SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 20, No. 11, pp. 2469–2474, 2001

Printed in the USA
0730-7268/01 $9.00 + .00

# POTENTIAL NITRIFICATION RATE AS A TOOL FOR SCREENING TOXICITY IN METAL-CONTAMINATED SOILS

ERIK SMOLDERS,* KRIS BRANS, FILIP COPPENS, and ROEL MERCKX
Laboratory of Soil Fertility and Soil Biology, Katholieke Universiteit Leuven, Kolsteelpark Arenberg, 20, 3001 Leuven, Belgium

(*Received* 13 *December* 2000; *Accepted* 11 *April* 2001)

**Abstract**—A potential nitrification rate test (PNR) was used to identify metal toxicity in field-contaminated soils. The test was applied to metal salt–spiked soils, to 27 uncontaminated soils, and to 15 soils that are contaminated by former metal smelting activities. Four agricultural soils (pH 4.5–6.6) were spiked with various rates of $CdCl_2$ (0–200 mg Cd/kg dry wt) or $ZnCl_2$ (0–3,000 mg Cd/kg dry wt) and were equilibrated more than nine months prior to testing. The soil Zn EC50s of the PNR were between 150 and 350 mg Zn/kg dry weight. No continuous decrease of the nitrification with increasing Cd application was observed. The nitrification rate was reduced by between 50 and 80% at the highest Cd application in all soils. The PNRs of 27 uncontaminated soils varied widely (0–21 mg N/kg/d), but most of this variability is explained by soil pH ($R^2$ = 0.77). The PNRs of the 15 contaminated soils were 0 to 44% of the values predicted for an uncontaminated soil at corresponding pH. Significant toxicity in field-contaminated soils was identified if the PNR was outside the 95% prediction interval of the PNR for an uncontaminated soil at corresponding pH and was found in seven soils. These soils contain 160 to 34,000 mg Zn/kg dry weight and 5 to 104 mg Cd/kg dry weight and had a pH >5.7. No toxicity could be detected below pH 5.6, where even a zero PNR value is within the 95%prediction interval of uncontaminated soils. It is concluded that the nitrification is sensitive to metal stress but that its power as a soil bioassay is low because of the high variability of the endpoint between uncontaminated soils. The ecological significance of the assay is discussed.

**Keywords**—Nitrification Cadmium Zinc Soil Soil solution

## INTRODUCTION

Nitrification is the conversion of ammonium ($NH_4^+$) to nitrite ($NO_2^-$) and then to nitrate ($NO_3^-$). Nitrification is performed by a specific group of microorganisms, that is, *Nitrosomonas* spp. for the oxidation to $NO_2^-$ and *Nitrobacter* spp. for the oxidation of $NO_2^-$ to $NO_3^-$. Nitrification is a process that is affected by metals in soil [1–5]. For example, zinc applications as low as 100 mg Zn/kg have been shown to reduce the initial nitrification rate to about 65% of that in the unamended soil [5].

Because of its sensitivity to metal contamination and its key role in nitrogen cycling, nitrification is a process that can be used in hazard assessment of chemicals in soil. The term "nitrification potential" is commonly used for nitrification in soil at saturating substrate concentrations, that is, the nitrification observed immediately after adding $NH_4^+$ as the substrate [2,3,5,6]. The soil nitrate concentrations are subsequently measured after incubation periods that can vary from a few hours to 50 d [1,6]. The accumulation of $NO_2^-$-N is also used as an endpoint, although this intermediate is found only in tests performed in suspensions [6] or in calcareous soils [2]. The International Organization for Standardization (ISO, Geneva, Switzerland) lists ISO 14238 [7] as a 28-d soil incubation test where the nitrification rate is used as an endpoint after adding 100 mg $NH_4^+$-N/kg soil as substrate.

The nitrification rate strongly varies with soil properties in uncontaminated soils. Laboratory tests show that nitrification rate is slow in acid soils [1,8]. The nitrification rate is also reduced at elevated soil nitrate content [9] and at high soil moisture content [10]. This variability is excluded when testing laboratory-contaminated soils relative to uncontaminated control samples at standard environmental conditions. For the purpose of screening soil quality, however, a need exists to know the expected value of the nitrification rate for uncontaminated soils. This can be achieved from a survey of this process in uncontaminated samples. Screening soil quality in the field could be a useful tool in risk assessment or in monitoring remediation activities.

This paper addresses the use of the potential nitrification rate (PNR) for screening toxicity in contaminated soils. The PNR is measured in a wide series of uncontaminated and contaminated soils and tested at standard conditions in the laboratory. The contaminated soils include soils that are spiked in the laboratory with Cd and Zn salts and field-contaminated soils (mixed metal pollution). The variability of the PNR in uncontaminated soils is related to soil properties, and this relationship is used to determine if the PNR of a contaminated soil indicates toxicity.

