# EFFECTS OF LAKE ACIDIFICATION ON MICROBIAL POPULATIONS AND PROCESSES

by

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March 1987
NWRI Contribution #86-224

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## ABSTRACT

Bacteriological data collected for water and sediment cores from some Ontario lakes receiving acidic deposition indicates that bacterial populations and activities were diminished by 20-30% under acidic conditions. A pH value below 5.5 appeared to be critical for active populations. Measurements such as direct counts of total and respiring bacteria, heterotrophic plate counts, nitrifying and sulfur cycle bacteria, microbial activities ($O_2$ consumption rates and organic substrate utilization), bacterial morphology and physiology were considered. An ultrastructural analysis is shown to differentiate between different levels of acid stress and combined acid/toxic metal stress under laboratory conditions. Data, methodology, and some implications of the studies are presented.

## INTRODUCTION

During the last two decades it has become evident that precipitation over the eastern U.S. and Canada has become increasingly acidic [1]. The concern for the impacts of acidic deposition on aquatic ecosystems has led to several biogeochemical studies in Ontario and eastern Canada [2]. Some of the lakes near Sudbury, Ontario exhibit wide diversity in geochemical and biological characteristics and show varying degrees of stress from acid precipitation [3-5]. Acid rain fall (pH 4.0-5.0) has become a common event and one which is deleterious to many aquatic ecosystems [6,7]. In lakes, bacteria are an important component of the biota in terms of biomass and nutrient regeneration activity [8]. Bacterial activity can, in some cases, regulate the rate of lake acidification [9]. The effects of acidity on microbial activity in aquatic ecosystems have generated a lot of interest recently [9-15] In general, the field surveys show that bacterial populations and activity were lower in acidified lakes than in non-acidified lakes [16]. Following such observations some laboratory studies were made to ascertain the effects of acid stress on bacterial isolates [17,18] or obtained from artifically acidified water [19]. Few previous studies have related the bacterial activity to the in situ pH of the sediments.

The goal of this article is to explore the nature and extent of the effects of acidic deposition on lake bacteria in some Ontario lakes

(Fig. 1) and the use of these populations from lake sediments as indicators of acid stress. An emphasis is placed on those parameters which correlate with the diminished capacity for degrading organic materials. The philosophy and ideas that form the basis for our studies here in Ontario may be applicable to developing studies on lakes receiving acidic deposition in Western Canada. The contents of this review article are extracted from previously published scientific articles [20-23] and unpublished data (Leppard and Rao).

## MATERIALS AND METHODS

### Study site

The lakes examined are located within a radius of 30 km from the smelters in Sudbury, Ontario (Fig. 1). These Precambrian shield lakes encompass a wide diversity of physical and chemical characteristics and show varying degrees of stress from acid rain [24]. Acidity of the lakes can be traced back to acid rain. The details of the geological settings and the general limnology of lakes in the region have been outlined [5].

### Sample collection

Surface water samples (1 litre) from a series of eight lakes (Fig. 1) were collected in sterile containers. These samples were packed in ice and transported within 48 h to our laboratory for further processing. This procedure did not affect the levels of bacterial populations [20]. The studies were usually carried out during May through October. Sediment cores (50 x 6 cm) were collected by means of a lightweight coring device [25]. Only cores that came up with their sediment-water interfaces intact and undeformed were subsectioned and processed within one hour after retrieval. Each core was subsectioned at 1-2 cm intervals up to 40 cm. The samples were collected in sterile plastic bags and refrigerated immediately. The samples were processed within 48 h in the shore laboratory.

The pH measurements were made using a portable pH meter with the electrodes inserted directly into the mud. Organic content of the sediments was measured by combustion [loss on ignition (LOI)] according to standard procedure [26,27].

## Bacteriological procedure

Counting of bacteria was performed using epifluorescence microscopy after staining with acridine orange. The staining of filters and solutions used follow Zimmerman et al.[27,28].

Total and actively respiring bacterial populations were estimated microscopically on all water samples using the combined acridine orange INT-formazan reduction technique[27]. Reduction of the tetrazolium dye in bacterial cells involves respiration. In actively respiring bacteria, deposits of accumulated reduced INT-formazan, seen as optically dense dark-red intracellular spots, which can be seen by light microscope observation[27]. Total bacteria were estimated on the same slide using UV light. Numbers of bacteria were determined in 20 ocular fields, resulting in a total count of 30-300 bacteria.

Aerobic heterotrophic bacteria were measured in all samples using the spread plate procedure and a low-nutrient medium. Incubation of the inoculated plates was for seven days at 20°C [28].

## Oxygen consumption rate

Oxygen consumption by the various sediment fractions from the 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 and oxygen uptake obtained by the KCN poisoning, microbial oxygen uptake was determined.

## Heterotrophic activity method

Heterotrophic activity measurements were made using modifications of Harrison et al.[29,30]. 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[23].

