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MICROBIOLOGICAL STUDIES OF LAKE ACIDIFICATION - TOXICOLOGICAL IMPLICATIONS

by
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MANAGEMENT PERSPECTIVE

An understanding of the events occurring in lakes due to acid precipitation could lead to necessary management remedial actions. Some of the events that occur in aquatic ecosystems include (among other effects) detrimental effects on microbial community structure, microbial processes and release of trace metals which have toxic effects on the ecosytem community and subsequently the 'health' of the lake. Therefore, it is important to understand the nature and extent of the effects of acidification on microbial communities, and their organic biodegradation and responses of lake ecosystem due to reduced acid-stress. Results of this study indicate that bacterial biodegradation of organic material was approximately 30% less in acid-stressed environments; a pH threshold of 5.5 is critical for certain bacterial activities; and that, although metals are very toxic to microbial processes, lakes have the ability to reverse the downward trend of metal toxicities when metal loadings are reduced.

The results of this study improve our understanding of lake acidification and resulting changes in microbial processes. The results also improve our ability to assess the impact of acid loadings on lake acidification processes.

PERSPECTIVE DE GESTION

La connaissance des répercussions des précipitations acides sur les lacs pourrait rendre nécessaire certaines mesures correctrices en matière de gestion. Certains des événements qui se passent dans les écosystèmes comprennent (entre autres) des effets néfastes sur la structure des communautés microbiennes, des processus microbiens et l'émission de métaux-traces qui ont des effets toxiques sur l'écosystème et, par conséquent, sur la "santé" du lac. Il est donc très important de comprendre la nature et l'étendue des effets de l'acidification sur les communautés microbiennes et leur biodégradation organique, ainsi que les réactions de l'écosystème du lac dues à un stress acide réduit. Les résultats de cette étude indiquent que la biodégradation bactérienne des matières organiques était d'environ 30 % inférieure dans les milieux agressés par la pollution acide; qu'un pH seuil de 5,5 est vital pour certaines activités bactériennes et que, bien que les métaux soient très toxiques pour les processus microbiens, les lacs ont la capacité d'inverser la tendance à la baisse des toxicités des métaux lorsque les charges métalliques sont réduites.

Les résultats de cette étude améliorent nos connaissances sur l'acidification des lacs ainsi que sur les changements qui en résultent au niveau des processus microbiens. Ces résultats améliorent également notre capacité d'évaluer l'impact des charges acides sur les processus d'acidification des lacs.

RÉSUMÉ

Les données bactériologiques et biochimiques recueillies dans les échantillons d'eau et les carottes de sédiments des lacs de l'Ontario sujets à des dépôts acides indiquent que les populations et les activités bactériennes peuvent être diminuées de 20 à 30 % dans des conditions acides. Un pH inférieur à 5,5 semble être une valeur critique pour les populations actives. La biodégradation bactérienne des matières organiques en milieu agressé par la pollution acide était d'environ 30 % inférieure à celle des milieux non agressés. Le ralentissement de la dégradation des matières organiques pourrait expliquer la teneur relativement élevée en matières organiques dans les sédiments de surface des lacs recevant des précipitations acides. Cela suppose que les matières résistantes trouvées dans ces écosystèmes persistent probablement plus longtemps, ce qui pourrait avoir des effets néfastes sur des niveaux trophiques globaux de l'écosystème. De plus, le dépôt atmosphérique augmente la charge de métaux polluants nocifs pour les communautés biologiques. Des mesures comme la numération directe des bactéries totales et des bactéries aérobies, la numération sur plaque des bactéries hétérotrophes, la mesure des bactéries de nitrification et du cycle du soufre, les activités microbiennes (taux d'absorption de O_2 et utilisation des substrats organiques), la physiologie et les concentrations de métaux-traces seront présentées en même temps que certaines implications des études traitées.

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Abstract: Bacteriological and biogeochemical data collected for water and sediment cores from some Ontario lakes receiving acidic deposition indicate that bacterial populations and activities can be diminished by 20-30% under acidic populations. Bacterial biodegradation of organic material in acid stressed environments was approximately 30% less than those from non-stressed environments. The decrease in the rate of organic matter degradation might explain the relatively high organic content at the surface sediments of lakes receiving acid precipitation. This suggests that recalcitrant materials found in these ecosystems probably persist for longer periods of time and this could have adverse effects on the overall trophic level in the ecosystem. In addition, atmospheric fallout results in increased loading of pollutant metals that are toxic to biological communities. 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), physiology, and trace metal concentrations will be presented, and some implications of the studies discussed.

Key Words: Acid stress, bacteria, activity, trace metal, organic matter, biodegradation

Introduction:

