Determination of Acute Zn Toxicity in Pore Water from Soils Previously Treated with Sewage Sludge Using Bioluminescence Assays

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The effects of increasing concentrations of Zn and Cu in soil pore water from soils of a long-term sewage sludge field experiment on microbial bioluminescence were investigated. Concentrations of total soluble Zn, free Zn^{2+}, and soluble Cu increased sharply in soil pore water with increasing total soil metal concentrations above 140 mg of Zn kg^{-1} or 100 mg of Cu kg^{-1}. Two luminescence bioassays were tested, based on two bacteria (*Escherichia coli* and *Pseudomonas fluorescens*) with the *lux* genes encoding bacterial luminescence inserted into them. The bioluminescence response of the two microorganisms declined as total soil Zn, soil pore water soluble Zn, and soil pore water free Zn^{2+} concentrations increased. The EC$_{25}$ values for *E. coli* and *P. fluorescens* were 1.3 ± 0.2 and 4.3 ± 0.5 mg L^{-1} on a free Zn^{2+} basis, respectively. The EC$_{50}$ values were 2.5 ± 0.2 and 9.6 ± 0.9 mg of free Zn^{2+} L^{-1}, respectively. Copper had no significant effect on bioluminescence in the two assays, even at the largest soil pore water concentration of about 620 μg L^{-1}, corresponding to a total Cu concentration in bulk soil of about 350 mg kg^{-1}. Thus, the decline in bioluminescence of the two assays was ascribed to increasing soil pore water free Zn^{2+} and not soluble Cu.

Introduction

One of the major sources of heavy metals to individual agricultural fields is through the use of sewage sludges as soil amendments. Sewage sludge additions, although carefully regulated by legislation formulated to avoid phytotoxic effects of metals on plants and possible entry into the human food

chain, vary from country to country (*1*). All the regulations that define a maximum concentration in the receiving soil are based on total soil metal concentrations, which take no account of the bioavailable fraction in the soil pore water. However, it is now generally accepted that plant uptake of metals depends on metal concentrations in soil pore water and particularly the free ionic form and that this is also the fraction which can be toxic to the soil microflora and fauna at larger concentrations (*2−4*). Hence, it is the concentration of a metal in its soluble bioavailable form that is crucial to understanding metal toxicity, and therefore, metal loading limits for agricultural soils receiving sewage sludges need updating to allow for this.

The growth of an organism follows a typical dose–response curve, where, if an essential element is deficient, a linear increase in growth occurs with increasing concentration of the element in the environment. At maximum growth, further increases in essential element concentration do not induce either beneficial or adverse effects on the organism. This lack of response is due to the organism actively and/or passively resisting further uptake of the element or tolerating increased uptake. In addition, the buffering capacity of the soil regulates the available fraction of the element through processes such as adsorption, desorption, and chelation. However, a point is reached where the intrinsic tolerance and/or exclusion by the organism and buffering by the soil begins to break down as further additions of the element are made, leading to toxicity.

There has been much research to determine the metal concentrations at which toxicity occurs to soil organisms (*5−7*). Bååth (*8*), in a comprehensive review, collated information from a large literature base and found wide variations in the concentrations at which metals caused toxicity to soil organisms. There are three main reasons that probably explain this variation: (a) in all cases, only total soil metal concentrations were measured; (b) literature studies consist of tests on many disparate types of organisms or microbial processes, which differ in their sensitivity to metals; and (c) microbial testing methods are poorly standardized. To be able to widely interpret toxic thresholds, standardized toxicity tests that have comparable responsiveness need to be employed, while at the same time making measurements of the toxic species of metals in the soil pore water. Further studies are therefore of paramount importance in order to formulate a rational and generic basis for assessing thresholds of toxicity for heavy metal pollutants in soils with differing physicochemical properties.

