THE UNIVERSITY OF MANITOBA

THE EFFECTS OF A NUMBER OF HERBICIDES UPON PHOTOSYNTHESIS AND HETEROTROPHY OF NATURALLY OCCURRING ALGAL AND BACTERIAL COMMUNITIES IN DELTA MARSH, MANITOBA.

BY

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ABSTRACT

The short term effects of a number of herbicides upon the photosynthetic rate of two algal communities, phytoplankton and periphyton, and heterotrophic assimilation of phyto- and bacterioplankton populations in the light and the dark were determined. All experimental work was conducted on samples of naturally occurring algal and bacterial communities in Delta Marsh, Manitoba and all such samples were treated in the laboratory under strictly controlled environmental conditions. Fourteen commercial grade herbicides from eight herbicidal groups: Phenoxyacetic Acids (2,4-D and MCPA), Benzoic Acids ("Amiben"), Aliphatic Acids (TCA and "Dalapon"), "N" Heterocyclics ("Simazine", "Atrazine" and "Amitrole"), Substituted Ureas ("Linuron"), Carbamates ("Barban", EPTC and "Triallate"), Bipyridyls ("Paraquat") and Inorganic (copper sulphate) were employed in this study.

Only three herbicides, "Linuron", "Simazine" and "Atrazine" were capable of totally inhibiting phytoplankton photosynthesis as opposed to seven for periphyton photosynthesis, "Linuron", "Simazine", "Atrazine", "Barban", "Paraquat", copper sulphate and "Dalapon". Excluding 2,4-D, MCPA and "Amiben", 50% reductions of algal photosynthesis occurred with all other herbicides

investigated in the experimental program.

The herbicides most toxic to planktonic heterotrophs were copper sulphate, "Paraquat", "Barban", "Triallate", EPTC and "Linuron"; the only herbicides capable of reducing heterotrophic assimilation of ^{14}C-glucose by photo- and bacterioplankton populations by 50%. 2,4-D and MCPA produced moderate inhibitions of planktonic heterotrophy whereas the six remaining herbicides exhibited slight or no inhibition. With the exception of "Barban", there appeared to be no apparent difference between illuminated and darkened samples of heterotrophs.

INTRODUCTION

Herbicides are chemicals that kill and/or inhibit unwanted vegetation. The means by which this is affected are diverse and in some cases unknown. With increased utilization of herbicides, there is a greater probability of herbicides infiltrating our water systems and in many instances resulting in as yet unpredictable consequences.

Algae are the primary producers (the first trophic level) in a body of water. The infiltration of foreign chemicals into the aquatic environment may threaten their existence and this in turn may have reprocussions on the other trophic levels in the food chain. By the same token any adverse effect of herbicides on micro-heterotrophs in the aquatic ecosystem may retard mineralization processes and consequently limit the productivity of the system.

Little published information exists on herbicidal effects upon algal photosynthesis and planktonic heterotrophy. Accordingly, in May 1974, a study was undertaken to investigate the effects of fourteen commonly used herbicides upon the photosynthetic rate of two algal communities and also to determine the effects, if any, upon

planktonic heterotrophy.

All experimental work was conducted on samples of naturally occurring algal and bacterial communities in the Delta Marsh, Manitoba. The experimental approach basically consisted of enriching aliquots of phytoplankton and bacterioplankton with known amounts of herbicide and monitoring the herbicidal effects by measuring relative rates of photosynthesis and heterotrophy.

The ^{14}C-method first proposed by Steeman Nielsen (1952) and modified by Schindler (1966) and Strickland and Parsons (1968) was utilized to monitor the herbicidal effects upon photosynthesis. Herbicidal effects upon planktonic heterotrophy were monitored by measurement of the assimilation of ^{14}C-labelled glucose (Parsons and Strickland 1962).

LITERATURE REVIEW

Today's emphasis on higher crop production and reduced processing costs have stimulated much research on the development of new herbicides and the greater utilization of existing herbicides. In 1962, in the United States alone, more than 95 million pounds of herbicides were applied to farmlands and in 1969 the amount almost quadrupled to 348 million pounds (Metcalf 1971). The Subcommittee on Weeds (1968) states that herbicides now account for approximately one-half of all pesticide usage and the relative usage is increasing. Büchel (1972) also points out the exceedingly high utilization of herbicides relative to insecticides and fungicides.

In conjuction with the increased emphasis on research and development of new herbicides, research upon the effects of herbicides in the environment was stimulated. Woodford et al. (1958) points out that during the four year period 1953-1957, approximately 10,200 papers on chemical weed control have appeared in the Weed Abstracts. Most of these papers relate to work on the evaluation of herbicides under field con-

ditions and have as their object the development of practical recommendations (Woodford et al 1958). Such proliferation of information on herbicides has continued.

Herbicide toxicological studies of algal and bacterioplankton populations have, however, been almost totally ignored in all these investigations and in investigations involving aquatic pollutants. Research on the effects of herbicides upon algal photosynthesis, and upon the assimilation of dissolved organic materials by heterotrophy is almost nonexistent.

The classification or grouping of herbicides is dependent upon their chemical structures. Only categories containing the selected herbicides in this experimental program will be discussed in this review.

The classification system adopted for the herbicides in this project is a hybrid of a number of workers' schemes (Klingman 1961; Crafts 1961; Hilton et al. 1963; Subcommittee on Weeds 1968; Büchel 1972; and, Ashton and Crafts 1973). The fourteen herbicides investigated in this experimental program are representatives of the following groups: Phenoxyacetic Acids, Benzoic Acids, Aliphatic Acids, "N" Heterocyclics, Substituted Ureas, Carbamates, Bipyridyls; and a single inorganic herbicide - copper sulphate.

A. Characteristics of the herbicides

1. Phenoxyacetic Acids

Many herbicides of this group exhibit general physiological properties of natural auxins (Klingman 1961; Crafts 1961; Hilton et al. 1963; Subcommittee on Weeds 1968) and demonstrate an ability to be translocated in plants (Subcommittee on Weeds 1968). 2,4-Dichlorophenoxyacetic acid (2, 4-D) and 4-chloro-2 methylphenoxyacetic acid (MCPA) are two members of this group which have this feature in common.

Two,4-D is a systemic herbicide used quite widely for control of broadleaf weeds in cereal and other crops. It is also effective against woody vegetation (MDA 1973; WSSA 1974). The herbicide is subject to microbial breakdown, in warm, moist soils; leaching, dependent upon soil type and herbicide formulations, and only slight photodecomposition in soils (WSSA 1974). The longevity of 2,4-D in soils is dependent upon soil conditions, and ranges approximately 1 month in warm, moist soils to six months in drier soils (WSSA 1974).

Two,4-D is one of the most extensively researched herbicides and is known to affect a number of plant processes; photosynthesis, respiration, protein synthesis, enzyme systems and mineral uptake, all dependent upon

concentrations of the herbicide and types of plants used for experimentation (Woodford et al. 1958; Klingman 1961; Crafts 1961; Hilton et al. 1963; Subcommittee on Weeds 1968).

The effects of 2,4-D upon lower plant forms in the aquatic environment may be quite different from effects on higher terrestrial plants. In the aquatic ecosystem 2,4-D may undergo photodecomposition. Aly and Faust (1964) demonstrated that within fifty minutes, 2,4-D sodium salt in aqueous solution, pH 7.0, underwent 50% photocecomposition when illuminated with ultraviolet light. Furthermore, 2,4-dichlorophenol, the degradative product of the above reaction was more photolabile than its predecessor, with a 50% loss in five minutes at pH 7.0. They also state that this process is pH dependent and that photolysis occurred faster at pH 9.0 than at pH 7.0. However, Aly and Faust (1964) also suggest that ultraviolet energy from natural solar radiation is not expected to decompose 2,4-D. Crosby and Tutass (1966) disagree with the latter statement, and state that in their experiments 2,4-D decomposition occurred with both natural and artificial light.

In simulated lake studies, Aly and Faust (1964) demonstrated that the amount of deactivation of 2,4-D,

due to sorption to clay particles, is insignificant and that 2,4-D persisted up to 120 days in lake waters aerobically incubated in the laboratory. Frank (1972) on the other hand states that herbicides applied to waters for aquatic weed control, are introduced initially at high levels, but then residues are often not detectable within a few days or weeks. He attributes the reduction of herbicide levels to be due to dilution in the water and adsorption to soil and aquatic plants (Frank et al. 1970).

MCPA is very similar to 2,4-D. It is also a systemic herbicide, but is more selective towards broadleaf weeds (WSSA 1974). Its herbicidal properties, length of residual toxicity in the soil and toxicity to man and animals are similar to 2,4-D (Klingman 1961). It differs from 2,4-D in that it is rapidly leached from soils and is relatively stable to light (WSSA 1974).

2. Benzoic Acids

Some members of the benzoic acid group exhibit hormone-like properties (Crafts 1961). These herbicides are relatively strong acids and thus form salts in plants and in soils (Subcommittee on Weeds 1968). These substances are systemic growth regulators, that inhibit root

development of seedling weeds, but their specific mode of action is still unknown (Subcommittee on Weeds 1968).

"Amiben" is the trade name for 3-amino-2,5-Dichlorobenzoic acid. It is a preemergent herbicide used for control of broadleaf weeds and annual grasses (Crafts 1961). According to the WSSA (1974), "Amiben" is readily leached in sandy soils, broken down by soil microorganisms, and is subject to some degree of photo-decomposition in aqueous solutions. Under normal soil conditions the life expectancy of "Amiben" is six to eight weeks (WSSA 1974).

3. Aliphatic Acids

The aliphatic acids are open chained acids with a carboxyl group (Klingman 1961). Two chlorinated aliphatic acids namely Trichloroacetic Acid (TCA) and "Dalapon" (2,2-dichloropiopionic acid) are widely employed as herbicides. These herbicides tend to be more toxic towards monocotyledenous than dicotyledononus plants. Both TCA and "Dalapon" act similarly upon weeds and often their effects are not distinguishable from each other (Woodford et al. 1958).

TCA is a strong acid, highly soluble in water and is readily leached from soils (Crafts 1961). Its resi-

dual toxicity in soils is three to ten weeks, depending upon soil conditions (WSSA 1974). TCA precipitates proteins and hence, once in the protoplast probably inactivates enzyme systems (Klingman 1961; Crafts 1961; Subcommittee on Weeds 1968).

"Dalapon" is also a strong acid, highly soluble in water and is readily leached from soils (WSSA 1974). It is a more effective grass killer than TCA and has a slightly shorter period of residual toxicity in soil, (two to four weeks) (Klingman 1961; WSSA 1974). The residual toxicity of "Dalapon" in water is dependent upon hydrolysis, photodecomposition and microbial breakdown. Smith et al. (1957) demonstrated that "Dalapon" salt undergoes hydrolysis to yield pyruvic acid. Kenaga (1974) points out that the chemical hydrolytic half-life of "Dalapon" is two months at temperatures less than 25°C. The hydrolytic life of "Dalapon" is also dependent upon pH. Kenaga (1974) makes reference to work by Tacey and Bellinger (1958) and states that at 60°C hydrolysis of "Dalapon" sodium salt was 20% complete in 30 hours at which time the pH was 2.3. By maintaining a pH of 12 during hydrolysis, hydrolysis was 40% complete in 30 hours.

"Dalapon" may also be photodegraded to pyruvate. Kenaga (1974) citing work by Tanaka (1972 b) states that "Dalapon" will be photodegraded quite quickly to pyruvate which in turn is decarboxylated to carbon dioxide and acetaldehyde at a much slower rate. As an illustration he states that 70% of a 0.25 M "Dalapon" solution was photodegraded in seven hours, as compared to 1% hydrolysis in the dark for seven hours.

Microbial breakdown of "Dalapon" occurs quite rapidly in the aquatic environments, and furthermore, degradive products such as pyruvate or acetaldehyde are nontoxic to aquatic flora and fauna (Kenaga 1974). Frank et al. (1970) reported that only trace amounts of "Dalapon", less than 10 ppb, were detected from water flowing from treated areas several hours after application of the herbicide.

4. "N" Heterocyclics

Cyclic compounds which contain in addition to carbon, one or more other atoms in the ring are called heterocyclic (Linstromberg 1966). In the case of "N" heterocyclics, ring structures are composed of nitrogen and carbon atoms. Two azines, (six membered rings with two or more N atoms), "Simazine" and "Atrazine",

commonly known as the "S" or symmetrical triazines and one azole, (five membered ring with two or more N atoms), "Amitrole-T" are reviewed here.

The "S" triazine herbicides are comprised of compounds which are direct inhibitors of plant photosynthesis (Klingman 1961; Crafts 1961; Hilton et al. 1963; Zewig 1969; Büchel 1972; WSSA 1974). It is postulated that these herbicides affect the Hill reaction of photosynthesis, (Good 1961; Moreland and Hill 1962).

"Simazine", 2-chloro-4,6-bis (ethylamino)-S-triazine, is a widely used selective herbicide for control of broadleaf and grassy weeds in a variety of crops (WSSA 1974). It is more readily adsorped to soils with high clay or organic content rather than soils of low clay and organic content and with its low water solubility, (5 ppm at 20^{o}C) it is not easily leached. As a result it is persistent in soils, for three to six months if applied as a preemergent herbicide; and for six to twenty-four months if applied as a soil sterilant (Klingman 1961).

"Simazine" rapidly blocks the Hill reaction of photosynthesis (Gysin and Knüsli 1960; Good 1961; Moreland and Hill 1962). Exer (1958) found a 50% inhibition of photosynthesis of isolated corn and spinach chloroplasts with a concentration of 7×10^{-7} M (0.141 ppm). Moreland

et al. (1959) observed that "Simazine" reduced the photosynthetic rate of isolated barley chloroplasts by 50% at a concentration of 4.6×10^{-6}M (0.928 ppm). Ashton et al. (1960) have reported total inhibition of photosynthesis of excised leaves from bean plants with a concentration of 1 ppm of "Simazine".

"Atrazine", 2-chloro-4-ethylamino-6-isopropylamino-S-triazine, is also used widely as a selective herbicide for control of broadleaf and grassy weeds in a variety of crops (WSSA 1974). It is structurally similar to "Simazine" as well as exhibiting similar herbicidal effects. "Atrazine" is slightly more soluble in water, (33 ppm at 25 C) and therefore its residual toxicity is shorter (Klingman 1961). Exer (1958) found that "Atrazine" was 1.8 - 2.4 times as effective of reducing photosynthesis of isolated corn and spinach chloroplasts when compared with the same concentration of "Simazine".

"Amitrole-T" consists of a mixture of "Amitrole" and ammonium thiocyanate. This mixture has proven to be far superior for herbicidal use than just "Amitrole" alone (Crafts 1961), although considerably more information is available on "Amitrole". "Amitrole" is the common name for 3-amino-1,2,4-triazole. The herbicide is utilized in control of broadleaf weed and grasses as well as control of aquatic vegetation. It is subject

to microbial breakdown taking approximately two to three weeks in warm, moist soils, and to minor photodecomposition. The resultant persistency of "Amitrole" in soils is approximately four weeks (WSSA 1974). In water "Amitrole" has been known to persist for up to a year (Subcommittee on Plants 1968).

"Amitrole" may affect plant processes in a number of ways. Crafts (1961) citing work by Hall et al. (1954) states that in low concentrations "Amitrole" stimulates growth whereas at higher concentrations it inhibits growth and causes chlorosis. The chlorosis may be temporary or permanent depending upon the concentrations applied for herbicidal control. He also points out that sprayed cotyledons of cotton seedlings in concentrations 650 ppm or greater of "Amitrole" remained permanently chlorotic whereas below this concentration chlorosis was only temporary. The chlorotic effect may be a result of chlorophyll breakdown or an inhibition of chloroplast formation (Klingman 1961). Hilton et al. (1963) state that "Amitrole" also affects cation exchange and purine synthesis.

5. Substituted Ureas

This group of herbicides are synthesized by replacing hydrogen atoms of urea with other elements or

groups of elements such as methyl, phenyl or chlorophenyl groups. The substituted urea herbicides like the "S" triazines are direct inhibitors of the Hill reaction of photosynthesis (Hilton et al. 1963; Subcommittee on Weeds 1968, Zewig 1969; Büchel 1972). Büchel (1972) has found these compounds to be inhibitors of the electron transport system in photosystem II of photosynthesis and Moreland et al. (1958) and Moreland (1967) pinpoint the site of inhibition of the substituted ureas, as being between plastoquinone and the quencher. Concentration between 10^{-7} - 10^{-5}M will inhibit the Hill reaction by 50% (Subcommittee on Weeds 1968).

"Linuron", 3(3,4-dichlorophenyl)-1-methoxy-1-methylurea, is a selective herbicide which controls germinating and newly established broadleaf and grassy weeds in a variety of crops. The adsorption of the herbicide increases as clay and organic content of soil increases; leaching and photodecomposition are insignificant. Phytotoxic levels disappear from the soil, in approximately four months from application (WSSA 1974).

6. Carbamates

The carbamates are derivatives of carbamic acid. These herbicides are usually highly selective and most

of them are for preemergence utilization (Subcommittee on Weeds 1968). They are readily hydrolyzed in soils or aqueous media (Metcalf 1971). The carbamate may be divided into two subgroups: aryl carbamates and thiocarbamates.

The aryl carbamates are derivatives of carbamic acid which possess aryl group substitutions. At lower concentrations, these herbicides generally cause a cessation of protein synthesis, thereby stopping cell division (Klingman 1961). At higher concentrations plant photosynthesis may be inhibited (Subcommittee on Weeds 1968).

"Barban", 4 chloro-2-butynyl N-(3 chlorophenyl) carbamate, represents this subgroup of herbicides. "Barban" is selectively used for the control of wild oats (MDA 1973; WSSA 1974). It is highly adsorped to soils and its main mode of deactivation is by microbial decomposition. Within 3 weeks only trace amounts may be left in the soil (WSSA 1974).

The thiocarbamates are derivatives of carbamic acid with sulphydral group substitutions. EPTC ("Eptam") and "Triallate" are representatives of this subgroup.

EPTC is a selective herbicide which provides effective preemergent control of a number of broadleaf

and grassy weeds. It is relatively soluble in water, (375 ppm at 20°C), and is readily leached in soils which are low in clay and organic matter (Klingman 1961). Residual phytotoxicity in moist, warm, loamy soils is approximately four to six weeks (Klingman 1961). The mode of action of this herbicide is not specifically known (Hilton et al. 1963; WSSA 1974), but at concentrations of a few ppm it inhibits growth in the meristematic region of grass leaves (WSSA 1974).

"Triallate" is the common name for S-(2,3,3, trichloroallyl) diisopropylthiocarbamate. It is a pre-emergent herbicide used for control of wild oats (MDA 1973). According to the WSSA (1974), "Triallate" has low water solubility, (4 ppm), is readily adsorped to soil colloids, and is resistant to photodecomposition. Volatilization only occurs if the herbicide remains on the surface of soils at high temperatures. The main degradation pathway of "Triallate" is by microbial breakdown, with resultant phytotoxicities in soils of approximately six weeks (Subcommittee on Weeds 1968; WSSA 1974). The mode of action of "Triallate" is by inhibiting cell division and cell elongation (WSSA 1974).

7. Bipyridyls

"Paraquat", 1,1'-dimethyl-4,4'-bipyridinium ion (as dichloride salts) is a contact or quick-kill herbicide, used for total eradication of terrestrial and aquatic weeds. The herbicide is completely water soluble, (70 g/100 ml of water at 20°C) (Brian et al. 1958) and is rendered biologically inactive when in contact with soil (Akhavein and Linscott 1968). When exposed to ultraviolet light or exposed to intense sunlight "Paraquat" will decompose (Slade 1965; WSSA 1974), but in aqueous solutions photodegradation does not occur (Slade 1965). Persistence in the soils is not known, but expected to be quite long (Akhavein and Linscott 1968; WSSA 1974). Persistence in water is quite short as it is bound to the soil particles (Coats et al. 1964). Frank et al. (1966) could not detect "Paraquat" twelve days after its application to several ponds, although an accumulation was detected in the top inch of the bottom sediments.

"Paraquat" is capable of undergoing reversible oxidation-reduction reactions within plants. This is the basis of its phytotoxicity (Hilton et al. 1963; Akhavein and Linscott 1968; Büchel 1972). The herbicide is reduced to its respective free radical, by accepting electrons from photosystem I, in place of ferredoxin, or

by pulling electrons from $NADPH_2$ (Black and Myers 1966), which accounts for its toxicity in light and dark. Büchel (1972) states that this process is too slow and does not account for the rapid action of bipyridyl herbicides upon plants. Akhavein and Linscott (1968) point out that the free radical is readily oxidized from the oxygen of photosystem II, resulting in the formation of hydrogen peroxide and oxidized "Paraquat", which may account for the quick death of the plants and may be the reason that many report (Büchel 1972, WSSA 1974 and so on) the herbicide as being light dependent.

8. Copper Sulphate

As early as 1896, Bonnet discovered that copper sulphate was effective in controlling weeds in cereals (Subcommittee on Weeds 1968). By 1904, Moore and Kellerman had reported successful control of unwanted algae with the utilization of copper sulphate as an algicide. Throughout the years, copper sulphate has been the safest, most effective, cheapest, and probably most utilized algicide (Subcommittee on Weeds 1968). The guide to Chemical Weed Control, MDA 1973), labels copper sulfate as the standard treatment for algae in water.

Copper sulphate (copper sulphate pentahydrate) is completely water soluble (Thomson 1973) and at recom-

mended levels, 0.1 to 0.5 ppm for control of planktonic algae and 0.5 and 1.0 ppm for control of filamentous algae, is nontoxic to fish and animals (Klingman 1961; Subcommittee on Weeds 1968). The specific mode of action of copper sulphate is not known, but it affects plant photosynthesis (Steeman Neilsen et al. 1969; Cendo-Maldonado and Swader 1972) and cell membrane permeabilities (McBrien and Hassall 1965).

B. Bioassay techniques

The bioassay techniques for the evaluation of the effects of herbicides upon algae may be divided into the two categories of the effects of herbicides upon algal growth and upon algal photosynthesis.

The monitoring of growth of algae in response to herbicides has been carried out in many ways. Fitzgerald et al. 1952; Palmer and Maloney 1955; Fitzgerald and Faust (1963) and Vance and Smith (1969) have visually compared with untreated algal samples and arbitrarily ranked the amount of growth inhibition or kill of treated algal samples. Other workers have monitored the effects of herbicides upon growth of algae by cell enumeration (Castelfranco and Bisalputra 1965; Shennan and Fletcher 1965; Fitzgerald 1957; Steenan Nielsen and

Kamp Nielsen 1970), by measuring volumes of packed cell volumes (Ashton et al. 1966), by spectrophotometric, turbidimetric or colorimetric techniques (Wolf 1962; Ukeles 1962; Loeppky and Tweedy 1969; Elder 1970; Walsh 1972; Voight and Lynch 1974). Chlorophyll and pigment determinations have also been utilized to monitor the effects of herbicides upon growth of algae (Castelfranco and Bisalputra 1965; Ashton et al 1966; Zewig et al. 1967; Kratky and Waven 1971).

The effects of herbicides upon algal photosynthesis have been monitored by assimilation of radioactive carbon ($^{14}CO_2$) (Butler 1965 a; Steeman Nielsen et al. 1969) or monitored by the evolution of oxygen utilizing Gilson or Warburg respirometers (Geoghegan 1957; Zewig et al. 1967; Walsh 1972; Hollister and Walsh 1973) or with oxygen electrodes (Overnell 1975).

C. Herbicidal effects upon algae

The herbicidal effects upon algae will be reviewed in terms of concentrations of herbicides which are inhibitory or stimulatory to algal growth and photosynthesis.

1. Phenoxyacetic Acids

Most research indicates that 2,4-D is not detrimental to growth and photosynthesis of algae. In toxicological studies carried out by Vance and Smith (1969), concentrations of 2,4-D as great as 200 ppm, showed no inhibition of growth of Scenedesmus quadricauda, Chlamydomonas eugametos and Chlorella pyrenoidosa. Elder (1970) demonstrated similar results with a number of species of algae. Culture media containing algae and 2,4-D, up to 220 ppm, produced no apparent difference in optical density when compared with control samples after an incubation period of three days. Poorman (1973) demonstrated that Euglena gracilis underwent morphological changes when subjected to 100 ppm of 2,4-D for 24 hours or for seven days. The changes were only temporary as the cell reverted back to their normal state after being transferred to herbicide-free medium for a period of seven days. Thomas et al. (1973) utilizing the "paper disc technique" found that Chlorella sp. was capable of normal growth in the presence of 1000 ppm of 2,4-D.

In short term studies, Butler (1965 a) investigating the effect of a number of pesticides upon the photosynthetic rate of estuarine phytoplankton, found no inhibition of photosynthesis at the end of a four hour period with one ppm of 2,4-D.

Other workers have shown that 2,4-D may be inhibitory to algal growth and photosynthesis. Walsh (1972) observed total inhibition of growth of Chlorococcum sp., Dunaliella tertiolecta, Isochrysis galbana and Phaeodactylum tricornutum, in concentrations of 2,4-D ranging between 75-100 ppm. Growth of the above species was reduced by 50% with concentrations ranging between 50-60 ppm. The photosynthetic rates of the four species of algae were also inhibited by 2,4-D. Total inhibition occurred with concentrations ranging between 85-95 ppm; 50% reduction of photosynthesis occurred between 50-60 ppm. Voight and Eynch (1974) also observed that the growth of the green alga Coelastrum microporum was completely inhibited at concentrations ranging between 60-65 ppm 2,4-D and, the blue-green alga Anacystis nidulans was inhibited with concentrations greater than 90 ppm 2,4-D.

Very little is known about the effects of MCPA upon algae, but algal responses are expected to be similar to 2,4-D. In reviewing the work by Shennan and Fletcher (1965) however, it appears that MCPA has only a slight effect, if any, upon algae. In their investigations, the growth of Chlamydomonas subangulosa and Dictyococcus terrestris were inhibited 17 and 4%, respectively with 500 ppm of MCPA and 42 and 11% respectively with 1000 ppm.

2. Benzoic Acids

Only extremely high concentrations of "Amiben" appear to be detrimental to algae. Chlorella pyrenoidosa exhibited no inhibition of growth in the presence of 1000 ppm of "Amiben" (Thomas et al. 1973). Walsh (1972) showed that the ammonium salts of "Amiben" were completely inhibitory to growth and photosynthesis of four species of algae, but only at high concentrations. Growth was completely inhibited with concentrations between 1500-5500 ppm and oxygen evolution inhibited with concentrations greater than 5000 ppm.

3. Aliphatic Acids

Repeated effects of aliphatic acids appear contradictory on one hand. Thomas et al. (1973) found no inhibition of growth of Chlorella pyrenoidosa when subjected to a concentration of 1000 ppm of "Dalapon". Walsh (1972) observed complete inhibition of growth of Chlorococcum sp., Dunaliella tertiolecta, Isochrysis galbana and Phaecodactylum tircornutum with concentrations of "Dalapon" greater than 750 ppm. Growth of the above species were reduced by 50% with concentrations ranging between 400-500 ppm.

With "Dalapon" additions of 1 ppm, Butler (1965 a) found no reduction in $^{14}CO_2$ uptake by estuarine phytoplankton in a four hour period. Walsh (1972) observed

total inhibition of algal photosynthesis, with concentrations of "Dalapon" exceeding 4000 ppm, and reduction by 50% with concentrations ranging between 2250-2500 ppm.

4. "N" Heterocyclics

Responses of algae to the "S" triazine herbicides, "Simazine" and "Atrazine" appear to be quite variable. Walsh (1972) found that 0.5 - 5.0 ppm "Simazine" reduced the growth of four algal species by 50% while 1.0 - 8.0 ppm affected total inhibition. Oxygen evolution of the same four species of algaewwas totally inhibited by concentrations ranging between 1.5 - 6.0 ppm and inhibited by 50% by concentrations of 0.6 - 4.0 ppm. Lower concentrations of "Atrazine" produced the same effects. Total inhibition of growth was affected by 0.2 - 1.2 ppm "Atrazine" and 50% inhibition by 0.1 - 0.3 ppm (Walsh 1972). 100% reduction of oxygen evolution was caused by 0.2 - 0.7 ppm and 50% reduction by 0.1 - 0.3 ppm (Walsh 1972). In an investigation of eighteen algal species representing 4 familees Hollister and Walsh (1973) demonstrated that the mean range of "Atrazine" concentrations required to reduce oxygen evolution by 50% was 0.079 - 0.265 ppm. There were, however, substantial differences among and within families. Ashton et al. (1966) demonstrated that in the presence

of 70 ppm of "Atrazine", growth of Chlorella vulgaris declined to 23.2% of untreated cells in 48 hours and to 14.6% after 72 hours. Chlorophyll levels of the cells steadily fell off; After 24, 48 and 72 hours chlorophyll concentrations were 24.6%, 19.6% and 13.8% respectively of control values. Ashton et al. (1966) also observed that the inhibitory effects of "Atrazine" upon Chlorella vulgaris were overcome if the growth medium was supplemented with a 2% concentration of glucose. Snow (1963) demonstrated that "Simazine" was toxic to algae, and that the effect of the chemical was rapid. With a concentration of 2 ppm, a bloom of green planktonic algae, was cleared from a bass pond within 24 hours. Hoeppky and Tweedy (1969) found "Atrazine" to be inhibitory to the growth of Chlamydomonas reinhardii at a concentration of 0.5 ppm whereas concentrations of 5.0 ppm did not affect the growth of Chlamydomonas eugametos. They also observed that 5 ppm "Atrazine", had no appreciable effects upon the heterotrophic growth of Chlamydomonas reinhardii. The manufacturers of "Simazine", (CIBA-Geigy), have demonstrated "Simazine" to be an effective algal toxin, and are currently trying to obtain government registration for its algicidal use in lakes at a recommended level of 0.5 ppm.

Other workers have, however, found that "Simazine" has no inhibitory effects upon a number of species of algae. Otto (1970) observed that "Simazine" concentrations of 0.5, 5.0 and 10 ppm had no effect upon the growth of the blue-green alga, Phormidium ambigum. The algal species, Scenedesmus quadricauda, Chlamydomonas eugametos and Chlorella pyrinoidosa exhibited no inhibition of growth with concentrations as high as 200 ppm of "Simazine" (Vance and Smith 1969). Thomas et al. (1973) demonstrated slight and no inhibition of growth of Chlorella pyrenoidosa with concentrations of 1000 ppm of "Simazine" and "Atrazine", respectively.

"Amitrole" and "Amitrole-T", act similarly, although the latter may be more effective at lower levels (Klingman 1961). Wolf (1962) investigating the effects of "Amitrole" upon the growth of Chlorella pyrenoidosa, found that 5.0 ppm inhibited growth by 50%. Castelfranco and Bisalputra (1965) observed similar results, but in this case total inhibition of Scenedesmus quadricauda was obtained with 10 ppm. They also found that the herbicidal effects on Scenedesmus were temporary, as cells washed and transferred to herbicide-free medium, reverted to normal growth patterns. Kratky and Warren (1971) showed that the growth of Chlorella pyrenoidosa was inhibited less than 50% with an "Amitrole" concentration of 20 ppm and greater than 50%

with 30 ppm. Vance and Smith (1969) demonstrated that complete growth inhibition of Chlorella pyrenoidosa, Scenedesmus quadricauda and Chlamydomonas eugametos occurred with concentrations of "Amitrole-T" greater than 150 ppm. Scenedesmus and Chlorella were not affected by concentrations of 20, 50 and 100 ppm, but growth was inhibited by 30% at 150 ppm, by 70% at 200 ppm and totally killed at concentrations of 250 ppm or more. Walker et al. (1973) found no adverse effects of "Amitrole" upon the growth of Chlorella pyrenoidosa, at concentrations as high as 1000 ppm.

5. Substituted Ureas

There is little documentation of the effects of "Linuron" upon algae. Consequently, references will be made to compounds which are closely related to this herbicide. Both "Linuron" and "Diuron" demonstrate similar herbicidal activity patterns (Evans of DuPont, Pers.Comm.). The substituted ureas appear to be one of the most toxic groups of herbicides to algal growth and photosynthesis. Fitzgerald (1957) demonstrated total inhibition of growth of Rhizoclonium sp., Cladophorora sp. and Spirogyra sp. with 1 ppm of "Diuron" and at 2 ppm for Microcystis aeruginosa. Geoghegan (1957) investigated the

effects of a number of substituted urea herbicides upon the growth of Chlorella vulgaris and found that "CMU", 3 para-chlorophenyl-1:1-dimethylurea; "PDU", 3 phenyl-1:1-dimethylurea; and "Diuron", 3(3':4'-dichlorophenyl-1:1 dimethylurea; all totally inhibited the growth of Chlorella vulgaris, but at different concentrations, these being 0.5, 5.0 and 0.1 ppm respectively. He also observed that 60, 25 and 250 times the amount of the above herbicides were required for equivalent inhibition of growth of Chlorella when the algal medium was supplemented with a 2% concentration of glucose. Ukeles (1962) also reports that the growth of Chlorella sp. was inhibited by 0.04 ppm of "Diuron", Protococcus sp. and Dunaliella tricornutum by 0.004 ppm and Monochrysis lutheri with concentrations of "Diuron" as low as 2.0×10^{-5} ppm. Walsh (1972) found that the growth of four species of algae was reduced by 50% with concentrations of "Diuron" ranging between 0.01 - 0.02 ppm. Total inhibition of the growth of the algal species occurred with concentrations of 0.03 - 0.05 ppm of "Diuron". "Diuron" also proved to be quite detrimental to Scenedesmus quadricauda. With concentrations of 0.1 ppm Stadnyk et al. (1971) observed that cell numbers drastically decreased from the second day of treatment to about the sixth day. Carbon assimilation ($^{14}CO_2$) was reduced by 90% within four days.

Thomas et al. (1973) observed no inhibitory effects upon the growth of Chlorella pyrenoidosa with concentrations of 100 ppm of "Linuron", but severe effects with 1000 ppm. It must be noted here that the alga was grown upon agar supplemented with a 1% glucose concentration so that the effects of the herbicide may not be fully exhibited (Geoghegan 1957).

The substituted ureas appear to be relatively toxic to algae in short periods of time. Zewig et al. (1967) found that oxygen evolution by Chlorella pyrenoidosa was totally inhibited within ten minutes of the addition of 7 ppm "Diuron". This was found to be a temporary effect as oxygen evolution could be restored by washing the cells. Butler (1965 a) noted a 87 - 94% decrease of carbon fixation by estuarine phytoplankton when subjected to concentrations of 1 ppm of "Diuron", "Neburon" and "Monuron" for a period of four hours. Walsh (1972) and Hollister and Walsh (1973) determined minumum concentrations of herbicides which reduced the oxygen evolution of a number of algal species by 50%. "Diuron" concentrations of 0.010 - 0.024 ppm, were required for members of the Chlorophyceae, Chrysophyceae and Rhodophyceae whereas concentrations of approximately 0.067 ppm were required for members of the Bacillariophyceae. Total inhibition of oxygen evolution occurred at concentrations of "Diuron" ranging from

0.02 to 0.05 ppm (Walsh 1972). Overnell (1975) observed that "Diuron" reduced the oxygen evolution of Chlamydomonas reinhardii by 50% with concentrations of 4.0×10^{-7}M (0.093 ppm).

6. Carbamates

Very little information is available about the toxicities of carbamic herbicides to algae. Since "Barban" has a low rate of terrestrial application and low leaching properties, its effects on algae have been little investigated (Riden, Pers. Comm.). The only investigation appears to be that of Kratky and Warren (1971). They found that growth of Chlorella pyrenoidosa was inhibited by less than 50% by 1 ppm and greater than 50% by 10 ppm.

From the work available on EPTC; it appears to be nontoxic to the growth and photosynthesis of algae. Kratky and Warren (1971) state that with concentrations of 1 and 10 ppm of EPTC, growth inhibition of Chlorella pyrenoidosa was less than 50%, although it is not clear by how much less than 50%. Thomas et al. (1973) demonstrated no inhibition of growth to the same species with 1000 ppm EPTC. Grigsby (1958) points out that EPTC, at a concentration of 5 ppm was unsuccessful in controlling the growth of Chlamydomonas sp. The photosynthetic rate of estuarine phytoplankton did not show any adverse

effects when subjected to 1 ppm of EPTC for a period of four hours (Butler 1965 a).

The only documented information available about the effects of "Triallate" upon algae is by Kratky and Warren (1971). Chlorella pyrenoidosa exhibited less than 50% inhibition of growth with 1 and 10 ppm of "Triallate", but how much less than 50% is not stated.

7. Bipyridyls

The bipridyls, "Diquat" and "Paraquat" exhibit similar phytotoxic characteristics upon algae (Walsh 1972).

Zewig et al. (1967) noted no adverse effects upon the oxygen evolution and chlorophyll concentrations of Chlorella pyrenoidosa over a 48 hour period, when subjected to 5.5 ppm of "Diquat". Walsh (1972) on the other hand, found that "Paraquat" totally inhibited growth of four species of algae, at concentrations ranging from 15 to 75 ppm and reduced it by half with concentrations between 5 - 50 ppm. Less than 50% growth inhibition was observed by Kratky and Warren (1971) when Chlorella pyrenoidosa was subjected to concentrations of 10 and 20 ppm of "Paraquat" - dichloride. Thomas et al. (1973) demonstrated that out of 20 herbicides tested at concentrations of 1000 ppm; "Paraquat" and "Diquat" were the most toxic to the growth of Chlorella pyrenoidosa.

In short term experiments, Zewig et al. (1967) observed no decrease in oxygen evolution by Chlorella pyrenoidosa, during 60 minutes exposure with "Diquat" at 5.5 ppm. Walsh (1972) found that extremely high concentrations of "Paraquat" were necessary to inhibit algal photosynthesis totally or by 50%. The four species of algae tested required concentrations greater than 5000 ppm for total inhibition and concentrations ranging from 2500 to greater than 5000 ppm for a reduction of 50%. Butler (1965 a) found that low concentrations of "Paraquat" were quite detrimental to estuarine phytoplankton. With a concentration of 1 ppm of "Paraquat", the photosynthetic rate of estuarine phytoplankton was reduced by 53% of control values within a four hour incubation period.

8. Copper Sulphate

The responses of algae to copper sulphate are quite variable. Microcystis aeruginosa was killed completely within twenty-four hours, after being exposed to 0.2 ppm of copper sulphate (0.051 ppm copper); (Fitzgerald et al. 1952). Similar results were obtained by Fitzgerald et al. (1963). Growth inhibition of the above algal species occurred with concentrations of copper sulphate ranging between 0.05 - 0.30 ppm (0.013 - 0.076) ppm copper). The span of herbicidal concentrations was dependent upon the constituents of the different growth media. Palmer and

Maloney (1955) observed total kill of Microcystis aeruginosa after 21 days incubation with 2 ppm copper sulphate (0.51 ppm copper). Possibly lower concentrations would have given similar results.

Some algae are more susceptible to certain concentrations of copper sulphate than are others. Palmer and Maloney (1955) found that the blue-green, Microcystis aeruginosa, two diatoms, Gomphonema parvulum and Nitzschia palea and one green alga, Chlorella variegata, were much more sensitive to 2 ppm of copper sulphate (0.51 ppm copper) than the blue-green, Cylindrospermum licheniforme and green algae, Scenedesmus obliquus. The more sensitive algal species showed no signs of growth throughout 3, 7, 14 and 21 days of incubation. The less sensitive algae, Cylindrospermum and Scenedesmus showed partial inhibition of growth for the first seven days, than Cylindrospermum exhibited total growth inhibition while Scenedesmus reverted to a normal, healthy state. Fitzgerald et al. (1963) also demonstrated differential responses of algae to copper sulphate. Chlorella pyrenoidosa appeared to be more resistant to higher levels of copper sulphate than Microcystis aeruginosa. Growth of Microcystis was inhibited by concentrations of 0.05 - 0.30 ppm (0.013 - 0.076 ppm copper) as opposed to 1 - 8.0 ppm (0.255 - 2.04 ppm copper) for Chlorella. Mandelli (1969) noted

that the growth of three dinoflagellates, Glenodinium toliaceum, Glenodinium sp. and Exuviaella sp., and four diatom species, Skeletonema costatum, Cyclotella nana, Thalassiosira fluviatilis and Nitzschia closterium, was inhibited in media containing 0.055 and 0.265 ppm of copper, respectively. The blue-green alga, Coccochloris elabans failed to grow in the presence of 0.03 ppm copper, whereas the green alga Dunaliella tertiolecta grew in medium containing 0.60 ppm of copper.

Steeman Nielsen et al. (1969); Steeman Nielsen and Kamp Nielsen (1970) and Steeman Nielsen and Wium Andersen (1970) have stated that the effects of copper upon algae are dependent upon illumination, pH, form of copper and chemical constituents of the medium or the environment. They found that concentration as low as 1 to 2 ppb of ionic copper were poisonous to growth and photosynthesis of algae.

MATERIALS AND METHODS

A. Description of the sampling area

Delta Marsh is located at the southern end of Lake Manitoba and covers an area of approximately 15,000 hectares. The marsh is separated from the lake by a barrier ridge with a number of channels connecting the marsh and the lake. Water levels of the marsh fluctuate with those of the lake, due primarily to wind action.

A permanent marsh channel, the Blind Channel, was selected as the sample site. Water depth at the site fluctuated between 70 and 100 cm throughout the sampling period. Maximum water temperature during the ice-free season (May to October), recorded at 10 cm below the surface, was 25.5C. Water pH ranged from 8.0 to 8.7 and alkalinity values from 250-365 mg $CaCO_3/\ell$. The surrounding vegetation was predominantly Typha latifolia with scattered areas of Phragmites communis and Scirpus spp. Submerged aquatic vegetation consisted predominantly Potamogeton spp. and Myriophyllum sp.

B. Experimental approach

All experimental work was conducted on samples of naturally occurring algal and bacterial communities in the Blind Channel. All such samples were treated in the laboratory under strictly controlled environmental conditions. The experimental program consisted of three series of bioassays on the effects of a selected number of herbicides upon the photosynthetic rate of phytoplankton; upon the photosynthetic rate of periphyton and upon the heterotrophic assimilation of glucose by phytoplankton and bacterioplankton.

Each set of experiments consisted of fourteen bioassays; one bioassay for each herbicide investigated in the experimental program. Commercial grade herbicides representing the following groups; Phenoxyacetic Acids, Benzoic Acids, Aliphatic Acids, "N" Heterocyclics, Substituted Ureas, Carbamates, Bipyridyls and inorganic herbicides were chosen for the study. Common, chemical and commercial names of the herbicides are presented in Table 1. Dates and types of experiments performed throughout the experimental period are listed in Appendix I.

Table 1. Common, chemical and commercial names of the herbicides investigated in the experimental program in Delta Marsh, Manitoba.

