ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 12, 53–69 (1986)

Comparison of Methods to Test Chemicals for Side Effects on Soil Microorganisms

L. ZELLES, I. SCHEUNERT, AND F. KORTE

Gesellschaft für Strahlen- und Umweltforschung mbH München, Institut für Ökologische Chemie, Ingolstädter Landstrasse 1, D-8042 Neuherberg, Federal Republic of Germany

Received December 3, 1985

The effects of the test chemicals pentachlorophenol (PCP) and $HgCl_2$ on the bioactivity of microorganisms in three different soils were studied in low and high concentrations (2 and 20 ppm). Bioactivity was measured in long-term experiments (18 weeks) by a threefold application of chemicals to soils of varying moisture content. The selected tests were measurements of ATP, of heat output under aerobic and anaerobic conditions and after amendment with glucose, of soil respiration after the addition of glucose, and of Fe(III) reduction. The suitability of each test depends on soil microorganisms, on environmental conditions, and on soil properties. The effects caused by the chemicals were as follows. For the low concentration, stimulation or inhibition were mostly repaired within the observation period. The high concentration mostly induced inhibitions which increased or decreased as a consequence of the repeated application of the chemicals. The effects of chemicals were strongly modified by the soil types: in a soil with high organic matter content, differences to the control were lower than in soils with lower organic matter content. These experiments also indicate that measurement of only one physiological parameter is not sufficient to characterize chemicals ecotoxicologically.

INTRODUCTION

Changes in the bioactivity of soil microorganisms due to chemical application have been described frequently. Most observations reported are based on measurements of the activity of some selected populations or functions (Domsch *et al.*, 1983) which should have indicative character on the whole activity of microorganisms. On the other hand, products or energy characteristic of life activity of all microorganisms or, at least, of important and large populations, such as ATP (Tu, 1982; Malkomes and Wöhler, 1983; Zelles *et al.*, 1985a), CO_2 (Anderson *et al.*, 1981; Jenkinson and Powlson, 1976; Zelles *et al.*, 1984), different enzymes (Schnürer and Rosswall, 1982; Beck, 1983; Zelles *et al.*, 1985b), and heat output (Ljungholm *et al.*, 1979; Sparling, 1983) have been determined. Physiological reactions of some microorganisms, such as the iron(III)-oxide reduction under anaerobic conditions (Pal *et al.*, 1979; Welp and Brümmer, 1985), were also taken as a measure of the activity of soil microorganisms. Nevertheless, the evaluation and interpretation of the effects of chemicals on nontarget microorganisms is difficult due to the difference of microorganisms in their sensitivity to chemicals, to the complexity of the population dynamics, to the multitude of species, and to the sensitivity of the tests under different conditions.

In the present paper, various methods are evaluated and compared with each other to detect pesticide damage on soil microbial activities: ATP measurement (Zelles *et al.*, 1985a), the measurement of heat production with and without glucose amendment under aerobic and anaerobic conditions, the measurement of CO_2 production (Anderson and Domsch, 1978), and of the reduction of iron(III) oxides.

0147-6513/86 $3.00

TABLE 1

CHARACTERISTICS OF SOILS USED

Soil	Particle size distribution (%) Clay 2 μm	Silt 2–63 μm	Sand 0.06–2 mm	Total organic matter (%)	Organic C (%)	N (%)	pH in $CaCl_2$	P_2O_5 (mg/100 g)	K_2O (mg/100 g)	Water holding capacity ($g \cdot 100\,g^{-1}$)
A	31	40	29	20.6	12.0	1.2	5.9	1	57	96
B	25	58	17	2.4	1.4	0.2	6.9	22	25	38
C	7	26	67	1.9	1.1	0.1	6.5	34	35	29

Two concentrations of the test chemicals pentachlorophenol and $HgCl_2$ were applied to three different soils. In order to study the population dynamics, the application of the chemicals was repeated periodically. The sensitivity of each method was also tested under different environmental conditions.

MATERIALS AND METHODS

1. SOILS AND CHEMICALS

Three different soils were used for the investigations: *A*, peat from grassland; *B*, clay (brown soil), and *C*, sand (para brown earth) from arable land. Their characteristics are shown in Table 1.

The soils were freshly sampled from 5- to 20-cm depths, sifted through a 5-mm sieve, and adjusted with H_2O to 55% of the maximum water holding capacity (WHC). One kilogram wet soil was placed in a desiccator (11-liter volume). A low (2 mg/kg) and a high (20 mg/kg) concentration of pentachlorophenol (Fluka, 99% purity) and of $HgCl_2$ (Merck, p.a.) were applied to soils. The pesticides were distributed in 10 g of fine sand and added to the soil samples. Controls were left without chemicals.

2. EXPERIMENTAL CONDITIONS

The desiccators were placed in a climate chamber with 12-hr daylight, 22°C during the day and 16°C during the night.

For the ATP, heat 1, heat 2, and Fe(III) tests (see Section 3, Applied Methods), the following measurement time schedule was used. The first measurement of bioactivity was carried out 3 days after the application of the chemicals, followed by four other measurements which were carried out successively once a week (section I). The soils were mixed well before the samples were taken for measurement. In the sixth week, the water content was adjusted to the original level and the application of the chemicals was repeated. The next measurement was carried out 3 days after the application, followed by three further measurements once a week. In the following weeks (10–13 weeks), the soils were allowed to lose about 50% of their water content (*A* 52%, *B* 51%, and *C* 48%, "dry stress") and were allowed to develop populations in this changed environment. Measurements were carried out in week 13 in homogeneously mixed soil samples. This section II was thus divided into two subsections (6–9 weeks and 10–13 weeks). After these measurements, the water content of the soils was again brought to the original level and the third application of chemicals was carried out.

In section III, three measurements were carried out: in soil *C* after 14, 15, and 16 weeks, in soil *B* after 14, 15, and 17 weeks, and in soil *A* after 14, 15, and 18 weeks.

For the CO_2 and hepo test (see Section 3, Applied Methods), determinations were started only at section II. Parallel to the measurements in week 15, the experiments for testing the influence of environmental parameters on the sensitivity of methods were started. Fifty grams of soil of the untreated samples were placed in polyethylene vials and then incubated for 2 weeks under different environmental conditions. Control samples (1) were compared with those of changed water content: 85% (2) and 25% (3) of the maximum water holding capacity. In other samples, pH was adjusted with acidic sand (H_2SO_4) to approximately 3.5 (4). Finally, the effect of temperature was tested: the polyethylene vials with soil were incubated at 38°C (5) and at 5°C (6) continuously in the dark. No restoration of humidity lost by evaporation was carried out.

