

## EFFECT OF HEAVY METAL CONTAMINATED SHOOTING RANGE SOILS ON MYCORRHIZAL COLONIZATION OF ROOTS AND METAL UPTAKE BY LEEK

A. MOZAFAR<sup>1</sup>, R. RUH<sup>1</sup>, P. KLINGEL<sup>2</sup>, H. GAMPER<sup>1</sup>, S. EGLI<sup>3</sup> and  
E. FROSSARD<sup>1\*</sup>

<sup>1</sup> Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH), Eschikon Experiment Station, Lindau, Switzerland; <sup>2</sup> Office of Biological Safety, Birmensdorfstrasse 55, Zürich, Switzerland; <sup>3</sup> Swiss Federal Research Institute WSL, Birmensdorf, Switzerland  
(\* author for correspondence, e-mail: emmanuel.frossard@ipw.agrl.ethz.ch)

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**Abstract.** We grew leek (*Allium porrum*) in soils of two shooting ranges heavily contaminated with heavy metals in the towns of Zuchwil and Oberuzwil in Switzerland as a bioassay to test the activity of arbuscular mycorrhizal (AM) fungi in these soils. Soil samples were taken from (1) front of the shooting house (HOUSE), (2) the area between house and target (FIELD) and (3) the berm (BACKSTOP). Samples of Ribwort plantain (*Plantago lanceolata*) growing naturally within the shooting ranges were also collected and the colonization of its roots by mycorrhizal fungi was measured. The number of AM spores in the soils was significantly reduced concomitant with the increase in the degree of soil contamination with metals. In Zuchwil, mycorrhizal fungi equally colonized roots of Ribwort plantain sampled from BACKSTOP and HOUSE. In Oberuzwil, however, plants from BACKSTOP had lower colonization when compared with those sampled from HOUSE. Colonization of leek was strongly reduced in the BACKSTOP soil of Zuchwil and slightly reduced in the BACKSTOP soil of Oberuzwil when compared with plants grown in respective HOUSE soil. Concentrations of Cd, Cr, Cu, Ni, Pb and Zn in the leaves of leek grown in the BACKSTOP soil was within the range considered toxic for human consumption. This points to the high degree of bio-availability of these metal in these soils. Significant decrease in the number of mycorrhizal spores in the BACKSTOP soils in Zuchwil and the low colonization of leek roots grown in these soils point to possible changes in the species diversity of mycorrhizal fungi in these soils.

**Keywords:** Cd, Cr, Cu, leek, metals, military shooting range, mycorrhiza, Pb, Sb, Zn

### 1. Introduction

In Switzerland there are more than 2000 shooting ranges for the regular shooting practice of civilian militia and sport clubs. The bullets used are 7.5 mm (GP11) and 6.5 mm (GW Pat 90). GP11 has a hard core composed of 98% Pb and 2% Sb as well as traces of Cd, Zn, Cu and As. Cu and Ni are also used in the shell casing. Bullets are often fragmented and pulverised upon impact with the BACKSTOP or bullet traps located at the range. In 1994, for example,  $90 \times 10^6$  rounds of ammunition were fired that released 400–500 tons of Pb into the environment (BUWAL, 1997), mostly into the soils of the shooting ranges. Soil in shooting



ranges is known to be high in Pb. Lead concentrations in soils of shooting ranges in the US state of Michigan, for example, have been noted to be as high as  $2256 \mu\text{g g}^{-1}$  (Murray *et al.*, 1997) and  $60\,000 \mu\text{g g}^{-1}$  in Switzerland (AUKSG, 1994). The degree of soil contamination in some shooting ranges in Switzerland has become very controversial, especially since some cows died several days after accidentally grazing in a shooting range (Braun *et al.*, 1997). A similar observation has been made in Denmark (Jorgensen and Willems, 1987). One of the shooting sites most studied in Switzerland is a 300 m long range with 50 targets in the town of Zuchwil in canton Solothurn that has been in operation since 1924. This is a range which is very frequently used and in 1992, for example, 150 000 rounds of ammunition were fired there (AUKSO, 1994). Measurements of soil and vegetation in this site have extremely high concentrations of Pb and several other metals, especially in the vicinity of the FIELD and BACKSTOP soil (AUKSO, 1994), and in the vegetation (AUKSO, 1995).