## MATERIALS AND METHODS

### *Soil sampling and treatment*

Two groups of soils were collected in this study: 27 uncontaminated soils and 15 soils contaminated by former smelter activities (field-contaminated soils). The uncontaminated soils were collected in Belgium in August 1997 (soils 1–4) or in August 1998 (soils 5–27). The soils include a variety of agricultural soils and forest soils (Table 1). Total Cd and Zn concentrations in these soils (Table 1) were below the baseline concentrations of Flemish soils, which are defined as 90th percentiles of >450 soil samples collected in arable land, forest, and fallow and pasture, well away from point metal sources [11]. Soils 12 and 14 have Zn concentrations above the base-

* To whom correspondence may be addressed (erik.smolders@agr.kuleuven.ac.be).

Table 1. Selected characteristics of the uncontaminated soils

| Sample | Land use | pH $CaCl_2$ (0.01 M) | % C | % Clay | CEC pH 7 ($cmol_c/kg$)[a] | Aqua regia soluble metals (mg/kg) Cd | Zn |
|---|---|---|---|---|---|---|---|
| 1 | Pasture | 4.4 | 2.8 | 1 | 7.8 | 0.36 | 32 |
| 2 | Pasture | 5.0 | 1.5 | 7 | 9.5 | 0.27 | 45 |
| 3 | Arable land | 6.6 | 1.1 | 10 | 6.5 | 0.10 | 28 |
| 4 | Arable land | 6.6 | 0.9 | 17 | 10.2 | 0.20 | 47 |
| 5 | Pine forest | 3.0 | 3.4 | NM[b] | 9.0 | 0.10 | 19 |
| 6 | Arable land | 7.0 | 1.6 | NM | 11.7 | 0.64 | 80 |
| 7 | Arable land | 6.6 | 1.0 | NM | 6.8 | 0.38 | 39 |
| 8 | Lawn | 7.0 | 1.5 | NM | 6.7 | 0.52 | 61 |
| 9 | Arable land | 4.8 | 1.1 | NM | 3.9 | 0.41 | 47 |
| 10 | Riverside | 6.6 | 2.5 | NM | 8.8 | 0.91 | 152 |
| 11 | Forest | 6.9 | 2.8 | NM | 12.6 | 0.31 | 44 |
| 12 | Riverside | 7.0 | 3.2 | NM | 11.8 | 0.96 | 166 |
| 13 | Arable land | 7.1 | 1.4 | NM | 9.4 | 0.29 | 41 |
| 14 | Grassland | 6.4 | 1.5 | NM | 7.8 | 0.32 | 77 |
| 15 | Pine forest | 3.1 | 3.1 | NM | 8.6 | 0.13 | 30 |
| 16 | Lawn | 6.2 | 2.9 | NM | 8.4 | 0.61 | 99 |
| 17 | Arable land | 5.6 | 0.9 | NM | 2.4 | 0.39 | 43 |
| 18 | Forest | 3.2 | 1.8 | NM | 5.6 | 0.14 | 31 |
| 19 | Forest | 3.5 | 1.4 | NM | 6.1 | 0.12 | 60 |
| 20 | Arable land | 7.5 | 2.4 | NM | 7.6 | 0.41 | 67 |
| 21 | Lawn | 5.1 | 1.7 | NM | 5.0 | 0.24 | 58 |
| 22 | Forest | 3.5 | 1.2 | NM | 5.4 | 0.05 | 24 |
| 23 | Forest | 3.7 | 1.3 | NM | 4.2 | 0.07 | 32 |
| 24 | Forest | 3.9 | 2.4 | NM | 6.7 | 0.13 | 34 |
| 25 | Grassland | 4.7 | 1.5 | NM | 7.4 | 0.49 | 58 |
| 26 | Grassland | 4.8 | 2.9 | NM | 6.3 | 0.80 | 65 |
| 27 | Arable land | 5.9 | 1.0 | NM | 7.2 | 0.29 | 59 |

[a] CEC = cation exchange capacity.
[b] NM = not measured.

line concentrations. These soils were sampled on riversides that typically have elevated metal concentrations [12]. It is unknown if the elevated Zn is from anthropogenic or from natural origin. The PNRs of both soils are among the highest among the uncontaminated soils at corresponding pH, indicating that Zn is not causing an adverse effect (details not shown). Soils 1 to 4 were used for dose–response analysis and were spiked with Zn and Cd in the laboratory (see the following discussion). The field-contaminated soils were collected in Belgium in September 1998 and include also a variety of agricultural soils and forest soils (Table 2).

