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Beneficial effects of the growth of metal tolerant grass on biological and chemical parameters in copper- and zinc contaminated sandy soils

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***Background*. Growth of metal tolerant grasses on two sandy soils contaminated with either copper (arable soil) or zinc (bare soil) resulted in a significant rehabilitation of soil chemical and biological properties.**
***Methods*. In the arable soil (Wageningen, the Netherlands), copper contamination caused a siginificant reduction of crop growth but the growth of a Cu-tolerant variety of *Agrostis capillaris* resulted in an increase in soil pH, DOC and dissolved Ca concentrations which caused a significant reduction of the free Cu^{2+} activity from initially toxic (between 10^{-5} to 10^{-6} M) to non-toxic levels (between 10^{-7} to 10^{-10} M). Also, bacterial growth and numbers of bacterivorous nematodes, which had been strongly suppressed as a result of high Cu levels (170 $mg \cdot kg^{-1}$) in combination with a low soil pH (4.7), normalized as an effect of grass growth. In the extremely Zn-polluted bare sandy soil of an old zinc smelter site (Maatheide, Belgium) with Zn levels up to 16.000 $mg \cdot kg^{-1}$, biological and chemical parameters also recovered due to the growth of Zn-tolerant varieties of *Agrostis capillaris* and *Festuca rubra* during a rehabilitation study on 3 hectares. To reduce metal availability, the experimental field on the Maatheide site was treated with beringite (modified aluminosilicate originating from fluidized bed burning of coal refuse), and composted municipal waste. A mixture of the two grass varieties was sown which resulted in a well developing grass cover within 4 months.**
***Results*. The combination of the reduced chemical availability due to addition of beringite and the gradual development of the vegetation, greatly reduced the toxicity of metals present in the soil solution. Extractable amounts of Zn in a 0.01 M $CaCl_2$ solution decreased from 525 $mg \cdot kg^{-1}$ to 16 $mg \cdot kg^{-1}$ in the treated plots which resulted in the normalization of the below-ground foodweb as expressed by the numbers and diversity of organisms and of metabolic functioning, such as bacterial growth and soil respiration. Under grass, approximately 10^9 bacteria, $6 \cdot 10^4$ protozoa, 5 m fungal hyphae and 27 nematodes were found per gram of dry soil, which was between 10 to 100 times higher than those obtained in the non-treated plots. Also the functional diversity of the soil bacterial populations, measured as the capacity to metabolize a number of different substrates, had almost doubled after soil treatment.**
***Conclusions*. Chemical (*i.e.* addition of beringite) and biological (growth of metal resistant crops) manipulation of soil resulted in a marked decrease of the toxicity of metals present in the soil and an increased availability of food for soil organisms that had been reduced to poverty for many years. Consequently, the soil food webs were restored.**

Key words: Phytostabilization - Soil ecosystem - Copper - Zinc - Beringite - Bioremediation.

Ever since the iron age, and in particular since the industrial revolution, heavy metal contents in soils have increased considerably, and have reached actual and potential toxic levels in industrialized and mining regions.[1] Although effects of contaminants on soil

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ecosystems are obscured by the habitat, negative effects on plant-growth and above-ground animals become visible more easily. Special metal vegetations, in particular consisting of metal-tolerant grass varieties, cover contaminated old mining areas. These grasses are useful for revegetation of contaminated areas where plant growth decreased or disappeared.[2]

Although sensitive soil ecosystems sometimes already respond to even slight increases in the heavy metal content, the system as a whole with its many interacting participants, is still functioning when vegetation gives up. Under laboratory conditions, small additions of heavy metals, for example, increase the lag period that preceeds the start of mineralization of certain substrates.[4] Under field conditions, the ratio of respired to biomass-incorporated substrate- C increases in metal- contaminated soils in particular when microbial biomass is decreased.[5-7] Cu-resistant bacteria already increased considerably (up to 1000-fold) at soil Cu-concentrations far below (up to 10 times) the current EU limit of 140 µg Cu•g^{-1} soil due to the addition of limited amounts of Cu-contaminated pig manure.[8] Also among consumers of microbes and plant-roots, and among their predators, such as protozoa and microbivorous, herbivorous and predatory nematodes, considerable changes in numbers and diversity occur as a result of increased heavy metal concentrations in the soil solution [9 10] with predatory and omnivorous nematodes being the most vulnerable in this respect.[12] As a result of changes in the soil ecosystem, important processes such as enzyme activity, organic matter decomposition, litter accumulation, N-fixation, may be altered.[7 13] However, until decrease or break-down of primary production, soil ecosystems are still provided with substrate and it is hypothesized that impoverished ecosystems in contaminated soils with reduced or no vegetation suffer more under lack of substrate than under toxicity due to the presence of heavy metals in the soil solution.