3. Applied Methods

(a) *ATP Measurements*

For each measurement, 0.75 g wet soil was used. The method applied has already been described (Zelles *et al.*, 1985a).

(b) *Measurements of Heat Output*

These measurements were performed in an LKB 2277 Bioactivity Monitor. Two different tests were carried out: the heat 1 test under aerobic conditions, and the heat 2 test under anaerobic conditions.

For the heat 1 test, 1.5 g wet soil were placed in a glass ampoule (3 ml) and incubated at 25°C for 24 hr. Loss of humidity was impeded by placing a metal cap on the ampoule, which was not crimped. After incubation, the ampoule was hermetically sealed and transferred into the measuring cylinder, followed by the reference ampoule in the twin position. The calorimeter reached thermal equilibrium 30 min after the introduction of the ampoules.

For the heat 2 test, the tightly closed ampoule used for the heat 1 test was incubated for another 72 hr; then the heat output was determined again.

(c) *Fe(III)-Reduction Test*

This test was adapted from techniques described by Pal *et al.* (1979) and by Welp and Brümmer (1985). A detailed description of the modification of this test has been given recently (Zelles *et al.*, 1986). Five grams wet soil was placed into polyethylene vials (25 ml), 5 ml glucose solution (20 mg/ml) was added, and the vials were hermetically sealed with a screw cap. The samples were shaken overnight (15 hr) and incubated anaerobically (153 hr) at room temperature. Five milliliters of 1 M KCl solution was added to the suspension which was then shaken vigorously and centrifugated after 15 min. An aliquot of the clear solution was acidified with concentrated HNO_3 (ratio 9:1 v/v) and the Fe^{2+} was determined quantitatively in an inductively coupled plasma emission spectrometer (ICP).

(d) *Measurement of CO_2 Production*

The measurement of CO_2 production was adapted from the technique described by Anderson and Domsch (1978). Glucose and talcum (ratio 1.5:1.0 wt) were placed

in a mortar and ground to a fine powder; 33 mg of this was poured onto 10 g wet soil. After mixing, 8.5 g of the amended soil was placed in an Erlenmeyer flask (250 ml) equipped with air inlet and outlet. CO_2 production was then determined with an infrared gas analyzator (Uras 7 N, Hartmann and Braun).

(e) *Measurement of Heat Potential (hepo) Output*

Measurement of hepo output after glucose amendment records the potential activity of microorganisms. The 1.5 g wet soil amended with glucose, left over from technique (d), was placed into a glass ampoule, and heat production was measured in the Bioactivity Monitor. For the measurements, the lowest position of the thermogram was taken before the heat output significantly increased due to the reproduction of microorganisms (Anderson and Domsch, 1978).

Three replicates were carried out for each measurement. Coefficients of variation were, in general, below 10%.

RESULTS

1. Bioactivity in Control Soils

The changes of the measured parameters in control soils during the time of observation are shown in Fig. 1.

The highest amount of ATP (Fig. 1a) was measured in peat soil (*A*), the clay soil (*B*) and sand (*C*) containing about only one-half and one-third of this amount, respectively. The most marked effect was obtained in peat soil after the decrease of humidity in section II (week 13): the amount of ATP was diminished to one-third of the original level. In the remaining soils the loss of ATP due to dryness was limited. Roughly, it can be stated that during the observation period the quantity of ATP remained constant when the environmental parameters were constant.

The time courses of heat output (Fig. 1b) after a resting period of 24 hr (heat 1) were different: heat output decreased up to the middle of section II and then remained on the same level, and, with the exception of the first measurement of soil *B*, no significant change during the whole observation period occurred. The measurements under anaerobic conditions (heat 2) support the finding of the former ones but the quantity of heat was lower and the differences between soils *A* and *B* were more pronounced. No particular effects caused by dryness were observed under anaerobic conditions.

In contrast to the other tests, the Fe(III)-reduction test (Fig. 1c) showed the most Fe^{2+} in soil *B*, followed by soils *C* and *A*. An increase up to the end of section II with interruptions followed by an extreme (*B*) and a small (*C*) increase was observed.

The measurements of CO_2-production (Fig. 1d) started at section II. A continuous decrease up to the middle of this section was followed by a constant level in soil *A*, whereas the other soils produced CO_2 permanently at a lower level without any remarkable alterations.

The heat production of potentially active microorganisms (hepo) showed a nearly constant level in all soils (Fig. 1e). In this test the differences between soils *A* and *B* were remarkably smaller than those between soils *B* and *C*.

