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Environmental Contamination and Toxicology

Automated Respirometer Method for Microbial Toxicity Assessment of Low-Level Zinc Contamination in Soil

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Zinc (Zn) is an essential trace element for all living organisms, including humans. Its toxicity is generally low, both in soil and water. More commonly, however, low levels of Zn in soils in many parts of the world leads to Zn deficiency in plants and animals which can result in nutritional deficiencies of Zn in humans. The background levels of Zn in soil range from 10 to 300 mg/kg soil worldwide, but concentrations of Zn above ambient levels are primarily a result of anthropogenic activity (Parsons 1977) which can adversely affect the surrounding ecology (Jordan and Lechevalier 1975). For example, microorganisms in the soil respond to Zn contamination in a variety of ways. These include population loss (Hicks et al. 1990), changes in the population structure (Chaudri et al. 1993), and physiological activity (Bitton and Dutka 1986); or, they may be tolerant of the chemical and exhibit no adverse effects (Babich and Stotzky 1985). Factors such as the metal's bioavailability, the numbers and species of microbes present, the organism's duration of exposure to the metal, and the physiochemical nature of the soil, control these responses (Bitton et al. 1989).

Because microbial-based toxicity approaches to assess the changes in ecosystem processes are not well defined for soil applications (Bitton et al. 1989), our laboratory has developed an automated respirometer capable of measuring responses of anthropogenic stress in microcosm systems. This procedure permits the analysis of many separate cultures with amendment variations and sampling intervals dictated by the experimental design. In this study, we report the utility of this respirometer to assess Zn induced changes in native bacterial and fungal populations and the comparative effect on soil respiration.

MATERIALS AND METHODS

The soil used in this study was a Veneta silt loam common to Benton County, OR, USA. The physical nature of this soil and its chemical content is reported elsewhere (Knezevich 1975; Hendricks and Rhodes 1992).

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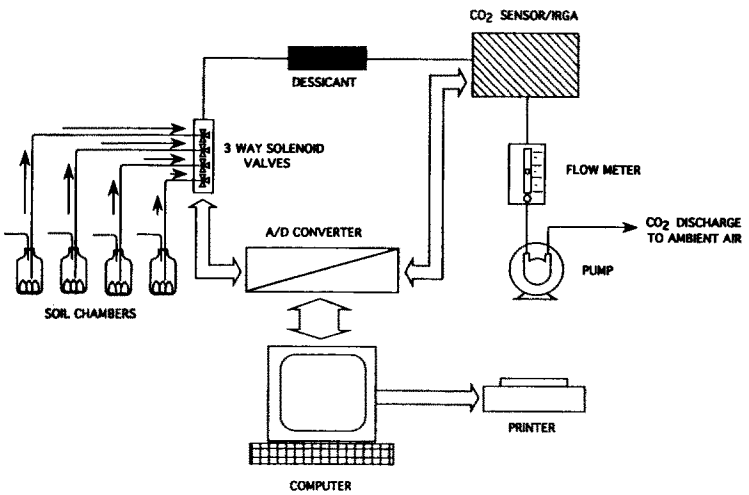

Figure 1. Schematic design of the automated respirometer.