To test this hypothesis, experiments were conducted focussed on soil ecosystems (organisms and metabolic processes) from two contaminated soils: 1) a moderately Cu-contaminated (13 years) sandy arable field with reduced crop production and 2) a heavily Zn-contaminated (dozens of years) bare sandy area where vegetation had disappeared since long. In both soils metal-tolerant grass was sown and the effects of increased or renewed vegetation on the soil ecosystem was established. In the Cu-contaminated soil this was monitored in a pot-experiment [15] and in the Zn-contaminated soil in a field experiment.[16-18] Measured changes in the soil ecosystem parameters due to the growth of the grass are indicators of the remediation of the soil and for increase in soil quality. In supplemented short-term incubation experiments the substrate induced response of the ecosystem in the bare Zn-contaminated soil was measured after lab incubation. This was done in an effort to separate the effects on the ecosystem of toxicity related to the presence of heavy metals in the soil solution from effects of lack of substrate.

Materials and methods

Soils and experimental design

The Cu-contaminated sandy arable soil was obtained from an experimental site near Wageningen, The Netherlands. In a randomised block design 4 Cu-treatments had been established and 0, 250, 500 and 750 kg Cu·ha-1, respectively, were added as $CuSO_4•5H_2O$. Within each Cu-treatment 4 pH levels, ranging from 4.0 to 6.1, were obtained by adding lime or elemental sulphur to the soil. A crop rotation with oat, maize and potato has been practised for 13 years. Soil from plots with a pH 4.7 and 6.1 in combination with the 0 and 750 kg•ha^{-1} Cu treatments (*i.e.* 4 combinations) was used for a pot experiment with a Cu-tolerant grass variety, *Agrostis capillaris* L. var. Parys Mountain. Previous to the experiment, the soil was fertilized with NH_4NO_3, $MgSO_4$, K_2O and P_2O_5. Twenty-four pots (11×9×9.5 cm) were placed at random in a greenhouse at ambient temperature (15±2°C) for 10 weeks. The experiment comprised 3 replicates of each pH/Cu-combination, with either bare soil or soil sown with the grass variety. Crop yields in the field plots (6×11 m) treated with 750 kg Cu•ha^{-1} at pH 4.7 were strongly reduced down to 15% of the original yield for maize (compared to the 0 Cu treatment at pH 6.1) and 42% for potatoes respectively. The impact, however, was less at pH 6.1 in the soil treated with 750 kg•ha-1 and ranged from 70% for maize to 91% for potato, respectively.[10] The actual toxicity of Cu in the soil samples used in the pot experiment was checked in a preliminary pot experiment using both a Cu-tolerant and a non-tolerant variety of *Agrostis capillaris.* The growth of the tolerant variety was uninhibited, even at pH 4.7 and in the soil treated with 750 kg•ha^{-1}, whereas the yield of the non-tolerant variety decreased by 63% in the same soil.

The Zn-contaminated sandy nature soil was obtained from the Maatheide (Kempen, Belgium) where a zinc-smelter, that has been in operation from 1904 to 1974, caused an extremely high contamination of the surrounding area with in particular Zn, but also with Cd, Cu, and Pb. This has resulted in a complete disappearance of the natural vegetation in an area of 135 ha. In a demonstration experiment that was established in 1990, 3 ha (100×300 m) heavily contaminated (up to 16.000 mg Zn·kg^{-1} soil) bare soil was rehabilitated. This was achieved by adding 120 tons•ha^{-1} of beringite and 100 tons•ha^{-1} of composted municipal waste to the upper 35 cm of soil. Also a mixture of two Zn-tolerant grass cultivars of *Agrostis capillaris* and *Festuca rubra* was sown which resulted in a closed, even grass cover within 4 months. Due to the addition of beringite, a modified aluminosilicate,[16 19] the pH of the soil solution increased with approx. 2 units from 5.5 to 7.5. This resulted in a drastic reduction of the solubility of all metals. Furthermore, the municipal waste not only provided the poor soil with nutrients for plant growth but also increased the water holding capacity of the soil. Beneficial effects of the beringite-compost addition, therefore resulted in *i)* a reduction in the heavy metal content in the plants (compared to those growing in non-treated soils),[16] *ii)* a reduction of the leaching losses of Cd and Zn of 85%,[17] *iii)* an increase in the water holding capacity, and *iv)* elimination of the vulnerability of the soil to wind erosion. Besides mosses and algae, 26 species of higher plants settled on the experimental field and AM-mycorrhiza developed on the grass roots in the course of the years.[18] Also, large numbers of sporulating puffballs showed up amidst the grass, while in addition 14 other, less conspicuous mushroom species were observed. Six years after the establishment of the experimental field, observations on the soil ecosystem in treated vegetated and in untreated bare soil were initiated.

