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Side-effects of organic and inorganic pollutants on soil nitrification and respiration

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Abstract — Side-effects of heavy metals (Cd and Zn) and organic pollutants (phenanthrene and paclobutrazol) on nitrification and respiration in a loam soil were investigated with its air-dried and fresh conditions. Critical levels for these test pollutants were derived from this investigation. Bioavailable concentrations of Cd and Zn were also determined. It was concluded that such critical levels as NOEL and LOEL of heavy metals should be expressed using bioavailable concentrations rather than total ones since different soil has different properties, especially different absorption ability to heavy metals.

Keywords: nitrification; respiration; organic pollutants; heavy metal; bioavailable concentration.

1 Introduction

Soil microorganisms play vital roles in affecting soil fertility and primary production through their roles in organic matter decomposition and nutrient cycling (Parkinson, 1991). Soil contain a large variety of microbial taxa with a wide diversity of metabolic abilities. Therefore, it is hardly surprising that for decades there has been consistent, sometimes heated, interest in studying various facets of the heterogeneous soil microbial community.

In recent decades the soils of industrial countries have been increasingly contaminated with inorganic as well as organic pollutants, mostly from deposit of exhaust gases, mine spills, application of sewage sludge, discharging of municipal and industrial wastewater, and widespread use of pesticides and other chemicals (Wilke, 1989; Babich, 1980; Gong, 1993). Numerous investigations have shown adverse effects of pollutants on soil microbial activities, such as C-mineralization, N-mineralization, nitrogen transformation, soil enzyme activity, microbial numbers and biomass, and species composition and diversity (Baath, 1989).

The present study is initially designed to investigate side effects of our different chemicals of 3 categories, that is , cadmium and zinc standing for heavy metals, phenanthrene for PAHs, and paclobutrazol for pesticides, which is popularly used as a plant growth regulator in southern China. Such microbial activities as nitrification and respiration which were concluded by Domsch *et al.* (Domsch, 1983) from 734 experiments to be rather sensitive indicators were chosen as indices.

2 Methods and materials

2.1 Test soil

A 0—20 cm top layer soil was collected twice from a trial field at LUFA-Augustenberg in Karlsruhe, Germany. The first time was in early December of 1993, and the second time in late March of 1994. The properties of the soil were shown in Table 1. From the total concentrations of some heavy metals in this soil, it could be derived that the soil was rather unpolluted. The levels of total Zn and Cd were only 65 and 0.2ppm respectively. All experiments employed both fresh and air-dried soils, which were sieved through A 2mm mesh. Sieving of the air-dried soil was done after two-days' drying in open air.

Table 1 Properties of the experimental soil

pH($CaCl_2$)	7.5	Heavy metals, ppm	
$CaCO_3$,	12%	Mn	636
N	0.11%	Cu	63
C	1.6%	Zn	65
CEC, mval/100g soil	13.8	Hg	0.4
Texture		Pb	43
<2μ	12.9%	Cd	0.2
2—6μ	6.3%	Cr	42
6—20μ	10.0%	Ni	20
20—63μ	34.4%		
>63μ	36.4%		

2.2 Test pollutants

Cd and Zn both as sulfate salts, phenanthrene, paclobutrazol [Chemical name: (2RS, 3RS)-1-(4-chlorophenyl)-4,4- dimethyl-2-(1H-1,2,3-triazol-1-yl)pentan-3-01,C. A.; 98% purity, code No. PP333] and multi-effect-triazole (MET, trade mark), which is one of its pesticide formulations, were used in this study. Both paclobutrazol and MET (a wettable powder) were purchased from the Jiangsu Pesticide Institute in Nanjing, China. MET contains 15% active ingredient (a. i.) of paclobutrazol. Pollutants were added to soil either in distilled water solutions or suspension for heavy metals and MET or in "stock soil" for phenanthrene, paclobutrazol and MET. "Stock soil" was prepared through mixing certain weights of the three organics evenly with a small amount of dry soil.