Common	Chemical	Commercial
2,4-D	2,4-dichlorophenoxyacetic acid	2,4-D Amine 80
MCPA	2-methyl-4-chlorophenoxyacetic acid	MCPA Amine 80
Chorambenl	3-amino-2, 5-dichlorobenzoic acid	Amiben (ammonium salt)
TCA	Trichloroacetic acid	NaTCA (84% acid equiv.)
Dalapono	2,2-dichloropropionic acid	Dowpon (74% acid equiv.)
Simazine	2-chloro-4,6-bis(ethylamino)-s-triazine	Princeps (50% w.p.)
Atrazine	2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine	AAtrex (80% w.p.)
Amitrole	(3-amino-1,2,4-triazole)	Amitrole-T (2.4lb a.i./Imp.gal)
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea	Lorox (50% w.p.)
Barban	(4-chloro-2-butynyl N-(3 chlorophenyl) carbamate	Carbyne (1.2 lb. a.i./ Imp. gal.)

Table 1. Continued

Common	Chemical	Commercial
EPTC	S-ethyl dipropylthiocarbamate	Eptam (8 lb. a.i./ Imp. gal)
Triallate	S-(2,3,3-trichloroallyl diisopropylthiocarbamate	Avedex BW (46.3% a.i.)
Paraquat	1,1'-dimethyl-4,4-bipyridinium ion (as dichloride salts)	Gramoxone (2 lb. a.i./Imp. gal)
Copper Sulphate	$CuSO_4.5H_2O$	Copper Sulphate Pentahydrate

acid equiv. - acid equivalent
a.i. - active ingredient
Imp. - Imperial
w.p. - wettable powder

C. Field procedure

Field work was restricted to algal collection and the monitoring of dissolved inorganic carbon in the marsh water.

1. Phytoplankton

Field procedures for collection of phytoplankton were identical for all experimental work involving this community. A single station in the mid region of the Blind Channel was subjectively selected as a sample site. For each collection a four liter-darkened carboy was submerged approximately 10 cm below the surface and allowed to fill. The water sample was filtered through 110 μ Nitex mesh net to remove any large zooplankters and transported to the laboratory for experimental purposes.

2. Periphyton

Procedures followed methods described by Hooper (1973). Cellulose acetate was utilized as an artificial substrate for periphyton attachment. Squares of cellulose acetate, measuring approximately 25 cm by 25 cm, were stapled to wooden frames and submerged in a sheltered area on the east side of the Blind Channel. Each of three wooden frames supported eight

acetate squares. A three week colonization period followed before the initiation of experimental work. For each experiment, an acetate square having a homogenous periphytic colonization was selected, detached from the wooden frame and transported to the laboratory in a covered pan containing marsh water. The square with attached periphyton was used for bioassay evaluations. Also for each experiment, a four liter-darkened carboy was filled with surface marsh water, from the same location of the wooden frames, and returned to the laboratory to be used as an incubation medium.

3. Chemical parameters

At weekly intervals water samples were taken to the laboratory from the sample sites for alkalinity determinations.

D. Laboratory procedure

The laboratory procedure consisted of dividing equal amounts of algae into sample bottles, enriching the samples with known amounts of herbicide and monitoring the herbicidal effect by measuring the assimilation of $^{14}CO_2$ and ^{14}C labelled glucose.

In each experiment nine concentrations of herbicide, ranging from 25 ppb to 250 ppm (Table 2) were used, and for each concentration, triplicate illuminated and duplicate darkened samples were utilized. For each experiment, control samples (with no added herbicide) and blanks (filtered water and isotope only) were established. All samples, including controls and blanks, were enclosed in 35 ml capacity clear and darkened glass bottles fitted with ground glass stoppers.

Isotope stock solutions of labelled sodium bicarbonate and uniformly labelled glucose were prepared as needed throughout the experimental program. Ampoules containing 1 mCi of ^{14}C-sodium bicarbonate or 1 mCi of ^{14}C (U) D glucose, were obtained from either Amersham/Searle or New England Nuclear. Preparation and standardization procedures of isotope are described in Appendix II.

1. Herbicidal effects upon the photosynthetic rate of phytoplankton

Water samples were shaken to ensure a homogenous suspension of phytoplankton. Fifty aliquots of 25 ml were placed in light and dark sample bottles. Herbicidal additions were made with sterile 1 ml syringes

Table 2. Herbicidal concentrations investigated in the experimental program in Delta Marsh, Manitoba.

Sample number	Herbicidal Level (ppm*)
1	0.025
2	0.250
3	0.625
4	1.25
5	2.50
6	25.0
7	75.0
8	125.0
9	250.0

* Concentration based upon actual herbicide, not herbicidal formulation.

or 10 λ Eppendorf Pipette so that final concentrations of sample bottles ranged from 25 ppb to 250 ppm (Table 2). Approximately 1 μCi of standardized ^{14}C sodium bicarbonate solution was added to each sample bottle. Control samples were identical to the above, but received no herbicidal additions. Blanks contained marsh water, which had been filtered through a 47 mm, 0.2 μ pore diameter Sartorius cellulose acetate filter and isotope. After herbicidal and isotope additions, samples were shaken to ensure even distribution. Samples were then incubated under constant conditions in a growth chamber (Controlled Environments Model ℓ 18L) at a temperature of 18C and light intensity of approximately 400 foot candles (4,300 lux). Illumination was provided by Cool White General Electric fluorescent fixtures. Samples were incubated for a period of four hours, a recommended incubation period for algal productivity studies (Ichimura and Saijo 1958; Vollenweider and Nauwerck 1961). After the incubation period, phytoplankton samples were filtered immediately through 25 mm Gelman, 0.45 μ pore diameter cellulose acetate filters, which prior to filtering had been pre-dampened for purposes of easy handling. Thirty ml capacity glass Sartorius filtration funnels, placed in 125 ml filtration flasks, were used. Eighteen funnels were utilized in an ex-

periment, thereby enabling the filtration of fifty-five samples within fourty-five minutes. The filters were decontaminated of inorganic carbon by fuming over concentrated HCl acid for one minute (Wetzel 1965). Vacuum pressure while filtering was not monitored, but was low and consistent for all experimental work. The filters while still damp (Wallen and Geen 1968; Ward and Nakanishi 1971), were placed directly into scintillation vials containing 10 ml of PCS scintillation fluor[1] (Amersham/Searle).

Scintillation counting was performed in a Picker Liquimat 220 liquid scintillation counter. Counting of each vial was for ten minutes at a preset statistic of 1.5 $\pm$ 2.0 standard deviations. Counts per minute (cpm) were converted disintegrations per minute (dpm) by the Channels Ratio Method (Wang and Willis 1965). Quenched standards were counted with each series of vials to ensure reliability of data.

Carbon uptake by phytoplankton for both light and dark bottles was calculated in the following manner which was modified from Strickland and Parsons (1968).

1. PCS is a highly efficient fluor, yielding 89% efficiency when containing 40% water (Amersham/Searle speculations).

Carbon uptake

$$(\text{mg C m}^{-3}\ 4\ \text{h}^{-1}) = 1.05 \cdot \frac{Y}{Z} \cdot W$$

where Z is the total dpm of radio-carbon solutions added to the sample bottle.

W is the total carbonate-carbon content of the lake water in mg C m^{-3}

Y is the uptake in dpm recorded from filtered mixed phytoplankton.

a. for light bottles - mean dpm of triplicate samples.

b. for dark bottles - mean dpm of duplicate samples.

1.05 is the correction factor to allow for the discriminative difference in uptake between ^{14}C and ^{12}C.

Statistical analysis was limited to the calculations of means and standard deviations of light bottles and means of dark bottles. Dark bottle values, taking into account passive diffusion of $^{14}CO_2$ by phytoplankton and bacterial chemotrophy were subtracted from values of light samples and final results are expressed in terms of carbon uptake $m^{-3}\ 4\ h^{-1}$.

2. Herbicidal effects upon the photosynthetic rate of periphyton

Phytoplankton was removed from the four liter water sample by filtering through two 47 mm, Sartorius glass fiber filters. Sufficient water was filtered to fill all sample bottles with 25 ml and to half fill a glass tray. The cellulose acetate square, colonized by periphyton was cut into squares approximately 1 cm x 1 cm. These squares were placed in a glass tray containing filtered marsh water and squares with even periphytic colonization subsequently selected and placed in sample bottles (1 square per sample bottle). Herbicide and isotope additions and incubation procedures were the same as those outlined in section D.1, above.

After incubation periphyton samples, both acetate square and incubation water, were filtered through Gelman 25 mm, 0.45 μ pore diameter cellulose acetate filters. Acetate squares on filters were removed from the filtration apparatus and fumed over concentrated HCl acid for one minute. Acetate squares were accurately measured and values recorded for each sample. Filters and acetate squares were then placed in scintillation vials containing 10 ml of Bray's fluor (Bray 1960). Bray's fluor was used to facilitate

the dissolving of the cellulose acetate, thereby releasing the periphyton into suspension (Hooper 1973).

The activity of the samples was determined by liquid scintillation counting procedures described above, and dpm were adjusted to provide a measure of radioactivity on a cm^2 of acetate. Carbon uptake by periphyton for both light and dark bottles was calculated in the following manner; modified from Strickland and Parsons (1968).

Carbon uptake

$$(\mu g\ C\ cm^{-2}\ 4\ h^{-1}) = 1.05 \cdot \frac{Y}{Z} \cdot W$$

where ZZ is the total dpm of radio-carbon solution added to the sample bottle.

W is the total carbonate carbon content of marsh water in each sample bottle, µg C/25 ml.

Y is the uptake in dpm recorded from filtered periphyton.

a. light bottles - mean dpm of triplicate samples.

b. dark bottles - mean dpm of duplicate samples.

1.05 is a correction factor to allow for the discriminative difference in uptake between ^{14}C and ^{12}C.

3. Herbicidal effects upon planktonic heterotrophy

In experiments on heterotrophy, 'in situ' levels of organic carbon, were assumed negligible relative to the added amounts of substrate under investigation (Parsons and Strickland 1962). The added amount of substrate in these experiments was 1 μCi (960 mg C/m^3) of uniformly labelled ^{14}C-glucose per experimental sample. The suitability of this level was determined by performing two saturation experiments, one at the beginning and one at the termination of this series of bioassays (Appendix I). Prior to each experiment ^{14}C-glucose was filter sterilized, by filtering through a Sartorius 47 mm, 0.2 μ pore diameter cellulose acetate filter, and standardized (Appendix II).

In each saturation experiment one of nine increasing concentrations of uniformly labelled ^{14}C-glucose, ranging from 9160 to 960 mg C/m^3, were added to triplicate light and duplicate dark bottles (Table 3). Each bottle contained a 25 ml mixed phytoplankton sample. Blank samples of 25 ml of 0.2 μ filtered marsh water were used for each concentration. Incubation, filtration and liquid scintillation counting procedures were as described for the herbicidal effects upon the photosynthetic rate of phytoplankton except for the following modifications. As the isotope was

Table 3. ^{14}C-glucose concentrations utilized in the saturation experiments.

Sample number	Glucose Concentrations (µg C/ℓ
1	9.6
2	28.8
3	48.0
4	67.2
5	96.0
6	288
7	480
8	672
9	960

organic, filters were not acid fumed. Additionally, although McMahon (1973) and Robinson et al. (1973) recommend a 100 ml post wash of filters to permit isotope not fixed by organisms to pass through filter papers, only a 10 ml post wash was used in these experiments. Post washes greater than this volume caused clogging of filters.

Procedures for the investigation of the effects of each herbicide upon the heterotrophic uptake of mixed phytoplankton and bacterioplankton followed those stated for the herbicidal effects upon the photosynthetic rate of phytoplankton. Modifications included the utilization of ^{14}C-glucose instead of ^{14}C-sodium bicarbonate and filtration changes as stated for the saturation experiments.

Calculations of uptake data followed the modified procedure of Parsons and Strickland (1962).

$$v \text{ (mg C } m^{-3} \text{ 4 } h^{-1}) = \frac{c \cdot f \cdot (Sn + A)}{C \quad \mu}$$

where v is the velocity of uptake in mg C m^{-3} 4 h^{-1}.

c is the radioactivity of filtered organisms in dpm.

a. Light bottles - mean dpm of triplicate samples.

b. Dark bottles - mean dpm of duplicate samples.

f is the correction factor (1.05) to allow for the discriminative difference in uptake between ^{14}C and ^{12}C.

Sn is the 'in situ' concentration of dissolved glucose in mg C/ℓ.

AA is the concentration of added ^{14}C-glucose in mg C/ℓ. This exact level was determined with standards for each experiment (Appendix II).

C is the dpm of 1 μCi of labelled ^{14}C glucose.

W is the quantity of ^{14}C-glucose added per experimental sample.

Blank values were subtracted from light and dark samples and results expressed in terms of mg C m^{-3} 4 h^{-1}.

4. Chemical parameters

Alkalinity determinations were performed as outlined in APHA (1971). The chemicals for the procedures were obtained from the Hach Chemical Co. The contents of one phenolphthalein powder pillow was added to a 250 ml Erlenmyer flask containing 100 ml of sample water. The sample was stirred, with a magnetic stirrer, for approximately five minutes and the contents of a second powder pillow, Brom Cresol Green-Methyl Red, was added. A greenish-blue colour developed and after

another five minutes of stirring, the sample was titrated to the end point with 0.02 N standard sulphuric acid. The amount of titrant was recorded and alkalinity calculated according to the following formula:

$$\text{Total alkalinity } (\text{mg}/\ell \text{ Ca } CO_3) = \frac{B \cdot N \cdot 50{,}000}{\text{ml sample}}$$

where B is the total ml of titrant

N is the normality of the acid

Only total alkalinity determinations were performed as no free CO_2, was present in the water, (phenolphthalein alkalinity equalled zero).

RESULTS

A. Herbicidal effects upon the photosynthetic rate of phytoplankton

1. Phenoxyacetic Acids

Phytoplankton samples responded differently to additions of 2,4-D and MCPA. Reduction of phytoplankton photosynthesis was noted with concentrations of 2,4-D between 0.025 and 0.625 ppm and between 25.0 and 250 ppm, with the maximum inhibitory responses noted with lowest and highest concentrations (Fig. 1). The photosynthetic rate was reduced by 34% with 0.025 ppm and by 43% with 250 ppm. Intermediate levels of 2,4-D (1.25 and 2.50 ppm) had no apparent effect upon the photosynthetic rate of natural phytoplankton samples.

MCPA, concentrations of 2.50 ppm and less, appeared to have no detrimental effects upon phytoplankton photosynthesis (Fig. 2). Concentrations of 25.0 ppm and greater significantly reduced $^{14}CO_2$ fixation. Photosynthesis was inhibited by 36-65% at MCPA concentrations of 25.0-250 ppm.

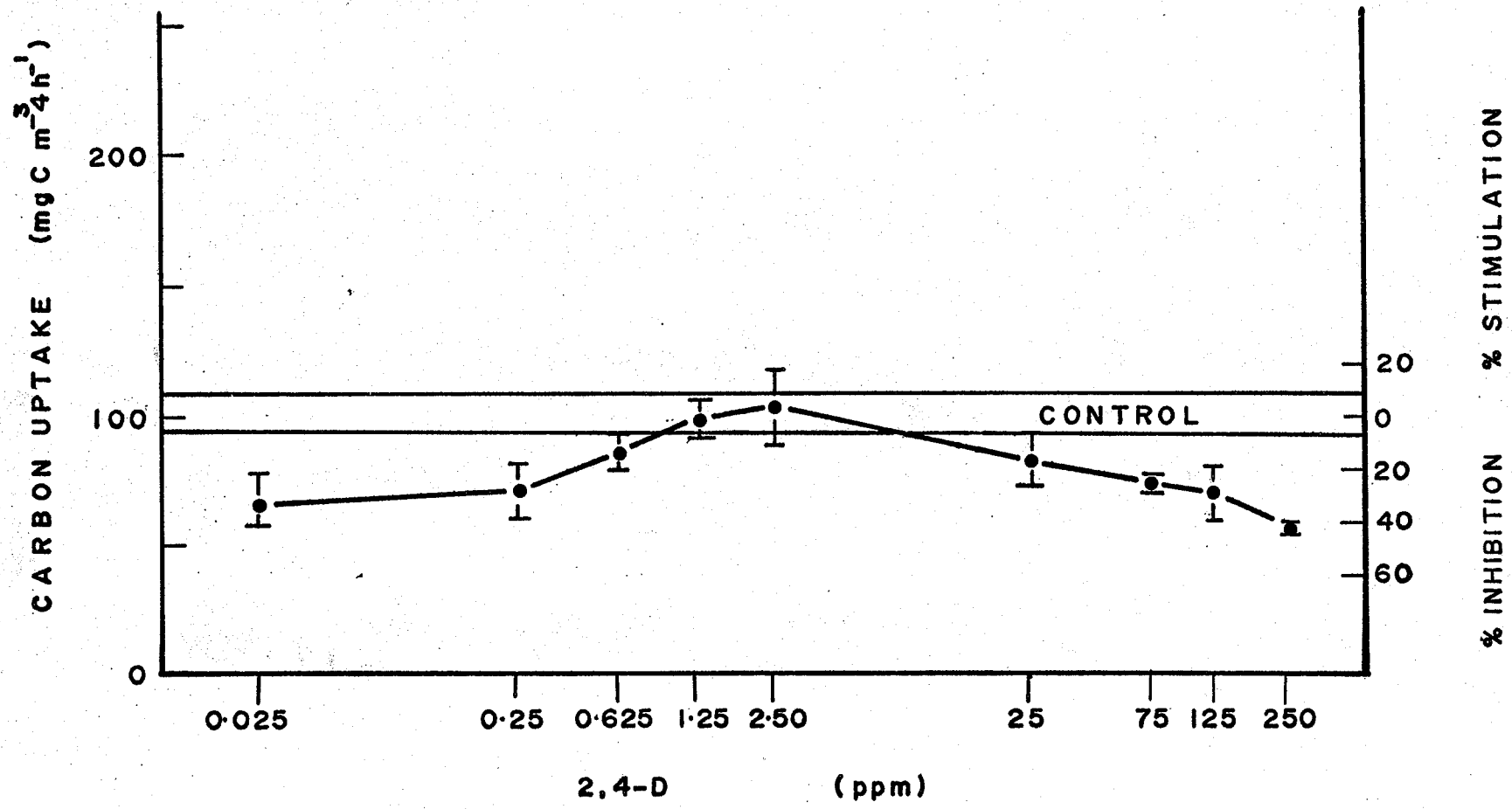

Fig. I. The effects of increasing concentrations of 2,4-D upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

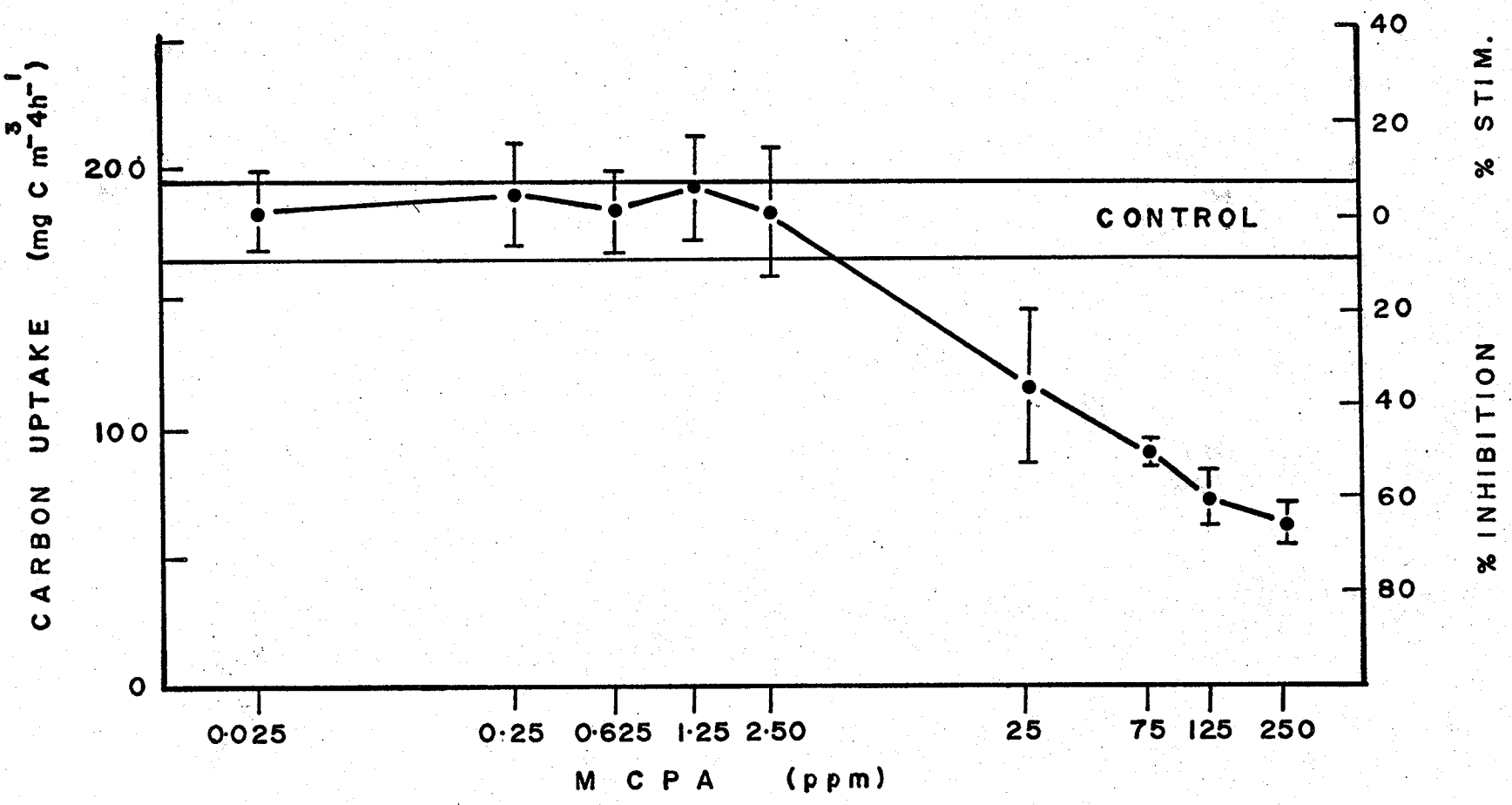

Fig. 2. The effects of increasing concentrations of MCPA upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

2. Benzoic Acids

"Amiben" in varying concentrations, had a number of effects upon phytoplankton photosynthesis (Fig. 3). A 4-53% stimulation of photosynthesis was noted at concentrations of 0.250-25.0 ppm whereas $^{14}CO_2$ fixation was reduced 14-30% by "Amiben" concentrations of 125 and 250 ppm. Concentrations of 0.025 and 75.0 ppm appeared not to affect photosynthesis.

3. Aliphatic Acids

TCA and "Dalapon" appear to have similar effects upon the photosynthetic rate of natural phytoplankton samples in that low concentrations apparently stimulated photosynthesis while high concentrations were inhibitory; although the stimulatory effect was marginal.

0.025-25.0 ppm TCA, were stimulatory (Fig. 4). Photosynthesis was increased by a maximum of 34% with a TCA concentration of 0.250 ppm. Reduction in carbon assimilation occurred at concentrations of 75.0 ppm and greater, with a maximum inhibition of photosynthesis of 52% at 75.0 ppm.

"Dalapon" appeared to be more effective than TCA in reducing phytoplankton photosynthesis (Fig. 5). With this herbicide, concentrations of 0.250-1.25 ppm were stimulatory to phytoplankton $^{14}CO_2$ fixation, whereas

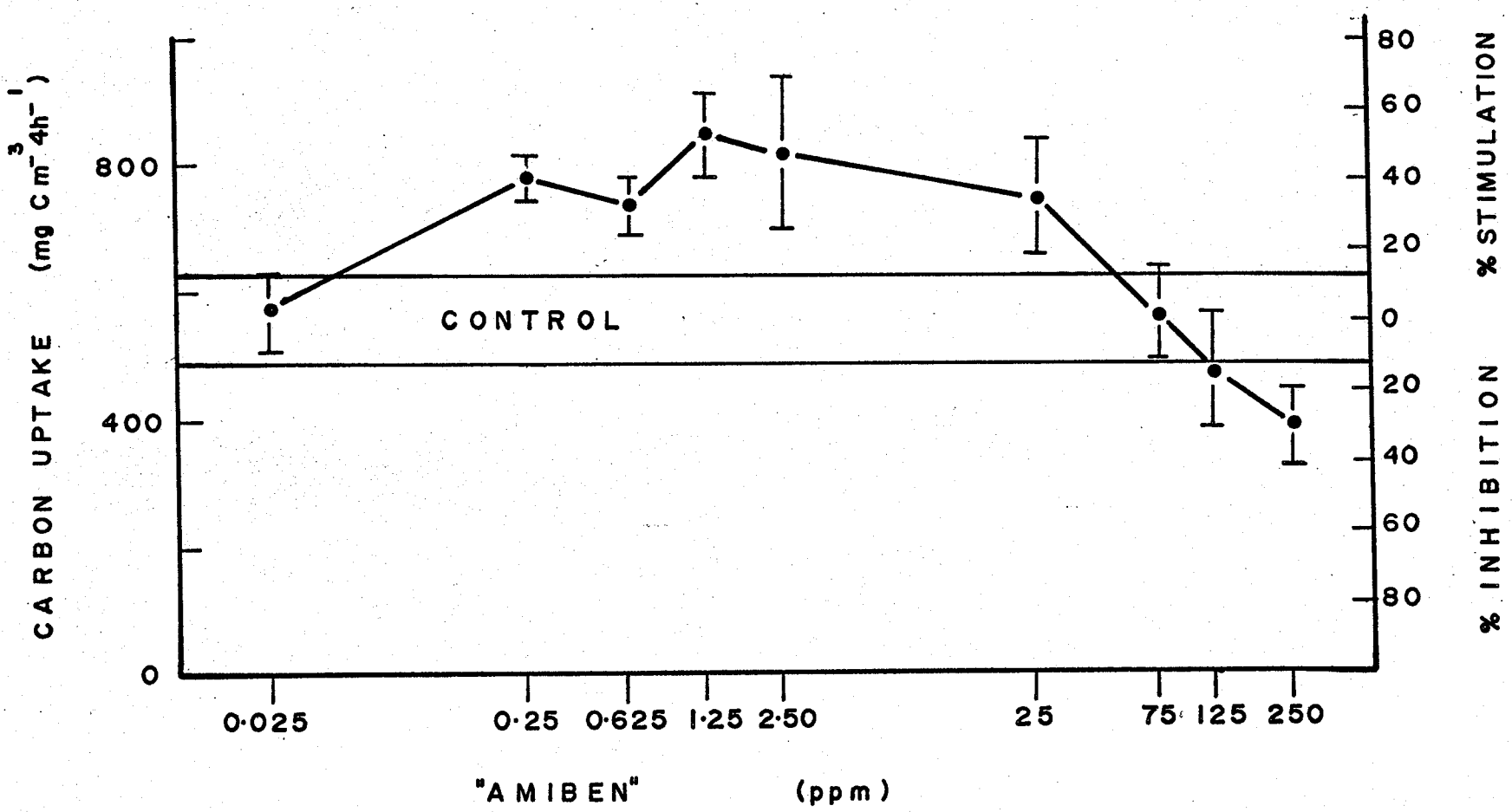

Fig. 3. The effects of increasing concentrations of "AMIBEN" upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

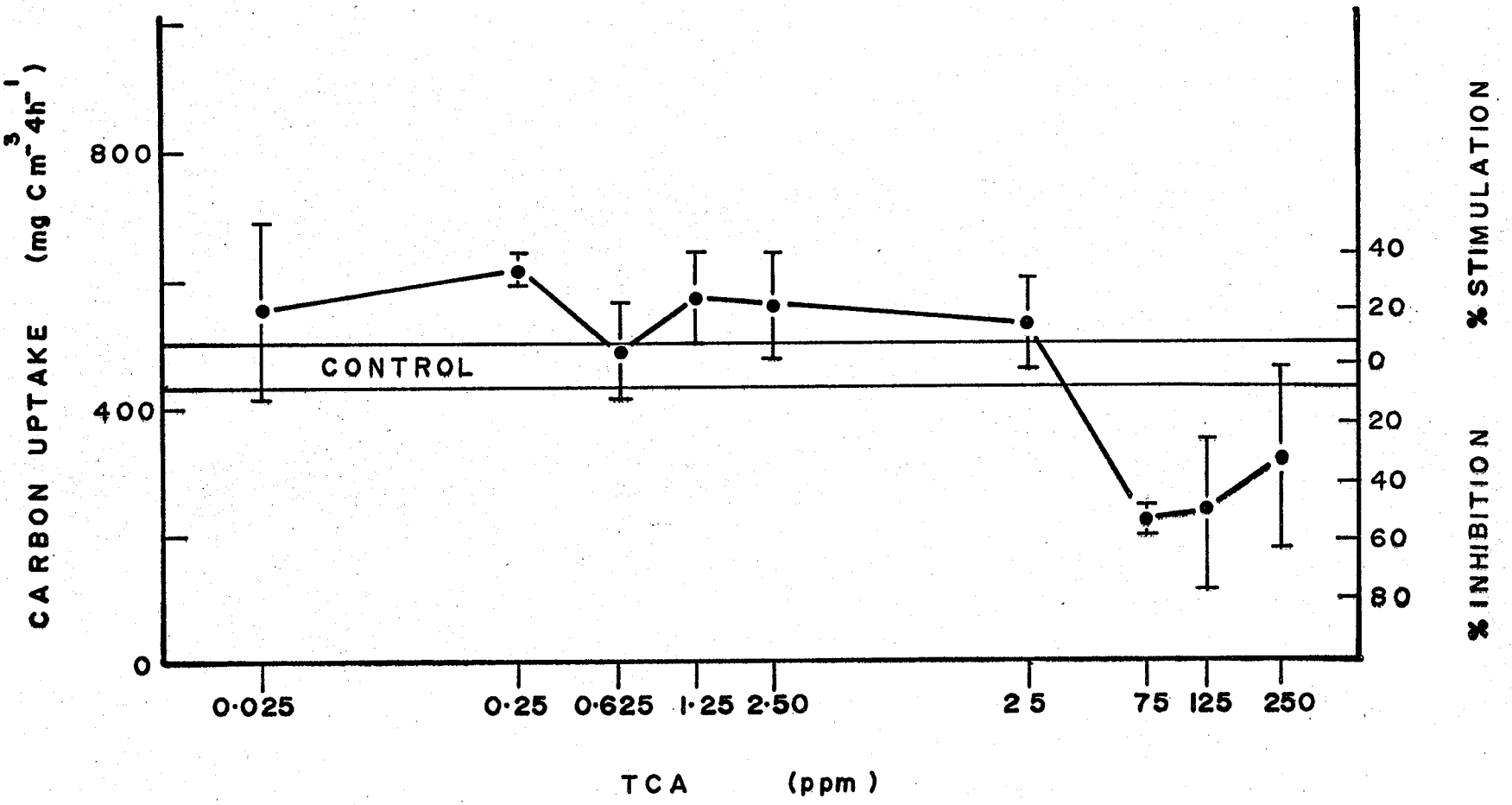

Fig. 4. The effects of increasing concentrations of TCA upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

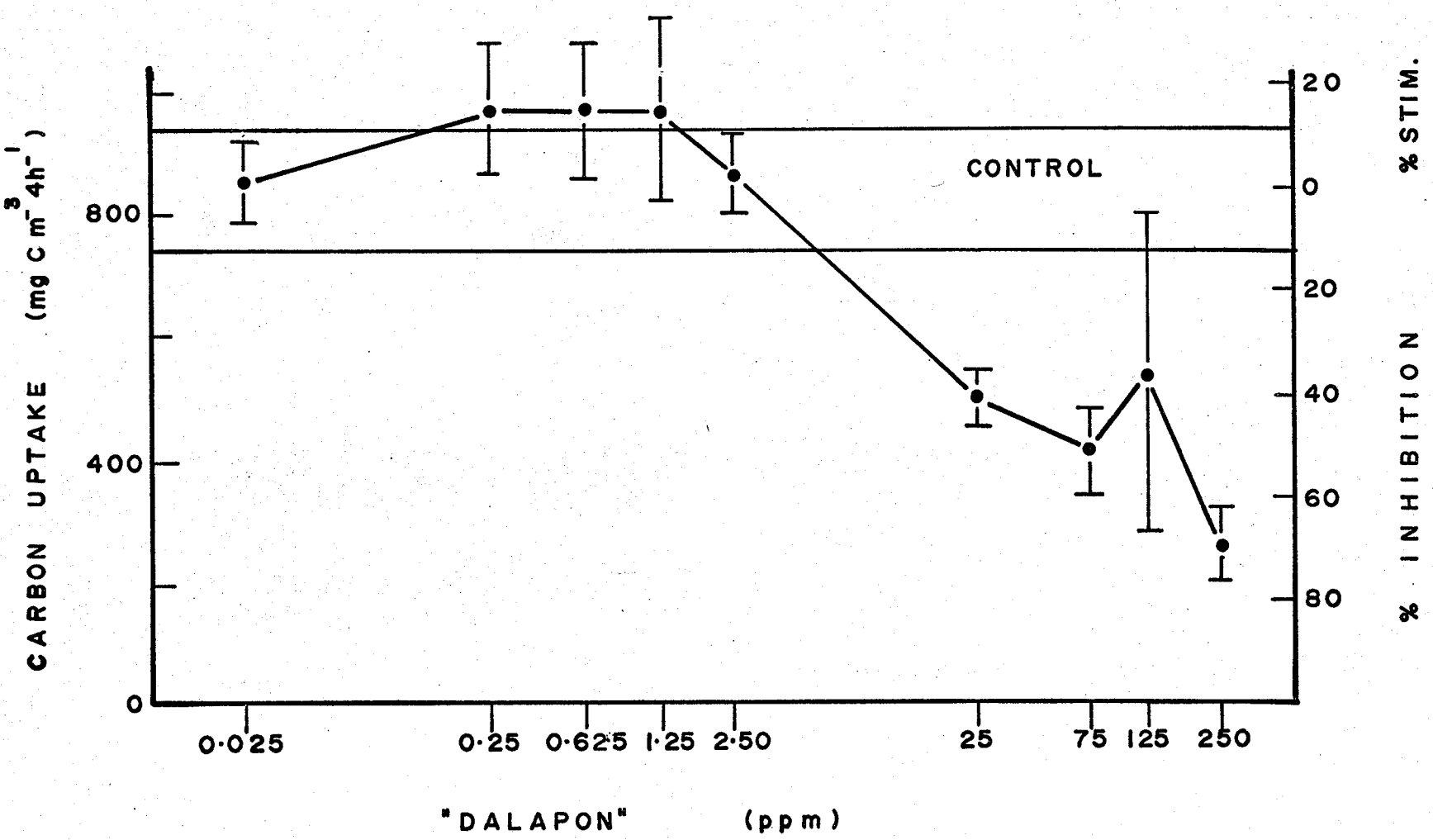

Fig. 5. The effects of increasing concentrations of "DALAPON" upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

concentrations between 25.0 and 250 ppm were inhibitory. Maximum stimulation of carbon uptake occurred with 0.25, 0.625 and 1.25 ppm, $^{14}CO_2$ fixation was increased to 16% above control values. Maximum inhibition of photosynthesis was observed with the highest concentration of "Dalapon", (250 ppm), where a 69% reduction of carbon uptake occurred.

4. 'N' Heterocyclics

The 'S' triazines, "Simazine" and "Atrazine" were extremely effective in reducing phytoplankton carbon assimilation (Fig. 6 and 7). At a concentration of 0.250 ppm, phytoplankton photosynthesis was inhibited by 48 and 74%, respectively. Concentrations greater than 0.250 ppm, progressively decreased $^{14}CO_2$ fixation to zero (Figs. 6 & 7).

"Amitrole-T", although related to the 'S' triazines herbicides, did not cause the same degree of inhibition of photosynthesis within the range of concentrations used (Fig. 8). The lowest concentration (0.025 ppm) appeared to have no effect upon phytoplankton photosynthesis; concentrations between 0.250 to 25.0 ppm caused a slight reduction in carbon uptake whereas concentrations between 75.0 - 250 ppm showed a significant reduction in phytoplankton photosynthesis.

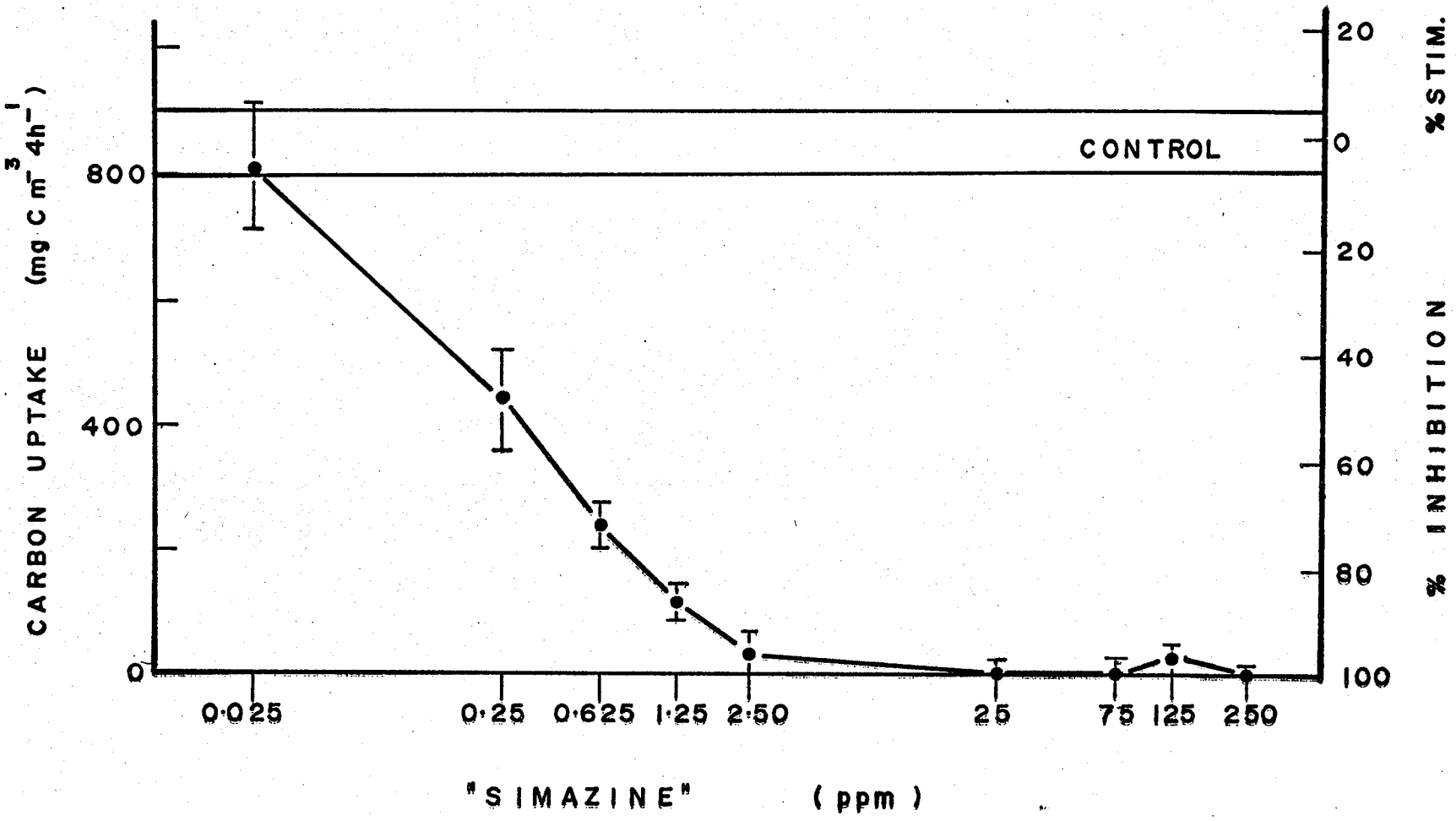

Fig. 6. The effects of increasing concentrations of "SIMAZINE" upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

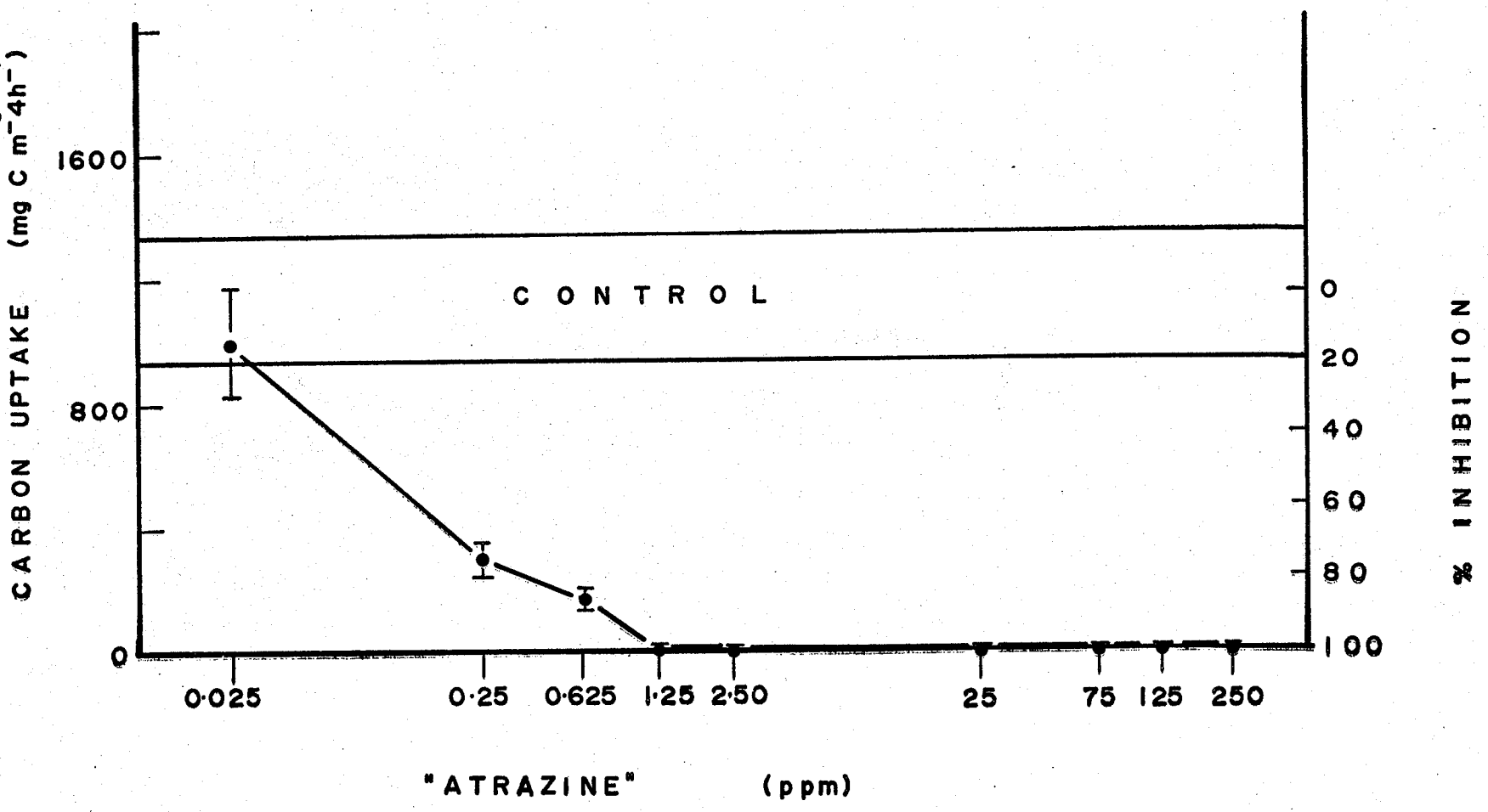

Fig. 7. The effects of increasing concentrations of "ATRAZINE" upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

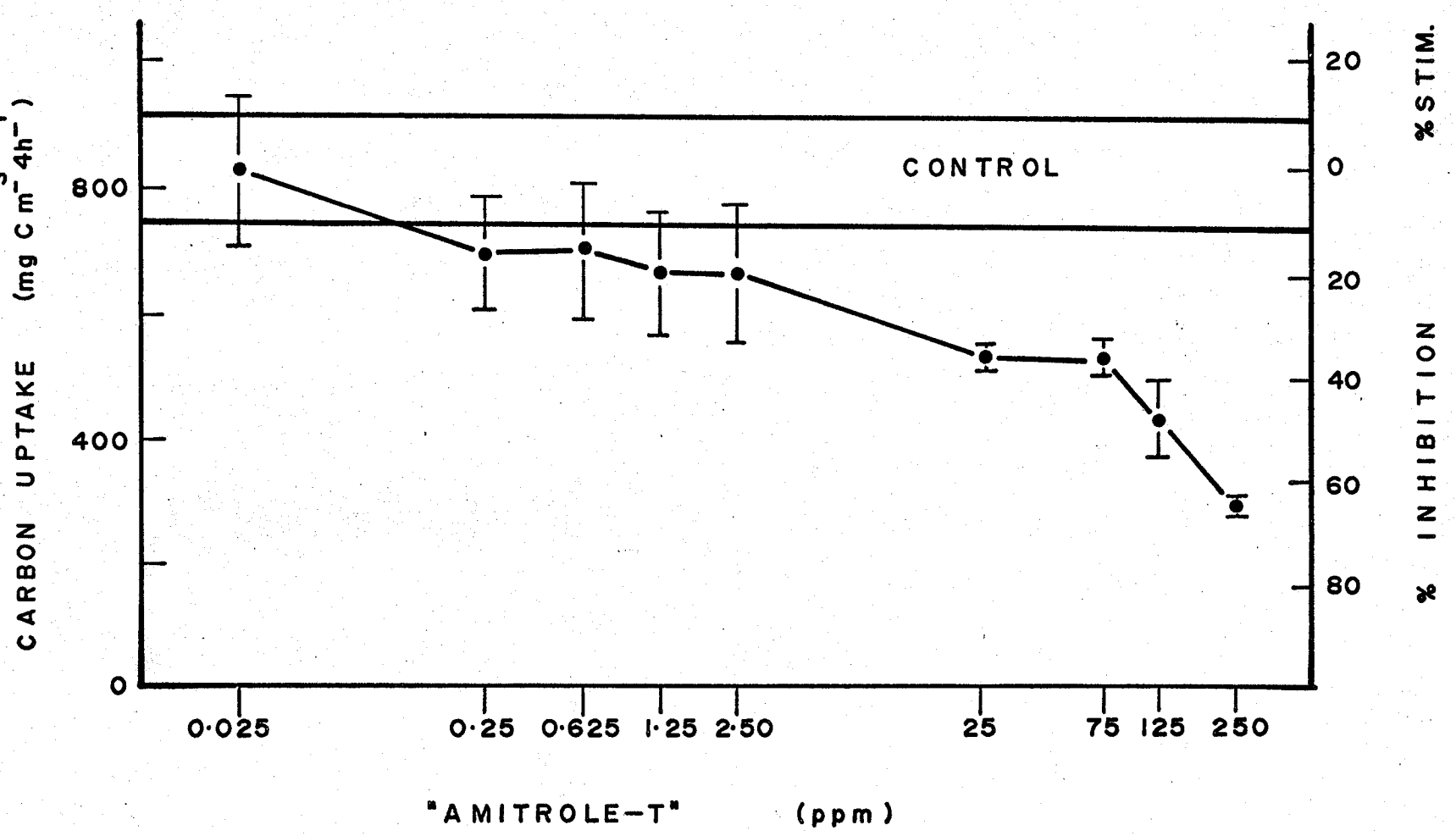

Fig. 8. The effects of increasing concentrations of "AMITROLE-T" upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

Carbon assimilation was reduced 15-20% with the former concentration range and a 36-65% reduction with the latter.

