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## LETHAL TOXICITY OF LEAD NITRATE TO *TETRAHYMENA PYRIFORMIS*

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LEAD is a common heavy metal and its toxicity to man has been known for centuries[1]. Lead accumulates in bones and tissues, and in high concentrations causes anemia[2], impairment of the function of liver, kidney and spleen, spinal deformities[3] and death[4]. Concentration of lead is steadily increasing in rivers, lakes and oceans. In view of the general interest of environmental contamination by heavy metals their effect on living cells is of interest[5,6]. Lead is very toxic to most plants, moderately toxic to animals[5,6] where it acts as a cumulative poison, and quite toxic to aquatic organisms[7]. Surprisingly, although aquatic micro-organisms are probably of greater value to industry than fish, pollutant-caused killing of fish attracts considerably more attention[8].

The waste assimilation capacity of a stream or lake depends in large part on the protozoan population since it is the protozoans that face the initial and most important attack upon wastes entering the water body. *Tetrahymena pyriformis* Ehrenberg, a ciliate protozoan, occurs world-wide in a variety of freshwater habitats. Its structure, physiology and biochemistry have been extensively studied[9].

As a part of a detailed eco-toxicological study of the effect of 13 heavy metals on *T. pyriformis* the effect of the pollutant lead nitrate was evaluated in terms of toxicity, stimulation, inhibition, destruction and alteration under conditions of short exposure of

**Table 1** *Changes in morphology and motility of T. pyriformis exposed to lead nitrate*

| Toxicant conc. (mg/l) | Exposure (min) | General appearance | Motility |
|---|---|---|---|
| 200 | 30 | 80% Lysed<br>20% Phase I | Increased and,<br>then decreased |
| 100 | 30 | 25% Phase I<br>75% Phase III | Increased, then<br>normal |
| | 60 | 15% Lysed<br>25% Phase I<br>60% Phases II & III | Normal |
| | 150 | 50% Lysed<br>40% Phase I<br>10% Phase III | 50% immobile<br>to reduced |
| | 300 | Occasional lysing<br>20% Phase I,<br>75% Phase III | Reduced |
| 80 | 30 | No change | Normal |
| | 60 | No change | Normal |
| | 150 | 25% Phase I<br>75% Phase III | Normal to<br>reduced |
| | 300 | 30% Lysed<br>20% Phase I<br>50% Phase III | 50% immobile<br>or else normal<br>and slightly<br>reduced. |

3–5 h. Survival time data were supplemented with morphological studies at intervals of minutes. Toxic and tolerated concentrations of lead nitrate were determined.

*T. pyriformis* was collected locally and cultured in hay infusion[10]. Stationary-phase organisms (3–4-day-old) were used throughout the study using 5 ml toxicant dilutions in cavity blocks (55 × 55 mm) for acute and chronic toxicity bioassay. For behavioural studies under short term exposure the methods followed were according to Schultz *et al*[11,12].

The duration of acute toxicity test was 5 h. Final concentrations for the test were 100, 200 and 400 mg/l. The samples were examined every 10 min during the first hour, and later every 30 min. Cell disintegration was taken as end-point in evaluating toxicity.

The appearance and motility of the cells exposed to different concentrations of lead nitrate ranging from 10 mg/l to 200 mg/l were examined under a phase contrast microscope and the percentage of animals altered was noted.

All experiments were carried out at room temperature and repeated at least thrice with not less than four replicates for each concentration.

The observations on appearance and motility of ciliates exposed to lead nitrate are summarized in table 1. The changes in cell morphology were continuous but the process can be subdivided into 3 main phases: (i) normal pear-shaped cells becoming angular and irregular in shape, (ii) irregular cells becoming spherical and varying in size, and (iii) spherical cells becoming uniform in size.

The ciliates were exposed over a range of concentrations from 10 mg/l to 400 mg/l. Motility of the organisms increased within a minute of exposure to 200 mg/l and then rapidly decreased and within 30 min all the cells lysed. A concentration of 100 mg/l produced marked increase in motility which returned to normalcy with increasing exposure. This was accompanied by reduction in size of the ciliate. There was slight alteration in morphology. Viable cells aggregated around detritus from lysed cells. Most of the cells lysed after 24 h exposure.