2.3 Nitrification

Nitrification was measured using a batch incubation procedure described by Stratton

(Stratton, 1990). Ammonium ion, supplied as ammonium sulfate, was added to 10g of test soil in serum bottles to get a concentration of 250 ppm and the accumulation of produced nitrate ion was analyzed on day 1, 3, 7, 10, 14, 21 and 28 during following incubation period. All samples were incubated in dark at 22℃ and adjusted to a 75% water holding capacity (allowing for subsequent addition of aqueous solutions). Treatments were prepared in enough replicates to allow five or six serum bottles within each group to have their contents harvested and assayed at each time interval. Treatments included a water blank (no NH_4^+-N added), an autoclaved blank, an autoclaved control, an untreated control, and treated systems containing various concentrations of pollutants. Autoclaving was carried out twice at 126.5℃ for 1 hour on two consecutive days before incubation. At each time interval bottles were sacrificed for analysis.

Nitrate was extracted from test soil by adding 50ml of distilled water to each serum bottle, followed by shaking for 1 hour on a mechanical shaker. After settling and filtration through blue-ribbon filter papers, 1.0 or 0.5 ml filtrate was removed and analyzed for NO_3^--N. Nitrate-N was quantitated colorimetrically following a German standard method (DEV, 1982). Absorbance values were converted to NO_3^--N concentrations ($\mu g \cdot g^{-1}$ dry soil) using regressed standard curves.

2.4 Respiration

Soil respiration was estimated using Sapromat, which was an equipment originally designed for BOD measurement by VOITH and first used by Beck (Beck, 1980; 1984) for estimating soil respiration by measuring oxygen uptake of soil samples incubated in it. 50g of dry soil was added into a 500 ml Erlenmeyer flask and fresh soda lime with indicator was added into a vessel built into the glass stopper for absorbing CO_2 produced by soil microorganisms. Oxygen consumed was replenished automatically by a pressure sensor and oxygen generator. Oxygen consumption was recorded on a colour plotter. An optimum concentration of glucose, which should be determined previously to a achieve a highest induced respiration rate, was added into the test soil as substrate, and pollutants were also amended before incubation. For the soil used in this experiment, 6250 μg glucose g^{-1} dry soil appeared to be the optimum concentration.

2.5 Bioavailable heavy metal concentrations

After 28 or 34 days of incubation, bioavailable Cd and Zn added into the 10 g test soil were extracted with 50 ml distilled water and filtrated through the blue-ribbon filter papers. Their concentrations in the filtrates were analyzed by a Furnace Atomic Absorption Spectrometer (Perkin Elmer 2100).

2.6 Statistical analysis

All results were analyzed using a two-tailed student's *t*-test where were applicable. All tests were performed at the 95% and 99% levels of confidence.

3 Results and discussion

If not mentioned, the following results were obtained from experiments with rewetted air-dried soil.

3.1 Nitrification

3.1.1 Heavy metals

The effect of zinc on soil nitrification over an incubation period of 4 weeks is shown in Fig. 1. In the first two weeks, all concentrations of Zn addition showed inhibitory effect. However, in the end, only 1000 and 2000 ppm Zn inhibited nitrification. Fig. 2 indicated that 5 to 100 ppm Cd addition caused no effect after 2 weeks incubation even though 5 ppm showed some inhibitory effect at day 7, and 25, or 50 ppm also caused inhibition at day 10. So the Cd concentration was increased up to 1000 ppm, then obvious inhibition at 250 to 1000 ppm addition from day 10 to 21 of incubation was observed(Fig. 3). But, again in the end, only 1000 ppm showed significant effect.