5. Substituted Ureas

"Linuron" appeared to have the greatest detrimental effect of all the herbicides tested upon phytoplankton photosynthesis (Fig. 9). Carbon assimilation was reduced by 59% with 0.025 ppm "Linuron", the lowest concentration tested. At 0.250 ppm, photosynthesis was reduced by 97% and at concentrations greater than 0.625 ppm photosynthesis was inhibited by 99-100%.

6. Carbamates

The effects of the aryl carbamate "Barban" and the thiocarbamates, EPTC and "Triallate" on phytoplankton photosynthesis were dissimilar.

0.025 ppm "Barban" caused a 12% stimulation of photosynthesis (Fig. 10). Concentrations of 0.250-2.50 ppm progressively inhibited photosynthesis to a reduction of 87%. Higher concentrations did not cause further inhibition (Fig. 10).

The thiocarbamates showed similar effects upon phytoplankton photosynthesis, with "Triallate" being slightly more effective in reducing carbon uptake than

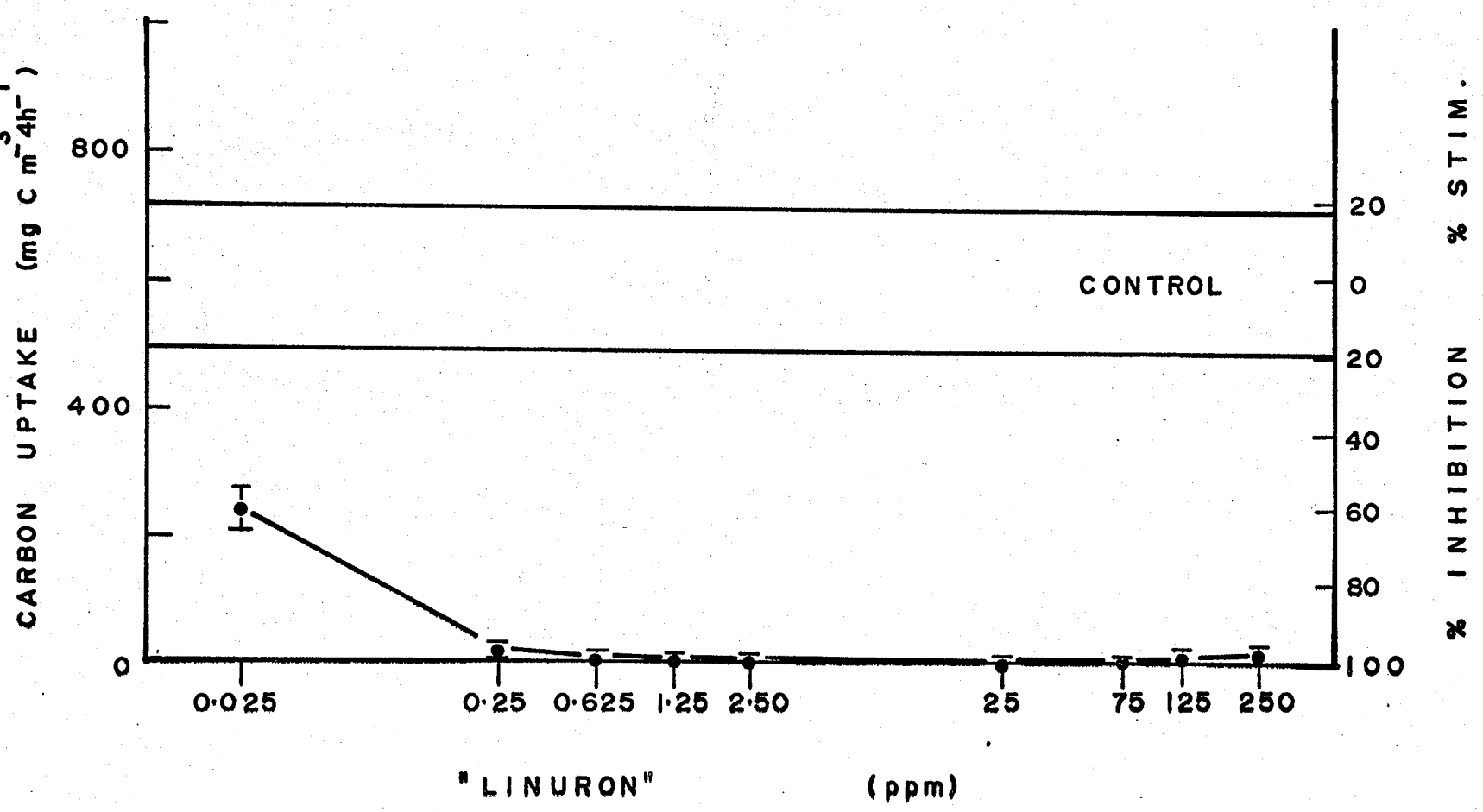

Fig. 9. The effects of increasing concentrations of "LINURON" upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

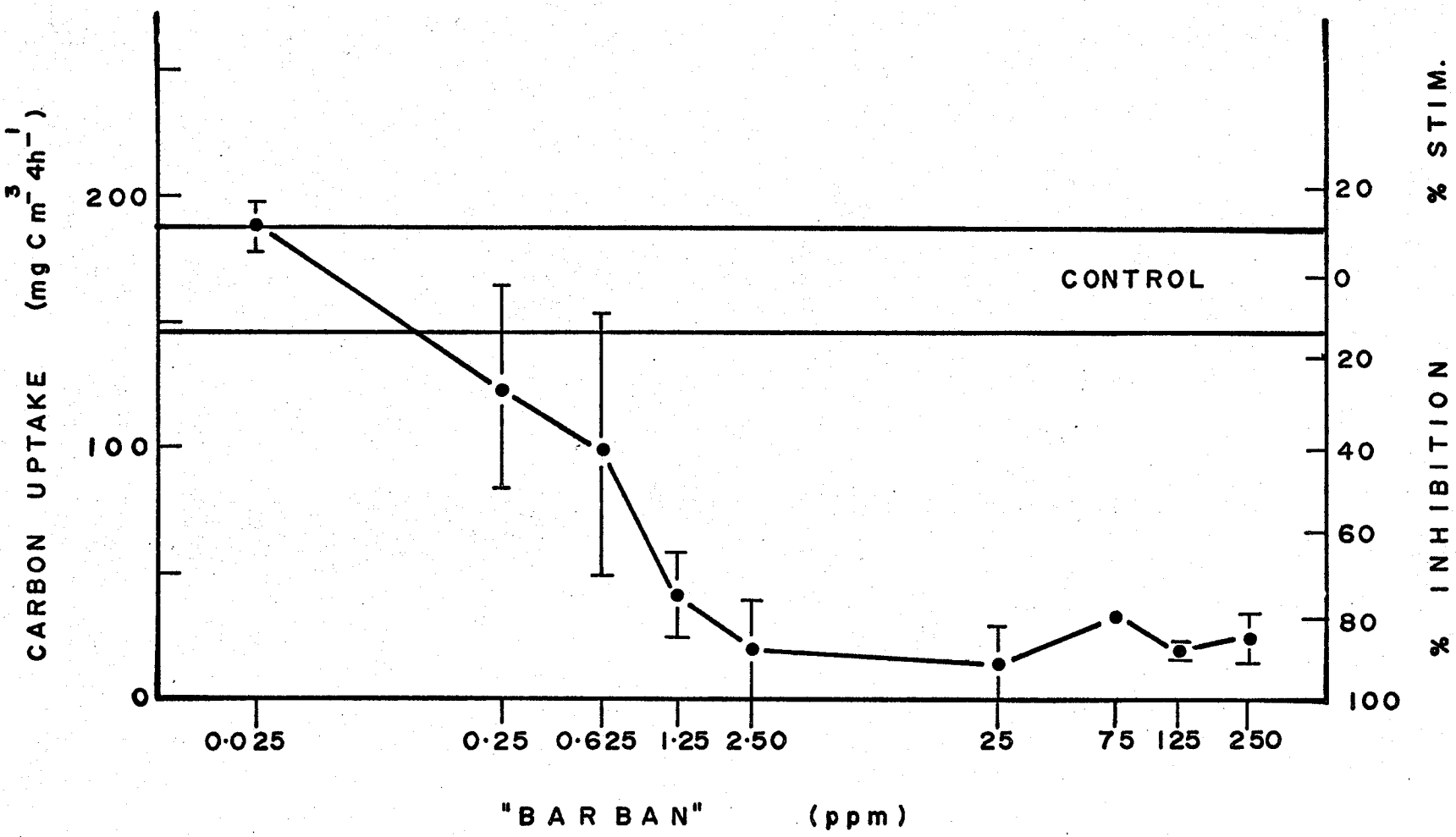

Fig. 10. The effects of increasing concentrations of "BARBAN" upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

EPTC (Figs. 11 and 12). At concentrations of 0.025-2.50 ppm both herbicides appeared to stimulate phytoplankton photosynthesis, whereas concentrations of 25.0 ppm and greater rapidly decreased $^{14}CO_2$ fixation. 0.025-2.50 ppm "EPTC" caused a 21-38% increase in the photosynthetic rate and the same range of "Triallate" caused a 10-40% increase. 25.0-250 ppm "EPTC" reduced carbon assimilation by 9-90% (Fig. 11). The same concentrations of "Triallate" caused a reduction of 63-94% (Fig. 12).

7. Bipyridyls

Photosynthesis was progressively inhibited by "Paraquat" as its concentration was increased from 0.025 ppm to 25.0 ppm (Fig. 13). The maximum inhibition reached was 99%. At herbicide concentrations above 25.0 ppm the rate of photosynthesis remained approximately constant.

8. Copper sulphate

Copper sulphate effectively reduced the photosynthetic rate of phytoplankton (Fig. 14). Concentrations of 0.025 to 25.0 ppm reduced the photosynthetic rate 10-65%. Carbon uptake values for phytoplankton samples treated with 25.0 ppm and greater appear to be

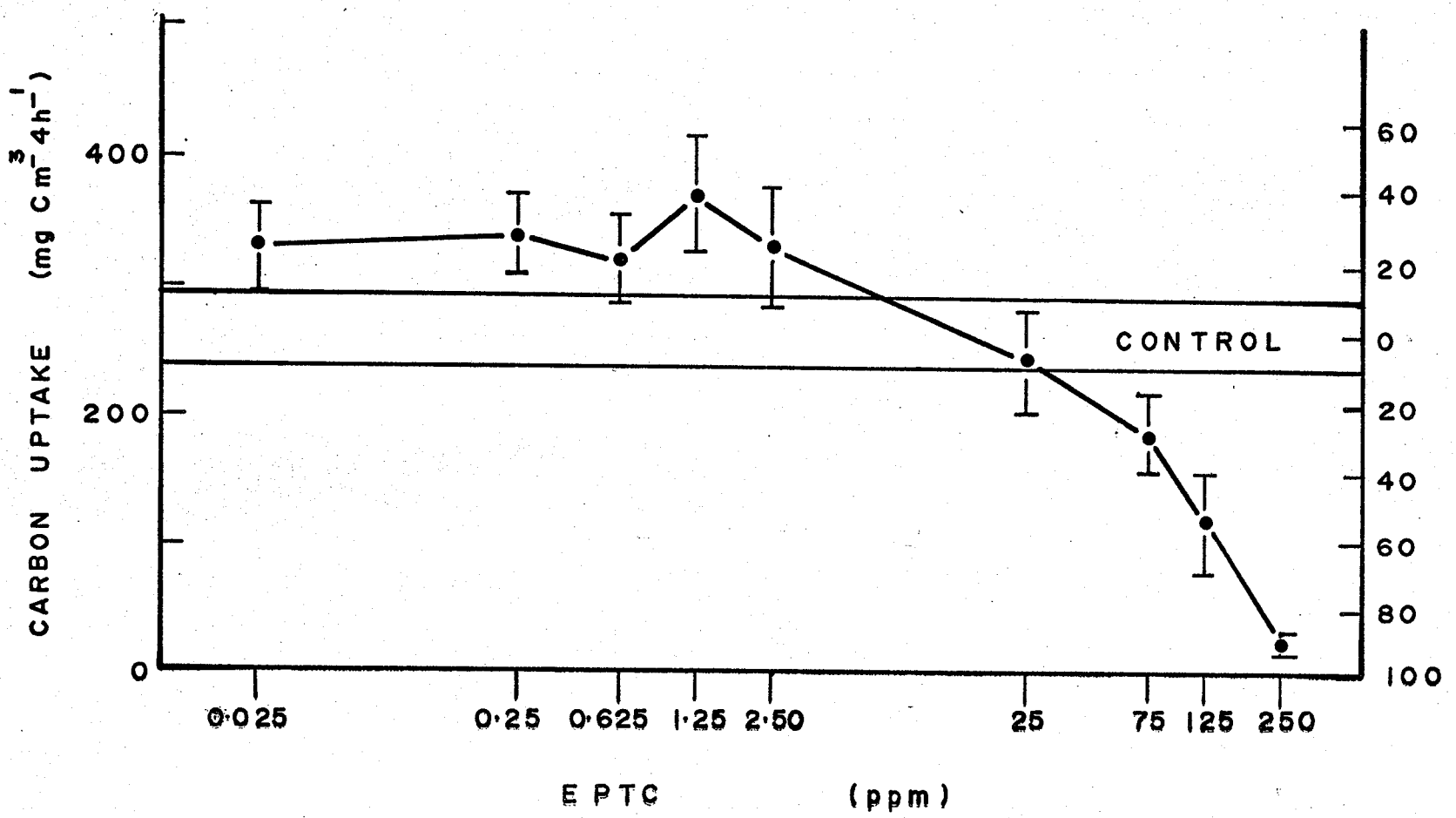

Fig. 11. The effects of increasing concentrations of EPTC upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

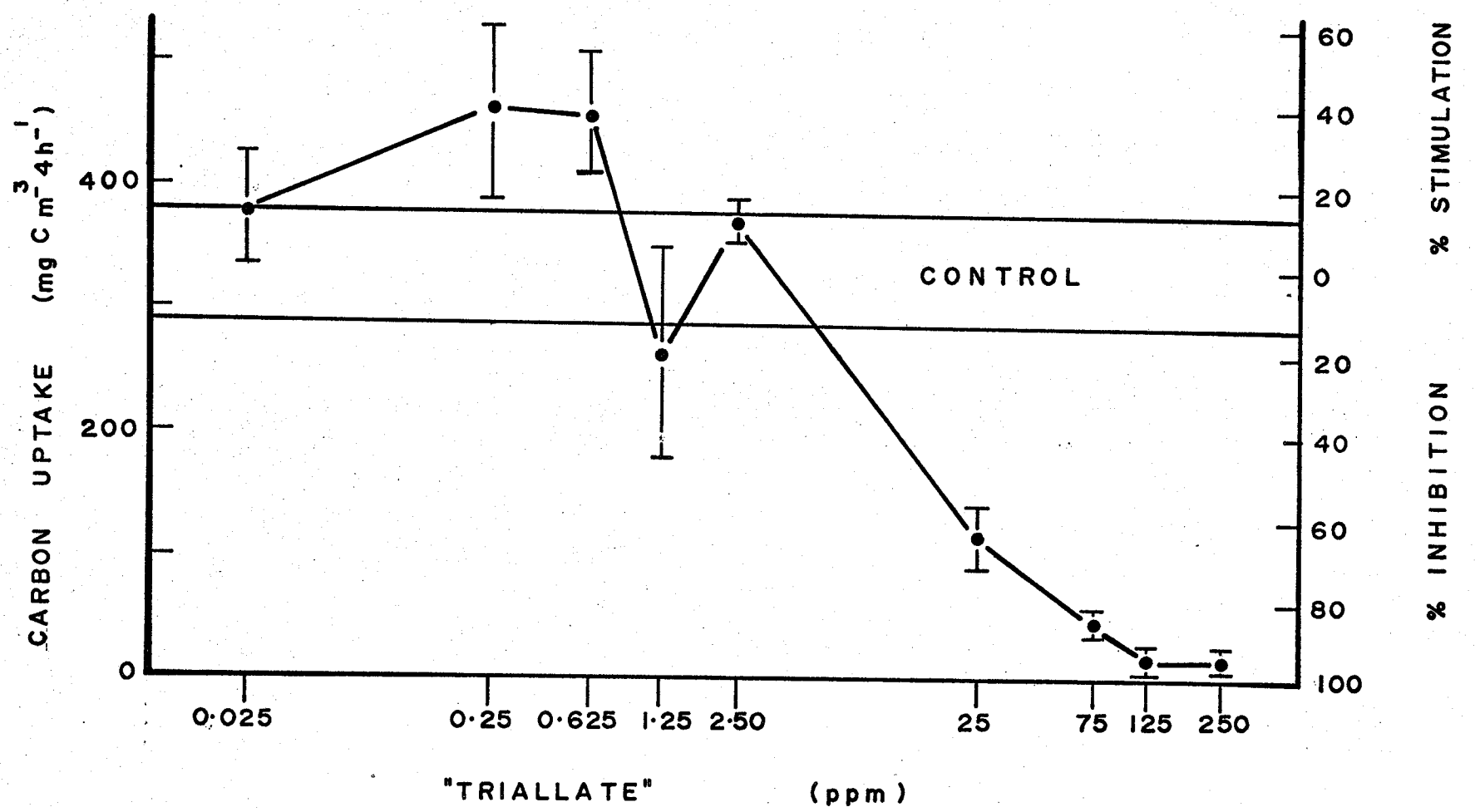

Fig.12. The effects of increasing concentrations of "TRIALLATE" upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

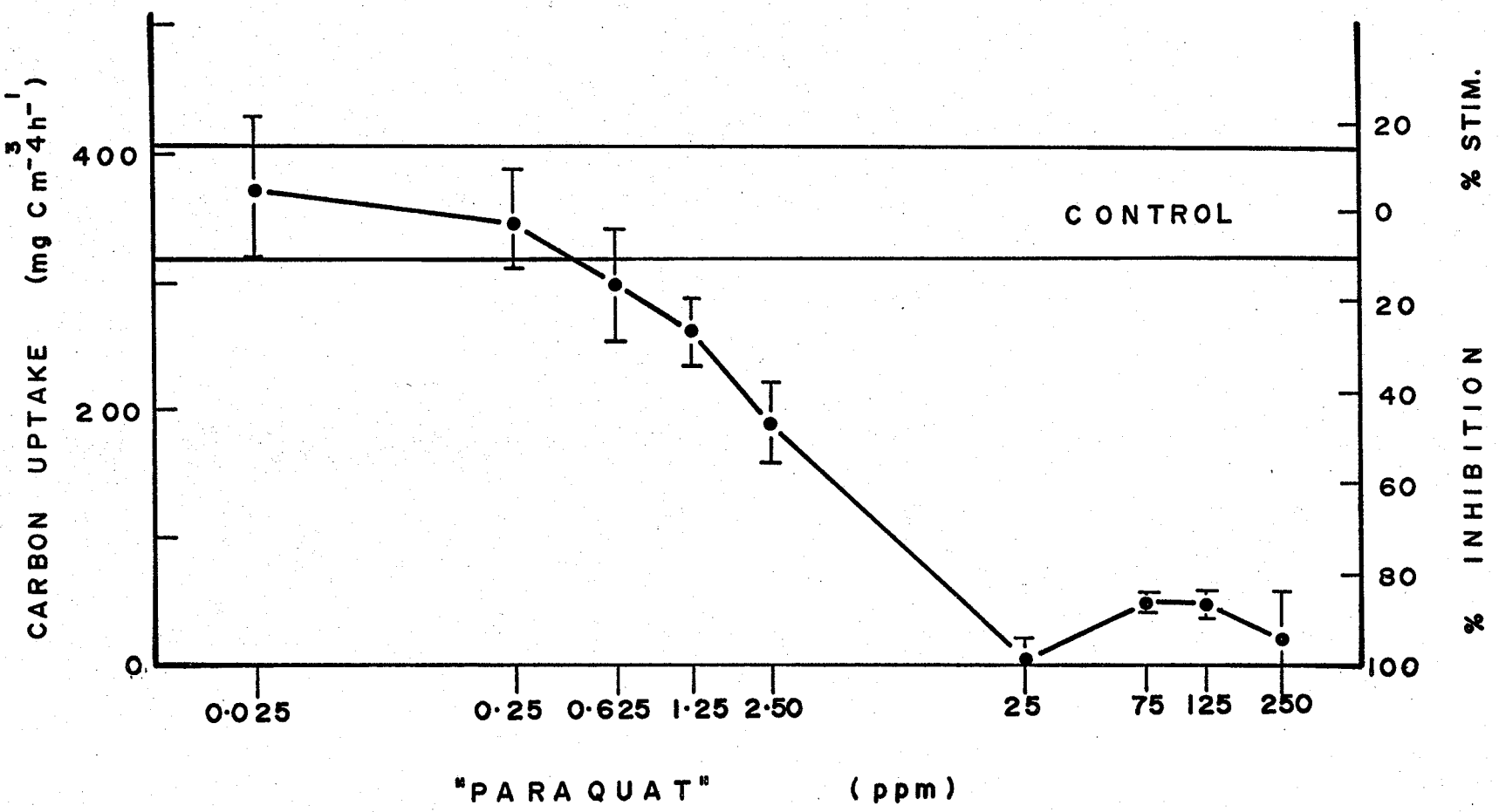

Fig. 13. The effects of increasing concentrations of "PARAQUAT" upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

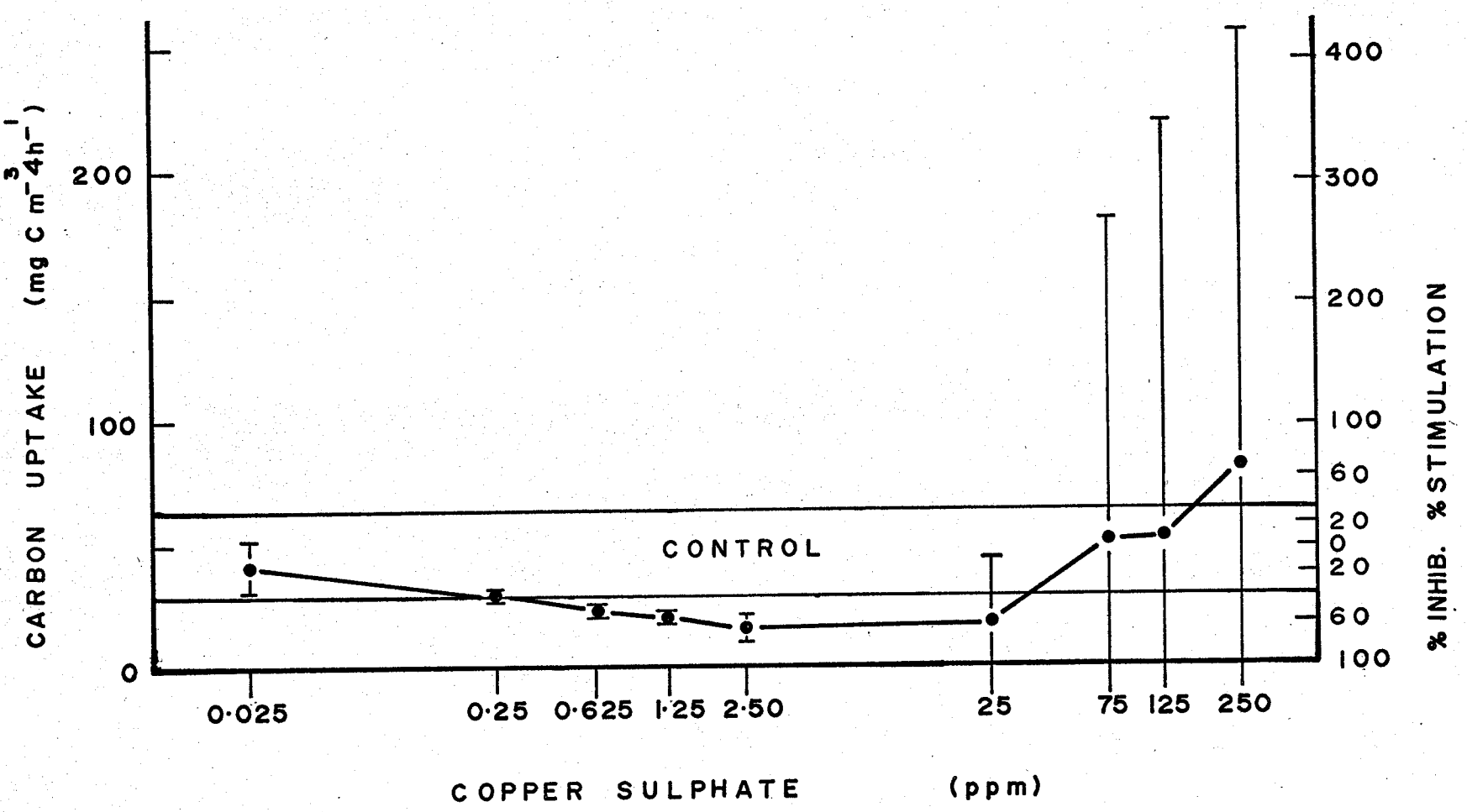

Fig. 14. The effects of increasing concentrations of COPPER SULPHATE upon phytoplankton photosynthesis in Delta Marsh, Manitoba.

unreliable, as the variability of triplicate samples was too great for comparative purposes.

The effects of the herbicides upon the photosynthetic rate of natural phytoplankton populations are summarized in Table 4. These may be arbitrarily ranked from the most effective at reducing phytoplankton photosynthesis to the least effective, but since "effectiveness" is dependent upon herbicidal concentrations such ranking has been conducted in terms of "effectiveness" at low concentrations (0.025-2.50 ppm) and at high concentrations (2.50-250 ppm).

The low concentration ranking from most effective to least effective is as follows: "Linuron", "Atrazine", "Simazine", copper sulphate, "Barban", "Paraquat", "Amitrole-T" and 2,4-D. The remaining herbicides tested were all stimulatory at these levels, but may be ranked from the least stimulatory to the most as follows: MCPA, "Dalapon", TCA, EPTC and "Triallate" are approximately equal and "Amiben".

The high concentration ranking from most "effective" to least "effective" is as follows: "Linuron", "Atrazine", "Simazine", "Barban", "Paraquat", "Triallate", "Dalapon", MCPA, "Amitrole-T", EPTC, TCA, 2,4-D and "Amiben" Copper sulphate has not been included in this latter ranking.

Table 4. The effect of a number of herbicides upon the photosynthetic rate of naturally occurring phytoplankton populations in Delta Marsh, Manitoba. Results expressed as % Stimulation (+) and % Inhibition (-).

Sample	Conc. (ppm)	Phenoxy-acetic Acids		Benzoic Acids	Aliphalic Acids		"N" Heterocyclics			Substituted Ureas	Carbamates			Bipyridyls	Inorganic
							"S" Triazines		Azole		Aryl-	Thio-			
		2,4-D	MCPA	Amiben	TCA	Dalapon	Simazine	Atrazine	Am-T	Linuron	Barban	EPTC	Triallate	Paraquat	$CuSO_4$
1	0.025	-34	+2	+4	+18	+2	-5	-11	0	-59	+12	+23	+13	+3	-10
2	0.250	-30	+5	41	+34	+16	-48	-74	-16	-97	-25	+28	+40	-5	-35
3	0.625	-15	+1	+32	+5	+16	-71	-89	-15	-99	-41	+21	+37	-18	-52
4	1.25	-2	+6	+53	+23	+16	-86	-98	-20	-100	-75	+38	-20	-28	-56
5	2.50	+3	+1	+48	+20	+4	-96	-98	-20	-100	-87	+23	+10	-47	-65
6	25.0	-16	-36	+35	+14	-41	-99	-98	-36	-100	-92	-9	-63	-99	-60
7	75.0	-26	-50	+2	-52	-51	-100	-99	-36	-100	-80	-28	-85	-86	+6
8	125	-29	-60	-14	-50	-35	-96	-100	-48	-100	-87	-55	-94	-86	+10
9	250	-43	-65	-30	-31	-69	-100	-100	-65	-98	-85	-90	-93	-94	+76

Comparisons of herbicidal concentrations, which effectively reduced phytoplankton photosynthesis totally (EC_{100}) or by 50% (EC_{50}) are presented in Table 5. According to EC100 values "Linuron" is the most effective herbicide and "Simazine" and "Atrazine" rank second. The other herbicides failed to reduce phytoplankton photosynthesis totally, with the range of concentrations tested. According to EC50 values, the herbicides may be ranked as follows: "Linuron", "Atrazine", "Simazine" and copper sulphate are approximately equal, "Barban", "Paraquat" and "Triallate" are approximately equal, "Dalapon", "MCPA", "EPTC" and TCA approximately equal and "Amitrole-T".

B. Herbicidal effects upon the photosynthetic rate of periphyton

Data on the effects of herbicides upon periphyton is often erratic. This may be due to the inherent difficulties in obtaining the necessary replicated samples of plant material. Consequently results in this section will be used as being supplementary to the previous section (A).

Table 5. Herbicidal concentrations (ppm) which effectively reduced the photosynthetic rate of natural phytoplankton samples by 50% (EC_{50}) and 100% (EC_{100}).

Herbicide	EC_{50}	EC_{100}
2,4-D	> 250	> 250
MCPA	75	> 250
Amiben	> 250	> 250
TCA	75.0-125	> 250
Dalapon	25.0-75.0	> 250
Simazine	0.250-0.625	75.0
Atrazine	0.025-0.250	75.0
Amitrole-T	125-250	> 250
Linuron	≤ 0.025	1.25
Barban	0.625-1.25	> 250
EPTC	75.0-125	> 250
Triallate	2.50-25.0	> 250
Paraquat	2.50-25.0	> 250
Copper Sulphate	0.250-0.625	———

1. Phenoxyacetic Acids

Various concentrations of 2,4-D had a number of effects upon periphyton photosynthesis (Fig. 15). Stimulation of photosynthesis occurred at concentratiosn of 2,4-D between 0.025 and 1.25 ppm with carbon uptake being increased by 28-57%. Increasing concentrations of 2,4-D of 2.50-125 ppm, had either a slight or no significant effect upon the photosynthetic rate of periphyton whereas 250 ppm reduced carbon uptake by 45%.

MCPA, in all concentrations tested, appeared to be stimulatory to the photosynthetic rate of periphyton (Fig. 16). Maximum stimulation of photosynthesis was noted at 25.0 ppm where carbon uptake was increased 118% above the mean control value.

2. Benzoic Acids

"Amiben" concentrations of 01025 and 0.250 ppm increased the fixation of $^{14}CO_2$ 29% and 11%, respectively above the mean control value (Fig. 17). 0.625 ppm "Amiben", had no significant effect upon the photosynthetic rate of periphyton samples, whereas concentrations between 2.50 and 75.0 ppm slightly inhibited photosynthesis by 8-15%. Maximum inhibition of photosynthesis was noted at concentrations of 125 and 250 ppm.

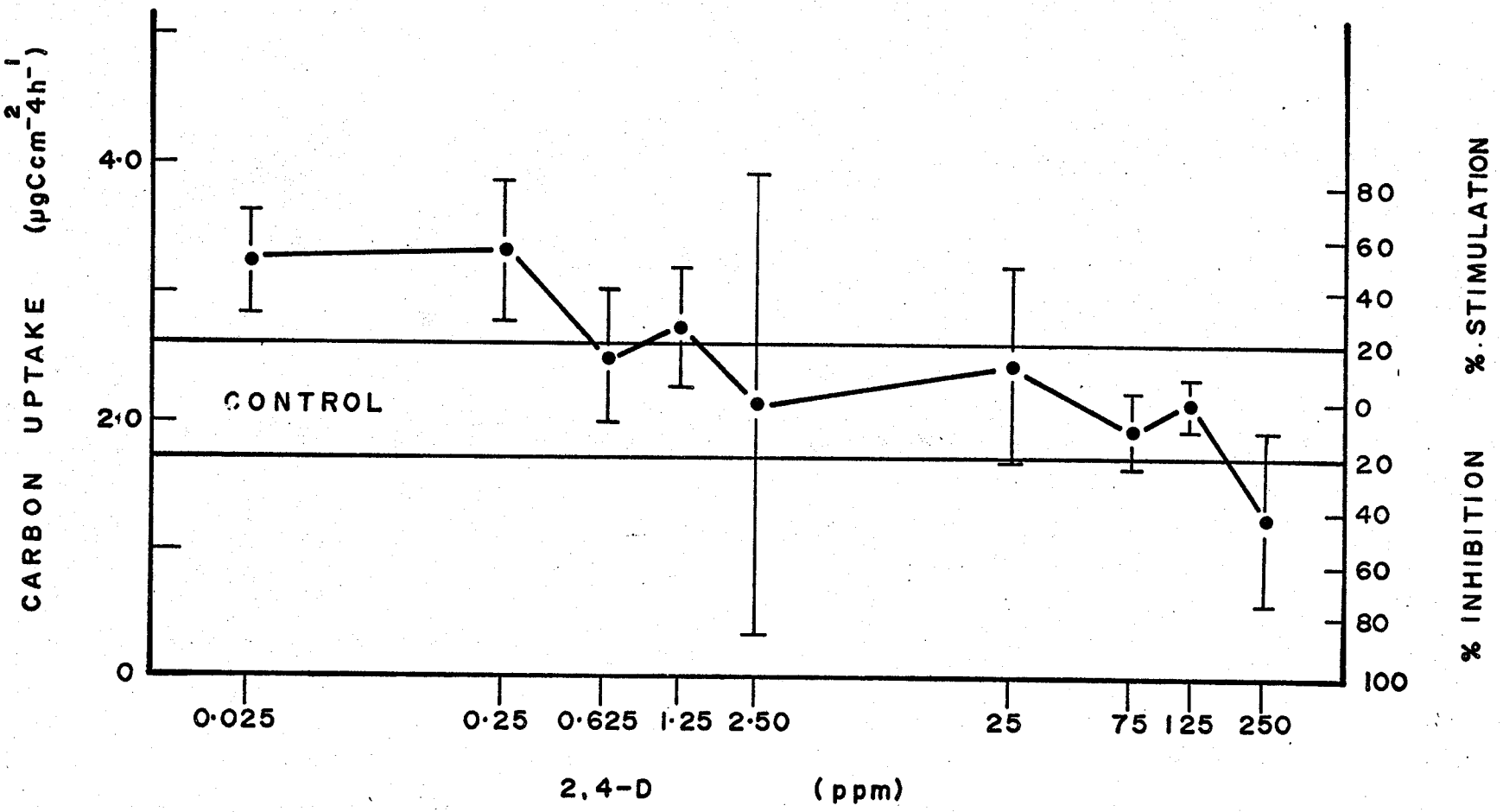

Fig. 15. The effects of increasing concentrations of 2,4-D upon periphyton photosynthesis in Delta Marsh, Manitoba.

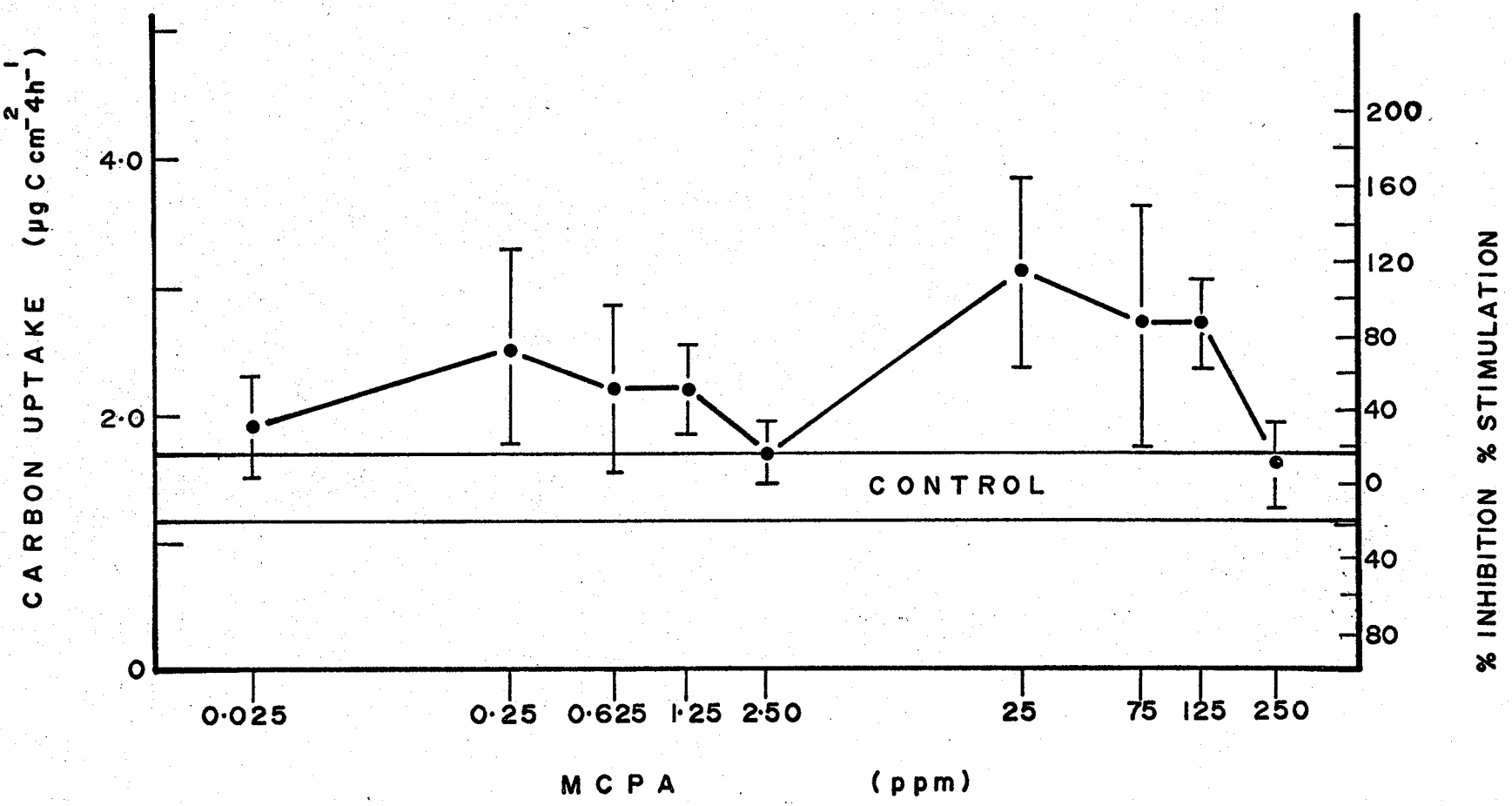

Fig. 16. The effects of increasing concentrations of MCPA upon periphyton photosynthesis in Delta Marsh, Manitoba.