All soils were air dried and sieved (2 mm) and were stored at least for one month prior to testing the PNR. The four soils used for dose–response analysis were rewetted with deionized

Table 2. The potential nitrification rate (PNR) in 15 contaminated soils, sorted by soil pH. The PNR is compared with the value expected for an uncontaminated soil at corresponding pH (regression model Eqn. 1)

| Sample | Land use | pH $CaCl_2$ (0.01 M) | % C | % Clay | CEC pH 7 ($cmol_c/kg$)[a] | Aqua regia soluble metals (mg/kg dry wt) Cd | Zn | PNR (mg N/kg d) Observed | Expected (95% PI) | % of expected |
|---|---|---|---|---|---|---|---|---|---|---|
| 28 | Bare | 3.6 | 2.7 | 10 | 2.0 | 7 | 129 | 0.4 | 0.8 (7.5) | 44[b] |
| 29 | Lawn (park) | 4.2 | 2.8 | 10 | 1.8 | 10 | 93 | 0.7 | 2.0 (7.4) | 33[b] |
| 30 | Roadside | 4.3 | 5.9 | 13 | 1.9 | 11 | 545 | −1.1 | 2.1 (7.4) | <0[b] |
| 31 | Pasture | 5.1 | 7.1 | 27 | 21.4 | 20 | 229 | 0.5 | 4.8 (7.5) | 10[b] |
| 32 | Pasture | 5.5 | 5.7 | 38 | 23.9 | 21 | 194 | 1.6 | 6.8 (7.5) | 23[b] |
| 33 | Natural grassland | 5.6 | 2.5 | 11 | 4.2 | 9 | 720 | 3.2 | 7.6 (7.4) | 41[b] |
| 34 | Pasture | 5.6 | 6.3 | 21 | 18.3 | 19 | 650 | 1.6 | 7.7 (7.4) | 21[b] |
| 35 | Natural grassland | 5.7 | 1.7 | 13 | 3.1 | 8 | 603 | 2.4 | 8.0 (7.4) | 30[b] |
| 36 | Arable land | 5.9 | 3.1 | 11 | 5.8 | 11 | 987 | 0.7 | 9.2 (7.4) | 7[c] |
| 37 | Arable land | 5.9 | 2.4 | 11 | 6.1 | 7 | 473 | −0.1 | 9.3 (7.4) | −1[c] |
| 38 | Lawn (residential) | 5.9 | 3.8 | NM[d] | 6.1 | 20 | 326 | −0.4 | 9.5 (7.4) | −4[c] |
| 39 | Pasture | 6.1 | 6.8 | 30 | 24.6 | 10 | 160 | 1.5 | 10.3 (7.4) | 14[c] |
| 40 | Forest | 6.2 | 3.1 | 10 | 3.1 | 104 | 34,100 | 1.2 | 10.8 (7.4) | 11[c] |
| 41 | Industrial site | 6.2 | 2.9 | 10 | 0.7 | 32 | 3,630 | 0.8 | 10.8 (7.4) | 7[c] |
| 42 | Pasture | 7.0 | 5.0 | 29 | 16.2 | 5 | 552 | 2.0 | 17.2 (7.5) | 11[c] |

[a] CEC = cation exchange capacity.
[b] Not significantly different from uncontaminated soil at same pH.
[c] Value outside the 95% prediction interval (PI) for uncontaminated soils.
[d] NM = not measured.

Table 3. Effect of soil properties on the potential nitrification rate (PNR, mg N/kg d) in 27 uncontaminated soils. Results of stepwise multiple regression; all variables are significant at the 0.05 level

| Step | Soil variable | Partial $R^2$ | Model $R^2$ |
|---|---|---|---|
| 1 | pH | 0.77 | 0.77 |
| 2 | % C | 0.10 | 0.87 |
| 3 | CEC ($cmol_c/kg$)[a] | 0.02 | 0.89 |
| | PNR = −23.4 + 4.2 pH + 2.0%C + 0.6 CEC | | |

[a] CEC = cation exchange capacity.

water containing appropriate amounts of $Cd^{2+}$ and $Zn^{2+}$ as their chloride salts to obtain a range of soil metal concentrations (control and six treatments). The metal salts were applied at a rate of 2, 20, and 200 mg Cd/kg, or 300, 600, and 3,000 mg Zn/kg dry weight. The control soils were rewetted with deionized water only and were further treated similarly as the metal-spiked soils. In soil 4, we included three additional pH treatments (the original pH and two lower pH values) using 35 and 60 mmol $H^+$/kg based on $H_2SO_4$. These three pH treatments were applied to all seven metal treatments. The final moisture contents of the soils were 21% (soil 1), 22% (soil 2), 20% (soil 3), and 21% (soil 4). All soils were mixed after spiking, and the samples were stored in darkness for 7 d at 25°C followed by a drying/rewetting cycle during 7 d (plant growth chamber). The soils were subsequently stored at 4°C. The toxicity tests were performed between 9 and 19 months after spiking the soils.

