-
Aug. 11-22	18.98	*	34.55	31.50	-	31.06	28.04	-	*	-
Aug. 22-Sept. 3	28.29	26.57	27.18	*	*	34.19	34.81	*	*	29.44
Sept. 3-13	20.93	25.60	49.83	54.79	*	41.00	36.43	46.51	182.83	48.01
Sept. 13-25	25.35	36.75	24.97	54.40	48.48	23.98	27.63	30.99	39.19	32.97

* Samplers at these stations were damaged during the accrual period.

- Samplers at these stations were not installed until August 22.

Table B5. Primary Productivity of Periphyton in Culpeper, Virginia, During the Period June Through September, 1981

Inclusive Dates, 1981	Stream Station and Corresponding Primary Productivity ($g/m^2/day$)									
	C1	D1	D2	D3	D4	D5	D6	C2	C3	C4
June 22-July 8	1.63	1.50	0.60	1.78	-	*	2.89	-	*	-
July 8-16	9.86	2.61	3.56	3.56	-	4.15	6.16	-	2.37	-
July 16-24	0.36	0.73	1.18	1.42	-	1.30	3.08	-	0.12	-
July 24-Aug. 3	5.82	*	*	1.94	-	1.55	3.63	-	0.39	-
Aug. 3-11	2.96	0.89	2.25	1.54	-	1.18	4.03	-	0.44	-
Aug. 11-22	16.00	*	2.36	1.60	-	1.89	2.38	-	*	-
Aug. 22-Sept. 3	2.36	1.23	1.56	*	*	1.52	2.46	*	*	1.23
Sept. 3-13	4.36	3.88	1.45	0.68	*	0.48	1.45	0.68	0.63	0.48
Sept. 13-25	2.30	1.15	3.20	0.44	1.89	1.64	2.63	1.80	0.78	0.78

* Samplers at these stations were damaged during the accrual period.

- Samplers at these stations were not installed until August 22.

Table B6. Chlorophyll-a Concentration in Periphyton Total Solids in Culpeper, Virginia, During the Period June Through September, 1981

Inclusive Dates, 1981	Stream Station and Corresponding Chlorophyll-a Concentration (mg/m^2)									
	C1	D1	D2	D3	D4	D5	D6	C2	C3	C4
June 22-July 8	*	*	*	*	-	*	*	-	*	-
July 8-16	0.00	4.49	0.78	0.50	-	0.43	1.39	-	0.86	-
July 16-24	0.37	1.43	0.76	2.41	-	1.37	1.26	-	0.25	-
July 24-Aug. 3	0.79	*	*	1.48	-	1.28	3.38	-	0.69	-
Aug. 3-11	2.18	1.16	3.61	1.24	-	1.32	0.71	-	2.05	-
Aug. 11-22	1.88	*	0.30	0.42	-	0.98	0.49	-	*	-
Aug. 22-Sept. 3	0.21	0.00	0.08	*	*	0.08	0.08	*	*	0.43
Sept. 3-13	0.00	0.20	0.28	0.13	*	0.00	1.90	0.10	0.23	*
Sept. 13-25	0.29	0.11	1.30	0.38	1.65	0.00	1.37	0.19	0.70	*

* Samplers or Samples at these sites were damaged during the accrual period.

\- Samplers at these sites were not installed until August 22.

Table B7. Autotrophic Indices of the Periphyton From Culpeper, Virginia, During the Period June Through September, 1981

Inclusive Dates, 1981	Stream Station and Corresponding Autotrophic Index									
	C1	D1	D2	D3	D4	D5	D6	C2	C3	C4
June 22-July 8	*	*	*	*	-	*	*	-	*	-
July 8-16	4,551	529	4,781	8,402	-	6,108	4,050	-	6,702	-
July 16-24	1,069	462	1,711	537	-	1,181	2,255	-	470	-
July 24-Aug. 3	17,812	*	*	1,524	-	3,284	2,844	-	720	-
Aug. 3-11	1,501	1,021	603	2,047	-	826	5,749	-	198	-
Aug. 11-22	10,161	*	11,472	6,414	-	2,218	12,659	-	*	-
Aug. 22-Sept. 3	20,323	*	10,280	*	*	11,486	*	*	*	6,396
Sept. 3-13	*	22,581	7,402	5,710	*	*	885	6,520	3,081	*
Sept. 13-25	10,198	12,484	3,225	1,580	1,490	*	2,490	14,222	1,635	*

* Samplers or samples at these sites were damaged or lost during the accrual period.

- Samplers at these sites were not installed until August 22.

able B8. Mean Total Arsenic Concentration in the Periphyton from Culpeper, Virginia, During the Period June Through September, 1981

Sampling Station	Mean Arsenic Concentration (ug/g)*
C1	4.35
D1	49.20
D2	39.92
D3	13.06
D4	10.91
D5	16.19
D6	9.01

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B9. Mean Total Chromium Concentration in the Periphyton from Culpeper, Virginia, During the Period June Through September, 1981

Sampling Station	Mean Chromium Concentration (ug/g)*
C1	34.15
D1	161.25
D2	63.39
D3	58.41
D4	64.93
D5	48.98
D6	48.18

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B10. Mean Total Copper Concentration in the Periphyton from Culpeper, Virginia, During the Period June Through September, 1981

Sampling Station	Mean Copper Concentration (ug/g)*
C1	33.65
D1	38.12
D2	52.95
D3	47.32
D4	48.48
D5	33.99
D6	31.41

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B11. Mean Periphyton Primary Productivity in Culpeper, Virginia, During the Period June Through September, 1981