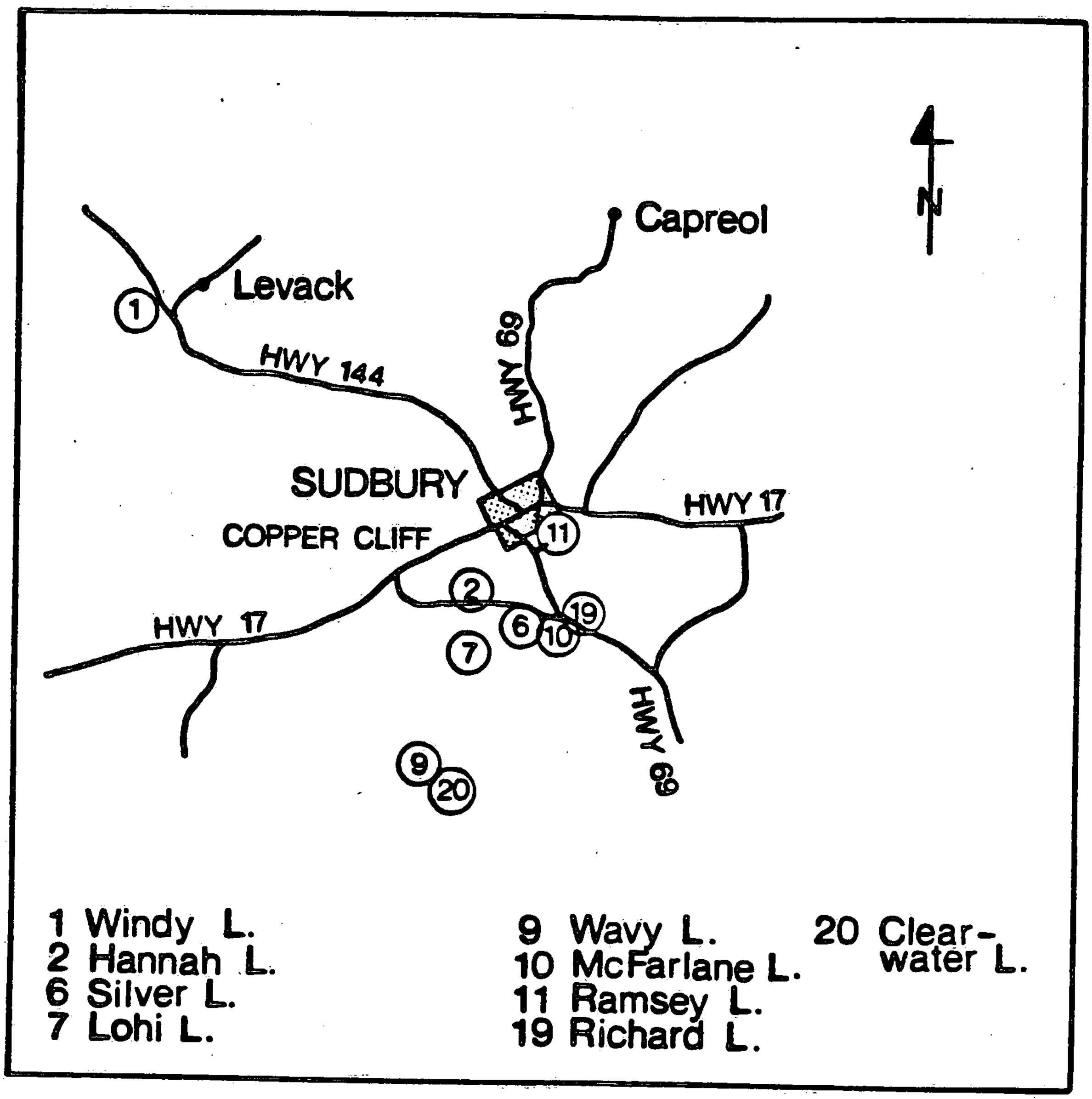
During the last two decades it has become evident that precipitation over the eastern U.S. and Canada has become increasingly acidic (Almer et al., 1978). The concern for the impacts of acidic deposition on aquatic ecosystems has led to several biogeochemical studies in Ontario and eastern Canada (Nriagu, 1984). Some of the lakes near Sudbury, Ontario exhibit wide diversity in geochemical and biological characteristics and show varying degrees of stress from acid precipitation (Gorham & Gordon, 1960: Beamish & Harvey, 1972; Conroy et al., 1978). Acidic rainfall (pH 4.0-6.0) has become a common event and one which is deleterious to many aquatic ecosystems (Haines, 1981; Wright & Gjessing, 1976). In lakes, bacteria are an important component of the biota in terms of biomass and nutrient regeneration activity (Wetzel, 1975). Bacterial activity can, in some cases, regulate the rate of lake acidification (Kelly et al., 1982). The effects of acidity on microbial activity in aquatic ecosystems have generated a lot of interest recently (Kelly et al., 1982; Leivestad et al., 1976; Alexander, 1978; Traan, 1980; Pagel, 1981; Fjerdingstad & Nilssen, 1982; Leduc & Ferroni, 1984). In general, field surveys show that bacterial populations and biodegradation activity were lower in acidified lakes than in non-acidified lakes (Babich & Stotsky, 1978). Following such observations some laboratory studies were made to ascertain the effects of acid stress on bacterial isolates (Baker et al., 1982, 1983) or obtained from artificially acidified water (Baath et al., 1979). 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 bacteria in some Ontario lakes (Fig. 1) and to use these populations from lake sediments as indicators of acid stress and relate the information to biota toxicity. 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, Canada may be applicable to developing studies for an understanding of some of the key events in lakes receiving acidic deposition anywhere in the world. The contents of this review article are extracted from previously published scientific articles (Rao & Dutka, 1983; Rao et al., 1984; Burnison et al., 1986; Nriagu & Rao, 1987).

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. 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 by Conroy et al., 1978).

Microbiological Procedures: Counting of bacteria was performed using epifluorescence microscopy after staining with acridine orange. The staining of filters and solutions used follow Zimmerman et al., 1978. Total and



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

Fig. 1.

actively respiring bacterial populations were estimated microscopically on all water samples using the combined acridine orange INT-formazan reduction technique (Zimmerman et al., 1978).

