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The use of sigmoidal dose response curves in soil ecotoxicological research

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Summary The advantages of a generalised logistic dose response model as compared to a linear model were demonstrated while following the inhibitory effect of Ni on soil respiration rates in a sandy loam. The biological meaning of the parameters to describe the logistic model was indicated. An Ecological Dose Range to describe the increased rate of inhibition upon increasing concentrations of a pollutant was proposed. Remarks were made about the way this model must be used together with applications in other fields of soil biological research.

Introduction

The effects of heavy metals as soil pollutants have been investigated frequently by measuring the decrease in the rate of soil respiration upon increasing the concentration of heavy metals. In laboratory studies heavy metals were added to soil samples and the effect on soil respiration was measured after a period of time. The number of concentrations used varied: one[4] (Pb, Zn), two[5] (Ag, Bi, Cd, Co, Cu, Hg, Ni, Pb, Sb, Sn, Tl, Zn), three[10] (Cd, Cu, Hg, Ni, Pb, Zn), four[3] (Cd, Hg, Zn) or seven[7] (Pb) different concentrations were used. The respiration in the presence of added heavy metals was mainly expressed as a percentage of the respiration of the unamended soil samples.

When soil respiration rates and enzymatic activities were measured in the vicinity of local pollution sources a negative correlation was found between the concentration of the pollutant and the microbial activity measured, Tyler[11] calculated such a negative correlation assuming a linear relationship between the logarithm of the concentration of heavy metals (Cu + Zn) and the rate of soil respiration and enzymatic activities (*e.g.* urease, fosfatase and β-glucosidase. Mathur[9] found linear correlations between the concentrations of extractable Cu and 8 different enzymatic activities in histosols. Linear relationships between log-concentrations of Cd, Pb, Zn and letter weight have also been suggested[6].

In our experiments we have more often found sigmoidal relations on a logarithmic scale rather than linear relations. Therefore, in this note a logistic response curve is proposed.

Materials and methods

Design of experiment

To field-moist sandy loam (pH-H_2O 6.0 organic matter content 5.7%, clay 9%, Cation Exchange Capacity 10–12 meq 100 g^{-1}) finely ground $NiCl_2$ was added. The concentrations used were 55, 100, 150, 250, 400, 650, 1000, 1500, 2000, 3000, 4000, 5500 and 8000 mg kg^{-1} and two untreated soil samples served as controls. Details of sample preparation and storage

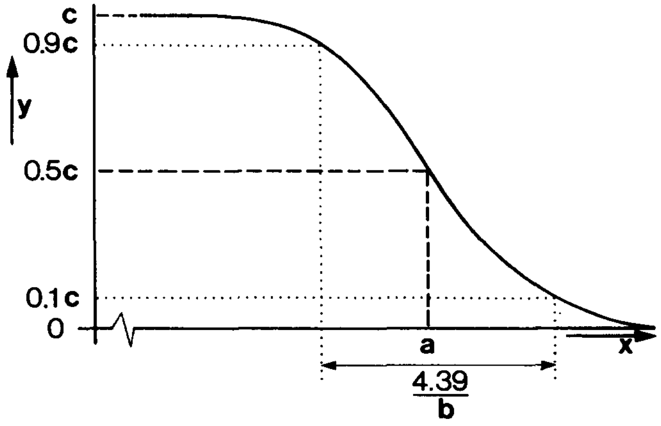

Fig. 1. The logistic response curve with parameters a, b and c.

are described elsewhere[8]. Starting from the twelfth day after mixing the soil samples with Ni, the respiration was measured in triplicate at 20°C with weekly intervals up to 92 days by measuring the rate of CO_2-production (in μl CO_2 day^{-1} 100g^{-1}) as described in an earlier paper[7].

Logistic response model

The search for a formula describing a sigmoidal curve soon turns into a selection problem, because there are several families of sigmoidal curves available. For technical and interpretative reasons we decided for a logistic curve and have fitted our observations to the following model

$$Y = \frac{c}{1+e^{b(X-a)}} + E$$

where Y is the observed respiration rate, X the (natural) logarithm of the concentration, c is the undisturbed respiration level, a the logarithm of the concentration at which the respiration was half the undisturbed level and b a slope parameter indicating the inhibition rate (see Appendix for exact description). The quantity E is the stochastic error term, assumed to have a normal distribution with mean zero and variance σ^2, independent of X; E describes the deviation of the observed value of Y from the value expected according to the model, *i.e.* the deviations of the observations around the model response curve. In our experiments we did not observe that σ^2 was dependent of X or Y. Figure 1 shows the logistic curve with parameters a, b and c.