The respirometer system (Fig. 1) was constructed from readily available laboratory glassware and electronic hardware. Six large-mouthed, 4 liter glass jars served as growth chambers. A series of four wide-mouthed glass bottles (110 ml) served as culture vessels. For a particular Zn concentration, 25.0 g (dry wt) of sifted soil was moistened to 55 % water holding capacity and placed into each bottle. The chambers were closed each with a two-hole rubber stopper fitted with polyethylene tubing used for sampling the gas phase. Laboratory air was drawn through each chamber only at the time of sampling by the systems pump (Ametek Inc., Pittsburgh, PA) and passed through a $CaSO_4$ desiccant (W. A. Hammond Drierite Co., Xenia, OH) to dry the respiratory gas before it was measured by the $C0_2$ sensor/ Infrared gas analyzer (Ametek Inc., Pittsburgh, PA.). Flow was controlled by a gas flow meter (Ametek Inc., Pittsburgh, PA) regulated at 0.27 standard liters per minute (slpm). Timing of the maximum CO_2 concentration, sampling of the gas phase and data calculation was accomplished automatically with a computer program written in BASIC. A personal computer (Leading Edge Inc., Canton, MA) fitted with a Dascon I control board (Metrabyte Corp., Stoughton, MA) controlled the timing of opening and closing of 3-way Minimatic solenoid valves (Clippard Instrument Co., Cincinnati, OH) so that the gas volume and CO_2 concentration of each culture vessel could be measured. At the time of sampling, the % CO_2 concentration was determined, and the maximum % CO_2 value was retained in the computer memory and used in the mg C g^{-1} Dry Soil calculations (Page et al., 1982). For each measurement, the date and time, sample number, flow rate, incubation temperature and the amount of CO_2 in each chamber were calculated, printed and also stored in computer memory for later statistical analysis.