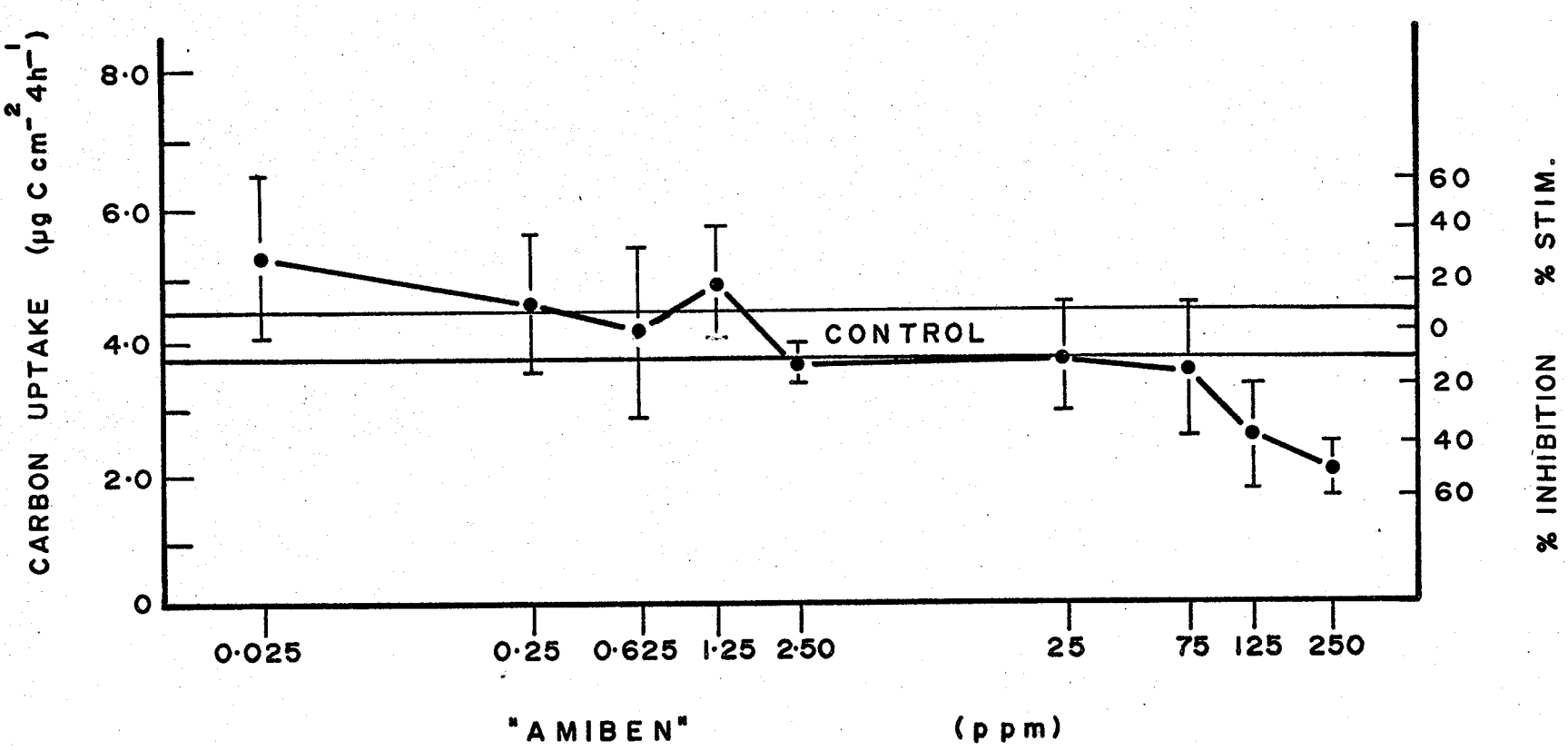

Fig. 17. The effects of increasing concentrations of "AMIBEN" upon periphyton photosynthesis in Delta Marsh, Manitoba.

Carbon uptake values were reduced by 38 and 50%, respectively.

3. Aliphatic Acids

The lowest concentration of TCA (0.025 ppm), inhibited periphyton photosynthesis by 7%, whereas slightly higher concentrations of 0.250 and 0.625 ppm increased $^{14}CO_2$ fixation by 12 and 8%, respectively (Fig. 18). Further increased concentrations of 1.25-25.0 ppm reduced carbon uptake by 24-72%, whereas reductions of carbon uptake at concentrations of 75.0-250 ppm, were 75-81%, respectively.

The effects of increasing concentrations of "Dalapon" upon the photosynthetic rate of periphyton appeared varied with a general trend towards a reduction in carbon uptake (Fig. 19). The maximum inhibitory effect was noted with 125 ppm, where photosynthesis was totally inhibited.

4. "N" Heterocyclics

The effects of the "N" heterocyclic herbicides upon the photosynthetic rate of periphytic samples, also appeared variable. Photosynthesis was inhibited 66% and 36% by "Simazine" concentrations of 0.025 and 0.250 ppm, respectively (Fig. 20). Zero photosynthesis was reached with "Simazine" concentrations of 0.625-

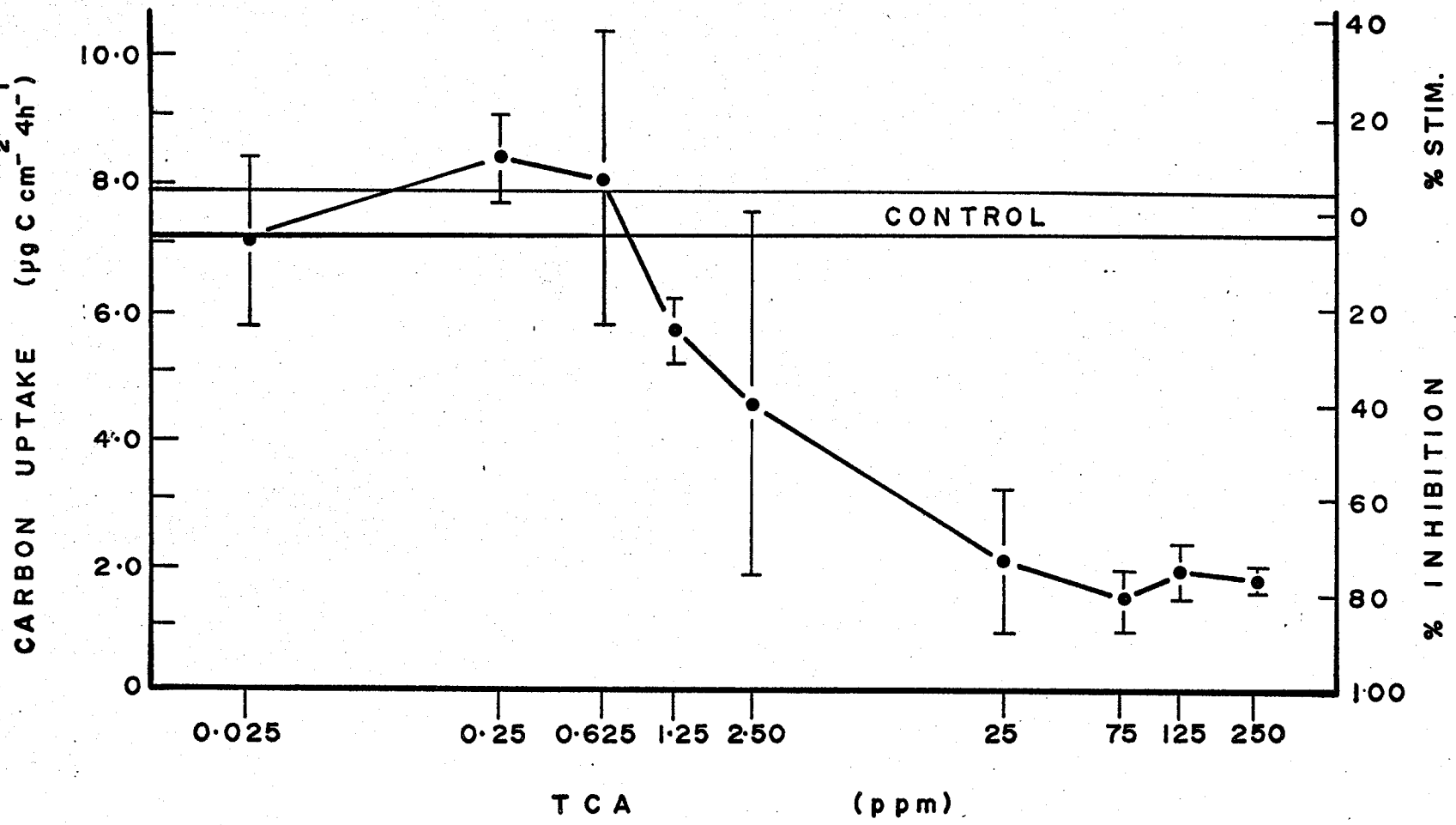

Fig. 18. The effects of increasing concentrations of TCA upon periphyton photosynthesis in Delta Marsh, Manitoba.

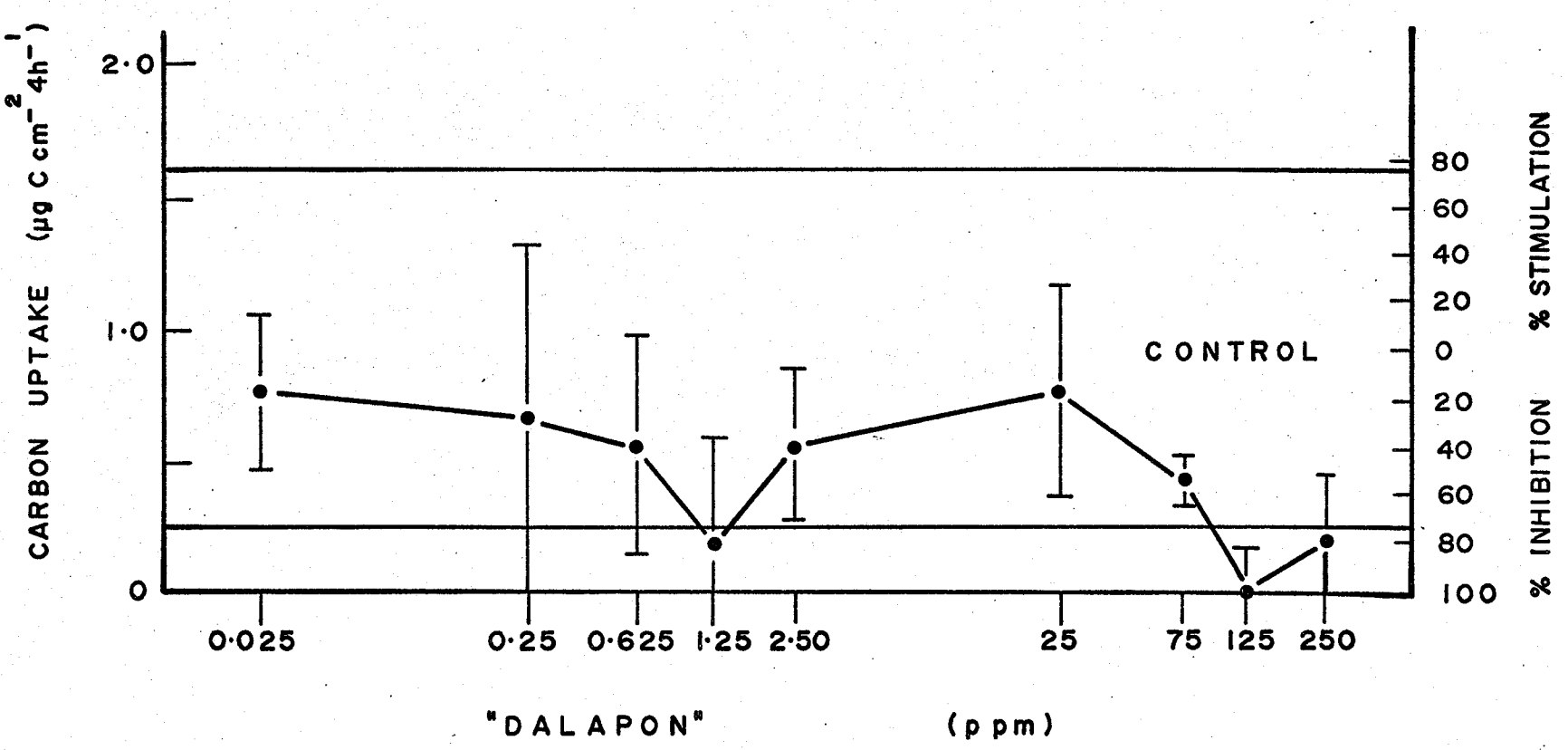

Fig. 19. The effects of increasing concentrations of "DALAPON" upon periphyton photosynthesis in Delta Marsh, Manitoba.

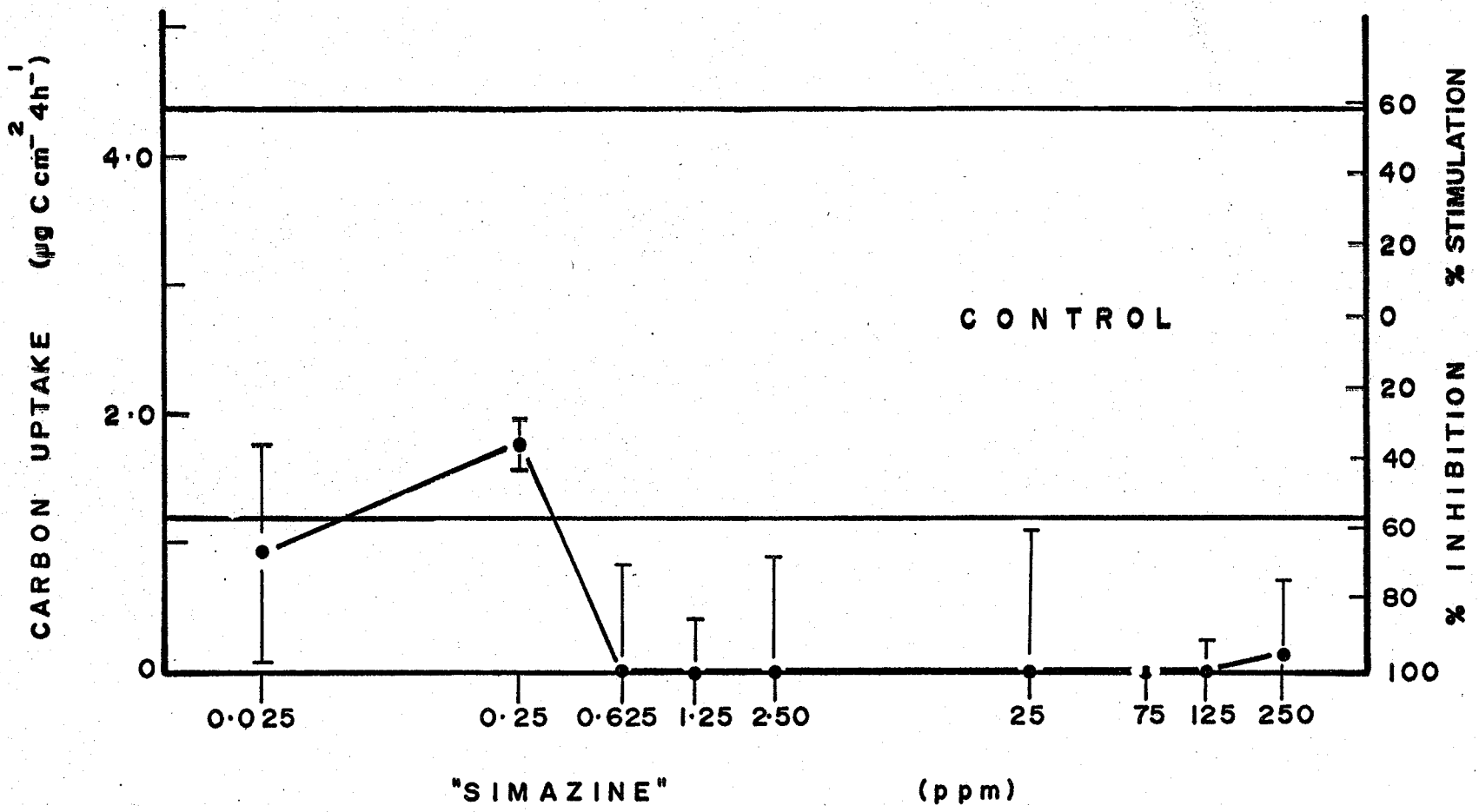

Fig. 20. The effects of increasing concentrations of "SIMAZINE" upon periphyton photosynthesis in Delta Marsh, Manitoba.

125 ppm.

The lowest concentration of "Atrazine" utilized (0.025 ppm), reduced carbon uptake by 72% (Fig. 21). At a concentration of 0.625 ppm, photosynthesis was reduced by 91% and at 1.25 ppm carbon uptake was totally inhibited. Carbon uptake values for periphyton samples treated with "Atrazine" concentrations of 2.50 ppm and greater appeared to be erratic. Photosynthesis was reduced by 78-90% whereas "Atrazine" concentrations of 1.25 and 250 ppm totally inhibited photosynthesis.

Experimental data for the effects of increasing concentrations of "Amitrole-T" upon the photosynthetic rate of natural periphyton samples have been ommitted from this section. Carbon uptake values of dark samples were much higher than light samples providing consistantly negative and therefore presumably meaningless data.

5. Substituted Ureas

The effects of increasing concentrations of "Linuron" upon the photosynthetic rate of natural periphyton samples were apparently variable. With the exception of 0.250 ppm, "Linuron" concentrations between 0.025-1.25 ppm stimulated periphyton fixation of $^{14}CO_2$ by 15-99% above the mean control value (Fig. 22). Photosynthesis was totally inhibited by concentrations

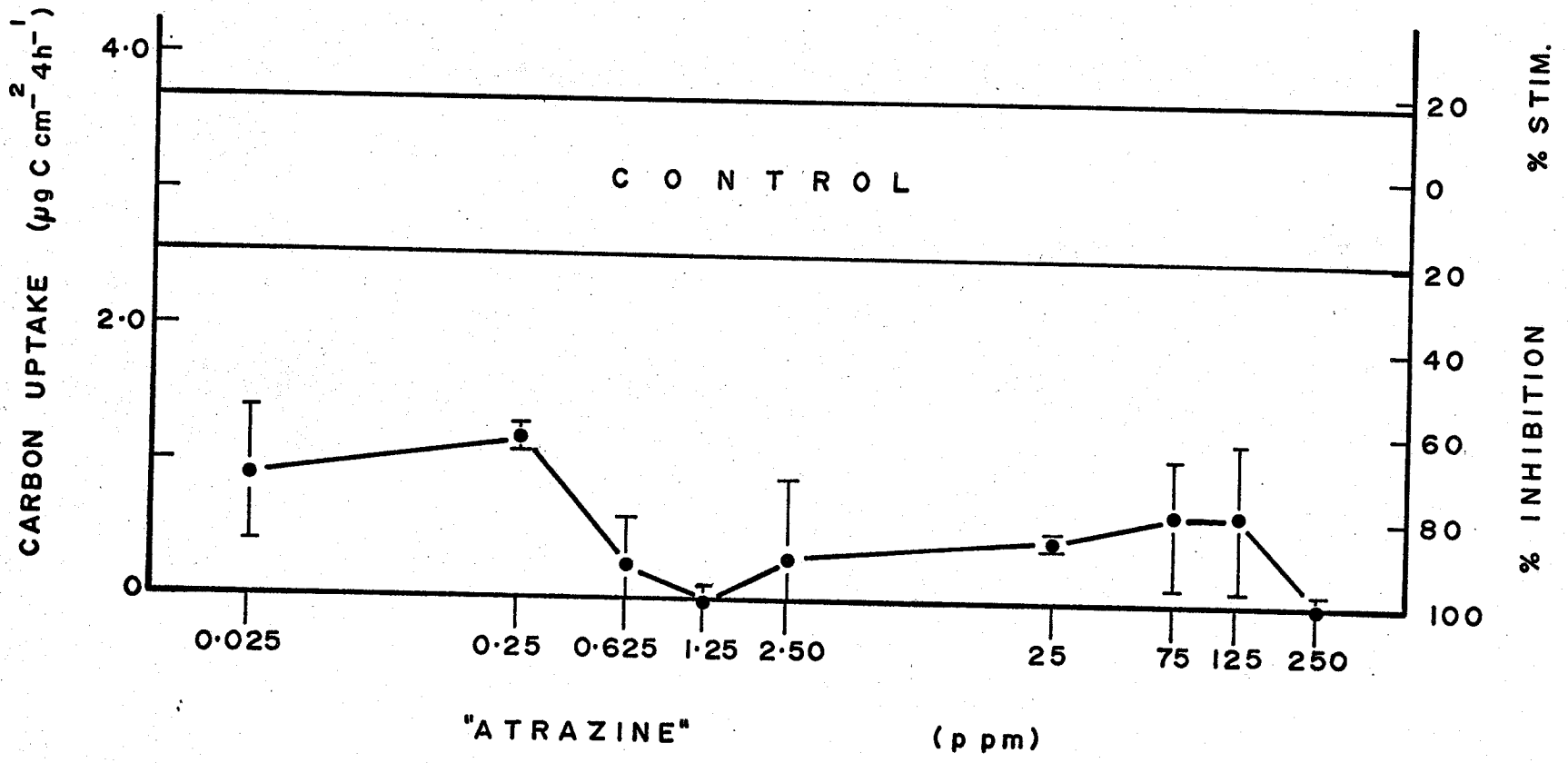

Fig. 21. The effects of increasing concentrations of "ATRAZINE" upon periphyton photosynthesis in Delta Marsh, Manitoba.

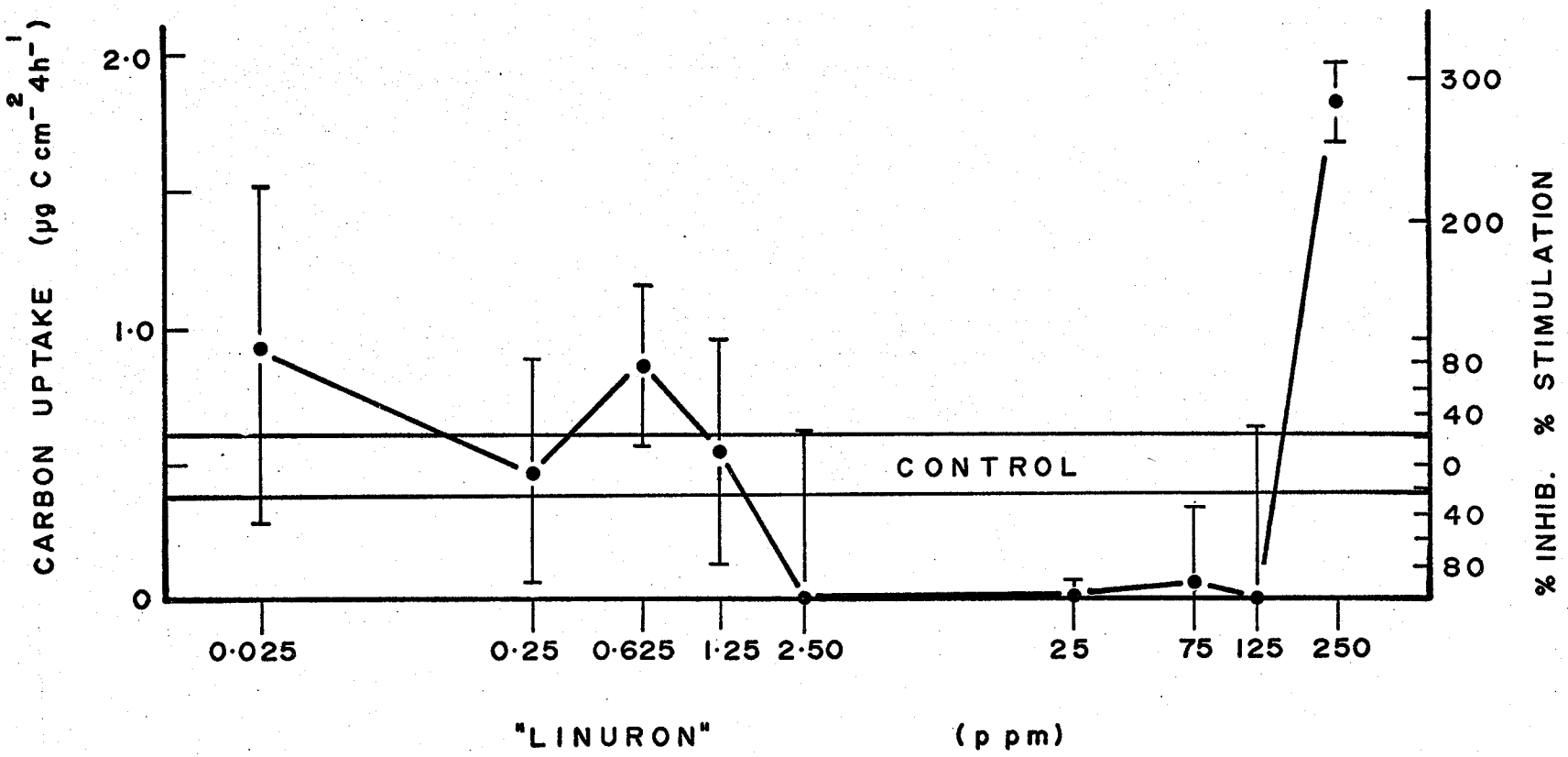

Fig. 22. The effects of increasing concentrations of "LINURON" upon periphyton photosynthesis in Delta Marsh, Manitoba.

of 2.50 and 125 ppm and almost totally inhibited by concentrations of 25.0 and 75.0 ppm (a reduction of 97 and 15%, respectively). Despite this apparent inhibitory effect 250 ppm caused a stimulation of 283% over the mean control value.

6. Carbamates

"Barban", in increasing concentrations, steadily decreased the photosynthetic rate of periphyton samples (Fig. 23). Concentrations of 0.025-2.50 ppm decreased carbon uptake by 5-45%, whereas concentrations of 25.0-250 ppm reduced carbon uptake by 89-100%.

"EPTC" at all concentrations tested, reduced periphyton photosynthesis, but concentrations of 0.025-0.250 and 25.0-250 ppm, were most detrimental (Fig. 24). Carbon uptake was reduced by 84% and 68% when treated with 0.025 and 0.250 ppm, respectively and by 74-81% when treated with concentrations of 25.0-250 ppm. Concentrations of 0.625-2.50 ppm were least detrimental to periphyton photosynthesis, carbon uptake being only reduced by 49-58%.

"Triallate", at low concentrations of 0.025 and 0.250 ppm, stimulated the photosynthetic rate of natural periphyton samples by 16-18% above the mean control value (Fig. 25), whereas increased concentrations of "Triallate" steadily decreased periphyton

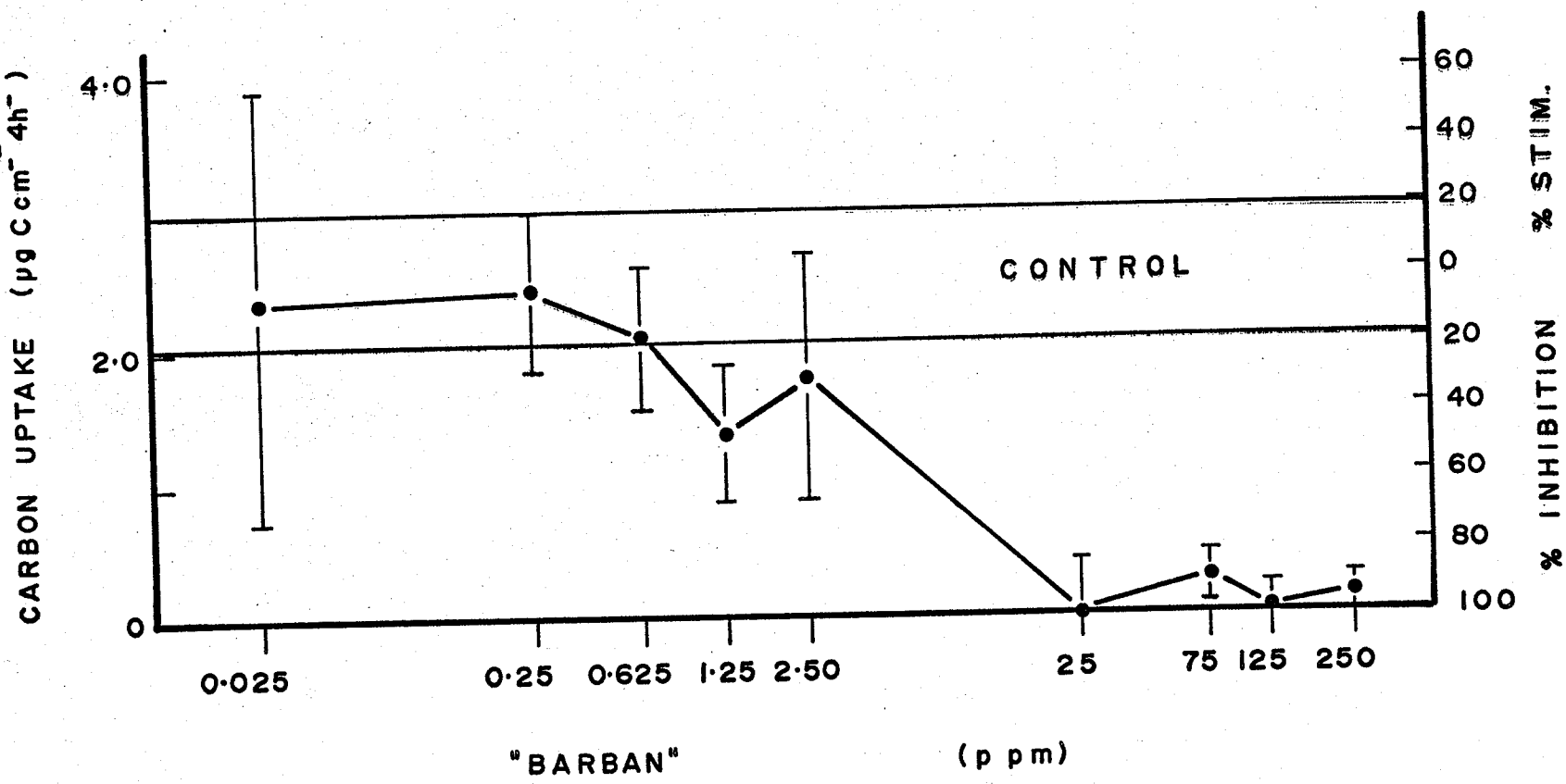

Fig. 23. The effects of increasing concentrations of "BARBAN" upon periphyton photosynthesis in Delta Marsh, Manitoba.

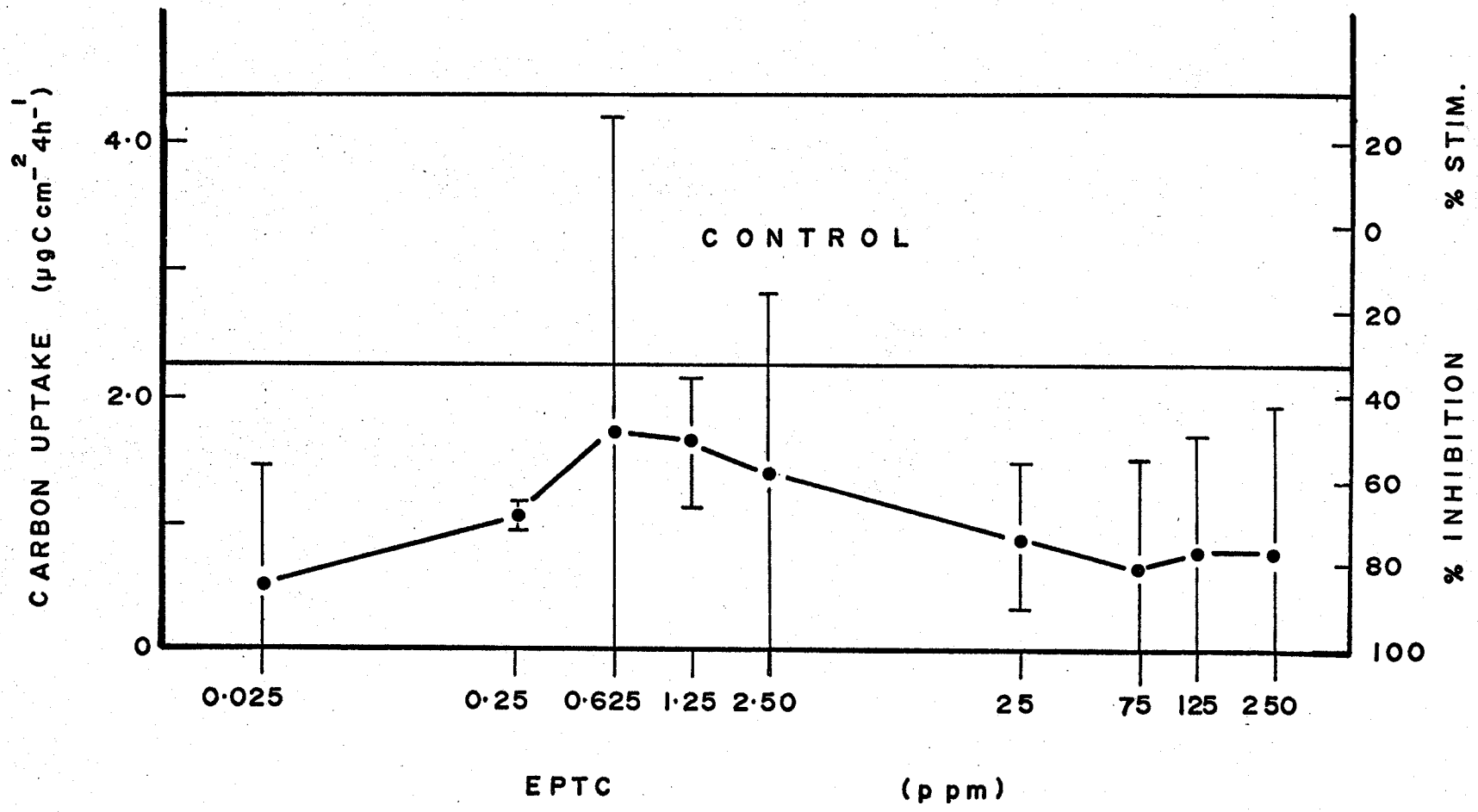

Fig. 24. The effects of increasing concentrations of EPTC upon periphyton photosynthesis in Delta Marsh, Manitoba.

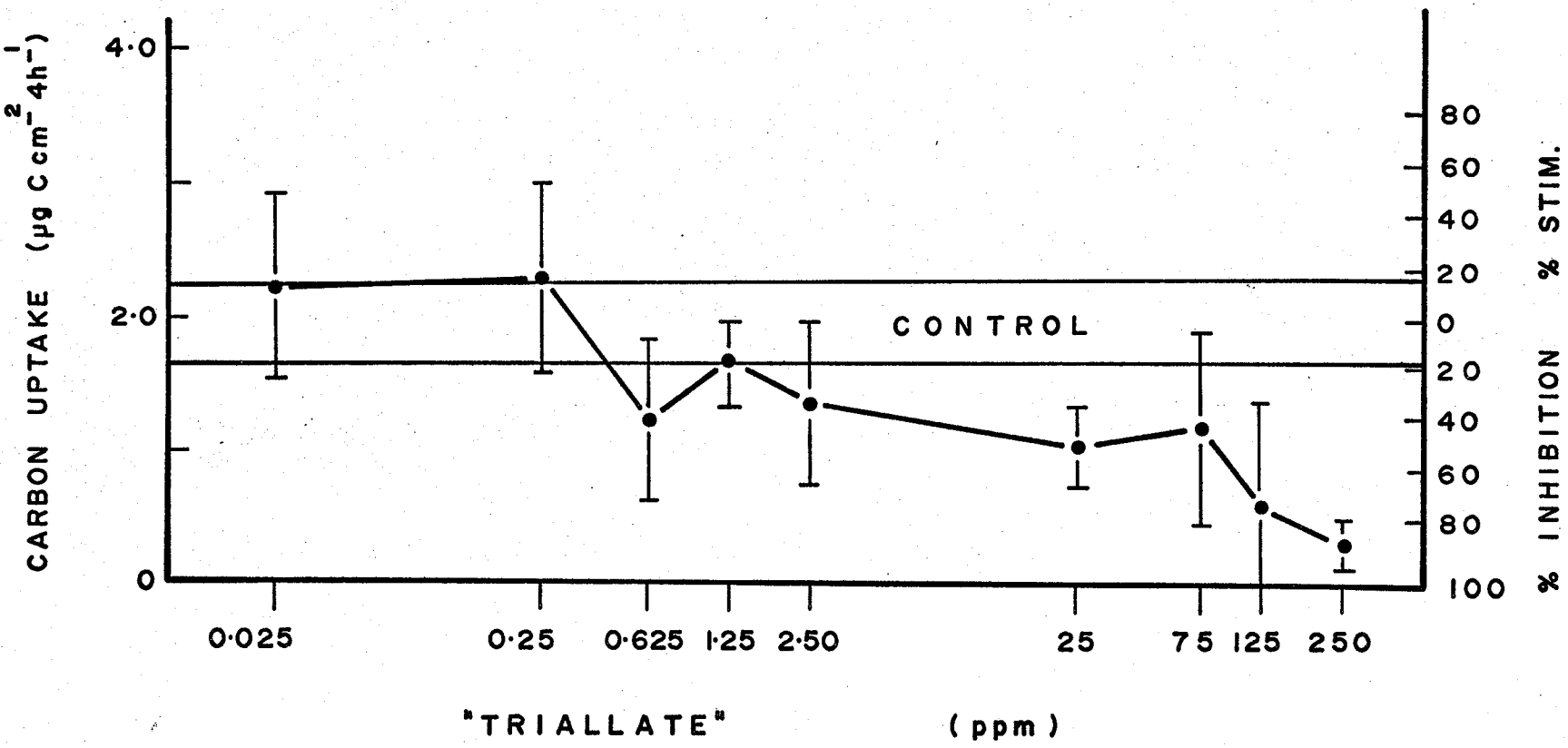

Fig. 25. The effects of increasing concentrations of "TRIALLATE" upon periphyton photosynthesis in Delta Marsh, Manitoba.

fixation of $^{14}CO_2$. At concentrations of 0.625-250 ppm, carbon uptake was reduced by 16-86%.

7. Bipyridyls

A slight to moderate reduction of photosynthesis was noted with periphyton samples treated with 0.025-2.50 ppm "Paraquat" (a reduction of 2-26%) (Fig. 26). "Paraquat" concentrations of 25.0, 75.0, 125 and 250 ppm greatly reduced carbon uptake values by inhibiting photosynthesis by 57, 97, 97 and 100%, respectively.

8. Copper Sulphate

The lowest concentration of copper sulphate utilized (0.025 ppm) had no affect upon the photosynthetic rate of natural periphyton samples, whereas all other concentrations tested reduced carbon uptake (Fig. 27). With concentrations of 0.250-250 ppm, carbon assimilation was reduced by 8-100%.

The effects of the herbicides upon the photosynthetic rate of natural periphyton populations are summarized in Table 6. Comparisons of the herbicidal concentrations which effectively reduced periphyton photosynthesis totally (EC_{100}) or by 50% (EC_{50}) are presented in Table 7. According to EC_{100} values "Simazine" is the most effective herbicide followed by

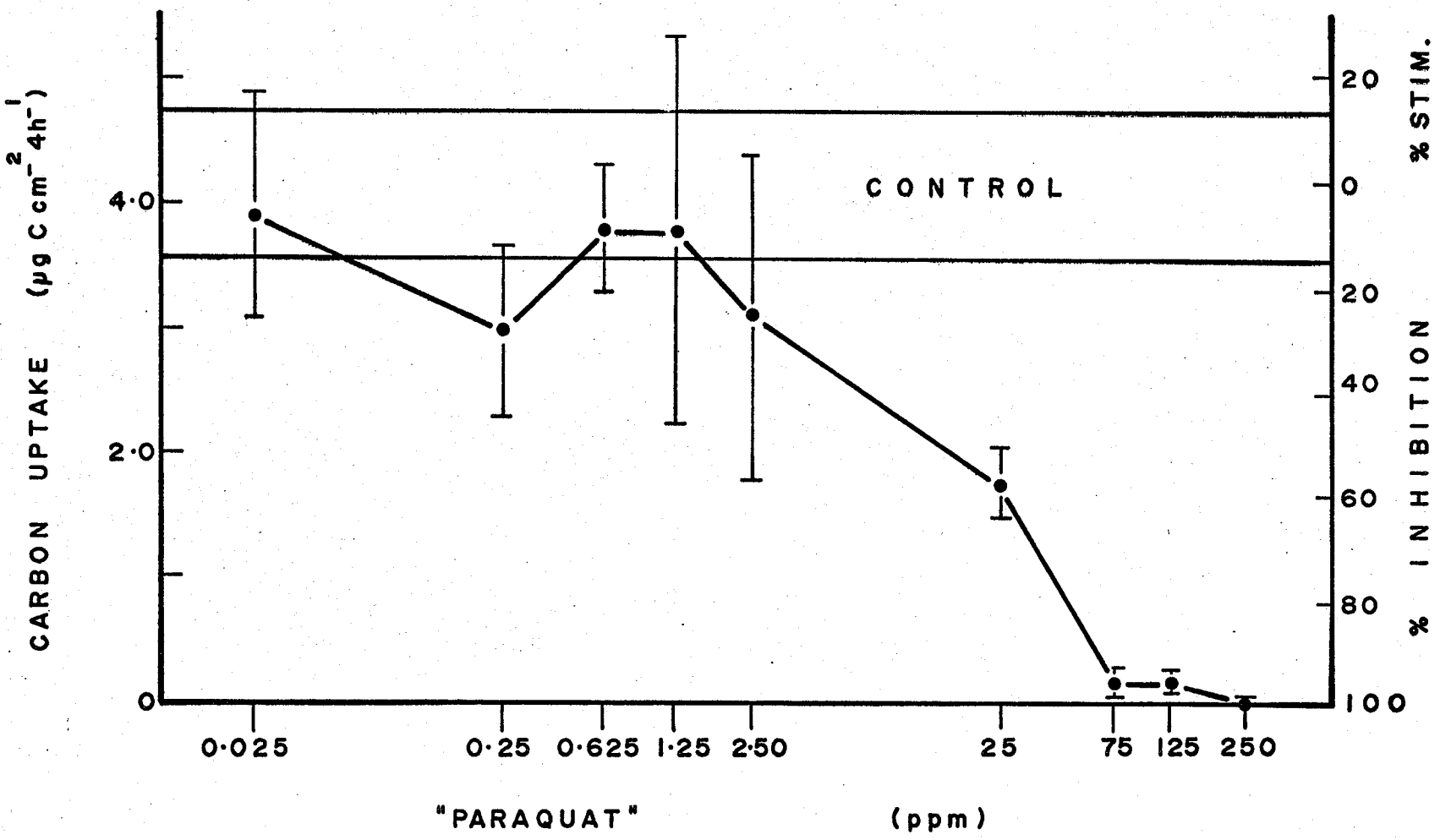

Fig. 26. The effects of increasing concentrations of "PARAQUAT" upon periphyton photosynthesis in Delta Marsh, Manitoba.