## Organic substrate utilization

Indigenous bacterial populations taken directly as inoculum from the two lakes at the extremities of our selected pH range (Silver Lake, ca. pH 4.0 and McFarlane Lake ca. pH 7.0) were harvested from 48 h batch cultures ( at room temperature) in one-half strength nutrient broth at pH 4.0 and pH 7.2. Cells were washed three times with filter-sterilized, low-response water and harvested by centrifugation at 10 x 1000 g for 20 min at 4°C. These washed cells were resuspended (40 ml) for the organic substrate utilization studies. Bacterial respiration using

three substrates ( glucose, glutamic acid, and sodium acetate) at 5 µmoles of substrate per flask was determined using a Gilson differental respirometer at 20°C. Respiration studies were performed in duplicate. Data were corrected for endogenous respiration. Respiration was used to infer the bacterial activity index.

### Morphological studies

The chemical preparation of the samples for electron microscopy and the analysis of sections was done according to Leppard et al.[30,31], but with one change; the phosphate buffer was replaced by cacodylate-HCl buffer. Counterstaining of epoxy-embedded samples[31,32] and the mode of analysis of ultrathin sections (50-70 nm) was done according to Burnison and Leppard[32,33]. Observations and photographs were made with a Philips 300 transmission electron microscope (TEM).

## RESULTS AND DISCUSSION

Figure 2 is a scattergram showing the representative relationship between bacterial populations and pH in waters of the eight lakes studied. The pH values of these lakes did not change significantly during the study period May-October. Total bacterial densities were in the $10^6$ $ml^{-1}$ range in all lakes examined. Acid stress had no apparent effect on total bacteria since lakes having a pH of 7.8 had total bacterial popu-

lations somewhat similar to lakes with pH 3.8. These data confirm earlier observations[12,33], which showed no consistent relationship between total bacteria counts and water acidity.

Respiring bacteria and aerobic heterotrophs in contrast showed a definite response to environmental pH changes. Generally, maximum respiring bacteria recorded in these lakes were in the $10^5$ $ml^{-1}$ range while aerobic heterotrophs recorded were in the $10^4$ $ml^{-1}$ range. The pH values below about 5.5 may be critical for these populations.

The aerobic heterotrophic count dropped significantly around pH 5.5. The low-nutrient media used a pH of 7.0, but we assumed that most bacteria would grow well at this pH. Boylen et al.[23] isolated bacteria from lakes of various pH values grew on media at pH 7.0 and 5.0 and found that all colonies grew best on the media of higher pH, irrespective of the pH of the lake from which they were collected. Bacteria present in our lakes which are at a pH lower than 5.0 may not necessarily grow at pH 7.0. The lower count which is observed at pH 4 (Fig. 2) must be viewed with this reservation.

The respiring bacteria densities also seem to decline around pH 5.5. Visual observations of the INT reduction slides indicate that the bacteria present in lakes with a low pH are smaller than those from a circumneutral pH. The observed decline may be caused by an actual reduction

of metabolic activity of bacteria at low pH or possibly their is a threshold visual limitation of observing a formazan structure inside a very small bacterial cell.

Figure 3 compares the pH profiles with changes in aerobic heterotrophs in the lake sediment cores from the acid-stressed Silver Lake and the non-acid stressed McFarlane Lake. The aerobic heterotrophic bacterial populations in the top 5-cm layer of sediment cores from Silver Lake (pH of 3.8-5.0) number in the $10^3$-$10^4$ $ml^{-1}$ range. However, their densities between 5 and 40 cm, where the recorded pH was about 5.5, were relatively larger ($10^5$-$10^6$ $ml^{-1}$). In contrast, the profile of these bacterial populations in the core from non-acid McFarlane Lake declined from about $10^7$ $ml^{-1}$ in the surficial sediments with a pH of about 7.1 to about $10^6$ $ml^{-1}$ in the deeper layers, where the pH is relatively constant at about 6.5. These data suggest that pH below about 5.5 appears to be critical for bacterial populations in the sediments.

## Morphological effects of low-pH on bacteria

A dominant mixed population of bacteria was isolated from non-acid stressed McFarlane Lake and subjected to low-pH stress (pH range of 3 to 7). At pH level of 3,4,5,6, and 7, the culture was repeatedly subcultured in order to permit its adjustment to the new pH environment, Analysis of structure for populations growing on agar and broth within

the pH range of 4 to 7 are summarized in Table 1. Growth was too sparse at pH 3.0 for proper microscopic analysis. Lowering of the pH of the aquatic environment seems to increase the complexity of the cell wall-environment interface and the abundance of extracellular materials[22].

Ultrastructural analysis has also been used to differentiate between different levels of both acid stress and combined acid/copper stress in laboratory experiments on bacterial enrichment cultures (Leppard and Rao, submitted for publication).

**Sediment microbial respiration and bacterial organic substrate utilization**

One of the effects of lake acidification is reported to be the increased accumulation of organic matter in the lake sediments [14,17,18,34]. Kelly et al.[35] has shown that decomposition rates of "old" organic carbon was uneffected by pH values as low as 4.0, but newly sedimented material decomposition rates started to decrease at pH 5.25-5.0. Figure 4 compares the concentration of organic matter in the sediments with the pH of the overlying water for the eight lakes studied. The strong relationship found confirms the results of the previous studies. The build-up of organic matter has been attributed to the inhibition of microbial decomposition processes and/or to a shift from bacterial to less efficient fungal mineralization[14,17,18,34]. The increased storage

of organic matter in the sediments is tied to the recycling of nutrients and hence can affect the behaviour of the whole lake ecosystem. This particular facet of lake acidification remains to be investigated in detail.

