SEDIMENT MICROBIAL ACTIVITY IN LAKES RECEIVING ACID PRECIPITATION

by

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MANAGEMENT PERSPECTIVE

A comparison of the sediment bacterial activities as measured by oxygen consumption rates , heterotrophic bacterial plate counts and the uptake and respiration of ^{14}C-labelled glucose and glutamic acid (heterotrophic activity) was made using the sediment of a clearly acid (Clearwater Lake), slightly acidic (Turkey Lake) and a non-acidic lake (McFarlane Lake). The oxygen consumption rates and the heterotrophic bacteria population estimates were well correlated and were always higher in the non-acidic lake by nearly 20-fold than the other two lakes. The heterotrophic activity of the bacteria gave conflicting results, usually the acidic lake had higher uptake values than the non-acidic lake. Rationale for these observations is discussed and the most likely explanation is that there is an sorption interference between glutamic acid and the sediment of the non-acidic lake. Further experimentation is needed to develop an appropriate <u>in situ</u> sorption control.

PERSPECTIVE GESTION

On a comparé l'activité bactérienne des sédiments, qui était mesurée par la vitesse de consommation d'oxygène, le nombre de bactéries hétérotrophes, la respiration et l'absorption de glucose et d'acide glutamique marqués au C-14 (activité hétérotrophe), en utilisant des sédiments provenant d'un lac très acide (lac Clearwater), d'un lac légèrement acide (lac Turkey) et d'un lac non acide (lac McFarlane). Il y avait une corrélation étroite entre la vitesse de consommation d'oxygène et le nombre de bactéries hétérotrophes; ces deux paramètres étaient toujours plus élevés (par un facteur de presque 20) dans le lac non acide que dans les deux autres lacs. L'activité hétérotrophe des bactéries donnait des résultats contradictoires; le lac acide présentait habituellement des valeurs plus élevées pour l'absorption que le lac non acide. Parmi les explications possibles, la perturbation du processus de sorption intervenant entre l'acide glutamique et les sédiments du lac non acide est la plus plausible. Il faudra effectuer d'autres travaux pour mettre au point une méthode appropriée permettant d'évaluer la sorption in situ.

SEDIMENT MICROBIAL ACTIVITY IN ACIDIC AND NON-ACIDIC LAKES

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ABSTRACT

The sediments from an acidic lake, a slightly acidic lake and a non-acidic lake were studied to determine the effect of lake acidification on organic biodegradation rates and bacterial numbers. Organic biodegradation was determined by manometric respirometry and mineralization of ^{14}C-labeled compounds. All measurements were normalized to the same temperature (20°C). The oxygen consumption rate and bacterial numbers (based on spread plate counts) were greater in the non-acidic lake sediment throughout the study period. The oxygen consumption rates in the other lake sediments were 18-20 times lower and the bacterial numbers were 15 times lower than the non-acidic lake. In contrast, the results from experiments using ^{14}C-labeled glucose and glutamic acid often showed that the acidic sediments had twice the heterotrophic potential of the non-acidic sediments. ^{14}C-labeled compounds should be used cautiously when measuring the acidification effects on organic matter decomposition.

ACTIVITE MICROBIENNE DANS DES SEDIMENTS DE LACS ACIDES ET NON ACIDES

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RESUME

On a étudié les sédiments d'un lac acide, d'un lac légèrement acide et d'un lac non acide, en vue de déterminer l'effet de l'acidification des eaux lacustres sur la vitesse de biodégradation des produits organiques et sur le nombre de bactéries. On a déterminé la biodégradation des produits organiques par respirométrie manométrique et par l'étude de la minéralisation de composés marqués au C-14. Toutes les mesures ont été ramenées à la valeur correspondant à 20 °C. La vitesse de consommation d'oxygène et le nombre de bactéries (établis par numération sur plaques) étaient plus élevés dans les sédiments du lac non acide tout au cours de la période d'étude. La vitesse de consommation d'oxygène dans les sédiments provenant des autres lacs était 18-20 fois plus faible, tandis que le nombre de bactéries était 15 fois inférieur à celui dans le lac non acide. Par contre, les résultats d'expériences avec du glucose et de l'acide glutamique marqués au C-14 ont souvent montré que le potentiel hétérotrophe des sédiments des lacs acides était deux fois plus élevé que celui des sédiments du lac non acide. Il faut fairepreuve de prudence lorsqu'on utilise des composés marqués au C-14 pour mesurer les effets de l'acidification sur la décomposition de la matière organique.

INTRODUCTION

The impact of acidity on the sediment microbial activity has been studied for the past several years. Since bacterial populations are one of the major biological communities participating in nutrient regeneration and assimilation processes, it is essential to ascertain the effects of low pH on their physiology. The most prevalent activity studied has been the effects on organic matter decomposition. Anderson et al. (1978) found that it took at least several months for lake sediments to adapt to a lower pH in the water, because of the strong buffering capacity of the sediments and that only the decomposition of organics at the sediment-water interface would be retarded by the low pH. Schindler et al. (1980) found that after experimental lake acidification, there was no evidence of reduced decomposition. Gahnstrom et al. (1980) observed that glucose turnover rates and sediment oxygen uptake rates were only slightly affected by acidification. However, others have shown there is a substantial difference in organic matter decomposition rates (Grahn et al. 1974; Hendrey et al. 1976; Baker et al. 1982, 1983; Phelps and Zeikus 1984; Kelley et al. 1984). Kelly et al. (1984) demonstrated that decomposition of organic matter that had been in the sediments for several months was unaffected by lower pH values (even pH 4.0), but the decomposition rates of newly sedimented material began to decrease at pH 5.2. Total planktonic bacterial populations in acidified lakes do not change appreciably from non-acidified lakes (Boylen et al. 1983; Rao et al. 1984) and are even more abundant in naturally acidic humic lakes (Traaen 1980). In some acid and non-acid lake comparisons, the acidic lake had a lower total cell count (Rao and Dutka 1983). However, heterotrophic bacteria populations and their activities are definitely lower in acidified lakes (Rao et al. 1984a, 1984b).

The mineralization of ^{14}C-labeled compounds has been used to compare the relative organic decomposition rates of heterotrophic microorganisms in acid and non-acid sediments (Baker et al. 1983, 1984) and lake water (Ferroni et al. 1983; Leduc and Ferroni 1984; Ferroni and Leduc 1984). Baker et al. (1983, 1984) and Ferroni et al. (1983) found the "heterotrophic activity method" very useful in simulated acid rain studies. In contrast, the same authors found the method to be unreliable in extremely acidic lakes (Leduc and Ferroni 1984) as well as in less acidic lakes (Ferroni and Leduc 1984).

We report here a comparison of the "heterotrophic activity method" (both mineralization and assimilation), heterotrophic bacterial populations, and oxygen consumption in acidic and non-acidic sediments.