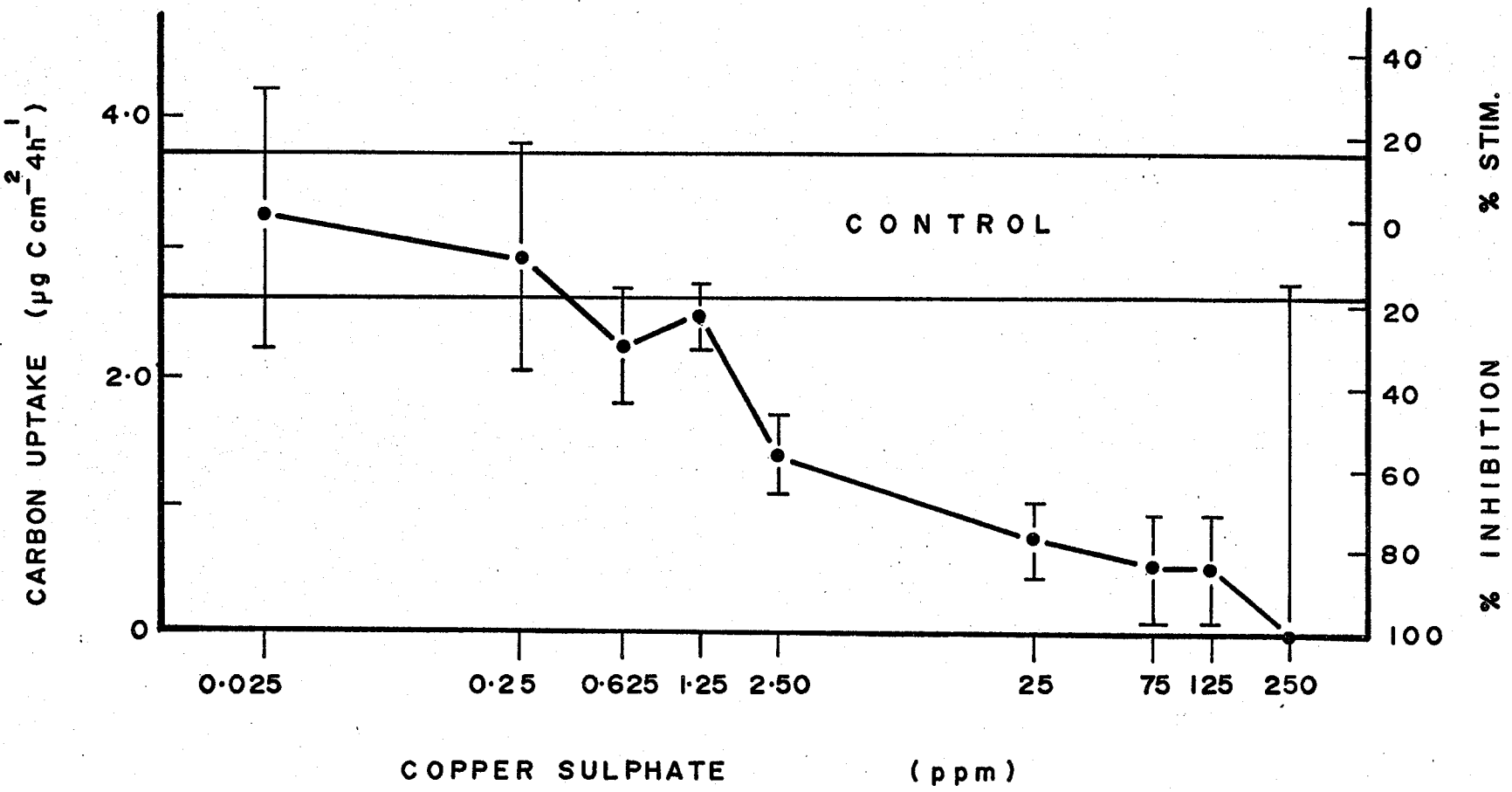

Fig. 27. The effects of increasing concentrations of COPPER SULPHATE upon periphyton photosynthesis in Delta Marsh, Manitoba.

Table 6. The effect of a number of herbicides upon the photosynthetic rate of naturally occurring periphyton populations in Delta Marsh, Manitoba. Results expressed as % Stimulation (+) and % Inhibition (-).

Sample	Conc.	Phenoxy-acetic Acids		Benzoic Acids	Aliphalic Acids		"N" Heterocyclics "S" Triazines		Substituted Ureas	Carbamates			Bipyridyls	Inorganic
	(ppm)	2,4-D	MCPA	Amiben	TCA	Dalapon	Simazine	Atrazine	Linuron	Aryl-Barban	Thio-EPTC	Triallate	Paraquat	$CuSO_4$
1	0.025	+52	+29	+29	-7	-16	-66	-72	+99	-7	-84	+16	-2	+2
2	0.250	+57	+71	+11	+12	-28	-36	-63	-5	-5	-68	+18	-26	-8
3	0.625	+15	+50	+2	+8	-44	-100	-91	+81	-18	-49	-36	-9	-31
4	1.25	+28	+53	+17	-24	-80	-100	-100	+15	-45	-50	-16	-8	-22
5	2.50	+1	+21	-12	-37	-39	-100	-90	-100	-31	-58	-30	-25	-57
6	25.0	+11	+118	-8	-72	-17	-100	-85	-97	-100	-74	-47	-57	-77
7	75.0	-8	+88	-15	-81	-53	-100	-78	-95	-89	-81	-41	-97	-84
8	125	0	+85	-38	-75	-100	-100	-79	-100	-100	-76	-71	-97	-85
9	250	-45	8	-50	-78	-80	-94	-100	+283	-95	-78	-86	-100	-100

Table 7. Herbicidal concentrations (ppm) which effectively reduced the photosynthetic rate of natural periphyton samples by 50% (EC_{50}) and 100% (EC_{100}).

Herbicide	EC_{50}	EC_{100}
2,4-D	> 250	> 250
MCPA	> 250	> 250
Amiben	> 250	> 250
TCA	2.50-25.0	> 250
Dalapon	0.625-75.0	125-250
Simazine	0.025-0.250	0.625
Atrazine	≤ 0.025	1.25
Linuron	2.50	2.50
Barban	1.25-25.0	25.0
EPTC	≤ 0.025	> 250
Triallate	75.0-125	> 250
Paraquat	2.50-25.0	250
Copper Sulphate	1.25-2.50	250

"Atrazine", "Linuron", "Barban", "Dalapon", "Paraquat" and copper sulphate are approximately equal. The other herbicides failed to reduce periphyton photosynthesis totally with the range of concentrations tested. According to EC_{50} values the herbicides may be ranked as follows: "Atrazine" and EPTC are approximately equal, "Simazine", "Dalapon", copper sulphate, "Barban", "Linuron", TCA and "Paraquat" are approximately equal, "Triallate and "Amiben" 2,4-D and MCPA failed to reduce periphyton photosynthesis by 50%.

C. Herbicidal effects upon planktonic heterotrophy

The relationship between velocity of uptake (v) of ^{14}C-glucose and substrate concentration (A) was approximately assymptotic for light and dark samples in both saturation experiments (Figs. 28 and 29). In both experiments uptake in the light was slightly higher than uptake in the dark.

1. Phenoxyacetic Acids

2,4-D and MCPA exhibited a moderate inhibition of planktonic heterotrophy. 2,4-D concentrations of 2.50, 25.0, 75.0, and 250 ppm effectively reduced organic carbon uptake of samples in the light by

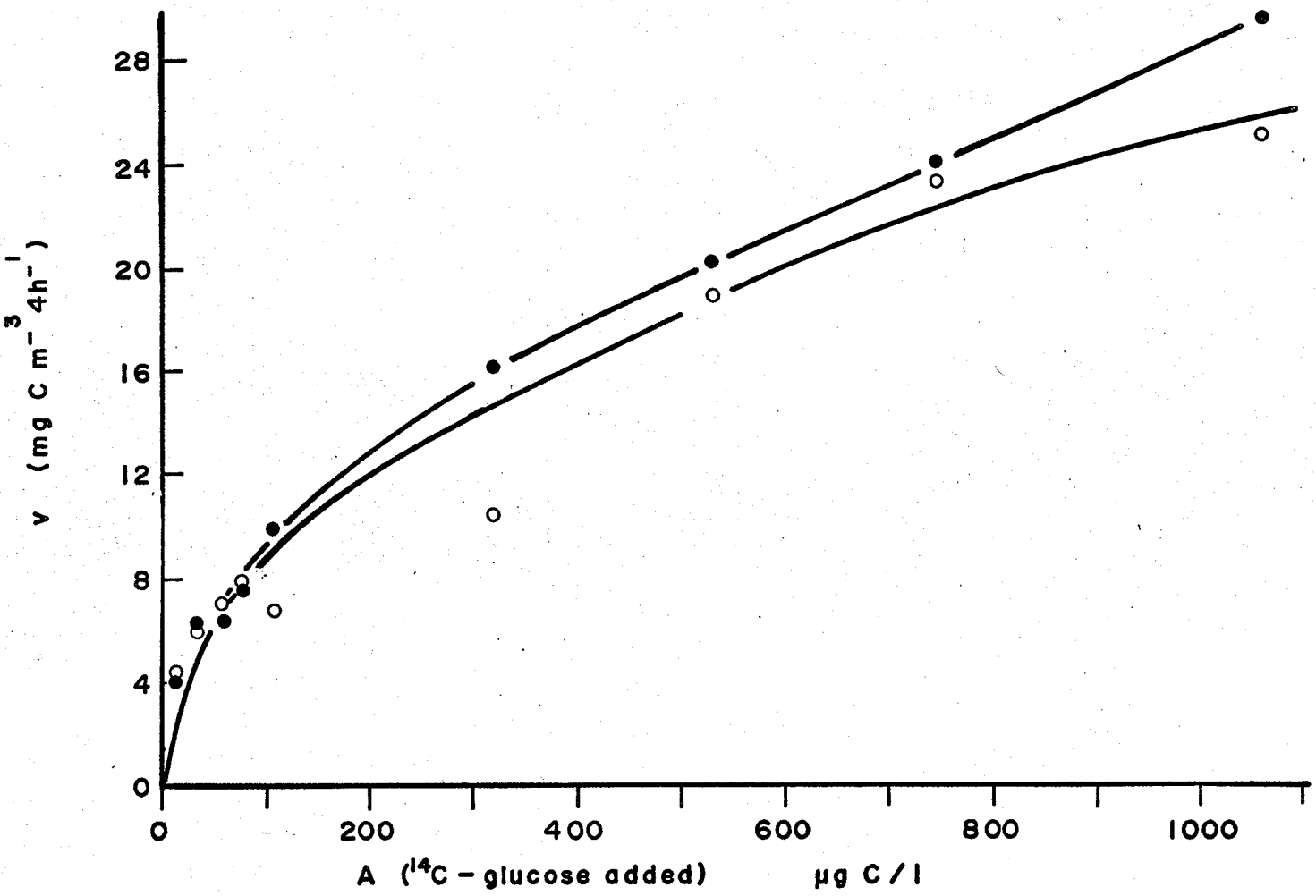

Fig. 28. Velocity of uptake (v) of ^{14}C-glucose, at increasing substrate concentrations (A) for plankton samples in the light (●) and the dark (○). July 29, 1974.

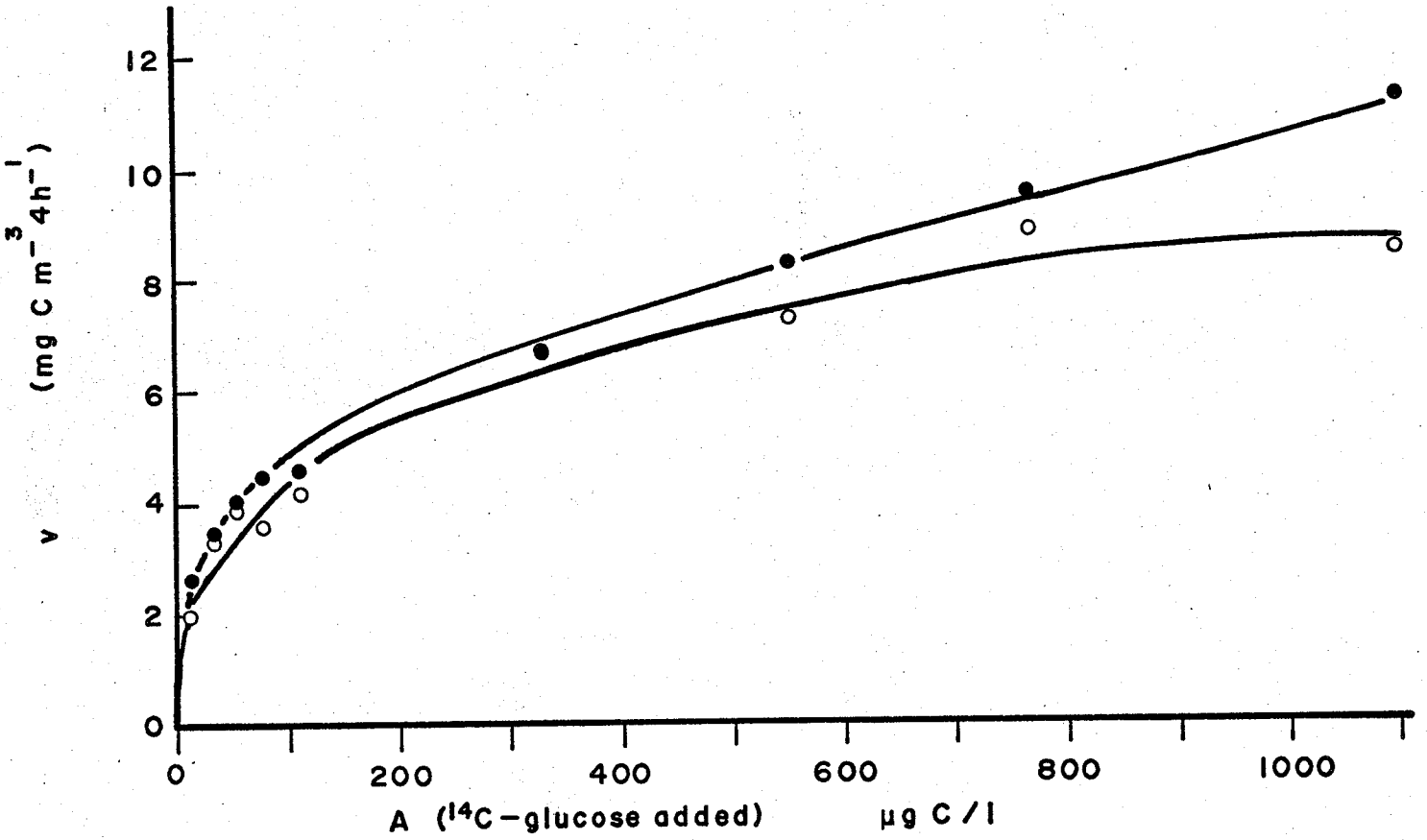

Fig. 29. Velocity of uptake (v) of ^{14}C glucose, at increasing substrate concentrations (A) for plankton samples in the light (●) and the dark (○). Sept. 7, 1974.

8-42% (Fig. 30). Dark heterotrophic samples, subjected to increasing concentrations of 2,4-D, had slightly higher uptake values than light samples, but dark planktonic samples, required slightly more 2,4-D initially, to yield similar patterns of inhibition of organic carbon uptake (Fig. 31). Concentrations of 25.0-250 ppm of 2,4-D reduced ^{14}C-glucose uptake by 5-42%.

Within 1 standard deviation, MCPA concentrations of 2.50 ppm and less, had no affect upon planktonic heterotrophic assimilation of ^{14}C-glucose in the light whereas concentrations of 25.0 ppm and greater reduced organic uptake by 13-34% (Fig. 32). Within the range of low and high dark values, MCPA concentrations less than 2.50 ppm had no affect upon dark planktonic heterotrophy whereas concentrations between 2.50-250 ppm reduced heterotrophic assimilation of ^{14}C-glucose by 7-51% (Fig. 33).

2. Benzoic Acids

"Amiben" appeared to exhibit no detrimental effects upon planktonic heterotrophy (Figs. 34 and 35). With the exception of dark samples treated with 0.025, 0.250, 0.625 and 1.25 ppm "Amiben", uptake values were within 1 standard deviation or within low and high

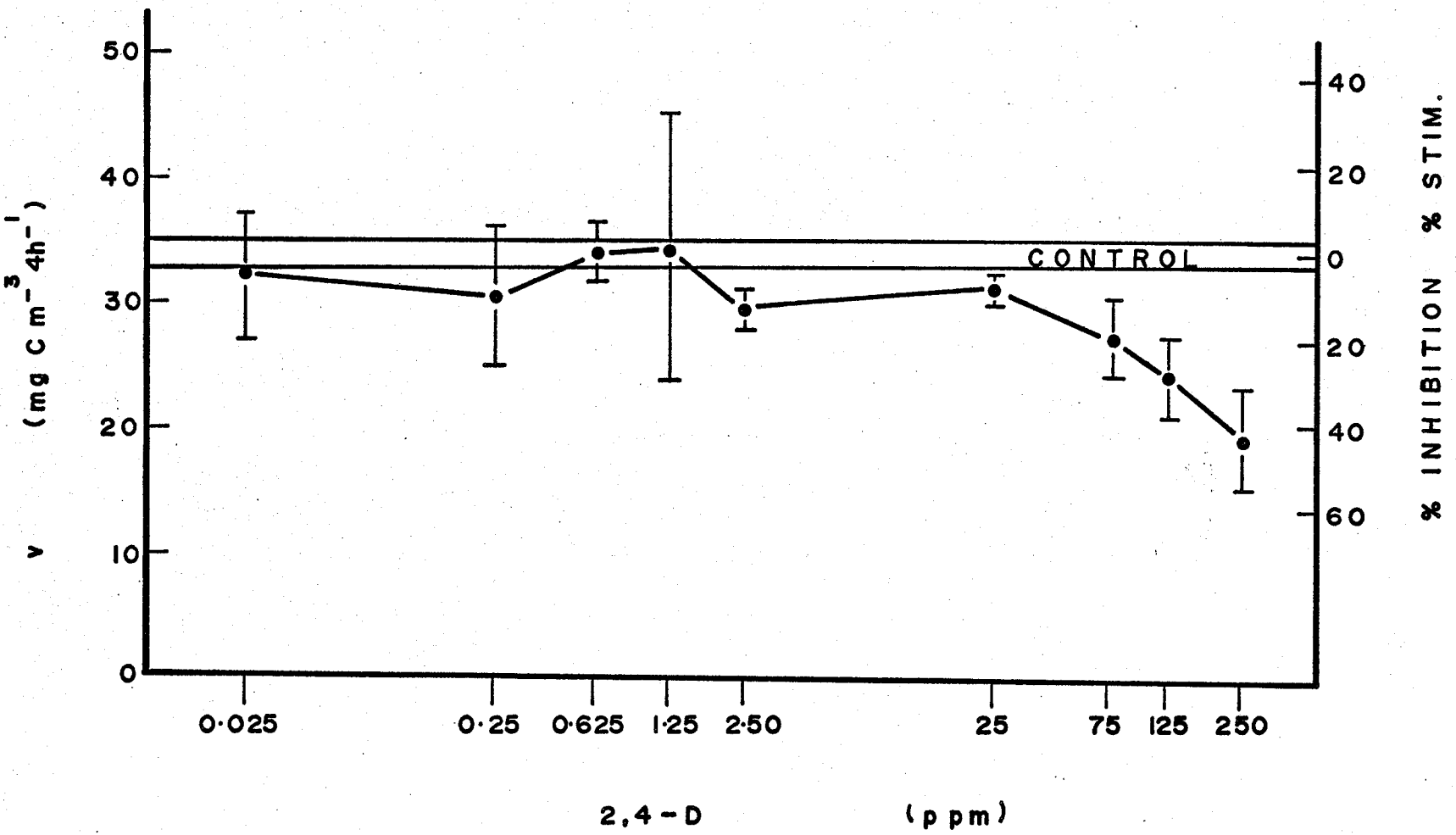

Fig. 30. The effects of increasing concentrations of 2,4-D upon planktonic heterotrophy in the light.

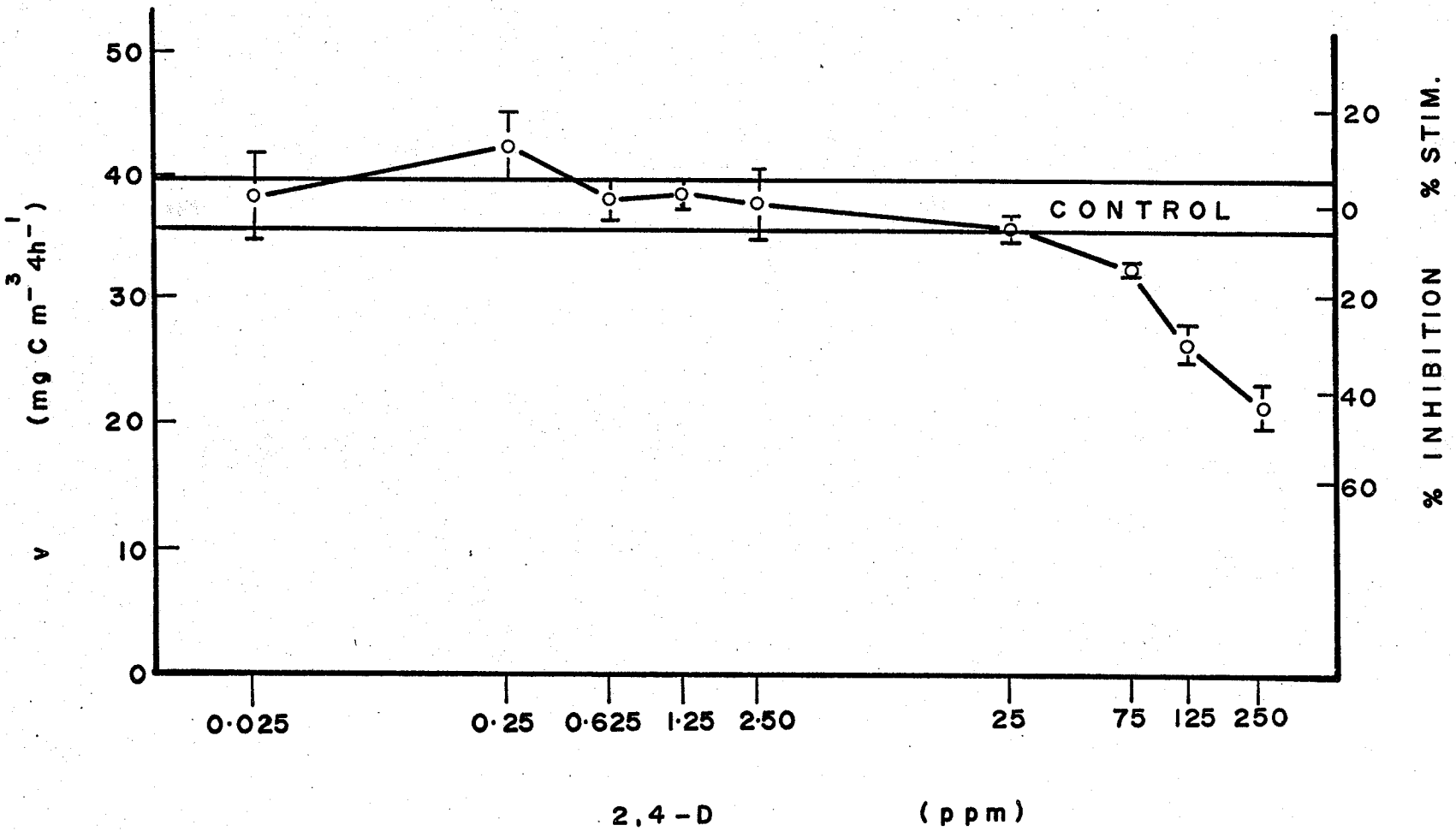

Fig. 31. The effects of increasing concentrations of 2,4-D upon planktonic heterotrophy in the dark.

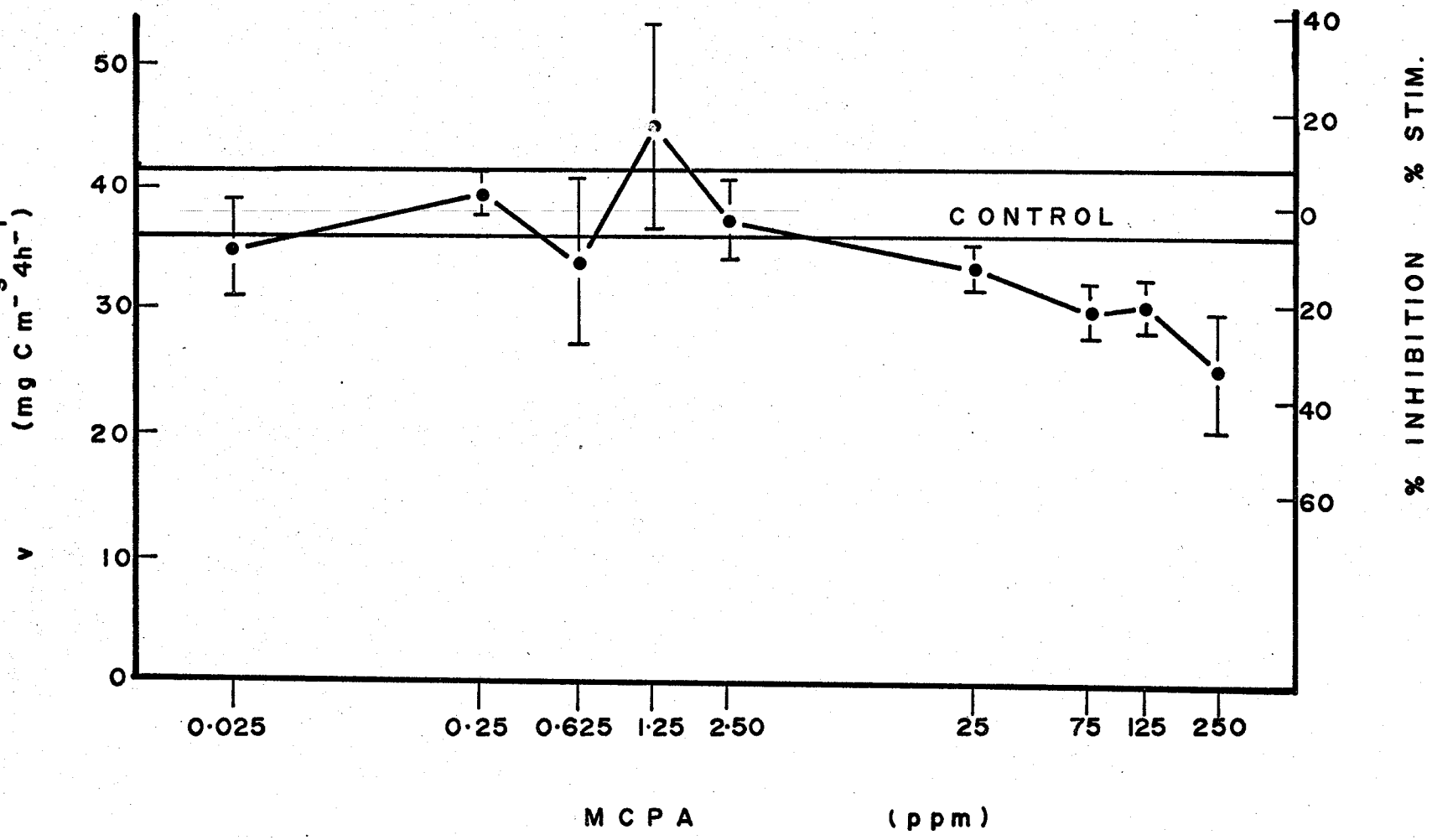

Fig. 32. The effects of increasing concentrations of MCPA upon planktonic heterotrophy in the light.

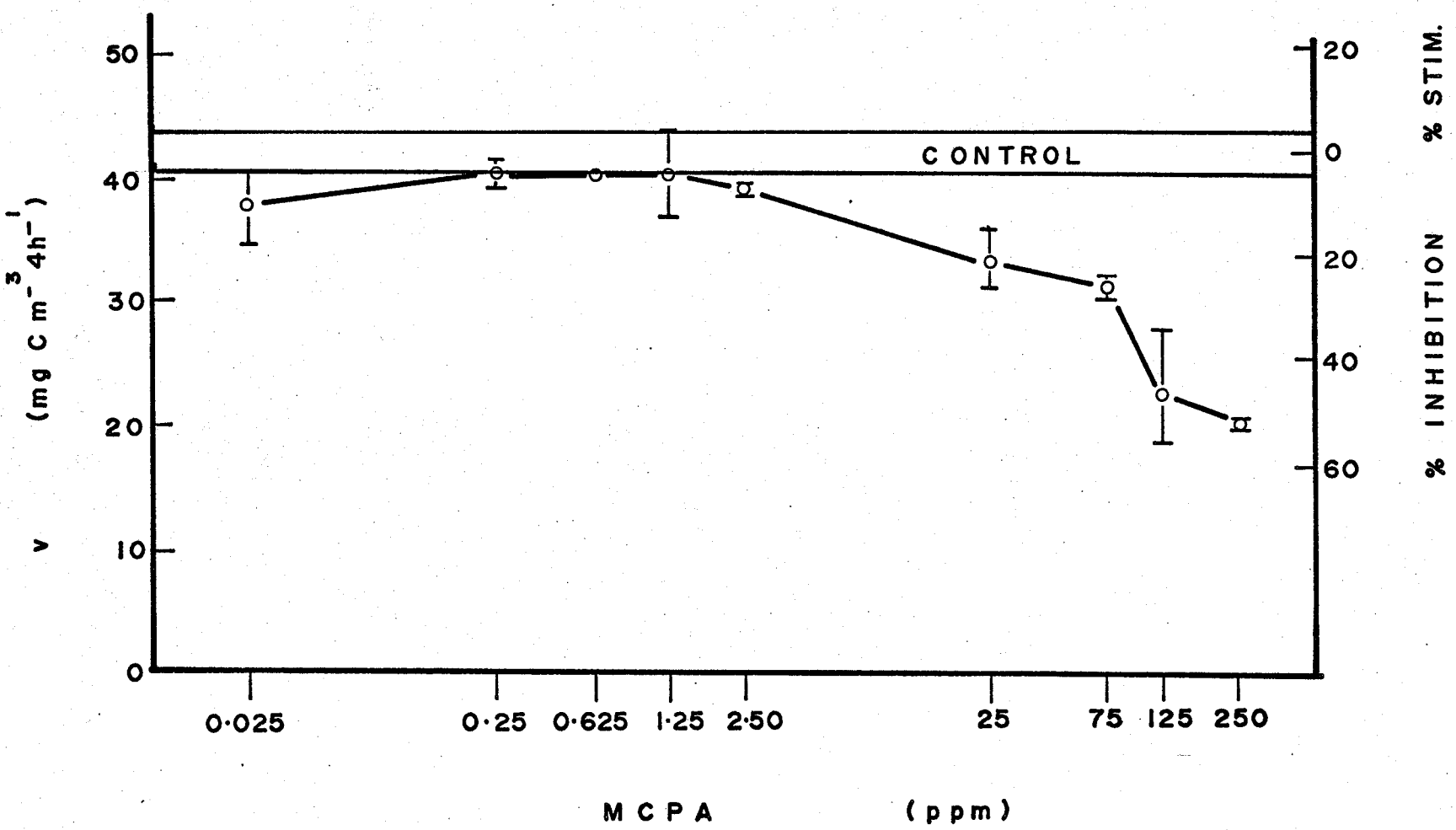

Fig. 33. The effects of increasing concentrations of MCPA upon planktonic heterotrophy in the dark.

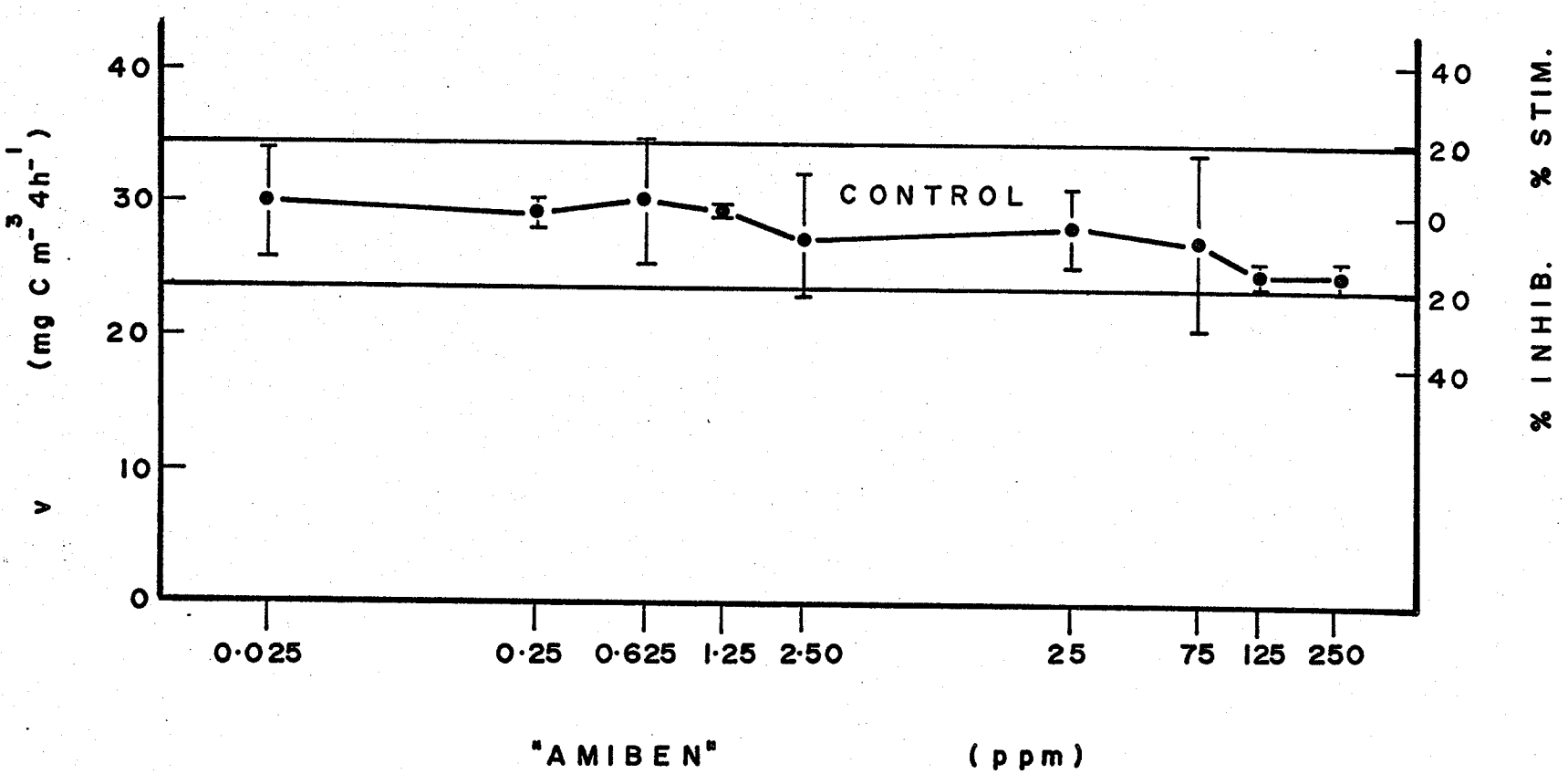

Fig. 34. The effects of increasing concentrations of "AMIBEN" upon planktonic heterotrophy in the light.

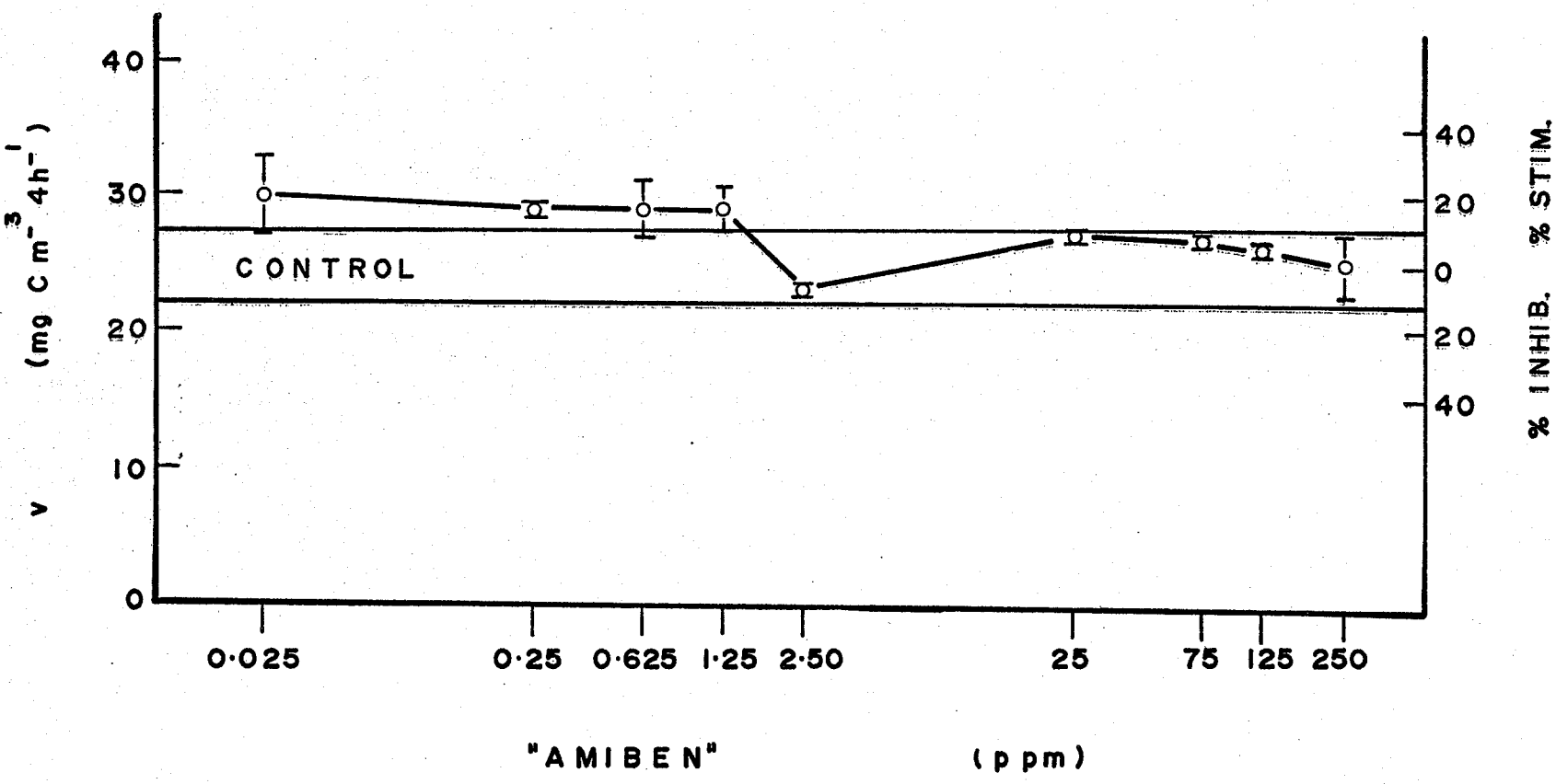

Fig. 35. The effects of increasing concentrations of "AMIBEN" upon planktonic heterotrophy in the dark.

values of light and dark control values. The prestated concentrations of "Amiben", 0.025-1.25 ppm stimulated heterotrophic assimilation of ^{14}C-glucose by 17-21% (Fig. 35).

3. Aliphatic Acids

With the exception of light samples treated with 0.025, 0.625 and 1.25 ppm, TCA did not appear to significantly affect planktonic heterotrophy (Figs. 36 and 37). TCA concentrations of 0.025 and 1.25 ppm, appeared to slightly increase uptake of organic carbon to a level of 6-9% above the mean control value, whereas a 10% reduction was noted with 0.625 ppm (Fig. 36). Similar uptake values were noted for samples in the light and the dark.

"Dalapon" appeared to be slightly effective in reducing planktonic heterotrophy, but only at concentrations of 75.0-250 ppm (Figs. 38 and 39). Dark heterotrophic assimilation was reduced 18-32% by "Dalapon" concentrations of 75, 125 and 250 ppm whereas light samples were only affected by 250 ppm; organic carbon uptake reduced 34%.

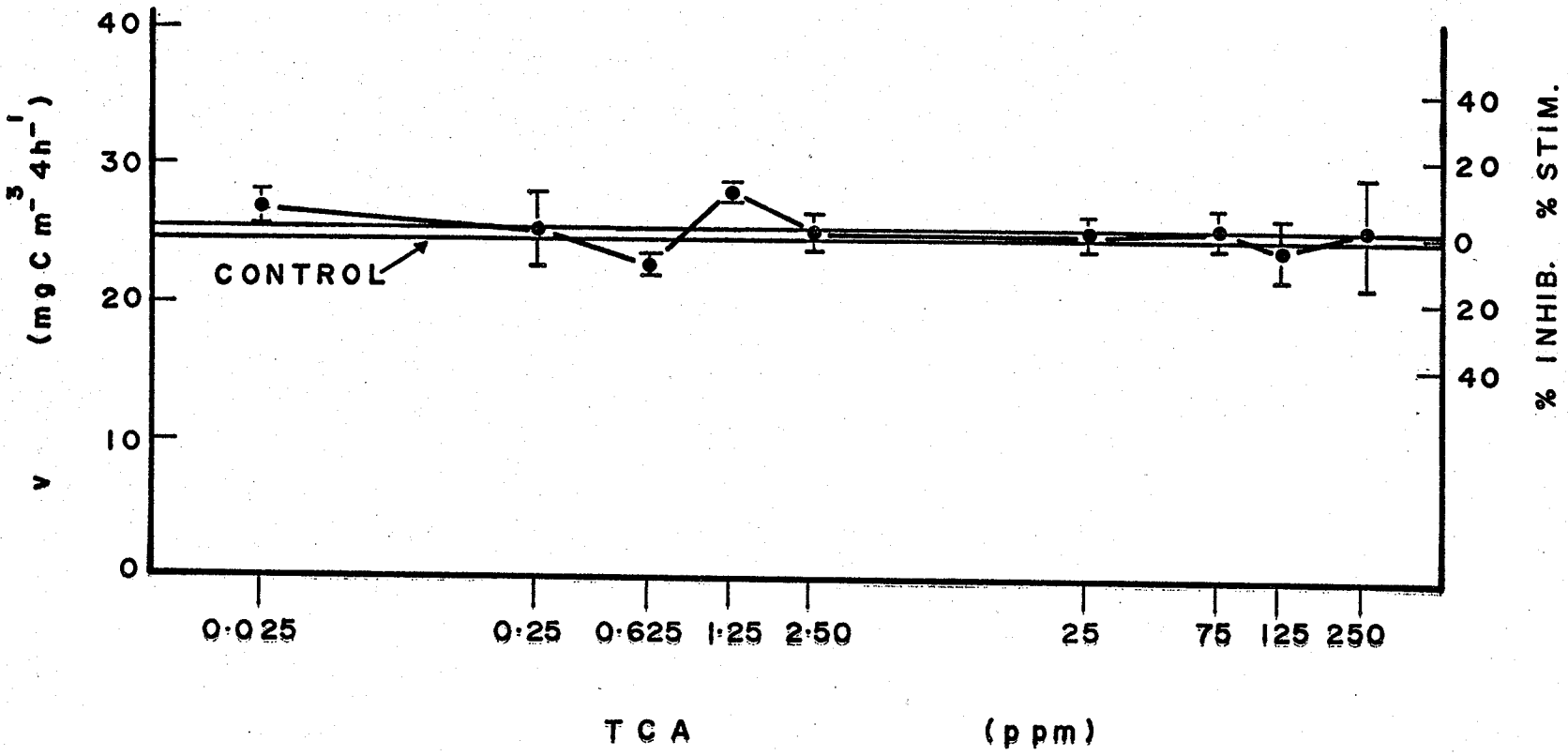

Fig. 36. The effects of increasing concentrations of TCA upon planktonic heterotrophy in the light.