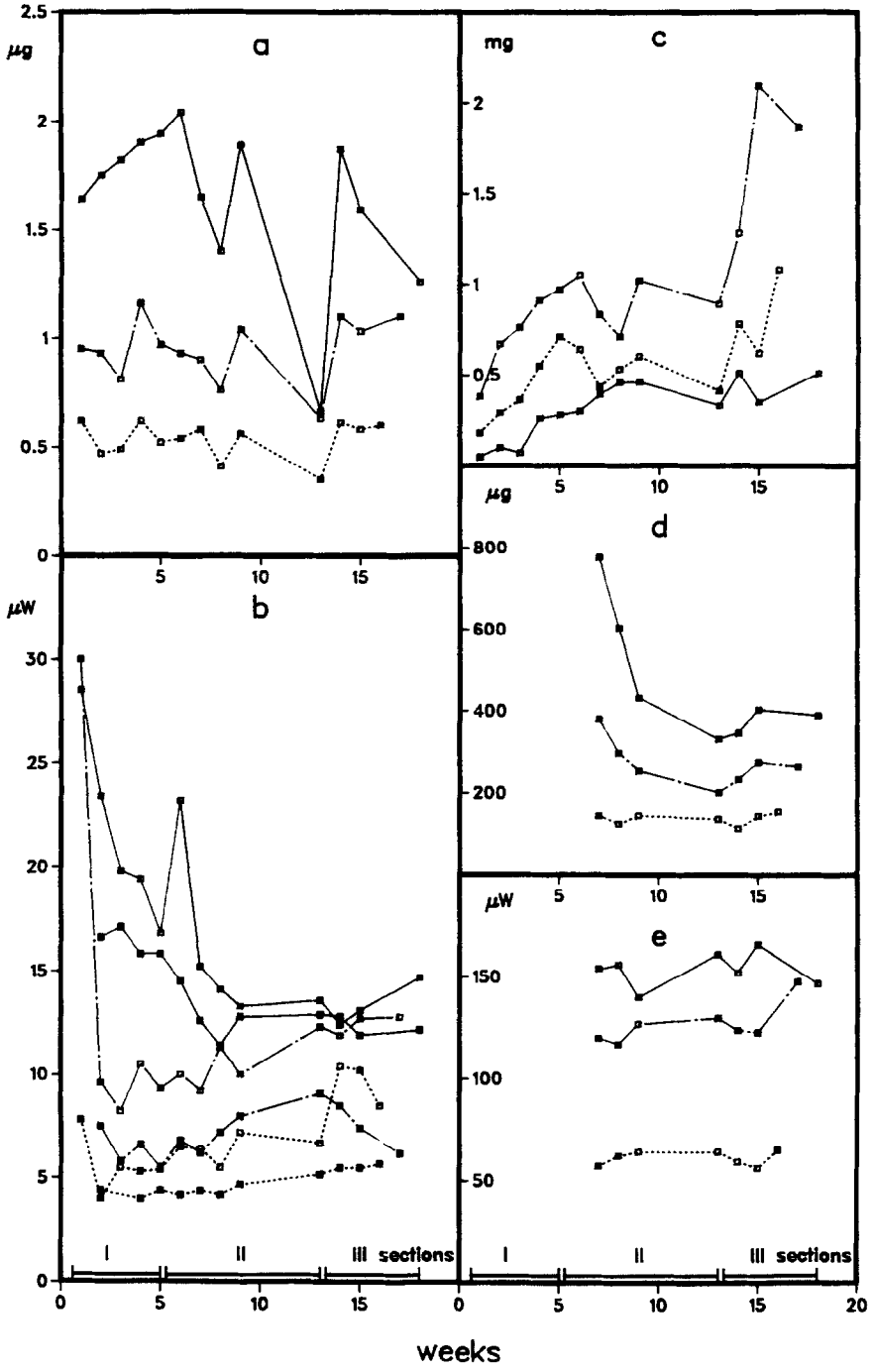

FIG. 1. The measured quantities in control soil, calculated for 1 g ovendried soil: (a) μg ATP, (b) μW heat, derived from aerobic and from anaerobic soils, (c) mg Fe^{2+}, (d) μg $CO_2 \cdot hr^{-1}$, and (e) μW heat after the application of glucose. Soil *A* (peat) □ – □, ■ – ■; soil *B* (clay) □ – · – · □, ■ – · – · ■; and soil *C* (sand) □ · · · □, ■ · · · ■. For Fig. 1b: Aerobic, open squares; anaerobic, filled squares.

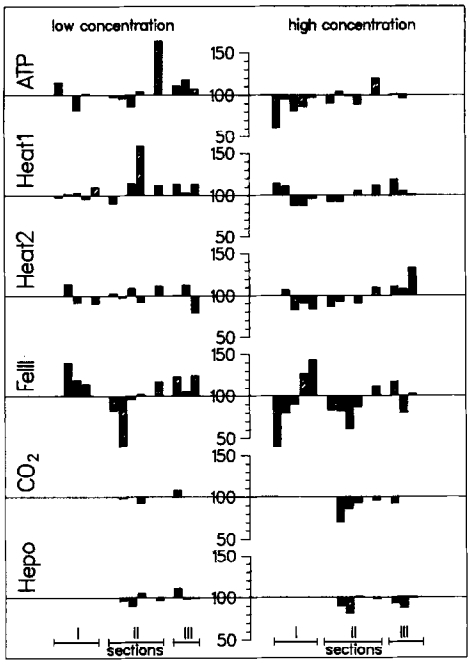

FIG. 2. The effect of a low (2 mg·kg^{-1}) and a high (20 mg·kg^{-1}) concentration of PCP on the bioactivity in peat (*A*) soil, expressed in percentage of control. Values of 100% (no effect) are not depicted. Section I: 1–5 weeks; section II: 6–13 weeks; section III: 14–18 weeks.

2. EFFECTS OF PCP ON THE BIOACTIVITY OF SOILS

(a) *Peat Soil (Fig. 2)*

The low concentration of PCP induced a small stimulation at the beginning of the observation, followed by an inhibition of 18%, as measured by the ATP test. The effects until the first part of section II were negligible. The measurement performed during dry stress (section II) showed a significant stimulation of 60%, whereas in section III ATP remained between 10 and 20% above that of the control. The higher concentration of pesticide effected a significant inhibition at the beginning of section I, which disappeared at the end of this section. Repetition of the chemical application did not cause new deviations from the control. The only stimulation effect observed was by the dry stress, in the second part of section II.

Only the second addition of PCP at the end of section II caused a significant stimulation measured by the heat 1 test. The effect in section III again was lower than that in section II. The higher concentration of PCP stimulated heat production slightly and then reduced it during the first section. This small inhibition continuously decreased and was followed by a significant stimulation in the middle of section III. The measurements with the low concentration of PCP (heat 2) did not show clear-cut effects during the whole observation period, whereas the high concentration caused a clear inhibition during section II, which continuously decreased. In section III a significant stimulation was observed.

With the Fe(III)-reduction test, a 40% stimulation by the low PCP concentration was obtained, which was reduced stepwise, so that the second addition of the chemi-

cals caused a strong inhibition (60%) at the middle of section II, which was again compensated for at the end of this section. The third addition of chemicals caused a clear stimulation. The higher concentration of PCP caused a 60% inhibition which changed, at the end of section I, to a 40% stimulation, whereas the following application caused clear inhibitions. The effect of the last application was not uniform.

No significant effects were obtained with the low concentration of PCP when the potential activity of microorganisms was measured (CO_2 and hepo techniques), and only a slight inhibition at the beginning of section II with the high concentration was observed.

(b) *Clay Soil (Fig. 3)*

The low concentration of PCP caused slight inhibitions at the end of each section, as measured by the ATP method.

Using the heat 1 technique, the measured bioactivity showed similarities between the samples with the low and high concentrations of PCP: the first application of the chemical caused strong inhibitions (40 and 30%) which, until the end of section I, disappeared (high concentration) or were even converted to a stimulation (low concentration). The following PCP applications inhibited the heat output. For the low concentration, the "dry stress" caused an increase in bioactivity. With the high concentration of PCP, the inhibitory effects increased with the last application. For the heat 2 technique, similarities to the heat 1 technique were observed: with the low concentration of PCP, in section I stimulations, in sections II and III inhibitions, and, during the dry stress, a stimulation was observed, whereas with the high concentration the inhibition increased with the observation period.