Ciliates exposed to 80 mg/l did not show any change in motility or cell shape. After 5 h of exposure many cells became immobile with slight alteration in morphology. Motility also decreased gradually with increasing exposure.

Exposure to lower concentrations, from 10 mg/l to 80 mg/l, did not result in cellular motility and all the ciliates remained normal. The number of cells affected was directly related to the concentration. In most cases swelling was followed immediately by lysis. In higher concentrations morphological changes were much more rapid than in lower concentration.

*T. pyriformis*, a holotrichous ciliate, shows a remarkable sensitivity to lead nitrate. The toxic effect of lead on ciliates depends on the concentration. In the present study a concentration of 250 mg/l killed the ciliates immediately, whereas 400 mg/l was the killing concentration for the hypotrichous ciliate *Oxytricha fallax*[13]. The response in the form of the observed morphological changes indicates the organism's attempt to reduce its surface area in contact with the toxicant. Similar observations were recorded in the earlier study on the ciliate *O. fallax* exposed to lead nitrate[13] and lead acetate[14].

The rounding up of cells and general mucocyst discharge have been observed in *T. pyriformis* exposed to a variety of biologically hazardous compounds[11,12,15,16]. These responses appear to be non-specific (defensive behaviour) at concentrations higher than those required to alter respiration. Thus increased respiratory activity becomes a direct reflection of increased cellular motility. The reduced $O_2$ uptake in higher concentrations of toxicant undoubtedly reflects a decrease in cell population due to lysis[12]. The lethal effect of lead nitrate to *T. pyriformis* appears to be cell lysis.

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# ANNOUNCEMENTS

## NATIONAL SYMPOSIUM ON RECENT DEVELOPMENTS IN MICROBIAL GENE TECHNOLOGY

The above symposium will be held at the Department of Microbiology, Osmania University, Hyderabad during 9–11 December 1988. The three day symposium will include the inaugural address, special lectures and presentation of papers on fundamental and applied aspects of microbial gene technology. The symposium will cover the following aspects: 1. Mutagenesis (site directed mutagenesis and gene replacement), 2. Protoplast fusion and hybridoma, 3. Gene amplification, 4. Strategies for gene cloning, 5. Diagnosis of microbial infections by *r*-DNA technology, 6. Novel approaches to anti biotic production.

Further particulars may be had from: Dr H. Polasa, Director of Symposium, Department of Microbiology, Osmania University, Hyderabad 500 007.

## WORKSHOP ON RANDOM VIBRATION AND CHAOS IN NONLINEAR SYSTEMS

The above workshop will be held during December 26–30, 1988 at the Department of Civil Engineering, Indian Institute of Science, Bangalore.

The main objective of the workshop is to expose scientists and engineers from the Universities and Research and Development Laboratories to the current state of the art and further enthuse them to investigate nonlinear phenomenon in their own field of interest.

Nonlinear behaviour of structural systems is of importance in the analysis and design of aerospace engineering, nuclear engineering and civil engineering systems. The workshop will deal with the analysis of nonlinear systems under random dynamic loading which model gusts the earthquake forces. An introduction to the recently emerging field of chaotic behaviour under deterministic inputs would also be presented at the workshop. The activity is sponsored by INSA, ARDB and NAL.

Further particulars may be had from: Prof. R. N. Iyengar, Convener, or Prof. S. Anantha Ramu, Co-convener, Department of Civil Engineering, Indian Institute of Science, Bangalore 560 012.

## INDIAN SOCIETY OF HYPERTENSION

Meetings are arranged on 'the Problems in drug treatment in hypertensive emergencies in developing countries' and 'Future of traditional (Herbal) medicines in hypertension' during the 4th International Conference on Hypertension in Blacks, Nairobi, Kenya from June 28 to July 2, 1989.

For abstract submission, travel grants, etc. please write to Dr Shailendra Vajpeyee, Indian Society of Hypertension, B. J. Medical College, Ahmedabad 380 016.