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 (Dutka, 1978). Oxygen consumption rates by the various sediment fractions from the lakes were measured at 20°C using a Gilson differential respirometer as outlined in Rao et al., 1984. Heterotrophic activity measurements were made using modifications of Harrison et al., 1971 as outlined in Burnison et al., 1987. Bacterial respiration using three substrates (glucose, glutamic acid, and sodium acetate) at 5 µmoles of substrate per flask was determined using a Gilson differential respirometer at 20°C. Respiration studies were performed in duplicate and the results were corrected for endogenous respiration. 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 10x1000 g for 20 min. at 4°C. These washed cells were resuspended (40 ml) for the organic substrate utilization studies.

The digestion of the sediment samples to trace metals was performed in Teflon-lined Par Digestion Bombs (Agemian and Chau, 1976) and the metal concentrations in the leachates were determined by atomic absorption spectrometry (Nriagu et al., 1982). 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 dry combustion [loss on ignition (LOI)] according to standard procedure (APHA, 1980).

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 populations somewhat similar to lakes with pH 3.8. These data confirm earlier observations (Traan, 1980; Boylen et al., 1983), which showed no consistent relationship between total bacteria counts and water acidity.

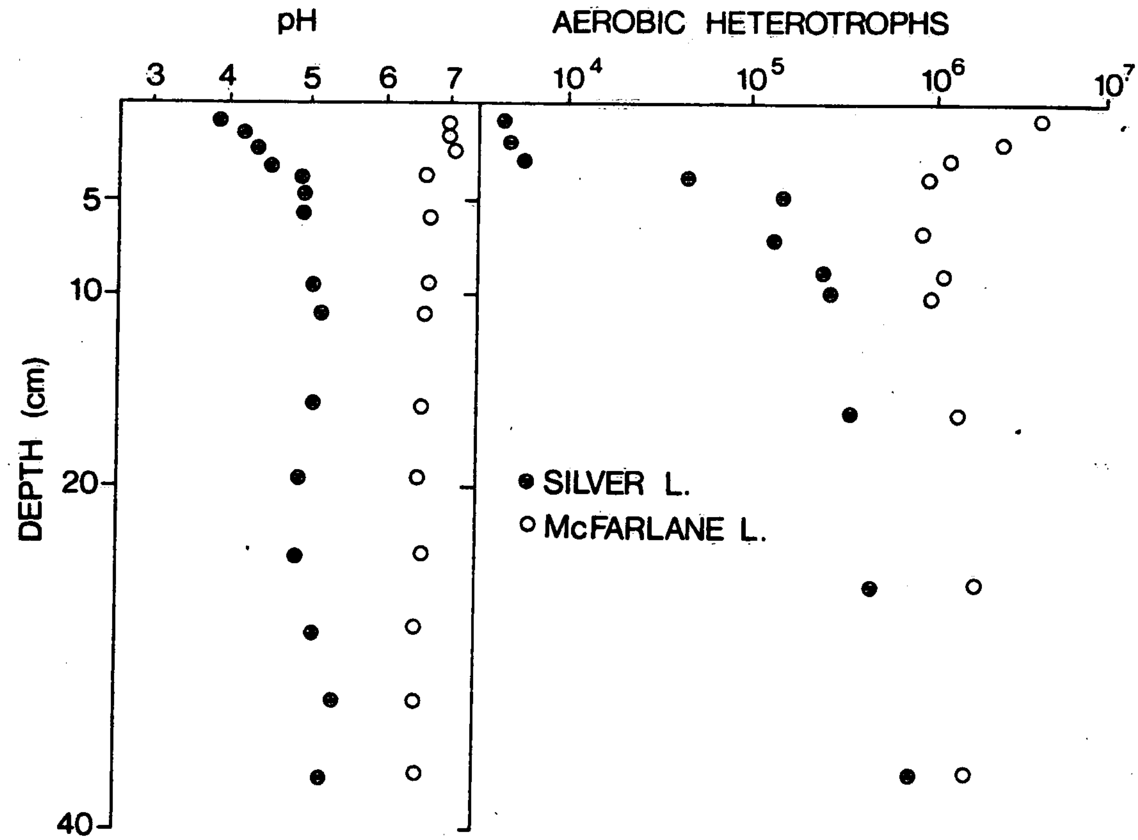
Respiring bacteria and aerobic heterotrophs in contrast showed a definite response to environmental pH changes (Fig. 2). Generally, maximum respiring bacteria recorded in these lakes were in the 10^5 ml^{-1} range while aerobic heterotrophs recorded were in the 10^4 ml^{-1} range. The pH values below 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. (1983) 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 (Fig. 2). 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 there is a threshold visual limitation of observing a formazan structure inside a very small bacterial cell.