From observations the parameters a, b, c and σ^2 can be estimated *e.g.* by the least squares method. For the computations we used the iterative least squares procedure supplied by the statistical program package GENSTAT[1].

As the logarithm of the added concentration of Ni in control samples could not be calculated, it has been substituted by the logarithm of a very small value (*e.g.* 10^{-3} mg kg^{-1}).

One should be convinced that for this value the curve reaches its asymptotic value, so the value of the substitution is not essential.

Results and discussion

The relationship between soil respiration and the logarithm of the concentration of Ni was approximately linear 19 days after mixing the soil with Ni (Fig. 2a) if a dosis of 1 mg kg^{-1} was assigned to the control observations.

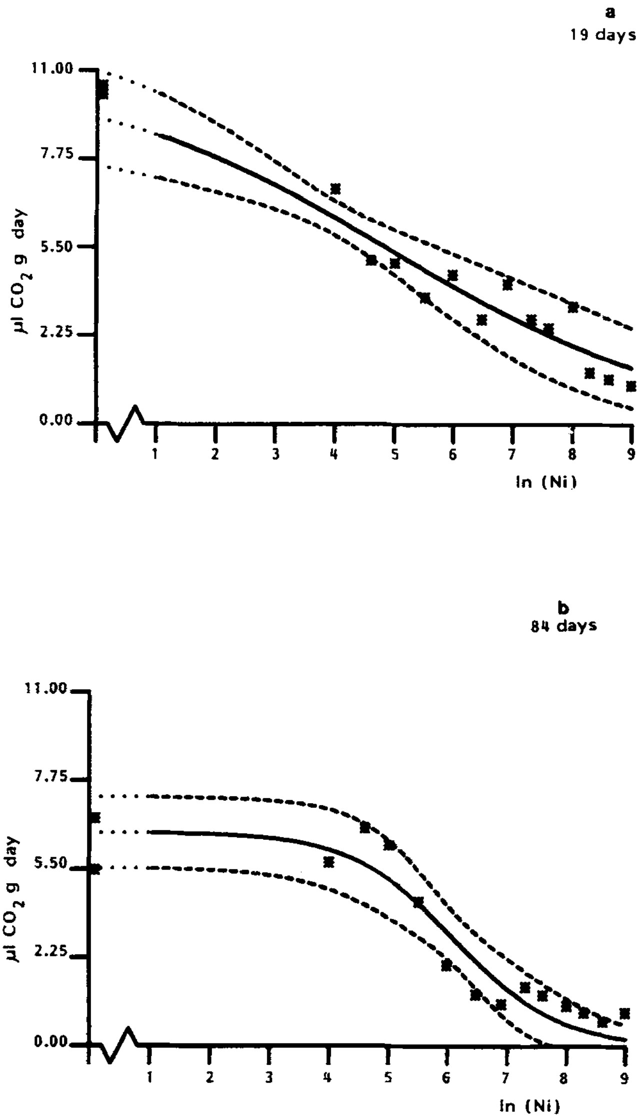

Fig. 2. The influence of Ni on soil respiration in a sandy loam 19 and 84 days after addition of $NiCl_2$ to the soil
* = observations
—— = estimated mean response
---- = 95% confidence bounds for the mean response curve

After 84 days the logistic curve fits the data much better than the linear curve (Fig. 2b).

The method proposed above implies a simultaneous estimation of the parameters a, b, c and σ^2. Another, perhaps more appearing estimation method is the following 2-steps procedure:

- estimate c from the control observations by taking the mean of their responses

– use this value for the estimation of a and b from the remaining observations.

We do not prefer this procedure because in the second step c is kept constant and hence the stochastic nature of the control observations is lost. Moreover the method we propose uses not only the control observations for the estimation of c but also the observations at low doses.

Most of the following remarks about the ecotoxicological meaning of the parameters a and b are hypothetical and need further experimental examination.

