THE RELATION BETWEEN EXTRAPOLATED RISK, EXPRESSED AS POTENTIALLY AFFECTED FRACTION, AND COMMUNITY EFFECTS, EXPRESSED AS POLLUTION-INDUCED COMMUNITY TOLERANCE

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Abstract—The results of toxicity tests can be used to calculate the potentially affected fraction (PAF) of species in an ecosystem at a given pollutant concentration using statistical extrapolation methods. The PAF curve indicates the fraction of species from the original community that may become inhibited at each elevated pollutant concentration and is a measure of the ecotoxicological risk. Pollution-induced community tolerance (PICT) is a true community response that is measured under controlled conditions in the laboratory, using organisms from contaminated field sites. Microorganisms from experimental field plots with added Zn were exposed to various concentrations of Zn in the laboratory and the mineralization of $^{14}$C acetate was monitored. Microorganisms from plots with Zn concentrations above 124 mg/kg showed a significant increase in the effect concentration 10% (EC10) and, therefore, had a significant PICT. The pore-water concentrations of Zn in these field soils were in the same magnitude as the EC10 of the microorganisms from these soils. The PAF curve was calculated from previously reported toxicity tests with five different microbial species using the average and the standard deviation of the logarithmically transformed EC10 values. The average sensitivity of this PAF curve was similar to the EC50 of the acetate mineralization curve from the field plot without added Zn$^{2+}$, but the PAF curve was less steep. Our experiments indicated that 27 to 84% of the original microbial species were inhibited at Zn concentrations from 334 to 1,858 mg/kg soil, respectively. Our results suggest that the PICT method can now also be used to quantify the fraction of the original species composition that is inhibited at a specific pollutant concentration.

Keywords—Toxicity Metals Microorganisms Community Tolerance

INTRODUCTION

Risk assessment of soil pollutants is usually based on a risk evaluation of human exposure and on an assessment of the ecotoxicological risks [1]. Ecological observations at polluted field sites can give important information on the severity of the effect of the pollution. However, correlating ecological effects with the different pollutants at the site is very difficult [2]. Soil is a very heterogeneous medium and the distribution of organisms in a landscape is often very patchy. Therefore, the absence of specific species might be attributed to ecological factors as well as to pollution at the site [2]. At present, two independent methods are often used to estimate the ecotoxicological risk of a pollutant at a polluted site. The first is the extrapolated risk approach (using the potentially affected fraction [PAF]), which is calculated from the species sensitivity distribution (SSD) derived from single-species laboratory toxicity tests [3]. The second is the pollution-induced community tolerance (PICT), which is measured in organisms isolated from the polluted site [4].

Because of the many problems that are encountered with the detection of toxic effects in the field, most ecotoxicological experiments are performed with single species in the laboratory. In these experiments the effect of polluted soils or the effect of added toxicants can be measured quite accurately. However, the prediction of effects in the field from single-species laboratory tests is difficult [5]. For each pollutant, exposure–effect relationships differ markedly among species [6]. An effect parameter, such as a no-observed-effect concentration (NOEC) or an effective concentration, 10% (EC10), can be derived from each exposure–effect relationship. The logarithmically transformed effect parameters of different species can be used to describe an SSD. The value of the NOEC is very much dependent on the accuracy of the test [7]. Therefore, the use of the EC10 is preferred over the NOEC [8–10]. A hazardous concentration for 5% of the species (HC5) can be calculated from the EC10 distribution of the test species using a statistical extrapolation method [5]. At present, this procedure is used in The Netherlands to set environmental quality guidelines [1]. Conversely, this method can also be used to calculate the PAF of all species at a specific pollutant concentration in the field [11].

The concept of PICT, which was introduced by Blank et al. [4], can be used to correlate a specific ecological effect to a specific pollutant at a site. When the concentration of a pollutant exceeds a critical value, the most sensitive species at the site will become inhibited. Eventually, only resistant species will be present at a site. For example, if only Zn-resistant species are present at a Zn-polluted site, the Zn pollution can be assumed to have eliminated the sensitive species from the site. Pollution-induced community tolerance is a general concept that has been used with very different organisms, such as algae from surface water [12] or nematodes from sediment [13]. Increased toxicant resistance has been observed with microorganisms [14–16], plants, and animals [17]. The increased tolerance for pollutants of microbial communities is accompanied by a disruption of sensitive functions [18–20] and a decrease in soil biomass [21,22].