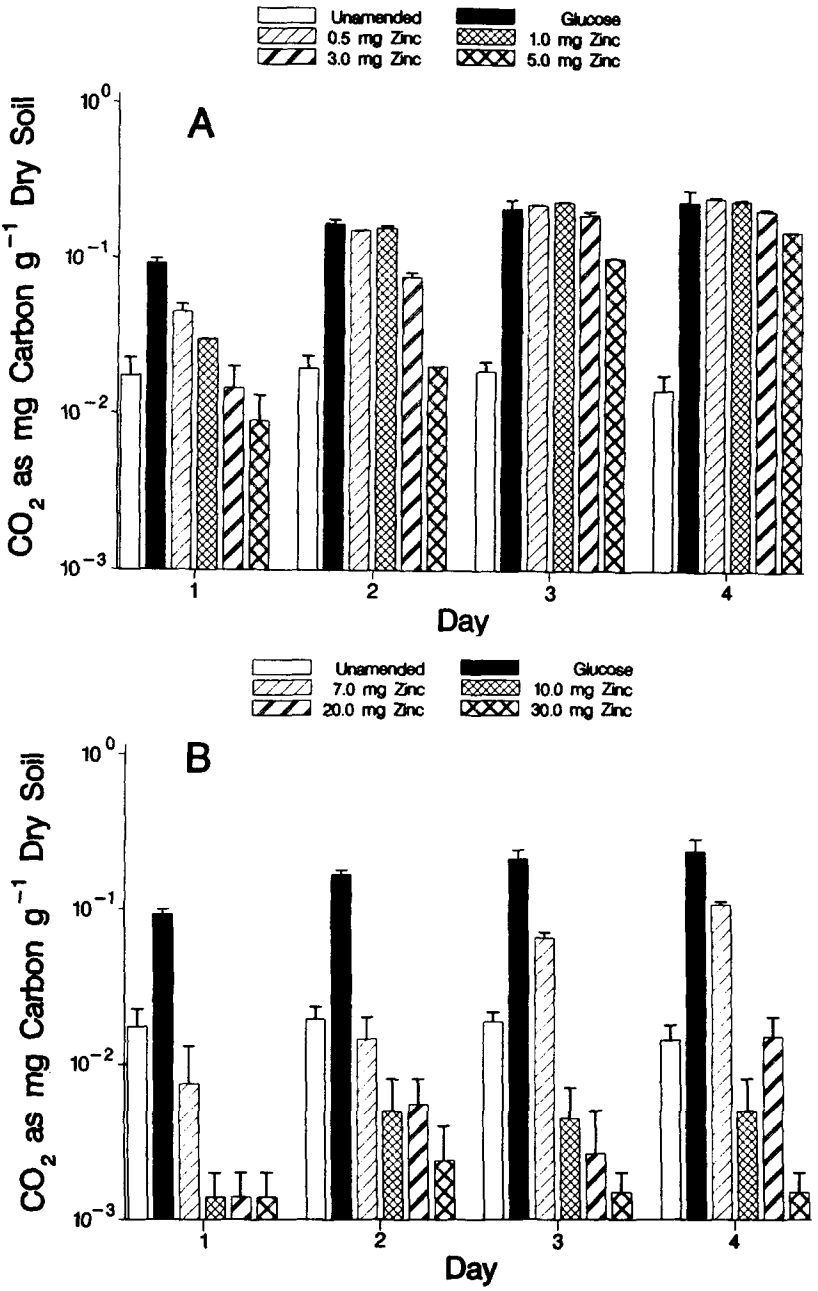

Figure 2. Daily respiration rates in the presence of low (A) and high (B) levels of zinc.

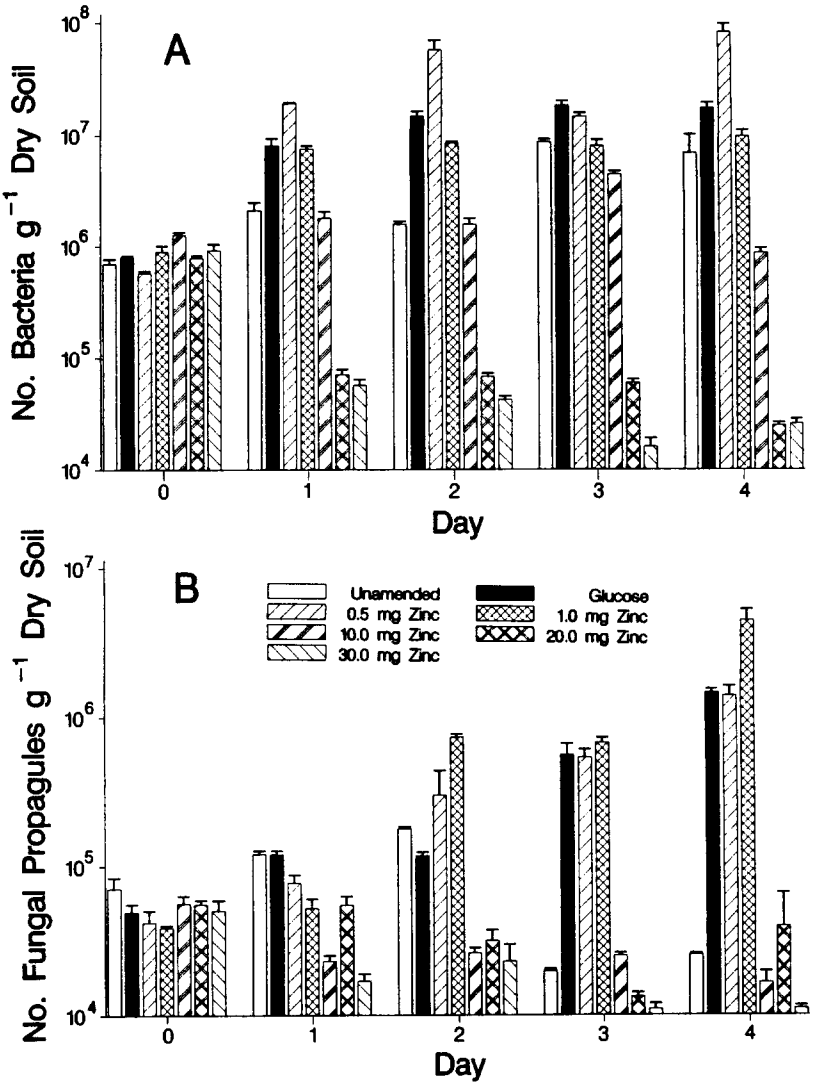

Figure 3. Daily viable bacterial counts (A) and fungal propagules (B) in the presence of various concentrations of zinc.