The use of microbial bioluminescence bioassays in conjunction with soil pore water free ion concentrations may be a reliable tool for determining thresholds of toxicity. Prokaryotic bioluminescence is the emission of light as a result of the oxidation of aldehyde (RCHO) and reduced flavin mononucleotides (FMNH$_2$) by molecular oxygen to the corresponding fatty acid (RCOOH), flavin mononucleotide (FMN), and water. The reaction is catalyzed by the enzyme luciferase and is intrinsically linked to cell metabolism (*9*). Naturally bioluminescent marine bacteria such as *Vibrio fisheri* have been used as toxicity tools in which a decrease in their luminescence reflects toxicity induced by pollutants (*10*). Commercially available biosensors, such as Microtox, are based on marine bacteria and are therefore not ecologically relevant for the soil environment.

In this study, we report on the effects of free Zn^{2+} and soluble Cu in soil pore water on the bioluminescence of the genetically modified microbial biosensors *Escherichia coli* HB101 pUCD607 (a representative microorganism in sewage

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TABLE 1. Mean Soil and Soil Pore Water Chemical Anaylsis from Plots of the Gleadthorpe Long-Term Field Experiment

| | | | total metal concn (mg kg^{-1}) | | | | soil pore water concn (mg L^{-1}) | | | | | |
| | soil pore water pH | DOC[c] (mg L^{-1}) | | | | | total pore water concn | | | | M^{2+} pore water concn | |
treatments[a]			Zn	Cu	Ni	Cd	Zn	Cu	Ni	Cd	Zn^{2+}	Cd^{2+}
no sludge	6.53	22	44	9	7	0.18	0.06	0.013	0.003	0.0001	0.053	0.00003
control uncomtd sludge	6.29	15	53	11	7	0.28	0.08	0.010	0.005	0.0002	0.029	0.00009
Zn treament 1	6.03	25	141	16	9	0.22	1.38	0.025	0.020	0.0009	0.657	0.00067
Zn treatment 2	5.72	29	278	15	8	0.24	5.47	0.030	0.025	0.0018	2.627	0.00110
Zn treatment 3[b]	5.66	29	291	21	9	0.80	10.91	0.045	0.055	0.0093	5.087	0.00575
Zn treatment 4[b]	5.42	29	441	22	9	0.87	19.71	0.045	0.075	0.0144	9.199	0.00868
Zn:Cu treatment 1[b]	6.21	31	94	58	9	0.24	0.68	0.120	0.020	0.0012	0.380	0.00086
Zn:Cu treatment 2[b]	5.31	28	189	86	9	0.52	5.77	0.175	0.070	0.0085	2.398	0.00459
Zn:Cu treatment 3[b]	5.28	30	230	159	9	0.54	10.85	0.360	0.115	0.0026	4.755	0.00146
Zn:Cu treatment 4[b]	5.43	37	328	223	9	0.60	18.69	0.600	0.155	0.0042	6.277	0.00228
Cu treatment 1[b]	5.95	26	75	150	8	0.24	0.40	0.250	0.040	0.0013	0.208	0.00090
Cu treatment 2	6.22	23	57	160	7	0.18	0.26	0.240	0.015	0.0006	0.108	0.00065
Cu treatment 3[b]	6.04	26	69	254	9	0.27	0.43	0.340	0.045	0.0015	0.184	0.00095
Cu treatment 4[b]	5.54	28	73	349	9	0.25	1.02	0.620	0.065	0.0023	0.399	0.00147

[a] All treatment values are means of two replicate plots, except for the no sludge control treatment where $n = 4$. [b] Treatments receiving a second application of naturally contaminated Zn and/or Cu sludges in 1986. [c] DOC, dissolved organic carbon in soil pore water.

sludge) and *Pseudomonas fluorescens* 10586rs pUCD607 (a rhizobacterium, ubiquitous in soil). The genes encoding bacterial bioluminescence (*lux*CDABE, originally isolated from *V. fisheri*) were inserted into *E. coli* and *P. fluorescens* using the multicopy plasmid pUCD607 (*11*). The soil pore water was extracted from soil samples taken from a long-term field experiment to which sludges contaminated with Zn, Cu, or Zn plus Cu were added in the past. The addition of these sludges at different rates has resulted in a range of soil metal concentrations, making this site a valuable resource for dose−response studies.