This study is a part of a large multidisciplinary project that has been executed to validate the ecotoxicological risk ex-
traplication methods based on SSDs [2]. This study focuses on microorganisms, whereas the other parts of the project focused on soil animals or plants [2]. Pollution-induced community tolerance was demonstrated using microbial metabolic activities (Biolog) from a Zn-amended test site [23] and a Zn pollution gradient near a smelter near Budel, The Netherlands [24]. The EC50 in the Biolog plates of microorganisms extracted from control soils had relatively high values of total Zn at approximately 100 mg/L [24]. The dissolved concentration in the Biolog plates was probably much lower because of precipitation and complexation of Zn [24]. The toxicity of dissolved Zn is dependent on environmental conditions such as pH and the presence of salts [14]. The 14C acetate mineralization test is used in our experiments because it does not require the growth of soil microorganisms [25] and is suited to measuring the sensitivity of soil microorganisms without interference by the chemicals present in growth media [14]. This paper correlates the PAF calculated from five single-species microbial toxicity tests with the PICT measured using microorganisms from a Zn-contaminated test site in Amsterdam, The Netherlands. This work combines the measurements of ecotoxicological effects in the field with laboratory toxicity data. This combination of two independent approaches gives a more reliable estimation of the ecotoxicological risk in the field than does either approach alone.

MATERIALS AND METHODS

Description of the Zn-amended field plots

A test site was constructed in July 1994 in Amsterdam with Zn additions using a randomized block design [26]. After establishment of the test site, rainwater seeped through the soil, reducing the Zn concentrations to approximately 70% of the initial value in the first months. After this period, Zn losses were small and the sorption of Zn increased in subsequent years [26]. In addition to the test site, a well-monitored Zn gradient in soil caused by a Zn smelter near Budel in The Netherlands was used as study location [2]. Even near the Budel smelter, attributing ecological differences along the gradient to the pollution at the sampling sites is complicated [2]. The field plots were constructed in July 1994 and consisted of 50 compartments of 0.5 m² separated by stainless steel walls. The ZnCl₂ was mixed with homogenized soil to yield nominal Zn concentrations of 0, 32, 56, 100, 180, 320, 560, 1,000, 1,800, and 3,200 mg/kg dry weight of soil and the soil was placed in the plots in 30-cm-deep layers in five replicated series [26]. The unamended soil contained Zn at 29 mg/kg soil dry weight. This sandy soil contained 2.0 to 2.9% clay, 6% silt (2- to 38-µm particle size), 89% sand, and 2.0 to 2.4% organic matter. The pH was 7.2 in the control; the pH dropped to 6.5 at the three highest concentrations of Zn [26,27]. The calcium content was 2.5 g/kg but was significantly decreased to 1.6 g/kg at the highest dose of Zn [27].

Measurement of acetate mineralization in diluted soil suspensions

In order to measure microbial PICT, a dilution method was developed to extract microorganisms from soil samples taken from the Zn-amended field plots. Four subsamples were taken per compartment with an auger. For each concentration, a mixed soil sample was made from five replicate compartments, which means that each mixed soil sample consisted of 20 subsamples. The mixed soil samples were sieved and stored according to standard methods (International Standard Organization 10381-6). Ten grams of soil were mixed in a blender with 0.5 L of 20 mM Tris buffer (pH 8) for 2 min. Highly diluted soil suspensions were made with 30 mg (dry weight) of soil and 15 ml of Tris buffer incubated in 60-ml bottles closed with rubber stoppers. A substrate solution containing 14C acetate was injected with a syringe to reach a final concentration of 1 ng/ml and 200 Bq per bottle as in Van Beelen et al. [25]. The acetate mineralization experiments were performed in duplicate. The bottles were incubated at 20°C in a shaker at 300 rpm. The microorganisms in the soil suspension were able to convert the 14C acetate into 14CO₂ during the incubation. After the incubation, 0.5 ml of 2 M sulfuric acid was added and the carbon dioxide formed was flushed with nitrogen and subsequently trapped and counted as in Van Beelen et al. [25].