MATERIALS AND METHODS

STUDY SITES AND SAMPLING PROCEDURES

Water and sediment cores were collected from three Shield lakes in northern Ontario. McFarlane Lake (pH 6.8) and Clearwater Lake (pH 4.5) are located within a radius of 30 km from the smelters in Sudbury. McFarlane Lake has a relatively small watershed compared to the 419 ha of Clearwater Lake (OME 1982). The surface deposit

geology around McFarlane Lake has calcareous clay content whereas Clearwater Lake is in a bedrock basin (Art Roy, Water Resources Branch, Ontario Ministry of the Environment, personnel communication). Turkey Lake (pH 6.2) is situated in Norberg and Wishart townships, 50 km east of Coppermine Point on Lake Superior. Geology of the Turkey Lakes Watershed can be found in Colwell and Wickware (1983). Water samples were collected two metres from the bottom in sterile containers, packed in ice, transported to the laboratory, and analysed within 48 hours. This procedure did not affect the bacterial population level (Rao and Dutka 1983). Sediment cores (50 X 6 cm) were collected by means of a light weight coring device (Williams and Pashley 1979). Only cores obtained with intact and undeformed sediment-water interfaces were sectioned and processed. These cores were sectioned at 1-2 cm intervals, and the sections collected in sterile plastic bags and then transported to the laboratory. The pH measurements were made using a portable pH meter with the electrode inserted directly into the sediment (Rao et al. 1984).

HETEROTROPHIC ACTIVITY

Heterotrophic activity measurements were made using modifications of Harrison et al. (1971). Sediment (0.5 ml) was suspended with 10 ml of filter (0.2 µm) sterilised lake water. Various aliquots of a 18.5 kBq/ml solution of uniformly labelled ^{14}C-glucose or ^{14}C-glutamic acid (Amersham, specific activity 10 and 10.4 GBq/mmol, respectively) were added to combusted (450 °C for 2 hours) glass serum bottles. All concentrations were run in duplicate. Five ml of filter sterilized lake water was

then added. At zero time, 0.5 ml of the diluted sediment slurry was added to each bottle. Appropriate killed-controls consisted of adding 100 µl of formaldehyde before the addition of sediment. The respiration cup assembly, with a 2.5 X 5.0 cm accordion-folded piece of filter paper (Whatman No. 1) in the cup, was used as the bottle stopper. The bottles were incubated in the dark for one hour at 20 °C. At the end of the incubation, 200 µl of 5 N H_2SO_4 was added to each bottle to stop further isotope uptake and release the $^{14}CO_2$ from the aqueous phase. ß-Phenethylamine (200 µl) was carefully added to the cup assembly. The CO_2 was allowed to evolve for 2 h, the bottles were uncapped, and the filter paper added to a scintillation vial containing 10 ml of ACS II (Amersham). The vials were counted on a liquid scintillation counter using an external standard for quench correction.

The suspended sediments were collected on 0.45 µm Sartorius membrane filters (25 mm). Distilled water (10 ml) was used to wash the residue from the bottles, rinse the filter funnels and rim the edges of the filters after the funnel had been removed. The filters were placed in glass scintillation vials, 10 ml ACS II added, and the filters were allowed to dissolve. The sediment was dispersed with a Sonic Dismembrator (Model 150, Artek Systems Corp.) at the full power setting. Five ml of water was then added to the sediment suspension, mixed vigorously, and counted as above.

OXYGEN CONSUMPTION

Oxygen consumption by the various sediment fractions from the three lakes was

measured at 20 °C using a Gilson differential respirometer. Each Warburg flask contained 3 g of sediment (wet weight), 2 ml of filter sterilized lake water, which served as diluent, and 0.2 ml of 20% KOH in the centre well for carbon dioxide absorption. A preincubation period of 15 min. was used in all experiments. No exogenous substrate was added. The Warburg flasks were shaken at a rate of 110 strokes per min. to ensure that the oxygen diffusion rate was not a limiting factor. KCN was used to stop microbial activity. By calculating the difference between total oxygen uptake obtained by the KCN poisoning, microbial oxygen uptake was determined.

HETEROTROPHIC BACTERIA

Aerobic heterotrophic bacterial populations were determined on all samples using the spread plate procedure and a low nutrient medium (Rao and Dutka 1983). This medium contained peptone, 3.0 g;K_2HPO_4, 0.2 g;$MgSO_4$, 0.05 g;$FeCl_3$, trace; agar, 20 g in one litre distilled water.

RESULTS AND DISCUSSION

OXYGEN CONSUMPTION RATES AND HETEROTROPHIC PLATE COUNTS

The oxygen consumption rate (Fig. 1) was the highest in the non-acidic McFarlane lake sediment and lowest in the sediment of the other two lakes, Clearwater and Turkey. The oxygen consumption which occurs in sediment can either reflect biological or chemical mediated processes. We have included potassium cyanide (KCN) controls to correct for oxygen consumed by abiotic processes. This procedure represents microbial oxygen uptake (Rao et al. 1984). However, we have not confirmed that the biological oxygen consumption was all bacterial. There is a possibility that the respiration of other benthic microorganisms such as protozoans may be included. If these organisms are present at the sediment-water interface, they would be more prevalent in the non-acidic lakes. Therefore, the high O_2 consumption rates at the surface Lake may not parallel bacterial activity.

The trend found in the heterotrophic bacteria plate count (Fig. 2) was almost identical to the O_2 consumption rate found in Fig. 1. Clearwater and Turkey Lake sediments had lower cell counts than McFarlane Lake sediment in the top 2 cm, not only in June, 1985 (Fig. 2) but throughout the sampling period. Bacterial plate counts are usually considered to underestimate total bacterial populations by at least three orders of magnitude. We do not know the extent they underestimate the heterotrophic population. Certainly, many researchers feel that all heterotrophs can not grow on relatively rich agar medium. In addition, the organisms which do form colonies may or may not be active in the sediment under natural conditions.

HETEROTROPHIC ACTIVITY

The heterotrophic activity method of Wright and Hobbie (1965) was used to estimate the level of heterotrophy in the sediments from the three Ontario lakes. We have assumed that all the activity was from bacterial origin since we used low levels of substrate. The limitations of the heterotophic activity procedure can be found in Wright and Burnison (1979). The most important drawback, under the conditions in which it has been used here, is that only one organic compound can be tested at a time and this does not reflect the actual flux of the entire dissolved organic carbon pool, which is a mixture of organic compounds.