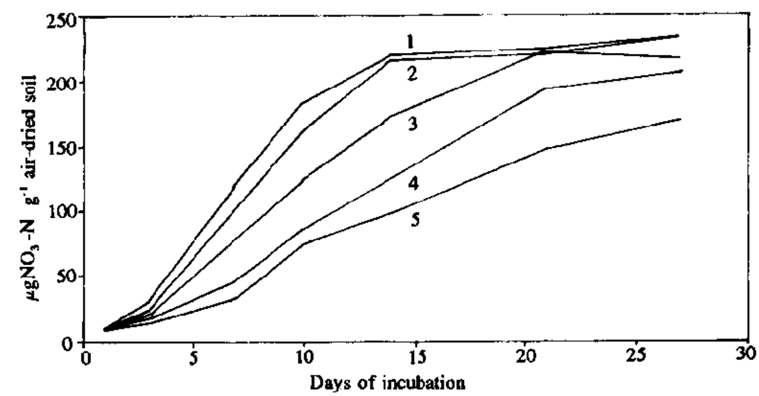

Fig. 1 Effect of Zn on nitrification in a rewetted air-dried soil
1. control 2. 250ppm 3. 500ppm 4. 1000ppm 5. 2000ppm

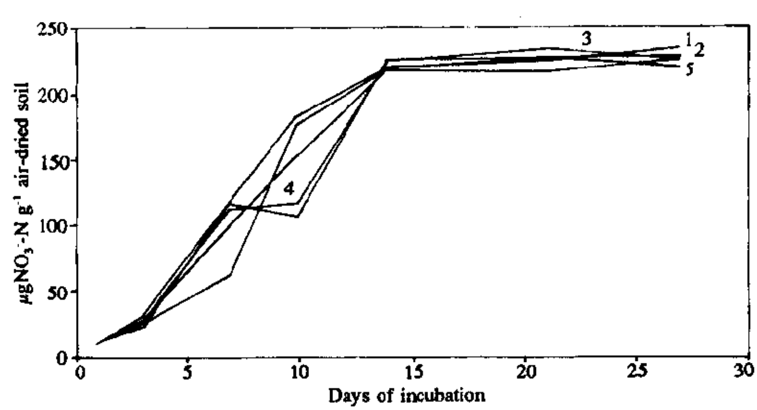

Fig. 2 Effect of Cd on nitrification in a rewetted air-dried soil
1. control 2. 5ppm 3. 25ppm 4. 50ppm 5. 100ppm

These results suggested the possible effects of high ion concentrations instead of heavy metal toxicity on soil nitrification. Thus 15 to 45μmol $K^{+} g^{-1}$ dry soil was tested, which were equivalent to ion concentrations of about 1500 ppm Cd or 850 ppm Zn to 4500 Cd or 2550ppm

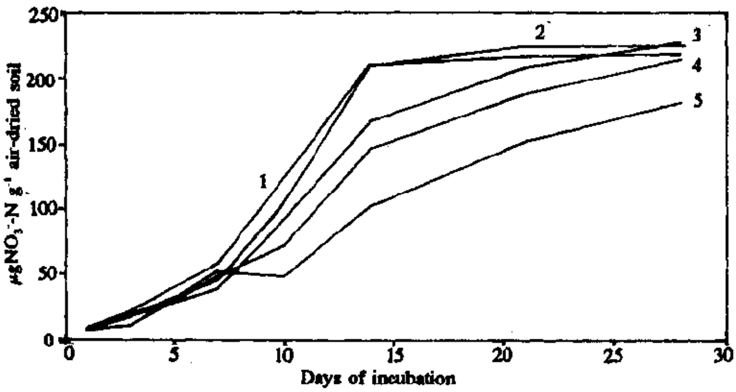

Fig. 3 Effect of Cd on nitrification in a rewetted air- dried soil

1. Control 2. 100ppm 3. 250ppm 4. 500ppm 5. 100ppm

Zn. This range covered the highest ion concentrations of two tested heavy metals. Nevertheless, only 45μmol K^+g^{-1} dry soil caused slight inhibition(Fig. 4). Therefore, it could be concluded that Cd and Zn do have some toxic effects on soil microbe if their concentrations are high enough.