*Soil characteristics*

The soil texture was determined with the pipette method after soil sample pretreatment and sand removal [13]. Soil pH was measured in $CaCl_2$ 0.01 M in a solid/liquid (S/L) ratio of 1:2.5 after overnight equilibration. The cation exchange capacity (CEC) was measured using silver thiourea as the index cation in a 0.1-M $NH_4OAc$ buffer at pH 7.0 [14]. Total carbon was measured by dry combustion (solid sampler analyzer CA 100, Skalar, Breda, The Netherlands). The soil metal concentrations were measured after an aqua regia digest and either flame atomic absorption spectroscopy (FAAS), graphite furnace atomic absorption spectroscopy (GFAAS, deuterium background correction and 17.4-mM $H_3PO_4$ as modifier), or inductively coupled plasma/optical emission spectroscopy. The water holding capacity was determined by submerging a soil-filled (50-g) cylinder with a perforated base (covered with a filter paper) in a water bath for 3 h at room temperature and subsequently allowing the water to drain overnight on a tray of sand. The water content was determined after drying at 105°C. All soil properties are expressed on an oven-dried (105°C) weight basis (Tables 1 and 3). Soil solution was separated by centrifugation (1,100 *g*, 1 h) and filtered (0.45 μm). The metal concentrations in soil solution and in soil digests were measured with FAAS. The Cd concentrations below 0.1 mg/L were measured using GFAAS. Soil properties of the spiked soils were measured after the spiking and a 14-week equilibration period.

*Potential nitrification rate*

Soil samples (~200 g) were incubated in darkness at 25°C for 7 d in closed 1.5-L containers prior to substrate addition. The soils were amended with 100 mg $NH_4$-N/kg dry weight [as $(NH_4)_2SO_4$] through a stock solution containing 80 mg $(NH_4)_2SO_4$/ml. Additional deionized water was added to adjust soil moisture content to 60% of water holding capacity. The soil was subsequently mixed and incubated as 200-g aliquots in 1.5-L closed containers at 25°C. Subsamples were taken at 0, 2, and 4 d after substrate addition (20 g, three replicates). The soil nitrate was measured colorimetrically in a filtered soil extract (1 M KCl, 1:5 solid:liquid ratio, 2 h end-over-end shaking).

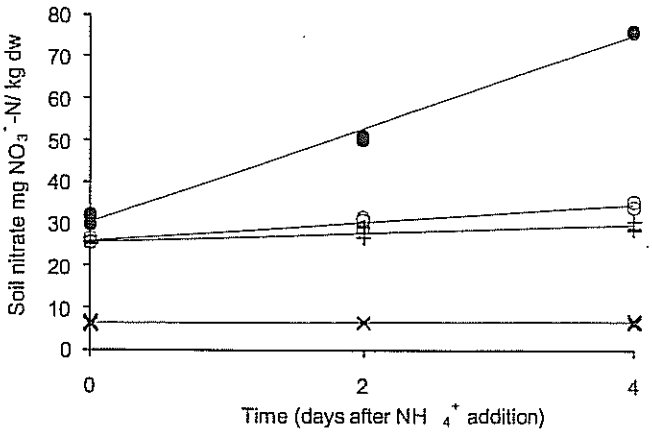


Fig. 1. Soil nitrate concentration after addition of 100 mg $NH_4$-N/kg dry weight in soil 4 at pH 6.6 and at three Zn addition rates. ● = control; ○ = 300 mg Zn/kg dry weight; + = 600 mg Zn/kg dry weight; × = 300 mg Zn/kg dry weight.

*Statistics*

The potential nitrification rate was calculated as the slope of the regression of soil nitrate to time. The lowest-observed-effect dose (LOED, the lowest added metal concentration significantly affecting the process) in the spiked soils was determined using a *t* test ($p < 0.05$) on the PNR between treatment and control. The standard error of the difference between PNR values was calculated from the standard errors of each slope. All PNR values above the LOED were always significantly lower than the control. A logistic equation [15] was fitted to the PNR-log(metal concentration) data (measured metal concentrations, not the dose) using the Marquardt method (proc NLIN, SAS® 6.12, Cary, NC, USA). The EC50 (effect concentration causing a 50% reduction in the PNR) and its 95% confidence interval were derived from the appropriate parameter of the logistic equation and its standard error, respectively. Significant toxic effects on PNR in the field-contaminated soils are determined as explained in the Results and Discussion sections.

## RESULTS

The soil nitrate concentration increases in a linear fashion after $NH_4^+$ addition in all soils, except at high metal application, where nitrification is completely impaired (e.g., soil 4, Fig. 1). The soil nitrate content at day 0 was larger in the control samples of soils 1, 2, and 4 than in corresponding metal-spiked soils (e.g., soil 4, Fig. 1). This variability is most likely the result of metal toxicity on N-mineralization and nitrification during the more than nine months of storage after metal spiking. No such trend was, however, found for soil 3. Nitrification rate was always measured from the increase in soil nitrate during the 4-d test and not based on the final nitrate content after 4 d. The highest differences in soil nitrate content at day

