

**MICROBIOLOGICAL STUDIES OF  
LAKES RECEIVING ACIDIC DEPOSITION**

by

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NWRI Contribution #86-98

# MICROBIOLOGICAL STUDIES OF LAKES RECEIVING ACIDIC DEPOSITION

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## ABSTRACT

A summary of 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 (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 and Gordon 1960, Beamish and Harvey 1972, Conroy et al. 1978). Acid rain fall (pH 4.0-5.0) has become a common event and one which is deleterious to many aquatic ecosystems (Haines 1981, Wright and Gjessing 1976). In lakes, bacteria are an important component of the biota in terms of biomass and nutrient regeneration activity (Wetzel 1983). 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 (Leivestad et al. 1976, Alexander 1978, Traaen 1980, Pagel 1981, Fjeringstad and Nilssen 1982, Kelly et al. 1982). In general, the field surveys show that bacterial populations and activity were lower in acidified lakes than in non-acidified lakes (Babich and Stotzky 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 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 the following scientific publications: Rao and Dutka (1983), Rao et al. 1984a, Rao et al. 1984b, Burnison et al. 1986, Leppard and Rao (1986).

## 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 (Nriagu et al. 1982). 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).

### 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 (Rao and Dutka 1983). 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 (Williams and Pashley 1979). 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 (American Public Health Association 1980).

### 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. (1978).

Total and actively respiring bacterial populations were estimated

microscopically on all water samples using the combined acridine orange INT-formazan reduction technique (Zimmerman et al. 1978). 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 (Zimmerman et al. 1978). 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 (Dutka 1978).

#### 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. (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  $^{14}\text{C}$ -glucose or  $^{14}\text{C}$ -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  $\text{H}_2\text{SO}_4$  was added to each bottle to stop further isotope uptake and release the  $^{14}\text{CO}_2$  from the aqueous phase.  $\beta$ -Phenethylamine (200 µl) was carefully added to the cup assembly. The  $\text{CO}_2$  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  $\mu\text{m}$  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 (Burnison et al. 1986).

#### 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  $10 \times 1000 \text{ g}$  for 20 min at  $4^\circ\text{C}$ . 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  $\mu\text{moles}$  of substrate per flask was determined using a Gilson differential respirometer at  $20^\circ\text{C}$ . 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. (1977), but with one change; the phosphate buffer was replaced by cacodylate-HCl buffer. Counterstaining of epoxy-embedded samples (Spurr, 1969) and the mode of analysis of ultrathin sections (50-70 nm) was done according to Burnison and Leppard (1983). 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<sup>-1</sup> 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 by Traaen (1980) and Boylen et al. (1983) who 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  $10^5 \text{ ml}^{-1}$  range while aerobic heterotrophs recorded were in the  $10^4 \text{ ml}^{-1}$  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 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  $10^3$ - $10^4 \text{ ml}^{-1}$  range. However, their densities between 5 and 40 cm, where the recorded pH was about 5.5, were relatively larger ( $10^5$ - $10^6 \text{ ml}^{-1}$ ). In contrast, the profile of these bacterial populations in the core from non-acid McFarlane Lake declined from about  $10^7 \text{ ml}^{-1}$  in the surficial sediments with a pH of about 7.1 to about  $10^6 \text{ ml}^{-1}$  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 sub-cultured 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 (Rao et al. 1984b).

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 (Grahn and Hultberg 1974, Grahn et al. 1974, Baker et al. 1982, 1983, Fjordingstad and Nilssen 1982). Kelly et al. (1984) has shown that decomposition rates of "old" organic carbon was unaffected 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 (Grahn et al. 1974, Baker et al. 1982, 1983, Fjordingstad and Nilssen 1982). 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  $\mu\text{l O}_2/10^9$  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  $10^7$ /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. 1984b).

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 (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 pool, which is a mixture of organic compounds.

Figure 6 illustrates calculated uptake velocities ( $V_{50}$ ) for  $^{14}\text{C}$ -glucose and  $^{14}\text{C}$ -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  $^{14}\text{C}$ -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 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 (Rao et al. 1984b).