Figure 5 shows that a strong relationship exists between low-pH stress and microbial activity. For example, as the pH decreased, a corresponding decrease in the rate and extent of oxygen utilization and bacterial organic substrate utilization was observed. Maximum uptake at the end of 3 h of incubation in the top 5 cm layer of acid stressed Silver Lake did not exceed 10 to 80 µl $O_2/10^9$ cells/ml. However, for the same incubation period in the top 5 cm layer of non-acid stressed McFarlane Lake, oxygen uptake was nearly 10 times more. Somewhat similar observations (3 to 30 times increase) were noticed with regard to indigenous bacterial respiration (organic substrate utilization) from the non-acid McFarlane Lake. Using the INT-formazan reduction technique, at pH 4 no respiring bacteria were detected at the end of 3 h using any of the three organic substrates. However, under the same conditions, the densities of respiring bacteria were in the range of $10^9$/ml when the pH was 7.2. No actively respiring bacteria were detected under severe acid stress conditions. However, this does not neccessarily mean such populations were absent; possibly the technique applied did not permit detection of such populations[22].

The heterotrophic activity method of Wright and Hobbie[36] was used to estimate the level of heterotrophy in the sediments from the three Ontario lakes[23]. 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[37]. 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.

Figure 6 illustrates calculated uptake velocities ($V_{50}$) for $^{14}$C-glucose and $^{14}$C-glutamic acid in the sediments of the three Ontario lakes studied. Usually glucose uptake is faster than glutamic acid uptake with the expection of Clearwater Lake (water column pH 4.5) sediment. Two possibilities for this observation are: 1) the bioavailability of the $^{14}$C-labelled compound 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 second possibility cannot be completely ignored, we feel that glutamic acid adsorption to calcareous minerals or clay in the circumneutral McFarlane Lake is the most likely explanation. Precise research on the proper adsorption control is still needed[23].

The various observations on the effects of acid precipitation on the microbial population and its activity in lake sediments are summarized in a conceptual model (Fig. 7). Lake acidification is surmised to reduce the bacterial activity and hence increase the organic content of the sediments. These parameters can, therefore, be used to trace the historical changes in the response of lakes to acid precipitation[22].

## CONCLUSIONS

The following are the major conclusions from our studies:

1. Bacterial populations and densities were lower in acid stressed lakes than in non-acid stressed lakes.
2. Respiring and heterotrophic bacterial populations in acid stressed lakes were nearly an order of magnitude less than in the non-acidified lake.
3. A pH value less than 5.5 may be critical for these bacterial populations in sediments and in the water column.
4. A relationship was found between low pH-stress and sediment microbial activity.
5. Diminished microbial activity in surface sediments resulted in a increased accumulation of organic matter.
6. The increased storage of organic matter in the sediments is tied into the recycling of nutrients and hence can affect the behaviour of the whole lake ecosystem.

7. The greatest complexity in terms of bacterial cell structure diversity and development of extracellular products is found at pH 5.0.
8. Low pH stress has a marked effect on the bacterial morphology per se and/or the selection of dominant types.
9. Low bacterial activity in acidified lakes may be the result of certain cell structural changes.
10. The physiological alterations in bacteria may be significant as stress indicates if related to cell surface exchange and/or membrane permeability properties associated with nutrient transport or transport of toxic substances.

FIGURE CAPTIONS

Fig. 1. Microbiology sampling sites in lakes receiving acid precipitation near Sudbury, Ontario. 1, Windy Lake; 2, Hannah Lake; 6, Silver Lake; 7, Lohi Lake; 9, Wavy Lake; 10, McFarlane Lake; 11, Ramsey Lake; 19, Richard Lake; 20, Clearwater Lake, (from Rao et al.[21]).

Fig. 2. Relationships between bacteria and pH in lake water receiving acid precipitation near Sudbury, Ontario, 1982, (from Rao et al.[21]).

