Bull Environ Contam Toxicol (2007) 78:112–117
DOI 10.1007/s00128-007-9045-6

Zinc and Copper Toxicity to Soil Bacteria and Fungi from Zinc Polluted and Unpolluted Soils: A Comparative Study with Different Types of Biolog Plates

B. Klimek · M. Niklińska

Published online: 5 April 2007

The negative effect of excessive concentrations of heavy metals on the soil organic matter mineralization rate and soil properties is well documented (Giller et al., 1998). Some of the heavy metals are required by organisms as nutrients and are essential, but in the larger doses, both essential and nonessential metals can exert a toxic effect on soil microorganisms (Bruins et al., 2000). The soil microbial properties are often used as soil quality and health indicators (Brookes, 1995). The reduction of microbial activity and biomass, and/or changes in the microbial community structure in metal contaminated soils, have been frequently reported (Frostergård et al., 1996, Giller et al., 1998). At the same time, microorganisms in long-term, polluted soils can be adapted even to high concentrations of heavy metals, and different mechanisms for these adaptations have been considered (Bruins et al., 2000). The resistant part can have a lower genetic diversity, that is a species richness. The resistant part can have a lower functional (metabolic) diversity, which means a lower microbial community capability to adapt to changes in environmental conditions and a lower ability in terms of degradation of the carbon substrates (Wang et al., 2004).

The use of Biolog microplates for assessing the functional diversity of soil microbial communities was originally described by Garland and Mills (1991). This technique is now widely used for studying the community-level physiological profile of soils and changes in soil microbial communities caused by different environmental stressors, such as heavy metal pollution (Rutgers et al., 1998; Niklińska et al., 2006). The multiwells Biolog plates contain different C substrates that are similar to various soil compounds and plant root exudates and the pattern of utilization of these substrates by soil microbes is specific for the soil microbial population (Preston-Mafham et al., 2002). The most common plates used in ecological studies are GN and Eco plates, which have been developed for the bacterial part of a microbial community, although new types known as SF and FF plates are especially recommended for fungi profile analyses (Classen et al., 2003).

It is well known that microorganisms from long-term metal polluted soils are more tolerant to metal than those from unpolluted soils (Blanck, 2002; Lock and Janssen, 2005). Some have also suggested that heavy metals have a different effect on bacteria than on fungi, and that the soil bacteria are usually more sensitive to heavy metals (Frostegård et al., 1996). However, Pennanen (2001) showed that copper is relatively more toxic to fungi than to bacteria.

The purpose of this study was to compare the acute toxic effect of high concentrations of zinc and copper on the physiological profiles and on the functional diversity of microorganisms, originating from long-term polluted and unpolluted soils.

Materials and Methods

The humus samples were collected in April 2005 from two forest sites in southern Poland. Three samples of polluted soil (P) were taken in the Olkusz area (N 50°18′; E 19°30′), in the vicinity of the former zinc and lead smelter, and three samples of unpolluted soil (UP) were collected near Nakło (N 50°41′; E 19°43′). Both sites were covered by Scots pine forest (*Pinus sylvestris*) with an admixture of

B. Klimek (✉) · M. Niklińska
Institute of Environmental Sciences,
Jagiellonian University, Gronostajowa 7,
30-387 Kraków, Poland
e-mail: klimek@eko.uj.edu.pl

birch (*Betula pendula*). The collected samples were immediately sieved at the plot with 1cm × 1cm mesh sieve to remove the green parts of plants, stocks, stones, and roots. Soils were incubated for two weeks in laboratory conditions prior to microbial analysis (field moisture, 10°C). At this time, the physicochemical parameters of the soils were measured, including the water content (drying at 105°C for 24 h), the organic matter content (loss of ignition at 550°C for 24 h), and the pH in H_2O (1:10 w:v). The total concentrations of heavy metals (Zn_t, Cu_t) were measured after wet digestion of dried and grinded subsamples in concentrated nitric acid. The water-soluble metal concentrations (Zn_w, Cu_w) were extracted by shaking samples with the deionized water (pH 5.5). The Zn and Cu concentrations (total and water-soluble) were measured by flame atomic absorption spectrometry (Perkin-Elmer, model AAnalyst 800), using the flame or graphite-furnace method. All chemical data were expressed per unit of dry weight soil. The chemical data for soils from UP and P sites data were analyzed using a one-way analysis of variance ANOVA (Statgraphics *Plus* v 5.0). Significant differences were tested by Tukey's HSD test. Results were considered significantly different at $p < 0.05$.