#### 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.



#### FUTURE STUDIES

Future studies should be sufficiently intensive to establish in detail the extent and the exact nature of the acidification effects on bacterial organic matter degradation rates in lake sediments. Studies on the effects of sulphate and nitrate inputs on microbial organic matter degradation in sediments of lakes receiving acid precipitation should be considered. Studies should be conducted on acidified lakes where low productivity and low sulphur concentrations exist and productive lakes receiving high sulphur inputs. Laboratory microcosm studies to ascertain the processes by which alkalinity is produced, after additions of sulphate and nitrate, thereby reducing the impact of acid rain. In addition, the value of refined microbiological techniques such as the use of  $^{14}\text{C}$ -labelled compounds to study various sediment microbiological processes need to be reassessed.

#### REFERENCES

- Alexander, M. 1978. Effects of acidity on microorganisms and microbial processes in soil. In Hutchinson, T.C. and M. Haves (eds.), Effects of acid precipitation on terrestrial ecosystem. Plenum, New York and London (published in co-ordination with NATO Scientific Affairs Division), pp. 363-374.
- Almer, B., W. Dickson, C. Ekstrom, and E. Hornstorm. 1978. Sulfur pollution and aquatic ecosystem. In Nriagu, J.O. (ed.) Sulfur in the Environment Part 2, Ecological Impacts. John Wiley and Sons, N.Y., pp. 273-311.
- American Public Health Association. 1980. Standard methods for the examination of water, wastewater and sediments, 15 ed. Washington, D.C., APHA-AWWA-WPCF. 1134 p.
- Baath, E., Lundgren, and B. Soderstrom. 1979. Effects of artificial acid rain on microbial activity and biomass. Bull. Environ. Contam. and Toxicol., 23, 737-740.
- Babich, H. and G. Stotzky. 1978. Influence of pH on inhibition of bacteria, fungi and coliphages by bisulfite and sulfite. Environ. Res., 15, 405-417.
- Baker, M.D., W.E. Inniss and C.I. Mayfield 1982. Effect of pH on the growth and activity of heterotrophic sediment microorganisms. Chemosphere, 11, 973-983.
- Baker, M.D., W.E. Inniss, C.I. Mayfield and P.T.S. Wong 1983. Effect of acidification, metals and metalloids on sediment microorganisms. Water Research, 17, 925-930.
- Beamish, R.J. and Harvey. 1972. Acidification of lakes in Canada by acid precipitation and the resulting effects on fishes. J. Fish. Res. Bd. Can., 29, 1131-1143.
- Boylen, C.W., M.O. Shick, D.A. Roberts and R. Singer 1983. Microbiological survey of Adirondack lakes with various pH values, 45, 1538-1544.
- Burnison, B.K. and G.G. Leppard. 1983. Isolation of colloidal fibrils from lake water by physical separation techniques. Can. J. Fish. Aquat. Sci., 40, 373-381.