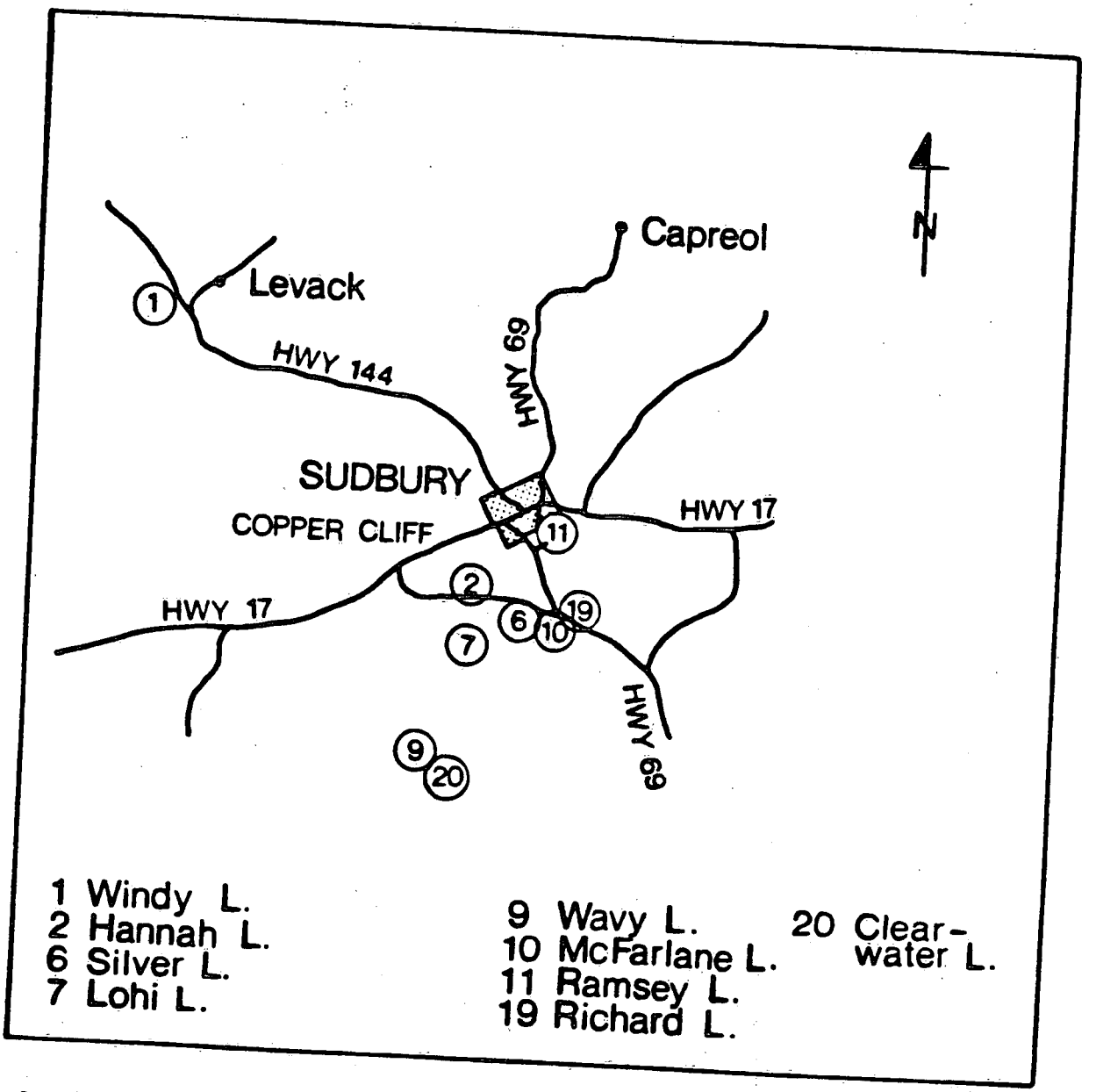
Fig. 3. Effects of acid stress on bacterial populations in lake sediments, (from Rao et al.[21]).

Fig. 4. pH and total organics in lake sediments, (from Rao et al.[21]).

Fig. 5. Microbial activity from acid stressed and non-acid stressed lakes, (from Rao et al.[22]).

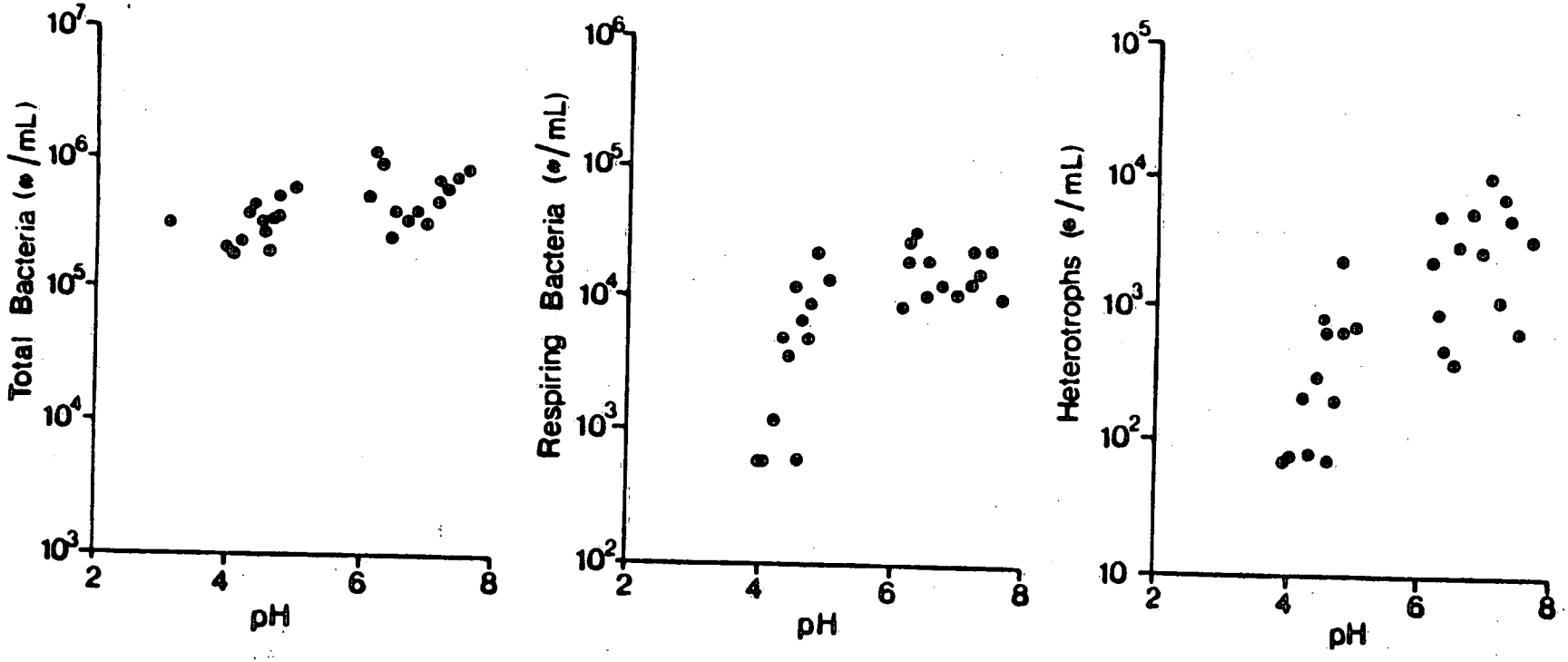
Fig 6. Calculated velocities for glucose ( ▼ ) and glutamate ( ● ) at an assumed concentration of 50 µg/L, (from Burnison et al.[23]).