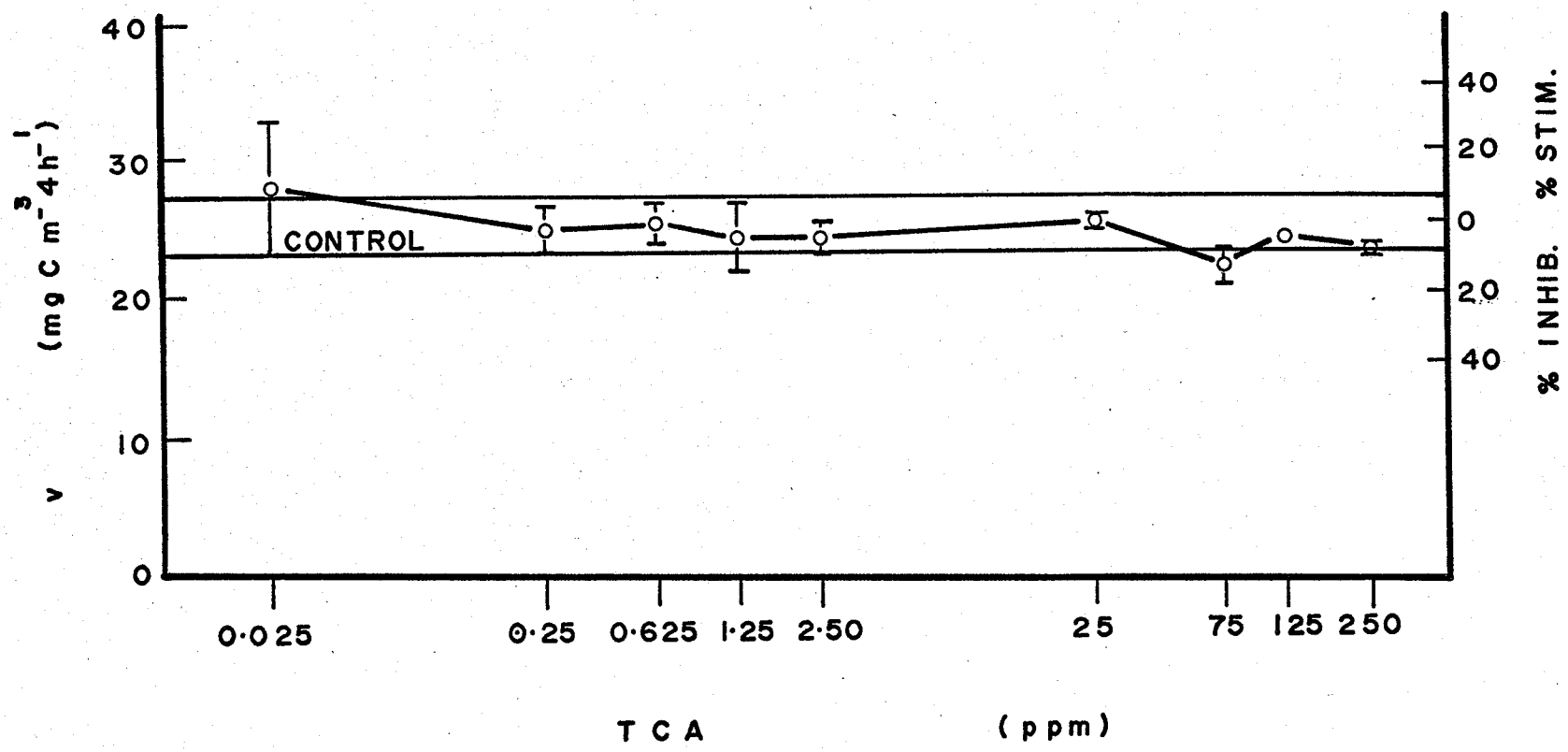

Fig. 37. The effects of increasing concentrations of TCA upon planktonic heterotrophy in the dark.

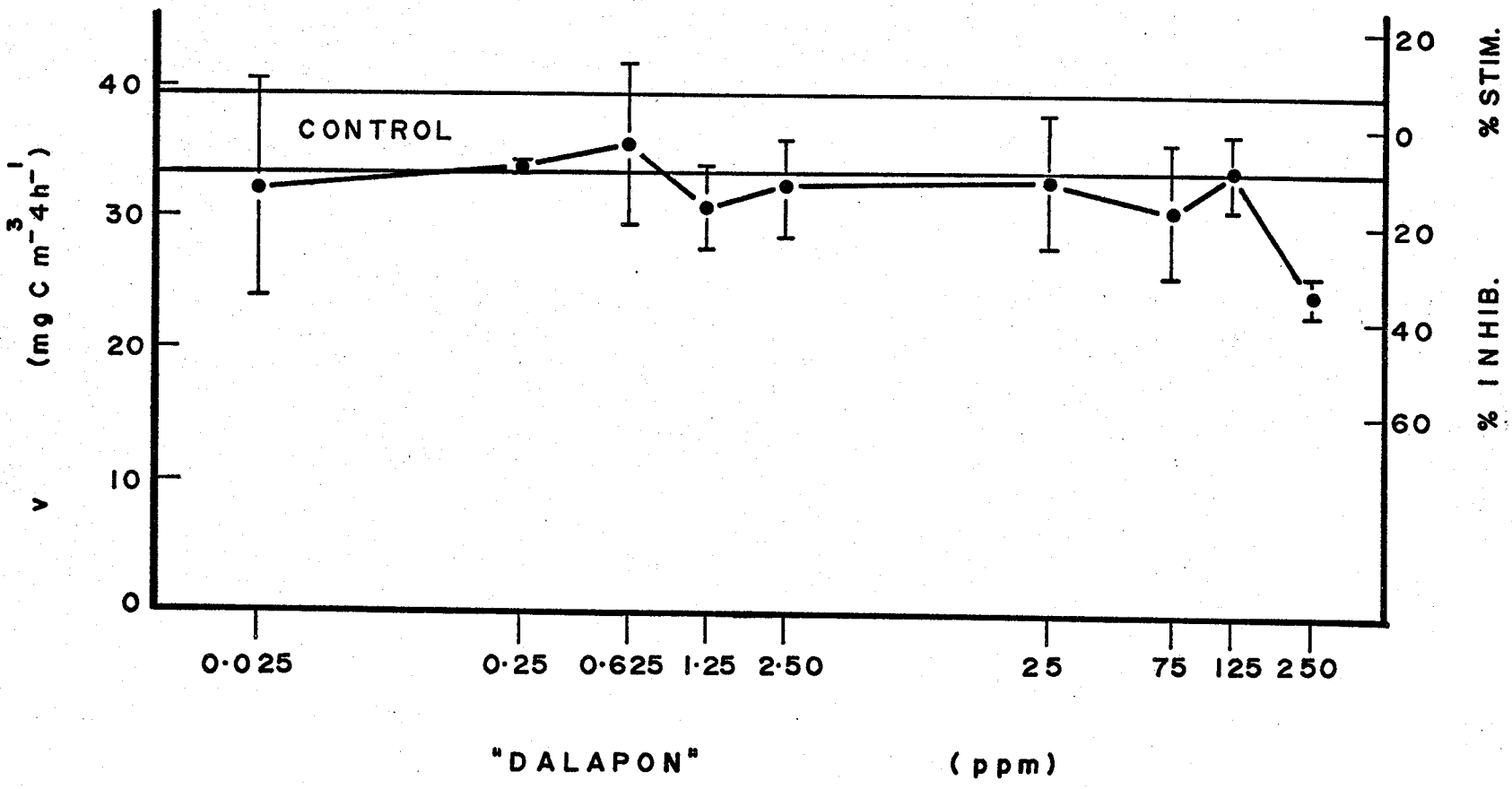

Fig. 38. The effects of increasing concentrations of "DALAPON" upon planktonic heterotrophy in the light.

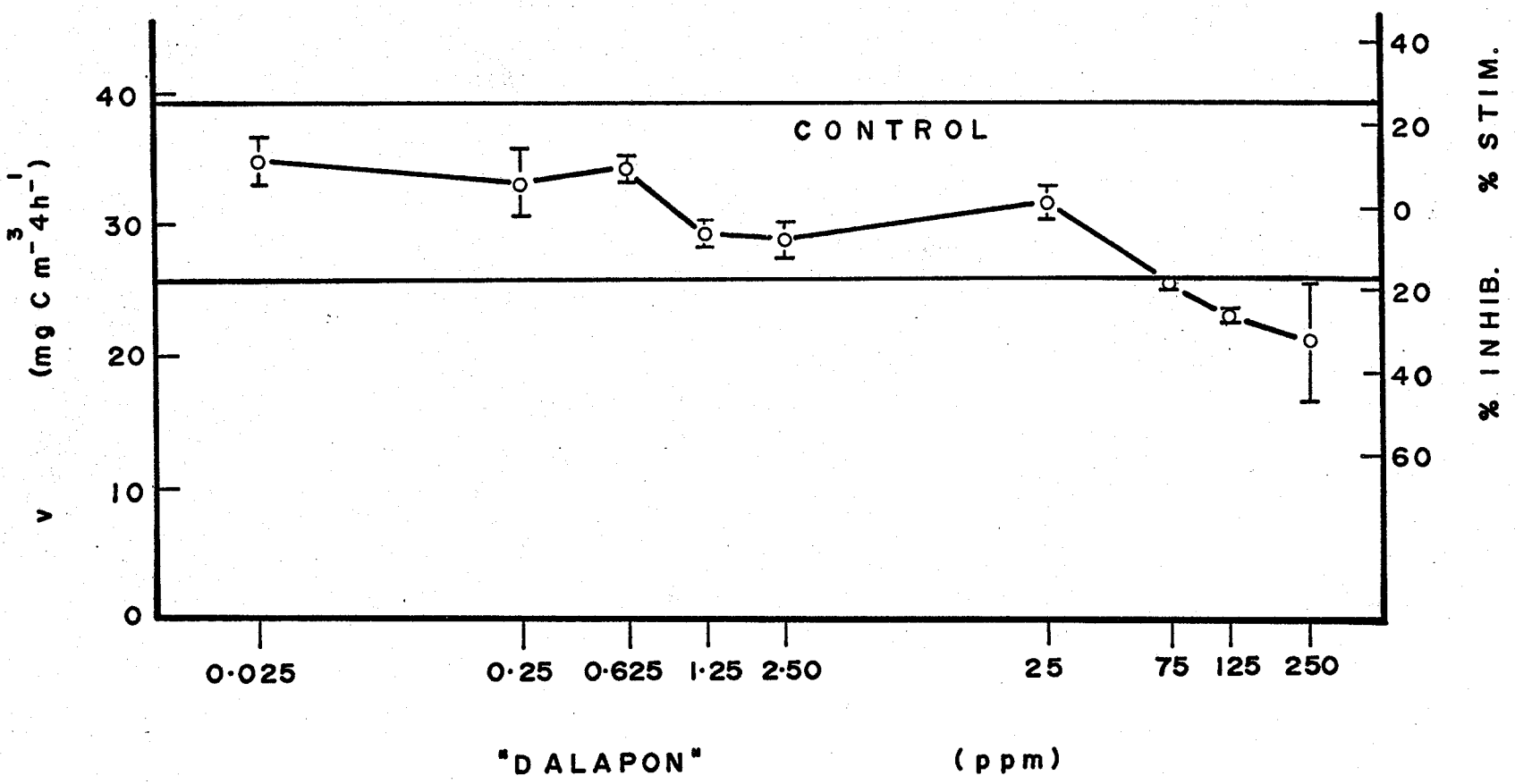

Fig. 39. The effects of increasing concentrations of "DALAPON" upon planktonic heterotrophy in the dark.

4. "N" Heterocyclics

The "N" heterocyclic herbicides appeared to have only a slight effect upon planktonic heterotrophy. "Simazine" concentrations of 2.50-250 ppm reduced heterotrophic assimilation of ^{14}C-glucose in the light by 18-22% (Fig. 40) whereas concentrations of 1.25-250 ppm reduced organic uptake of darkened samples by 12-21% (Fig. 41).

"Atrazine" concentrations of 0.250-250 ppm decreased planktonic uptake of ^{14}C-glucose for samples in the light by 7-20% (Fig. 42). With the exception of 1.25 ppm of "Atrazine", dark heterotrophic assimilation was increased 1-23% above the mean control value by concentrations of 0.025-75.0 ppm. Concentrations of 1.25, 125 and 250 ppm had no effect upon dark planktonic heterotrophy (Fig. 43).

With the exception of darkened samples treated with 2.50 and 250 ppm of "Amitrole-T", this herbicide had no significant effect upon either light or dark planktonic heterotrophy (Figs. 44 and 45). Concentrations of 2.50 and 250 ppm reduced dark heterotrophic assimilation by 13%. Organic carbon uptake values appeared to be slightly higher for samples in light.

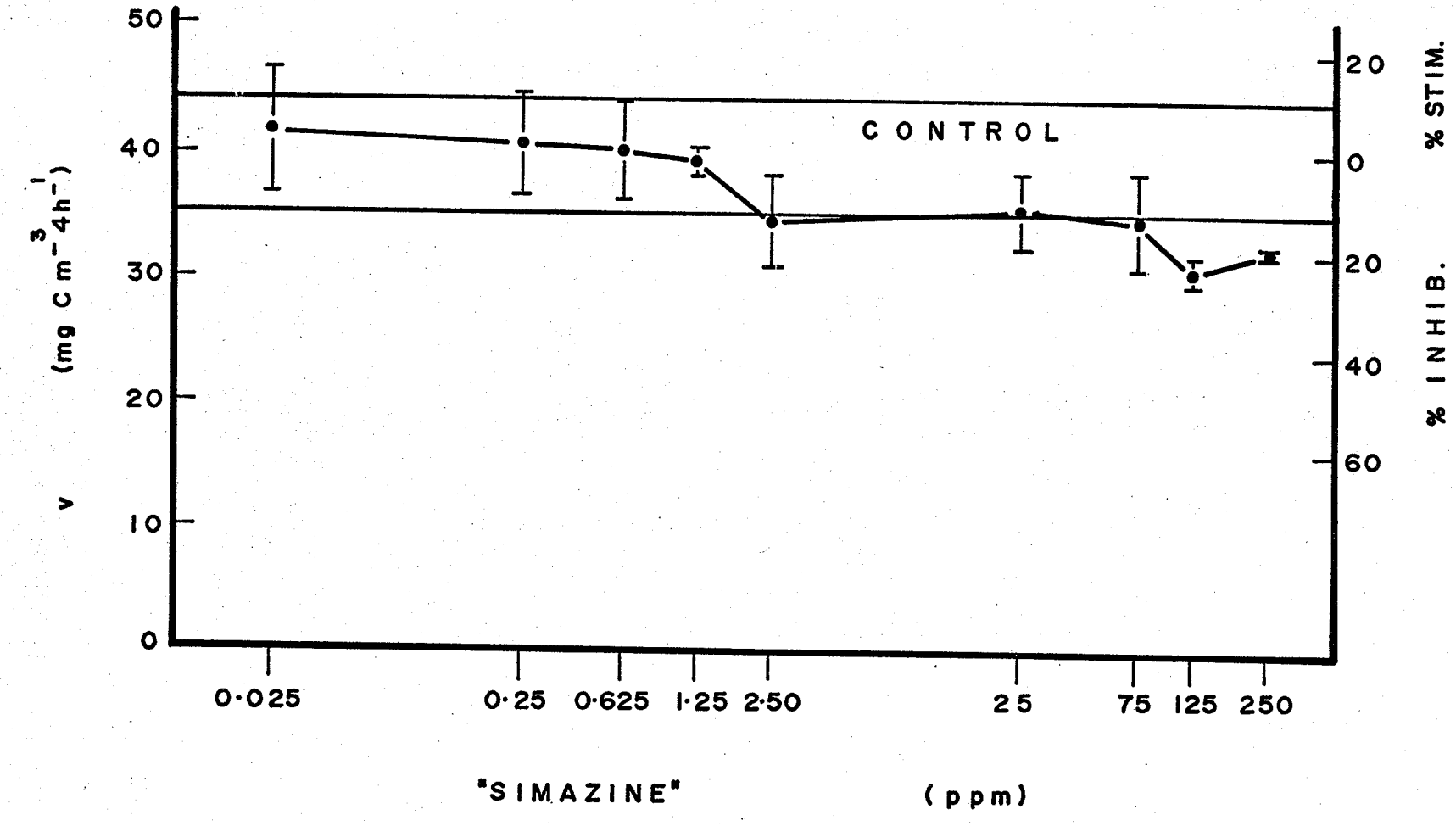

Fig. 40. The effects of increasing concentrations of "SIMAZINE" upon planktonic heterotrophy in the light.

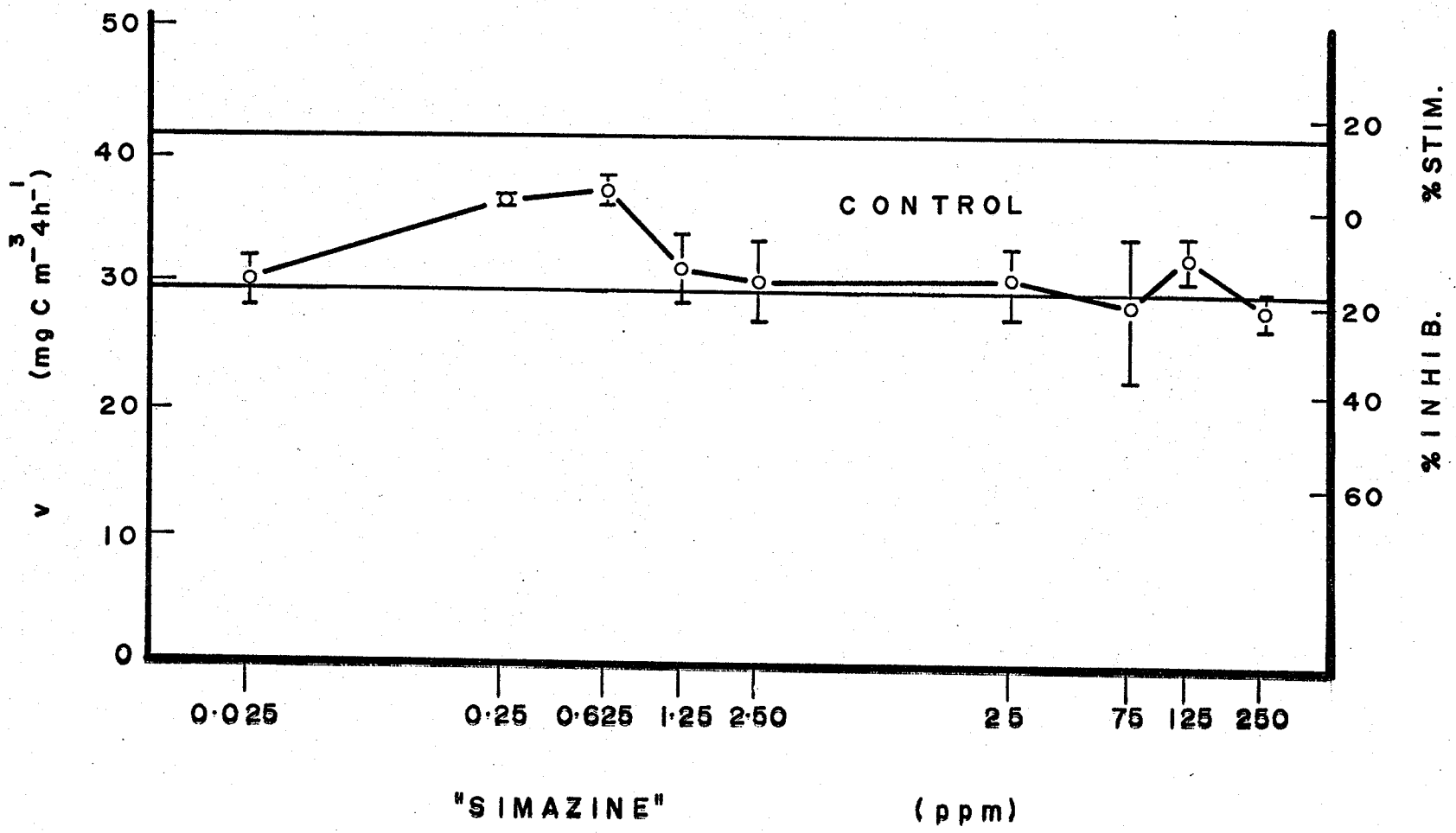

Fig. 41. The effects of increasing concentrations of "SIMAZINE" upon planktonic heterotrophy in the dark.

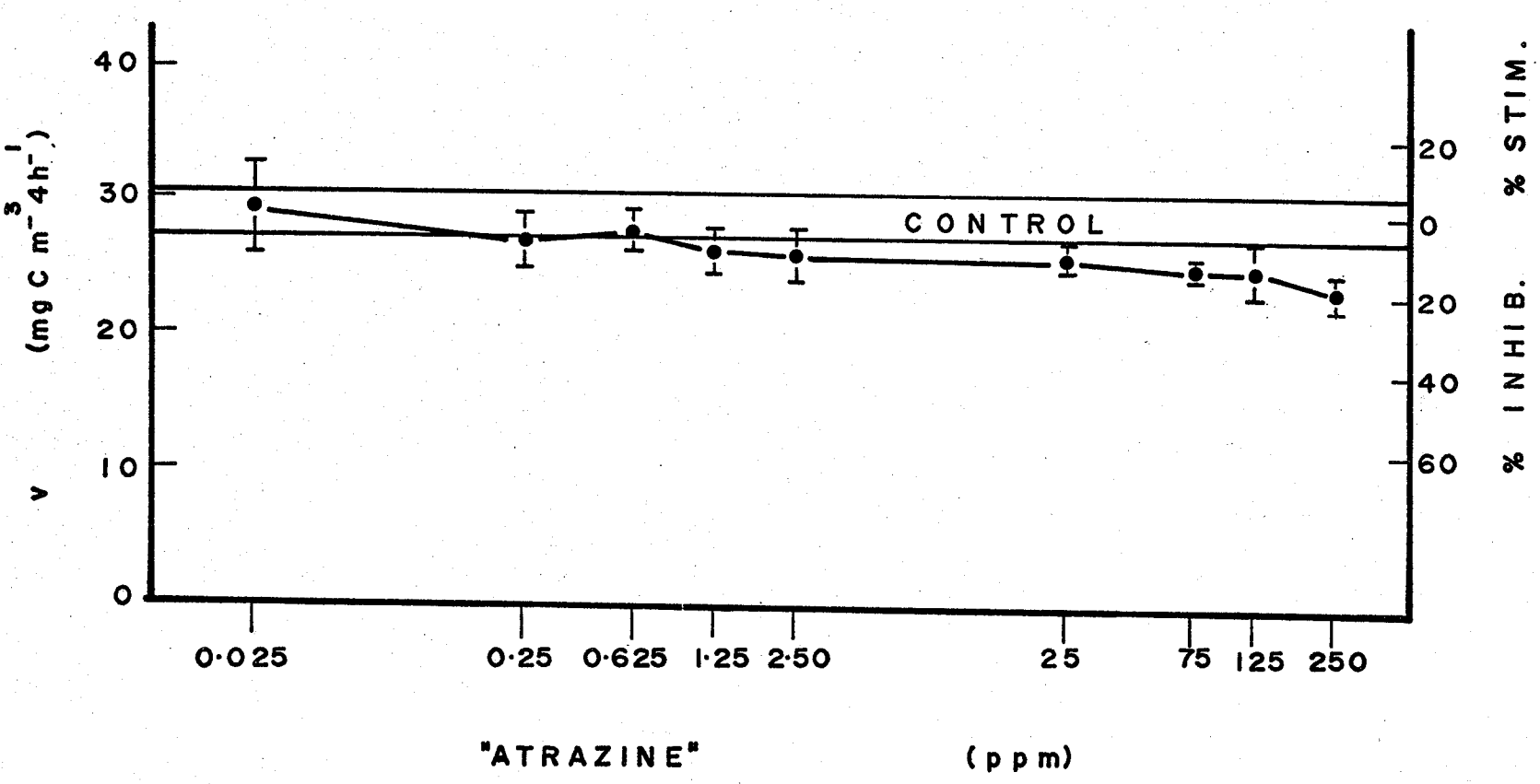

Fig. 42. The effects of increasing concentrations of "ATRAZINE" upon planktonic heterotrophy in the light.

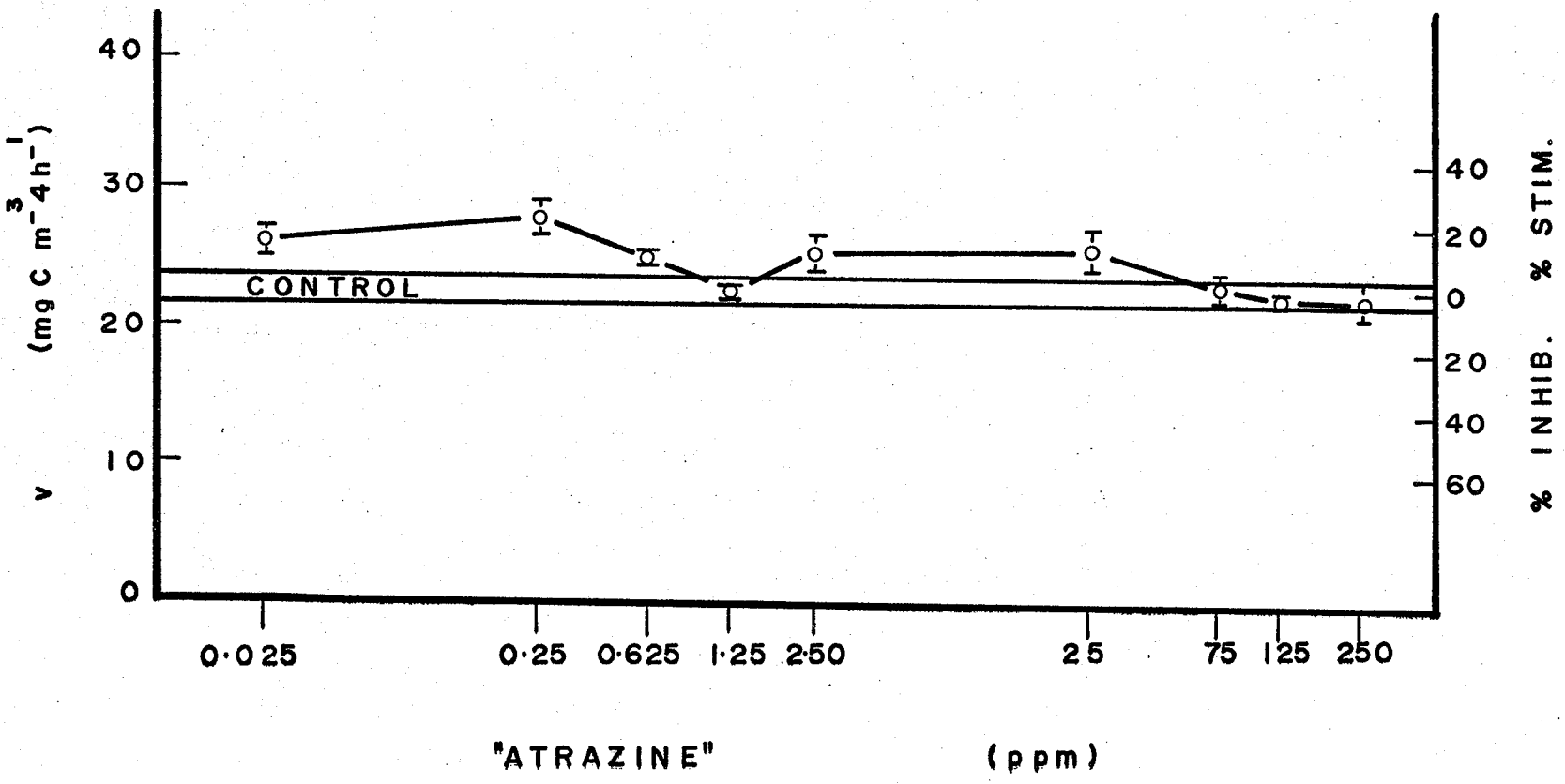

Fig. 43. The effects of increasing concentrations of "ATRAZINE" upon planktonic heterotrophy in the dark.

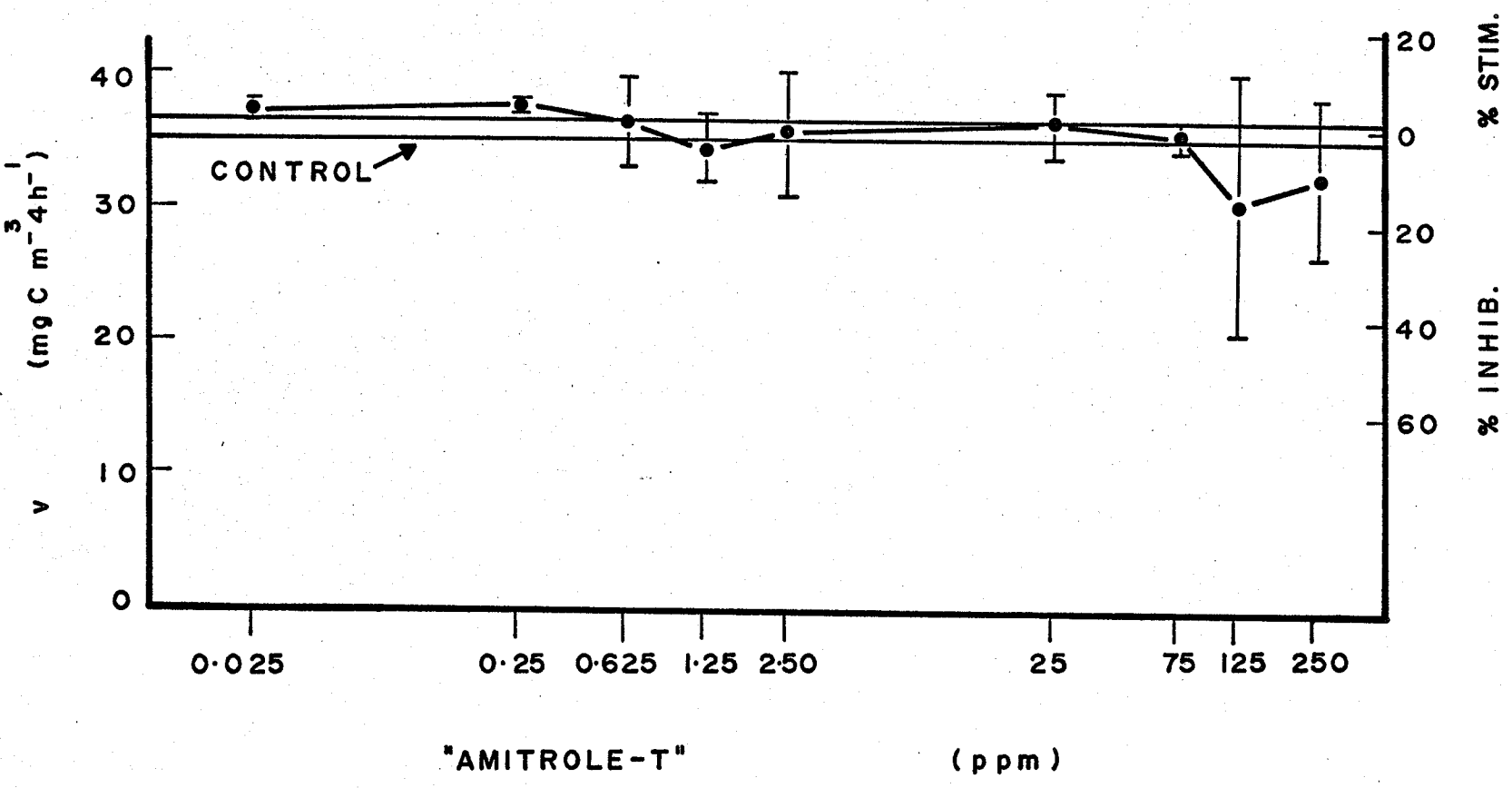

Fig. 44. The effects of increasing concentrations of "AMITROLE-T" upon planktonic heterotrophy in the light.

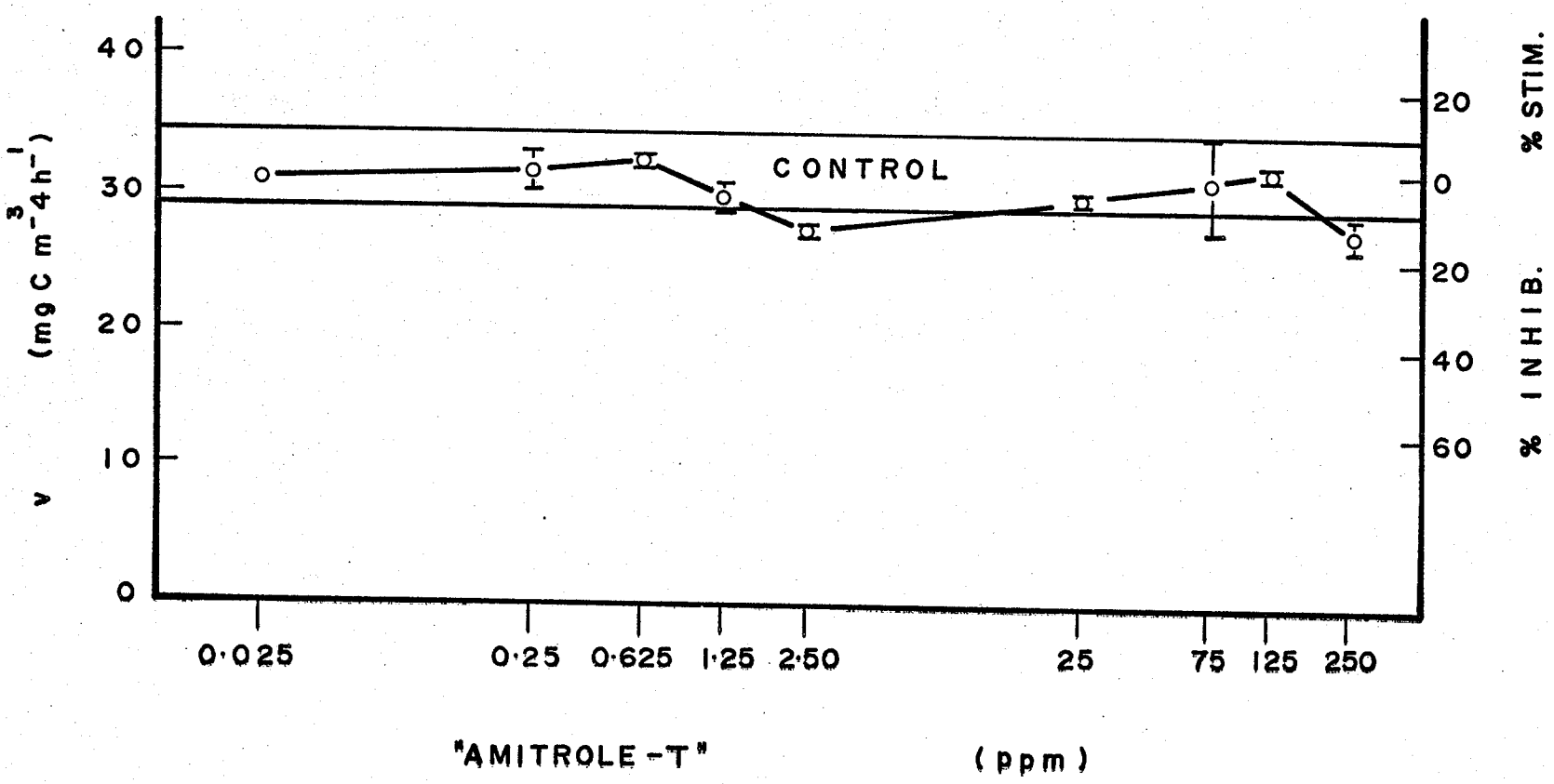

Fig. 45. The effects of increasing concentrations of "AMITROLE-T" upon planktonic heterotrophy in the dark.

5. Substituted Ureas

"Linuron" concentrations of 0.025-2.50 ppm and 0.025-1.25 ppm, had no significant effect upon light and dark planktonic heterotrophy, respectively (Figs. 46 and 47). Concentrations of 25.0-250 ppm reduced organic carbon uptake of light planktonic samples by 36-63% whereas ^{14}C-glucose uptake by darkened samples was reduced 27-63% by "Linuron" concentrations of 2.50-250 ppm.

6. Carbamates

A profound reduction of planktonic heterotrophy was caused by the carbamate herbicides. The lowest concentration of "Barban" (0.025 ppm) slightly stimulated organic carbon uptake of light and darkened samples by 17 and 8%, respectively whereas concentrations of 0.250 ppm and greater drastically reduced heterotrophic assimilation (Figs. 48 and 49). Uptake of ^{14}C-glucose was reduced by 11-99% for light samples and by 27-99% for darkened samples by "Barban" concentrations of 0.250-250 ppm. Uptake values for light and darkened samples were dissimilar in that light uptake was approximately 25-50% greater than dark for samples treated at concentrations of 0.025-2.50 ppm.

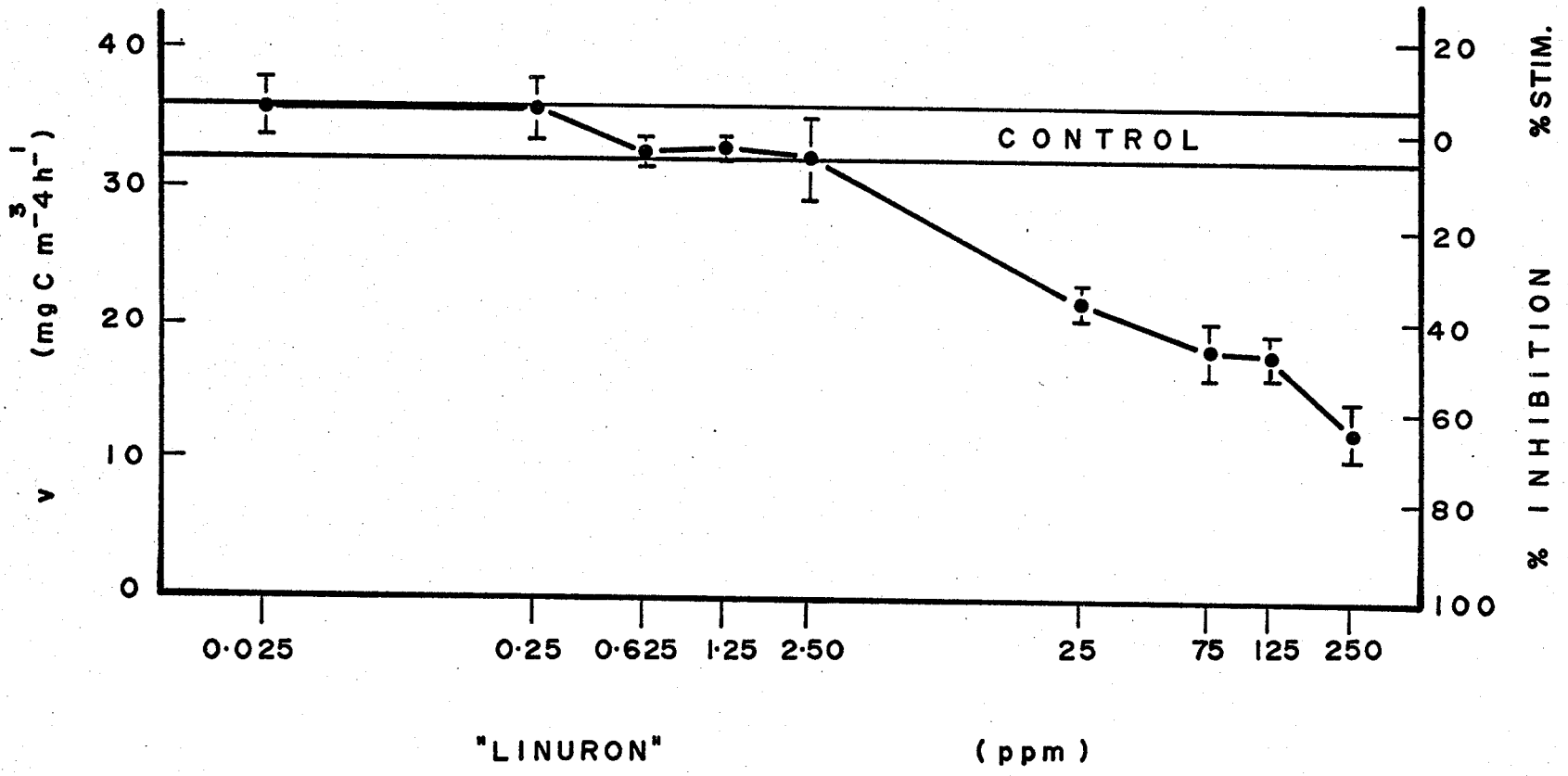

Fig. 46. The effects of increasing concentrations of "LINURON" upon planktonic heterotrophy in the light.