- Burnison, B.K., S.S. Rao, A.A. Jurkovic and D.J. Nuttley. 1986. Sediment microbial activity in acidic and non-acidic lakes. *Water Poll. Res. J. Can.*, (in press).
- Conroy, N.I., K. Hawly and K. Keller. 1978. Extensive monitoring of lakes in the greater Sudbury area. Technical report. Ontario Ministry of the Environment.
- Dutka, B.J. 1978. Methods for microbiological analysis of water, waste water and sediments. Burlington, Ontario. IWD.
- Fjerdingsstad, E. and J.P. Nilssen. 1982. Bacteriological and hydrological studies on acidic lakes in Southern Norway. *Arch. Hydrobiol.*, 4,443-483.
- Gorham, E. and A.G. Gordon. 1960. The influence of smelter fumes upon the chemical composition of lake waters near Sudbury, Ontario and upon the surrounding vegetation. *Can. J. Bot.*, 38,477-487.
- Haines, T.A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: A review. *Trans. Amer. Fish. Soc.*, 110,669-707.
- Harrison, M.J., R.T. Wright and R.Y. Morita. 1971. Method for measuring mineralization in lake sediments. *Appl. Microbiol.*, 21,698-702.
- Kelly, C.A., J.W.M. Rudd, R.B. Cook, and D.W. Schindler. 1982. The potential importance of bacterial processes in regulating rate of lake acidification. *Limnol. Oceanogr.*, 27,868-882.
- Kelly, C.A., J.W.M. Rudd, A. Furutani and D.W. Schindler 1984. Effects of lake acidification on rates of organic matter decomposition in sediments. *Limnol. Oceanogr.*, 29, 687-694.
- Leduc, L.G. and G.D. Ferroni 1984. Glucose mineralization activity and the use of the heterotrophic activity method in an acidified lake. *Water Research*, 18, 609-612.
- Leivestad, H., G. Hendrey, I.P. Muniz and E. Shekvik. 1976. Effects of acid precipitation on freshwater organisms. In Summary report on research results from the phase I (1972-75) of the research project on acidification - Effects on forest and fish. F.N. Braekke (ed.). *In Impact of acid precipitation on forest and freshwater ecosystems in Norway*, 87-111. SNSF Project F.R 6/76, Oslo.
- Leppard, G.G., A. Massalski and D.R.S. Lean. 1977. Electron-opaque microscopic fibrils in lakes: their demonstration, their biological derivation and their potential significance in the redistribution of cations. *Protoplasma*, 92,289-309.
- Leppard, G.G. and S.S. Rao. 1986. Acid stress and lake bacteria: An ultrastructural analysis of some combined acid/copper stresses. (submitted to *Env. Poll. Ser. A*).
- Nriagu, J.O. 1984. (ed.) *Environmental impacts of smelters*. John Wiley and Sons, New York. 608 p.
- Nriagu, J.O., H.K.T. Wong and R.D. Coker. 1982. Deposition and chemistry of pollutant metals in lakes around the smelters at Sudbury, Ontario. *Environ. Sci. & Technol.*, 16,551-560.
- Pagel, J.E. 1981. A review of microbiological processes relevant to the effects of acid precipitation. Technical report. Ontario Ministry of the Environment, June, 1981.
- Rao, S.S. and B.J. Dutka 1983. Influence of acid precipitation on bacterial populations in lakes. *Hydrobiologia*, 98, 153-157.

- Rao, S.S., A.A. Jurkovic and J.O. Nriagu 1984a. Bacterial activity in sediments of lakes receiving acid precipitation. *Environ. Pollut. Ser. A.*, 36, 195-205.
- Rao, S.S., D. Paolini and G.G. Leppard 1984b. Effects of low-pH stress on the morphology and activity of bacteria from lakes receiving acid precipitation. *Hydrobiologia*, 114, 115-121.
- Spurr, A.R. 1969. A low-density epoxy resin embedding medium for electron microscopy. *J. Ultrast. Res.*, 26, 31-34.
- Traan, T.S. 1980. Effects of acidity on decomposition of organic matter in aquatic environments. In Drablos, D. and A. Tollan (eds.) *Proc. of the Int. Conf. on the Ecological Impact of Acid Precipitation*. SNSF Project, Oslo-Aas, Norway, pp. 340-341.
- Williams, J.D. and A.E. Pashley 1979. Light-weight corer designed for sampling very soft sediments. *J. Fish. Res. Bd. Can.*, 36, 241-246.
- Wright, R.T. and B.K. Burnison. 1979. Heterotrophic activity measured with radiolabelled organic substrates. In Costerton, J.W. and R.R. Colwell (eds.), *Native Aquatic Bacteria: Enumeration, Activity, and Ecology*. ASTM STP 695, pp. 140-155.
- Wright, R.F. and E.T. Gjessing. 1976. Changes in the chemical composition of lakes. *Ambio*, 5, 219-223.
- Zimmerman, R., R. Iturriaga and J.B. Brick. 1978. Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. *Environ. Microbiol.*, 36, 926-935.