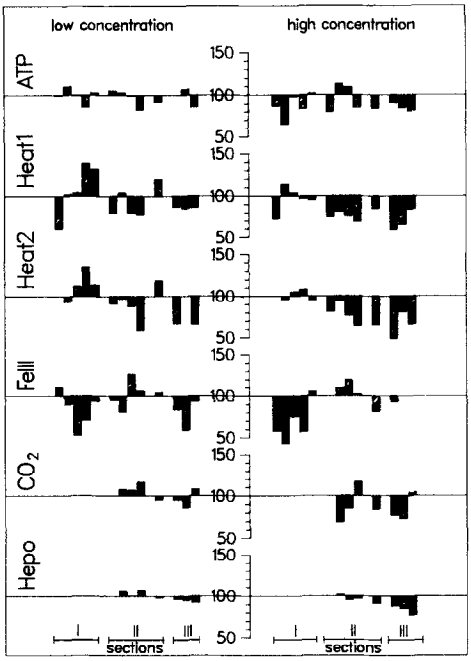

FIG. 3. The effect of a low (2 mg·kg^{-1}) and a high (20 mg·kg^{-1}) concentration of PCP on the bioactivity in clay (*B*) soil, expressed in percentage of control. Values of 100% (no effect) are not depicted. Section I: 1–5 weeks; section II: 6–13 weeks; section III: 14–18 weeks.

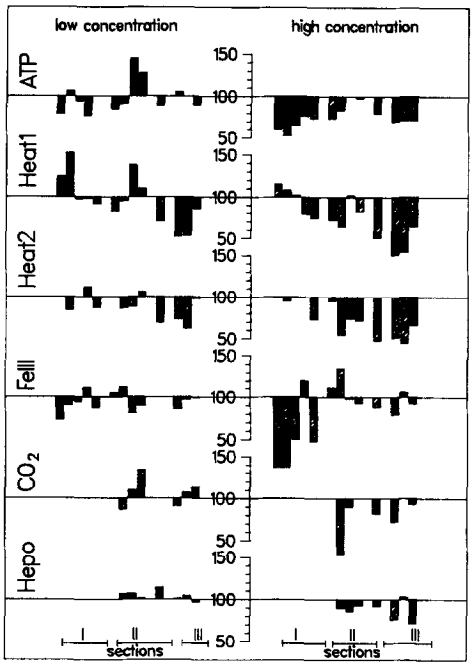

FIG. 4. The effect of a low (2 mg·kg^{-1}) and a high (20 mg·kg^{-1}) concentration of PCP on the bioactivity in sand (*C*) soil, expressed in percentage of control. Values of 100% (no effect) are not depicted. Section I: 1–5 weeks; section II: 6–13 weeks; section III: 14–18 weeks.

The reactions of soil microorganisms to the low concentration of PCP, as measured by Fe(III) reduction, were similar in all three sections: retarded inhibitions were compensated for in a different way. The high concentration caused a strong inhibition in section I and was slightly overcompensated for (stimulation) in section II. No clear effects were observed in section III.

The measurement of carbon dioxide showed, with the higher concentration of PCP, an inhibition which was compensated for at the end of the sections, whereas with the low concentration the effects were less pronounced (stimulation in section I, retarded inhibition with compensation in section II).

The hepo technique showed no effects in samples with a low amount of PCP but a continuous increase of damage in higher concentrated samples.

(c) *Sand Soil (Fig. 4)*

For the ATP test, an inhibition of 20% was observed in samples with the small concentration of PCP, which was compensated for at the end of section I. The effects were more extreme in section II and fully adapted in section III. The high concentration caused a strong inhibition (32–58%) which was reduced in section II. In the dry period (end of section II) and after the third application of the chemical, the inhibition (30%) seemed to be constant and irreversible.

With the heat 1 technique, an increased stimulation followed by a full compensation could be detected in section I. Section II was similar to that of ATP measurement, whereas section III showed a significant inhibition which was reduced at the end of the section. The higher concentration of PCP damaged the microorganisms

more with each new application. The heat 2 technique indicated, for the low concentration of PCP, mostly clear inhibitions; the tendency to damage, however, was not constant. For the high concentration, in sections II and III strong inhibitions (about 50%) were observed with no tendency to compensation.

The Fe(III)-reduction test, for the low concentration of PCP, revealed a small effect: inhibitions measured at the beginning of sections I and II were reduced until the end, whereas those in section III were fully compensated for. The high concentration of PCP caused, at the beginning of section I, an enormous inhibition (more than 90%). Even such a strong effect was adapted in the following sections, so that the effects at the end of section III had nearly disappeared.

The presentation of the CO_2 test with the low concentration of PCP in sections II and III was similar: the inhibition was changed to stimulation in section II more clearly than in section III. The higher concentration of PCP inhibited bioactivity at the beginning of each section; at the end, however, nearly no differences between these values and those of the control were observed.

The effects of the small PCP concentration measured by the hepo test were small and not significant, whereas the higher concentration caused inhibitions mainly in section III.

3. Effects of $HgCl_2$ on the Bioactivity of Soils

(a) *Peat Soil (Fig. 5)*

The small concentration of $HgCl_2$ did not cause a significant tendency in bioactivity alteration, as measured by the ATP method. Stimulations and inhibitions alter-

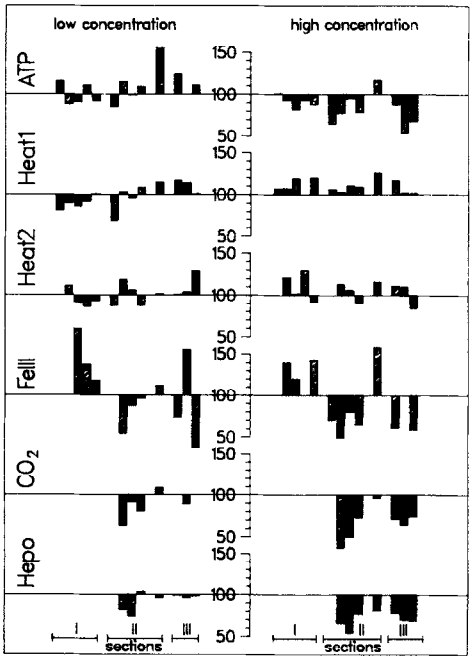

FIG. 5. The effect of a low (2 mg·kg^{-1}) and a high (20 mg·kg^{-1}) concentration of $HgCl_2$ on the bioactivity in peat (*A*) soil, expressed in percentage of control. Values of 100% (no effect) are not depicted. Section I: 1–5 weeks; section II: 6–13 weeks; section III: 14–18 weeks.

nated in sections I and II, whereas in section III there were only stimulatory effects. With one exception (dry stress), the higher concentration of the chemical caused an increasing inhibition. The dry stress caused a separate effect which did not fit the general tendency.

The inhibitions at the beginning of sections I and II and the stimulation in section II were compensated for at the end of each section with small amounts of PCP, when the observation was carried out with the heat 1 test. The higher concentration caused stimulations until the beginning of section III. The results obtained with the heat 2 test were similar to those measured with the heat 1 test: the inhibitions in section I were compensated for mainly in section III (low concentration), or stimulations were followed by an inhibition at the end of each section (high concentration).