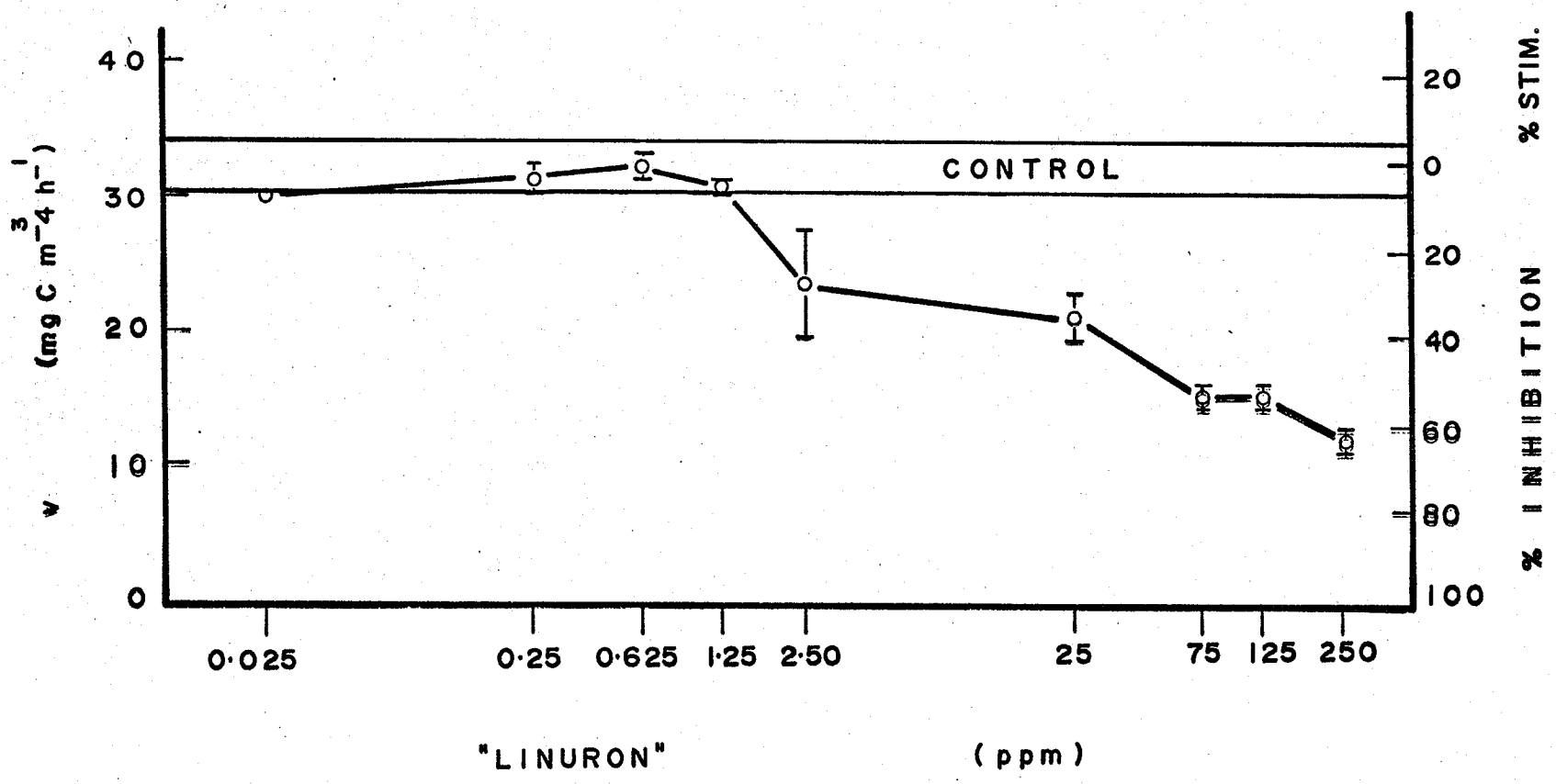

Fig. 47. The effects of increasing concentrations of "LINURON" upon planktonic heterotrophy in the dark.

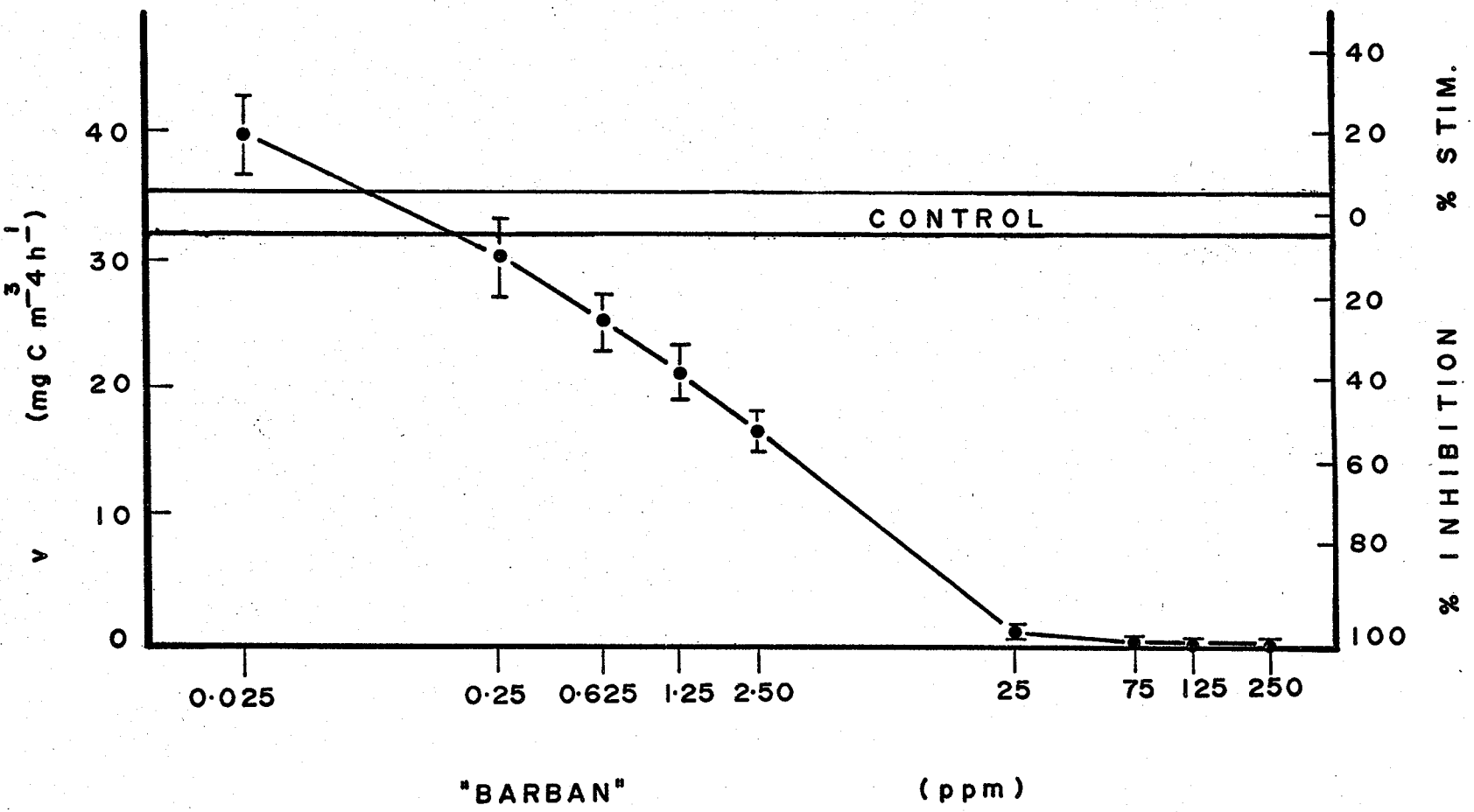

Fig. 48. The effects of increasing concentrations of "BARBAN" upon planktonic heterotrophy in the light.

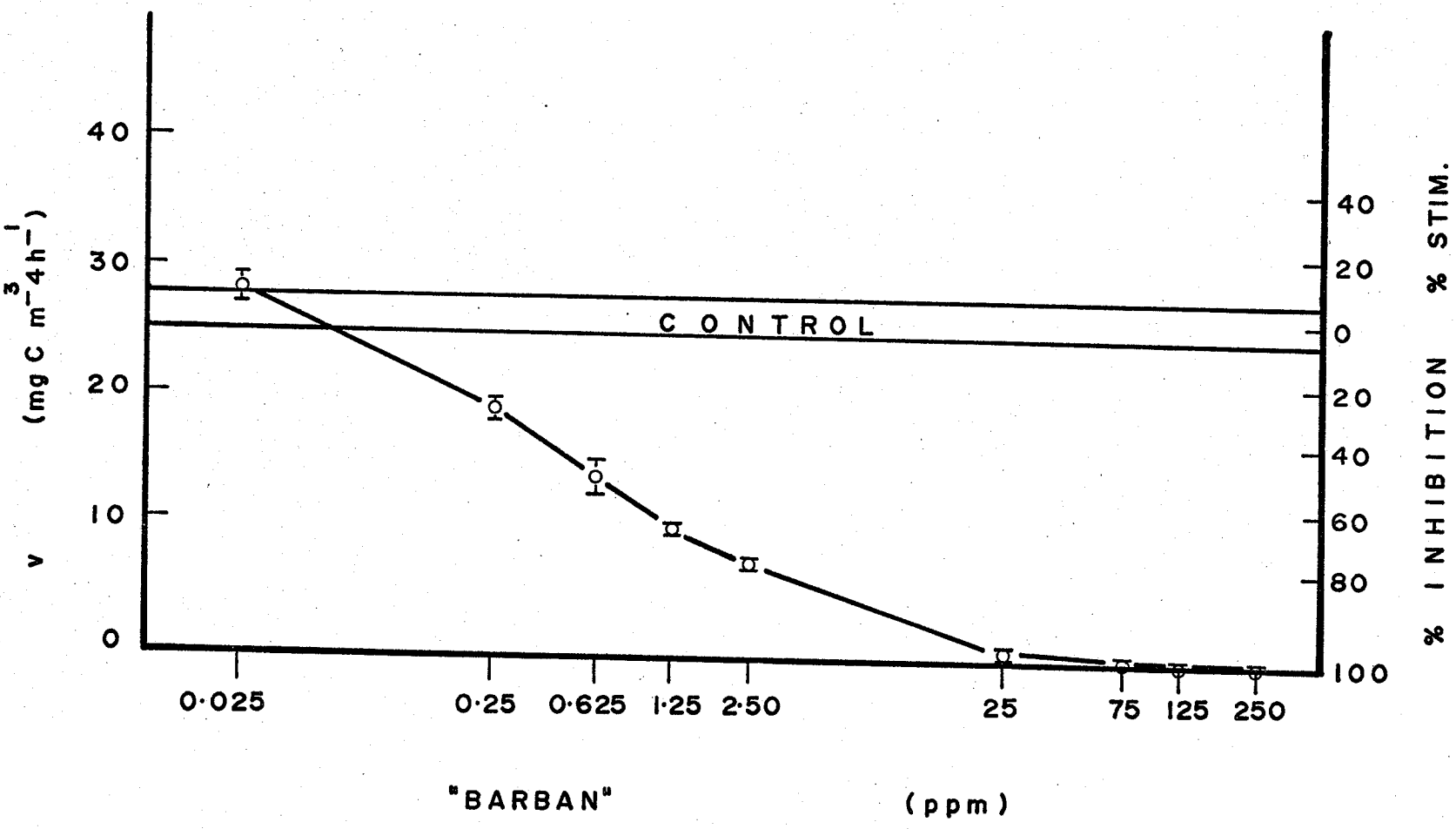

Fig. 49. The effects of increasing concentrations of "BARBAN" upon planktonic heterotrophy in the dark.

EPTC concentrations of 1.25 ppm and less, had no apparent effect upon planktonic heterotrophy, whereas concentrations of 2.50-250 ppm significantly reduced heterotrophic assimilation (Figs. 50 and 51). Organic carbon uptake of samples in the light, was reduced by 28-96%, whereas heterotrophic assimilation by samples in the dark was reduced by 29-98%.

"Triallate" was also detrimental to planktonic heterotrophy. At concentrations as low as 1.25 and 0.250 ppm for light and darkened samples, respectively uptake of ^{14}C-glucose was reduced 21% for light samples and 10% for dark samples (Figs. 52 and 53). 250 ppm, "Triallate", totally inhibited organic carbon uptake.

7. Bipyridyls

Organic carbon uptake of light samples was reduced significantly by all concentrations of "Paraquat" (Fig. 54). Heterotrophic assimilation was reduced by 27-96% at concentrations of 0.025-250 ppm. "Paraquat" concentrations of 0.025-0.625 ppm affected a 5-38% reduction of heterotrophic assimilation of ^{14}C-glucose in darkened samples, whereas 1.25-250 ppm affected a 27-94% reduction (Fig. 55).

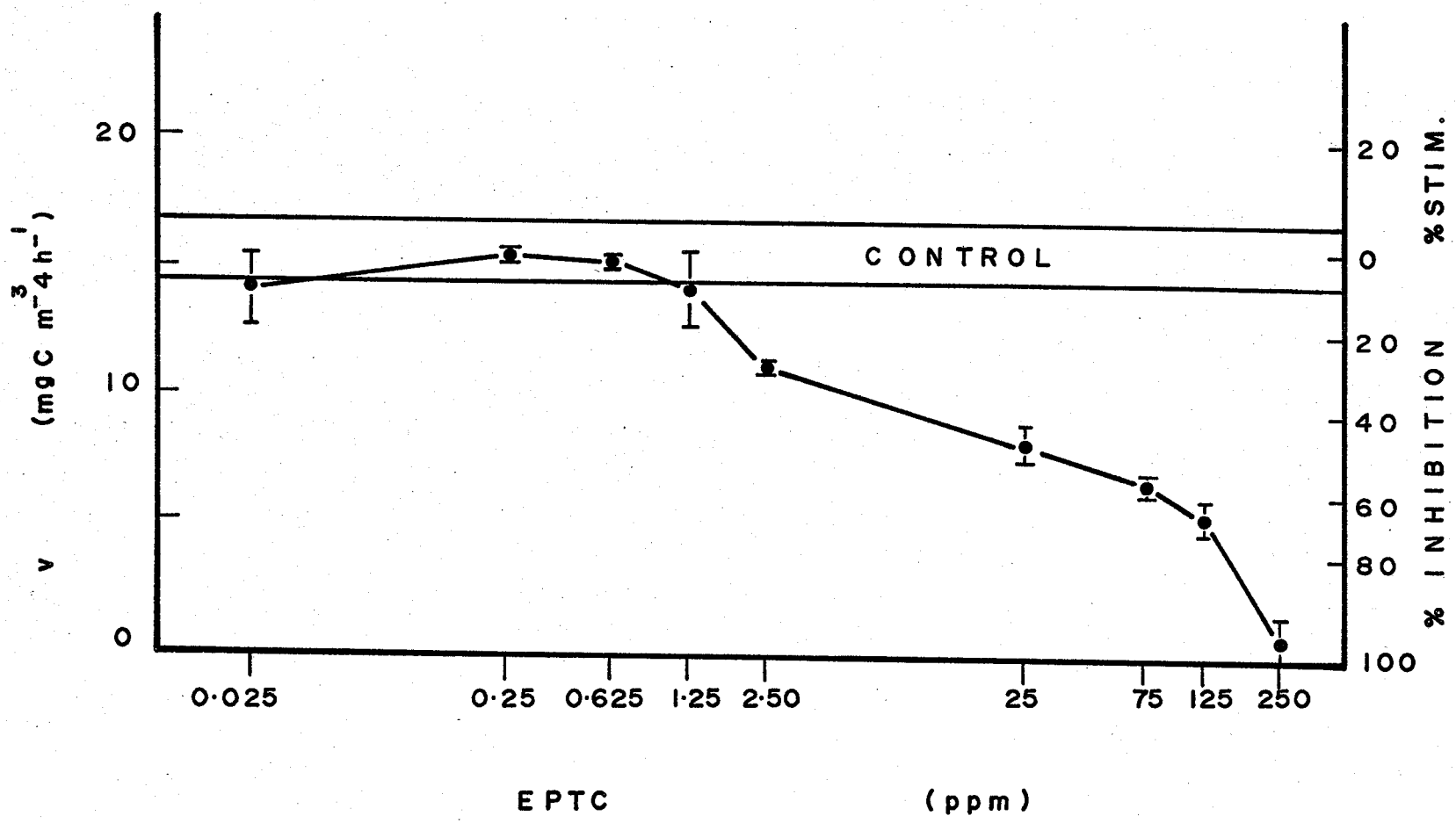

Fig. 50. The effects of increasing concentrations of EPTC upon planktonic heterotrophy in the light.

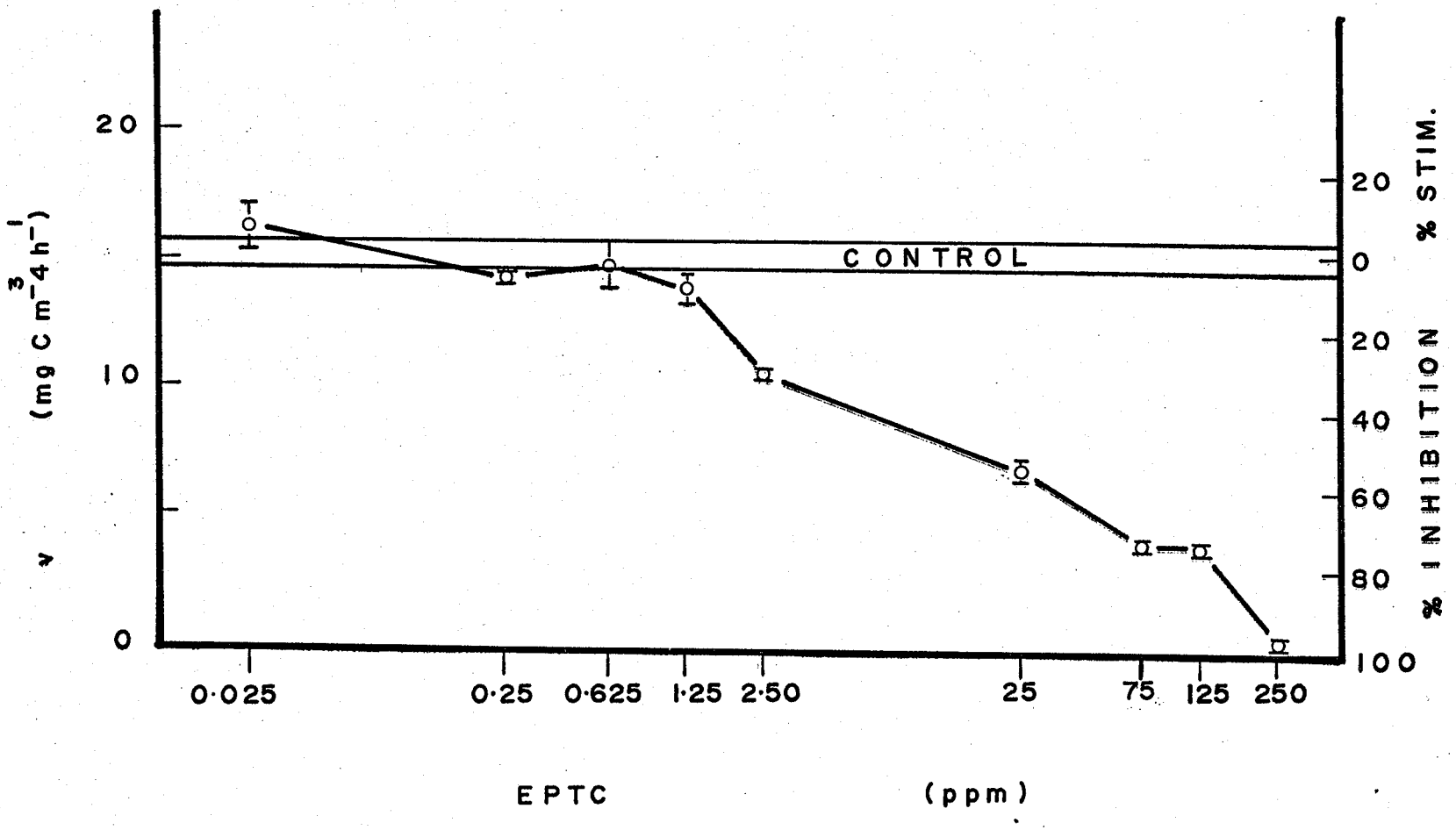

Fig. 51. The effects of increasing concentrations of EPTC upon planktonic heterotrophy in the dark.

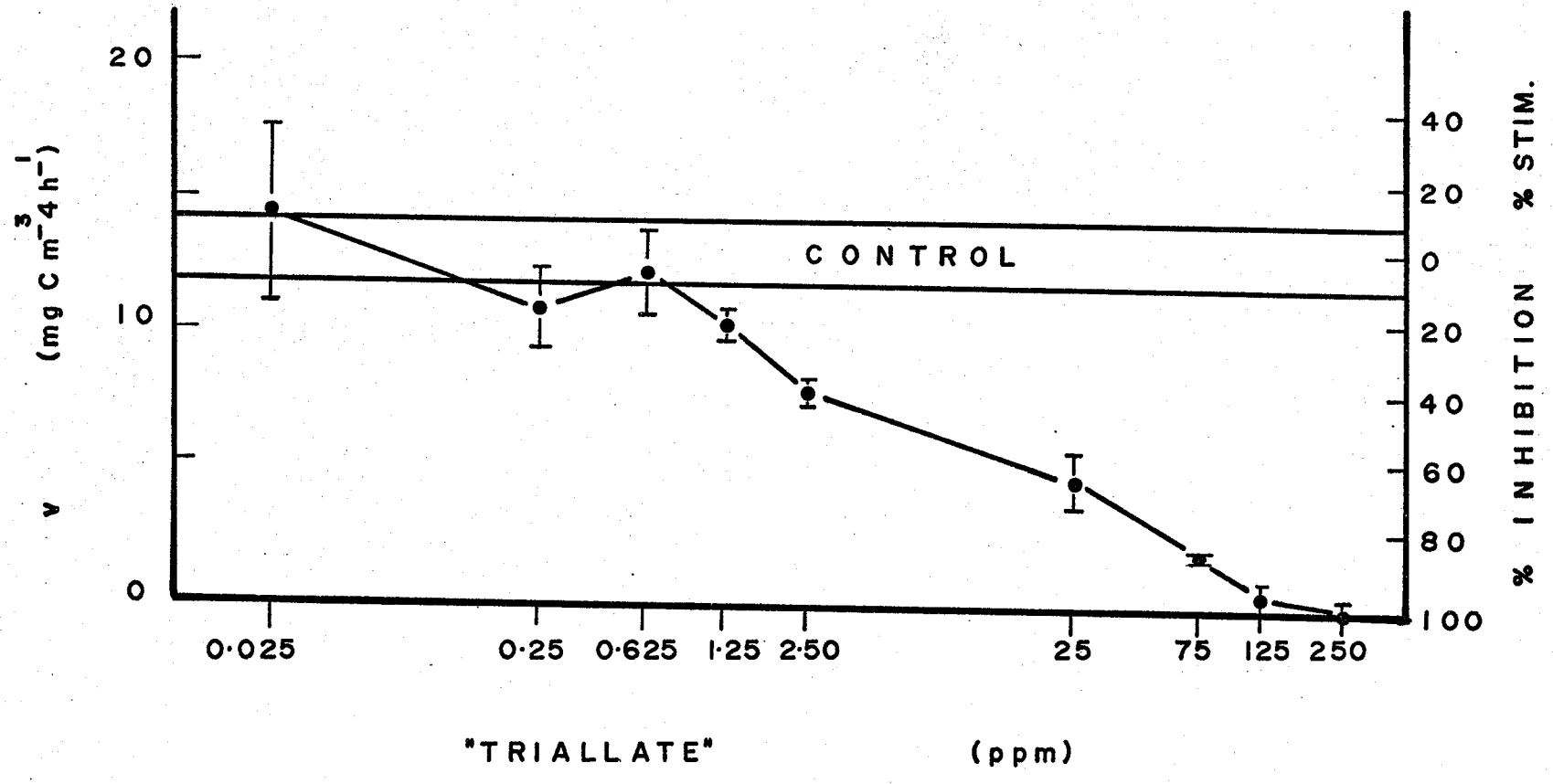

Fig. 52. The effects of increasing concentrations of "TRIALLATE" upon planktonic heterotrophy in the light.

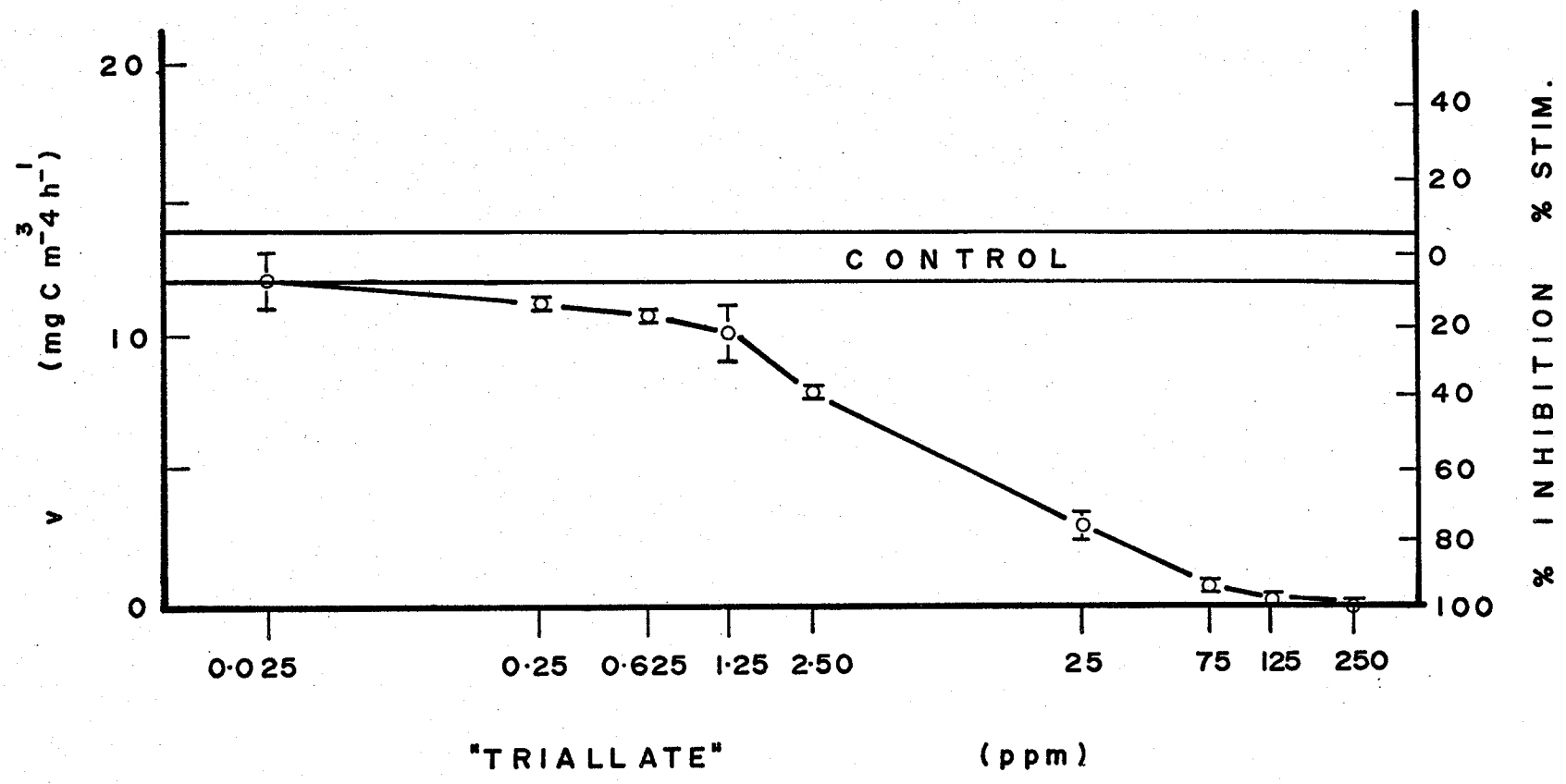

Fig. 53. The effects of increasing concentrations of "TRIALLATE upon planktonic heterotrophy in the dark.

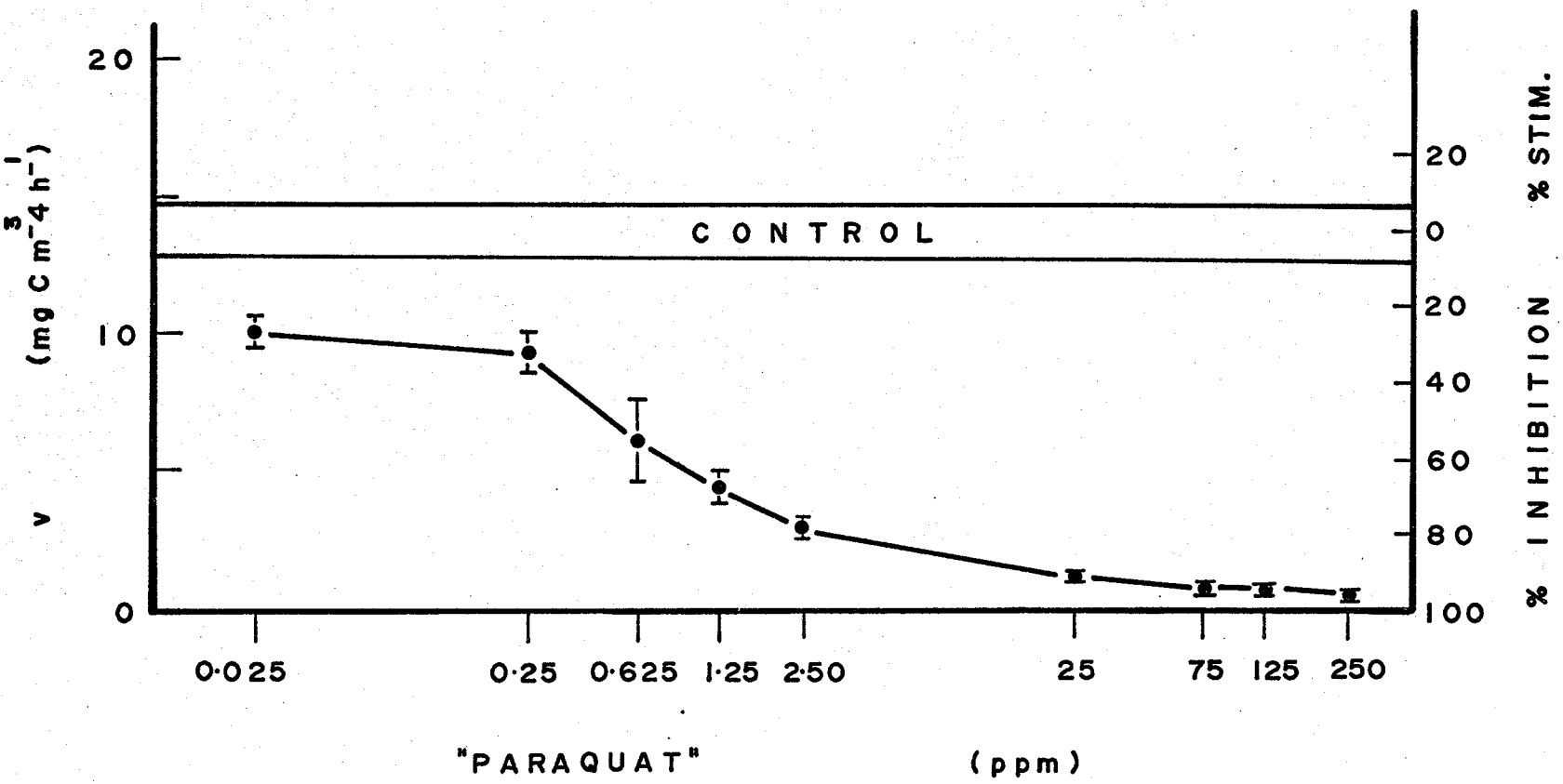

Fig. 54. The effects of increasing concentrations of "PARAQUAT" upon planktonic heterotrophy in the light.

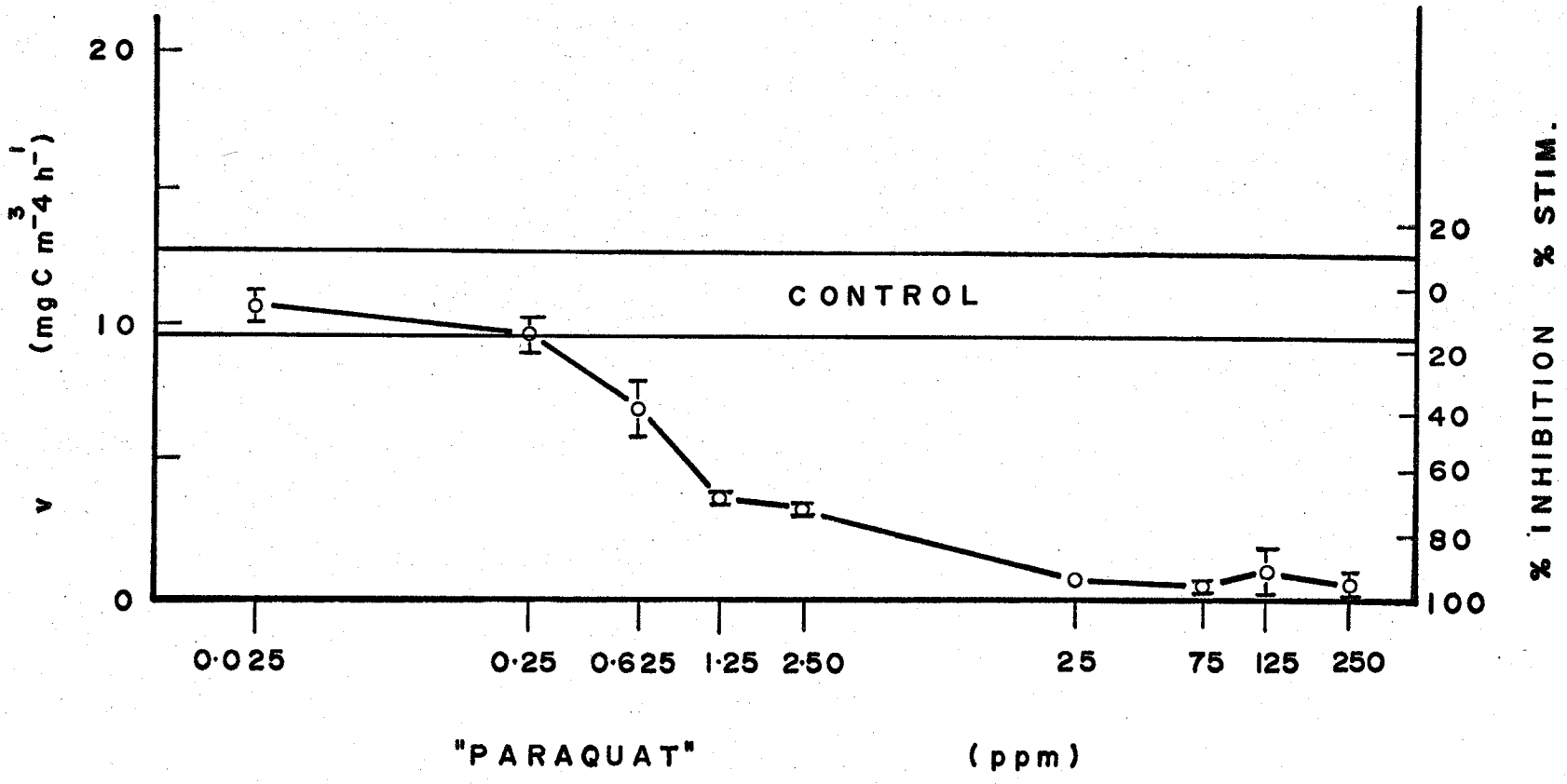

Fig. 55. The effects of increasing concentrations of "PARAQUAT" upon planktonic heterotrophy in the dark.

8. Copper Sulphate

Similar patterns of inhibition of ^{14}C-glucose uptake were noted for both light and darkened plankton samples (Figs. 56 and 57). Copper sulphate concentrations as low as 0.250 ppm, reduced light and dark heterotrophy by 75%. Maximum inhibition of heterotrophic assimilation of ^{14}C-glucose occurred at a concentration of 2.50 ppm of copper sulphate where a reduction of 96 and 97% for light and darkened samples, respectively occurred. A general increase in organic carbon uptake values was noted with planktonic samples treated with 25.0-250 ppm of copper sulphate. At 250 ppm, heterotrophic assimilation was increased 6% and 22% above the mean control value for light and darkened samples respectively.

The effects of the herbicides upon heterotrophic assimilation of ^{14}C-glucose by natural phytoplankton and bacterioplankton samples, are summarized in Tables 8 and 9. At low levels (0.025-2.50 ppm) the herbicide most effective at reducing heterotrophic activity was copper sulphate, followed by "Paraquat" and "Barban", "Triallate", and EPTC. With higher concentrations (2.50-250 ppm) "Barban" was the most effective herbicide followed by "Paraquat","Triallate", EPTC and "Linuron". Copper sulphate was excluded from this

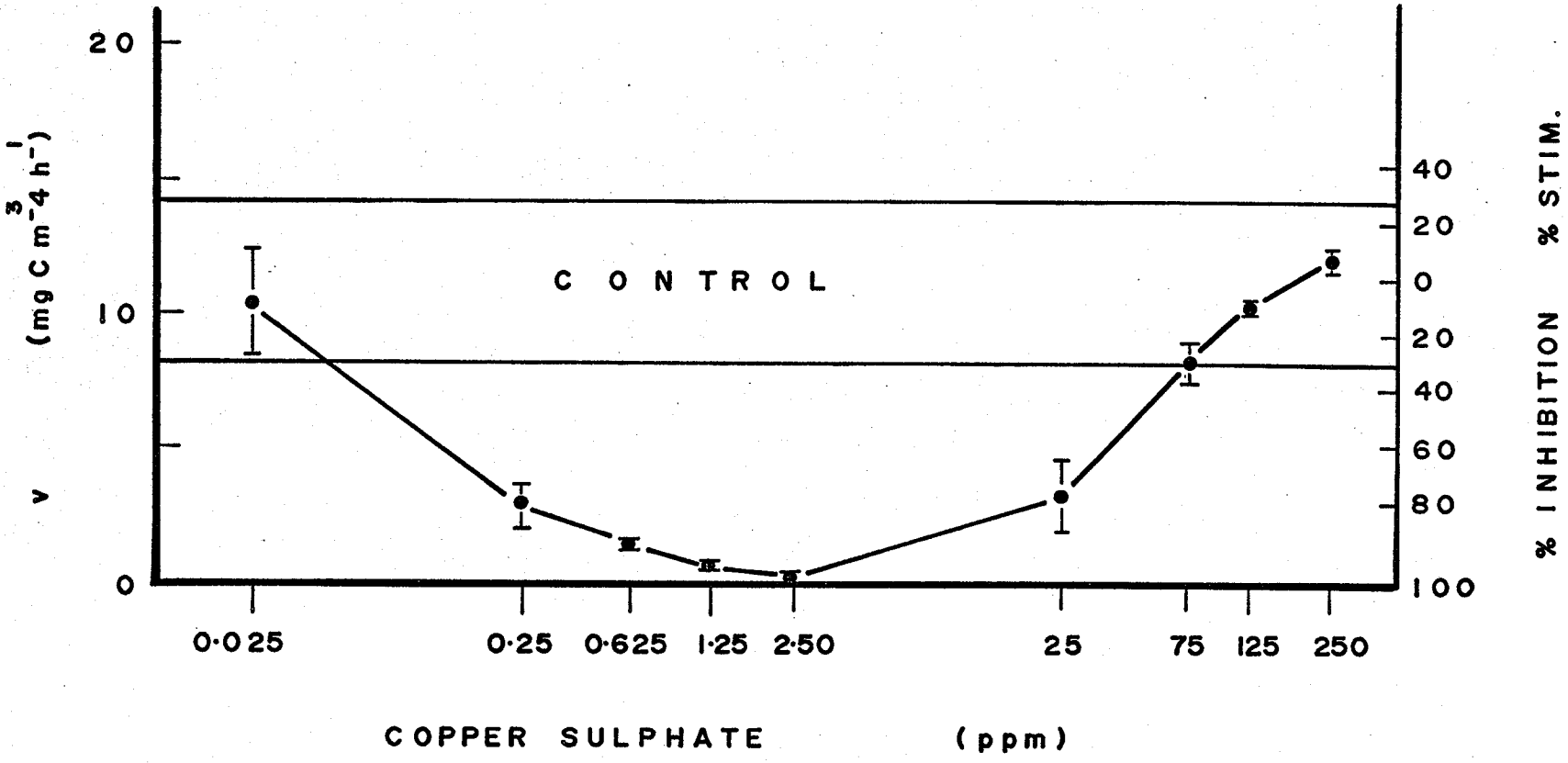

Fig. 56. The effects of increasing concentrations of COPPER SULPHATE upon planktonic heterotrophy in the light.

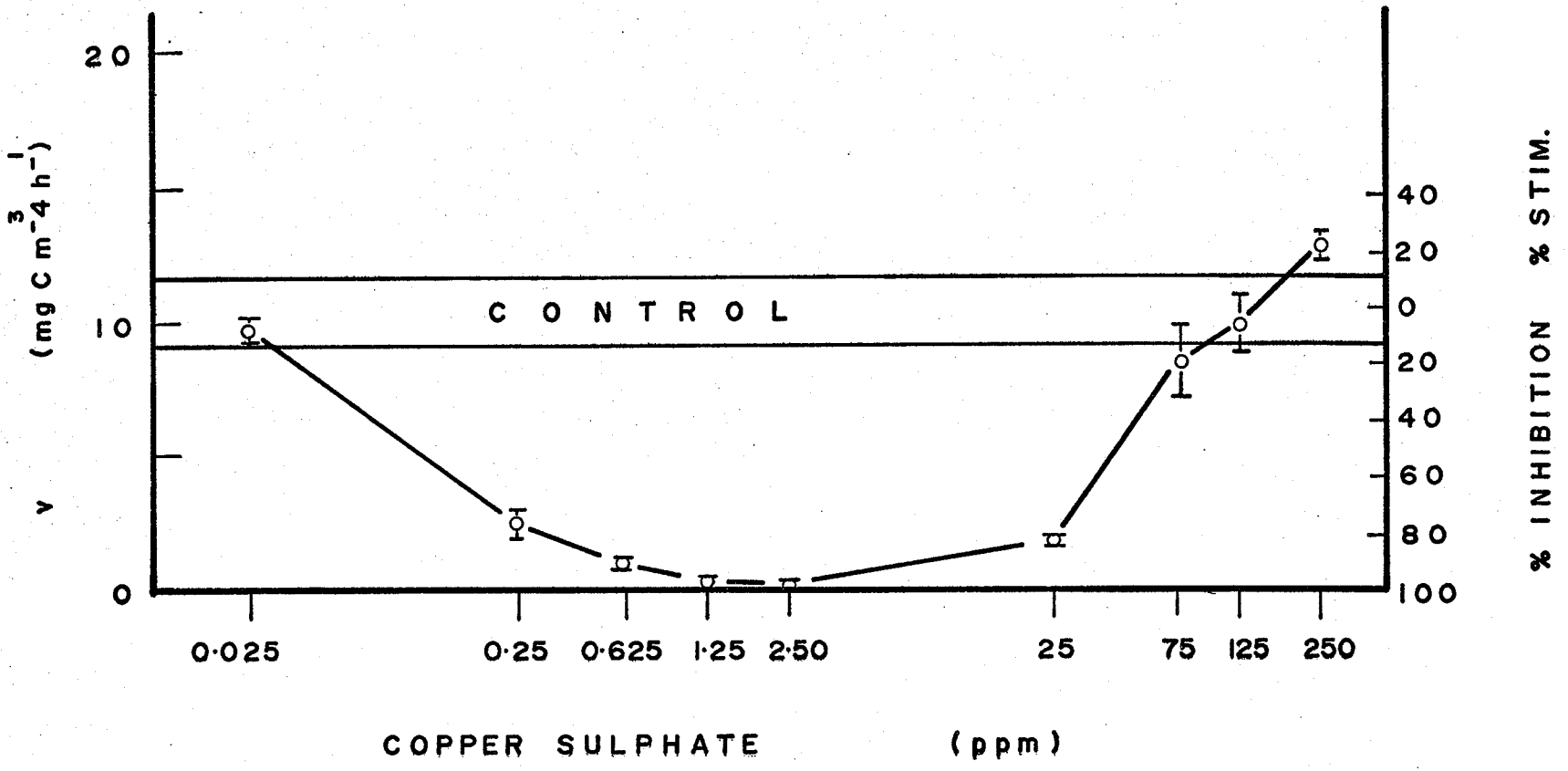

Fig. 57. The effects of increasing concentrations of COPPER SULPHATE upon planktonic heterotrophy in the dark.