To measure the soil microbial physiological profiles and functional diversity of the microbial community, four types of 96-well Biolog® microplates: Eco, GP, FF, and SFN were used (http://www.biolog.com). The Ecoplates were designed especially for ecological applications, and they contain three replicate sets of 31 carbon substrates (with tetrazolium dye), and they are degradable by different soil bacteria. GP plates, developed particularly for Gram-positive bacteria, contain 95 sole carbon substrates with tetrazolium dye. FF plates contain 95 sole carbon substrates and modified tetrazolium dye, which allows for assessment of the fungal activity. In turn, SFN fungal plates with 95 sole carbon substrates have no redox indicator.

The samples of each soil (10g d.w.) were shaken for 1 h in 100 mL of 10 mM Bis Tris buffer (pH = 7) and were allowed to settle for ½ h. The supernatants containing microbes were decanted and diluted (10^{-2}) in a Bis Tris solution, and then concentrated solutions of zinc or copper chloride were added to achieve, finally, a 300 mg Zn l^{-1} or 300 mg Cu l^{-1} concentration in the soil solution. Additionally, soil solutions without any metal addition were prepared as a control for both soil types (UP C and P C). Furthermore, the separate soil extracts, for both types of fungal plates, were diluted in a Bis Tris buffer with antibiotic oxytetracycline (concentration 100 mg l^{-1}) (Sigma-Aldrich). The antibiotic addition is recommended to prevent bacterial growth and development; oxytetracycline binds to the 30S ribosome subunit and inhibits protein synthesis (Bailey et al., 2002). All prepared solutions were left overnight. The following day, the solutions were inoculated into the Biolog plates (125 µl per well) and were incubated at 22°C. All chemical solutions, transfer equipment, and glassware were sterilized prior to use on microplates, and inoculations were completed under a laminar-flow chamber to minimize the risk of contamination. Substrate utilization was measured as light absorbance: at 590 nm for the Eco and GP plates, at 490 nm for the FF plate as color development, and at 750 nm for the SFN plate as turbidity development (mycelium growth). The first measurement was carried out 4 h after inoculation, and the subsequent readings were taken at 12 h intervals for 168 h (μQuant spectrometer; BIO-TEK Instruments). The absorbance and turbidity measurements for individual wells were corrected against the control well containing only water.

The following microbial indices were calculated for each plate and sample: AWCD or AWTD (Average Well *Color* Development for the ECO, GP, and FF plates, and the Average Well *Turbidity* Development for SFN plates), AUC (Area Under the Curve), R_s (substrate richness), H′ (functional diversity index), and I′ (index of evenness). AWCD was calculated for the measurements at 168 h as: $AWCD = \Sigma A_n/n$, where A_n is the absorbance of each individual well and n represents the number of substrates. AUC was calculated as: $AUC = \Sigma (A_n + A_{n+1})/2 \times (t_{n+1} - t_n)$, where A_n and A_{n+1} is the absorbance of each individual well at two consecutive measurements at two different measurement times for t_n and t_{n+1}. R_s (substrate richness) represents the number of degraded substrates. The H′ functional diversity index (based on the Shannon-Wiener biodiversity index) is derived from the total and a relative number of substrates used on the plates: $H' = -\Sigma p_n (\log 10\ p_n)$, where p_n is the proportion of use of an individual substrate, calculated as a proportion of well absorbance, divided by sum of absorbance for all wells. The I′ index of evenness was calculated as: $I' = H'/R_s$. Each index describes the different aspect of diversity and they may correlate together, but these correlations are not really obvious. For example, similar AUC or AWCD can be reached when a lot of substrates (R_s) are used with a low microbial activity or when small numbers of substrates are intensively utilized (Duelli and Obrist, 2003). Then H′ and I′ yield additional information about the microbial functional diversity based on the structure of the substrates used. AWCD and AUC are mostly used as indicators of general microbial activity (Kong et al., 2006).