Fig. 7. Influence of acid precipitation on bacterial activity in lake sediment (conceptual model), (from Rao et al.[22]).



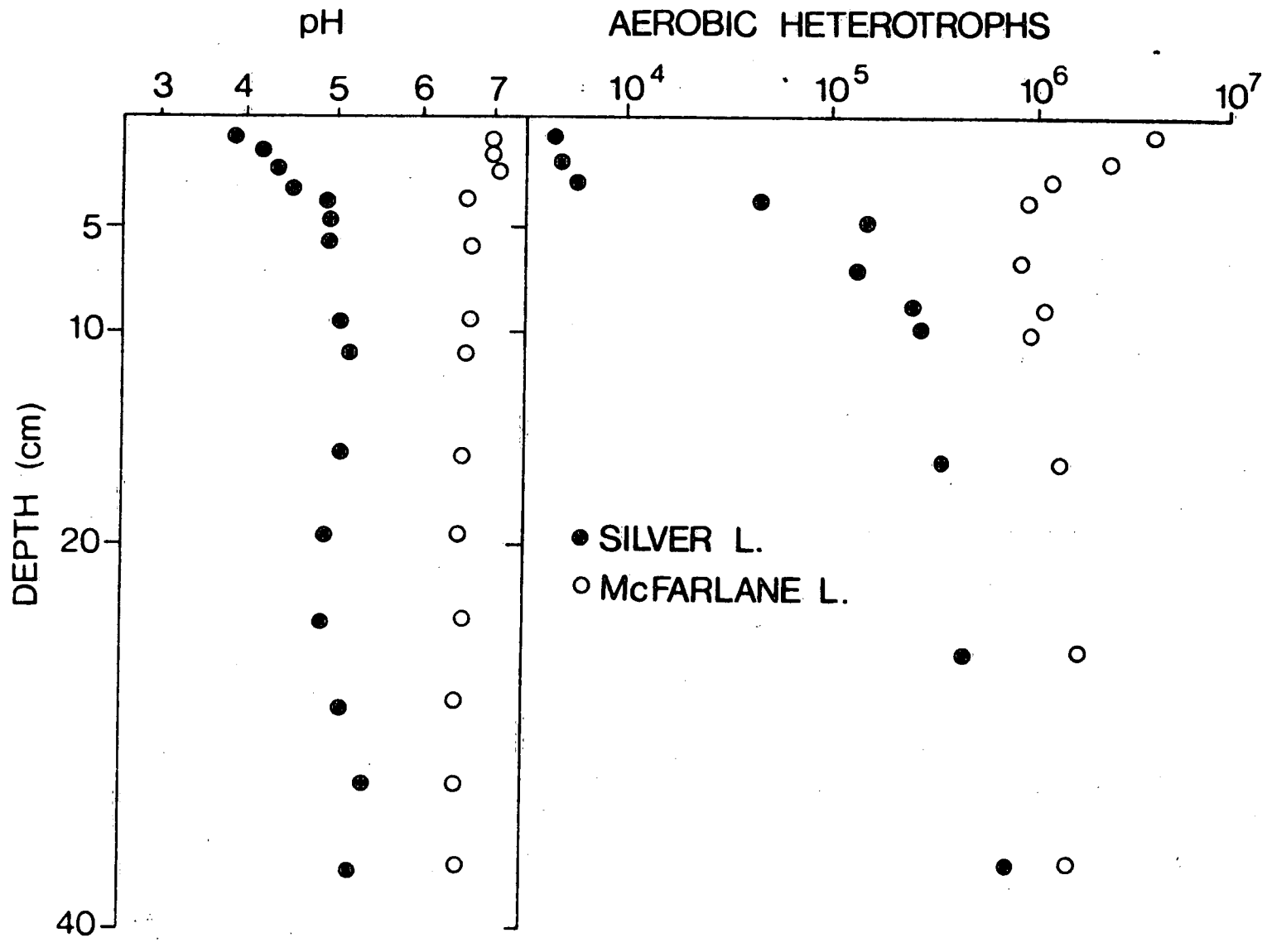

MICROBIOLOGY SAMPLING SITES IN LAKES RECEIVING ACID PRECIPITATION NEAR SUDBURY, ONTARIO.