Maatheide soil was sampled in June 1996. Two samples of 2 kg each were taken from the upper 20 cm of the soil; one from the vegetated experimental field ("grass" sample) and one from the neighbouring bare area (bare sample). Three samples of each treatment of 150 g of sieved, moistened (15% w/w) soil were incubated in stoppered 0.5 l jars for one month at 20° C in the dark. Stoppers were provided with a gas septum to facilitate extraction of gas samples. In the same way also a series with 150 g soil from the bare sample was incubated after addition of 80 mg C per jar, added as 100 mg glucose and 125 mg glutamic acid (substrate induced bare sample).

Biological measurements

After one month incubation of the Maatheide soil samples the following biological parameters were measured (the parameters marked with '*' were also established in the pot-experiment with Cu-contaminated soil).

Bacterial* numbers were enumerated by automatic image analysis and hyphal (fungi) lengths were measured by epifluorescence microscopy;[20] protozoa* (flagellates, amoebae, ciliates) were counted by the MPN-method;[21] numbers of colony forming bacteria, fungi and streptomycetae (CFU) were counted after inoculation of soil suspensions on trypton-soybean agarplates (TSA, oxoid, 1/10); nematodes* (feeding guilds and taxa) were counted under the microscope, after elutriation from the soil;[22] bacterial activity* was measured as incorporation of ^{14}C-labelled leucine into bacterial proteins and in Cu-contaminated soil as ^{3}H-thymidine incorporation into bacterial DNA;[23] soil respiration* was measured as oxygen consumption or carbondioxide production by gaschromatography;[14] functional diversity of the soil microbial population was measured by the Biolog method.[24-26]

Chemical measurements

Analytical data of the Cu-contaminated arable soil have been described by Korthals *et al.*[10] In the present experiment, the pH, the Ca-, Cu-, NO_3^--N, NH_4^+-N, and DOC concentrations in the soil solution from the various soil samples were determined as were the total copper- and organic matter content of the soil.[15] The free Cu^{2+}-activity in the solution was calculated using a recently developed [27] thermodynamic equilibrium model.

Analytical data of the Maatheide nature soil have been described by Vangronsveld *et al.*[16-18] In the present experiment Zn, Cu, Pb and Cd availability were measured after extraction of the soil samples with H_2O (1:5) and with 0.01 M$CaCl_2$ (1:10). Also $pH_{H_2O,CaCl_2}$ and DOC-concentration were measured. Effects of grass development and of substrate addition were tested by analysis of variance (ANOVA): all pair wise multiple comparison procedures were carried out according to the Student Newman Keuls method.

TABLE I.—*Effects of the growth of Cu-tolerant* Agrostis capillaris *(10 weeks) on soil chemical parameters at two pH levels, averaged over two copper levels (0 and 750 kg·ha^{-1}).*

Soil Parameter	Incubation time (weeks)		
	0	10 F**)	10 P**)
pH - L*) pH_{H_20}	4.7	3.7	4.7
- H -	5.3	4.5	6.1
- L Ca (mM)	9.9	16.9	4.2
- H -	13.4	28.5	4.6
- L DOC (mM-C)	4.8	12.4	13.9
-H -	4.1	10.9	11.8

*) pH_{kcl} - L = 4.7, H = 6.1 (initial target pH); **) F = fallow pot, P = planted pot.