The Fe(III)-reduction test presented, in samples treated with the small concentration of $HgCl_2$, an 80% stimulation in the middle of section I and a 50% inhibition at the beginning of section II. The retarded high stimulation and inhibition in these sections were compensated for, whereas the effect in section III was an alternation of strong inhibitions and stimulations.

The higher concentration of $HgCl_2$ caused, in section I and in the second part of section II, significant stimulations, whereas the values of the remaining measurements showed only significant inhibition.

The CO_2 and hepo tests indicated inhibitory effects in sections II and no further effects for the low concentration, and, for the high concentration, strong inhibitions with decreasing tendency in section I and moderate inhibitions with constant level in sections II.

(b) *Clay Soil (Fig. 6)*

After the addition of the small concentration of $HgCl_2$, no clear tendency was observed with the ATP method. Sections I and III tended to inhibition, whereas section II showed mostly no significant deviation from the controls. For the high concentration, all values were under the level of the controls. The inhibition was between 20 and 30% and increased with each application.

The strong inhibition (50%) detected by the heat 1 technique was soon neutralized, and a significant stimulation was the consequence of the second application. The third application caused constant inhibitions. For the high concentration of $HgCl_2$, the values of heat production showed no clear tendency. The heat 2 method showed, for the low concentration, an increasing stimulation in sections I and II (with the exception of the last measurement in section II). In section III, inhibition changed to stimulation. For the high concentration of $HgCl_2$, stimulations at the beginning of section I and at the end of section III and inhibitions in the remaining observation period were significant.

For the low concentration of $HgCl_2$, the Fe(III)-reduction test showed, until the second part of section II, no clear tendency, whereas the third application caused clear inhibitions. The higher concentration induced, after the first application, a strong inhibition (30–50%) with a decreasing tendency; the second and third applications caused higher inhibitions (40–70%) without clear tendencies of repair.

The CO_2 measurement showed, for the low concentration, that the inhibitions obtained at the start of sections II and III changed to stimulation; for the high concentration, the inhibitions were significant, and no tendency toward improvement could be observed.

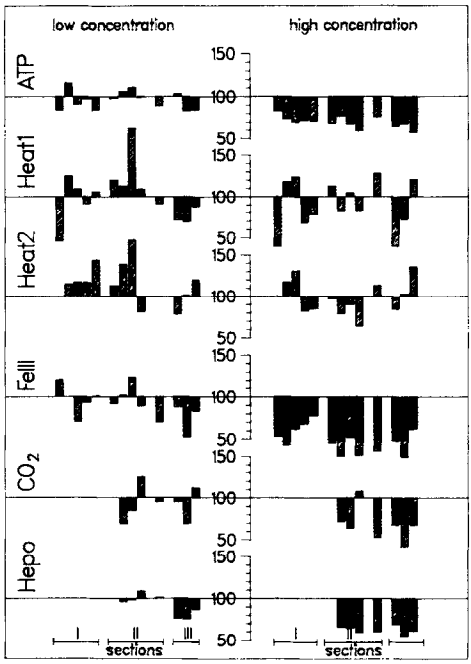

FIG. 6. The effect of a low (2 mg · kg^{-1}) and a high (20 mg · kg^{-1}) concentration of $HgCl_2$ on the bioactivity in clay (*B*) soil, expressed in percentage of control. Values of 100% (no effect) are not depicted. Section I: 1–5 weeks; section II: 6–13 weeks; section III: 14–18 weeks.

With the hepo method no significant inhibition was detected before section III in the low concentration samples; then, an inhibition of about 20% was observed. In the high concentration samples, only inhibitions of a constant level of 36–50% were detected.

(c) *Sand Soil (Fig. 7)*

The ATP test showed, in samples treated with a low dose of $HgCl_2$, no effects in section I and significant inhibitions in sections II and III. In samples treated with high doses of $HgCl_2$, the inhibition surpassed 60% and seemed to be irreversible.

The heat 1 and 2 techniques showed similar effects: the more low doses of $HgCl_2$ were applied, the higher was the inhibition (except for the last value measured by heat 2). The high concentration of $HgCl_2$ induced strong stimulations (section I), alternating stimulation and inhibition (section II), and inhibition (section III), as measured by the heat 1 technique, a single stimulation (section I), and inhibitions overcompensated for by stimulation (section III), as measured by the heat 2 technique.

By the Fe(III)-reduction method, using small quantities of $HgCl_2$, mostly small inhibitions in section III were visualized. The high concentration, on the contrary, caused inhibitions between 60 and 90% during the whole observation period.

The CO_2 test showed, for the low concentration of chemical, a strong stimulation in section II, and no significant effects in section III, whereas, for the high concentration, inhibition was strong and increasing in section III.

The hepo technique induced, in samples treated with the low concentration of $HgCl_2$, strong (section II) and light (section III) inhibitions, whereas in those samples

with the high concentration, a constant inhibition between 30 and 50% could be seen during the whole observation period.

4. Dynamics of Bioactivity

For studying the dynamics of microorganisms in different soils, the averages of bioactivities measured by different methods were calculated and expressed as a percentage of the controls (Fig. 8).

The small concentration of PCP, in soil *A*, caused only a slightly retarded stimulation in section I. The second application resulted in inhibition, which was then overcompensated for to stimulation, the maximum of which was reached in the second part of section II. After this maximum, the stimulation effect was stepwise reduced. In soil *B*, the first application caused a clear inhibition (section I) which was overcompensated for to stimulation. In section II, the effects changed from stimulation to inhibition, whereas in section III inhibition was significant. In soil *C*, with two exceptions, only inhibitions were measured.

The higher concentration of PCP caused, in all soils, clear inhibitions with tendencies to recovery in soil *A* (sections I and III) and in soil *B* (section I). The second application of PCP to soil *B* again caused inhibitions which, however, were less than in section I. The inhibitory effects after the third application of the chemical were higher in soil *C* than in soil *B*.

The low concentration of $HgCl_2$ induced, in soil *A*, a retarded stimulation in section I, and an inhibition in section II; both were reduced at the end of each section. The second part of section II and the middle of section III showed stimulative effects.

