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## The effect of soil amendments on plant performance in an area affected by acid mine drainage

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### Abstract

A field experiment was carried out in order to assess the effect of urea, topsoil and municipal compost addition on plant performance and on the uptake of heavy metals by plants from contaminated soil. The experimental site was a disturbed soil in spite of remediation actions after several decades of uranium leaching (Eastern Thuringia, Germany) in 2004. Two plant species, *Lupinus angustifolius* L. and *Secale cereale* L. were grown at the site in a controlled experiment. We estimated the differences in soil pH, soil humidity, metal content, S-SO<sub>4</sub>, P-PO<sub>4</sub>, nitrogen (ammonium, nitrate and nitrite), plant growth, and plant uptake of metals between the experimental variants.

The study indicated that the most efficient treatment (in terms of biomass increase) was compost, followed by topsoil and urea addition. The mechanism underlying this effect was the change in soil parameters leading to higher availability of nutrients. There was, however, a depletion of the soil nitrogen in the amended variants at the end of the growing season.

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**Keywords:** Heavy metal; *Lupinus angustifolius*; Municipal compost; Remediation; *Secale cereale*; Soil; Topsoil; Acid mine drainage

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## 1. Introduction

Uranium mining in former East Germany was extensive during the period 1946–1990 with a total production of over 200 kt (Jakubick et al., 1997). This production established East Germany as the third producer after Canada and Australia in total volume, still 14 years after production stopped. An area of 460 ha was used in uranium mining operations at Ronneburg and the dumps had a volume of  $125 \times 10^6 \text{ m}^3$  (Jakubick et al., 1997). Since the beginning of the 1990s a substantial remediation effort has been ongoing (Jakubick et al., 1997; Hockley et al., 1997; Smolensky et al., 1997; Jahn et al., 2002; Hockley et al. 2003; Paul et al., 2003) and the remediation will continue for several decades.

The need to remediate is caused by the ecotoxicological risk from acid mine drainage, having a low pH and enriched concentrations of heavy metals, as well as from the risk of radon exhalation (Jakubick et al., 1997).

At the site of the former Gessenhalde the residual contamination as well as the properties of the soil affects the re-establishment of vegetation. The area was vegetated after completed remediation using a mixed grassland seed preparation. The current plant cover is however heterogeneous and patchy. The naturally immigrating plants include a substantial fraction of nitrogen fixing species such as *Trifolium* (*Trifolium pratense* L., *Trifolium repens* L., *Melilotus albus* L., *Trifolium arvense* L.) but also other species belonging to Fabaceae. (e.g. *Medicago lupulina* L.)

The aim of this study was to see which treatment is most favourable for plant development and performance at the site. Spray fertilization as well as topsoil and compost addition was evaluated. The results presented should be seen as preliminary as the evaluation covers only a single growing season.

## 2. Material and methods

### 2.1. Area description and experimental design

The Gessenhalde heap was located in the Ronneburg mining area, Thuringia, Germany. Gessenhalde was, as the single one in the area, constructed for leaching of low-grade ore, 1970–1989. To increase the efficiency of the leaching operation acid mine drainage (AMD) and/or diluted sulphuric acid were distributed on top of the leaching dump (Beleites, 1992; Schippers et al., 1995; WISMUT, 1997). During the screening prior to the remediation it was recognized as the most acid-generating dump (WISMUT, 1994a, b) and thus relocated to the lowermost part of the Lichtenberg open pit and mixed with granulated anhydrous lime (Jakubick et al., 1997; Paul et al., 2003).

The leaching heap consisted of three lifts of 15 m. The lower two levels were Silurian siliceous shale (Kieselschiefer), and the uppermost which was added later consisted of Ordovician shale (Lederschiefer) (Beleites, 1992). Thus, all three layers were consisting of what generally is referred to as black shale. According to Schippers et al. (1995), leached ore sampled within the heap contained per kg dry weight: ~250 mg Fe, 75 mg Mn, 20 mg Cu and, 18 mg Zn. The redox potential in a mixed slurry was about +600 mV with the average radioactivity level higher than 1000 nGy/h. Based upon drillings of the dump the average

pyrite concentration in the leaching material was 0.7 wt%, uranium 70 g/ton, and <3.40 Bq/g radium prior to the remediation according to WISMUT (1994a, b).

In order to investigate the effects of various soil amendments three Latin square lattices were set up. Areas of 20 × 20 meters were chosen for lattice plot in the experiment. To one 5 cm compost was added, the compost used was commercially available municipal compost. A topsoil amendment was likewise added to the second plot with a thickness of 5 cm. Both these plots, as well as the control (without any amendment) were mechanically mixed to a depth of approximately 20 cm. In the centre of each plot a 12 × 12 m Latin square was laid out, hence each of the nine subplots measure 4 × 4 m.

Plants chosen were rye (*Secale cereale* L.) and lupine (*Lupinus angustifolius* L.). They were sowed, with a seed mixture of 60 kg rye and 100 kg lupine/ha, in early June 2004. In order to assess the effects of fertilization urea was added as a solution (20 kg N/ha). This application added once in one third of the Latin squares (1 month after sowing but after the first plant collection) and twice (1 and 3 months after sowing and before the second plant collection) in another third; the last third was kept unfertilized as control.

Plants were sampled (5 individuals from each subplot) after one month, in July, and at the end of experiment, in October. Soil samples were collected from all sub-plots at the start of the experiment, at the time of sowing, as well as in the end of the experiment in October.