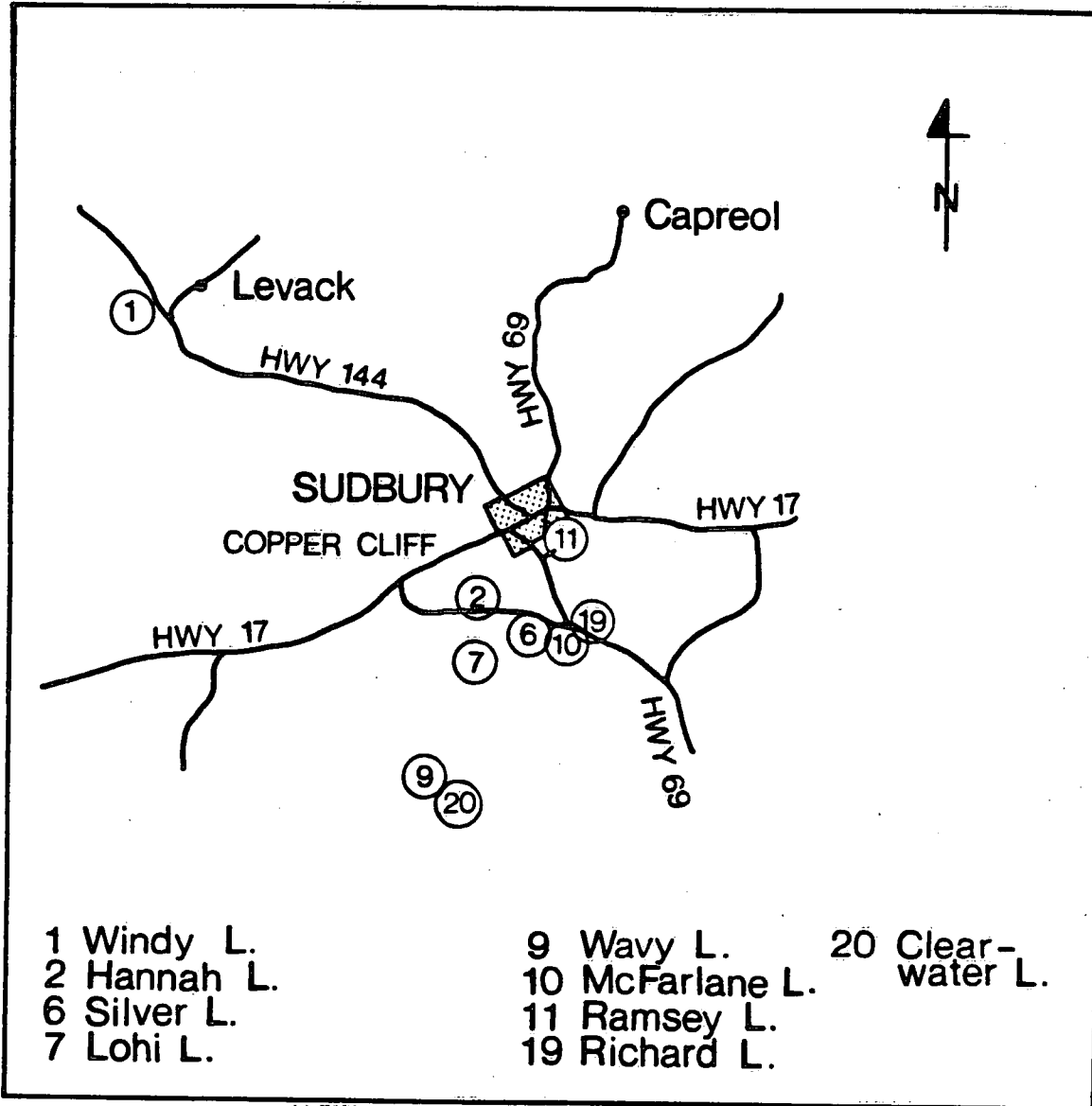
Table 1. The cytological effects of acidity on populations of bacteria isolated from McFarlane Lake.