Table 8. The effect of a number of herbicides upon planktonic heterotrophic assimilation of ^{14}C-glucose in the light. Results expressed as % Stimulation (+) and % Inhibition (-).

Sample	Conc. (ppm)	Phenoxy-acetic Acids		Benzoic Acids		Aliphalic Acids	"N" Heterocyclics "S" Triazines			Substituted Ureas	Carbamates Aryl-	Carbamates Thio-		Bipyridyls	Inorganic
		2,4-D	MCPA	Amiben	TCA	Dalapon	Simazine	Atrazine	Am-T	Linuron	Barban	EPTC	Triallate	Paraquat	$CuSO_4$
1	0.025	-4	-10	+3	+6	-12	+5	+1	+3	+5	+17	-9	+9	-27	-9
2	0.250	-10	+2	+1	-1	-8	+3	-7	+5	+4	-11	-1	-16	-32	-75
3	0.625	+1	-12	+5	-10	-3	+1	-5	+1	-3	-26	-3	-5	-54	-87
4	1.25	+2	+16	+1	+10	-16	-1	-10	-4	-3	-38	-10	-21	-67	-94
5	2.50	-11	-3	-5	-1	-11	-13	-11	0	+5	-51	-28	-40	-78	-96
6	25.0	-8	-13	-2	-1	-11	-11	-12	+1	-36	-98	-47	-65	-89	-71
7	75.0	-19	-22	-7	0	-15	-13	-14	-1	-46	-99	-57	-86	-95	-29
8	125	-28	-20	-15	-6	-9	-22	-14	-15	-47	-99	-66	-96	-94	-8
9	250	-42	-34	-15	-1	-34	-18	-20	-10	-63	-99	-96	-100	-96	+6

Table 9. The effect of a number of herbicides upon planktonic heterotrophic assimilation of ^{14}C-glucose in the dark. Results expressed as % Stimulation (+) and % Inhibition (-).

Sample	Conc. (ppm)	Phenoxy-acetic Acids		Benzoic Acids	Aliphalic Acids		"N" Heterocyclics "S" Triazines			Substituted Ureas	Carbamates Aryl-	Carbamates Thio-		Bipyridyls	Inorganic
		2,4-D	MCPA	Amiben	TCA	Dalapon	Simazine	Atrazine	Am-T Azole	Linuron	Barban	EPTC	Triallate	Paraquat	$CuSO_4$
1	0.025	+1	-10	+21	+10	+12	-15	+14	-2	-8	+8	+8	-5	-5	-6
2	0.250	+13	-4	+17	-3	+6	+2	+23	0	-4	-27	-6	-10	-14	-75
3	0.625	+1	-2	+18	0	+10	+5	+10	+3	-1	-47	-1	-16	-38	-89
4	1.25	+2	-2	+18	-4	-6	-12	-1	-6	-5	-62	-8	-20	-67	-96
5	2.50	0	-7	-6	-5	-8	-15	+11	-13	-27	-72	-29	-38	-70	-97
6	25.0	-5	-20	+10	0	+2	-15	+13	-6	-35	-97	-54	-75	-93	-81
7	75.0	-13	-24	+9	-13	-18	-21	+1	-2	-53	-98	-72	-92	-94	-18
8	125	-29	-45	+6	-4	-26	-9	-2	+1	-54	-99	-73	-97	-90	-6
9	250	-42	-51	+2	-8	-32	-21	-3	-13	-63	-99	-98	-100	-94	+22

ranking.

Comparisons of herbicidal concentrations, which effectively reduced planktonic heterotrophy totally (EC_{100}) or by 50% (EC_{50}) are presented in Table 10. Only one herbicide successfully reduced planktonic heterotrophy by 100%, namely "Triallate" at a concentration of 250 ppm. "Barban" reduced heterotrophic assimilation by 99% at 125 and 250 ppm whereas EPTC and "Paraquat" treated samples were reduced by 98 and 94%, respectively at a concentrations of 250 ppm.

According to the EC_{50} values, the herbicides may be ranked as follows: copper sulphate, "Paraquat", "Barban", "Triallate", EPTC and "Linuron". The other herbicides failed to reduce the planktonic heterotrophic assimilation of ^{14}C-glucose by 50%, with the range of concentrations tested.

Table 10. Herbicidal concentrations (ppm) which effectively reduced planktonic heterotrophic assimilation of ^{14}C-glucose by 50% (EC_{50}) and 100% (EC_{100}). Given concentrations are applicable to both light and dark samples.

Herbicide	EC_{50}	EC_{100}
2,4-D	> 250	> 250
MCPA	> 250	> 250
Amiben	> 250	> 250
TCA	> 250	> 250
Dalapon	> 250	> 250
Simazine	> 250	> 250
Atrazine	> 250	> 250
Amitrole-T	> 250	> 250
Linuron	25.0-250	> 250
Barban	0.625-2.50	> 250
EPTC	2.50-75.0	> 250
Triallate	2.50-25.0	250
Paraquat	0.250-1.25	> 250
Copper Sulphate	0.025-0.2500	——

DISCUSSION

In interpreting the foregoing results several points should be considered. Firstly, in an attempt to simulate real conditions, commercial grade herbicides with their associated impurities were utilized. Therefore the apparent herbicidal effects upon the algal and bacterial populations may be due, in part, to impurities in the herbicidal formulations. Secondly, it is quite possible that the species composition and relative abundance within the populations or organisms used for the assay experiments may have changed during the period of each set of experiments. Thirdly, the foregoing results may only be characteristic of a marsh environment. They may not necessarily be applicable to other aquatic ecosystems. Fourthly, for logistic reasons, only one experiment was conducted for each herbicide in each of the three series of bioassays. Although replicate samples were employed for all such experiments, the experiments themselves were not replicated. Finally these investigations did not take into account the different rates of decay and degradation which are exhibited by the herbicides. These have been investigations of the immediate effects of freshly prepared herbicides which consequently most closely simulate

effects of direct application of such components to water. Direct application may occur from purposeful application for control of aquatic vegetation, drift from agricultural spraying and accidental spillage. The above results may not always be applicable to indirect application of herbicides to water by agricultural runoff and leaching.

From the following discussion it will be apparent that there is little published information on herbicidal effects upon aquatic micro-heterotrophs. Geoghegan (1957), Ashton et al (1966) and Thomas et al (1973) have noted that the effects of herbicides upon algal growth could be partially or completely masked by the presence of glucose in the medium. Such does not, however, provide any direct indication of any possible herbicidal effects upon planktonic heterotrophy.

Since samples utilized for measurement of heterotrophic assimilation of ^{14}C-glucose were whole water samples, they contained both phytoplankton and bacterioplankton. Most of this assimilation was undoubtedly attributable to the latter population (Hobbie and Wright 1965a, 1965b; Munro and Brock 1968), but a degree of algal heterotrophy cannot be excluded.

From the results obtained in this study, only three herbicides, "Linuron", "Simazine" and "Atrazine" were capable of totally inhibiting phytoplankton photo-

synthesis. Periphyton appeared to be more sensitive to a greater number of herbicides. In addition to the three mentioned above, periphyton photosynthesis was also totally inhibited by "Barban", "Paraquat", copper sulphate and "Dalapon". With the exceptions of 2, 4-D, MCPA and "Amiben", 50% reductions of algal photosynthesis occurred with all other herbicides at one or more of the concentrations utilized.

In contrast, the herbicides most toxic to planktonic heterotrophy were copper sulphate, "Paraquat", "Barban", "Triallate", EPTC and "Linuron". These alone were capable of reducing ^{14}C-glucose assimilation by as much as 50%. 2, 4-D and MCPA were less toxic and the remaining six herbicides had either no effect at all on heterotrophy or caused a very minor inhibition. With the exception of "Barban" both light and darkened samples appeared to respond similarly to all the herbicides investigated in the experimental program.

1. Phenoxyacetic Acids

The phenoxyacetic acids were one of the least toxic groups of herbicides to algal photosynthesis. 2, 4-D failed to reduce both phytoplankton and periphyton photosynthesis by 50% whereas MCPA was successful in reducing photosynthesis of the former algal population by 50%, but

not the latter. Their effects upon planktonic heterotrophy were more pronounced, than those of other groups of herbicides. A moderate inhibition (maximum inhibitory effects of 34-51% noted at 250 ppm) of planktonic heterotrophy in both light and darkened samples was induced by 2, 4-D and MCPA.

Butler (1965a), investigating the effects of a number of pesticides upon the photosynthetic rate of estuarine phytoplankton, found no inhibition of photosynthesis at the end of a four hour period with 1 ppm of either 2, 4-D technical acid or 2, 4-D dimethylamine salt. However, other formulations did produce detrimental effects upon algal photosynthesis. 2 ethylhexyl ester; propyleneglycol butyl ether ester and butoxy ethanol ester formulations of 2, 4-D reduced phytoplankton photosynthesis by 49, 44, and 16%, respectively (Butler 1965a). In this study 1 ppm was not utilized as an experimental level, but 0.625 and 1.25 ppm of 2, 4-D dimethylamine salt reduced phytoplankton photosynthesis by 2% and 15%, respectively and stimulated periphyton photosynthesis by 15% and 28%, respectively. Walsh (1972) found total inhibition of photosynthesis of four algal species at 2, 4-D (technical acid) concentration of 85-95 ppm and inhibition of 50% at concentrations of 50-65 ppm. 2, 4-D did not reduce phytoplankton photosynthesis totally or by 50% in this study,

but concentrations of 2, 4-D dimethylamine salt similar to those used by Walsh (1972) reduced phytoplankton photosynthesis by only approximately 16-26%.

The effects of MCPA (dimethylamine salt) upon phytoplankton and periphyton photosynthesis appeared to be quite different. Inhibition of phytoplankton photosynthesis occurred at MCPA concentrations of 25.0- 250 ppm, with a 50% reduction at 75.0 ppm and a maximum inhibition of 65% at 250 ppm. Inhibition of periphyton photosynthesis did not occur within the span of concentrations tested in this study, yet all concentrations tested apparently stimulated periphyton photosynthesis. Maximum stimulation (118%) of periphyton photosynthesis was noted at a concentration of 25.0 ppm.

Since the effects of MCPA upon algal photosynthesis have not previously been investigated, comparison of these above results with those of others is not possible.

Both, 2 4-D and MCPA act as natural auxins (Klingman 1961; Crafts 1961; Hilton et al 1963; Subcommittee on Weeds 1968) and are known to affect photosynthesis, respiration, protein synthesis, enzyme systems and mineral uptake. The differences in the effects of the two algal communities may be explained by one plant process being affected more than another or possibly by a difference of sensitivities in the algal communities. The partial effect upon

planktonic heterotrophy may reflect the approximate proportion of the heterotrophic community that is sensitive to such compounds.

Neither direct nor indirect application of 2, 4-D or MCPA (dimethyl salts) would appear to pose a serious threat to algal and bacterial populations in water particularly since presumably 2, 4-D and presumably MCPA are quickly rendered inactive by photodecomposition and adsorption to soil particles (Aly and Faust 1964; Frank et al, 1970).

2. Benzoic Acids

The ammonium salt formulation of "Amiben" was one of the least toxic herbicides to algal photosynthesis and planktonic heterotrophy. This herbicide failed to reduce phytoplankton photosynthesis by 50%, and only reduced periphyton photosynthesis by 50% at the maximum concentration (250 ppm) tested in the study. "Amiben" also appeared to have no detrimental effects upon planktonic heterotrophy.

The effects of increasing concentrations of this herbicide upon the photosynthetic rate of phytoplankton and periphyton appeared dissimilar. "Amiben" concentrations of 0.250-25.0 ppm appeared to stimulate phytoplankton photosynthesis with a maximum increase of 53%

noted at 1.25 ppm whereas inhibition occurred at concentrations of 125 and 250 ppm (reductions of 14 and 30%, respectively). Periphyton, on the other hand, appeared to be more sensitive to increasing concentrations of "Amiben". 8-50% inhibitions of periphyton photosynthesis occurred at "Amiben" concentrations of 2.50-250 ppm.

Walsh (1972), on the other hand, found that only extremely high (1500-5500 ppm) concentrations of "Amiben" (ammonium salt formulation), were capable of inhibiting algal photosynthesis totally or by 50%. Much less "Amiben" technical acid or methyl ester were required for similar inhibition patterns (115-175 ppm and 1.7-5.0 ppm, respectively) (Walsh 1972). In this study phytoplankton and periphyton photosynthesis was reduced to a maximum of 30 and 50%, respectively at 250 ppm. This contradiction may be related to the different experimental procedures and assay organisms used by Walsh's (1972) investigation. It is possible that the chemical properties of the herbicide in sea water may differ from its properties in fresh water.

The specific mode of action of "Amiben" is not known (Subcommittee on Weeds 1968), but it may possess hormone-like properties (Crafts 1961). The slight toxicity to algal photosynthesis and lack of effect upon planktonic heterotrophy may be a result of such hormone-

like properties.

"Amiben" may enter water systems, as it is readily leached from soils (WSSA 1974), but as demonstrated by the results in this study, and coupled with the fact that it is subject to rapid photodecomposition in aqueous solutions (EPA 1972), it does not appear to be a threat to algae and bacteria in aquatic ecosystems.

3. Aliphatic Acids

Klingman (1961) stated that "Dalapon" was a more effective grass killer than TCA. It also appeared to be a more effective inhibitor of algal photosynthesis and planktonic heterotrophy. Inhibition of phytoplankton photosynthesis commenced at a TCA (sodium salt) concentration of 75.0 ppm whereas 25.0 ppm of "Dalapon" (sodium salt) affected inhibition.

Periphyton photosynthesis appeared to be reduced more than that of phytoplankton by equivalent concentrations of the aliphatic acids. TCA (sodium salt) inhibited periphyton photosynthesis at a concentration as low as 1.25 ppm with a maximum inhibition of 75-81% reduction in carbon uptake being noted at concentrations of 75.0-250 ppm. All levels of "Dalapon" (sodium salt) reduced periphyton photosynthesis. The values obtained for periphyton samples treated with "Dalapon" (sodium salt) were, however, in some

instances very erratic and the variability amongst replicates was extremely high.

TCA (sodium salt) had no detrimental effects upon planktonic heterotrophy; whereas "Dalapon" (sodium salt) slightly reduced planktonic assimilation of ^{14}C-glucose, but only at higher concentrations (18-34% reduction at "Dalapon" concentrations of 75.0-250 ppm).

Butler (1965a) found that 1 ppm of "Dalapon" (sodium salt) had no detrimental effects upon the photosynthetic rate of estuarine phytoplankton. From the results obtained in this study, phytoplankton photosynthesis was also not inhibited by 1 ppm of "Dalapon" (sodium salt), but rather stimulated by 16%. Butler (1965a) did not record any stimulation of phytoplankton photosynthesis. "Dalapon" (sodium salt) concentrations exhibited different effects upon periphyton photosynthesis by causing 44 and 80% reductions at concentrations of 0.625 and 1.25 ppm.

Walsh (1972), on the other hand, showed that the photosynthetic rate of four marine algal species was totally inhibited at "Dalapon" (sodium salt) concentrations exceeding 4000 ppm and by 50% at concentrations of 2250-2500 ppm. In this study total inhibition of phytoplankton photosynthesis did not occur within the span of concentrations tested, but concentrations between 25.0 and 75.0 ppm reduced photosynthesis by 50%. Periphyton,

again, appeared more sensitive to equivalent concentrations of "Dalapon". A total inhibition of periphyton photosynthesis was affected at concentrations of 125-250 ppm and a 50% reduction at 0.625-75.0 ppm. It should also be noted that Walsh (1972) found that equivalent concentrations of "Dalapon" technical acid were much more toxic to algal photosynthesis than "Dalapon" sodium salt. Total inhibition of algal photosynthesis occurred at concentrations of 35-55 ppm (technical acid) and 50% inhibition at 25-40 ppm. These values appear to be more closely related to the results of this study.

TCA (sodium salt) and "Dalapon" (sodium salt) appear to affect plants by precipitating proteins (Klingman 1961; Crafts 1961; Subcommittee on Weeds 1968). Their effects upon algal photosynthesis and planktonic heterotrophy are likely attributable to inactivation of enzyme systems. The more pronounced effects of TCA and "Dalapon" upon periphyton photosynthesis may be related to possible differences of cell wall permeabilities between phytoplankton and periphyton.

Infiltration of the aliphatic acids into water systems is a possibility as both herbicides are highly soluble in water and readily leached from soils. In the aquatic environment it appears that "Dalapon" does not threaten the existence of algal and bacterial populations

as it is rapidly degraded by hydrolysis, photodecomposition and microbial action (Smith et al 1957; Kenaga 1974). Also to substantiate this, Frank et al (1970) reported that only trace amounts of "Dalapon", less than 10 ppb, were detected from water flowing from treated areas several hours after application of the herbicide.

4. "N" Heterocyclics

The "S" triazines, "Simazine" and "Atrazine" ranked second in effectiveness at reducing phytoplankton photosynthesis and first in reducing periphyton photosynthesis, but only had a minor effect upon planktonic heterotrophy. The toxic effects of these herbicides upon algal photosynthesis might have been anticipated as the "S" triazine herbicides are direct inhibitors of the Hill Reaction (Klingman 1961; Crafts 1961; Hilton et al 1963; Zewig 1969; Büchel 1972; WSSA 1974).

An agreement exists between concentrations required to reduce the photosynthetic rate of selected chloroplasts of higher plants and that required to reduce the photosynthetic rate of algae. Exer (1958) and Moreland et al (1959) noted that the photosynthetic rate of isolated corn, spinach and barley chloroplasts was reduced by 50% at "Simazine" concentrations of 0.141-0.928 ppm.

In this study, phytoplankton photosynthesis was reduced 50% at Simazine concentrations of 0.250-0.625 ppm and periphyton photosynthesis by concentrations of 0.025-0.250 ppm. Ashton et al (1960) reported total inhibition of photosynthesis of excised bean leaves at 1 ppm "Simazine". One ppm was not utilized as an experimental level in this study, but 0.625 and 1.25 ppm reduced phytoplankton photosynthesis by 71 and 86%, respectively whereas both concentrations totally inhibited periphyton photosynthesis.

Walsh (1972) found that oxygen evolution of four algal species was reduced by 50% at "Simazine" (technical acid) concentrations of 0.6-4.0 ppm and by 100% at concentrations of 1.5-6.0 ppm. In this study $^{14}CO_2$ fixation by phytoplankton was reduced 50% at "Simazine" concentrations (actual "Simazine" levels not including the carrier) of 0.025-0.250 ppm and totally at 75.0 ppm, although at concentrations of 2.50 ppm and greater, inhibition was within one standard deviation of being total. Equivalent concentrations of "Simazine" appeared to be more detrimental to periphyton photosynthesis. A 50% reduction of periphyton photosynthesis was affected by "Simazine" concentrations of 0.025-0.250 ppm and a 100% inhibition by 0.625 ppm.

Lower concentrations of "Atrazine" appeared to be more toxic to algal photosynthesis. "Atrazine" concentrations of 0.025, 0.250, 0.625, 1.25 and 2.50 ppm were

2.2, 1.5, 1.2, 1.1 and 1.0 times as effective as "Simazine" at reducing phytoplankton photosynthesis. Exer (1958) also found that "Atrazine" was 1.8-2.4 times as effective in reducing the photosynthetic rate of isolated corn and spinach chloroplasts. Walsh (1972) noted that less "Atrazine" was required for 50% and 100% reductions of algal photosynthesis. "Atrazine" (technical acid) concentrations of 0.1-0.3 ppm and 0.2-0.7 ppm affected 50% and 100% reductions of algal photosynthesis, respectively as opposed to "Simazine" (technical acid) concentrations of 0.6-4.0 ppm and 1.5-6.0 ppm, respectively. This trend did not hold true for the periphyton data. "Simazine" appeared to be more effective than "Atrazine", but it should be noted that these results were again highly variable.

Walsh (1972) found a 50% inhibition of algal photosynthesis at "Atrazine" (80% wettable powder) concentrations of 0.2-0.6 ppm and total inhibition at 0.5-1.0 ppm. This partially agrees with the results obtained in this study. Phytoplankton and periphyton photosynthesis were inhibited by 50% at concentrations of 0.025-0.250 ppm and concentrations less than 0.025 ppm, respectively and by 100% at "Atrazine" concentrations of 75.0 ppm and 1.25 ppm, respectively. The 75.0 ppm, concentrations of "Atrazine" required for total inhibition of phytoplankton

photosynthesis is rather high and is atrributed to its low solubility in water (33 ppm at 25C). "Atrazine" concentrations of 1.25-75.0 ppm affected 98-99% reductions of photosynthesis.

The "S" triazine herbicides appeared to have only minor effects upon planktonic heterotrophy. Slight inhibitions or stimulations of heterotrophic assimilation of ^{14}C-glucose by phyto- and bacterioplankton populations were in most cases within one standard deviation of control values.

The "S" triazines, "Simazine" and "Atrazine" are widely used for control of broadleaf and grassy weeds in a variety of crops (WSSA 1974). The possibility of their indirect infiltration into aquatic ecosystems is slight as these herbicides tend to adsorb to soils with high clay and organic content and with their low water solubilities (5 and 33 ppm at 25C for "Simazine" and "Atrazine, respectively) are not easily leached. However direct application of these herbicides to water, would appear to significantly reduce algal photosynthesis and ultimately kill algal populations.

The effects of "Amitrole-T" upon algal photosynthesis, were not as severe as its related "N" heterocyclic compounds, even though all concentrations, with the exception of 0.025 ppm, reduced phytoplankton photosynthesis.

However, its effects upon light and dark planktonic heterotrophy were quite similar. The less severe effects of "Amitrole-T" upon phytoplankton photosynthesis and minor effects upon planktonic heterotrophy, may be related to its mode of action and experimental procedures. "Amitrole-T" does not affect the photosynthetic mechanism directly, but rather causes cllorosis of plant tissue (Klingman 1961). The herbicide may also affect cation exchange and purine synthesis (Hilton et al 1963). Accordingly, an incubation period of four hours may have been insufficient time to demonstrate the full effect of the herbicide.

"Amitrole-T" is utilized for control of broadleaf and grassy weeds in non-cropped areas as well as control of aquatic vegetation (WSSA 1974). The herbicide is subject to microbial breakdown in warm, moist soils and to minor photodecomposition with a resultant persistency in soils of approximately four weeks (WSSA 1974). The chemical is highly water soluble, but the literature surveyed reports nothing about its leaching properties. Once in the water "Amitrole" has been known to persist for up to a year (Subcommittee on Plant 1968).

5. Substituted Ureas

"Linuron" appeared to be the most toxic herbicide to phytoplankton photosynthesis. Although this was not

apparent, for periphyton, these results were again highly variable. Others have found that substituted urea herbicides are detrimental to algal photosynthesis. Walsh (1972) found that in experiments involving thirty herbicidal formulations, the urea and triazine herbicides were most toxic to the oxygen evolution of four algal species. The Subcommittee on Weeds (1968) stated that substituted urea herbicides were capable of inhibiting the Hill Reaction of photosynthesis at very low concentrations (a 50% reduction of photosynthesis at 10^{-7}-10^{-5}M concentrations). In this study 59-100% inhibition of phytoplankton photosynthesis was noted at equivalent concentrations of "Linuron" (100% "Linuron" based upon "Lorox" formulation) whereas a 99% stimulation to 100% inhibition of photosynthesis was noted for periphyton. Carbon uptake by estuarine phytoplankton was reduced 87, 90 and 94% when exposed to 1 ppm of the substituted urea herbicides, "Diuron", "Neburon" and "Monuron", for four hours (Butler 1965a). In this study 1 ppm of "Linuron" (100% "Linuron" based upon "Lorox" formulation) was not utilized as an experimental level, but 0.625 and 1.25 ppm reduced phytoplankton photosynthesis by 99 and 100% respectively.

The results in this study partially agree with those of Walsh (1972) and Hollister and Walsh (1973); although "Diuron" (a compound closely related to "Linuron")

was used in their study. They found that 0.010-0.024 ppm of "Diuron" was required to reduce oxygen evolution by 50% for members of the Chlorophyceae, Chrysophyceae, and Rhodophyceae and approximately 0.067 ppm for Bacillariophyceae. Total inhibition of oxygen evolution occurred at "Diuron" levels of 0.02-0.05 ppm (Walsh 1972). In this study, less than 0.025 ppm was required for 50% inhibition of phytoplankton photosynthesis whereas total inhibition occurred at concentrations of 0.625-1.25 ppm.

The erratic nature of the results obtained for the effects of increasing concentrations of "Linuron" upon periphyton photosynthesis are most likely attributable to the inherent variability amongst replicates of periphyton samples. Furthermore, samples at concentrations greater than 75.0 ppm (the solubility of "Linuron" at 25C) may have had "Linuron" particles in suspension. Binding of $^{14}CO_2$ to such particles, and insufficient fuming over concentrated acid may have yielded rather high carbon uptake values, such as exemplified at 250 ppm, where a 283% increase of photosynthesis was noted.

"Linuron" appeared to be toxic to planktonic heterotrophy, but only at relatively high concentrations. 25.0-250 ppm and 2.50-250 ppm affected inhibitions of 36-63% and 27-63%, respectively for light and darkened

planktonic samples. The solubility of "Linuron" in water is 75.0 ppm at 25C, yet the maximum herbicidal effects (63% reductions) of the herbicide occurred at 250 ppm.

Similar to the "S" triazines, herbicides of this group are also direct inhibitors of photosynthesis (Hilton et al 1963; Subcommittee on Weeds 1968; Zewig et al 1969; Büchel 1972), by inhibiting the electron transport chain in photosystem II (Büchel 1972). It is reasonable to assume that this herbicide is toxic to other energy producing electron chains within algal and bacterial cells; therefore accounting for its toxicity to planktonic heterotrophy.

The possibility of "Linuron" entering water systems by leaching is slight as it is readily adsorbed to soils of high clay and organic content and its solubility in water is low (75 ppm at 25C). Direct application or infiltration of "Linuron" into aquatic ecosystems, might however, result in substantial reductions in algal and bacterial populations.

6. Carbamates

The effects of the aryl carbamate, "Barban" upon algal photosynthesis were dissimilar to the effects exhibited by its related compounds the thiocarbamates EPTC and "Triallate".

"Barban", at most concentrations tested, had detrimental effects upon the photosynthetic rate of algae. Only "Linuron", the "S" triazines and copper sulphate were more effective at reducing algal photosynthesis. With the exception of the lowest concentration tested, 0.025 ppm, phytoplankton photosynthesis was reduced at all levels of "Barban". The maximum inhibition (92%) of photosynthesis was noted at 25.0 ppm; concentrations greater than this produced no further reductions in algal photosynthesis.

Inhibition of periphyton photosynthesis occurred at all levels of "Barban", with total inhibition of photosynthesis occurring at 25.0 ppm.

"Barban" also appeared to be one of the most toxic herbicides to planktonic heterotrophy. Only copper sulphate and "Paraquot" were more effective at reducing heterotrophic assimilation of ^{14}C-glucose. Increasing "Barban" concentrations (0.250-25.0 ppm) produced a rapid decline (11-99% and 27-99% for light and darkened samples respectively) of organic carbon uptake by planktonic heterotrophs. Uptake values for light and darkened samples were, however, dissimilar in that light uptake was approximately 25-50% greater than dark uptake for samples treated with 0.025-2.50 ppm herbicide. No explanation is offered for this difference at the present time as the phytotoxicity of "Barban" does not appear to be light dependent and both light and darkened samples were treated in the identical manner. As with phytoplankton and periphyton, "Barban"

concentrations greater than 25.0 ppm had, within one standard deviation, no further effects upon planktonic heterotrophy. This may be attributed to the solubility of the herbicide in water (11 ppm at 25C).

Investigations of the effects of Barban upon higher plants indicate that the herbicide affects protein synthesis and at higher concentrations plant photosynthesis (Klingman 1961; Subcommittee on Weeds 1968; WSSA 1974). Its effects upon algal photosynthesis and planktonic heterotrophy may be similar.

The possibility of "Barban" entering the aquatic ecosystem by indirect means is slight as it is highly adsorbed to soils and is resistant to leaching. Direct application of this herbicide to water may, however, result in significant effects upon algal and bacterial communities. EPTC concentrations of 75.0-125 ppm and "Triallate" concentrations of 2.50-25.0 ppm reduced phytoplankton photosynthesis by 50%. Butler (1965a) found that 1 ppm of EPTC had no effect upon the photosynthetic rate of estuarine phytoplankton after a four hour exposure period. One ppm was not utilized as an experimental level in this study, but 0.625 and 1.25 ppm had no detrimental effects upon phytoplankton photosynthesis; indeed a 21 and 38% stimulation of photosynthesis was noted at these concentrations.

Periphyton appeared to be more sensitive to the thiocarbamates than phytoplankton. Periphyton photosynthesis was reduced at all concentrations of EPTC and at "Triallate" concentrations of 0.625-250 ppm, but it should be noted again that the validity of the results for the effects of EPTC upon periphyton photosynthesis is somewhat doubtful. Carbon uptake values appeared to be erratic with much variability among replicate samples.

The thiocarbamates also inhibited planktonic heterotrophy, but these effects at lower concentrations, were not as great as "Barban". EPTC concentrations of 2.50-250 ppm reduced organic carbon uptake by 28-96% and 29-98%, respectively for light and darkened samples "Triallate" was slightly more effective at reducing planktonic heterotrophy. Heterotrophic assimilation in the light was reduced 10-96% at concentrations of 1.25-250 ppm and darkened samples by 6-98% at concentrations of 0.250-250 ppm.

The solubility of "Triallate" in water is 4 ppm at 25 C. The maximum effects of "Triallate" upon algal photosynthesis and planktonic heterotrophy would be expected at the concentration that approximates its water solubility. This did not appear to be the case. The maximum inhibition of phytoplankton and periphyton photosynthesis and planktonic heterotrophy was produced at the highest herbicidal levels (250 ppm). A possible explana-

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tion for this phenomenon is that "Triallate" is readily adsorbed to soil colloids (WSSA 1974) and this may have occurred in samples used for this study.

EPTC and "Triallate" inhibit cell division in higher plants (WSSA 1974); and "Triallate" also inhibits cell elongation (WSSA 1974). Considering the shortness of the experimental period in this study, it is unlikely that either cell enlargement or division were inhibited. It is more likely that some preceding metabolic event(s) was inhibited.

EPTC may infiltrate water systems by leaching as its solubility is relatively high (375 ppm at 20 C), although loss by volatilization is also a possibility. whereas this is unlikely for "Triallate" because it is readily adsorbed to soils and has a low water solubility (4 ppm at 25 C). The residual phytotoxicities of these herbicides in water do not appear to be documented, but once in the natural environment, may be toxic to both algal and bacterial populations.

7. Bipyridyls

"Paraquat" proved to be extremely toxic to algal photosynthesis and planktonic heterotrophy. Phytoplankton photosynthesis was reduced by concentrations of 0.250-250 ppm and periphyton photosynthesis by all concentrations tested. Butler (1965a) noted a 53% reduction of phyto-

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EPTC may infiltrate water systems by leaching as its solubility is relatively high (375 ppm at 20 C), although loss by volatilization is also a possibility. This is unlikely for "Triallate" because it is readily adsorbed to soils and has a low water solubility (4 ppm at 25 C). The residual phytotoxicities of these herbicides in water do not appear to be documented, but once in the natural environment, may be toxic to both algal and bacterial populations.

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"Paraquat" proved to be extremely toxic to algal photosynthesis and planktonic heterotrophy. Phytoplankton photosynthesis was reduced by concentrations of 0.250-250 ppm and periphyton photosynthesis by all concentrations tested. Butler (1964a) noted a 53% reduction of phyto-

plankton photosynthesis at a "Paraquat" concentration of 1 ppm. In this study 1 ppm was not utilized as an experimental level, but "Paraquat" concentrations of 0.625 and 1.25 ppm reduced phytoplankton photosynthesis by 18 and 28%, respectively.

The results obtained in this study appear to contradict those of Walsh (1972) and Zewig et al (1967). Walsh (1972) found that inhibition of photosynthesis of four algal species occurred at concentrations greater than 5000 ppm "Paraquat" and a 50% reduction at concentrations ranging from 2500 to greater than 5000 ppm. In this study, however, a 99% inhibition of phytoplankton photosynthesis and total inhibition of periphyton photosynthesis were attained by a herbicidal concentration of 250 ppm and concentrations of only 2.50-25.0 ppm were required for 50% reductions of phytoplankton and periphyton photosynthesis.

Zewig et al (1967) concluded that the bipyridyl herbicide, "Diquat" (a compound closely related to "Paraquat"), was nontoxic to algal photosynthesis, since no decrease in oxygen evolution of Chlorella pyrenoidosa was observed during a 60 minute exposure to 5.5 ppm of the herbicide. In this study 5.5 ppm was not utilized as an experimental level, but "Paraquat" concentrations of 2.50 ppm reduced phytoplankton and periphyton photosynthesis

by 47 and 25%, respectively. Such contradictory results may be related to the experimental procedures and differing environmental conditions. "Paraquat" is rendered biologically inactive under certain environmental conditions (Akhavein and Linscott, 1968). The work performed by Walsh(1972) and Zewig et al (1967) was upon algal cultures in synthetic media as opposed to natural algal communities in natural waters used by Butler (1965a) and the present author.

Only copper sulphate appeared to be more effective than "Paraquat" at reducing planktonic assimilation of ^{14}C-glucose. The herbicide at lower concentrations appeared to be slightly more effective on illuminated sampled. Organic carbon uptake by planktonic samples in the light was reduced significantly at all "Paraquat" concentrations whereas only concentrations of 0.625-250 ppm significantly inhibited planktonic heterotrophy in the dark.

The toxic effects of this herbicide upon algal photosynthesis and planktonic heterotrophy may be similar to effects upon higher plants. "Paraquat" is capable of undergoing reversible oxidation-reduction reactions within plants (Hilton et al, 1963; Akahavein and Linscott, 1968; Buchel, 1972). The herbicide is reduced to its respective free radical, by accepting electrons from photosystem I (Black and Meyers, 1966) which accounts for its toxicity

in the light and the dark. The free radical is also capable of being oxidized from the oxygen of photosystem II, resulting in the formation of hydrogen peroxide and oxidized "Paraquat", which may account for the quick or contact kill of plants. This herbicide may act in a similar fashion upon algal and bacterial populations. Although the photosynthetic mechanism is absent in heterotrophic bacteria, the herbicide may affect other energy producing electron transport systems, thereby inhibiting the active uptake of ^{14}C-glucose.

The possibility of indirect infiltration of "Paraquat" into aquatic ecosystems appears slight as it is readily adsorbed to soils (Akhavein and Linscott 1968). Since the herbicide is used for control of aquatic vegetation and as indicated by the above experiments, direct application of "Paraquat" to water, would have significant effects upon algal and bacterial communities. However, the toxicity of this herbicide in water is short lived. Coates et al (1964) point out that "Paraquat" in water is rapidly bound to soil particles. Frank et al (1966) could not detect "Paraquat" twelve days after its application to several ponds, although an accumulation was detected in the top inch of bottom sediments.

8. Copper Sulphate

Copper sulphate appeared to be one of the more toxic herbicides to algal photosynthesis and the most toxic herbicide to planktonic heterotrophy. Only "Linuron", "Simazine" and "Atrazine" appeared more effective at reducing phytoplankton photosynthesis; whereas in addition to the three herbicides stated above, "Barban" and "Dalapon" appeared more effective in reducing periphyton photosynthesis.

Copper sulphate was the only chemical investigated in this study that is widely recommended and utilized as an algicide. The recommended levels for control of algae (0.1-1.0 ppm (Klingman 1961)) were not utilized as experimental levels in this study, but 0.250 and 1.25 ppm reduced phytoplankton photosynthesis by 35-56% and periphyton photosynthesis by 8-22%. The maximum detrimental effects upon phytoplankton photosynthesis were noted at 2.50 ppm where a 65% reduction of photosynthesis occurred, and at 250 ppm for periphyton, where total inhibition of photosynthesis was observed. Steemann Nielsen et al (1969) have found that concentrations as low as 1 to 2 ppb of ionic copper were poisonous to algal photosynthesis, however, they also noted that the degree of toxicity of copper upon algae was dependent upon illumination, pH, form of copper and chemical constituents of the medium

or the environment.

Heterotrophic assimilation of ^{14}C-glucose in light and darkened samples was reduced 75% at concentrations of 0.250 ppm, whereas maximum inhibition (96 and 97%, respectively) of planktonic heterotrophy occurred at 2.50 ppm. High uptake values noted for samples treated with copper sulphate concentrations of 25.0 ppm and greater appear to be a result of retention of unincorporated isotope by filters and assay organisms. This may occur with insufficient rinsing of filters and filtered organisms (McMahon 1973, Robinson et al 1973).

The specific mode of action of copper sulphate is unknown, but it has been documented to affect algal photosynthesis (Steemann Nielsen et al 1969; Cendo-Malonado and Swader 1972) and cell membrane permeabilities (McBrien and Hassall 1965).

The threat of indirect infiltration of copper sulphate into the aquatic ecosystems does not appear likely as its only herbicidal use at present is in the control of unwanted algae. Direct application of the chemical into water would have significant effects upon algal and bacterial communities.

SUMMARY AND CONCLUSIONS

1. The substituted ureas and the "S" triazines appear to be the most toxic groups of herbicides to algal photosynthesis.
2. The herbicidal formulations of "Linuron", "Simazine" and "Atrazine" were the only herbicides capable of totally inhibiting phytoplankton photosynthesis.
3. 2,4-D and "Amiben" were the only herbicides that failed to inhibit phytoplankton photosynthesis by 50%.
4. Periphyton appeared to be more sensitive than phytoplankton to equivalent concentrations of the herbicides investigated in this study.
5. The herbicidal formulations of "Linuron", "Simazine", "Atrazine", "Barban", "Paraquat", copper sulphate, and "Dalapon" were capable of totally inhibiting periphyton photosynthesis.
6. 2,4-D and MCPA were the only herbicides that failed to inhibit periphyton photosynthesis by 50%.
7. Herbicides investigated in this study which are commonly used in the control of unwanted vegetation in aquatic environments, "Amitrole-T", "Paraquat" and copper sulphate, did not produce the greatest inhibitory effects to algal photosynthesis.

8. The herbicides most toxic to planktonic heterotrophy appeared to be copper sulphate, "Paraquat", "Barban", "Triallate", EPTC and "Linuron". These were the only only herbicides capable of reducing heterotrophic assimilation of ^{14}C-glucose of phyto- and bacterio-plankton populations by 50%.
9. 2,4-D and MCPA produced moderate inhibitions of planktonic heterotrophy.
10. "Amiben", TCA, "Dalapon", "Simazine", "Atrazine" and "Amitrole-T" had no effect at all on planktonic heterotrophy or caused only a very minor inhibition.
11. Two of the three herbicides investigated in this study which are commonly used in the control of unwanted vegetation in aquatic environments, "Paraquat" and copper sulphate, were the most toxic herbicides to planktonic heterotrophy.
12. With the exception of "Barban" there appears to be no significant difference between light and dark planktonic heterotrophy.

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Appendix I. Dates and types of experiments conducted during the experimental program in Delta Marsh, Manitoba. 1974.

A. Herbicidal effects upon the photosynthetic rate of phytoplankton

B. Herbicidal effects upon planktonic heterotrophy

C. Herbicidal effects upon the photosynthetic rate of periphyton

Herbicide	A	B	C
2,4-D	30 May	10 Aug.	25 Sept.
MCPA	5 June	11 Aug.	25 Sept.
Amiben	11 June	12 Aug.	26 Sept.
TCA	13 June	13 Aug.	30 Sept.
Dalapon	19 June	17 Aug.	1 Oct.
Simazine	20 June	17 Aug.	1 Oct.
Atrazine	22 June	18 Aug.	2 Oct.
Amitrole-T	23 June	18 Aug.	3 Oct.
Linuron	28 June	19 Aug.	3 Oct.
Barban	2 July	19 Aug.	8 Oct.
EPTC	11 July	5 Sept.	9 Oct.
Triallate	12 July	6 Sept.	9 Oct.
Paraquat	13 July	7 Sept.	10 Oct.
Copper Sulphate	14 Oct.	14 Oct.	13 Oct.

Saturation Experiments: 29 July; 7 Sept.

Appendix II. Preparation and standardization procedures for isotopes.

Preparation

1. Glass distilled water was filtered through a 47 mm 0.2 μ pore diameter Sartorius cellulose nitrate filter and buffered to pH 9.6.

2. The contents of a ^{14}C-sodium bicarbonate ampoule was added to the buffered distilled water.

3. The ampoule was washed out repeatedly with buffered distilled water.

4. The diluted isoltope was stirred and then dispensed in aliquots, large enough for one experiment, which were subsequently frozen until required.

^{14}C (U) D glucose

The preparation was the same as above, but the glass distilled water did not require buffering.

Standardization

1. The isotope was removed from the freezer and allowed to equilibrate to room temperature.

2. To safeguard against contamination the isotope was filtered through a 25 mm, 0.2μ Gelman cellulose acetate filter.

3. Ten 10λ aliquots of isotope were dispensed into scintillation vials containing scintillation fluor.

4. Vials were counted in a Picker Liquimat 220

liquid scintillation counter and activity determined per ml of isotope.