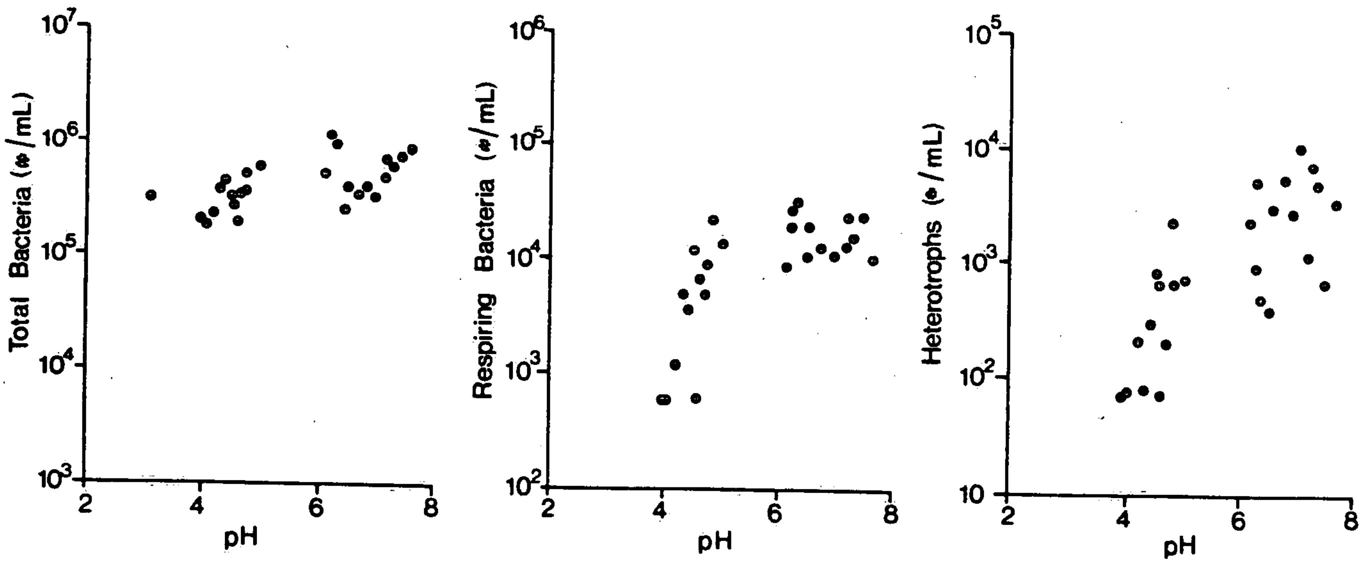
Figure 3 compares the pH profiles with changes in aerobic heterotrophs in the lake sediment cores from the acid-stressed Silver Lake and the non-acid stressed McFarlane Lake. The aerobic heterotrophic bacterial populations in the top 5-cm layer of sediment cores from Silver Lake (pH of 3.8-5.0) were in the 10^3-10^4 ml^{-1} range. However, their densities between 5 and 40 cm, where the recorded pH was above 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. Again, the data suggest that pH below about 5.5 appears to be critical for sediment bacterial populations.

<u>Sediment Microbial Respiration and Bacterial Organic Substrate Utilization</u>: One of the effects of lake acidification is reported to be the increased accumulation of organic matter in the lake sediments (Fjerdingstad & Nilssen, 1982; Baker et al., 1982, 1983; Grahn et al., 1974). Kelly et al. (1984) has shown that decomposition rates of "old" organic carbon was uneffected by pH values as low as 4.0, but recently



EFFECTS OF ACID STRESS ON BACTERIAL POPULATIONS IN LAKE SEDIMENTS

Fig. 3.



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

Fig. 2.

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 (Fjerdingstad & Nilssen, 1982; Baker et al., 1982, 1983; Grahn et al., 1974). 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 necessarily mean such populations were absent; possibly the technique applied did not permit detection of such populations (Rao et al., 1984).

The heterotrophic activity method of Wright and Hobbie (Wright & Hobbie, 1966) was used to estimate the level of heterotrophy in the sediments from the three Ontario lakes (Burnison et al., 1986). We have assumed that all the activity was from bacterial origin since we used low levels of substrate. The limitations of the heterotrophic 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 ppol, which is a mixture of organic compounds.