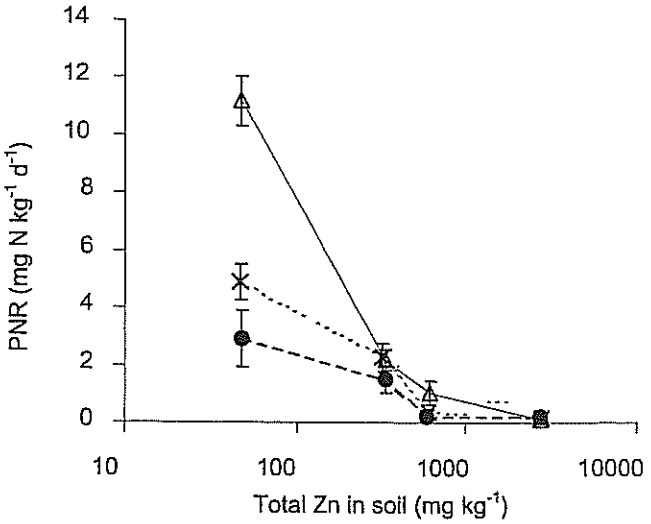


Fig. 2. The potential nitrification rate (PNR) in the control and Zn-spiked treatments of soil 4 at natural soil pH (Δ = pH 6.6) and at two adjusted pH values (× = pH 6.3; • = pH 5.9). The soil pH refers to that of the unspiked soil.

0 between contaminated soils and control soils amounted to 122 mg $NO_3^-$-N/kg dry weight (soil 1, difference between control and highest Zn rate). Soil 1 (control) also contained the highest nitrate concentrations (~130 mg $NO_3^-$-N/kg dry wt) of all 27 uncontaminated soils. The nitrification rate was more difficult to quantify in that soil, as soil nitrate concentration increased only by about 20 mg $NO_3^-$-N/kg dry weight during the 4 d of incubation.

The nitrification rate was reduced through the addition of nitrate in preliminary experiments. The addition of 80 mg $NO_3^-$-N/kg dry weight (as $KNO_3$ in solution) to soil 4 reduced the PNR by 38%. In soil 2, PNR was reduced by 34% after addition of 78 mg $NO_3^-$-N/kg dry weight.

Preliminary experiments showed that the sensitivity of the test decreases if the nitrification test is measured in a 0- to 10-d interval. This may be related to the limitations in $NH_4^+$ substrate. Analyses of the summed $NH_4^+$-N and $NO_3^-$-N contents in soil showed significant detectable ammonification starting from 6 d after $NH_4^+$ addition. In the 4-d test, less than 56% of the added $NH_4^+$-N was transformed into nitrate-N. Wilson [5] monitored nitrification in three soils for seven weeks after adding 100 mg N/kg as $NH_4Cl$. The soil nitrate content was significantly lower at 100 mg Zn/kg during weeks 2 and 3 after substrate addition, but this effect was not observed at the end of the incubation, when most of the substrate was depleted in all treatments.

Nitrification rate is sensitive to metal stress, but the variability in PNR between control soils may exceed the effects of metal stress. Figure 2 shows the effect of added Zn on PNR for soil 4 at three soil pH values (pH 6.6–5.9). The PNR of the soil at highest pH was significantly reduced by 81% at the lowest Zn rate (300 mg/kg dry wt). The PNR of the unspiked soil that was acidified to pH 5.9 was also reduced by 74%, significantly lower than the unspiked soil at pH 6.6, clearly illustrating the well-known effect of soil pH on nitrification [1,8]. The metal application, however, also reduced soil pH, and this was most pronounced in the Zn treatments of soil 3, where pH was reduced from 6.6 (control) to 5.3 at the highest Zn rate. The decrease of the PNR can partly, but not completely, be related to the decrease in the soil pH on metal addition. Soil pH in soil 4 (not acidified) was reduced from pH 6.6 to 6.4 at the lowest Zn rate (300 mg/kg), at which the PNR was 81% lower than in the control. The PNR in the control soil acidified to pH 6.3 was 56% lower than at pH 6.6 (Fig. 2).

Significant effects of Zn on PNR were found at the lowest (soils 2–4) or second-lowest (soil 1) Zn rate at which PNR was over 50% lower than in control treatments (Table 4). There was a dose–response relationship between PNR and soil Zn concentrations in all four soils (details not shown). The estimated EC50 values are between 150 and 350 mg Zn/kg dry weight, or, on a pore-water basis, between about 2 and 200 mg Zn/L. No indication existed that Zn was more toxic in the most acid soil 1 than in soils with pH 6.6 (soils 3 and 4). The Zn median effective concentration (EC50) values, expressed on a pore-water basis, were highest in the most acid soils because total Zn EC50 values were similar across soils and pore-water Zn markedly increases with reducing soil pH.