TABLE II.—*Effects of the growth of Cu-tolerant* Agrostis capillaris *(10 weeks) on the copper speciation at combinations of different pH and copper levels in the soil.*

pH/Cu treatment*)		Incubation time (weeks)		
		0	10 F**)	10 P**)
pH 6.1/Cu 0	Cu_t	0.48		
	Cu_s	0.05	2.0	3.0
	Cu^{++}	$10^{-9.0}$	$10^{-7.5}$	$10^{-9.5}$
pH 6.1/Cu 750	Cu_t	2.94		
	Cu_s	4.0	18	8
	Cu^{++}	$10^{-7.5}$	$10^{-6.0}$	$10^{-8.5}$
pH 4.7/Cu 0	Cut	0.47		
	Cu_s	2.0	7	10
	Cu^{++}	$10^{-7.5}$	$10^{-6.5}$	$10^{-8.5}$
pH 4.7/Cu 750	Cut	2.42		
	CU_s	11	135	17
	Cu^{++}	$10^{-7.0}$	$10^{-5.0}$	$10^{-7.0}$

*) Cu_t = total copper in m.mol.k^{-1}; Cu_s = dissolved copper concentration in µM; Cu^{++} = copper activity in soil solution .
**) F = fallow pot, P = planted pot

Results and discussion

Cu-contaminated soil

Chemical measurements

In all pH/Cu-treatments, soil pH decreased with approx. 1.3 unit during the first 5 weeks of incubation due to the nitrification of the added NH_4^+, but increased again in the last 5 weeks after depleltion of NH_4^+. The measured increase during the final 5 weeks was strongest in the planted pots (Table I). In the planted pots only, the Ca^{2+}-concentration in the soil solution dramatically decreased during the last 5 weeks of incubation (from approx. 17 mM to 4 mM). In all treatments, DOC-concentrations gradually increased (from 4.5 mMC to 12.3 mMC) to similar levels after 10 weeks of incubation, but the increase in the planted pots started sooner. Over the whole incubation period, dissolved Cu-concentrations (Cu_s) increased in all treatments (Table II). In the Cu-0 treatments dissolved Cu-concentrations increased to 10 µM, in the Cu-750 treatment to ± 125 µM (pH4.7) and ± 17.5 µM (pH 6.1) in the fallow pots, and up to 7.5 µM in the planted pots. In the pots with a grass cover, the dissolved Cu-concentration was considerably reduced in the Cu-750 treatment compared to the bare pot. This was mainly a result of the increase in pH in combination with a decrease of the dissolved Ca-concentration. The free Cu-activity (Cu^{2+}) in the soil solution was also significantly reduced in all planted treatments compared to the fallow soil which was mainly due to the increase in the concentration of DOC. In the bare pots, Cu^{2+}-levels were potentially toxic, but were reduced to non-toxic levels in the pots with a grass cover. The impact of the vegetation on the concentrations of the various Cu-species in solution, however, was indirect and brought forth by changes in pH and in Ca^{2+}- and DOC-concentrations, that resulted from grass-root development.

Biological measurements

At the start of the experiment the various biological parameters were measured in all pH/Cu combinations (results not shown). No significant differences were observed for bacterial numbers, oxygen consumption and protozoan numbers. Bacterial activity and nematode numbers (all feeding types), however, were significantly lower in the pH 4.7, Cu 750 treatment as compared to the other treatments (Fig. 1). During 10 weeks of incubation, with measurements after 3, 5 and 10 weeks, similarities and differences between the various parameters in the fallow soil did not change, with the exception of oxygen consumption which experienced a strong negative pH 6.1 effect during the last 5 weeks of incubation. Development of bacterial numbers did not differ between planted and fallow treatments. Bacterial activity in the planted pots increased in particular during the first 5 weeks of incubation and consequently stabilized. In the fallow pots bacterial activity decreased after 5 weeks of incubation, following an initial rise. Differences between initial bacterial activities in the various planted treatments were neutralized during incubation. After 10 weeks of incuba-