Materials and Methods

Soil Used. The soil samples were from a long-term sewage sludge field experiment at ADAS Gleadthorpe in Nottinghamshire, U.K. This site was first established in February 1982 on a loamy sand textured soil (6% clay, 2% organic matter) of the Newport association (Typic Quartzipsaments). Pressed sludge cakes from a single source were enriched with metal salts of Zn, Cu, or Zn plus Cu and applied in appropriate mixtures to attain a range of soil metal concentrations (Table 1). Control (non-metal-enriched) sludges were used where necessary to make up quantities so that all sludged plots received 100 t ha^{-1} dry solids in 1982. Twelve metal treatments were created along with an uncontaminated sludge cake control and a no-sludge control to give a total of 14 treatments. The design was a randomized block with two replicate plots of each treatment. The no-sludge control treatment had four replicate plots. Topsoil metal concentrations after 1982 were found to be lower than anticipated due to horizontal dispersion of the metals caused by cultivation and also by ploughing to 30 cm depth. So, in 1986, pressed sludge cakes from two sources naturally rich in Zn and Cu were added to selected treatments (different quantities of dry solids were added to different treatments to achieve the required metal levels). To prevent further horizontal soil movement during cultivation, the plots were isolated in October 1993 using oil-tempered hardboard to a depth of 0.4 m. In 1996, the total soil organic carbon content in all plots of the experiment was between 1 and 1.5%, which is near background levels for this particular soil type.

A variety of arable and grass crops have been grown on the site since 1982. Phosphorus and potassium were applied to all treatments at recommended rates based on soil analysis. When cereals were grown, all treatments received inorganic fertilizer N (as ammonium nitrate) at recommended rates; no inorganic fertilizer N was applied to legumes (*12*). Lime or elemental sulfur has been applied where necessary to maintain the soil pH at about 6.5.

Soil Pore Water Extraction. Moist soil samples were collected from selected treatments using a Dutch auger to 25 cm depth in November 1996. The samples were sieved moist to <3 mm, thoroughly mixed, and separated into 1 kg (oven-dried basis) portions to give triplicate samples for each plot. Rhizon soil moisture samplers from Rhizosphere Research Products (Wageningen, Holland) were used to extract soil pore water. These samplers consist of a length of inert porous (0.2 μm) plastic tubing capped with nylon at one end through which the soil pore water is extracted. The other end is attached to a 5 cm length of polyethylene tubing joined to a female luer lock. One sampler was placed diagonally from the lip of the pot to the base into each of three replicate 1.0 kg (dry weight) pots of the chosen soils. The soil was then made up to 50% water holding capacity (WHC) by adding deionized water to the saucer of the pot. Two weeks prior to extraction, the soils were made up to 75% WHC. Acid-washed disposable syringes, attached to the luer lock, were used to extract soil pore water from the soil. This method had been detailed elsewhere (*13*).

Chemical Analysis of Soil and Soil Pore Water. The soil samples were digested with concentrated nitric and hydrochloric acids, following the method of McGrath and Cunliffe (*14*). "Total" metal concentrations were determined by inductively coupled-plasma atomic emission spectrometry (ICP-AES; Accuris) and graphite furnace-atomic absorption spectrometry (GF-AAS) for Cd. Each extract of soil pore water was separated into several subsamples for analysis. Two aliquots were acidified with 5% (v/v) HCl and used for determining total solution metal concentrations by ICP-AES and GF-AAS for Cd. Other aliquots were used for determining pH and dissolved organic carbon (DOC).

Free Zn^{2+} and Cd^{2+} concentrations in soil pore water were determined using a calcium-saturated cation-exchange resin method. In this method, metal concentrations are determined before and after equilibrium is reached with the cation-exchange resin. The proportion of total metal in soil pore water present as free metal can be calculated by comparison with a reference experiment. This method is described in detail elsewhere (*15*). Because much of the Cu in soil pore water is complexed with DOC, it was not possible to estimate small concentrations of free Cu^{2+} by this method. However, as the toxic threshold for Cu was not reached, speciation analysis of Cu was unnecessary.