Effects of heavy metals on the activity of mycorrhizal fungi are contradictory. For example, although plants growing in heavy-metal-contaminated substrate were found to be strongly colonized by native mycorrhizal fungi (Gildon and Tinker, 1983b; Shetty *et al.*, 1994; Dev *et al.*, 1998; Koomen *et al.*, 1990), numerous reports point to the detrimental effect of heavy metals on mycorrhizal activity in soil (Gildon and Tinker, 1983b; Diaz and Honrubia, 1993; Leyval *et al.*, 1997; Sainz *et al.*, 1998; Thorne *et al.*, 1998; Kelly *et al.*, 1999; Val *et al.*, 1999).

The role of mycorrhizal colonization of plant roots in uptake of heavy metals and/or alleviation of metal toxicity is also not clear. For example, there are reports that mycorrhiza may have no effect (Ietswaart *et al.*, 1992), decrease (Galli *et al.*, 1994; Joner and Leyval, 1997; Helal *et al.*, 1998) or increase the uptake of toxic metals by plants (Gildon and Tinker, 1983a; Killham and Firestone, 1983; Loth and Höfner, 1995). Mycorrhizal fungi are also reported to protect plants from the toxic effect of high external concentration of several heavy metals (Wilkins, 1991; Tuner, 1994; Jentschke *et al.*, 1999), possibly by binding the metals in their hyphae (Denny and Wilkins, 1987; Tuner, 1994; Marschner *et al.*, 1998; Brunner and Frey, 2000) or by reducing the translocation of metals to the plant tops (Burke *et al.*, 2000).

Shooting ranges are locations with extremely high concentrations of several potentially toxic metals and thus provide a unique opportunity to study the effect of extreme multi-metal soil pollution on the activity of a group of soil microorganisms such as mycorrhizal fungi. The objective of this investigation was thus to study the impact of the particular combination of heavy metal contamination of shooting ranges on mycorrhizal fungi activity in soil by using two shooting ranges with different extents of metal contamination.

TABLE I

Some physical properties and the total heavy metal concentrations measured in soils of locations HOUSE, FIELD and BACKSTOP in the shooting ranges Zuchwil and Oberuzwil<sup>a</sup>

Parameters	Zuchwil			Oberuzwil			Limits <sup>b</sup>
	HOUSE	FIELD	BACKSTOP	HOUSE	FIELD	BACKSTOP	
Vegetation present	Artificial meadow	Natural vegetation	Natural vegetation	Artificial meadow	Artificial meadow	Natural vegetation	
pH	7.2	7.5	7.7	6.8	6.0	6.8	
Organic C (%)	1.5	2.1	4.5	4.2	2.7	23.5	
Cd ( $\mu\text{g g}^{-1}$ )	0.37	0.29	0.43	– <sup>c</sup>	–	–	0.8
Cu ( $\mu\text{g g}^{-1}$ )	128	84	771	75	17	1250	40
Hg ( $\mu\text{g g}^{-1}$ )	4.55	0.15	0.24	–	–	–	0.5
Ni ( $\mu\text{g g}^{-1}$ )	23	38	143	–	–	–	50
Sb ( $\mu\text{g g}^{-1}$ )	2.4	5.3	208	1.36	0.13	831	NA <sup>d</sup>
Pb ( $\mu\text{g g}^{-1}$ )	164	3110	33600	322	44	26000	50
Zn ( $\mu\text{g g}^{-1}$ )	296	161	431	424	63	974	150

<sup>a</sup> AUKSO, 1995; AUKSG, 1994.

<sup>b</sup> Official Swiss tolerance limits in soil (VBBo, 1998).

<sup>c</sup> Not measured.

<sup>d</sup> Not available.