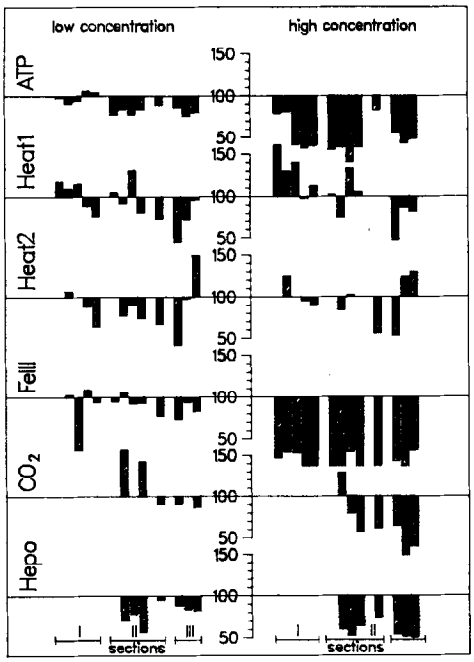

FIG. 7. The effect of a low (2 mg·kg^{-1}) and a high (20 mg·kg^{-1}) concentration of $HgCl_2$ on the bioactivity in sand (*C*) soil, expressed in percentage of control. Values of 100% (no effect) are not depicted. Section I: 1–5 weeks; section II: 6–13 weeks; section III: 14–18 weeks.

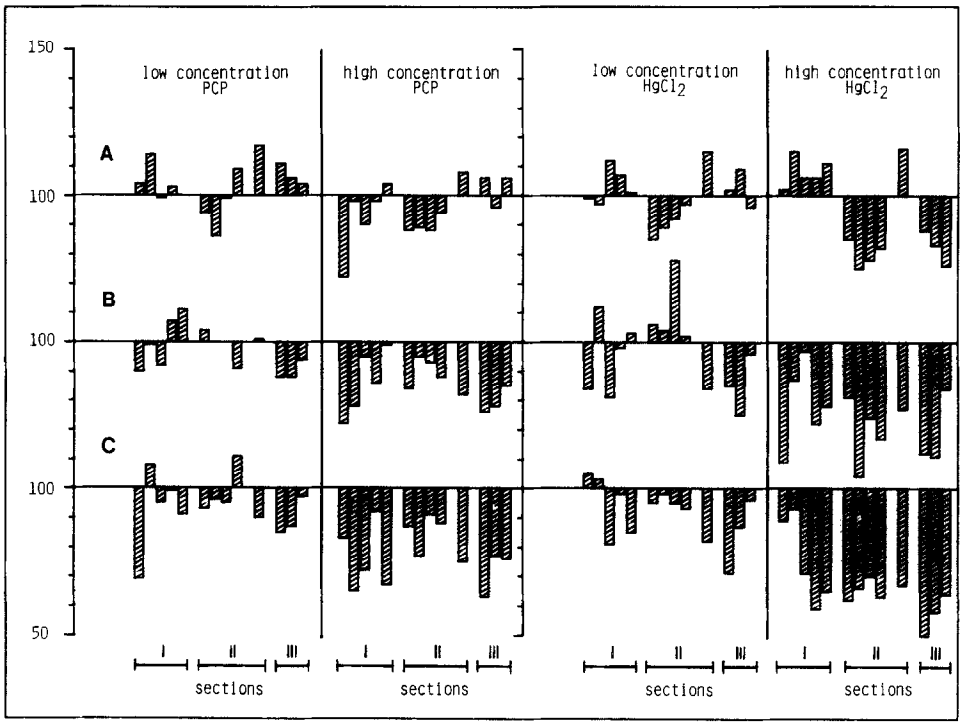

FIG. 8. The effects of PCP and $HgCl_2$, in a low (2 $mg \cdot kg^{-1}$) and in a high (20 $mg \cdot kg^{-1}$) concentration, on the bioactivity, calculated as the average of the percentage of controls, derived from the measurements of ATP, from heat 1 and heat 2 techniques, from the Fe(III)-reduction test, from respiration test, and from the heat potential (hepo) test, carried out in soils *A* (peat), *B* (clay), and *C* (sand). Section I: 1–5 weeks; section II: 6–13 weeks; section III: 14–18 weeks.

In soil B, section I indicated a rapid change of stimulation and inhibition, which was reduced at the end of the section. Section II showed slight and strong stimulations, whereas in section III significant inhibitions were observed. Soil *C* exhibited a retarded inhibition (section I) followed by an insignificant one (section II) and a clear but repaired inhibition (section III).

The high concentration of $HgCl_2$ in soil *A* stimulated bioactivity in section I and in the second part of section II (dry stress), whereas in the first part of section II and in section III only significant inhibitions could be observed, which were similar to those in soils *B* and *C*.

4. Effect of Environment on the Bioactivity of Soils as Determined by Various Tests

Artificial changes in environmental parameters such as temperature, pH, and water content influence the activity of microorganisms and the sensitivity of the tests. Therefore, experiments with untreated soil and the six test methods were carried out by varying these parameters. The results are shown in Table 2.

Only the temperature remained constant during the observation period (high 38°C, low 5°C), whereas pH and water content changed significantly during the experimental time. Therefore, the average of actual data determined during the observation period are also included in Table 2 (columns 3 and 4).

The ATP content was negatively influenced by low water content and lower pH in all of the soils investigated. The heat 1 test also showed smaller values in samples with low water contents (all soils), higher temperature (soil *B*), and low pH (soils *B* and *C*). The heat 2 test gave the lowest data in soils with low water content (*B* and *C*). The highest differences from the controls were obtained in measurements with the Fe(III)-reduction test when the pH of the soils was lower than 4.5 (*A* and *C*). The method for determining CO_2 and the hepo technique yielded lower results in soils with low water content and high temperature (all soils) and low pH (soils *A* and *C*).

DISCUSSION

The critical examination of techniques applied to the measurement of bioactivity in soils is an indispensable prerequisite for the assessment of the ecotoxicological behavior of chemicals. The specificity of each method and the limit of its applicability, in relation not only to the microorganisms in their environmental conditions, but also to the effects of physical parameters such as pH, humidity, and soil constituents on the sensitivity of the tests, have to be determined.

The methods applied in this work measure metabolic processes quantitatively and thus act as indicators of physiological conditions of soil microorganisms. In the most ideal case, the measured metabolic products are obtainable from all living microorganisms in balanced quantities and can be determined quickly and easily by simple methods unsensitive to environmental disturbances. However, this ideal case does not exist, and each method available today has its shortcomings.