Measurement of the effect of Zn²⁺ on acetate mineralization (PICT)

For the PICT measurements, slightly more concentrated soil suspensions were used with 60 mg of soil in 15 ml of Tris buffer. The suspensions were preincubated without acetate for 4 d. During this incubation the microbial activity increased although no nutrients were added. This increase might be attributed to the growth of microorganisms on nutrients that were present in the soil suspension. In a series of toxicity experiments, an extra amount of a concentrated ZnCl₂ solution was added to the highly diluted soil suspensions from the Zn-amended field plots before the incubation with acetate until a final concentration of Zn of 0.1, 0.3, 1, 3, 10, 30, and 100 mg/L was reached. The pH was not decreased, even at the highest Zn concentration. The chloride concentration of the Tris buffer was about 20 mM and was increased by 3 mM from the ZnCl₂ at the highest Zn addition. At the highest Zn concentration of 100 mg/L, only 23 to 32 mg/L were dissolved, whereas the remainder of the Zn was in suspension and could be removed after centrifugation and filtration over a 0.2-µm filter. Between 4.6 and 6 mg Zn/L was dissolved after the addition of 10 mg Zn/L. The Zn concentration without added Zn was below 0.1 mg/L in all highly diluted soil suspensions. Acetate mineralization in these suspensions was measured as described in the previous paragraph. The percentage of 14C acetate left and 14CO₂ formed at a given incubation time was plotted against the logarithm of the Zn concentration and the EC50 and EC10 were estimated using nonlinear regression as in Van Beelen et al. [28]. The statistical analysis of the data on the tested species or microbial community, leading to the EC50 and the EC10 and their standard errors, were also described previously [28].

Soil and pore-water analysis

Zinc analyses in soil were carried out on 0.5 g of dried and homogenized soil that was digested with 4 ml of concentrated nitric acid plus 12 ml of concentrated hydrochloric acid. The digestion was performed in a microwave oven at 190°C in a closed vessel. The digested mixture was diluted 100-fold and measured with atomic absorption spectroscopy (Perkin-Elmer AAnalyst, Norwalk, CT, USA). For Zn analysis in pore water, a total amount of 4 kg of soil was centrifuged for 20 min at 8,000 g in centrifuge tubes with a filter to separate soil from water. The natural humidity of the soil in February 1995 permitted the extraction of a few milliliters of pore water per kilogram soil. All the bottles and tubes used for Zn analysis were rinsed with nitric acid before use. The Zn in the extracted...
pore water was kept in solution by the addition of 0.7 ml of concentrated nitric acid per 100 ml of sample. The metals were analyzed in a graphite oven (Perkin Elmer 5100 ZL). The cations Mn, Na, K, Al, Fe, Cu, Pb, Cr, and As and the anions chloride, nitrate, sulfate, and phosphate were present in similar concentrations in all the field plots. The anion bicarbonate could not be measured in the pore water because of the addition of nitric acid. In contrast, the Zn, Ca, and Mg concentrations in pore water and also the conductivity were dependent on the amount of added Zn. Table 1 shows the relationship between the amount of added Zn and the pore-water concentrations of Zn, Ca, and Mg in the Zn-amended field plots. The speciation of Zn did not change significantly between February 1995 and October 1995. In May 1995, the Freundlich sorption constant was 586 L/kg [2]. At the three highest Zn concentrations a considerable fraction of the initial amount of Zn was eluted from soil by the first rains after the construction of the experimental plots. A pH drop to between 7.3 at the lower Zn additions and 6.5 (see Fig. 1, Zn100, Zn180, and Zn320). When one looks at the carbon dioxide production after 30 h, Zn0 shows 52% and 54% production, Zn32 shows 9% and 17% production, and Zn56 shows 11% and 18% production, whereas Zn100, Zn180, and Zn320 show less than 2% production (see Fig. 1). Therefore, a strong inhibition of the acetate mineralization clearly occurred in the Zn32 soil and the soils with a higher Zn content.

The data from Figure 1 can also be analyzed in a more elaborate manner in order to derive an exposure-effect curve. The ascending lines in Figure 1 are fitted by the least-squares procedure to the carbon dioxide production data using an equation for exponential growth

\[ \%P(t) = X \cdot e^{R \cdot t} \]  

where \( X = \) the initial carbon dioxide production expressed as percentage \( \text{CO}_2 \) formed; \( R = 0.12/h \), the growth rate that corresponds to a doubling time of 5.8 h; and \( t = \) time in hours. This growth rate gave the best fit with the Zn0 data and was used further for the data of the other field plots.