Experiments were initiated by adding a known concentration of Zn and a glucose amendment (10 mg glucose g^{-1} dry soil) to 25 g of soil in each bottle contained in two chambers. Two additional chambers served as unamended controls without either Zn or glucose to measure endogenous soil respiration, and the other two contained soil amended only with glucose. These controls were ran for each Zn concentration tested. Concentrations of Zn (as $ZnCl_2$) tested in this study for potential effects on respiration were 0.5, 1.0, 3.0, 5.0, 7.0, 10.0, 20.0, 30.0 mg Zn g^{-1} dry soil. This design permitted duplicate analyses to be made from

Zn-treated soil at each concentration tested. CO_2 measurements were obtained every 6 h and summed to provide a 24 h value. Two culture bottles at each Zn concentration, including the endogenous and glucose controls, were removed daily from each chamber at random and analyzed for wet and dry soil weight and pH using methods of Page et al. (1982). Viable bacterial and fungal propagule determinations were made in triplicate on Soil Extract and Rose Bengal-Streptomycin Agars respectively (Stotzky et al. 1993). In this study, we report the effect of 0.5, 1.0, 10.0, 20.0, and 30.0 mg Zn g^{-1} on bacterial and fungal populations.

The results of all experiments were analyzed using SAS (SAS Institute., Cary, NC) statistical analysis software. A repeated measures analysis was used to test for significant differences between groups and the standard error was calculated for each sampling. Unless otherwise noted, the decision level for hypothesis testing was 0.05.

RESULTS AND DISCUSSION

While Bååth (1989) has reported that low concentrations of most heavy metals do not significantly affect respiration, we did see adverse effects on soil respiration but it was not apparent for all concentrations tested (Fig. 2). The effect of Zn was not noticeable at concentrations of 0.5 to 5.0 mg (Fig. 2A), but at concentrations of 10.0, 20.0, and 30.0 mg, Zn significantly inhibited respiration (Fig. 2B). At very low levels (0.5 and 1.0 mg), Zn appeared to be beneficial to microbial respiration, as indicated by increases in CO_2 evolution beyond the glucose control. Because Zn is an essential micronutrient, it was expected that the metal would be benefit cellular processes at very low concentrations, though the sample size used in this study was not sufficient to show these values to be significant. In comparing daily CO_2 values for Zn levels ranging between 0.5-5.0 mg Zn (Fig 2A), it is apparent that the initial lag in respiration on Day 1 was not maintained throughout the four day incubation period. By Day 4, there was no significant difference between the cultures containing only glucose and those treated with Zn. At higher concentrations (7.0-30.0 mg Zn), recovery was not apparent (Fig. 2B).

Native bacterial populations were not effected by Zn levels up to 1 mg Zn as compared to the glucose control (Fig. 3). This further suggests that growth of the community was stimulated at the 0.5 mg Zn level, but a concomitant stimulation of respiration was not observed (Fig. 2A). Our laboratory has previously observed that physiological process activity can be shut down in the presence of certain herbicides while portions of the microbial population remained viable (Hendricks and Rhodes 1992). Since various native populations of micro-organisms lack a uniform sensitivity to heavy metals and some may be tolerant to Zn (Babich and Stotzky 1985), it is not unreasonable to expect a portion of the community in a mixed culture system be able to respire in the presence of the metal. A reduced level of respiration equal to or less than the glucose control