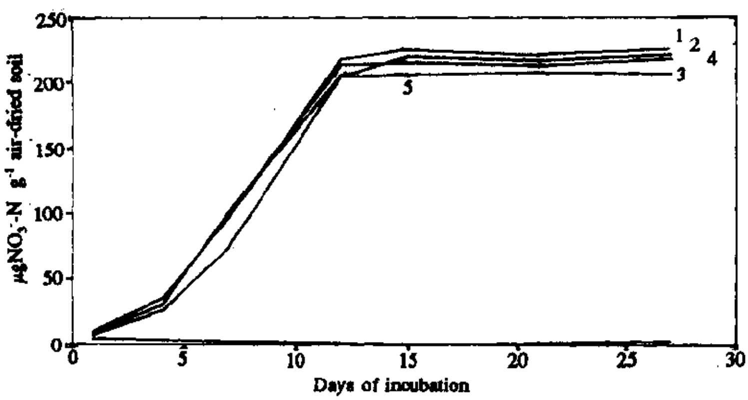

Fig. 4 Effect of K_2SO_4 on nitrification in a rewetted air-dried soil

1. [K^+]15μmol. g^{-1} 2. 30μmol. g^{-1} 3. 45μmol. g^{-1} 4. DCM 1 ml 5. distilled water control

Table 2 summarizes the influence of three sulfate salts on nitrification in dry as well as fresh soils after 4-week or 34 days incubation. It is indicated that in dry soil, 45μmol. g^{-1} K^+, 1000 and 2000 ppm, Zn and 1000 ppm Cd showed significant inhibition while all other levels no effect. In fresh soil, nitrate produced on day 34 was compared to that on day 28. It was found that from day 34 to day 28 nitrite in control samples declined from a mean of 236 ppm to 210 ppm while treated samples almost had no change (data not shown). This caused a decrease of inhibition rate from 49% to 41% for 2000 ppm Zn and from 38% to 31% for 1000 ppm Cd. No significant effect was observed in other treated samples.

Table 2 Effect of fortified pollutants on nitrification in a loam soil

Pollutant	Concentration, μg g^{-1}	Inhibition rate^{+}, %	
Air-dried soil		Day 28	
Zn^{2+}	250	(−)	
($ZnSO_4$)	500	(−)	
	1000	12(**)	
	2000	29(**)	
Cd^{2+}	5	(−)	
($CdSO_4$)	25	(−)	
	50	(−)	
	100	(−)	
	250	(−)	
	500	(−)	
	1000	18(**)	
K^{+}, μmol. g^{-1}	15	(−)	
(K_2SO_4)	30	(−)	
	45	6(*)	
Fresh soil		Day 28	Day 34
Zn^{2+}	250	(−)	(−)
($ZnSO_4$)	500	(−)	(−)
	1000	(−)	(−)
	2000	49(**)	41(**)
Cd^{2+}	100	(−)	(−)
($CdSO_4$)	250	(−)	(−)
	500	(−)	(−)
	1000	38(**)	31(**)

Notes: Student's *t*-test is used for statistical analysing of all results. Negative results mean stimulatory effect while positive ones inhibitory. Inhibition rates are calculated with means of 5—6 replicates.
(−)not significant at 95% confidence; (*) significant at 95% confidence; (* *) significant at 99% confidence

3.1.2 Organic pollutants

One of the major problems with organics is their low solubility in water, which makes it hard to apply them into soil to as high levels as needed. In this study, "stock soil" was tried to be used as the media for applying organic pollutants. The definition of "stock soil" was given as above. For MET, which is usually sprayed to farm land in water suspension, both suspension solution and stock soil were used for application. Suspension solutions of 0.30 to 3.00 ppm MET in distilled water were tested, while in farm land it is commonly applied at a level of 0.15ppm. So the lowest level used here is still twice as high as that in field application. However, even the highest level only showed slight stimulatory influence (Fig. 5). It could be seen in Fig. 4 and Fig. 5 that 1 ml of such organic solvents as acetone (ACT) and dichloromethane (DCM) absolutely inhibited the nitrification activity. This means that it is improper to apply organic pollutants through organic solvents. Stock soil method seems to be a right choice.