Fig. 1.



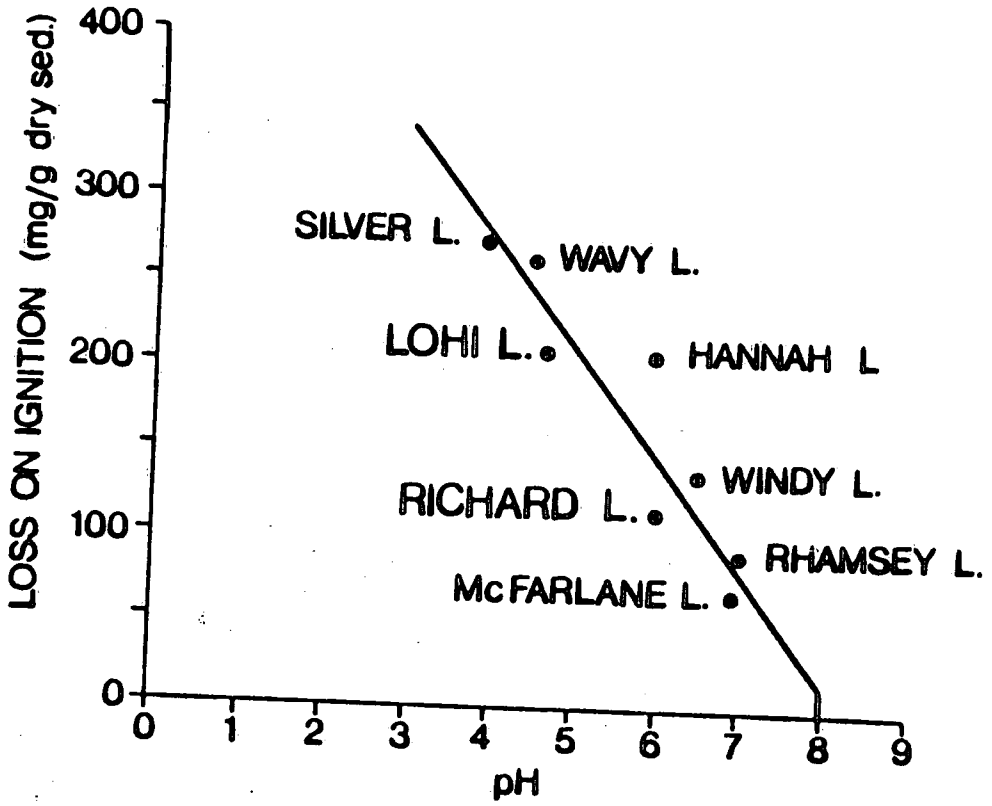

RELATIONSHIPS BETWEEN BACTERIA AND pH IN LAKE WATER RECEIVING ACID PRECIPITATION NEAR SUDBURY, ONTARIO 1982

Fig. 2.