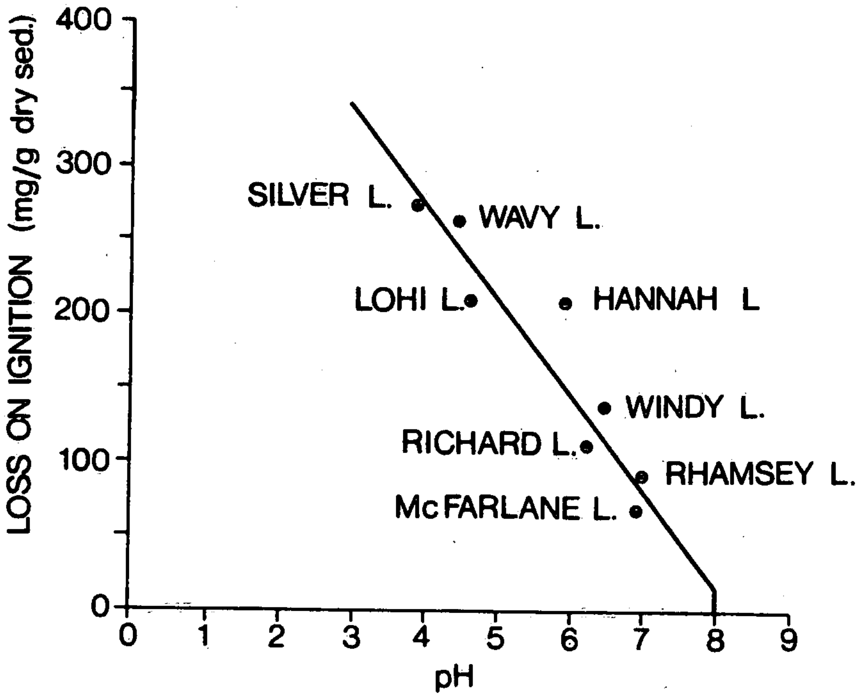


Fig. 4. pH AND TOTAL ORGANICS IN LAKE SEDIMENTS

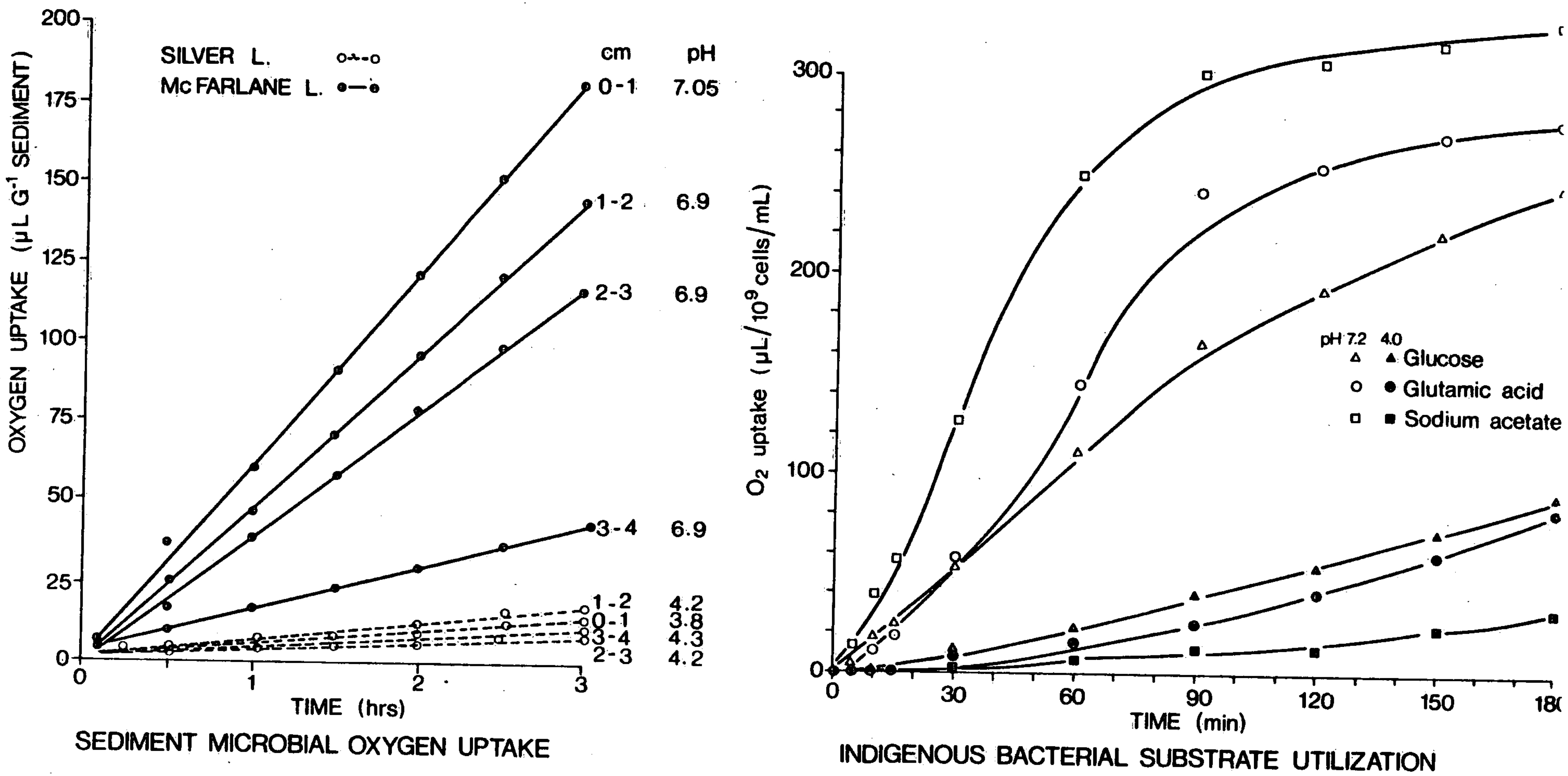


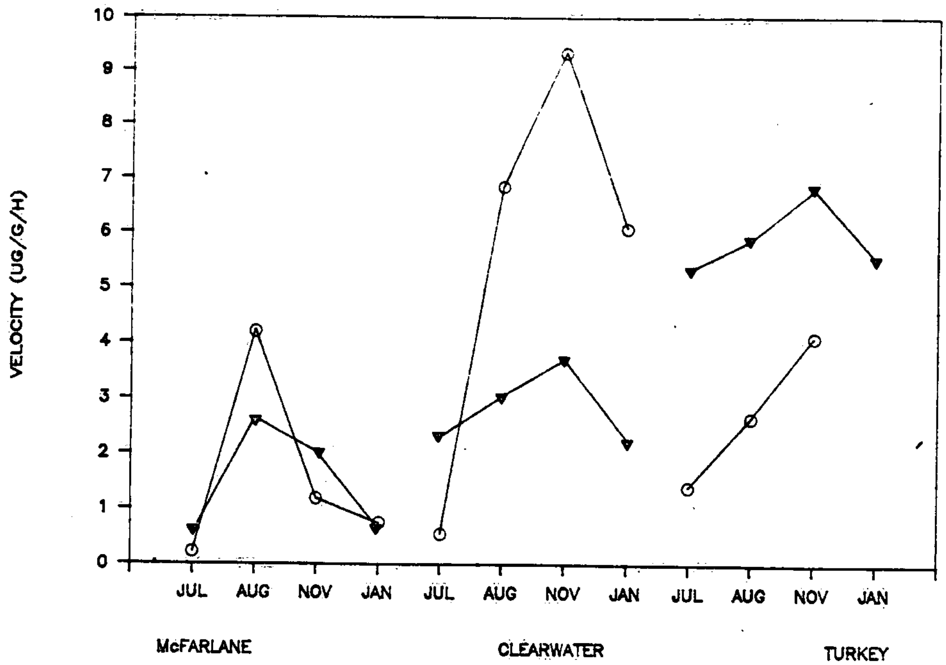
Fig. 5. MICROBIAL ACTIVITY FROM ACID STRESSED AND NON-ACID STRESSED LAKES

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 exception 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 (Burnison et al., 1986).