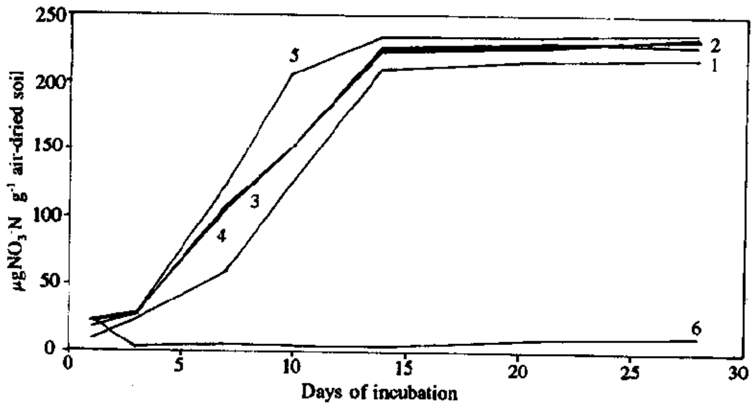

Fig. 5 Effect of MET on nitrification in a rewetted air-dried soil
1. Control 2. MET(a. i.) 0.30ppm 3. 0.60ppm
4. 1.50ppm 5. 3.00ppm 6. 2.94ppm(in 1ml ACT)

Table 3 provides the inhibition rates of all three organics including MET. Inhibition rates were calculated on the basis of average accumulation of nitrate produced after 4 weeks incubation. Since the produced nitrate could possibly reach a relatively stable level only after 4 weeks incubation, other data during incubation were ignored for further statistical analysis. As mentioned above, a two-tailed student's *t*-test was used for statistical analysis, because effects could probably be inhibitory or stimulatory.

In Table 3, only 250 ppm phenanthrene showed significant inhibition while 6.25 paclobutrazol and 0.6, 1.5 and 3.0 ppm(a.i.) MET stimulated soil nitrification by 5%—8%. All others showed no significant difference from control untreated soil.

Table 3 Effect of fortified organic pollutants on nitrification in a rewetted air-dried loam soil

Pollutant	Concentration, μg·g^{-1}	Inhibition rate$^+$, %	Pollutant	Concentration, μg·g^{-1}	Inhibition rate$^+$, %
Phenanthrene	5	(−)	MET	0.3	(−)
	25	(−)	(Active ingredient)	0.6	−6(*)
	125	(−)		1.5	−5(*)
	250	6(*)		3.0	−8(**)
Paclobutrazol	6.25	−6(*)		6.0	(−)
	12.5	(−)		12.0	(−)
	25.0	(−)		24.0	(−)
	50.0	(−)		48.0	(−)

+The same as Table 2

3.2 Respiration

Respiration of rewetted dry soil with Zn and Cd fortification was measured. Fig. 6 shows the oxygen consumption curve and derived respiration rate. Only the maximum rate was used as the index, which took place on the third day of incubation. Effect of heavy metals on soil respiration was summarized in Table 4. In this table, inhibition rates were calculated with the mean values of 5 or 6 replicates, and it could be seen that even though inhibition can be observed from −16% to 37%, only 2000 ppm Zn addition with a 37% inhibition cause significant effect on soil respiration. This result suggests that the deviation of samples of each treatment was very large. However, from nitrification results with dry soil, addition of up to 1000 ppm Zn or 500 pm Cd could not give rise to any effect significantly observable either. So respiration results show good agreement with nitrification results in this study.