The response of the PNR to Cd was more variable between soils and rates than in the case of Zn. The highest Cd dose of 200 mg Cd/kg dry weight reduced the PNR by between 50 and 80% compared to that in control samples (not significant for soil 1). A 10 to 20% reduction was observed in the PNR at 20 mg Cd/kg dry weight (significant only in soil 3) and an insignificant stimulation in soil 1. At 2 mg Cd/kg dry weight, a significant but small reduction was observed in PNR for soil 3, whereas no effects were found in the three other soils. The logistic dose–response curves could not be fit to most of the Cd treatments (Table 4).

Table 4. The potential nitrification rate (PNR) in Zn- and Cd-spiked soils: the lowest-observed-effect dose (LOED) of Zn and the Zn concentration at which the PNR is 50% lower than in the control soil[a]

| | | | | EC50 (95% CI) | | | |
|---|---|---|---|---|---|---|---|
| | PNR of control (± 95% CI) | LOED (% inhibition) (mg/kg dry wt) | | Total metal in soil (mg/kg dry wt, measured concn.) | | Soil solution concn. (mg/L) | |
| Soil | (mg N/kg d)[b] | Zn | Cd | Zn | Cd | Zn | Cd |
| 1 | 5.6 (3.5) | 600 (91) | >200 | 351 (224–563) | ND[c] | 184 (110–311) | ND |
| 2 | 6.4 (0.7) | 300 (58) | 200 (68) | 291 (291–292) | ND | 99 (98–99) | ND |
| 3 | 8.8 (0.4) | 300 (79) | 2 (14) | 307 (247–383) | 304 (9–1,000) | 20 (19–20) | ND |
| 4 | 11.2 (0.9) | 300 (81) | 200 (77) | 147 (81–337) | 105 (12–946) | ND (1.9 at 81% inhibition) | ND |

[a] Lowest-observed-effect dose is the lowest metal dose at which the PNR was significantly ($p < 0.05$) different from the control soil. Metal rates were 300, 600, and 3,000 mg Zn/kg dry weight and 2, 20, and 200 mg Cd/kg dry weight.
[b] CI = confidence interval.
[c] ND = not determined.

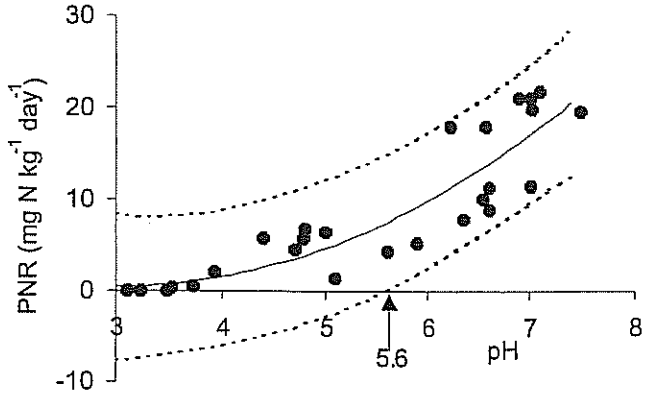


Fig. 3. The potential nitrification rate (PNR) in 27 uncontaminated soils as a function of soil pH. Arrow indicates the soil pH below which the PNR test cannot be used since even a zero PNR is within the 95% prediction interval of uncontaminated soils.

The PNR was measured in the 27 uncontaminated soils to evaluate its variability. The PNR ranged from values not significantly different from 0 to 21 mg N/kg/d. This large range of the PNR hampers detection of toxic effects in field-collected soils. A relationship between soil properties and the PNR of uncontaminated soil is a possible way to identify a predicted reference value of the PNR. Stepwise multiple regression of the PNR to soil pH, carbon content, and CEC showed that pH is the dominant factor controlling the PNR and that marginal but significant effects of the carbon content and CEC exist (Table 3). Below pH 3.5, no significant nitrification was detected within 4 d. The PNR increase about 3.5-fold between pH 5.0 and 7.0. A quadratic regression curve was fitted to the PNR-pH data yielding

$$\text{PNR} = 9.1 - 5.9\ \text{pH} + 1.01\ \text{pH}^2\ (R^2 = 0.81) \qquad (1)$$

This curve and its 95% prediction interval are given in Figure 3. A PNR value below the lower prediction interval is defined here as a value indicating toxicity. Figure 3 shows that no toxicity can be detected below pH 5.6 because even a zero PNR value is within the 95% prediction interval. The reference PNR values can also be predicted using the multiple regression model based on C, pH, and CEC (Table 3). The prediction intervals of this model are smaller than those of the pH-based model. This model was not used for identifying toxicity in the field-contaminated soils because the C contents of these soils exceed the range on which the multiple regression model was fitted.