The data from the bottles incubated for 37 and 48 h of Zn0, and incubated for 46 h of Zn56 were excluded from the fitting procedure because these bottles were incubated too long and all the acetate was depleted in these bottles. The \( R^2 \) value of the least-squares fit of the curves through the data points was 0.9 for both the acetate consumption and the carbon dioxide production in the upper three graphs of Figure 1. In the lower three graphs the fit was poorer and showed \( R^2 \) values of 0.6, 0.8, and 0.7 for Zn100, Zn180, and Zn320, respectively. The initial carbon dioxide production \( X \) calculated from these lower three graphs is close to zero.

Figure 2 (left) plots the initial rate of carbon dioxide production and of acetate consumption from the data in Figure 1 against the measured total Zn concentration. The upper initial acetate consumption curve and the lower initial carbon dioxide production curve in Figure 2 (left) can be described with logistic curves with an EC50 for Zn of 55 mg/kg and an EC10 of 32 mg/kg, which are about equal to the 29 mg/kg in the control. However, considerable uncertainty exists in the EC10 because its 95% confidence limits range from 6 to 170 mg/kg. The 95% CI of the EC50 was 22 to 140 mg Zn/kg for the acetate curve and 23 to 133 mg Zn/kg for the carbon dioxide curve in Figure 2 (left). Therefore, both the simple analysis

**Table 1. The chemical analysis of the total Zn content of the soil and the pore-water concentrations of Zn, Ca, and Mg in the Zn-amended field plots in February 1995**

<table>
<thead>
<tr>
<th>Soil</th>
<th>Total Zn (mg/kg)</th>
<th>Zn (mg/L)</th>
<th>Ca (mM)</th>
<th>Mg (mM)</th>
<th>Conductivity (μS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn0</td>
<td>28</td>
<td>0.08</td>
<td>1.0</td>
<td>0.13</td>
<td>225</td>
</tr>
<tr>
<td>Zn32</td>
<td>61</td>
<td>0.14</td>
<td>1.1</td>
<td>0.16</td>
<td>256</td>
</tr>
<tr>
<td>Zn56</td>
<td>85</td>
<td>0.14</td>
<td>1.1</td>
<td>0.13</td>
<td>232</td>
</tr>
<tr>
<td>Zn100</td>
<td>124</td>
<td>0.18</td>
<td>1.4</td>
<td>0.17</td>
<td>301</td>
</tr>
<tr>
<td>Zn180</td>
<td>186</td>
<td>0.36</td>
<td>0.9</td>
<td>0.11</td>
<td>191</td>
</tr>
<tr>
<td>Zn320</td>
<td>334</td>
<td>1.3</td>
<td>1.2</td>
<td>0.13</td>
<td>237</td>
</tr>
<tr>
<td>Zn560</td>
<td>551</td>
<td>2.5</td>
<td>0.70</td>
<td>0.08</td>
<td>157</td>
</tr>
<tr>
<td>Zn1000</td>
<td>898</td>
<td>3.8</td>
<td>0.63</td>
<td>0.06</td>
<td>148</td>
</tr>
<tr>
<td>Zn1800</td>
<td>1,260</td>
<td>9.3</td>
<td>0.47</td>
<td>0.04</td>
<td>123</td>
</tr>
<tr>
<td>Zn3200</td>
<td>1,858</td>
<td>13</td>
<td>0.43</td>
<td>0.04</td>
<td>137</td>
</tr>
</tbody>
</table>

* The Zn0 soil did not receive a Zn addition, whereas the soils Zn32 to Zn3200 received Zn at 32 to 3,200 mg/kg soil.
Fig. 2. The effect of the measured total Zn concentration in the field plot soils in May 1995 and in January 1996 (placed on the horizontal axis) on the initial acetate consumption □ and on the initial carbon dioxide production ●.

Fig. 3. The effect of freshly added Zn to diluted and preincubated suspensions from soils from the Zn-amended field plots in January 1996. The microbial suspensions from each soil are labelled Zn0 to Zn3200 after the corresponding nominal concentrations in the field plots and the formed percentage of carbon dioxide □ and the remaining percentage of acetate ● were measured.