Table 4 Effect of heavy metals on respiration in a rewetted air-dried loam soil

Pollutant	Concentration, $\mu g \cdot g^{-1}$	Inhibition rate*, %
Zn^{2+}	250	−8(−)
($ZnSO_4$)	500	15(−)
	1000	30(−)
	2000	37(**)
Cd^{2+}	100	−11(−)
($CdSO_4$)	250	−16(−)
	500	not test
	1000	not test

* The same as Table 2

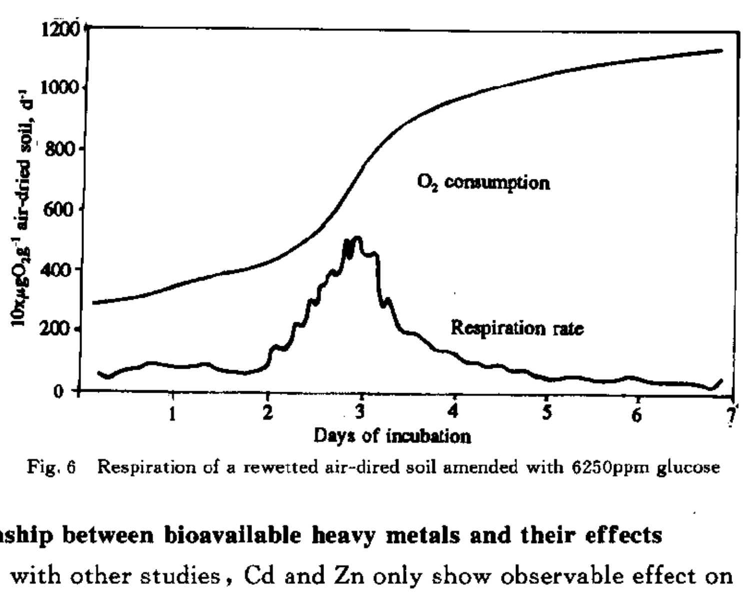

Fig. 6 Respiration of a rewetted air-dired soil amended with 6250ppm glucose

3.3 Relationship between bioavailable heavy metals and their effects

Compared with other studies, Cd and Zn only show observable effect on this test soil at much higher added concentrations. So it could be derived naturally that the test soil has extremely strong ability to absorb or deposit heavy metals, which might be due to too much carbonate and high pH in it. Its background levels of Zn and Cd, as shown in Table 1, were just 65 and 0.2 ppm, respectively. So it is necessary and also interesting to examine how much heavy metals is absorbed or deposited by soil and how much is releasable and bioavailable. Now there are two ways to extract bioavailable heavy metals in soil (Hornburg, 1993). One uses distilled water, and the other uses 1 mol/L NH_4NO_3 solution. The former one was employed in the present study.

Table 5 shows the relationship between bioavailable heavy metals and their effect on nitrification. In fresh soil, 2000 ppm Zn addition resulted in a bioavailable concentration of 112 ppm, correlating with a 41% inhibition rate, and 1000 ppm Cd addition resulted in a concentration of 21.7 ppm, correlating with a 31% rate. In air-dried soil, 26.5 ppm bioavailable Cd could be obtained by adding 1000 ppm Cd and caused 18% inhibition. Concentration of Zn in air- dried soil was not determined. Therefore, due to too few data available, critical levels like LOEL (lowest observed effect level) could not be derived from this experiment. But the NOELs (non observed effect level) for the two metals could be found in Table 5. That is, 20 ppm for Zn in fresh soil, 5.77 ppm and 9.59 ppm for Cd in fresh and air-dried soils respectively. A comparison of response to heavy metal pollution also can be made between fresh and air-dried soil. And it could be easily seen from this table that fresh soil is much more sensitive than dry soil, which might be due to the disappearance of sensitive microorganisms during the process of air-drying.