## 2.2. Soil characterization

Soil analysis were focused on the upper 10 cm assumed to be the most active zone in a biological sense (i.e. maximum root concentration) and also to the zone which is the most subject to the processes of erosion and atmospheric deposition. The brown-yellow loamy sand (10–20% loam) was analysed with regard to soil moisture, pH, organic matter, mineral-N concentrations, sulphate, phosphate, and heavy metals.

Soil moisture was computed after drying soil samples at 105 °C until constant weight. Soil pH was determined by mixing soil and 0.01 M CaCl<sub>2</sub> (1: 2.5 v/v) to a slurry and after shaking for 15 min it was reposed 1 h for the equilibration with carbon dioxide and reading after preliminary shaking.

Organic matter content of soil by loss-on-ignition (LOI) was determined by disintegration of organic material in an oven at 600 °C. The loss of weight gives an indication of the organic matter. At high temperature CaCO<sub>3</sub> and NaCl is disintegrated and structural water (which is part of the crystal lattice) lost. Furthermore, losses due to volatilization of sulphides and hydrated iron oxides and other volatile constituents were not accounted by this method (Carlsson, 2002 after Rowell, 1994). The amount of carbonates in the soil was likely to be very low as indicated by the low pH of the soil water, around 4 in untreated areas.

### 2.2.1. Mineral nitrogen concentrations, sulphate, phosphate

Soil samples were stored at 4 °C and processed within 24 h of retrieval. Twenty grams of each soil sample were extracted with 100 ml solution of 0.2 M KCl for nitrogen compounds as well as sulphate, and 5 g with 100 ml 0.5 M NaHCO<sub>3</sub> for phosphate analyses, filtered with glass filter (Whatmann GF/C) and analyzed by colorimetric methods: ammonium by sodium nitroprussid method, nitrate by sulphosalicylic acid method, nitrite with N1-naphthylethylenediamine and sulphanilamide method, sulphate by turbidmetrical method with barium chloride and phosphate with mix solution using molid-ammonium and malachite green reagents.

### 2.2.2. Chemical analyses

Elements analyses were performed on an ICP-AES (Liberty 150, Varian) after digestion with aqua regia (Hoffmann, 1991). The analysed elements were: Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, Ti, V, U, and Zn.

### 2.3. Plant analysis

After sampling, the plants were weighed (w.w.) and partitioned for analysis into above- and underground parts. The plant roots were briefly washed (repeated five time) with tap and finally with distilled water. All plant material was freeze-dried (d.w.), grounded and stored at  $-20^{\circ}\text{C}$  until further processing.

#### 2.3.1. Preparation of extracts for enzyme assay and determination of proteins

The powdery plant material (100 mg) was homogenized in 5 ml cold 0.1 M potassium phosphate buffer containing 2% (w/v) polyvinylpyrrolidone and 2 mM dithioeritriol. The supernatant was dialysed against a 5 mM potassium phosphate buffer at  $4^{\circ}\text{C}$  for 8 h and used for further analyses. The protein concentration was determined after precipitated with 10% trichloroacetic acid and solubilized with 1N NaOH, according to Lowry et al. (1951) using bovine serum albumin as standard.

#### 2.3.2. Enzyme assays

Superoxide dismutase (SOD) activity was measured by the ferricytochrome *c* method using xanthine/xanthine oxidase as the source of superoxide radicals, and one unit of activity was defined as the amount of enzyme necessary to inhibit the reduction of cytochrome *c* in a ratio of 50% according to McCord and Fridovich (1969).

Peroxidase (POD) activity was determinate at  $25^{\circ}\text{C}$  with guaiacol (Mascher et al., 2002, after Lagrimini, 1991). In the presence of  $\text{H}_2\text{O}_2$ , POD catalyses the transformation of guaiacol to tetraguaiacol. The reaction mixture contained 100 mM citric acid/potassium phosphate buffer (pH 5.0), 33 mM guaiacol and 0.3 mM  $\text{H}_2\text{O}_2$ . Horse-radish POD [guaiacol] was used as standard enzyme.

#### 2.3.3. Determination of chlorophyll and carotenoid content

Concentration of chlorophyll and carotenoids were determined by homogenizing 100 mg (d.w.) plant material in 80% acetone (80% acetone: 15% water: 5% conc.  $\text{NH}_3$ -solution [25%], v/v). The homogenate was centrifuged to remove the residue. The colour intensity of clear supernatant was spectrophotometrically measured at 480, 645, 647, 652, 663, 664 and 750 nm for chlorophyll *a*, chlorophyll *b* and carotenoids, respectively, as described by Schöpfer (1989).

#### 2.3.4. Element analyses

Element content was determined from plant samples after nitric acid microwave digestions, and the analysis were performed on an ICP-AES (Liberty 150, Varian). The analysed elements were the same as for soil characterization.

### 2.4. Statistical analysis

The data set acquired for evaluation represent averages calculated from nine subplots and two parallel analytical replicates values. A Mann–Whitney test was used for comparison of

change between the experimental variants at  $p < 0.05$ . The program SPSS (2002) for Windows was used for the statistical evaluation.

### 3. Results and discussion

The combined effect of amendment with compost or topsoil with the addition of urea is not further discussed as no additional effects from the urea were seen. Both, the discussion and the presentation of the result will focus on the three ways (adding compost, top soil or urea) to improve plant performance in the Gessenhalde area.