Luminescence-Based Bioassays. The luminescence-based bioassays consisted of two microorganisms with the genes encoding bacterial luminescence inserted into them. The organisms used were *E. coli* HB101 pUCD607 and *P. fluorescens* 10586rs pUCD607. The *E. coli* and *P. fluorescens* biosensors were resuscitated from freeze-dried vials following the protocols of Duffy (*16*) and Paton et al. (*11*), respectively. All bioassays consisted of adding 50 μL of the appropriate cell suspension to 450 μL of the test solution in a cuvette at 15 s intervals. The luminescence of the cells was then measured in a Bio-Orbit 1253 luminometer after exposure times of 145 and 15 min for *E. coli* and *P. fluorescens*, respectively. These exposure times were chosen because, in preliminary studies (data not shown), they gave optimum response from the biosensors to pollutants in soil pore water. All bioassays were carried out in triplicate, and the luminescence was expressed as a percentage of the luminescence measured in soil pore water from control treatments, which received uncontaminated sludge.

Statistical Analyses. Genstat 5 (*17*) for Windows (3rd edition) was used to carry out all statistical analysis. All metal concentrations were log transformed to normalize the data. Multiple linear regression analysis (MLR) and then stepwise multiple linear regression (SMLR) analysis were carried out in order to predict the response of the bioassays to the mixture of heavy metals in soil pore water. Results of the regression analysis are expressed as the proportion of variance accounted for (i.e., adjusted R^2). Nonlinear regression analysis was carried out using the Gompertz model, with four parameters, for *E. coli* and using the exponential model for *P. fluorescens* after log transformation of the soil pore water Zn and free Zn^{2+} data. For total soil Zn, the exponential model was used as the Gompertz model was found to be inappropriate. Curves were fitted to the bioluminescence data with Zn as the independent variable (i.e., *x* values). The Gompertz and exponential equations used were $y = y_0 + a \exp[-\exp\{-b(x - x_0)\}]$ and $y = y_0 + ab \exp[x]$, respectively, where *y* is the percent of control bioluminescence, *x* is Zn, and y_0, x_0, *a*, and *b* are parameters used in the equations. The adjusted R^2 values for the fitted curves were also determined using the equations.

Results and Discussion

Chemical Analysis. Compared to the no-sludge control, plots receiving control sludges contained slightly elevated Zn and Cu but not Ni concentrations (Table 1). This was because the uncontaminated sludges contained some metals at low concentrations. There were no Cd treatments in this experiment, and the sludges applied contained little Cd. This resulted in relatively low total soil Cd concentrations and very low soil pore water Cd and free Cd^{2+} concentrations in all treatments (Table 1). The addition of sludges at different rates gave total soil Zn and Cu concentrations up to 10 times the concentration of the control treatment for Zn and more than 50 times the concentration of the control treatment for Cu at their largest additions (Table 1). At total soil Zn concentrations less than about 140 mg kg^{-1}, there were only small increases in total soil pore water Zn or free Zn^{2+} with increasing total soil concentrations (Table 1). However, at total soil concentrations above 140 mg of Zn kg^{-1}, there was a linear increase in free Zn^{2+} in soil pore water ($R^2 = 0.90$, $P < 0.001$; Table 1). Similarly, at total soil pore water Zn concentrations <2 mg L^{-1}, there were only small increases in soil pore water free Zn^{2+} with increasing soil pore water soluble concentrations. However, at total soil pore water concentrations >2 mg L^{-1} there was a linear increase in free Zn^{2+} in soil pore water ($R^2 = 0.97$, $P < 0.001$; Figure 1). There were only small increases in soil pore water Cu at total soil Cu concentrations <100 mg kg^{-1}, but above this concentration, a linear increase in pore water Cu occurred with