Mineralization of the ^{14}C-labeled organic is usually used by researchers as an indicator of sediment heterotrophic activity (Harrison et al. 1971; Baker et al. 1982, 1983; Phelps and Zeikus 1984). In lake water, the amount respired does vary with season and probably is caused by shifts in bacterial populations (Burnison and Morita 1974). Therefore, radioactivity in the respired CO_2 and the particulate was measured in the three Ontario lake sediments. Figure 3 shows the percent respired for glucose and glutamic acid. Fifteen percent of the glucose was usually respired by the bacteria in all three sediments with slight variation over the sampling period. Glutamic acid respiration varied by sediment and season from a low of 52% in Clearwater Lake in Nov. to a high of 75% for the same lake in Jan. The average for all the sediments was around 60%, which is a common value for glutamic acid when it is used as a substrate for planktonic bacteria (Burnison and Morita 1974, Hobbie and Crawford 1969).

As examples, the kinetic plots for Turkey Lake and McFarlane Lake sediments are given in Fig. 4. On August 28, 1985 the sediment glucose uptake (V_{max}) for Turkey Lake was three times higher than in the non-acidic McFarlane Lake, whereas glut-

amic acid uptake was only half of the V_{max}. This latter result is clearly the exception when a comparison of V_{max} values is made over the entire sampling period (Fig. 5). The Turkey Lake and Clearwater Lake sediment V_{max} values for both glucose and glutamic acid are usually higher than values determined for McFarlane Lake sediment (glutamate uptake in Clearwater Lake sediments did not follow Michaelis-Menten saturation kinetics, see below). These results are contrary to the O_2 consumption rate and the bacterial heterotrophic plate count data (Figs. 1 and 2). The increased sediment glucose uptake rate in Turkey Lake may be the result of a greater population of glucose-utilizing bacteria. Turkey Lake has more vegetation in its watershed than the relatively barren surroundings of the Sudbury Lakes. In addition, the pH of the Turkey Lake surface sediment averaged about 6.5 whereas McFarlane Lake sediment was 7.3. A difference of $\pm$ 0.5 pH units around neutrality shouldn't significantly effect organic substrate uptake. We have concluded that the difference observed between these two sediments reflects the differences in bacterial species composition.

When ^{14}C-glutamic acid is used as the substrate in Clearwater Lake sediment, the data did not follow Michaelis-Menten saturation kinetics (Fig.6) and gave a plotted line parallel to the x-axis. Replotting the data according to the first-order kinetic model [concentration (µg/g) against velocity (µg/g/h)], there appears to be direct proportionality between uptake and the substrate concentration (Wright and Hobbie 1966). The "diffusion turnover time" (T_d) was calculated to be 4.8 h. This type of uptake does not become significant, in planktonic systems, until the substrate concentration is about ca. 100 µg/L (Wright and Hobbie 1966). The highest added substrate concentration in our sediments was only 16 µg/L. The T_t (y-intercept on the Cµt/c versus concentration plot) in McFarlane Lake sediment on this date was 4.3 h for glutamate. Turnover times (T_t and T_d), of course,

are directly influenced by the S_n, the *in situ* substrate concentration and the uptake velocity. Sediments were diluted 250 times with filtered lake water, so the sediment S_n was greatly lowered. However, the S_n was not directly measured during this study in any sediment or filtered lake water and concentrations certainly will vary between lakes and season. Turnover times (Fig. 7) varied 10-fold in McFarlane Lake sediments for glutamic acid and about 6-fold for glucose; the shortest T_t was in August for both compounds. With the exception of the July glutamic acid sample for Clearwater Lake sediment, the turnover times for both compounds for Clearwater and Turkey Lake sediments were under 10 hours.

The uptake velocities for both glucose and glutamic acid were calculated according to the equation from Wright and Hobbie (1966):

$$v = \frac{c}{C\mu t} \quad (S_n + A)$$

Since the sediment was diluted, the S_n was taken to be zero (there probably is a small unknown S_n in the diluent lake water). The velocities were plotted against added substrate concentration and the equation of the logarithmic curve calculated. For sediment comparison, an arbitrary substrate concentration of 50 µg/g was chosen. Figure 8 illustrates the uptake velocities (V_{50}) derived by substituting this concentration into the velocity equations for each sediment. Uptake of the two organic compounds peaked in August in McFarlane Lake sediments and in November for the two

acidic lake sediments. Usually the glucose uptake was faster than the glutamic acid uptake. Clearwater Lake sediment, however, is still the exception. The reason why glutamic acid uptake is so high in the most acidic lake is unknown. Two possibilities are: 1) the bioavailability of the ^{-14}C-labeled glutamic acid is higher in Clearwater Lake sediment than in the other two sediments and 2) there is an acidophilic bacterial population which is more efficient at taking up glutamic acid. Although the presence of particulate carbonates in the sediments of the three lakes was not measured, data on the concentration of pertinent ions in porewater is known. At a water depth of 20 m, the porewater of Clearwater Lake sediment (0.5 cm depth) has relatively lower concentrations of dissolved inorganic carbon (810 µmol/L), Ca (149 µmol/L), and PO_4-P (0.9 µmol/L) than McFarlane lake sediment (1 cm depth) which has 1760 µmol/L, DIC; 8.7 µmol/L, PO_4-P; and 464 µmol/L, Ca (Carignan and Nriagu 1985). We have assumed that the higher porewater concentrations of DIC and Ca in McFarlane Lake sediments reflect a higher particulate concentration in the sediment as compared to the Clearwater Lake sediments. The surficial soils in the Turkey Lakes Watershed are acidic (pH 4.0-4.1) and have approximately a 0.4% inorganic carbon content (Colwell and Wickware 1983). Whether the surficial sediments in Turkey Lake have particulate carbonates is not known. Amino acids are rapidly and strongly adsorbed to colloidal and particulate monocarbonates (Wetzel and Allen 1972). In addition, aspartic and glutamic acids are thought to form a monolayer on calcareous mineral surfaces (Jackson and Bischoff 1971). When organics are adsorbed to particulate surfaces the rate of uptake and respiration are significantly decreased (Gordon and Millero 1985). Wood and Chua (1977) state that adsorption to particulate material was highly significant in the sediments but not important in the water column. However, their sorption controls were fixed with an acidic mixed reagent and we feel that this is not a proper control to measure how much of the

substrate is adsorbed under in situ conditions. Dunstall and Schwarz (1983) heated their water samples to 95° C to correct for abiotic adsorption. We feel that a hot water extraction of the sediment would lead to rupture of most living cells and release of cellular contents into the sample and possibly binding to adsorption sites before the ^{14}C-labeled compound is added. Further experimentation is needed to determine an appropriate control to assess the degree of abiotic adsorption. The possibility that there is an acidophilic bacterial population can not be totally neglected. However, the nonconformity of the Clearwater Lake sediment results with glutamic acid leds to the conclusion there is a physiochemical interference in the initial setup of the incubations with the non-acidic sediment, probably because of the presence of calcareous minerals or clay.