All PNRs are small in the 15 contaminated soils (Table 2). All these soils contain Zn or Cd at concentrations well above background. Soil 40 contains excessively high Zn concentrations, and it is likely that this soil has been contaminated by smelter ashes. The PNR values, expressed as a percentage of the PNR of an uncontaminated soil at corresponding pH, are 0 to 44%, indicating that the PNR is affected in all soils. In one soil (soil 30), a significant decrease was observed in soil nitrate from 15 to 10 mg $NO_3^-$-N/kg, in 4 d after $NH_4^+$ addition. Significant toxicity, defined as given previously, was detected in 7 out of the 10 soils with a soil pH ≥5.6 and where toxicity can be identified. The three other soils in which toxicity can be detected but in which the PNR was not significantly different from that in uncontaminated soils have a soil pH close to 5.60, that is, the threshold pH below which no toxicity can be identified.

The PNRs in the 15 field-contaminated soils are not significantly related to total Cd or Zn concentrations, soil solution Zn concentration (all concentrations tested after logarithmic transformation), or to soil pH, percentage C, or CEC. A significant negative relationship was found between the PNR and the logarithm of the soil solution Cd concentration ($R^2 = 0.35$, $p < 0.05$). The PNR value, expressed as a percentage of the PNR of an uncontaminated soil at corresponding pH, were unrelated to any of these parameters.

Toxicity in the field-contaminated soils is most likely due to elevated Zn and not Cd based on the following analysis. In the metal-spiked soils, the PNR was similarly reduced by 50 to 80% at the highest dose of 200 mg Cd/kg dry weight (1.8 mmol Cd/kg dry wt) or the lowest dose of Zn 300 mg Zn/kg dry weight (4.6 mmol/kg dry wt). This indicates that the adverse effects of Cd and Zn to nitrification rate are not extremely different on a weight or molar basis. The average Zn:Cd total concentration ratio in the smelter affected soils is 80 (minimum 9, wt basis). The total Cd concentrations in environmental samples with Zn concentrations around the Zn EC50 values (150 and 350 mg Zn/kg dry wt) range between 2 and 4 mg Cd/kg dry weight (assuming a Zn:Cd weight ratio of 80). In that concentration range, Cd has little effect on PNR in the spiked soils. Similar conclusions can be drawn on the basis of soil solution Cd and Zn concentrations in the metal-spiked and field-contaminated soils (details not shown).

Total Zn concentrations in the field-contaminated soils where toxicity was found were 160 to 34,100 mg Zn/kg dry weight, and most samples contained more Zn than the EC50s of the Zn-spiked soils (150–350 mg/kg dry wt). Inhibition of the PNR in metal-spiked soils is, however, likely to be higher than in soils where metals have equilibrated for a much longer period. This may be related to the limited reaction time of metals in the freshly spiked soils or to the potential adverse effects of the salts in these soils that have not been leached prior to testing. It has been demonstrated that the EC50 of Zn (total metal in soil) for reproduction of *Folsomia candida* is twofold higher in a soil that has been leached after $ZnCl_2$ spiking than in a soil that was not leached after spiking [16]. Metal concentrations in pore water may be a better basis to compare toxicity between spiked soils and field-contaminated soils [17]. The Zn concentrations in the pore water of the field-contaminated soils, where toxicity was identified, range between 1.4 and 24 mg Zn/L. These concentrations should be compared with pore-water concentrations of metal-spiked soils at similar pH values as the field-contaminated soils (pH 5.9–6.2 and one soil at pH 7.0). Pore-water Zn in the control soils 3 and 4 were 0.16 to 0.21 mg/L (pH 6.6) and increased at the first Zn rate to 29 mg/L (soil 3, pH 6.0) or 1.9 mg/L (soil 4, pH 6.4), at which about 80% inhibition of the PNR in both soils was observed. This analysis indicates that the pore-water Zn concentrations of the field-contaminated soils are in the range where toxic effects in metal-spiked soils were identified.

## DISCUSSION

Even under controlled laboratory conditions, the PNR proved to be a parameter too variable to effectively identify toxic effects in field-contaminated soils. As an example, the predicted PNR (±95% prediction interval) at pH 6.2 is 10.3 ± 7.4 mg N/kg/d. The lower boundary of the prediction interval is 28% of the expected value (toxicity can be identified only if more than 72% inhibition occurs).

The Cd- and Zn-spiked soils (soils 1–4) have been subjected

to 30 different bioassays (microbiological tests, invertebrate tests, and phytotoxicity tests). The PNR was the most sensitive bioassay; however, the PNR was also the most variable endpoint between control soils (J. Bierkens et al., unpublished results). An overall positive correlation was observed between variability of the endpoint among control soils and the metal sensitivity of the test (J. Bierkens et al., unpublished results).

The lack of a consistent response in uncontaminated soils strongly reduces the power of the PNR as a bioassay for screening metal-contaminated soils. This power could be increased if uncontaminated samples are found with properties as close as possible to that of the test samples. Correspondence in soil pH is a minimal prerequisite. The use of a pH buffer to test all soils at identical pH will likely increase the sensitivity of the assay but decreases its ecological relevance since metal availability is strongly pH dependent.