The profiles of Ni, Pb, Co and Zn in sediments show that the sharp increase in deposition rates started at a depth of 13-14 cm in the two lakes (Fig. 7). This implies that the rates of sediment accumulation in the two lakes are very similar. The accelerated flux of metals into the lake sediments in the Sudbury basin has been linked to the local smelting of Ni/Cu ores which commenced around 1890 (Nriagu et al., 1982; Dillon & Smith, 1984). If the 13-14 cm horizon corresponds to 1890, the sedimentation rate is estimated to be 1.4 mm y^{-1}. This value is in reasonable agreement with the rate of 1.00 mm y^{-1} (uncompacted) obtained by lead-210 geochronology for McFarlane Lake sediments (Nriagu et al., 1982).

Previous studies have already noted that the Ni contents of recent lake sediments in the Sudbury basin (Fig. 1) are among the highest in North America. The slopes of the Ni profiles during the period of rapid increase are remarkably similar in the two lakes. The actual amount of Ni stored, however, is always greater in McFarlane Lake compared to Silver Lake sediments. From the sedimentation rate of 157 g m^{-2} y^{-1} (Nriagu et al., 1982) and the concentrations given in Fig. 7, it can be shown that until very recent years, the flux of Ni into McFarlane Lake sediments increased at the rate of about 1.3 mg m^{-2} y^{-1}. The increased loading of Ni into the sediments thus seems to reflect the expanding production of Ni and Cu from Sudbury.

From 1890 until very recently, the accumulation of Co in McFarlane Lake sediments increased at the rate of about 0.6 mm m^{-2} y^{-1}. This heavy influx is rather unexpected since the mining and smelting operations in Sudbury have yet to be widely recognized as a major source of pollutant Co in the Canadian environment. These operations do not emit large quantities of Zn and the gradual increase in the Zn content of the sediments can be attributed to general environmental pollution in the neighbourhood of an urban centre (Sudbury). At present



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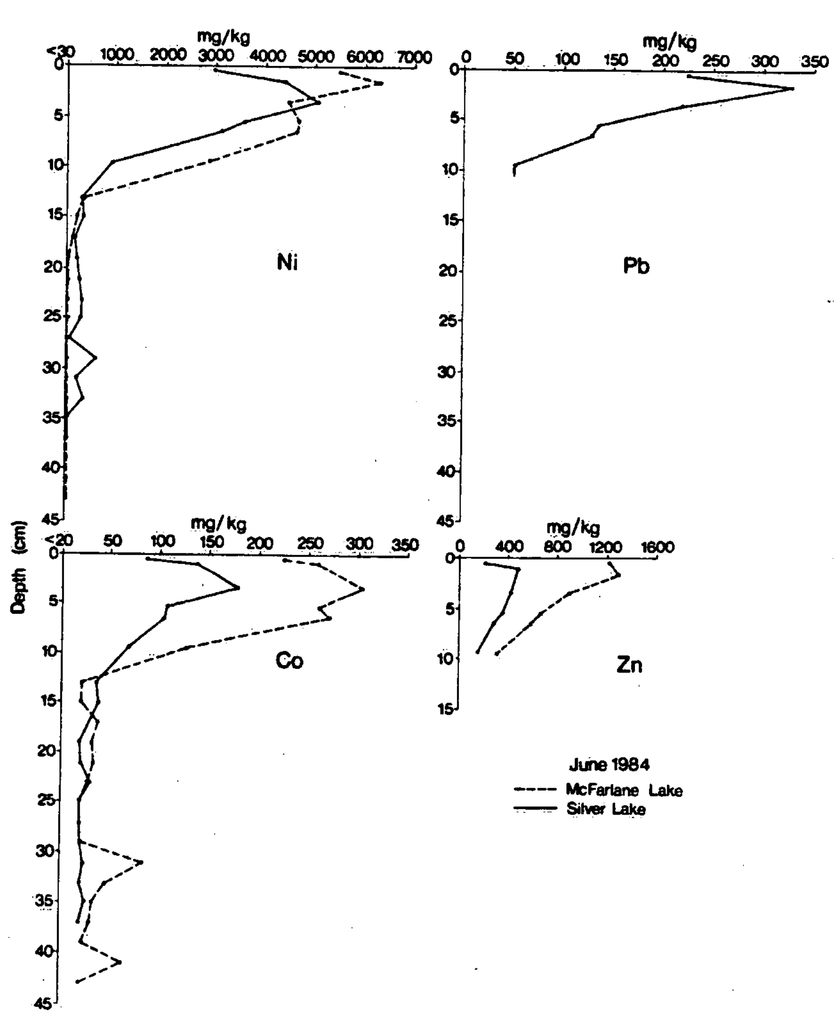
McFARLANE
CLEARWATER
TURKEY

Fig. 6.



mg/kg
<30 1000 2000 3000 4000 5000 6000 7000
Ni
mg/kg
0 50 100 150 200 250 300 350
Pb
mg/kg
<20 50 100 150 200 250 300 350
Co
mg/kg
0 400 800 1200 1600
Zn
Depth (cm)
June 1984
McFarlane Lake
Silver Lake

Fig. 7.

emissions from the smelters in the Sudbury basin include: Cu, 670; Ni, 500; Pb, 204; Zn, 66 and Cd, 32 tonnes y^{-1} (Chan & Lusis, 1985). These emission rates match the observed relative flux of metals into the sediments.