pH	Shape	Extracellular materials	Interface cell wall-environment <sup>a</sup>	Cytoplasm	Nuclear region
7	Nearly spherical	Rare	Naked	Extremely dense in agar, various densities in broth	Extremely dense
6	Nearly spherical	Fibrillar structures common	Electron-dense granules	Varied, with many cells having a dense peripheral cytoplasm	Condensed in agar, dispersed in broth
5	Many kinds	Fibrillar structures common, particularly in broth	Electron-dense granules	Varied	Varied
4	Many kinds	Rare	Electron-dense granules	Varied, with many cells having a dense peripheral cytoplasm	Varied

<sup>a</sup> At pH 5 and pH 4, many cells had an outermost, electron-dense, 'extra layer' on the cell wall; it was particularly evident at pH 4. (Table taken from Rao et al. 1984b).

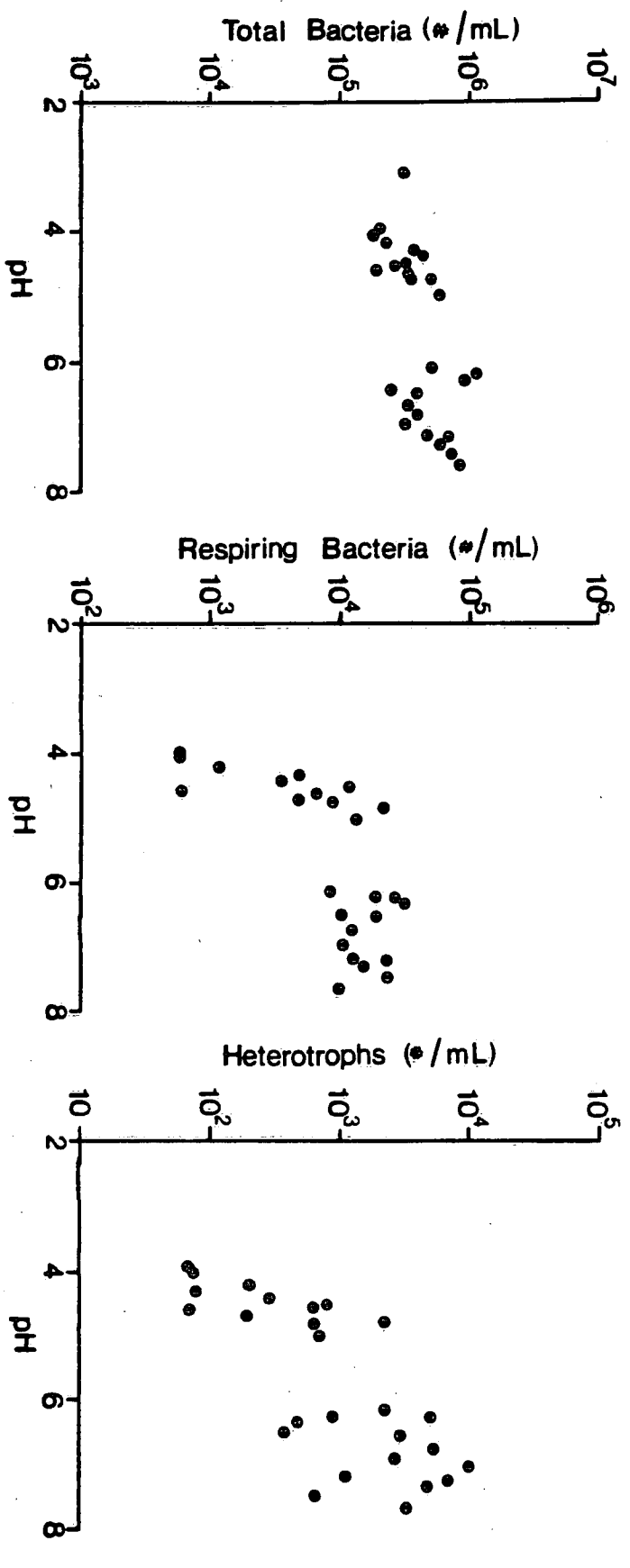
## FIGURE CAPTIONS

- 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., 1984a)