CONCLUSIONS

Aerobic heterotrophic plate count and oxygen consumption are correlated and show decreased values in acidic lake sediments. Analyses by the "heterotrophic activity method" show that the acidic lake often had higher uptake rates than the non-acidic lake. The "heterotrophic activity method" is a sensitive procedure to measure the uptake of specific organic substrates by sediment microbes. However, the use of ^{14}C-labeled organic compounds to access microbial activity in sediments of non-acidic and acidic lakes should be done cautiously. A major "pitfall" in the methodology is the potential adsorption of the ^{14}C-substrate to the sediment. An appropriate control must be included with every series to just determine sediment

adsorption. This a particularly important when using charged compounds such as amino acids.

KEY WORDS microbial activity, acidification, heterotrophic activity, sediments, sediment oxygen uptake

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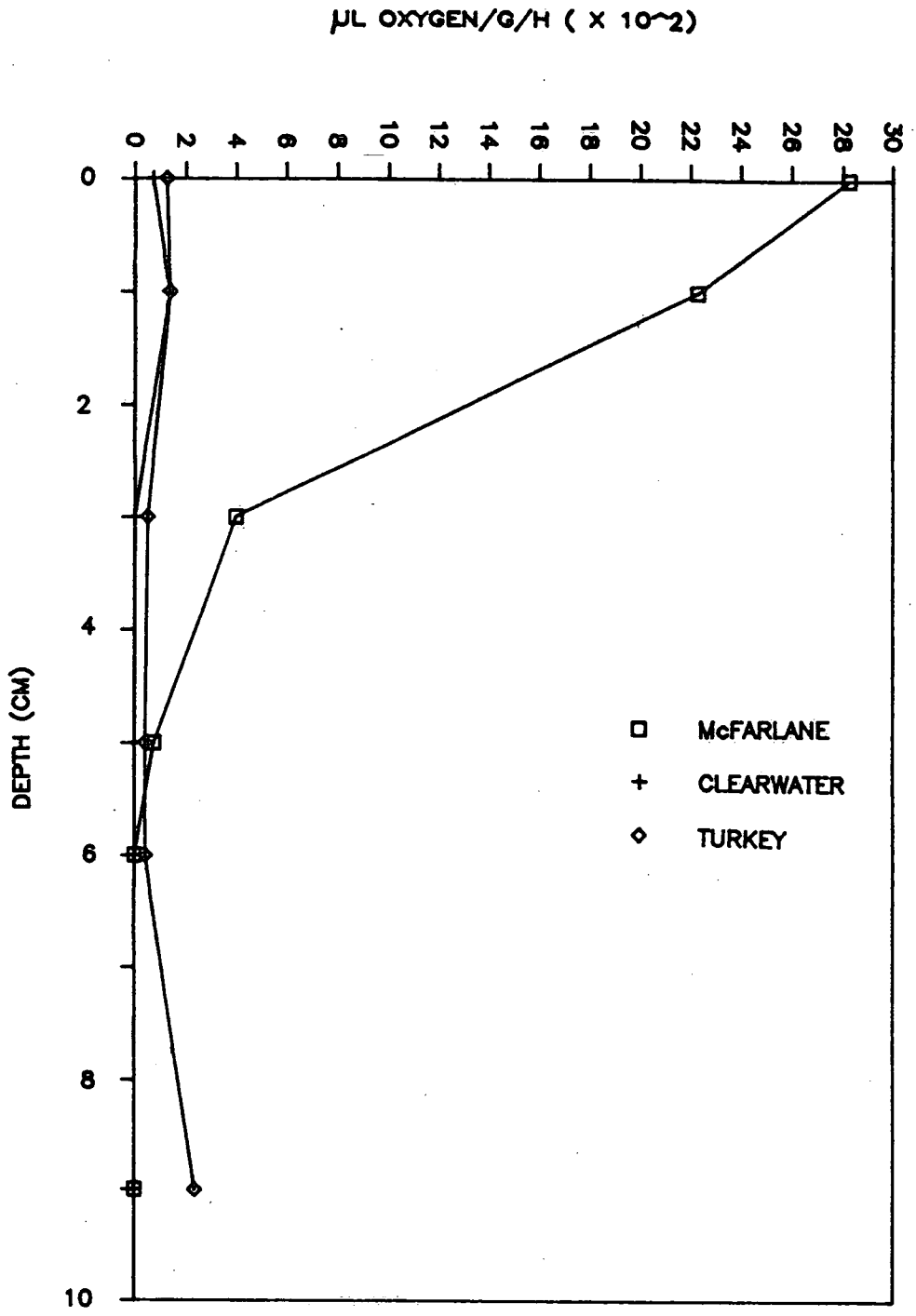


Figure 1. Typical depth profile of oxygen consumption rates in the sediments of McFarlane, Clearwater and Turkey Lakes.