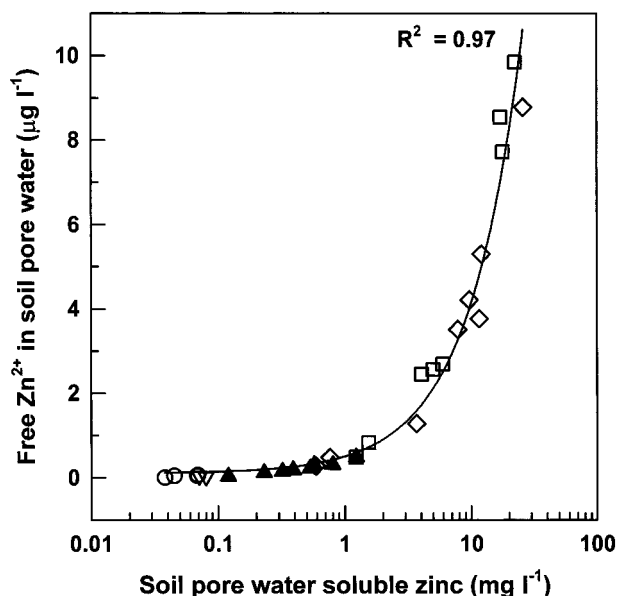FIGURE 1. Relationship between soil pore water soluble zinc and free Zn^{2+} in soil pore water. Treatments: ○, no sludge control; ▽, uncontaminated control sludge; □, Zn-contaminated sludge; ◇, Zn-plus Cu-contaminated sludges; ▲, Cu-contaminated sludge.

increasing soil concentration (Table 1; graph not shown). The very small increase in soil pore water Zn and Cu with increasing soil metal concentrations in the lower range was almost certainly due to the buffering effect of the soil. However, a point was reached where soil buffering no longer regulated pore water concentrations and an increase in soil pore water Zn and Cu occurred with increasing soil concentrations (Table 1).

The soil pore water pH values and dissolved organic carbon (DOC) concentrations ranged from 5.31 to 6.53 and from 15 to 37 mg L^{-1}, respectively (Table 1). The DOC concentrations in the soil solutions were generally higher in treatments that received a second sludge application in 1986 (Table 1). However, all DOC concentrations were of the same order of magnitude, reflecting oxidation of the added sludge organic matter during the 10 year period since sludge was last applied. Total soil organic carbon content was also similar (1−1.5%) in all plots of the experiment, again reflecting on its long-term nature.

Effect of Free Zn^{2+}, Cd^{2+}, Cu, and Ni in Soil Pore Water on Bioluminescence. The *E. coli* biosensor was more sensitive to metals in soil pore water than the *P. fluorescens* biosensor (Tables 2 and 3; Figures 2−4). Some differences are to be expected since *E. coli* is a gut dwelling organism, whereas *P. fluorescens* is found in soil. MLR analysis pointed to soil pore water pH and free Zn^{2+} as the main predictors of the bioluminescence response from *E. coli* and *P. fluorescens* (Table 2). Dissolved organic carbon (DOC) also had a significant effect, although the effect was not as large as that of pH and free Zn^{2+} (Table 2). Soil pore water pH, free Zn^{2+}, and DOC together accounted for 82% and 66% of the variance in bioluminescence of *E. coli* and *P. fluorescens*, respectively. SMLR analysis confirmed soil pore water pH and free Zn^{2+}, but not DOC, as the best predictors of the luminescence response from the two biosensors (Table 2). These two variates accounted for 81% and 66% of the variance in the data sets for *E. coli* and *P. fluorescens*, respectively. In contrast, soil pore water Cu, Ni, and free Cd^{2+} had no significant effect on the luminescence of the two biosensors.

The apparent dominant effect of soil pore water pH on the decline in luminescence of the two biosensors, as predicted by MLR and SMLR analysis, can be explained by

TABLE 2. MLR and SMLR Analysis of Luminescence from Biosensors against Soil Pore Water Chemical Properties

	multiple linear regression variance ratio[a]						% variance accounted for	stepwise multiple linear regression variance ratio[a]		% variance accounted for
biosensor	pH	DOC[b]	free Zn^{2+}	Cu	free Cd^{2+}	Ni		pH	free Zn^{2+}	
E. coli	79*	9*	25*	0.03	3.0	1.00	82	78*	33*	81
P. fluorescens	33*	9*	10*	1.50	0.6	0.97	66	32*	18*	66