Figure 4 plots the microbial tolerance evolution (PICT expressed as EC50 of added Zn in mg/L Tris buffer derived from Fig. 3) as function of the soil Zn concentration that was present in each compartment of the experimental field site. The tolerance is quantified by plotting the EC50 and EC10 values derived from Figure 3 on a logarithmic scale on the vertical axis. The EC50 and EC10 values are expressed as milligrams of added Zn per liter of Tris buffer. The tolerance of the microorganisms from the most polluted soil was increased significantly compared to the tolerance of the microorganisms from clean soil.

A detailed statistical analysis on the experimentally determined carbon dioxide production data presented in Figure 3 was carried out to determine the soil concentration at which a significant PICT starts. Two-sample t tests assuming equal variances were performed between the percentages of carbon dioxide from the control soils and the polluted soils (shown in Table 2). The Zn0 and Zn32 soils were used as control and compared with the Zn320, Zn1000, and Zn3200 soils. This statistical analysis shows that a significant tolerance increase (PICT) occurred in the Zn320, Zn1000, and Zn3200 soils.

after 30 h of incubation and this more elaborate analysis show that the acetate mineralization is sensitive to Zn concentrations above 140 mg/kg soil.

**Effect of aging and elution in Zn-polluted soils**

The experiments described in Figures 1 and 2 (left) were repeated on February 26, 1996, with soil samples taken on January 16, 1996. Figure 2 (right) shows that the EC50 increased significantly (Zn at 660 mg/kg soil with 95% CI from 340 to 1,285) compared to the EC50 of Zn at 55 mg/kg (with 95% CI from 23 to 133) measured at the same site six months earlier, as shown in Figure 2 (left). A decreased toxicity because of aging at this site was also shown with plants, springtails, worms, and glutamate mineralization [2].

**Measurement of PICT**

The PICT experiment was performed on February 5, 1996, with soil samples taken on January 16, 1996. When $^{14}$C acetate was added after the preincubation period, the mineralization showed first order kinetics with half-lives of 20 to 60 min (data not shown) whereas these suspensions did not show any acetate mineralization for more than 20 h when the preincubation was not performed. After this preincubation period the suspensions from different soils were exposed to a number of different Zn concentrations in the Tris buffer and exposure--effect relations were obtained based on the acetate mineralization incubated for 3 h (Fig. 3). The concentration of added Zn is plotted in mg/L Tris buffer on the x-axis on a logarithmic scale. The control without added Zn is placed arbitrarily at 0.001 mg Zn/L buffer on the horizontal axis. The acetate mineralization curves did not always show a good fit to the data points and, therefore, the acetate mineralization curves were only used to check the carbon dioxide production curves. In the absence of carbon dioxide production, 100% acetate remained. The carbon dioxide curves show $R^2$ values of 0.6, 0.4, 0.6, 0.9, 0.6, and 0.6 for the Zn0, Zn32, Zn100, Zn320, Zn1000, and Zn3200 curves, respectively. In this case hortesis occurs, which means that the carbon dioxide production is stimulated at low doses of Zn. This requires more elaborated curve-fitting procedures to get a better fit [29], but has little influence on the outcome of the EC10 and EC50 determinations. The microorganisms from the control soil (Fig. 3, Zn0) obviously were much more sensitive to added Zn than were the microorganisms from the soil with highest Zn concentration (Fig. 3, Zn3200).
be a better parameter than the pore-water concentration itself, for the bioavailable fraction of the pollutant in soil. This might from polluted soils can thus be used as a surrogate parameter concentrations. If this relationship holds true, then the EC10 mea-

sured that the level of tolerance may follow pore-water concentration of Zn2 above 1 mg/L pore water, the EC10 will be in the same order magnitude as the pore-water concentration of Zn2.

microorganisms from soils with more than 1 mg Zn/L pore water have a higher EC10. The increase in the EC10 is sufficiently large to allow for survival in the corresponding soil.

**DISCUSSION**

Use of the EC10 from a PICT experiment as a surrogate parameter for the bioavailable fraction of the pollutant in soil

One might speculate that at elevated Zn concentrations above 1 mg/L pore water, the EC10 will be in the same order of magnitude as the pore-water concentration of Zn2+, which means that the level of tolerance may follow pore-water concentrations. If this relationship holds true, then the EC10 measured under control conditions with (micro-) organisms taken from polluted soils can thus be used as a surrogate parameter for the bioavailable fraction of the pollutant in soil. This might be a better parameter than the pore-water concentration itself, because a number of factors such as soil water content, dissolved organic carbon, salt concentration, and pH can modify the toxicity of a pollutant in pore water [30,31].