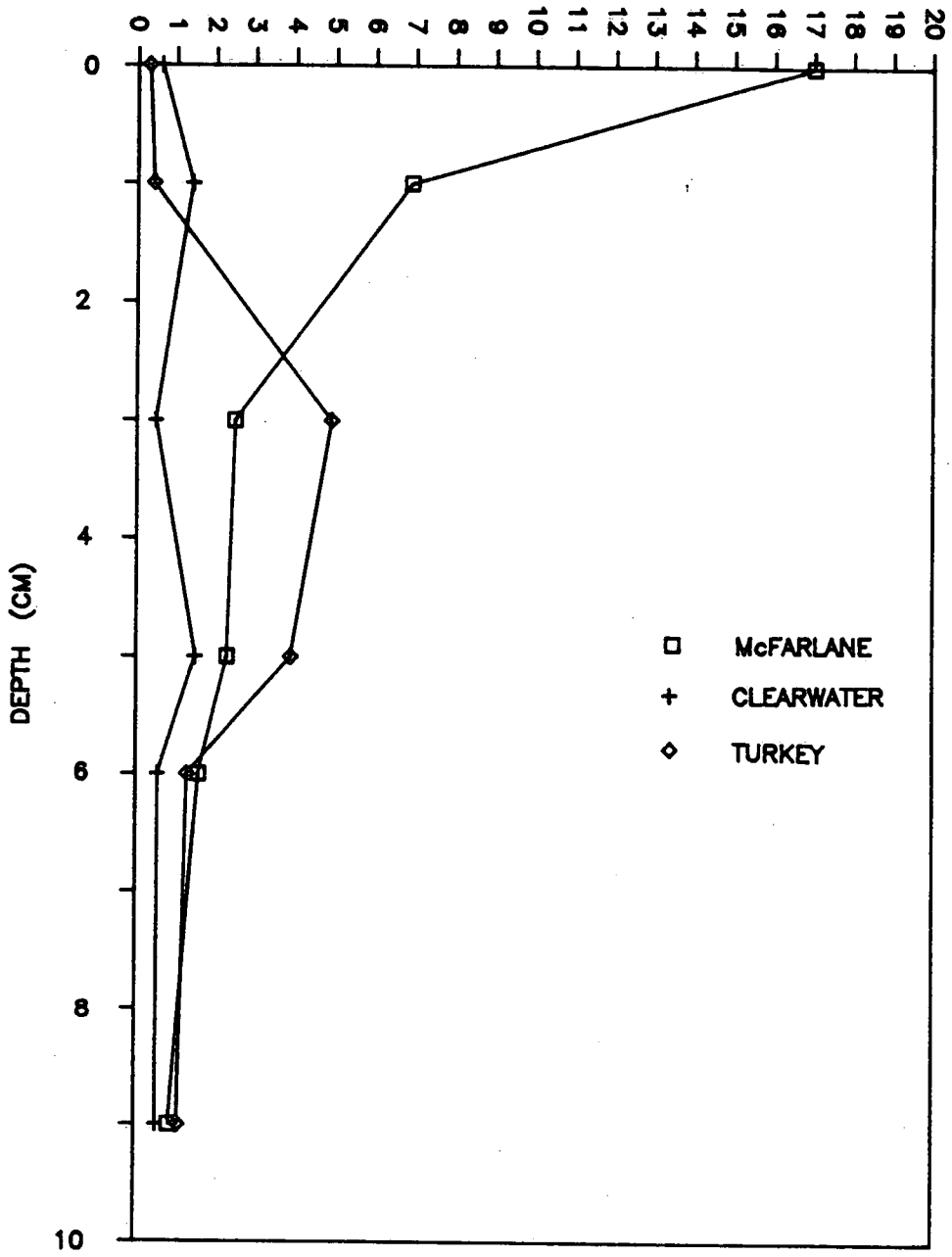


Figure 2. Typical depth profile of heterotrophic bacterial plate counts in the sediments of McFarlane, Clearwater and Turkey Lakes.

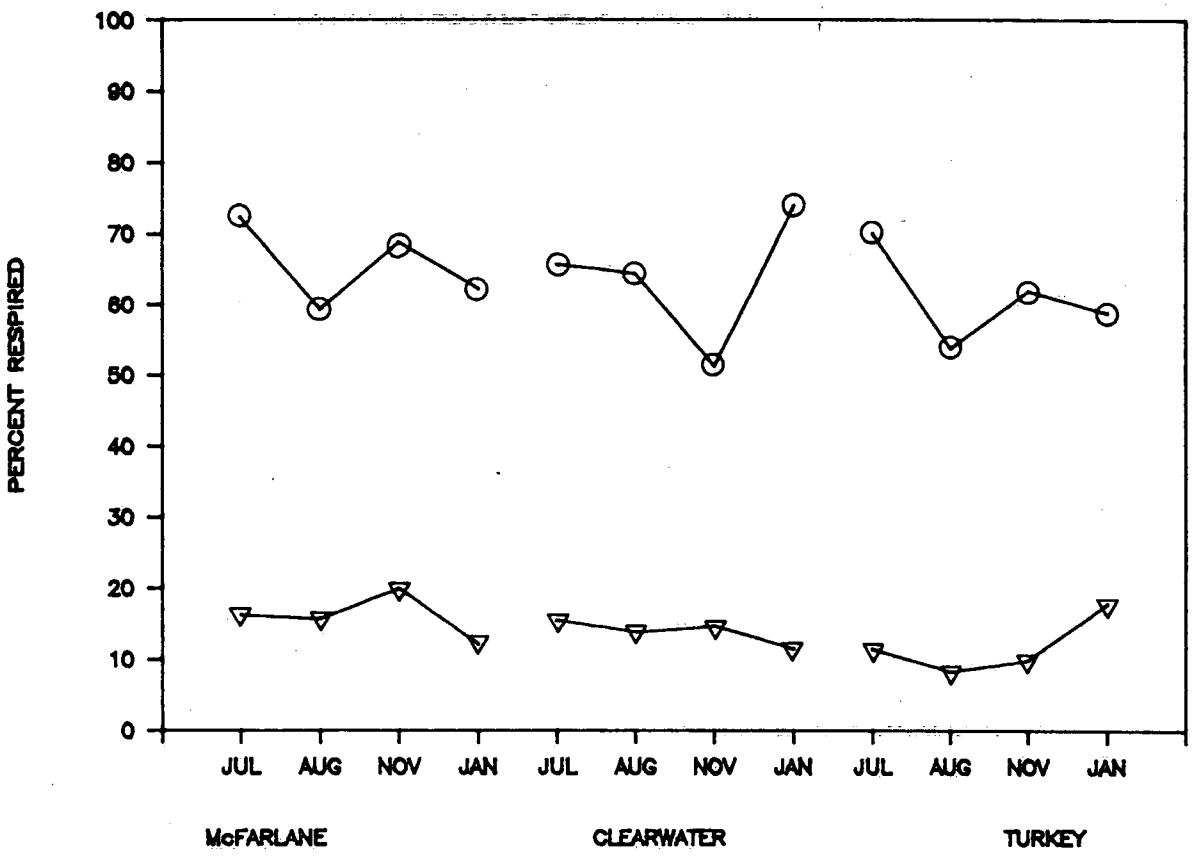
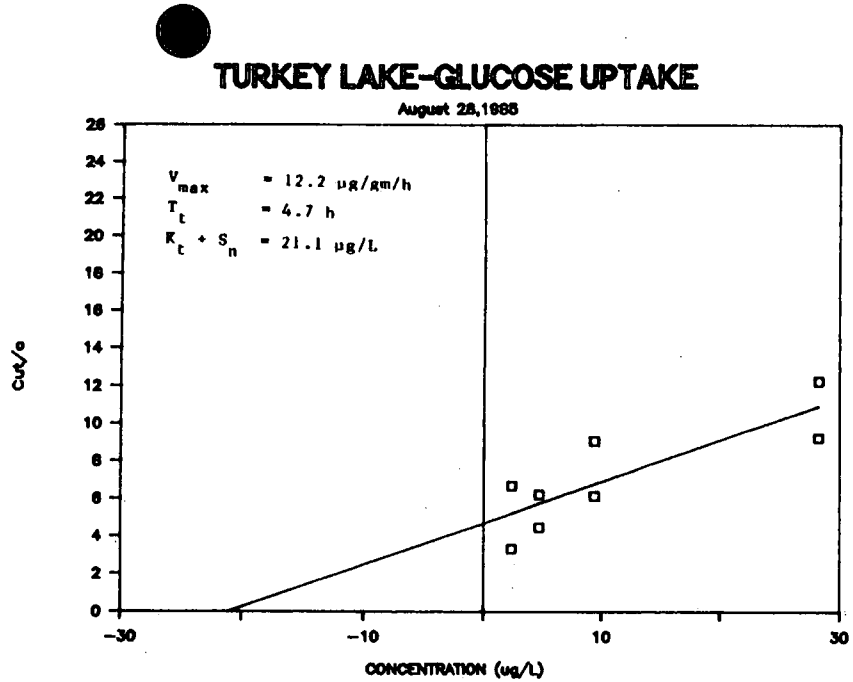
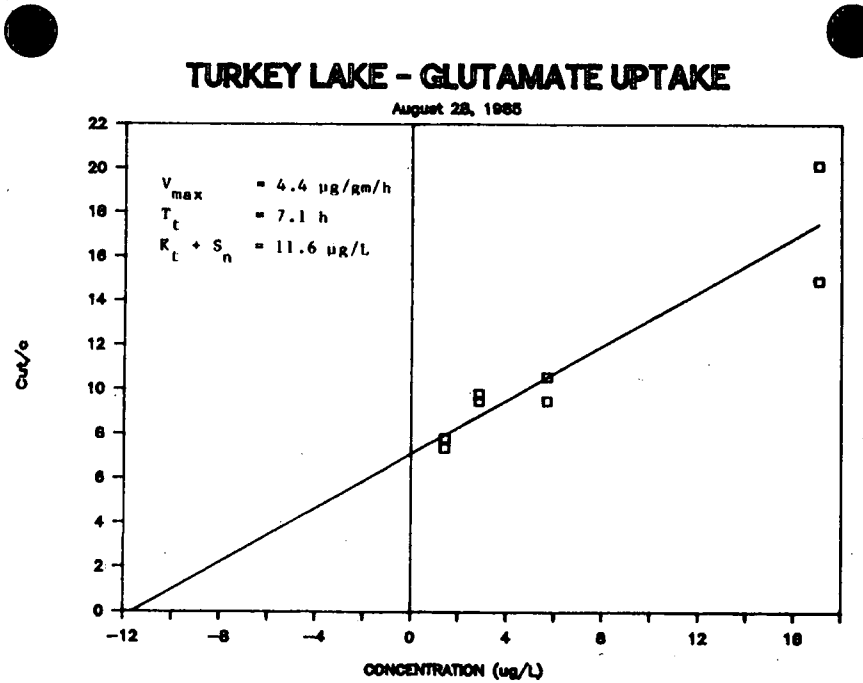
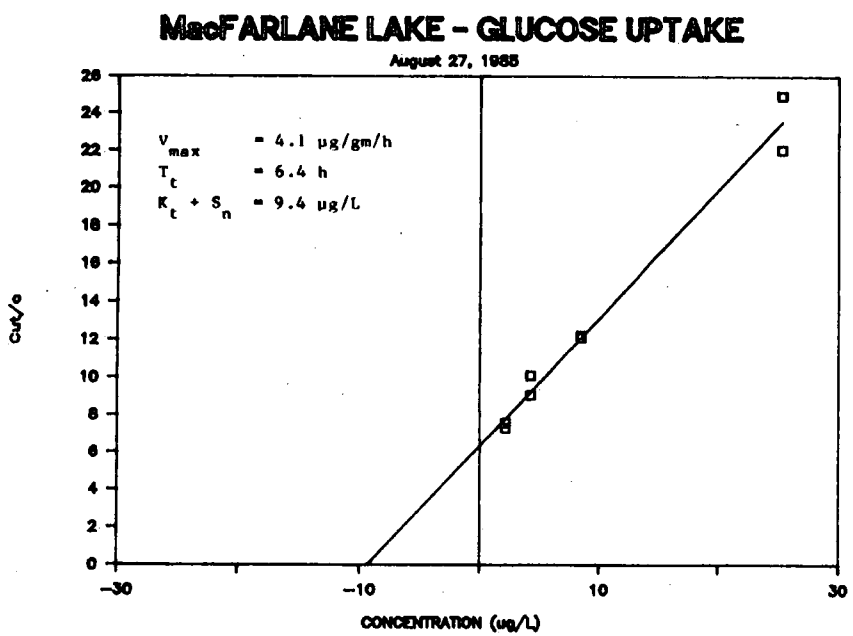