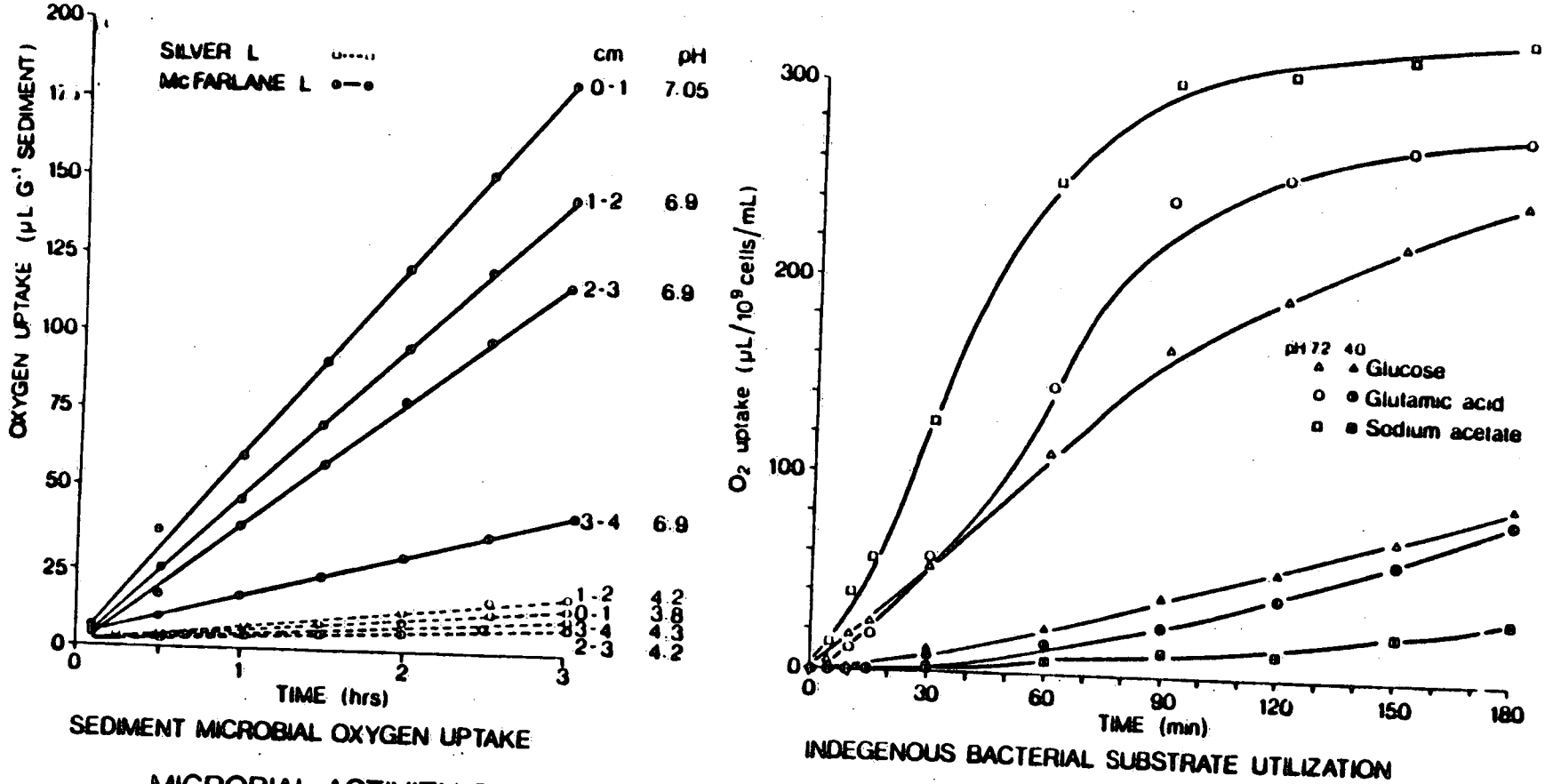

EFFECTS OF ACID STRESS ON BACTERIAL POPULATIONS IN LAKE SEDIMENTS

Fig. 3




pH AND TOTAL ORGANICS IN LAKE SEDIMENTS

Fig.4.




MICROBIAL ACTIVITY FROM ACID STRESSED AND NON-ACID STRESSED LAKES

Fig. 5.