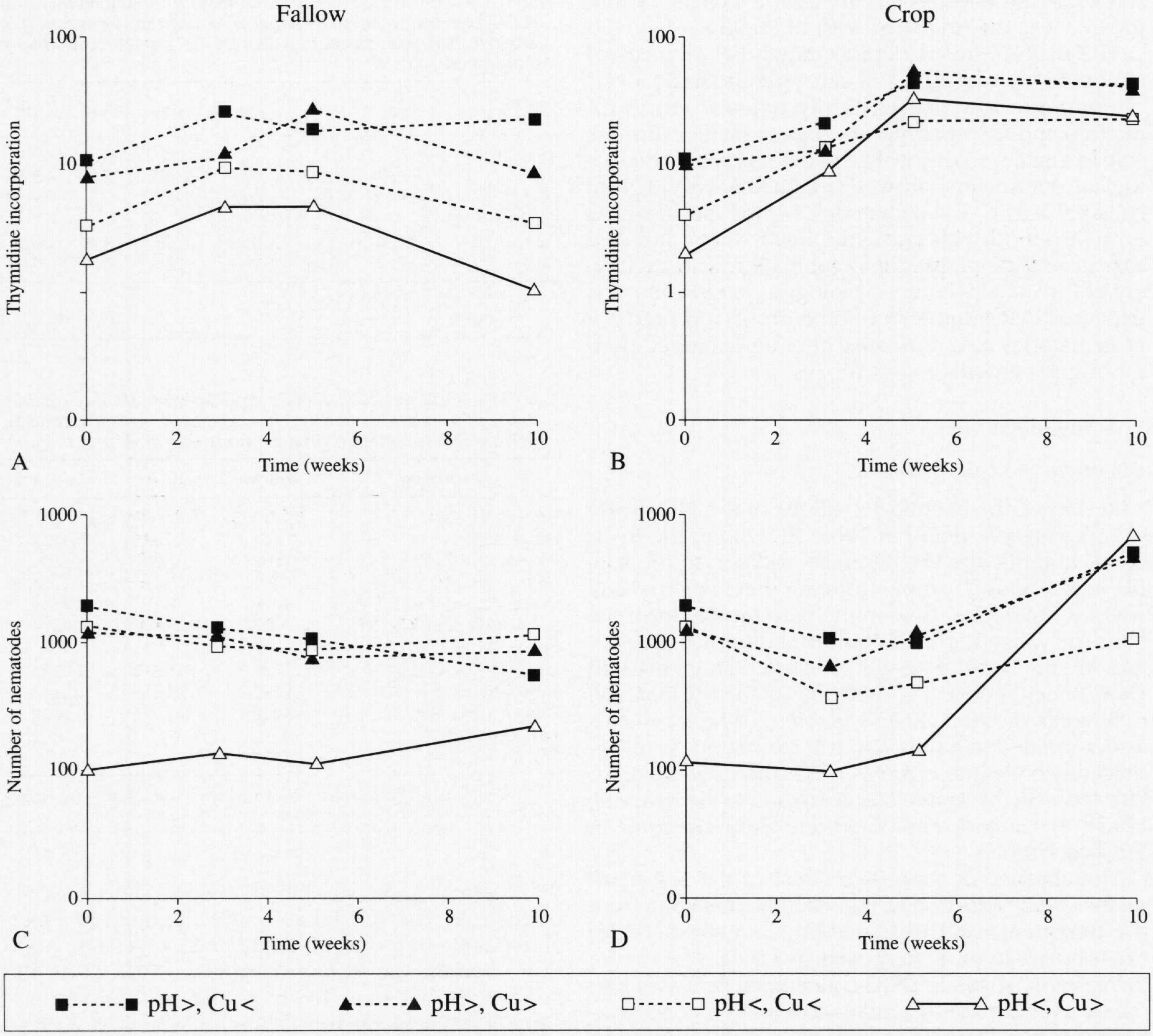

Fig. 1 A-D.—Pot experiment with and without *Agrostis capillaris* var. Parys Mountain. Dynamics of labelled thymidine incorporation (pmol/h* g dry soil) and numbers of nematodes per 100 g dry soil with (>) and without (<) copper added, at high (>) and low (<) pH.

tion higher numbers of protozoa and nematodes were observed in the planted pots compared to the fallow pots. Initial differences between nematode numbers among the planted treatments were neutralized. In particular bacterivorous nematodes - which increased from approx. 300 to 7400 in 100 g dry soil - in the pH 4.7, Cu 750 treatment had drastically recovered due to the growth of the grass although bacterial numbers nor activity increased in the last 5 weeks. Also the fungivores in the planted pH 4.7, Cu 750 treatment increased considerably during incubation from approx. 200 to 1500 in 100 g dry soil. However, also in the fallow pH 4.7, Cu0 treatment fungivores increased from 500 to 1800 per 100 g dry soil. From these results it is not clear if increase of fungivores was due to the growth of the grass.