Table 1 shows the effect of the amendments during the study period increasing in the following order: urea 1X < urea 2X < top soil < compost. There are no data for urea 1X and urea 2X in July; as it was applied after the plant collection. The biomass increase was statistically significant in most cases. Interestingly, while in July 2004 the effect can be observed both in the case of lupine and rye in all plots, in October 2004 the biomass of lupines decrease in control as well as in the top soil and, in the compost amendment is virtually missing. One plausible explanation would be the different life time expectancy of lupines which can change with the amendment application. However, acidic soils are an important constrain to crop production.

In order to investigate the causes of this biomass increase the soil was characterized. The concentration of most metals in the soil did not change significantly ( $p > 0.05$ ), so it is not possible to allocate the biomass increase to such a cause. Table 2 shows the elements concentration in soil identified for the 0–2, 3, and 4 soil variants (the addition of urea is assumed not to change the metal concentration, so the 0–2 variants was treated as one).

If looking at soil parameters (Table 3), an important difference between the experimental variants can be seen. In July 2004 there is a strong increase in inorganic N and phosphate, and a decrease in sulphate after the addition of top soil or compost. This trend is still present in October in the case of phosphate and sulphate, but not in the case of N. This may be explained by the higher mobility and bioavailability of the N. The decrease of N in compost was stronger than in top soil, probably related to the higher mobility of N in this organic mixture. Besides the transfer to plants, some immobilization in microbial biomass, as well as leaching to lower soil horizons could have occurred. The large increase in biomass is, however, the most likely cause to this depletion of N. Denitrification would be limited by the high redox conditions associated with the low soil humidity.

Soil humidity (Table 3) as well as L.O.I. (from 2.91% in the case of control to 5.27% in top soil and 11.40% in compost) significantly increased with the addition of compost and top soil, with a more pronounced effect of the compost addition. This increase was present still in October 2004, suggesting that the organic material absorbing water change that was responsible for the higher water retention capacity was preserved during the growing season. This clearly showed the importance of these amendments in order to improve soil quality. The addition of urea did not change significantly the nitrate concentrations.

**Table 1.** The effect of addition of one or two times urea, top soil and compost on biomass of plants (g d.w.) compared to the plants in the control treatment ( $n = 5$ , g)

		July			Mann–Whitney $p < 0.05$	October					Mann–Whitney $p < 0.05$	
		0 Control	3 Top soil	4 Compost		0 Control	1 Urea 1	2 Urea 2	3 Top soil	4 Compost		
Lupine	Ab. Ground	Mean	2.00	4.07	4.62	0–3, 0–4, 3–4	1.18	1.64	1.07	2.92	n.m.	
		SD	0.26	0.57	0.42		0.70	0.63	0.23	1.69	n.m.	
	U. Ground	Mean	1.14	2.23	2.59	0–3, 0–4, 3–4	0.31	0.34	0.37	0.52	n.m.	(0–3), (0–4), (1–3), (1–4)
		SD	0.24	0.37	0.32		0.13	0.08	0.15	0.19	n.m.	
	Total	Mean	3.14	6.29	7.21	0–3, 0–4, 3–4	1.49	1.98	1.44	3.44	n.m.	
		SD	0.48	0.93	0.69		0.82	0.72	0.38	1.82	n.m.	
Rye	Ab. Ground	Mean	1.59	2.98	4.92	0–3, 0–4, 3–4	12.70	13.51	23.20	29.60	103.90	0–2, (0–3), 0–4, 1–2, 1–3, 1–4, 2–4, 3–4
		SD	0.23	0.52	0.42		9.04	7.66	1.00	9.02	22.00	
	U. Ground	Mean	1.13	3.38	5.02	0–3, 0–4, 3–4	2.41	3.35	4.87	4.50	14.60	0–2, (0–3), 0–4, (1–3), 1–4, 2–4, 3–4
		SD	0.16	0.58	0.57		1.10	1.83	0.97	1.54	2.80	
	Total	Mean	2.73	6.35	9.94	0–3, 0–4, 3–4	15.10	16.80	28.10	34.10	118.60	0–2, (0–3), 0–4, 1–2, 1–4, 2–4, 3–4
		SD	0.39	1.09	0.99		9.88	9.47	0.61	10.30	24.20	
All	Total	Mean	5.87	12.70	17.20	0–3, 0–4, 3–4	16.60	18.81	29.50	37.50	118.60	0–2, 0–3, 0–4, 1–2 1–3, 1–4, 2–4, 3–4
		SD	0.67	1.33	1.41		10.50	9.21	0.66	10.10	24.20	

Mann is indicating whether there was a significant difference according to Mann–Whitney; n.m. indicate that parameters were not measured. Brackets indicate a  $p$  level between 0.1 and 0.05.

**Table 2.** Effect of treatments on the element concentration in soil (mg/kg)

Elements	Experimental variants			Elements	Experimental variants		
	0 Control	3 Top soil	4 Compost		0 Control	3 Top soil	4 Compost
Al	7370	9540	9720	Mg	2100	4180	2810
As	17.00	23.00	24.00	Mn	365.0	429.0	414.0
Ba	60.00	135.0	78.00	Mo	2.00	2.00	1.00
Ca	660.0	15900	806.0	Ni	40.00	37.00	55.00
Cd	2.00	2.00	3.00	P	321.0	1490	414.0
Co	13.00	11.00	19.00	Pb	n.d.	30	n.d.
Cr	22.00	28.00	29.00	Sr	10.00	73.00	13.00
Cu	9.00	29.00	23.00	Ti	318.0	286.0	399.0
Fe	22300	21600	28800	V	30.00	31.00	39.00
K	696.0	4620	871.0	Zn	44.00	117.0	61.00

No significant differences between the treatments were found. n.d. indicate that the metal was not detected.