Differences between particular parameters were compared separately for plate types. Two-way ANOVAs were use to compare differences between soils (UP and P), between metal treatment (control, Zn, and Cu), and the interactive effect between soil and metal treatment. When significant differences between soils or metal treatment were found, the means were compared using the Tukey's HSD test.

Results and Discussion

The results of physicochemical soil analysis are shown in Table 1. The total and soluble Zn concentrations in polluted soils (P) were higher than in unpolluted soils (UP). The total concentration of copper was also higher in P than in the UP soils, but similar reasonably low concentrations of soluble Cu were determined in polluted (P) and unpolluted (UP) soils. The polluted soils have a significantly higher pH than the unpolluted, which can influence the proportion of solubility of metals (Martinez and Motto, 2000).

Generally, the microbial indices calculated for different microplate types separately did not differ significantly between the UP control and P control soils. The only exception was AUC for the FF plates, which was significantly higher for UP soils (Tables 2–6). This exception indicates that the fungi from long-term polluted soils (P), cultured on FF plates, are probably less active than bacteria and other fungi cultured on SFN plates. The comparison of different microbial indices showed that AUC seems to be the most valuable measure of microbial activity on Biolog plates, as it combines both maximum values of absorbance attained by particular substrates and the time at which maximum color development values are achieved (Preston-Mafham et al., 2002). A lack of differences between other microbial indices for P and UP soils indicates similar microorganism activity in the P and UP soils, which may perform the same metabolic functions based on the similar functional diversity. This suggests that such long-term metal pollution may have shifted microbial communities to more heavy-metal–tolerant species or that the zinc available fraction in polluted soils is too low to affect microbial metabolic functions (Giller et al., 1998).

The acute additional laboratory metals treatment caused significant reduction on the microbial parameters measured on the bacterial Eco plates (Tables 2–6). Generally, copper seams to be more toxic for bacteria than zinc (the stronger reduction of AWCD, H′, R_s, and I), although there were no differences in AUC between zinc and copper treatment. The number of substrates used (R_s) was similar in control and zinc treatment, and indicates changes in structure of substrates used. The influence of both metals was different: zinc addition decreased the rate of utilization of many substrates but not number of substrates used when copper decreased number and also rate of substrates utilization. The influence is visible in the reduction of H′ and I′ indices, which decreased in order: control, zinc treatment, and copper treatment.

The results obtained from bacterial GP plates showed that additional laboratory metals treatment caused significant effects on the measured microbial parameters (Tables 2–6). Both metals similarly affected the Gram-positive bacteria, but the number of substrates used was significantly reduced only after copper treatment. This effect might suggest that Gram-positive bacteria, representing only part of the soil bacterial community, are probably similarly sensitive to both heavy metals.

The acute metals treatment caused different effects on the microbial parameters measured on the fungal FF plates (Tables 2–6). Zinc treatment did not change AWCD, H′, R_s, and I′ in comparison to the control. The higher AUC calculated for zinc-treated soil suspension inoculated on the FF plates, in comparison to the control untreated samples, confirmed the well-known phenomenon that soil fungi are less sensitive to some metals (e.g., zinc) than bacteria (Tables 2–6). The results based on FF plates showed that some fungi species might even better perform their metabolic function after additional zinc treatment. These results could be caused by the unlimited, casual growth of fungi on FF plates with a lack of competition with the soil bacteria for the available carbon substrates. Copper significantly reduced all the microbial indices measured on FF plates, indicating that fungi are especially sensitive on this metal.

Using SFN plates, the significant effects of metal were observed only for fungal activity parameters AWCD and AUC (Tables 2–6). AWCD values were different only

Table 1 The chemical characteristic of unpolluted (UP) and polluted (P) soils

Soil	OM	pH*	Zn_t**	Zn_w**	Cu_t**	Cu_w
UP1	44.9 (2.1)	4.93 (0.24)	100.0 (11.5)	2.5 (0.2)	46.8 (4.1)	0.3 (0.0)
UP2	57.9 (1.3)	4.54 (0.17)	164.2 (5.1)	6.7 (3.9)	39.4 (3.0)	0.3 (0.1)
UP3	67.8 (2.1)	4.56 (0.02)	157.8 (56.8)	2.2 (0.3)	33.4 (4.4)	0.3 (0.0)
P1	54.0 (2.6)	5.38 (0.07)	5636.5 (527.0)	93.6 (7.8)	80.6 (31.1)	0.2 (0.0)
P2	45.3 (2.6)	5.59 (0.04)	4500.8 (344.3)	69.8 (5.4)	67.1 (3.1)	0.2 (0.0)
P3	62.3 (5.2)	4.81 (0.04)	4915.6 (623.6)	83.5 (10.1)	102.5 (5.1)	0.3 (0.2)