The most interesting feature of the profiles is the sharp decline in the metal content of the most recent (surficial) sediments. The decline in relation to the observed maximal concentrations vary from over 40% (Ni in Silver Lake and Co in McFarlane Lake) to about 15% (Zn in McFarlane Lake). The erection of the 381 m 'superstack' in 1972 has been shown to significantly reduce local deposition of metals and other pollutants released from the smelters in the Sudbury basin (Kramer, 1976; Semkin & Kramer, 1976; Nriagu et al., 1982). The observed decrease in metal flux to the sediments obviously must be related to improvements in local air quality brought about by the superstack. This means that these sediments are extremely sensitive to short-term changes in rates of metal deposition into the lakes' basins. In fact, the 15-40% decrease derived from the sedimentary record is in reasonable agreement with estimates based on changes in local atmospheric fallout measurements (Kramer, 1976; Chan & Lusis, 1985).

There are, however, unresolved problems in the actual interpretation of the historical record depicted by the sedimentary profiles. For example, the decline in both the Ni and Co accumulation in Silver Lake sediments began at about 4.0 cm below the sediment-water interface; this corresponds to about 27 years ago considering the sedimentation rate of 0.15 cm y^{-1}. According to the sediment record also, the onset of reduced metal inputs into McFarlane Lake dates from 10 (Zn & Ni) to 27 (Co) years ago. It should further be noted that the decline in Ni accumulation seems to have started much earlier in Silver Lake compared to McFarlane Lake (Fig. 7).

These inter-element and inter-lake differences suggest that: (a) once deposited, the rates at which the metals are redistributed depends on their biogeochemical properties; or (b) the profiles are primarily an expression of the mechanisms of diffusion into and precipitation of each metal in the sediments (Carignan & Nriagu, 1985). The dissolution and removal of metals from the surficial sediments in response to increased lake acidification cannot explain the principal features of the profiles since the pH of McFarlane Lake has remained basically unaffected by the acid rain deposition. The point that needs to be emphasized, however, is that while the sediments are highly sensitive to changes in smelter emissions of metals, the relationship between the intensity of metal input and the records in sediments is quite complex and strongly influenced by the physico-chemical properties of both the sediments and the overlying water (Nriagu & Wong, 1986).

The pH of McFarlane Lake sediments decreases from about 7.6 at the sediment-water interface to slightly under 7.0 below the 6-7 cm horizon (Fig. 8). By contrast, the pH of Silver Lake increases from about 4.0 at the interface to a little over 5.0 below 5-6 cm. The differences in pH values and profiles may explain the observed higher rates of metal accumulation (or retention) in McFarlane compared to Silver Lake sediments (Fig. 7). It has been shown that pH values lower than 4.5 (typical of surficial sediments of Silver Lake) greatly reduce the retention of metals by sediment particles (Nriagu & Gaillard, 1984), and destabilize any metal compounds precipitated in such lake sediments (Arafat & Nriagu, 1986).

The effect of the reduced metal burden on microbial populations and microbial respiration in the surficial sediments is difficult to resolve. There is a marked increase in biomass of aerobic heterotrophs and sediment oxygen demand (McFarlane only) in the most recent sediments (Fig. 7). This increase apparently is not supported by a corresponding enrichment in the organic matter content of the sediments. If anything, the observed decline in the organic matter content in surficial sediments of Silver Lake may be attributed to enhanced biodegradation stemming from the recent increase in microbial populations. It should nevertheless be noted that the population of aerobic heterotrophs is about two orders of magnitude lower in the acid-stressed Silver Lake compared to the subalkaline McFarlane Lake and other oligotrophic lakes and that the change in bacterial activity with depth does not exactly parallel the metal profiles in the Silver Lake sediments (Fig. 8).

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. 9). Lake acidification is surmised to reduce the bacterial activity and hence increase the organic content of the sediments. The parameters can, therefore, be used to trace the historical changes in the response of lakes to acid precipitation (Rao et al., 1984).