**Estimation of the PAF from the general SSD of microorganisms**

The calculation of an SSD from the results of single-species toxicity tests is now a relatively standard procedure in The Netherlands [1,32]. The results of five different single-species toxicity tests are used here for this procedure. The effect of dissolved Zn2+ on the 14C acetate mineralization of microbial species was measured in buffer at different pH values in a previous study [28]. The EC10 values at pH 8 were selected because pH 8 is similar to the pH of the test site [26]. For the actinomycete Streptomyces lividans and the fungus Aspergillus niger the EC10 at pH 7 was used because these species were inhibited at pH 8. An EC10 for Zn of 3,000 mg/L Tris buffer was taken because the fungus was not inhibited by Zn at 1,000 mg/L, whereas the use of higher concentrations gave problems with solubility and with maintenance of the pH and the salt concentration in the test medium. This is a common problem in ecotoxicological testing and risk evaluation [1]. Omitting the Aspergillus data is not feasible because the statistical extrapolation method requires that the tested species are a random sample from all species. This is not the case when the least sensitive species is omitted. The EC10 of Pseudomonas putida MT2, P. putida DSM 50026, Rhodococcus erythropolis, Streptomyces lividans 66, and A. niger are 0.005.

**Comparison between PICT and pore-water concentrations**

The exposure of microorganisms in the Zn320, Zn1000, and Zn3200 field soils took place at Zn concentrations of 1.3, 3.8, and 13 mg/L pore water, respectively. These values are higher than the EC10 of 0.166 mg Zn/L measured for the acetate mineralization of the microorganisms from the unpolluted soil Zn0 listed in Table 3. Taking the data of Table 2 into account, this suggests exposure at toxic concentrations to Zn3200 (controls are indicated in italic within the same row).

**Table 2.** Carbon dioxide (%) formed from acetate in diluted soil suspensions (2 g/L) sampled in January 1996 from the field plots marked Zn0 to Zn3200 (controls are indicated in italic within the same row)

<table>
<thead>
<tr>
<th>Zn concn.</th>
<th>Nominal Zn concn. in soil a</th>
<th>Zn0</th>
<th>Zn32</th>
<th>Zn100</th>
<th>Zn320</th>
<th>Zn1000</th>
<th>Zn3200</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>8.2</td>
<td>15.9</td>
<td>18.5</td>
<td>9.0</td>
<td>23.1</td>
<td>9.8</td>
<td>24.6</td>
</tr>
<tr>
<td>0.001</td>
<td>9.4</td>
<td>13.9</td>
<td>18.5</td>
<td>9.0</td>
<td>23.1</td>
<td>9.8</td>
<td>24.6</td>
</tr>
<tr>
<td>0.1</td>
<td>27.6</td>
<td>34.4</td>
<td>15.5</td>
<td>16.0</td>
<td>24.4</td>
<td>21.6</td>
<td>30.8</td>
</tr>
<tr>
<td>0.3</td>
<td>32.5</td>
<td>31.6</td>
<td>24.2</td>
<td>25.5</td>
<td>23.9</td>
<td>14.4</td>
<td>42.6</td>
</tr>
<tr>
<td>1</td>
<td>9.0</td>
<td>6.8</td>
<td>22.9</td>
<td>31.6</td>
<td>9.5</td>
<td>20.2</td>
<td>23.5</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>3.9</td>
<td>4.6</td>
<td>3.5</td>
<td>2.3</td>
<td>5.4</td>
<td>14.5</td>
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<td>1.6</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
</tr>
</tbody>
</table>

a Six soils with their nominal Zn concentrations.

b The Zn concentrations added to the Tris buffer.

c Significantly higher percentages (p = 0.01) compared to the controls (shown in italic within the same row).

d SE = standard error.

e The Zn concentration in pore water of the field soils.