The addition of topsoil or compost strongly increased the soil pH towards neutrality, thus theoretically reducing metal mobility, which would lower the bioavailable metal fraction. Lower metal content in the plants would be expected in plants grown under these experimental conditions.

Indeed, if looking at the metal concentrations in plants, a pattern of decreasing concentrations from control (0) to compost (4) is valid for many elements. [Tables 4 and 5](#) exemplify this situation for rye and lupine in the following metals: Al, As, Cd, Co, Cu, Cr, Fe, Mg, Mn, Ni, Pb (only in case of rye), V, and Zn. This decrease can be explained by the higher soil pH, but also to the better soil nutrient status, as it is present not only in the case of top soil and compost, but also in the case of urea addition.

On the other hand, some metal concentrations range in both plant species and almost all experimental variants in/or over excessive or toxicity range. It is documented that the primary limitations on acid soils are the toxic levels of aluminium (Al) and manganese (Mn), as well as suboptimal levels of phosphorus (P) ([Kochian et al., 2004](#); [Jansen et al., 2002](#)). Aluminium and sulphuric acid are released from inactive mine tailings due to weathering of sulphide ores. At pH level of 4.5 to 6.5 Al tends to have a toxic effect, which increases with decreasing pH levels. Terrestrial plants have potentially greater risk of increased Al exposure due to the higher Al concentration of soil pore water and its lower pH. The adverse effects of Al include inhibited plant growth as well as decreases root elongation and weight. There are approximately 25 plant families with members that strongly bioaccumulate Al at concentrations exceeding 1000 mg/kg in their leaves ([Yokel, 2004](#) after [Jansen et al., 2002](#)). However, micromolar Al concentrations are toxic to many plants ([Yokel, 2004](#)). In [Tables 4 and 5](#) it can be observed an exceeding amount of aluminium uptake in lupine harvested in October, as well as in rye in control variants at both collection times.

**Table 3.** Soil parameters in the experimental variants (humidity in %, the chemical parameters as mg/kg d.w.)

		July			Mann–Whitney $p < 0.05$	October					Mann–Whitney $p < 0.05$
		0 Control	3 Top soil	4 Compost		0 Control	1 Urea 1	2 Urea 2	3 Top soil	4 Compost	
pH	Mean	4.63	6.01	7.39	0–3, 0–4, 3–4	4.70	4.69	4.66	6.04	7.49	0–3, 0–4, 1–3, 1–4, 2–3, 2–4, 3–4
	SD	0.03	0.11	0.15		0.06	0.08	0.00	0.11	0.15	
Humidity	Mean	6.78	7.43	14.90	0–4, 3–4	9.81	9.64	7.67	13.78	18.00	0–3, 0–4, 1–3, 1–4, 2–3, 2–4, 3–4
	SD	0.67	2.36	4.22		0.64	0.80	2.69	0.82	4.07	
N-NH <sub>4</sub>	Mean	2.76	5.65	16.10	0–3, 0–4, 3–4	2.43	1.95	2.17	2.37	0.86	(1–4), 3–4
	SD	1.55	1.29	5.20		1.63	1.23	2.74	1.84	0.42	
N-NO <sub>3</sub>	Mean	1.46	21.30	92.70	0–3, 0–4, 3–4	2.26	2.32	2.93	0.94	2.99	0–3, 1–3, (2–3), 3–4
	SD	0.76	9.59	45.50		0.71	0.48	1.67	0.45	2.11	
N-NO <sub>2</sub>	Mean	0.02	0.06	0.24	0–3, 0–4, 3–4	0.02	0.02	0.02	0.07	0.10	(0–3), 0–4, 1–3, 1–4, 2–3, 2–4, (3–4)
	SD	0.01	0.05	0.12		0.03	0.01	0.01	0.03	0.03	
P-PO <sub>4</sub>	Mean	6.91	25.00	76.50	0–3, 0–4, 3–4	12.90	19.20	8.24	27.00	106.0	0–2, 0–3, 0–4, 1–2 1–4, 2–3, 2–4, 3–4
	SD	2.01	6.57	22.50		0.40	9.72	0.81	5.04	37.30	
S-SO <sub>4</sub>	Mean	1944	375.0	845.0	0–3, 0–4	3010	1790	1860	443.0	648.0	0–3, 0–4, 2–3, 2–4
	SD	1002	171.0	705.0		780.0	1460	815.0	174.0	547.0	

Mann is indicating whether there was a significant difference according to Mann–Whitney. Brackets indicate a  $p$  level between 0.1 and 0.05.