Values are means with a standard deviation (n = 3). Organic matter content (OM) is given as a % of dry weight, and total and water-soluble zinc and copper concentrations (Zn_t, Zn_w, Cu_t,and Cu_w) are given as mg kg^{-1} d.w. Significant differences between UP and P soils (one-way ANOVAs) are marked by asterisks (*$p < 0.001$, **$p < 0.0001$)

Table 2 AWCD (Average Well Color Development) values (Eco, GP, FF plates) and AWTD (Average Well Turbidity Development) values (SFN plates) for unpolluted (UP) and polluted (P) soils for control, Zn, and Cu treatment and in the lower line results of two-way ANOVAs

Soil/treatment	Plate type			
	Eco	GP	FF	SFN
UP control	1.762 (0.215)	1.573 (0.353)	0.555 (0.068)	0.298 (0.102)
UP Zn	0.815 (0.549)	0.322 (0.177)	0.721 (0.098)	0.329 (0.025)
UP Cu	0.196 (0.006)	0.023 (0.007)	0.036 (0.150)	0.178 (0.025)
P control	1.608 (0.135)	1.426 (0.128)	0.372 (0.303)	0.220 (0.184)
P Zn	0.664 (0.121)	0.025 (0.005)	0.554 (0.060)	0.145 (0.035)
P Cu	0.191 (0.020)	0.175 (0.063)	0.021 (0.008)	0.294 (0.067)
Soil	$p = 0.4016$	$p = 0.2512$	$p = 0.0807$	$p = 0.2896$
Metal	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p = 0.0440$
	Control **a**	Control **a**	Control **a**	Control **ab**
	Zn **b**	Zn **b**	Zn **a**	Zn **a**
	Cu **c**	Cu **b**	Cu **b**	Cu **b**
Interaction	$p = 0.8418$	$p = 0.6945$	$p = 0.5135$	$p = 0.8418$

Values are means with standard deviations (n = 3). In the lower line results of two-way ANOVAs, p values for both factors and interaction, and significant differences between groups, are marked as **a**, **b**, **c**

Table 3 AUC (Area Under the Curve) values for unpolluted (UP) and polluted (P) soils for control, Zn, and Cu treatment and in the lower line results of two-way ANOVAs

Soil/treatment	Eco	GP	FF	SFN
UP control	222.416 (36.004)	521.671 (145.224)	108.828 (16.248)	92.544 (35.286)
UP Zn	85.425 (63.443)	105.753 (43.322)	181.948 (10.059)	113.783 (16.950)
UP Cu	19.929 (2.923)	13.718 (1.916)	19.227 (10.425)	37.950 (6.339)
P control	198.986 (23.919)	450.515 (52.898))	70.953 (48.535)	64.381 (52.473)
P Zn	48.013 (11.091)	43.490 (20.480)	130.871 (13.849)	78.317 (16.636)
P Cu	18.550 (2.646)	14.825 (4.436)	12.899 (6.589)	30.664 (4.466)
soil	$p = 0.1358$	$p = 0.1193$	$p = 0.0115$	$p = 0.1074$
			UP **a**	
			P **b**	
metal	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0083$
	control **a**	control **a**	control **b**	control **a**
	Zn **b**	Zn **b**	Zn **a**	Zn **a**
	Cu **b**	Cu **b**	Cu **c**	Cu **b**
interaction	$p = 0.5681$	$p = 0.4659$	$p = 0.2514$	$p = 0.7454$

Values are means with standard deviations (n = 3). In the lower line results of two-way ANOVAs, p values for both factors and interaction, and significant differences between groups, are marked as **a**, **b**, **c**

between zinc and copper treatment. Zinc treatment enhanced the AUC values similarly as on FF plates. SFN plate type seems to be less effective to study the soil fungi probably because of the other set of carbon substrates and the less sensitive turbidity measurement.