## 2. Material and Methods

We selected two shooting ranges in Switzerland with different kinds and degrees of soil pollution. These were two 300 m shooting ranges in the towns of Zuchwil (47°12'12"N, 7°33'24"E) in canton Solothurn, and Oberuzwil (47°25'51"N, 9°7'12"E) in canton St. Gallen. The soil in the Zuchwil range is contaminated to above the tolerance limit by Cu, Zn, Pb, Ni, Hg and Sb and that in Oberuzwil with the elements Cu, Zn and Pb (Table I). In both ranges the targets are located at 300 m from the shooting HOUSE. During the shooting practice, the bullets lodge into the soil mound (BACKSTOP), located behind the targets, either directly or after passing through the target sign. The BACKSTOP is usually covered with sawdust to prevent the bullets from ricocheting. At the time of soil sampling in Zuchwil barley was planted on the central portion of the range and the rest was covered with a clover-grass meadow. In Oberuzwil the whole range was covered with natural meadow except the BACKSTOP which was covered with natural vegetation.

Soil samples were taken in 1998 (Zuchwil) and in 2000 (Oberuzwil) at three locations within the shooting range: (1) 1–2 m in front of the shooting house (HOUSE), (2) in the field between the house and target (FIELD); and (3) in the berm (BACKSTOP). At each location five soil cores were taken perpendicular to the length of the shooting range. These were considered five replications samples.

Also at each location, Ribwort plantain (*Plantago lanceolata*), which was the only plant growing naturally in all three locations, was randomly sampled and their roots were tested for colonization by mycorrhizal fungi.

Thirty seeds of leek (*Allium porrum* L.) variety 'Dubouchet selma' were first sterilised for 10 min in 5% calcium hypochlorite, washed and soaked for 12 hr in distilled water, and then planted in pots containing the soil samples. After 2–3 weeks, the number of seedlings was thinned to 10 plants per pot. Greenhouse conditions were: 35 and 20 °C day/night temperature, 16 hr photoperiod and light intensity of ca. 300  $\mu\text{mol s}^{-1} \text{m}^{-2}$ ). Plants were irrigated twice weekly with half strength Hoagland solution that had low phosphorus concentration (1/100 of Hoagland solution). At 60 days after planting, plants were harvested, roots were separated and their sub-samples were placed in 50% alcohol for further measurements. Leaves were dried at 85 °C for 48 hr prior to metal analysis. Roots were stained by the method of Phillips and Hayman (1970) and the presence of hyphae, arbuscules and vesicles in the roots was quantified using a microscope by the magnified intersection method of McGonigle *et al.* (1990). Brown-colored hyphae and those with obvious septa were considered non-mycorrhizal and were not counted. Leaves were powdered in a ceramic mortar. Samples of 0.2 g leaf powder were placed in vessels and 2 mL of 40%  $\text{HNO}_3$  and 80  $\mu\text{L}$  of concentrated HF were added and predigested at room temperature for 30 min. Digestion was performed in a closed-vessel microwave digester (MLS Ultraclav, max. pressure: 10 Mpa; max temperature: 240 °C; total digestion time: 150 min). Metals were measured with an ICP-AES or ICP-MS. Experimental design was a factorial with three locations (HOUSE, FIELD and BACKSTOP), two soil preparations (intact and sifted), two harvesting dates and five replications. PCA (principal component analysis) was performed using Statgraphics<sup>®</sup> software. Pearson's correlation coefficients between variables and P according to Bonferroni test were calculated using Systat<sup>®</sup> software.

### 3. Results

#### 3.1. GROWTH OF LEEK

Growth of leek was strongly reduced in the heavily polluted BACKSTOP soil of Oberuzwil but was not affected by the degree of soil pollution in the soils taken from Zuchwil shooting range (Figure 1).

#### 3.2. ROOT COLONIZATION WITH MYCORRHIZA

##### 3.2.1. Ribwort Plantain

Plantain collected from the BACKSTOP soil of Oberuzwil showed significantly lower colonization by mycorrhizal fungi than those collected from the vicinity of the HOUSE. In Zuchwil, however, roots of plantain showed equal colonization at all three locations within the shooting range (Figure 2).