[a] Variance ratio >5.0 indicates a significant effect at $P \leq 0.05$. An asterisk (*) indicates $P < 0.001$. [b] DOC, dissolved organic carbon.
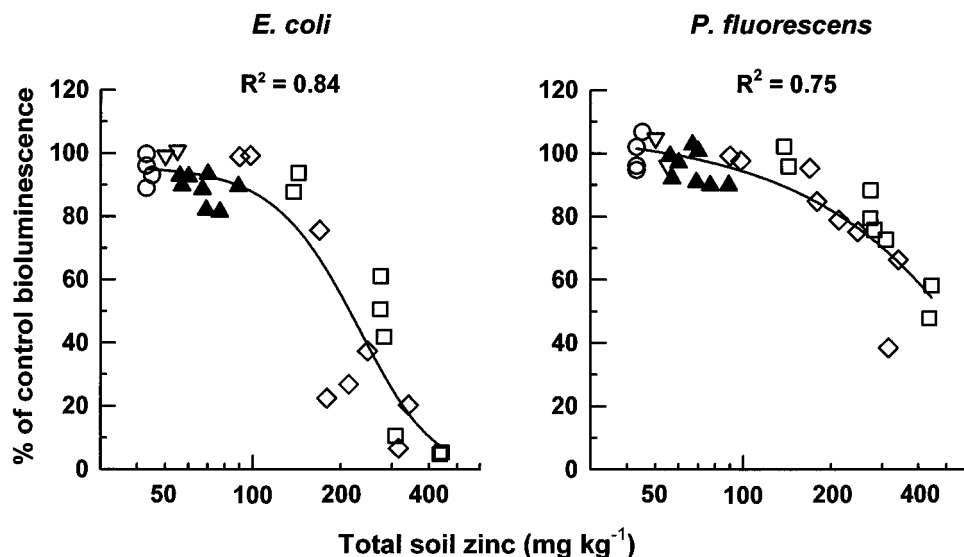

FIGURE 2. Relationship between total soil zinc and percent of control bioluminescence for the two biosensors. Treatments: ○, no sludge control; ▽, uncontaminated control sludge; □, Zn-contaminated sludge; ◇, Zn- plus Cu-contaminated sludges; ▲, Cu-contaminated sludge.
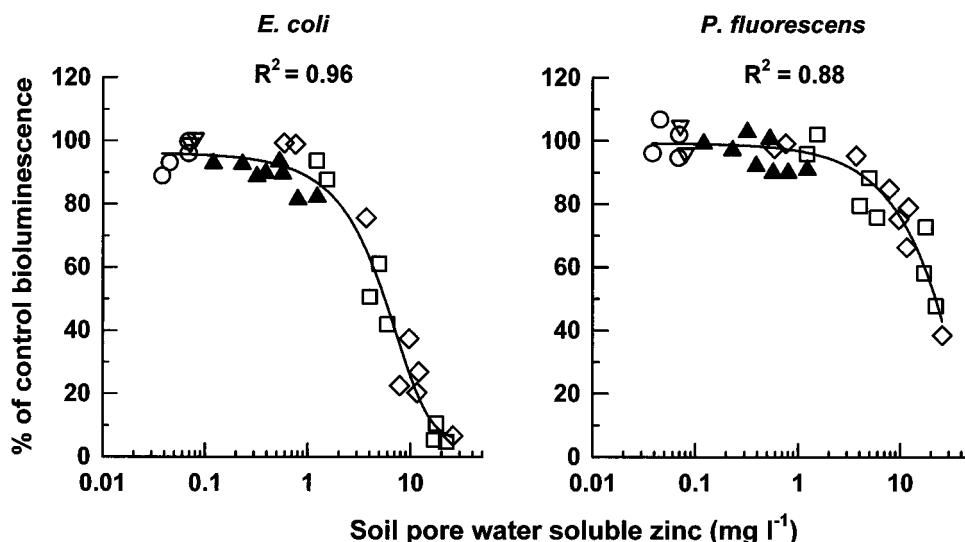

FIGURE 3. Relationship between soil pore water soluble zinc and percent of control bioluminescence for the two biosensors. Treatments: ○, no sludge control; ▽, uncontaminated control sludge; □, Zn-contaminated sludge; ◇, Zn- plus Cu-contaminated sludges; ▲, Cu-contaminated sludge.

the fact that both pore water pH and metal concentration were autocorrelated in the analysis. The luminescence response from the two biosensors used here has been shown to be stable across the pH range of 4.5−7.0 (*18, 19*). Therefore, the decrease in bioluminescence was likely to be due to increased free Zn^{2+} concentration in soil pore water and not a simple pH effect.