The measurements demonstrated that 13 years of exposure of crops and soil ecosystem to toxic levels of copper at low pH drastically reduced crop production and incorporation of organic matter into the soil. In contrast to this, only 2 of the measured soil biological parameters showed a significant reduction: bacterial activity and nematodes. During the 10 weeks growth period with grass, the heavy metal induced toxicity was neutralized in combination with a recovery of the initially reduced biological parameters. It is expected that numbers of herbivorous and predatory nematodes also normalize after an extended period of grass growth.

TABLE III.—*Concentrations (availability) of heavy metals and DOC, and pH, as measured after H_2O and $CaCl_2$ extraction in treated (beringite, municipal waste, grass growth) and in bare Maatheide soil.*

Sample/ Extractant	Zn	Cu	Pb	Cd	DOC	pH
"Grass" Soil						
H_2O *)	8.87**)	0.89	1.19	0.05	36.80	8.00
$CaCl_2$	15.57	0.29	0.67	0.35		7.10
Bare Soil						
H_2O	52.80	0.03	0.23	0.26	3.49	6.80
$CaCl_2$	525	0.26	7.47	3.27		5.80

*) H_2O: (1:5); $CaCl_2$: 0.01 M (1:10).
**) in $mg \cdot kg^{-1}$.

TABLE IV.—*Organisms, metabolic process-rates and Zinc-availability in incubated (30 days) soil samples from the Maatheide: bare soil, bare soil plus organic substrates and "grass" soil.*

Parameters	Bare Soil	B.S.+S	"Grass" soil
Bacteria $x10^9.g^{-1\ a,b}$	0.12±0.02	0.42±0.09	0.99 ± 0.32
Fungi $m.g^{-1\ b}$	0.0	0.4±0.6	5.4±2.6
Protozoa $x10^3.g^{-1\ a,b}$	2.5±0.75	104±47	63±30
Nematodes $.100\ g^{-1\ b}$	13±12	13±4	3416 ±233
^{14}C Leucine $ngC.g^{-1}.h^{-1\ a,b}$	8±3.5	49±8	73±15
Respiration $ppmCO_2.30d^{-1}$	50	6000	4200
Biolog % (95)	46	-	83
Bacteria $^{10}log\ CFU.g^{-1\ a,b}$	5.6±0.2	7.8±0.2	7.5±0.1
Streptomycete $^{10}log\ CFU.g^{-1\ ab}$	4.8±0.2	6.5±0.3	7.4±0.1
Fungi $^{10}log\ CFU.g^{-1\ a,b}$	5.0±0.1	6.4±0.4	6.0±0,1
$ZnH_2Omg.kg^{-1}$	53	—	9
$ZnCaCl_2\ mg.kg^{-1}$	525	—	16

[a,b] Significantly different figures between bare soil and bare soil plus substrate (a) and between bare soil and grass soil (b), ($p < 0.001$).

Zn-contaminated soil

Chemical measurements

Results of the chemical analyses of the Maatheide samples are presented in Table III. Due to the treatment, in particular the beringite addition to the soil, pH in the "grass" sample from the grass covered soil was 1.2 unit higher than in the bare sample from the bare soil. Together with compost addition and plant growth, the increase of the pH caused an increase in DOC-concentration. The effects of the various soil treatments on the actual availability of heavy metals as determined in a 0.01 M $CaCl_2$ extraction were significant: concentrations of Zn, Cd, and Pb were up to 90% lower in the "grass" soil compared to the bare soil. However, Cu-concentrations were about the same in the two samples.

The absence of a positive effect of the soil treatments (*i.e.* a reduction of the solubility resulting from the treatment) on the available ($CaCl_2$-extractable) Cu-concentration is supposed to be caused by the increase in pH and in DOC-concentration. This is also the case for the non-decreased Cu- and Pb-concentrations measured after H_2O-extraction. With an increase in DOC, both Cu and Pb are mobilized and remain in solution due to their high affinity to form dissolved metal-organic complexes. This effect is reduced by the presence of Ca (*e.g.* in the 0.01 M CaCl2 extraction). However, due to the higher DOC levels in solution, the free metal ion activity is reduced.

Thus, chemical analysis indicates that the toxicity of the soil solution was drastically reduced as a result of the soil treatments and consequently also the potential effects of heavy metals on the soil ecosystem and development of the vegetation. In this context it should be realized, however, that for development of a metal tolerant – or a – non-tolerant grass vegetation addition of municipal waste (compost) suffices on the short-term, but most likely not on the long-term [16] since it will not prevent leaching losses. Moreover, compost will gradually mineralise. Beringite which specifically immobilizes free metal ions is not able to inactivate the metal-organic complexes.