**Table 4.** Metal concentrations in rye

	Aboveground				Belowground				p<0.05											
	July		October		July		October													
	0	3	4	p<0.05	0	1	2	3		4	p<0.05									
Al	Mean 1250	703.0	396.0	0–4	1690	1600	729.0	395.0	264.0	0–2, 0–3, 0–4, 3–4	2420	3350	1880.0	3–4	8830	7390	7990	4360	1010	2–3, 2–4, 3–4
	SD	309.0	364.0	223.0	285.0	1780	137.0	111	97.30		1150	788	625.00		1010	821.0	1560	1860	445.0	
As	Mean	2.59	0.79	n.d.	3.45	3.71	2.40	0.23	n.d.	4.01	5.72	4.25		0.71	0.14	3.80	9.44	1.54		
	SD	0.85	1.37	n.d.	0.89	1.61	0.37	0.69	n.d.	2.99	2.10	0.93		0.71	0.14	3.80	9.44	1.54		
Ba	Mean	14.90	21.30	8.90	16.30	17.40	8.55	24.50	9.88	24.40	41.50	36.14		87.00	68.50	81.00	64.30	22.20	2–4, 3–4	
	SD	5.64	16.74	2.00	1.92	11.90	1.58	9.71	4.61	10.40	6.35	17.80		18.00	9.62	26.00	18.20	7.07		
Ca	Mean	388.0	204.0	305.0	282.0	251.0	209.0	250.0	208.0	132.0	301.0	416.0	0–3, 0–4	172.0	132.0	137.0	245.0	299.0	2–3, 2–4, 3–4	
	SD	122.0	94.00	35.30	85.00	60.00	53.30	46.20	35.00	22.90	86.20	58.00		58.10	4.04	8.53	31.90	63.30		
Cd	Mean	0.20	0.22	n.d.	0.76	0.66	0.44	0.06	n.d.	1.10	0.91	n.d.	0–4, 3–4	0.28	0.14	n.d.				
	SD	0.15	0.38	n.d.	0.15	0.37	0.41	0.18	n.d.	0.28	0.14	n.d.		0.18	0.53	0.51	1.77	0.21	(2–3), 2–4, 3–4	
Co	Mean	51.0	381	0.50	6.16	5.94	4.74	0.37	0.69	19.40	4.21	370.0	0–4	3.70	1.05	0.60				
	SD	2.06	6.03	0.79	1.31	4.02	1.76	0.67	0.40	2.37	1.05	0.60		3.50	15.60	1.96	1.83	1.10		
Cr	Mean	4.04	3.71	3.18	4.03	3.42	1.76	1.17	1.13	6.19	16.74	10100–3		60.20	61.70	53.05	17.40	2.172	2–3, 2–4, 3–4	
	SD	1.30	1.18	1.88	1.36	3.21	0.41	0.31	0.47	3.52	6.84	8.49		38.10	34.80	31.00	20.60	1.702	2–3, 2–4, 3–4	
Ca	Mean	14.80	8.40	10.50	9.31	9.53	9.89	7.69	8.62	12.00	18.00	14.60	0–3, 3–4	2.30	3.63	5.63	5.81	2.68		
	SD	1.91	1.92	1.00	1.70	1.25	1.68	1.45	1.16	3.00	0.62	1.64		2.30	3.63	5.63	5.81	2.68		
Fe	Mean	7280	9630	46200–4, 3–4	9485.0	11880	7770	2720	2930	2110	2810	1170		11690	13100	11880	5310	7680	2–3, 2–4, 3–4	
	SD	650	8300	n.d.	1740	6900	3060	1040	1070	400	610	1170		400	610	1170	1170	1170		
K	Mean	2300	2900	3640	15700	15800	17700	20400	28200	9230	2300	23900	0–3, 0–4	6760	5100	6540	7130	10800	2–4, 3–4	
	SD	1890	7530	4620	5680	3380	1400	6010	6130	1740	1920	2170		1240.0	1220.0	1840.0	860.0	1150.0		
Mg	Mean	3980	2170	1960	2410	2340	2630	1870	1610	4070	323.0	338.0		3530	3580	2710	1450	1410	2–3, 2–4	
	SD	635	1280	554.0	460.0	776.0	1930	453.0	395.0	6080	1610	1110	0–3, 0–4, 3–4	671.0	492.0	333.0	361.0	205.0		
Mn	Mean	9870	2970	1340	2690	3370	1510	788.0	804.0	808.0	358.0	93.10		2140	1110	1250	8370	1820	852.0	2–3, 2–4, 3–4
	SD	549.0	377	297.0	0.65	0.52	0.19	1.53	6.74	0.90	1.75	2.37		2.95	3.50	2.90	1.57	3.472	2–3, 2–4	
Mo	Mean	0.91	1.30	5.70	0.04	0.52	0.33	1.02	2.56	0.25	0.87	1.08		1.46	1.02	1.18	1.67	1.33		
	SD	0.10	0.29	1.02	0.04	0.52	0.33	1.02	2.56	82.20	64.60	46.00		173.0	26.50	36.10	44.10	5.77		
Ni	Mean	33.80	22.80	12.10	9.70	21.60	10.30	2.41	1.72	21.40	7.53	27.30		1000	835.0	893.0	1400	1700	2–3, 2–4, 3–4	
	SD	6.49	17.40	5.94	185.0	136.0	336.0	626.0	929.0	536.0	526.0	334.0	0–3, 0–4	158.0	59.40	186.00	168.0	332.0		
P	Mean	2250	3610	5970	2320	2320	4000	5080	9290	n.d.	n.d.	n.d.		5.19	7.80	7.86	2.33	1.19		
	SD	302.0	1280	1360	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		8.99	6.76	6.93	6.98	3.58		
Pb	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		19.00	15.80	16.80	21.20	15.90	3–4	
	SD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		5.34	2.40	3.73	3.59	2.85		
Sr	Mean	8.85	3.44	13.20	9.29	9.02	7.13	15.80	9.01	8.84	21.70	25.50	0–3, 0–4	71.90	76.10	87.30	52.50	15.10	2–4	
	SD	1.18	7.28	0.38	1.62	3.28	1.22	3.16	1.62	21.14	40.80	16.40		29.80	37.50	40.80	42.20	11.20		
Ti	Mean	16.40	21.50	7.00	15.10	20.70	15.50	10.00	8.03	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.		
	SD	3.82	11.32	1.19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.		
U	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.		
	SD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.		
V	Mean	2.55	1.87	1.23	4.17	4.22	2.13	0.99	0.66	6.32	7.76	3.79		20.20	19.00	22.00	10.30	2.56		
	SD	0.47	1.34	0.82	0.66	3.04	0.32	0.22	0.45	3.03	2.08	0.23		5.44	10.13	9.55	7.14	1.02		
Zn	Mean	45.10	38.20	67.20	45.50	41.90	43.30	38.00	40.10	76.20	107.0	91700–3		114.0	69.70	69.30	67.50	58.10	(2–3), 2–4	
	SD	14.60	7.76	17.50	7.17	9.33	4.07	15.60	5.84	19.00	13.70	12.90		38.50	11.30	19.00	25.60	15.60		