From the nonlinear regression analysis, total soil Zn explained 84% and 75% of the variance in bioluminescence of *E. coli* and *P. fluorescens*, respectively, (Figure 2). Soil pore water Zn accounted for 96% and 88% of the variance in the

luminescence data from the two biosensors, respectively (Figure 3). Similarly, pore water free Zn^{2+} accounted for 96% and 84% of the variance in the bioluminescence data from *E. coli* and *P. fluorescens*, respectively (Figure 4). The relationship between total soil metal concentrations and luminescence from the two biosensors was not as good as that between total pore water soluble Zn and luminescence and pore water free Zn^{2+} and luminescence, although all three relationships gave high R^2 values (Figures 2−4).

It is thought that the free metal ion is the toxic species (*4, 20*), and Knight et al. (*13*) have shown that soils with
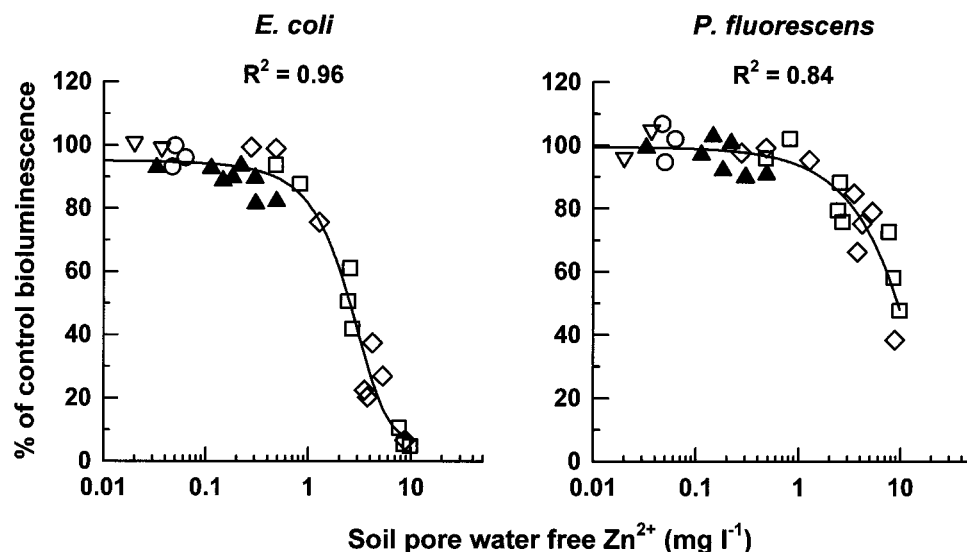
FIGURE 4. Relationship between soil pore water free Zn^{2+} and percent of control bioluminescence for the two biosensors. Treatments: $\bigcirc$, no sludge control; $\bigtriangledown$, uncontaminated control sludge; $\square$, Zn-contaminated sludge; $\diamond$, Zn- plus Cu-contaminated sludges; $\blacktriangle$, Cu-contaminated sludge.

TABLE 3. Calculated Critical Zn Values for Reduction in Bioluminescence of the Two Biosensors

biosensor		soil pore water (mg L^{-1})		total in bulk soil (mg kg^{-1})
		Zn	free Zn^{2+}	Zn^b
E. coli^a	EC$_{25}$^{c}	2.6 (0.3)^e	1.3 (0.2)	127 (14)
	EC$_{50}$^{d}	5.6 (0.4)	2.5 (0.2)	223 (17)
P. fluorescens^b	EC$_{25}$	10.2 (0.9)	4.3 (0.5)	275 (19)
	EC$_{50}$	22.5 (1.6)	9.6 (0.9)	438 (31)

^{a} Calculated using the Gompertz model. ^{b} Calculated using the exponential model. ^{c} EC$_{25}$, effective concentration causing 25% reduction. ^{d} EC$_{50}$, effective concentration causing 50% reduction. ^{e} Standard errors of the EC values.