The most unexpected results of this study were that, even using such differently polluted soils and two different metals (UP and P, Cu and Zn), we did not find any significant interactions between soils and metal treatment for any microbial indices (Tables 2–6). However, the same effects caused by laboratory Cu treatment in P and UP soils are easily understandable because of the low concentration of copper in both studied soils and the relatively high concentration used as a Cu treatment. But the same zinc effects in P (Zn polluted) and UP soils should be recognized as a lack of microbial tolerance for Zn in P soils, a

Table 4 H′ (functional diversity index) values for unpolluted (UP) and polluted (P) soils for control, Zn, and Cu treatment and in the lower line results of two-way ANOVAs

Soil/treatment	Eco	GP	FF	SFN
UP control	1.454 (0.010)	1.912 (0.028)	1.799 (0.042)	1.824 (0.113)
UP Zn	1.361 (0.045)	1.595 (0.079)	1.866 (0.005)	1.748 (0.021)
UP Cu	1.160 (0.063)	1.428 (0.1000)	1.259 (0.076)	1.753 (0.065)
P control	1.447 (0.008)	1.882 (0.018)	1.529 (0.427)	1.799 (0.049)
P Zn	1.316 (0.035)	1.566 (0.128)	1.811 (0.028)	1.660 (0.186)
P Cu	1.129 (0.070)	1.526 (0.100)	1.031 (0.341)	1.790 (0.023)
Soil	$p = 0.2210$	$p = 0.7463$	$p = 0.1098$	$p = 0.9250$
Metal	metal $p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p = 0.3857$
	control **a**	control **a**	control **a**	$p = 0.4003$
	Zn **b**	Zn **b**	Zn **a**	
	Cu **c**	Cu **b**	Cu **b**	
Interaction	$p = 0.7799$	$p = 0.3626$	$p = 0.6894$	

Values are means with standard deviations (n = 3). In the lower line results of two-way ANOVAs, p values for both factors and interaction, and significant differences between groups, are marked as **a**, **b**, **c**

Table 5 R_s (substrate richness) values for unpolluted (UP) and polluted (P) soils for control, Zn, and Cu treatment and in the lower line results of two-way ANOVAs

Soil/treatment	Eco	GP	FF	SFN
UP control	31 (0)	95 (0)	88 (4)	94 (17)
UP Zn	30 (2)	81 (7)	93 (2)	80 (3)
UP Cu	23 (2)	56 (25)	37 (9)	88 (4)
P control	31 (0)	90 (2)	68 (35)	94 (1)
P Zn	20 (2)	75 (11)	93 (3)	88 (6)
P Cu	29 (1)	74 (30)	29 (20)	74 (6)
Soil	$p = 0.0679$	$p = 0.7518$	$p = 0.2532$	$p = 0.8369$
Metal	$p < 0.0001$	$p = 0.0440$	$p = 0.0001$	$p = 0.5316$
	control **a**	control **a**	control **a**	$p = 0.2198$
	Zn **a**	Zn **ab**	Zn **a**	
	Cu **b**	Cu **b**	Cu **b**	
Interaction	$p = 0.2724$	$p = 0.3818$	$p = 0.6143$	

Values are means with standard deviations (n = 3). In the lower line results of two-way ANOVAs, p values for both factors and interaction, and significant differences between groups, are marked as **a**, **b**, **c**

phenomenon which for bacteria was found in an earlier study using only Eco plates (Niklińska et al., 2006).

We have to remember that the Biolog technique allow us to culture on the plates only a small percentage of the whole microbial community (Preston-Mafham et al., 2002). The culturable fraction of soil microorganisms may not reflect proper changes in whole community, although each part of the community is exposed to these same stress conditions (Niklińska et al., 2006). Furthermore, the similarity between soils could be caused by the small number of samples comparing such a heterogenous soil environment. In testing four different types of microplates, we used only three replicates for each of the soils, probably restricting the possibility of finding differences between fungi and/or bacterial tolerance in both the studied soils. But we still believe that the opportunity for separate incubation of bacteria and fungi, given by different types of Biolog plates – especially the Eco and FF plates – may be a useful tool to study changes in microbial structure and functions in heavily polluted soils.