Table 5 Relationship between bioavailable concentrations of heavy metals and their effects on nitrification in a loam soil

Pollutant	Concentration, $\mu g \cdot g^{-1}$			Inhibition rate+, %	Pollutant	Concentration, $\mu g \cdot g^{-1}$			Inhibition rate+, %
	FT	BA	AD			FT	BA	AD	
Fresh soil				Day 34	Air-dried soil				Day 28
Zn^{2+}	250	ND	250	(−)	Zn^{2+}	250	NM		(−)
($ZnSO_4$)	500	4.44	496	(−)	($ZnSO_4$)	500	NM		(−)
	1000	19.81	980	(−)		1000	NM		12(**)
	2000	112.1	1888	41(**)		2000	NM		29(**)
Cd^{2+}	100	0.50	99.5	(−)	Cd^{2+}	100	0.67	99.3	(−)
($CdSO_4$)	250	2.03	248	(−)	($CdSO_4$)	250	2.97	247	(−)
	500	5.77	494	(−)		500	9.59	490	(−)
	1000	21.71	978	31(**)		1000	26.51	937	18(**)

+The same as Table 2. FT: fortified; BA: bioavailable; AD: absorbed or deposited; ND: not detectable; NT: not measured

Baath (Baath, 1989) summarized that the difference between the highest and the lowest reported values for both the NOELs and LOELs of Cu, Zn and Pb spaned a range by a factor of 100 to 1000 for the mineral soils. The total range of the LOEL for Cd is 2—560 ppm addition (Witter, 1992). The large observed variation in metal sensitivity may be due to lots of environmental factors, such as clay minerals, organic matter, soil pH, inorganic anionic/cationic composition, CEC and ion exchange capacity (Babich, 1980). In precious studies, heavy metal toxicity toward microorganisms and microbially mediate processes in soil has usually been expressed using total concentration, rate of addition or relative increase over background instead of bioavailable one. This makes it impossible to compare results from different studies, since different soils have different properties.

According to a recent review by Witter(Witter, 1992), the LOELs for Cd derived from field and laboratory studies are more than 5-fold relative increase over background level and 14-fold increase or 2 ppm addition separately; for Zn, 3-fold relative increase or 100 ppm addition and 2.8-fold increase or 50 ppm addition(Table 6).

Table 6 NOEL and LOEL values derived by Witter, E(1992)

Critical levels	Laboratory studies		Field studies	
	Cd	Zn	Cd	Zn
NOEL				
Rate of addition, $\mu g\ g^{-1}$ soil	1.5	10	—	—
Relative increase over background	4	1.2	—	—
LOEL				
Rate of addition, $\mu g\ g^{-1}$ soil	2	50	—	100
Relative increase over background	14	2.8	>5	3
Total concentration in soil, $\mu g.\ g^{-1}$ soil	—	—	—	150

In this study, only addition of up to 1000 ppm Zn or Cd exerted significant inhibition, which sounds a bit incredible. However, if we use the NOELs derived from bioavailable concentrations, i.e. 20ppm for Zn in fresh soil, 5.77 ppm and 9.59 ppm for Cd in fresh and air-dried soils respectively(Table 5), then, the results appear to be reasonable and comparable. Therefore, it is highly recommended to use bioavailable concentrations for expressing critical levels.

4 Conclusion

The following could be concluded from this investigation:

1. The test soil was rather insensitive, which might be attributed to its high content of carbonate;

2. Nitrification and respiration were not very sensitive indicators to soil pollution, especially to heavy metals;

3. Fresh soil was more sensitive than air-dried soil, which might be due to the disappearing of sensitive classes of microorganisms during air-drying;

4. It should be highly recommended to use bioavailable concentrations of heavy metals for expressing critical levels in soil;

5. The NOELs, non observed effect levels (not the highest), were 20 ppm for Zn in fresh soil, 5.77 ppm and 9.59 ppm for Cd in fresh and air-dried soils separately (on the basis of bioavailable concentrations); 50 ppm for paclobutrazol in dry soil, and 48 ppm (a.i.) for MET in dry soil;

6. The highest NOEL for phenanthene was 125 ppm, and the LOEL (lowest observed effect level) for it was 250 ppm;

7. 6.25 ppm paclobutrazol and 0.6 to 3.0 ppm (a.i.) MET show a little stimulatory effect on nitrification.

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