differing physicochemical properties can have high total soil metal concentrations that give high total soil pore water concentrations but very low free ionic concentrations that are not likely to be toxic. So, to enable comparisons with other experiments and soil types, the free Zn^{2+} concentrations are also given in this paper and are regarded as being of more general significance for comparison between soils. However, because this experiment is on a single soil type and DOC concentrations did not vary greatly when Zn was toxic, total soil Zn, pore water Zn, and pore water free Zn^{2+} are correlated with each other, so any could be a reasonable predictor of toxicity. But pore water free Zn^{2+} is the fundamental cause of toxicity and should be used in comparing sites and could also form a common basis for soil protection regulations. Table 3 gives the calculated soil pore water soluble Zn and pore water free Zn^{2+} effective concentrations that gave a 25% (EC$_{25}$) and 50% (EC$_{50}$) reduction in luminescence of the biosensors along with the associated total soil Zn for comparative purposes.

The effect of Cu in soil pore water from plots that received Cu-contaminated sludges on the two luminescence bioassays was not significant. Even at the largest total soil Cu concentration (349 mg kg^{-1}), giving soil pore water concentrations of 620 μg L^{-1}, the percentage decreases in bioluminescence as compared with the uncontaminated sludge controls were only 18% and 9% for E. coli and P. fluorescens, respectively (Figures 2−4).

The response of the two luminescence-based bioassays to soil pore water from plots that received a mixture of Zn-plus Cu-contaminated sludges were similar to that from plots receiving only Zn-contaminated sludges with comparable concentrations. Thus, the decline in bioluminescence in soil pore water containing free Zn^{2+} plus Cu was similar to that obtained from soil pore water with comparable free Zn^{2+} concentrations, suggesting that the decline was due to pore water free Zn^{2+} and not soluble Cu (Figure 4). Both MLR and SMLR analysis indicated that pore water Cu had no effect on luminescence of the two biosensors.

Chaudri et al. (7) enumerated the indigenous population of *Rhizobium leguminosarum* bv. *trifolii* in soils from plots of two field experiments at Braunschweig in Germany to which metal-contaminated sewage sludge was added in the past. They concluded that the reduction in numbers of rhizobia, with increasing soil metal concentrations, were due to Zn and not the other metals present in addition to Zn. Total soil Cu concentrations reported in the Braunschweig soils ranged from 40 to 120 mg kg^{-1}, whereas in this study the concentrations ranged from 9 to 349 mg kg^{-1}. However, even though the highest total soil Cu concentration in our study was about three times that at Braunschweig, Cu had no effect on bioluminescence. Copper in its free ionic form is known to be very toxic to microorganisms (20). But in our study, it is likely that the concentrations of free ionic Cu were very low since, at the largest Cu addition (349 mg kg^{-1}), the pore water Cu represented <0.18% of the total soil concentration. Also, processes in soil pore water such as chelate formation, binding to soluble organic compounds, microbial binding, and metal uptake by cells in the bioassays would all have further reduced the free Cu^{2+} concentration. Metals are generally less toxic when complexed with organic compounds than in the free ionic form (21).

Our study identified acute free Zn^{2+} toxicity to bioluminescence, even though other metals were present in the soil pore water. However, in the field, both acute and chronic toxicity occur. Generally, studies looking at effects of metals on indigenous microbial populations often report short-term acute effects rather than long-term chronic effects. These chronic affects take many years, if not decades (6−8), to occur even in highly polluted soils, making early detection of problematic soils difficult. If microbial biosensors can be shown to be proxy indicators of chronic effects, then they will be extremely useful tools in the identification of potentially toxic soils, providing an early warning system. Any of these could be coupled with the simple technique

used here to collect clean soil pore water to provide rapid dose−response studies on different soils and with other metals. This information, once collected, will be valuable for use in systems that use basic ecotoxicological dose−response data on a uniform basis (i.e., soil pore water or free metal species in soil pore water). In the future, these approaches could be very powerful for regulating metal loads in soils based on the toxic bioavailable concentrations.

Acknowledgments

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