Acknowledgments This work was supported by the EU 6th Framework Project ALARM (contact No 506675) and the Polish State Committee for Scientific Research (Grant No 4650/P04/2005/29).

Table 6 I′ (index of evenness) values for unpolluted (UP) and polluted (P) soils for control, Zn, and Cu treatment and in the lower line results of two-way ANOVAs

Soil/treatment	Eco	GP	FF	SFN
UP control	0.975 (0.007)	0.967 (0.014)	0.913 (0.012)	0.925 (0.018)
UP Zn	0.921 (0.016)	0.836 (0.026)	0.947 (0.003)	0.921 (0.001)
UP Cu	0.0857 (0.018)	0.837 (0.046)	0.812 (0.087)	0.902 (0.025)
P control	0.970 (0.006)	0.962 (0.008)	0.850 (0.109)	0.914 (0.021)
P Zn	0.903 (0.017)	0.837 (0.042)	0.921 (0.010)	0.921 (0.003)
P Cu	0.873 (0.027)	0.832 (0.052)	0.742 (0.061)	0.894 (0.033)
Soil	$p = 0.7749$	$p = 0.8644$	$p = 0.754$	$p = 0.6919$
Metal	$p < 0.0001$	$p < 0.0001$	$p = 0.0028$	$p = 0.1645$
	control **a**	control **a**	control **a**	$p = 0.9524$
	Zn **b**	Zn **b**	Zn **a**	
	Cu **c**	Cu **b**	Cu **b**	
Interaction	$p = 0.2347$	$p = 0.9884$	$p = 0.7565$	

Values are means with standard deviations (n = 3). In the lower line results of two-way ANOVAs, p values for both factors and interaction, and significant differences between groups, are marked as **a**, **b**, **c**

References

Bailey VL, Smith JL, Bolton H Jr (2002) Fungal-to-bacterial ratios in soil investigated for enhanced C sequestration. Soil Biol Biochem 34:997–1007

Blanck H (2002) A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. Hum Ecol Risk Assess 8:1003–1034

Brookes PC (1995) The use of microbial parameters in monitoring soil pollution by heavy metals. Biol Fertil Soils 19:269–279

Bruins MR, Kapil S, Oehme FW (2000) Microbial resistance to metals in the environment. Ecotoxicol Environ Safety 45:198–207

Classen AT, Boyle SI, Haskins KE, Overby ST, Hart SC (2003) Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences contrasting soils. FEMS Mic Ecol 44:319–328

Duelli P, Obrist MK (2003) Biodiversity indicators: the choice of values and measures. Agri Ecosyst Environ 98:87–98

Frostergård Å, Tunlid A, Bååth E (1996) Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biol Biochem 28:55–63

Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Environ Microbiol 57:2351–2359

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol Biochem 30:1389–1414

Kong WD, Zhu YG, Fu BJ, Marschner P, He JZ (2006) The veterinary antibiotic oxytetracycline and Cu influence functional diversity of the soil microbial community. Environ Pollut 143:129–137

Lock K, Janssen CR (2005) Influence of zinc concentrations on zinc sensitivity and functional diversity of microbial communities. Environ Pollut 136:275–281

Martinez CE, Motto HL (2000) Solubility of lead, zinc and copper added to mineral soils. Environ Pollut 107:153–158

Niklińska M, Chodak M, Laskowski R (2006) Pollution-induced community tolerance of microorganisms from forest soil organic layers polluted with Zn or Cu. Appl Soil Ecol 32:265–272

Pennanen T (2001) Microbial communities in boreal coniferous forest humus exposed to heavy metals and changes in soil pH-a summary of use phospholipid fatty acids, Biolog® and ^{3}H-thymidine incorporation methods in field studies. Geoderma 100:91–126

Preston-Mafham J, Boddy L, Randerson PF (2002) Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles – a critique. FEMS Microbiol Ecol 42:1–14

Rutgers M, van't Verlaat IM, Wind B, Posthuma L, Breure AM (1998) Rapid method for assessing pollution-induced community tolerance in contaminated soil. Environ Toxicol Chem 17:2210–2213

Wang A, Chen J, Crowley DE (2004) Changes in metabolic and structural diversity of soil bacteria community in response to cadmium toxicity. Biol Fertil Soils 39:452–456