- Fig. 2. Relationships between bacteria and pH in lake water receiving acid precipitation near Sudbury, Ontario, 1982. (From Rao et al., 1984a).
- Fig. 3. Effects of acid stress on bacterial populations in lake sediments. (From Rao et al., 1984a).
- Fig. 4. pH and total organics in lake sediments. (From Rao et al., 1984a).
- Fig. 5. Microbial activity from acid stressed and non-acid stressed lakes. (From Rao et al., 1984b).
- Fig 6. Calculated velocities for glucose ( $\blacktriangledown$ ) and glutamate ( $\bullet$ ) at an assumed concentration of 50  $\mu\text{g/L}$ . (From Burnison et al., 1986).
- Fig. 7. Influence of acid precipitation on bacterial activity in lake sediment (conceptual model). (From Rao et al., 1984a).



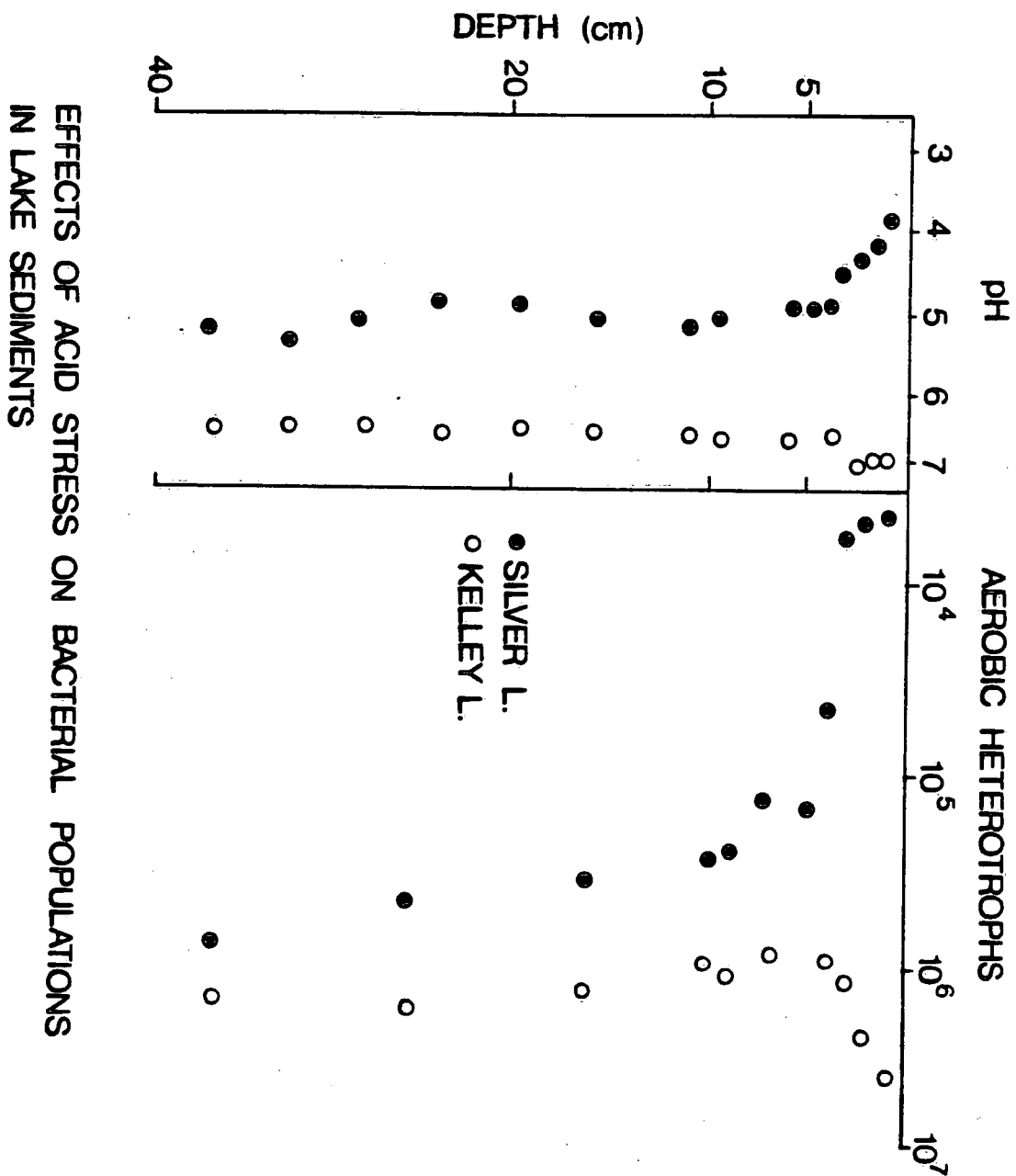
MICROBIOLOGY SAMPLING SITES IN LAKES RECEIVING ACID PRECIPITATION NEAR SUDBURY, ONTARIO.