The parameter c, the value of the undisturbed level of activity, is easy to understand and does not need further comment. The parameter a, the dose at which activity is half maximal (0.5 c), is equivalent to the Ecological Dose – 50% (ED-50)[2]. The use of the ED-50 in studies of inhibition of microbe-mediated processes has recently been discussed by Babich *et al.*[2]. This parameter is strongly influenced by environmental conditions like pH, buffer capacity or the presence of chelating compounds, which may prevent the pollutant from inhibiting the processes. The more these compounds are capable of eliminating the influence of a pollutant, the higher the ED-50 will be.

Parameter b is thought to indicate the rate of increase of inhibition with increasing concentrations around the ED-50 concentration. The higher the value of b, the more abruptly the activity decreases, which would mean that the pollutant strongly acts on the organisms involved with the measured activity. On the other hand a low value of b does not necessarily mean that a pollutant does not act strongly on these organisms. If the environmental conditions in the soil gradually loose their protecting capacities, due to the increasing concentration of the pollutant, the inhibition will increase proportionally to the amount of unbound pollutant. As these factors not only influence parameter b, but also parameter a, correlation between a and b may be expected.

The ED-50 is a useful measure in terrestrial ecotoxological research, but as it does not provide information about the 'suddenness' of the decrease in activity we suggest the use of an Ecological Dose Range (EDR) as a measure of the rate of increase of inhibition on increasing toxicant concentrations. This Ecological Dose Range is defined as the doses at which activity decreases from 90% to 10% of the undisturbed activity. Together with the ED-50 and the EDR the time between the addition of the pollutant and the measurement of the activity must be taken into account as the ED-50 and the EDR may change with time. Our experimental data show a significant increase of the ED-50 in time (160 and 450 mg kg^{-1} after 19 and 84 days respectively) indicating an increasingly effective protection. Parameter b increased in the same period of time from 0.37 to 1.14. This could mean that a part of the metal has been turned into a form which is more toxic to microorganisms. The effect of Ni in the soil used 84 days after mixing can be described as an ED-50 of 450 mg kg^{-1} Ni and an EDR ranging from 65 to 3090 mg kg^{-1} Ni.

Once a, b and c have been estimated, they may in turn serve as a basis for estimation of ED values at any level (not just 50 per cent), together with their confidence intervals (see Appendix for formules for estimator and confidence intervals). It is also possible to estimate the expected response for any arbitrary dose inside the range of the experiment. Formulas for this estimator and the corresponding confidence interval are also given in the Appendix.

A necessary condition for the application is the use of sufficient doses (we suggest at least 6) properly spread over the dose-scale. This is necessary for the estimation of the parameters and also for checking the appropriateness of the sigmoidal response curve. A blind application is not recommended neither for a logistic nor a linear model.

Not only for soil respiration but also for enzymatic activities we have observed the sigmoidal type of dose-response relation. Also the effects of chemicals on reproduction, food consumption and growth of earthworms, observed in our laboratory, could be described with a logistic model.

A copy of the GENSTAT-program used is available upon request.

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Appendix

In these Appendix the formulas used are given.

Another description of b is given by the formula

$$b = 4.39/(\ln(\text{ED-}90) - \ln(\text{ED-}10))$$

So b is inversely proportional to the difference on log scale between ED-10 and ED-90.

The logarithm of ED-100 p (where p has an arbitrary value between 0 and 1) can be estimated by:

$$a + \frac{1}{b} \cdot [\ \ln p/(1-p)] \qquad [1]$$

An estimate for ED-100 p is found simply by taking the exponential of this expression.

The mean response for an arbitrary dose d is estimated by:

$$\frac{c}{1+e^{b(\ln d - a)}} \qquad [2]$$

Approximate confidence intervals for the logarithm of ED-100 p and for the mean response for an arbitrary dose are given by the expression

$$\text{estimator} \pm t \cdot \sqrt{\text{var (estimator)}}$$

where the estimator is given by formula [1] or [2], t is two-sided critical value of the Student distribution with n-3 degrees of freedom (n = number of observations) and the variance of the estimator is defined by

$$\text{var (estimator)} = f'Vf$$

Here V is the variance-covariance matrix of a, b and c (this matrix is also supplied by the program package GENSTAT) and f is the 3-dimensional vector of partial derivatives of formula [1] or [2] with respect to a, b and c. The vector f' is the transpose of f.