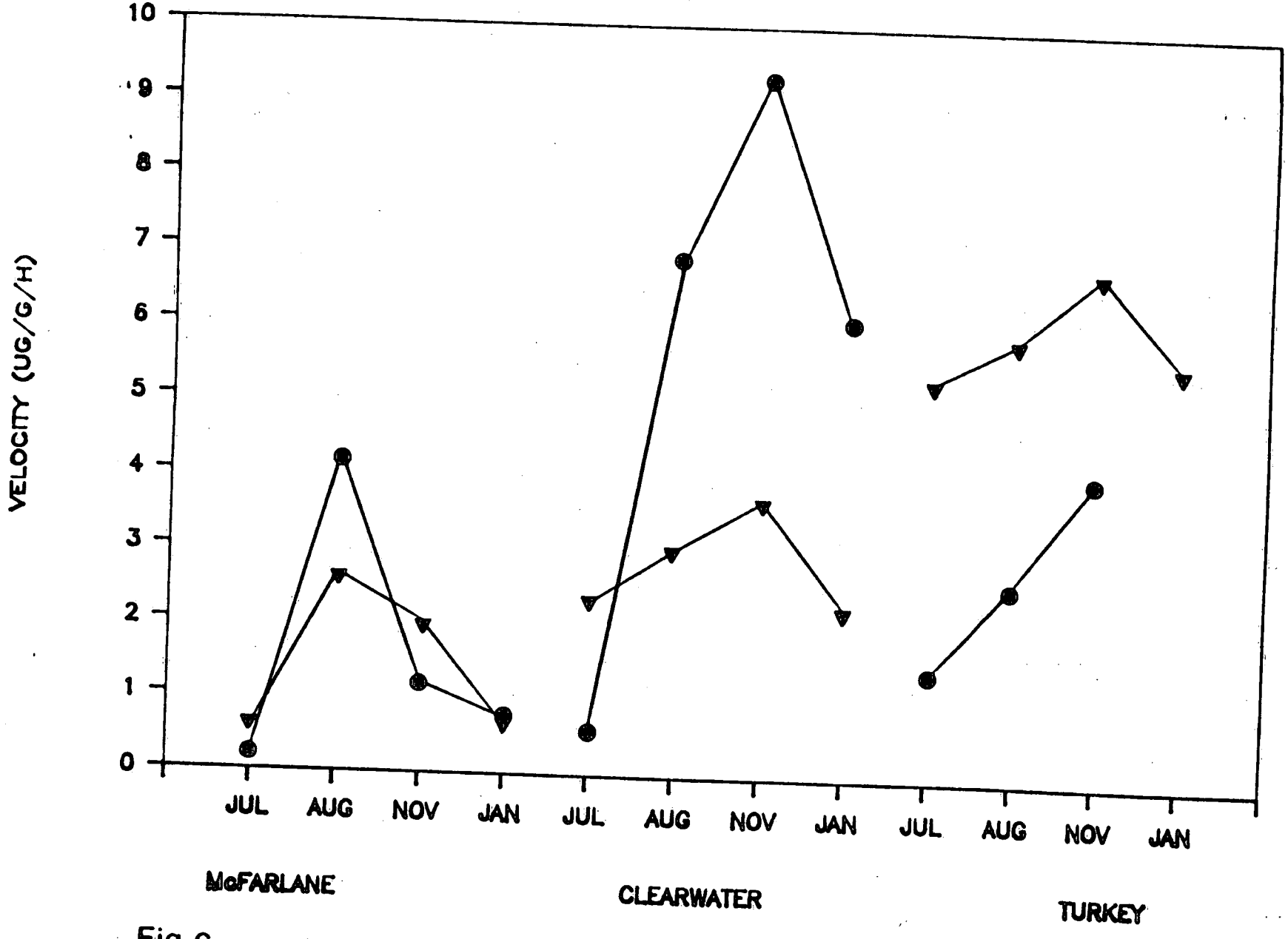
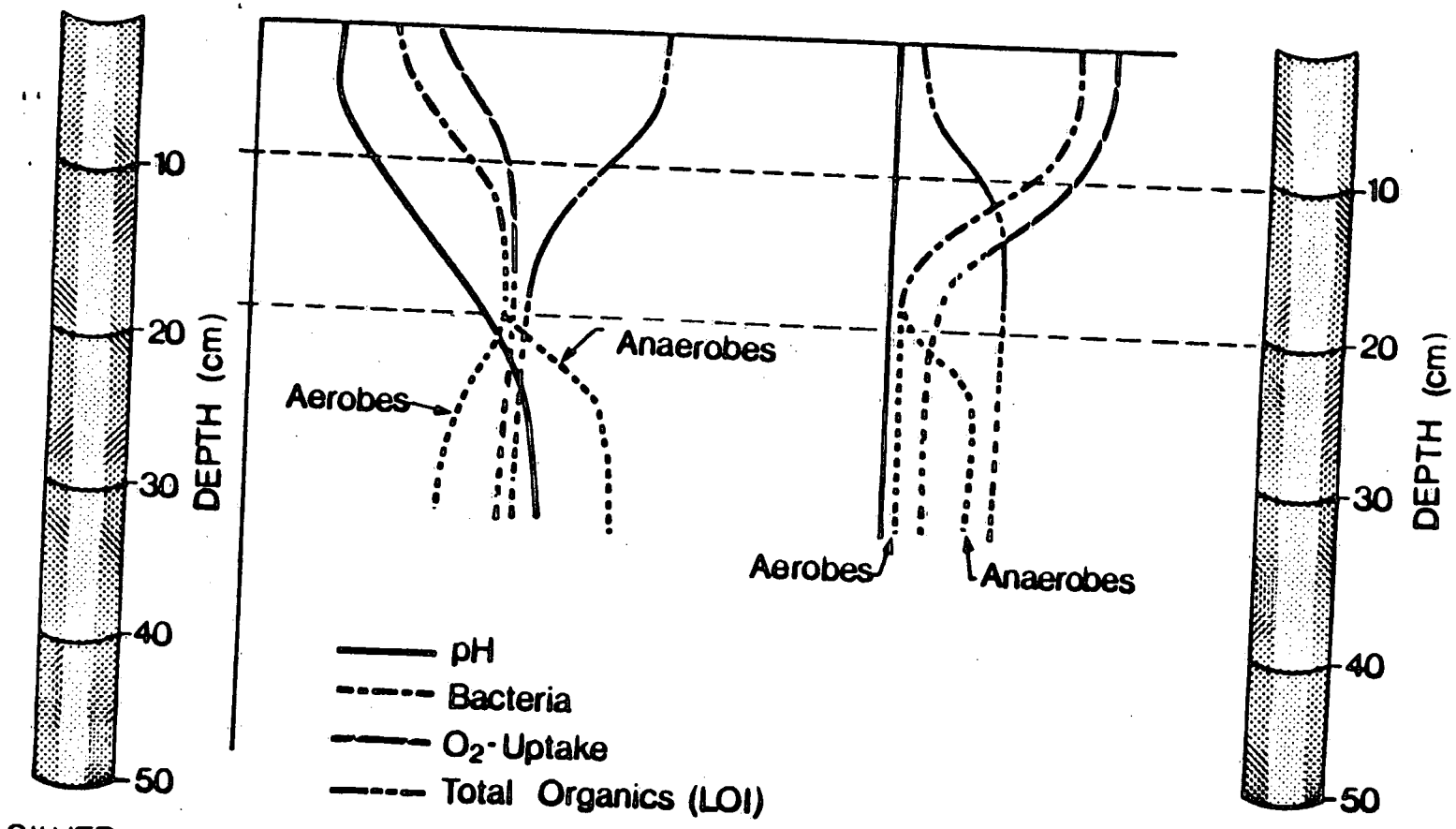

VELOCITY (UG/G/H)
10
9
8
7
6
5
4
3
2
1
0
JUL
AUG
NOV
JAN
JUL
AUG
NOV
JAN
JUL
AUG
NOV
JAN
McFARLANE
CLEARWATER
TURKEY

Fig.6.




INFLUENCE OF ACID PRECIPITATION ON BACTERIAL ACTIVITY IN LAKE SEDIMENT (CONCEPTUAL MODEL)

Fig. 7.

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