Conclusions

The following are the major conclusions from our studies:

1. Aerobic heterotrophic 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.

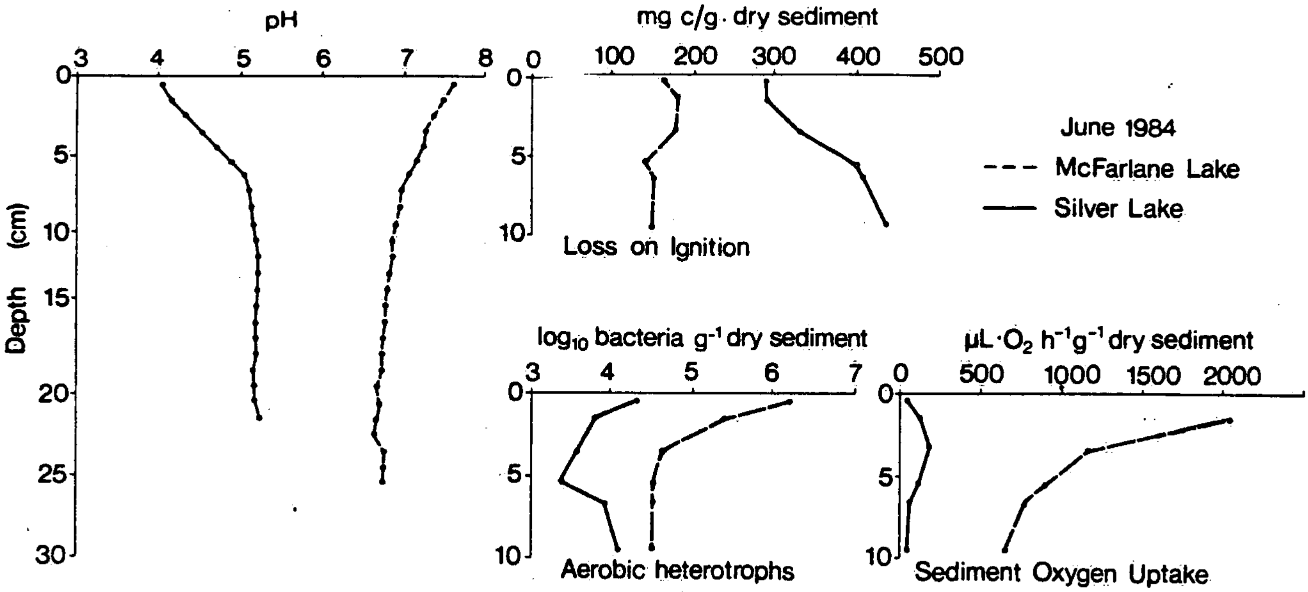
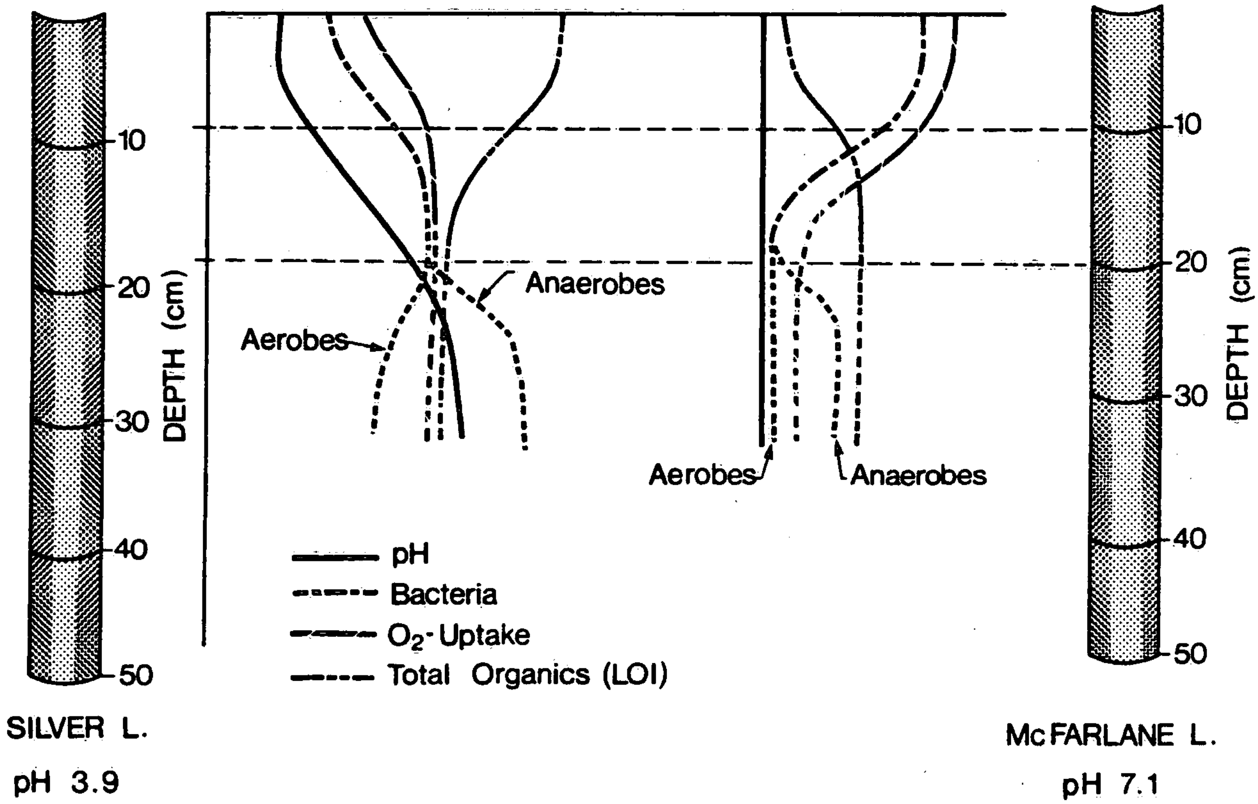


Fig. 8



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

Fig. 9

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 an 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 effect the behaviour of the whole lake ecosystem.
7. Lake sediments are extremely sensitive to changes in the emissions of metals from smelters.
8. Observed historical records provide <u>de facto</u> evidence that much of the damage suffered by aquatic ecosystems may be reversible.

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