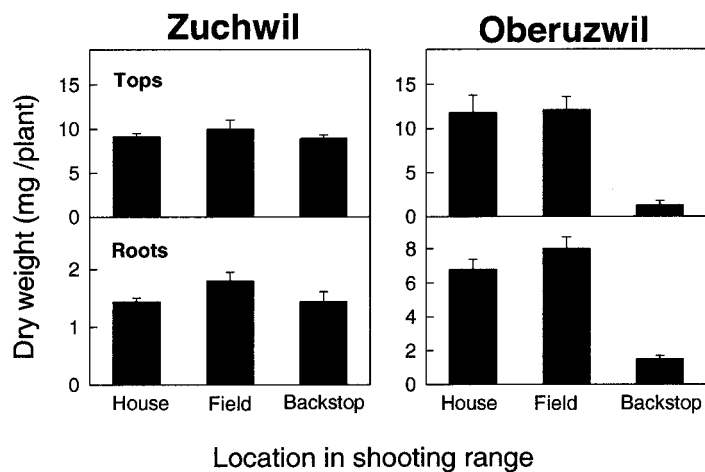


Figure 1. Growth of leek in soils from locations HOUSE, FIELD, and BACKSTOP in the shooting ranges Zuchwil and Oberuzwil. Bars denote standard errors.

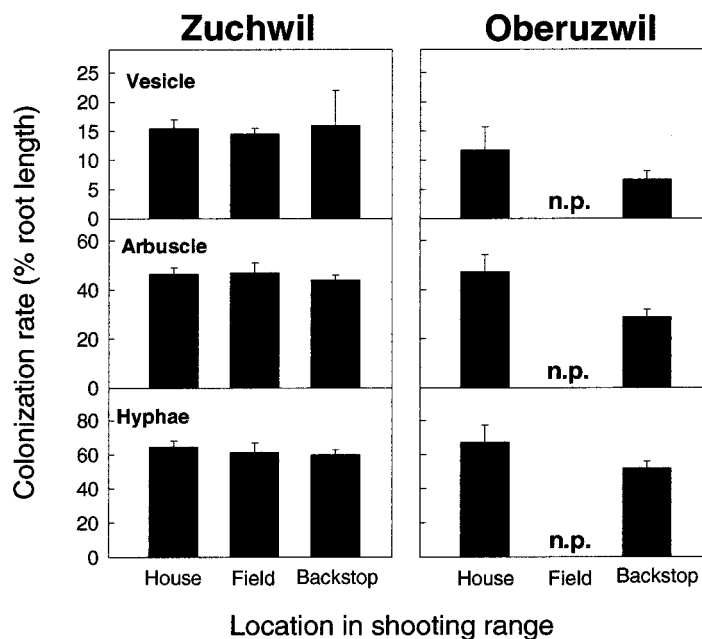


Figure 2. Colonization of Ribwort plantain roots collected from different locations (HOUSE, FIELD and BACKSTOP) in the shooting ranges Zuchwil and Oberuzwil. In location Oberuzwil no Ribwort plantain could be found in the location FIELD (n.p. = not present). Bars denote standard errors.

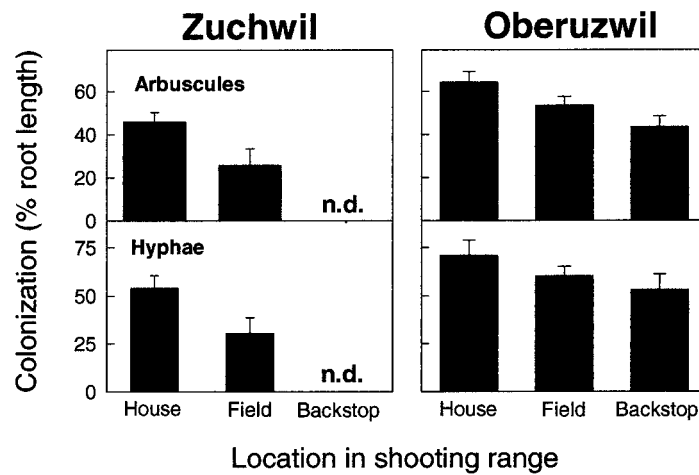


Figure 3. Colonization of leek roots grown in soils from locations HOUSE, FIELD, and BACKSTOP in the shooting ranges Zuchwil and Oberuzwil (n.d. = not detected). Bars denote standard errors.