Figure 3 Percent respiration of the ^{14}C-labeled glucose (▽) and glutamic acid (○) by the sediment microorganisms in McFarlane, Clearwater, and Turkey Lakes.







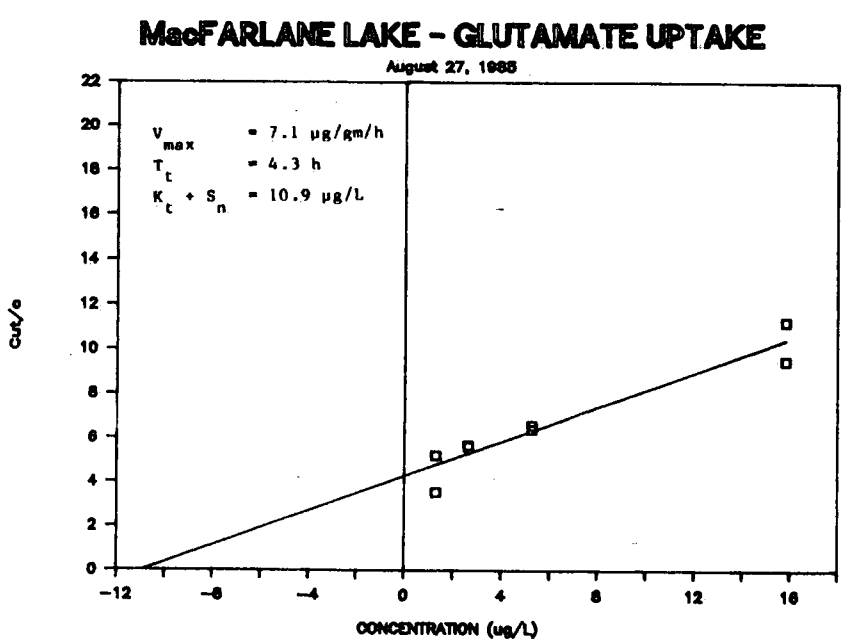


Figure 4. Modified Lineweaver-Burke plots for the uptake of ^{14}C-labelled glucose and glutamate in Turkey and McFarlane Lake sediments.