Grey areas show decreasing concentration from control (0), one (1) or two times (2) urea application, top soil (3) to compost (4), while bold and underlined values the increase of concentration from control to the amendments. n.d. indicates that the metal was not detected; p<0.05 indicates significant difference according to Mann-Whitney.

**Table 5.** Metal concentrations in lupine

	Aboveground										Belowground										
	July					October					July					October					
	0	3	4	$p < 0.05$	0	1	2	3	4	$p < 0.05$	0	1	2	3	4	$p < 0.05$	0	1	2	3	4
Al	Mean	848.0	314.0	374.0	0.3, 0.4	471.0	561.0	362.0	198.0	n.m.	0.3	1373	1099	817.0	0.4	n.m.	n.m.	3300	1870	n.m.	2-3
	SD	260.0	185.0	86.30		1690	1050	643.0	553.0	n.m.		399	182.0	241.0		n.m.	n.m.	1690	371.0	n.m.	
As	Mean	3.41	n.d.	3.00	0.3, 3.4	12.00	11.10	7.20	3.73	n.m.	0.3	4.52	5.16	n.d.	0.4	n.m.	n.m.	5.04	3.99	n.m.	
	SD	0.87	n.d.	0.67		7.12	0.82	1.30	n.m.		0.59	1.36	n.d.			n.m.	n.m.	1.91	1.41	n.m.	
Ba	Mean	11.70	12.30	16.00		39.60	49.00	29.00	27.40	0.3	13.40	24.30	18.00	0.3	n.m.	n.m.	21.70	26.00	n.m.		
	SD	3.40	5.83	12.40		14.00	9.93	5.50	3.53	n.m.	4.01	7.50	5.95		n.m.	n.m.	9.43	5.49	n.m.		
Cu	Mean	13.60	101.70	13380		17100	21800	9680	13400	0.2	15900	17380	4830	0.3, 0.4	n.m.	n.m.	4400	5710	n.m.		
	SD	3590	5350	4410		4950	3230	1270	4470	n.m.	967.0	1739	546.0		n.m.	n.m.	1620	1840	n.m.		
Cd	Mean	2.59	1.05	0.87	0.3, 0.4	9.37	11.10	11.70	4.36	n.m.	0.3	4.22	1.62	1.19	0.3, 0.4	n.m.	n.m.	6.98	4.43	n.m.	
	SD	0.96	0.53	0.24		1.36	2.57	1.62	1.55	n.m.	1.52	0.66	0.69		n.m.	n.m.	2.12	2.27	n.m.		
Co	Mean	19.80	6.41	6.47	0.3, 0.4	41.80	57.20	61.10	20.40	n.m.	0.3, 0.2	36.40	6.36	6.04	0.3, 0.4	n.m.	n.m.	5.43	1.80	n.m.	2, 3
	SD	4.19	1.98	4.33		16.00	26.10	18.40	7.37	n.m.	8.90	1.63	2.16		n.m.	n.m.	3.27	6.07	n.m.		
Cr	Mean	0.83	0.58	1.87	3-4	11.60	13.29	7.13	10.60	n.m.	0.2	21.30	18.00	8.32	0.4	n.m.	n.m.	18.70	59.00	n.m.	2-3
	SD	0.25	0.45	2.50		5.58	5.80	2.78	3.30	n.m.	19.60	5.47	4.89		n.m.	n.m.	5.33	24.00	n.m.		
Cu	Mean	13.40	12.00	9.46	0.4	31.80	37.10	33.80	20.40	n.m.	0.3	13.80	13.60	13.40		n.m.	n.m.	29.80	13.90	n.m.	2-3
	SD	0.45	5.00	1.53		2.55	7.36	0.98	3.62	n.m.	6.58	4.46	2.96		n.m.	n.m.	15.80	3.20	n.m.		
Fe	Mean	769.0	331.0	345.0	0.3, 0.4	4420	6690	2650	1740	n.m.	0.3	1350	1030	798.0	0.4	n.m.	n.m.	2290	1780	n.m.	2, 3
	SD	300.0	26.30	170.0		1280	3040	397.0	960.0	n.m.	238.0	36.80	273.0		n.m.	n.m.	164.0	40.80	n.m.		
K	Mean	15700	20200	32400	0.4, 3-4	6410	5600	4000	16000	n.m.	0.3	8090	35000	51300	0.3, 0.4	n.m.	n.m.	1780	19200	n.m.	2-3
	SD	12180	2530	1410		3500	3500	4100	3860	n.m.	7180	9320.0	12500		n.m.	n.m.	3970	6560	n.m.		
Mg	Mean	3270	74.0	1580	0.3, 0.4	18550	5930	9320	5860	n.m.	2020	1895	1280	3-4	0.3, 0.4	n.m.	n.m.	1790	6600	n.m.	
	SD	2060	761.0	586.0		4820	5590	5260	2510	n.m.	764.0	336.0	283.0	0.4	n.m.	n.m.	1680	681.0	n.m.	2-3	
Mn	Mean	40.0	1190	1370	0.3, 0.4	511.0	440.0	1870	567.0	n.m.	0.2	109	2.46	3.93	0.4	n.m.	n.m.	1.06	3.20	n.m.	