TABLE 2

MEASUREMENTS OF SOME PHYSIOLOGICAL PARAMETERS (ATP, HEAT OUTPUT, Fe(III) REDUCTION, AND RESPIRATION) AFTER ARTIFICIAL CHANGES OF ENVIRONMENTAL PARAMETERS

Soil	Changes[a]	H_2O content (% of max WHC)	pH	Measurement					
				ATP (μg/g)	Heat 1 (μW/g)	Heat 2 (μW/g)	Fe(III) (mg Fe^{2+}/g)	Co_2 (μg/hr)	Hepo (μW/g)
A	1	55	6.6	1.4 ± 0.2	18 ± 1.1	14 ± 0.3	0.47 ± 0.2	438 ± 55	160 ± 23
	2	64	6.8	2.0 ± 0.3	29 ± 3.4	14 ± 0.5	0.28 ± 0.1	496 ± 105	92 ± 22
	3	25	6.6	0.7 ± 0.2	13 ± 2.2	13 ± 3.9	1.21 ± 0.3	179 ± 32	77 ± 5
	4	45	3.8	0.5 ± 0.1	21 ± 1.9	15 ± 2.5	0.09 ± —	160 ± 23	96 ± 5
	5	49	6.7	0.9 ± 0.4	24 ± 3.2	14 ± 0.4	0.52 ± 0.1	148 ± 34	83 ± 12
	6	51	6.5	1.7 ± 0.2	21 ± 2.7	15 ± 2.3	0.95 ± 0.1	436 ± 70	175 ± 19
B	1	55	8.4	1.1 ± 0.2	12 ± 2.1	6 ± 2.2	6.3 ± 1.8	246 ± 42	116 ± 19
	2	76	8.2	1.1 ± 0.2	15 ± 2.5	7 ± 2.7	4.0 ± 0.6	193 ± 32	104 ± 16
	3	10	8.3	0.1 ± 0.1	1 ± 0.5	1 ± 0.4	4.4 ± 2.6	5 ± 7	2 ± 1
	4	43	6.8	0.6 ± 0.1	6 ± 1.9	6 ± 3.0	8.5 ± 1.2	200 ± 27	72 ± 7
	5	43	8.3	0.4 ± 0.1	3 ± 2.0	6 ± 7.4	10.1 ± 1.2	48 ± 19	25 ± 6
	6	55	8.5	0.9 ± 0.1	12 ± 2.1	7 ± 0.4	6.9 ± 1.2	264 ± 40	107 ± 14
C	1	55	7.3	0.5 ± 0.1	7 ± 1.1	6 ± 1.6	5.2 ± 1.5	124 ± 40	50 ± 13
	2	85	7.3	0.4 ± 0.1	7 ± 1.6	6 ± 1.5	3.0 ± 1.0	124 ± 28	61 ± 13
	3	13	7.4	0.1 ± 0.1	2 ± 0.1	1 ± 0.6	28.0 ± 16.0	25 ± 34	2 ± 1
	4	55	4.5	0.2 ± 0.1	3 ± 2.0	4 ± 2.4	0.2 ± 0.1	46 ± 13	23 ± 4
	5	53	7.4	0.4 ± 0.1	5 ± 3.0	3 ± 2.0	5.9 ± 1.6	96 ± 46	20 ± 8
	6	53	7.4	0.4 ± 0.1	9 ± 1.4	5 ± 1.0	4.0 ± 1.5	143 ± 53	48 ± 17

[a] 1, Control; 2, H_2O content 85% of maximum capacity; 3, H_2O content 25% of maximum capacity; 4, pH adjusted to 3.5; 5, temperature 38°C; 6, temperature 5°C.

ATP is present in all living microorganisms and is decomposed quickly if it occurs extracellularly or in dead organisms. The main difficulty of the ATP test lies in the variation of the extractability of the ATP and of the relation between biomass and ATP quantity (Jenkinson and Oades, 1979). An incomplete extraction of ATP due to the sorption on colloids of the soil (Ausmus, 1973) was also observed.

The application of heat measurement by a microcalorimetric technique is a good measure of overall soil catabolism and is largely independent of organisms or intermediate reactions. The low actual microbiological activity in soil is due to the fact that only part of the total microorganisms, the autochtonous bacteria, develop activity, whereas the greater part (zymogenous bacteria) are activated only after application of organic compounds (Beck, 1968). The main interference in measurements by this method is heat production of nonbiological origin; for example, the redistribution processes of moisture within unsaturated soil. Most error sources are eliminated by performing a comparison of test sample and control in a twin measurement. By using hermetically sealed vials kept at constant temperature (heat 2 test), redistribution of humidity is widely prevented and the measurements are carried out under anaerobic conditions. The samples of the heat 1 measurement are incubated in vials in such a way that the gas exchange is guaranteed and the evaporation of humidity is limited, so that this measurement represents heat production under aerobic conditions.

As a consequence of oxygen exhaustion, in each soil with sufficient organic compound content, reduction phenomena develop, in which a number of facultative and obligate anaerobic bacteria use the easily reducible Fe(III) oxides as hydrogen acceptors (Ottow, 1982). The limit of the applicability of the Fe(III)-reduction test (Welp and Brümmer, 1985) is a low soil pH, because then the microbial Fe^{2+} liberation is superimposed by a pure abiotic dissolution of Fe(III) oxides. The low values obtained at pH 4.5 in soils *A* and *C* are probably due to the low biological activity at this pH.

After the addition of a suitable carbon energy substrate to the soil samples, such as glucose, CO_2 production (Anderson and Domsch, 1978) and heat output (Sparling, 1983) increase significantly. The additional activity of microorganisms is derived from zymogenous bacteria (Beck, 1968) which are a potential component of the soil biomass.

Generally, most CO_2 is delivered by the aerobic heterotrophic organisms. The main sources of the inaccuracy of the CO_2 method are those abiotic processes which form CO_2, those biological processes in which degradation and reconstruction occur without CO_2 production, and the assimilation of CO_2, for example, by autotrophic organisms. In this work, the substrate-induced maximal initial respiration rates (Anderson and Domsch, 1978) were determined, and thus most disturbances were limited.

The Fe(III)-reduction test, the CO_2 test, and the heat potential output of microorganisms (hepo test) measure the potential activity of the soil microorganisms. Tests without the addition of glucose indicate the actual activity.

It is difficult to distinguish the proportion of the real activity of microorganisms measured by each method from the unability of the methods due to the limited sensitivity of the test. Some simple extreme environmental parameters were tested for the sensitivity of the methods and compiled in Table 2. The main problem of the experiment was to keep the soil humidity at a constant level without reducing the O_2 supply. Restoration of water which has been lost by evaporation causes an enhanced CO_2 production due to the liberation of amino acids and of carbohydrates as a consequence of drying (Beck, 1968). The addition of water also requires its homogeneous distribution; thus, the soil is artificially reactivated. In order to avoid such distur-

bances, the soil humidity was only measured and not adjusted. Consequently, in some soils the water content decreased to such a low level that the microbial activity was seriously limited (soil *B* 10% and soil *C* 13% of maximum WHC), and thus no useful values could be obtained from these experiments. On the contrary, in soil *A* the water content remained at 25% of the maximum WHC, and it was shown that, except for the heat 2 and Fe(III)-reduction tests, a significantly lower bioactivity was measured than in the control samples. With the Fe(III)-reduction test, in samples with a high water content, a low bioactivity was measured in all three soils. Here, what is influenced primarily by the high water content is also uncertain: the microorganisms generally, or a few of them, or the test itself.