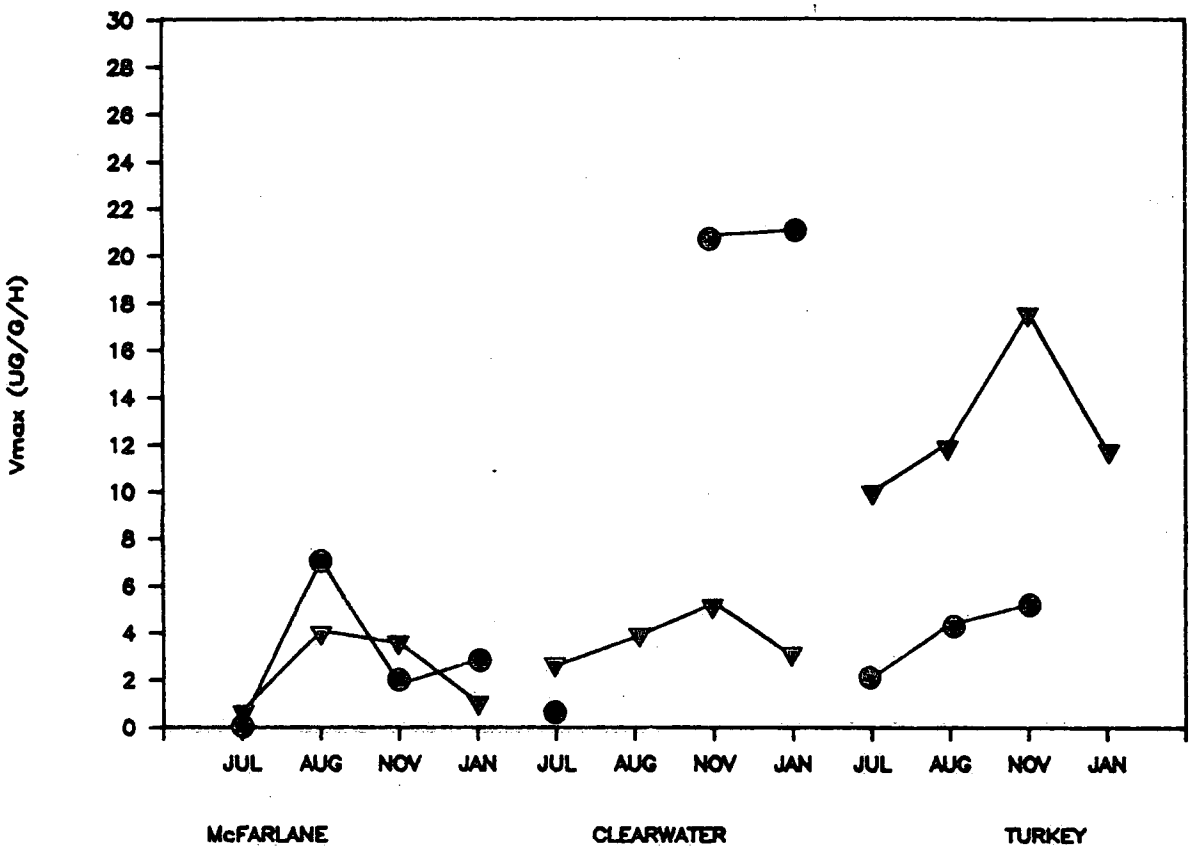


Figure 5 V_{max} values for the uptake of glucose (▼) and glutamic acid (●) on the four sampling dates.

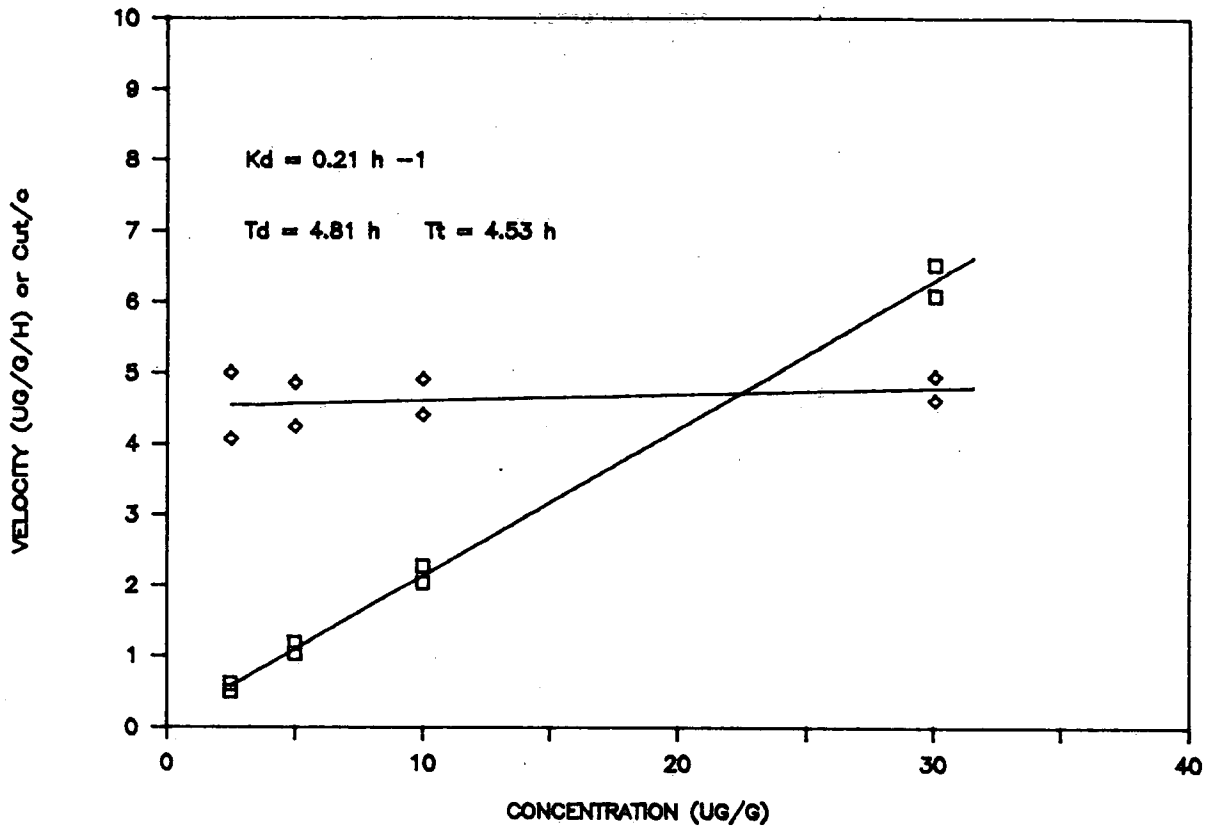


Figure 6 Kinetic analyses of the ^{14}C-glutamic acid uptake by Clearwater Lake sediment (August 28, 1985) via modified Lineweaver-Burke plot (◇) and velocity (□).