	SD	1.97	6.62	0.36		1.51	0.43	0.40	0.39	n.m.	0.27	0.28	1.47		n.m.	n.m.	0.04	1.48	n.m.		
Ni	Mean	90.43	25.20	36.60	0.3, 0.4	223.0	288.0	274.0	102.0	n.m.	0.3	126.0	39.70	23.10	0.3, 0.4	n.m.	n.m.	318.0	109.0	n.m.	2-3
	SD	18.40	14.00	29.40		30.00	77.50	69.40	27.20	n.m.	44.10	7.92	7.17		n.m.	n.m.	139.0	26.80	n.m.		
P	Mean	2160	2710	2710		3110	3240	2080	2960	n.m.	0.2	1050	2590	1640	0.3, 3.4	n.m.	n.m.	814.0	1350	n.m.	2, 3
	SD	146.0	572.0	423.0		264.0	803.0	190.0	430.0	n.m.	124.0	341.0	172.0		n.m.	n.m.	152.0	429.0	n.m.		
Pb	Mean	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.m.	n.d.	n.d.	n.d.		n.m.	n.m.	n.d.	n.d.	n.m.		
	SD	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.m.	n.d.	n.d.	n.d.		n.m.	n.m.	n.d.	n.d.	n.m.		
Sr	Mean	30.30	48.80	5.82	0.4	37.60	49.90	27.40	65.50	n.m.	0.3	10.40	58.10	39.60	0.3, 0.4	n.m.	n.m.	17.30	41.90	n.m.	2-3
	SD	5.99	24.10	2.36		9.08	11.13	3.37	17.70	n.m.	4.72	12.60	5.67	3.4	n.m.	n.m.	5.00	11.30	n.m.		
Ti	Mean	19.10	8.26	9.90	0.3, 0.4	54.40	80.20	24.90	31.40	n.m.	0.2	30.10	32.80	27.50		n.m.	n.m.	44.40	44.20	n.m.	
	SD	7.14	3.10	3.17		16.10	45.70	4.64	17.50	n.m.	7.54	3.55	6.60		n.m.	n.m.	27.50	13.20	n.m.		
U	Mean	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.m.	n.d.	n.d.	n.d.		n.m.	n.m.	n.d.	n.d.	n.m.		
	SD	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.m.	n.d.	n.d.	n.d.		n.m.	n.m.	n.d.	n.d.	n.m.		
V	Mean	1.54	0.84	0.63	0.3, 0.4	15.40	15.00	12.00	4.63	n.m.	0.3	2.78	1.96	2.20		n.m.	n.m.	7.14	4.21	n.m.	
	SD	0.46	0.60	0.60		9.09	4.30	1.81	1.33	n.m.	0.79	0.43	0.34		n.m.	n.m.	3.04	0.77	n.m.		
Zn	Mean	76.30	71.10	68.10		136.0	156.0	149.0	121.0	n.m.	48.10	62.20	41.20		n.m.	n.m.	76.00	58.90	n.m.		
	SD	4.50	22.80	14.60		15.40	21.60	21.60	15.30	n.m.	12.20	6.05	6.23		n.m.	n.m.	29.10	13.70	n.m.		

Grey areas show decreasing concentration from control (0), one (1) or two times (2) urea application, top soil (3) to compost (4), while bold and underlined values the increase of concentration from control to the amendments; n.d. indicate that the metal was not detected; n.m. the metal was not measured;  $p < 0.05$  indicates significant difference according to Mann-Whitney.

The manganese content of terrestrial plants depends on the species, the stage of growth, and the part of the plant involved. There is a variety of distribution among the different tissues (Anke et al., 1999b). Background contents of manganese, range from 17 to 334 mg/kg d.w. in grass and from 25 to 119 mg/kg d.w. in clover. By contrast, cereal grains show a relatively small variation in manganese content: between 15 and 80 mg/kg d.w. (Kabata-Pendias and Pendias, 2001). The generally higher manganese concentration may be related to the lower soil pH, which has not been treated with lime (Anke et al., 1999c). In our case manganese concentration in lupine as well as in rye are far over the excessive or toxicity level which is approximated in mature leaf tissue for various species between 400–1000 mg/kg d.w.