An increase of the power of the PNR assay by including better or more control samples effectively means that adverse effects of metals could then be identified at lower levels of metal contamination. However, the ecological significance of this statistical approach then becomes also more questionable. Without elaborating on the potential ecological effects of reduced nitrification in soil, it should already be recalled that the effect of metals on the PNR is small compared with the effect of soil properties (mainly pH) on the PNR in otherwise uncontaminated soils (Fig. 3 and Sauvé et al. [6]). Moreover, an adverse effect on the potential nitrification rate measured under laboratory conditions does not infer a similar adverse effect on the nitrification in the field. The potential nitrification rate, as tested here, reflects the nitrification rate at saturating substrate concentrations ($V_{max}$). This situation rarely occurs in most aerobic soils since it is known that $NH_4^+$-N usually represents a minor fraction of the total mineral N in soils ($NH_4^+ + NO_3^-$); that is, the nitrification potential usually exceeds the ammonification rate in soil. No field data are available indicating that field nitrification rates are impaired because of metals. The PNR assay should therefore be considered as a test that indicates the existence of a stress factor in soil. However, no basis exists by which to predict ecological effects of a reduced PNR in soil. Validation of this assay with field nitrification rate is at least required before the PNR assay data can be adopted for the derivation of soil quality guidelines.

*Acknowledgement*—We are indebted to the Openbare Afvalstoffenmaatschappij voor het Vlaamse Gewest and the Fund for Scientific Research, Flanders. Statistical advice from Wim Coucke is greatly appreciated.

## REFERENCES

1. Bewley RJF, Stotzky G. 1983. Effects of cadmium and simulated acid-rain on ammonification and nitrification in soil. *Arch Environ Contam Toxicol* 12:285–291.
2. Dušek L. 1995. The effect of cadmium on the activity of nitrifying populations in two different grassland soils. *Plant Soil* 177:43–53.
3. Liang CN, Tabatabai MA. 1978. Effects of trace elements on nitrification in soils. *J Environ Qual* 7:291–293.
4. Premi PR, Cornfield AH. 1969. Effects of addition of copper, manganese, zinc and chromium compounds on ammonification and nitrification during incubation of soil. *Plant Soil* 31:345–352.
5. Wilson DO. 1977. Nitrification in three soils amended with zinc sulfate. *Soil Biol Biochem* 9:277–280.
6. Sauvé S, Dumestre A, McBride M, Gillett JW, Berthelin J, Hendershot W. 1999. Nitrification potential in field-collected soils contaminated with Pb or Cu. *Appl Soil Ecol* 12:29–39.
7. International Organization for Standardization. 1997. Soil quality—Biological methods—Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes. ISO 14238. Geneva, Switzerland.
8. Dancer WS, Peterson LA, Chesters G. 1973. Ammonification and nitrification of N as influenced by soil pH and previous N treatments. *Soil Sci Soc Am J* 37:67–69.
9. Azam F, Mulvaney RL, Simmons FW. 1995. Effects of ammonium and nitrate on mineralization of nitrogen from leguminous residues. *Biol Fertil Soils* 20:49–52.
10. Sabey BR. 1969. Influence of soil moisture tension on nitrate accumulation in soils. *Soil Sci Soc Am Proc* 33:262–266.
11. Tack FMG, Verloo MG, Vanmechelen L, Van Ranst E. 1997. Baseline concentration levels of trace elements as a function of clay and organic carbon contents in soils in Flanders (Belgium). *Sci Total Environ* 201:113–123.
12. De Temmerman LO, Istas JR, Hoenig M, Dupire S, Ledent G, Van Elsen Y, Baeten H, De Meyer A. 1982. Onderzoek naar de "normale" gehalten aan spoorelementen in een aantal Belgische bodems als basis voor de detectie en het onderzoek van bodemvervuiling. *Landbouwkd Tijdschr* 35:1883–1911.
13. De Leenheer L, Van Hove J. 1956. Werkwijze voor mechanische behandeling met de kettinghydrometer. *Meded Landbouwhogesch Opzoekingsstatn Staat Gent* 21:249–274.
14. Chhabra R, Pleysier J, Cremers A. 1975. The measurement of the cation exchange capacity and exchangeable cations in soils: A new method. *Proceedings,* International Clay Conference, Mexico City, Mexico, July 16–23, pp 439–449.
15. Doelman P, Haanstra L. 1989. Short- and long-term effects of heavy metals on phosphatase activity in soils: An ecological dose response model approach. *Biol Fertil Soils* 8:235–241.
16. Smit CE, van Gestel CAM. 1998. Effects of soil type, prepercolation, and ageing on bioaccumulation and toxicity of zinc for the springtail *Folsomia candida. Environ Toxicol Chem* 17:1132–1141.
17. Smit CE, van Gestel CAM. 1996. Comparison of the toxicity of zinc for the springtail *Folsomia candida* in artificially contaminated and polluted field soils. *Appl Soil Ecol* 4:93–94.