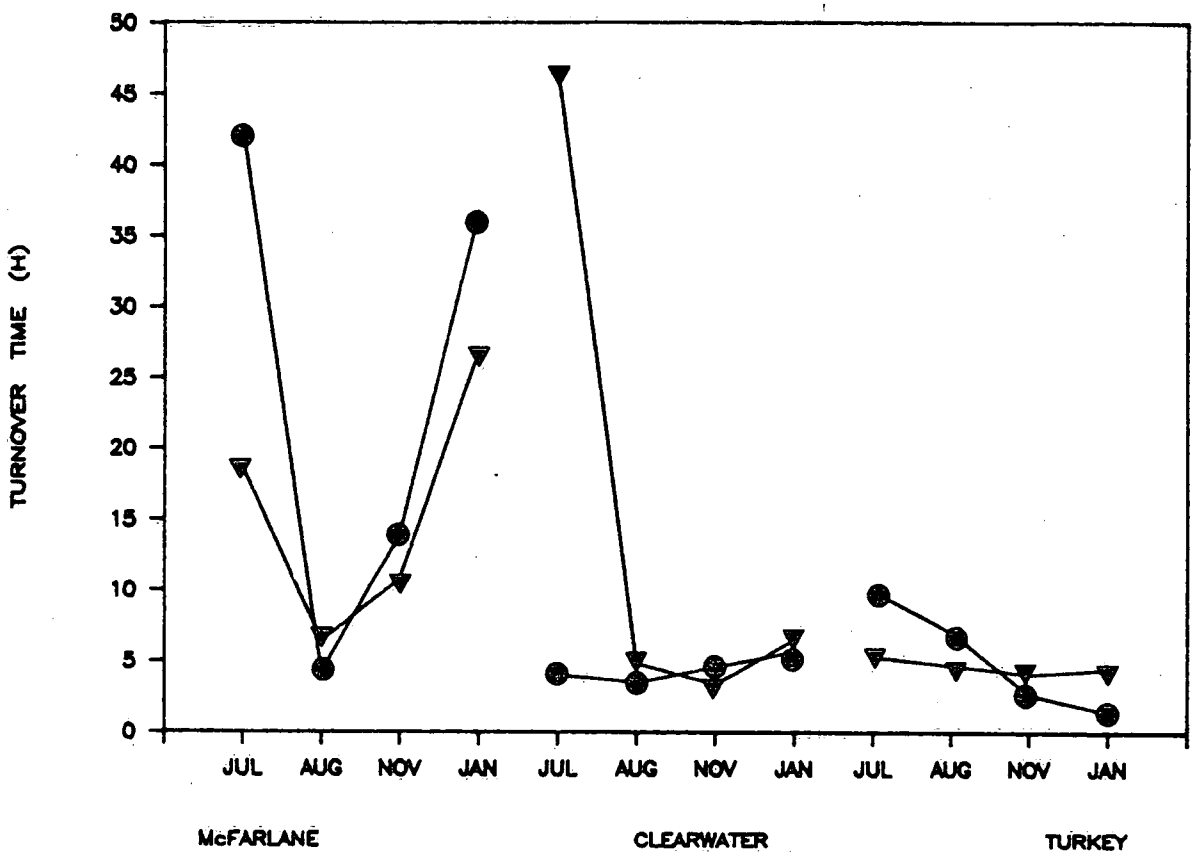


Figure 7. Turnover times, T_t and T_d (for glutamate in Clearwater Lake sediment), for glucose (▼) and glutamate (●)

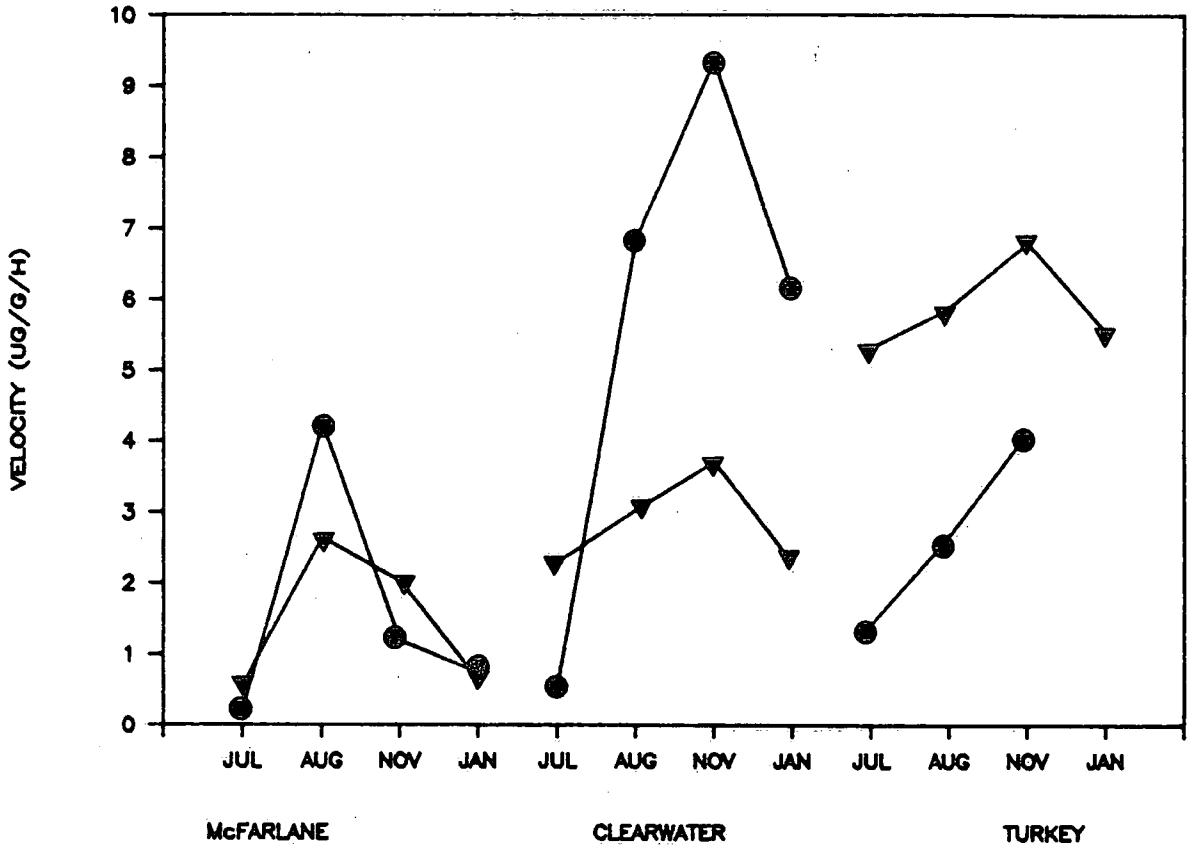


Figure 8. Calculated velocities for glucose (▼) and glutamate (●) at an assumed concentration of 50 µg/L