**Table 3.** Potentially affected fraction of microbial species from the test field soils with various Zn concentrations

<table>
<thead>
<tr>
<th>Soil concentration</th>
<th>Compartment</th>
<th>Zn concn. (mg/kg) a</th>
<th>Zn concn. (mg/L) b</th>
<th>EC50 (mg/L)</th>
<th>SE c (log EC50)</th>
<th>EC10 (mg/L)</th>
<th>SE d (log EC10)</th>
<th>Percent PAF e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn0</td>
<td>28</td>
<td>0.08</td>
<td>1.26</td>
<td>0.27</td>
<td>0.166</td>
<td>0.39</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>Zn32</td>
<td>61</td>
<td>0.14</td>
<td>1.8</td>
<td>0.22</td>
<td>0.35</td>
<td>0.33</td>
<td>37</td>
<td>20</td>
</tr>
<tr>
<td>Zn100</td>
<td>124</td>
<td>0.18</td>
<td>1.1</td>
<td>0.28</td>
<td>0.16</td>
<td>0.40</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>Zn320</td>
<td>334</td>
<td>1.30</td>
<td>2.9</td>
<td>0.11</td>
<td>0.50</td>
<td>0.14</td>
<td>40</td>
<td>27</td>
</tr>
<tr>
<td>Zn1000</td>
<td>1,898</td>
<td>3.5</td>
<td>3.2</td>
<td>0.17</td>
<td>3.69</td>
<td>0.23</td>
<td>42</td>
<td>34</td>
</tr>
<tr>
<td>Zn3200</td>
<td>1,858</td>
<td>13.2</td>
<td>27.3</td>
<td>0.20</td>
<td>5.84</td>
<td>0.29</td>
<td>58</td>
<td>84</td>
</tr>
</tbody>
</table>

a The nominal Zn concentration.

b The actual Zn concentration (on January 16, 1996).

c The Zn concentration in pore water of the field soils.

d SE = standard error.

e The percent potentially affected fraction (PAF) was calculated from the corresponding EC10 from column 6 and the corresponding species sensitivity distribution (SSD) (shown in Fig. 5).
The use of a general SSD for the risk evaluation of a specific pollutant at a local site is not the best choice when detailed information is available on the sensitivity of the species present at the local site. Figure 3 provides information on the sensitivity of the species present in the control soil Zn0, which can be used to estimate a local SSD. Figure 5 shows a steep bold curve, which represents a log–logistic exposure–effect curve with an EC50 of 1.26 and the EC10 of 0.166. These are derived from the sensitivity of the microorganisms from the control Zn0 soil shown in Table 3. In a previous paper, the argument was made that the log–logistic exposure–effect curve of a mixture of the five different microbial species listed in the above paragraph was very similar to a cumulative SSD of the log-transformed EC10 values of the same five species [34]. An inverse cumulative normal SSD with an average of log(1.26) = 0.100 and an SD of 0.687 was also fitted through the EC50 and the EC10 as described in the Appendix. Figure 5 indicates that the difference between the log–logistic curve with a log EC50 of 0.1 and a log EC10 of log(0.166) = −0.78 and the normal distribution with an average of 0.1 and an SD of 0.687 is indeed very small because both curves overlap completely and are presented as one steep curve passing through 0.166 and 1.26. The similarity between the log–logistic exposure–effect curve and the cumulative SSD does not hold when different species are used in the SSD and in the exposure–effect curve. The steep exposure–effect curve in Figure 5 falls outside the very broad confidence range of the general SSD for a number of Zn2+ concentrations. This indicates that sensitive species such as P. putida MT2 and very Zn-resistant microbial species such as A. niger are not very dominant in the control soil. Table 3 shows a large difference in the percent PAF estimated from the general SSD (calculated from the sensitivity of five single-species tests) in the eighth column and the percent PAF calculated from the local SSD (obtained from the exposure–effect curve from the control field plot). Despite these uncertainties, a considerable percentage of the microbial species clearly is inhibited at the three highest Zn concentrations at which a significant PICT was measured.

Estimation of the PAF from the local SSD

The SD of the SSD determines the probability of detecting PICT in the field. For compounds with a large SD the probability of the occurrence of PICT in the field is much larger than for compounds with a small SD, which show little variation in sensitivity from one species to another. When little variation occurs, the chances of finding a number of more resistant species are small. For nonpolar narcotic compounds the SD of the log LC50 varies from 0.23 to 0.35 [35], whereas the SD varies over a much wider range for metals and for biocides [35]. This indicates that the probability of finding PICT for metals or for biocides is indeed larger than for nonpolar narcotic compounds.