Sampling Station	Mean Periphyton Productivity (g ash-free wt/m^2/day) *
C1	5.10
D1	1.72
D2	2.02
D3	1.34
D4	1.89
D5	1.71
D6	3.19

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B12. Mean Chlorophyll-a Concentration in the Periphyton from Culpeper, Virginia, During the Period June Through September, 1981

Sampling Station	Mean Chlorophyll-a Concentration (mg/m^2)*
C1	0.86
D1	1.34
D2	1.09
D3	0.98
D4	1.65
D5	0.99
D6	1.15

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B13. Mean Autotrophic Index of Periphyton from Culpeper, Virginia, During the Period June Through September, 1981

Sampling Station	Mean Autotrophic Index *
C1	8,863
D1	7,416
D2	9.436
D3	3,911
D4	1,490
D5	3,221
D6	4,419

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B14. Mean Total Arsenic Concentration in Periphyton Total Solids During Each of the Nine Accrual Periods

Accrual Period, Inclusive Dates, 1981	Mean Arsenic Concentration (ug/g)*
June 22-July 8	34.19
July 8-16	34.37
July 16-24	30.34
July 24-Aug. 3	13.37
Aug. 3-11	14.80
Aug. 11-22	18.61
Aug. 22-Sept. 3	8.52
Sept. 3-13	17.14
Sept. 13-25	25.75

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B15. Mean Total Chromium Concentration in Periphyton Total Solids During Each of the Nine Accrual Periods

Accrual Period, Inclusive Dates, 1981	Mean Chromium Concentration (ug/g)*
June 22-July 8	98.44
July 8-16	60.92
July 16-24	85.87
July 24-Aug. 3	44.00
Aug. 3-11	51.92
Aug. 11-22	45.88
Aug. 22-Sept. 3	49.13
Sept. 3-13	57.64
Sept. 13-25	79.76

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B16. Mean Total Copper Concentration in Periphyton Total Solids During Each of the Nine Accrual Periods

Accrual Period, Inclusive Dates, 1981	Mean Copper Concentration (ug/g)*
June 22-July 8	71.64
July 8-16	50.56
July 16-24	38.53
July 24-Aug. 3	28.68
Aug. 3-11	31.87
Aug. 11-22	28.83
Aug. 22-Sept. 3	30.21
Sept. 3-13	38.10
Sept. 13-25	34.51

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B17. Mean Primary Productivity of the Periphyton During Each of the Nine Accrual Periods

Accrual Period, Inclusive Dates, 1981	Mean Primary Productivity ($g/m^2/day$)
June 22-July 8	1.68
July 8-16	4.98
July 16-24	1.34
July 24-Aug. 3	3.23
Aug. 3-11	2.14
Aug. 11-22	4.85
Aug. 22-Sept. 3	1.88
Sept. 3-13	2.05
Sept. 13-25	1.89

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B18. Mean Chlorophyll-a Concentration in the Periphyton During Each of the Nine Accrual Periods

Accrual Period, Inclusive Dates, 1981	Mean Chlorophyll-a Concentration (mg/m^2)*
June 22-July 8	-
July 8-16	1.47
July 16-24	1.27
July 24-Aug. 3	1.31
Aug. 3-11	1.71
Aug. 11-22	0.75
Aug. 22-Sept. 3	0.11
Sept. 3-13	0.59
Sept. 13-25	0.82

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

Table B19. Mean Autotrophic Index of Periphyton During Each of the Nine Accrual Periods.

Accrual Period, Inclusive Dates, 1981	Mean Autotrophic Index*
June 22-July 8	-
July 8-16	4,849
July 16-24	1,203
July 24-Aug. 3	5,294
Aug. 3-11	1,958
Aug. 11-22	8,585
Aug. 22-Sept. 3	14,030
Sept. 3-13	9,145
Sept. 13-25	5,373

* Means connected by the same bracket are not significantly different from one another at the 0.05 level.

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HEAVY METAL CONCENTRATIONS, PRIMARY PRODUCTIVITY, CHLOROPHYLL-A LEVELS, AND THE AUTOTROPHIC INDICES OF STREAM PERIPHYTON SUBJECTED TO WOOD PRESERVATION WASTE

by

Mary Ellen Heppner

(ABSTRACT)

A study was undertaken during June through September, 1981, in which the periphyton from ten locations in Culpeper, Virginia, were analyzed for arsenic, chromium, and copper, both upstream and downstream from the source of contamination. The periphyton primary productivity, chlorophyll-a content, and autotrophic index were also determined. Water samples were analyzed for the three metals; arsenic, chromium, and copper.

The highest mean periphyton arsenic and chromium concentration occurred at the station 1.2 miles below the site of contamination. The copper concentration in the periphyton was highest 2.7 miles below the area of contamination. All three periphyton metal concentrations were dependent upon the location of the sampling site, but only the periphyton copper content was dependent upon the date of sampling. All three periphyton metal concentrations below the spill site were higher than normal concentrations found in uncontaminated periphyton.

Periphyton primary productivity was greatest at the station above the spill site and lowest at the site 1.2 miles below the site of contamination. A negative correlation was observed between the productivity

and the periphyton chromium concentration. No other relationships were noted. The chlorophyll-a level in the periphyton was dependent only upon the date of sampling, not on the location of the sampling site. No relationships were observed between the chlorophyll-a levels and any of the three metals' concentrations in the periphyton.

The autotrophic indices, the ratio of the organic matter to the periphyton chlorophyll-a concentration, were very high at all stations, indicating organically polluted water. Both the location and date of sampling significantly affected the autrophic index.