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.**



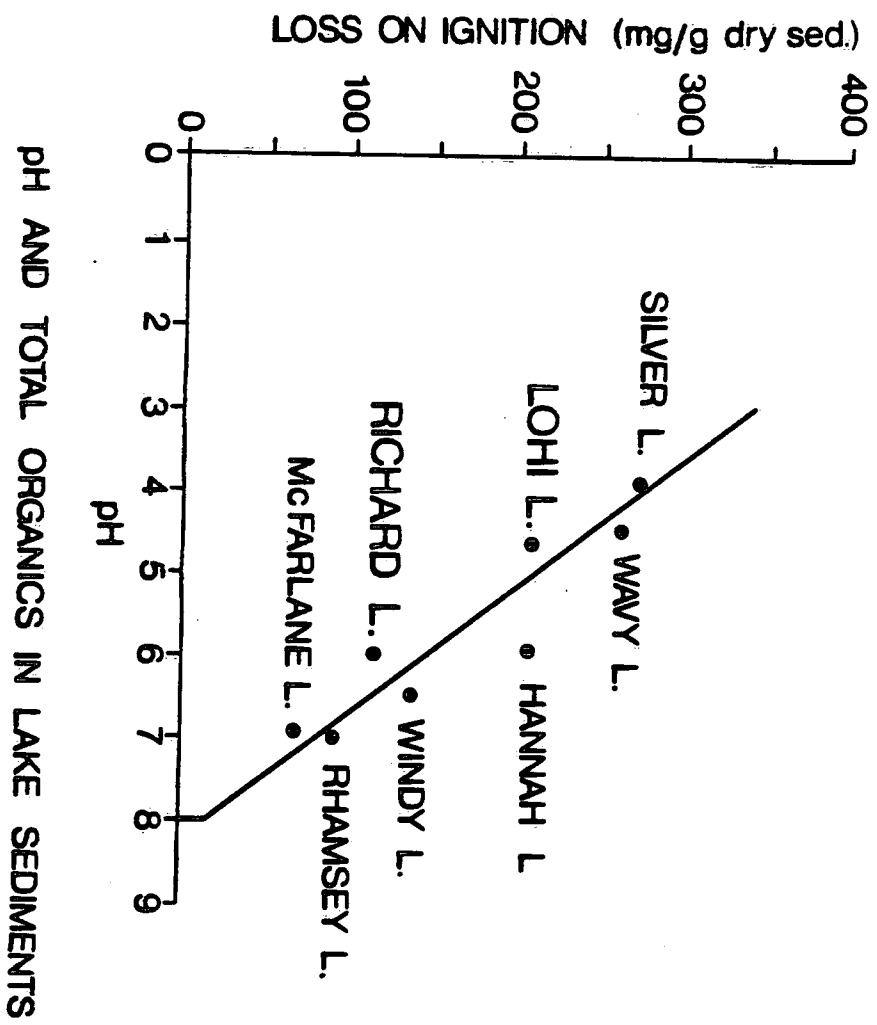


Fig.4.