Influence of the SSD on the detection of PICT in the field

The SD of the SSD determines the probability of detecting PICT in the field. For compounds with a large SD the probability of the occurrence of PICT in the field is much larger than for compounds with a small SD, which show little variation in sensitivity from one species to another. When little variation occurs, the chances of finding a number of more resistant species are small. For nonpolar narcotic compounds the SD of the log LC50 varies from 0.23 to 0.35 [35], whereas the SD varies over a much wider range for metals and for biocides [35]. This indicates that the probability of finding PICT for metals or for biocides is indeed larger than for nonpolar narcotic compounds.

CONCLUSIONS

Analysis of our results shows that the PICT principle can be used to pinpoint ecological differences between polluted sites to a specific pollutant. The magnitude of the induced tolerance can be used to quantify the ecological effect. The quantification of ecotoxicological effects is still subjected to considerable statistical and conceptual uncertainty. More experimental research is needed to reduce the statistical uncer-
tainty by acquiring more data and to reduce the conceptual uncertainty by validating the assumptions used for the setting of environmental quality standards.

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REFERENCES


APPENDIX

Calculation of the potentially affected fraction (PAF) from a species sensitivity distribution (SSD)

An SSD can be obtained from a number of EC10 or NOEC values derived from single-species toxicity tests. This distribution can be estimated through the average A and the standard deviation SD of n logarithmically transformed EC10 values. The ETX program uses the following equation to calculate the percent PAF from A and SD:

\[
PAF = 100 \times \frac{A}{A + SD}
\]
\[
\log(\text{HC}_p) = A - K(p, n) \cdot \text{SD}
\]  \hspace{1cm} (2)

where \(\text{HC}_p\) = the pollutant concentration at \(p\) percent PAF and \(K(p, n)\) = a statistical extrapolation constant that is dependent on \(p\) and \(n\).

Because the hazardous concentration, 5% (HC5) is commonly used for the estimation of environmental quality standards, the extrapolation constant at 5% PAF is the most important. When an infinite number of EC10 values are available, \(K(5, \infty) = 1.64\) is the fifth percentile of the normal standard distribution. Because only five EC10 values were used, \(K(5, 5) = 1.78\) must be used instead of 1.64 [36] in Equation (2). The gently sloping PAF curve in Figure 5 can be described with a cumulative normal distribution that is normalized to 100%.

\[f(x, \mu, \sigma) = \frac{100}{\sigma \sqrt{2\pi}} \int_{-\infty}^{x} \exp\left(-\frac{1}{2} \left(\frac{x - \mu}{\sigma}\right)^2\right) dx\]  \hspace{1cm} (3)

where \(x\) = the logarithm of the Zn concentration in mg/L, \(\mu = A = 0.282\), and \(\sigma = \text{SD} \cdot K(5, 5)/K(5, \infty) = 2.09\cdot1.78/1.64 = 2.27\).

The correction factor of the standard deviation was necessary because only five EC10 values were used instead of an infinite number. Confidence limits of the shallow PAF curve are derived from Aldenberg and Jaworska [36] and are plotted as the thin lines in Figure 5. Aldenberg and Jaworska use the term Fraction Affected for PAF. The horizontal intersection at percent PAF = 5 yields the median estimate and confidence limits of log HC5. These values range from an unrealistically low 3 ng/L up to 37 \(\mu\)g/L. The confidence interval was calculated using \(k5(\text{lower}) = 4.20\) and \(k5(\text{upper}) = 0.818\) as statistical extrapolation constants [36].

The steep curve in Figure 5 can be described by a log–logistic exposure–effect curve [34], which is identical to the descending carbon dioxide production curve in Figure 3, Zn0, except that it is normalized to end at 100%.

\[y = \frac{100}{1 + e^{s \cdot \log(x/\text{EC50})}}\]  \hspace{1cm} (4)

where the slope parameter \(s = \ln 9/\log(\text{EC10}/\text{EC50})\), EC50 = 1.26 mg Zn/L, and EC10 = 0.166 (see Table 3). Alternatively, this curve can also be fitted with Equation (3) using \(\mu = 0.100\) and \(\sigma = 0.687\). The difference between the calculated \(y\) values is \(<1.1\), which is so small that the steep curve in Figure 5 that goes through the EC10 of 0.166 and the EC50 of 1.26 represents both the log–logistic exposure–effect curve and the local sensitivity distribution.