Changes in temperature influence the bioactivity of the soil more than the effectiveness of the test itself. The differences between the activities obtained from control and test samples suggest that certain temperature-sensitive species are involved in the measured processes.

The experiments with the low pH suggest that the Fe(III)-reduction test should be applied only in soils whose pH is higher than 4.5.

From a wealth of data which resulted from measurements of side effects of chemicals on populations and functions of soil microorganisms, Domsch *et al.* (1983) concluded that reversible side effects causing delays in the restitution of microbial parameters up to 30 days are normal, up to 60 days are tolerable, and more than 60 days are critical. Most agrochemicals are applied in concentrations which probably do not affect the microorganisms critically in one application, but presumably the effects can be classified as tolerable or even as normal. A repetition of the so-called normal effects, however, impairs the microbial population differently. The changes can be measured either by finding a sensitive species or function which is also an indicator for the soil and condition of the microorganisms, or by methods which cover the total or at least a great portion of the physiological activities of the microorganisms.

The results obtained from only one species or function whose relation to the total population and bioactivity is undefined probably contain more uncertainties and also are more accidental than those obtained from activities of a number of microorganisms measured by different methods. In the first case, the sensitivity, and in the second one, the certainty is emphasized. A combination of both procedures delivers more satisfactory results.

The application of the test chemicals (PCP and $HgCl_2$) caused mostly inhibition or stimulation in soils as compared to untreated controls, either directly or retarded. These effects either increased or decreased with time, depending on the soil and the chemical applied. The effects of low concentration of the chemicals were mostly small and were reduced within the first section; the second application at Day 36 was mostly not affected by the first one.

It is assumed that small concentrations of chemicals are either utilized by the microorganisms (PCP) or sensitive species are eliminated and replaced by less sensitive ones. With the exception of the third application of PCP and also partly of $HgCl_2$ in peat soil, the measured effects are mostly inhibitory ones. Peat soil possesses a high content of organic matter, which gives more support to restoration either by the affected microorganisms repairing the relatively small injuries themselves or by the replacement of these microorganisms by other organisms.

The effects caused by high concentrations of chemicals, also, are less remarkable in peat soils than in other soils. The difference in the effects observed between soils *B* and *C* is a consequence of variations in the population of microorganisms and in the

physical conditions of the soil. The higher adsorption of chemicals on the colloids of soil *B* may also be a reason for the smaller effects of the chemicals in this soil.

CONCLUSION

It may be concluded that various effects of PCP and $HgCl_2$ on the microflora of soils can be measured by different methods. Since each method has its limitations, only a combination of several methods should be used to evaluate the ecotoxicological behavior of chemicals in soils.

REFERENCES

ANDERSON, J. P. E., ARMSTRONG, R. A., AND SMITH, S. N. (1981). Methods to evaluate pesticide damage to the biomass of the soil microflora. *Soil Biol. Biochem.* **13,** 149–153.

ANDERSON, J. P. E., AND DOMSCH, K. H. (1978). A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* **10,** 215–221.

AUSMUS, B. S. (1973). The use of the ATP-assay in terrestrial decomposition studies. *Bull. Ecol. Res. Commun. (Stockholm)* **7,** 223–234.

BECK, T. (1968). *Mikrobiologie des Bodens.* Bayer. Landwirtschaftsverlag, Munich, Vienna, Basel.

BECK, T. (1983). Mikrobiologische und biochemische Charakterisierung landwirtschaftlich genutzter Böden. I. Mitteilung: Die Ermittlung einer bodenmikrobiologischen Kennzahl. *Z. Pflanzenernähr. Bodenk.* **147,** 456–466.

DOMSCH, K. H., JAGNOW, G., AND ANDERSON, T. H. (1983). An ecological concept for the assessment of side effects of agrochemicals on soil microorganisms. *Residue Rev.* **86,** 65–105.

JENKINSON, D. S., AND OADES, J. M. (1979). A method for measuring adenosine triphosphate in soil. *Soil Biol. Biochem.* **11,** 193–199.

JENKINSON, D. S., AND POWLSON, D. S. (1976). The effect of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biol. Biochem.* **8,** 209–213.

LJUNGHOLM, K., NOREN, B., AND WADSÖ, J. (1979). Microcalorimetric observations of microbial activity in normal and acidified soils. *Oikos* **33,** 24–30.

MALKOMES, H. P., AND WÖHLER, B. (1983). Testing and evaluating some methods to investigate side effects of environmental chemicals on soil microorganisms. *Ecotoxicol. Environ. Safety* **7,** 284–294.

OTTOW, J. G. G. (1982). Bedeutung des Redoxpotentials für die Reduktion von Nitrat und Fe(III)oxiden in Böden. *Z. Pflanzenernähr. Bodenk.* **145,** 91–93.

PAL, S. S., SUDHAKAR-BARIK, AND SETHUNATHAN, N. (1979). Effects of benomyl on iron and manganese reduction and redox potential in flooded soil. *J. Soil Sci.* **30,** 155–159.

SCHNÜRER, J., AND ROSSWALL, T. (1982). Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Appl. Environ. Microbiol.* **43,** 1256–1261.

SPARLING, G. P. (1983). Estimation of microbial biomass and activity in soil using microcalorimetry. *J. Soil Sci.* **34,** 381–390.

TU, C. M. (1982). Influence of pesticides on activities of invertase, amylase and level of adenosine triphosphate in organic soil. *Chemosphere* **11,** 909–914.

WELP, G., AND BRÜMMER, G. (1985). Der Fe(III)-Reduktionstest, ein einfaches Verfahren zur Abschätzung der Wirkung von Umweltchemikalien auf die mikrobielle Aktivität in Böden. *Z. Pflanzenernähr. Bodenk.* **148,** 10–23.

ZELLES, L., BAHIG, M. E., SCHEUNERT, I., KLEIN, W., AND KORTE, F. (1984). Measurement of bioactivity based on CO_2-release and ATP content in soils after different treatments. *Chemosphere* **13,** 899–913.

ZELLES, L., SCHEUNERT, I., AND KORTE, F. (1985a). ATP-measurement in soil: A combination between the TCA und NRB extraction methods. *Chemosphere* **14,** 139–148.

ZELLES, L., SCHEUNERT, I., AND KORTE, F. (1985b). Side effects of some pesticides on non-target soil microorganisms. *J. Environ. Sci. Health* **B20** (5), 457–488.

ZELLES, L., SCHEUNERT, I., AND KORTE, F. (1986). Determination of the effect of pentachlorophenol on the bioactivity of soils by the iron-reduction test. *Chemosphere,* **15,** 309–315.