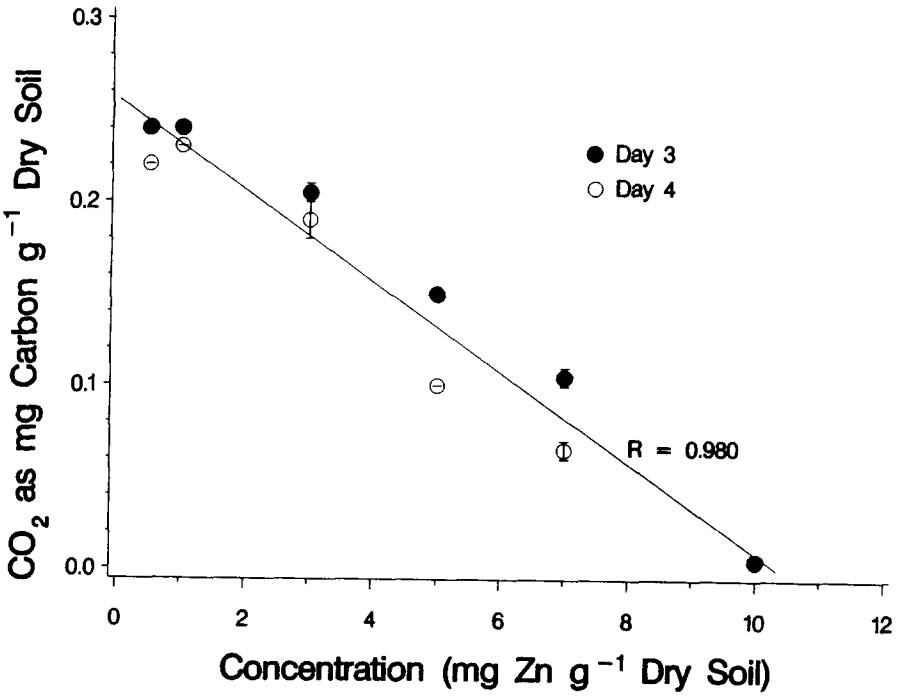

Figure 4 . Regression analysis of the effect of zinc on native bacterial and fungal respiration.

might be expected as well. In our system, maximum respiration is maintained for Zn concentrations through about 5.0 mg Zn (Fig. 2A) while showing a steady decline in bacterial and fungal numbers (Figs. 3A and 3B). When the concentration of Zn reached 10.0 mg, respiration and the microbial populations decrease dramatically. The major impact was on the bacterial component, but the bacterial and fungal community stabilized at about 1 x 10^4 Colony Forming Units (CFU) g^{-1} dry soil. For low levels of Zn, respiration was considerably more sensitive endpoint to measure ecological stress.

The overall sensitivity of the assessment procedure is shown in Fig. 4. Respiration rates increased throughout the course of the experiments for Zn concentrations ranging between 0.5 to 7 mg g^{-1} dry soil. This is shown by the striking linear correlation $R^2 = 0.98$ between the concentrations of Zn and the corresponding day 3 and 4 respiration rates. It is significant that respiration did not change with time for the higher concentrations of Zn although Zn-resistant bacteria do begin to appear after day 4 (Fig. 3A). This suggests that after four days incubation, Zn-tolerant forms may begin to affect the interpretation of results.

In conclusion, over the last several years a variety of environmental protection legislation has been developed world wide that requires information be determined about the safety of anthropogenic chemicals and wastes to the public health and the environment. To assess the safety of these chemicals, efficient methods are needed that can develop toxicological information as rapidly and economically as possible while maintaining the desired level of precision and accuracy (Bitton and Dutka 1986). Microorganisms are especially suited for use in rapid screening procedures, because of their unique biochemistry and their ease of handling (Cooney and Pettibone 1986). These characteristically result from the capability of microorganisms to produce successive generations that can uniformly respond to changes in their environment in a predictable fashion (Bitton et al. 1989). Moreover, animal species have physiological and metabolic properties related more to individually discrete organisms and generally possess large interspecies differences which, in addition to their care, add considerably to laboratory costs (Nyholm et al. 1992).

In this study, we found the automated respiration method to be rapid, easy to use, and reliable. The screening potential of the automated respiration system has a variety of environmental and public health applications, and can effectively be used as a viable alternative to more traditional methods of toxicity testing that can be expensive and cumbersome.

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