Biological measurements

Table IV presents the results of the biological measurements on the incubated soil samples. In the "grass" samples approximately 1×10^9 bacteria, 6×10^4 protozoa, 5 m fungal hyphae and 27 nematodes were found per gram soil. Protozoa consisted for ± 90% of amoebae and ± 10% flagellates whereas ciliates were rare. Nematodes comprised bacterivorous *Acrobeloides buetschlii* (± 50%) and *Rhabditis sp.* and fungivorous *Aphelenchoides saprophilus* (± 40%). In the untreated, bare soil these figures were 90 to 99% less. For metabolic activities such as bacterial growth and soil respiration, normal rates were encountered in the grass-covered soil. These rates were approximately 10 (for bacterial growth) to 85 times (for soil respiration) higher than those found in the bare soil. The Biolog measurement on the functional diversity of populations of soil bacteria gives an indication for differences between populations. Results of this measurement corroborate the figures on numbers of organisms. Due to the soil treatments, functionality increases considerably. The countings of CFU's on agar plates are also indicative for the much higher densities of bacteria ($\times 10^2$), streptomycetae ($\times 10^3$), and fungi ($\times 10^1$) in the "grass" soil. Whereas no fungal hyphae were observed in the bare soil, fungi developed as CFU's on agar, probably originating from germinated spores. The CFU measurements indicate that in the bare soil viable micro-organisms are still present and react immediately on substrate addition in agar; the same holds for the Biolog measurements with respect to the functionality of low activity microbes that practically do not respire. These observations suggest that the organisms survived prolonged times of deprevation (toxicity, lack of substrate).

Addition of glucose and glutamic acid to the bare soil resulted in a drastically increased biological response, exept for the nematodes. Protozoan numbers and soil respiration exceeded values found in "grass soil" considerably but other parameters like bacterial numbers and activity increased to approximately half the values found in the "grass" samples. The incubation time probably was too short to obtain a substantial increase of fungal hyphae. In the "grass" samples, bacterivorous *Acrobeloides buetschlii* represented 50% of the total nematode population; among the 45 specimens identified in the bare samples 8 specimens *A. buetschlii* were identified. This species with a parthenogenetic way of reproduction and a short (up to 6 days) life cycle and high rate of reproduction under conditions of ample food did not increase in the substrate amended bare samples. As protozoa, also bacterivorous organisms, increased considerably due to substrate addition, lack of food was not the factor preventing nematodes to increase in this incubated soil sample. Toxicity induced by metals in the soil solution or low initial nematode numbers could have been the reason for nematodes not to recover. Due to the addition of beringite the toxicity of metals in the soil solution was greatly reduced. Moreover, the successful development of the vegetation on the Maatheide experimental site resulted in a renewed availability of food for a soil ecosystem that had been reduced to poverty for dozens of years. Under these influences the soil food web and its functioning largely normalized. As most of the biological parameters also recovered under the influence of substrate addition, even though toxicity had not been reduced, it is concluded that the soil ecosystem suffers more from lack of substrate than from toxicity of the soil solution, probably with the exception of nematodes.

In the moderately Cu-contaminated soil with reduced production of crops the toxicity of the soil solution decreased due to the growth of the Cu-tolerant grass and, as a result of the grass growth, the soil ecosystem also rehabilitated. In the heavily Zn-contaminated soil without any vegetational production, the toxicity due the presence of metals in the soil solution had to be reduced before a closed and sustainable vegetation of metal tolerant grasses could develop, which, however, did not colonize the neighbouring untreated area. This suggests that, also for rehabilitation of the soil ecosystem in the Maatheide area, grass growth is essential which can be achieved by addition of beringite (or other metal immobilizing compounds) and compost.

Conclusions

It is concluded that many biological parameters can be used as an indicator for the successful rehabilitation of heavily Zn-contaminated sandy soil where natural vegetation had disappeared. Only a few sensitive parameters (bacterial growth, nematodes) are indicators for rehabilitation of moderately Cu-contaminated soil. For the recovery of soil ecosystems in contaminated soils, restoration of vegetative productivity should be the first target as soil food webs survive toxicity better than non-tolerant crops do.

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