It is documented that the extractable nickel content of soil affects the uptake of nickel by plant roots. Extractability of Ni from soil is influenced by physical factors (e.g. texture, water content), chemical factors (pH, organic constituents, redox potential) and biological factors (e.g. plants species variability, microbial activity (Sunderman, 2004 after NAS, 1975, Wallance et al., 1977; Heale and Ormond, 1982; Hazlett et al., 1983). The excessive or toxicity level of Ni in mature leaf tissue generalized for various species range between 10 and 100 mg/kg d.w. (Kabata-Pendias and Pendias, 1992). This level was slightly exceeded in case of rye only in control variants but, very high in case of lupine especially in October 2004, in all experimental variants. Other metals such Cd, Co, Cr, Cu, V and Zn have been found in excessive or toxicity level in case of lupine. The excessive or toxicity level for these metals are: Cd 5–30, Co 15–50, Cr 5–30, Cu 20–100, V 5–10 and Zn 100–400 mg/kg d.w. (Kabata-Pendias and Pendias, 1992). Thus, at the end of the experiment, the growth of lupine was severely affected for the toxicity stress causing the disappearance of lupine in some experimental variants.

Another remarkable effect which underlines the role of the soil nutrients status is the increase in P and K concentration in plants from minimum in the control to maximum in the compost. The effects are not similar in lupine in case of P, which was stable in October 2004 in treatment [0] and [3].

The lowered concentration of so many metals in plants, as well as the mitigation of drought, as reflected by the increase in soil humidity, should have led to a reduced oxidative stress and more energy for investing in photosynthetic pigments and in proteins. SOD and POD activities in case of both plant species decreased from control to compost amendments, chlorophyll significantly increased (Tables 6A and B).

It is documented that Zn, Fe, Cu, Mn or Ni are directly involved in protein synthesis (Marschner, 1995). In spite of toxic level of manganese found in the of compost and top soil experimental variants, they do show a significantly higher protein production than in the control. The data from Tables 6A and B show that plants did not suffer from metal toxicity. The decrease of SOD and POD activity and the increase in protein levels are an indication that the plants did not suffer from metal stress in July. Normally, nutrient toxicity influence SOD and POD activities, but the changes in the enzyme activities depend on the type and duration of the stress.

**Table 6.** Microparameters in lupine (A) and rye (B)

A		Lupine, July, Aboveground			Mann–Whitney $p < 0.05$
		0	3	4	
Protein	Mean	<b>3540</b>	<b>4540</b>	<b>6190</b>	0–3, 0–4, 3–4
	SD	754.0	533.0	1190	
SOD	Mean	899.0	713.0	539.0	0–3, 0–4, 3–4
	SD	134.0	79.90	91.00	
POD	Mean	1.91	1.79	1.00	0–4, 3–4
	SD	0.49	0.89	0.20	
Chl total	Mean	<b>1.78</b>	<b>3.16</b>	<b>3.81</b>	0–3, 0–4
	SD	1.03	0.65	2.32	

  

B		Rye, July, Aboveground			Mann–Whitney $p < 0.05$
		0	3	4	
Protein	Mean	<b>1340</b>	<b>5240</b>	<b>6060</b>	0–3, 0–4
	SD	60.0	213.0	701.0	
SOD	Mean	599.0	153.0	133.0	0–3, 0–4
	SD	27.0	6.0	15.0	
POD	Mean	100	6.0	69.0	
	SD	8.90	0.90	0.31	
Chl total	Mean	<b>1.30</b>	<b>7.60</b>	<b>8.00</b>	0–3, 0–4, 3–4
	SD	0.07	0.66	1.44	

The units are:  $\mu\text{g}$  proteins /g d.w above ground, mg total chlorophyll/g d.w leaves, Units SOD/g protein,  $\mu\text{Units}$  POD/g protein. Grey areas show decrease from control to top soil to compost and bold/underline values increase.

However, although the doubling of biomass in the compost field is not balanced by a decline in metal concentration, the total amount of metals extracted into aboveground plant parts is not controlled by biomass production alone, but also in principle by the size of the plant available fraction. This is implicating that the quality of the vegetation cover could be addressed, through improving the soil quality by amendments. It would be possible without risking a massive transfer of metals from the soil into the plants, since the total amount of metals extracted from the soil is not correlated to the biomass production.

The most efficient amendment (in terms of increase in biomass production) for the period studied was compost, followed by topsoil and urea addition. The mechanisms underlying this effect was change in soil parameters leading to lower bioavailability of metals and increased nutrient availability. Further studies are needed to investigate how the nitrogen depletion can be best compensated in the long term. Such studies could involve beside nitrogen addition, the inoculation with

microorganisms, coupling thus plant performance (e.g. phytoremediation) with remediation based on bacteria and fungi (Neagoe et al. 2004).

The lack of relationship between production and metal concentration in biomass is demonstrating the need for a deeper understanding of the different pools of metals in this disturbed soil. The transfer of metals between the pools is crucial in this understanding and has to be addressed. Naturally, one has to take both biological and geochemical factors into consideration when describing the element fluxes between different compartments in the soil, similar to the conceptual model found in Ebenå (2003). Metals present in the surface soil at the site do not seem to be increasingly mobilized to any higher degree by the introduction of vegetation, nor fertilizers or other amendments necessary to increase plant performance. The effects from the plants are judged to be minor and the integrated effects from the amendments applied might actually lower the available pool for the elements having a phytotoxic effect.

This means that if there are severe risks of erosion, or loss of material from the area, means to improve the vegetation cover would be recommended. The choice of amendment, or fertilizer, is rather a matter of cost. Therefore, if there is severe risk of erosion from the area this can be prevented by introducing plants to stabilize the upper soil without risking increased metal fluxes within the area.

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