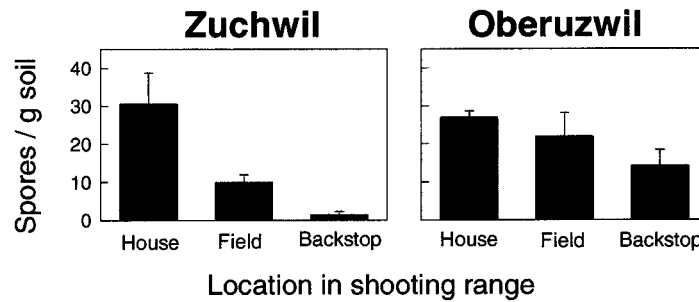


Figure 4. Total number of mycorrhizal spores found in soil from locations HOUSE, FIELD, and BACKSTOP in the shooting ranges Zuchwil and Oberuzwil. Bars denote standard errors.

### 3.2.2. Leek

Colonization of leek root by mycorrhiza was consistently and progressively lower as the degree of soil pollution with metals increased; less colonization in soils from FIELD and BACKSTOP as compared with that in HOUSE soil. This was especially true for Zuchwil where leek growing in the BACKSTOP soils was fully void of mycorrhiza (Figure 3).

### 3.3. NUMBER OF MYCORRHIZAL SPORES IN SOIL

The number of mycorrhizal spores in the soil was progressively lower in locations FIELD and BACKSTOP as compared with location HOUSE. This trend was more pronounced in Zuchwil (Figure 4). Microscopic examination spores showed that they belong exclusively to the genus *Glomus*.

TABLE II

Analysis of variance significance levels for the effect of location in the shooting range (HOUSE, FIELD and BACKSTOP) on the colonization of root of test plant (leek grown on these soils) by mycorrhizal hyphae (Hyp.), arbuscule (Arb.), vesicle (Ves.), and the metal concentrations in its leaves

Shooting range	Tops	Roots	Hyp.	Ves.	Arb.	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Zuchwil	***	***	***	*/**	***	NS <sup>a</sup>	*	NS	*	NS	*	***	***
Oberuzwil	***	***	***	NS	***	***	*	***	***	**	***	***	***

\*, \*\*, and \*\*\* indicate significant at 0.05, 0.01 and 0.001 levels, respectively.

<sup>a</sup> NS = Not significant.

TABLE III

Average metal concentration ( $\mu\text{g g}^{-1}\text{DW}$ ) observed in leek leaves grown in soils of locations HOUSE, FIELD and BACKSTOP in the shooting ranges in Zuchwil and Oberuzwil

Element	Zuchwil			Oberuzwil			Toxicity limits ( $\mu\text{g g}^{-1}\text{DW}$ ) <sup>a</sup>
	HOUSE	FIELD	BACKSTOP	HOUSE	FIELD	BACKSTOP	
Cd	0.18	0.21	0.55	0.13	0.16	0.91	0.5–1
Co	0.35	0.38	1.37	0.17	0.166	0.41	0.5–1
Cr	5.48	4.66	11.21	7.61	6.67	23.45	1.0–1000
Cu	5.85	8.16	168.88	7.00	6.62	20.59	100.0–200
Mn	44.41	32.12	44.12	20.44	29.72	37.29	200.0–400
Ni	5.93	5.92	25.44	2.32	3.29	16.68	10.0–50
Pb	6.42	45.13	1151.50	0.87	0.28	114.13	10.0–30
Zn	39.67	41.43	236.32	54.62	25.97	70.98	150.0–250

<sup>a</sup> Metal concentration in plant tissues considered being too high for human consumption (Lindt *et al.*, 1990).

### 3.4. METAL ABSORPTION BY LEEK

The concentrations of Co, Cu, Ni, Pb and Zn were significantly higher in the leaves of leek grown in the soil of BACKSTOP as compared with soil collected from the vicinity of HOUSE in the shooting range Zuchwil. Plants grown in the BACKSTOP soil of Oberuzwil contained significantly higher concentrations of Cd, Co, Cr, Cu, Ni and Pb than plants grown in HOUSE soil (Table II and Figure 5).

### 3.5. PRINCIPAL COMPONENT AND REGRESSION ANALYSIS

Three axes were significant in the PCA of both Zuchwil and Oberuzwil and explained 73.3 and 81.5% of the variability, respectively (results not shown). As expected, percentage colonization of roots by hyphae, arbuscules and vesicles of

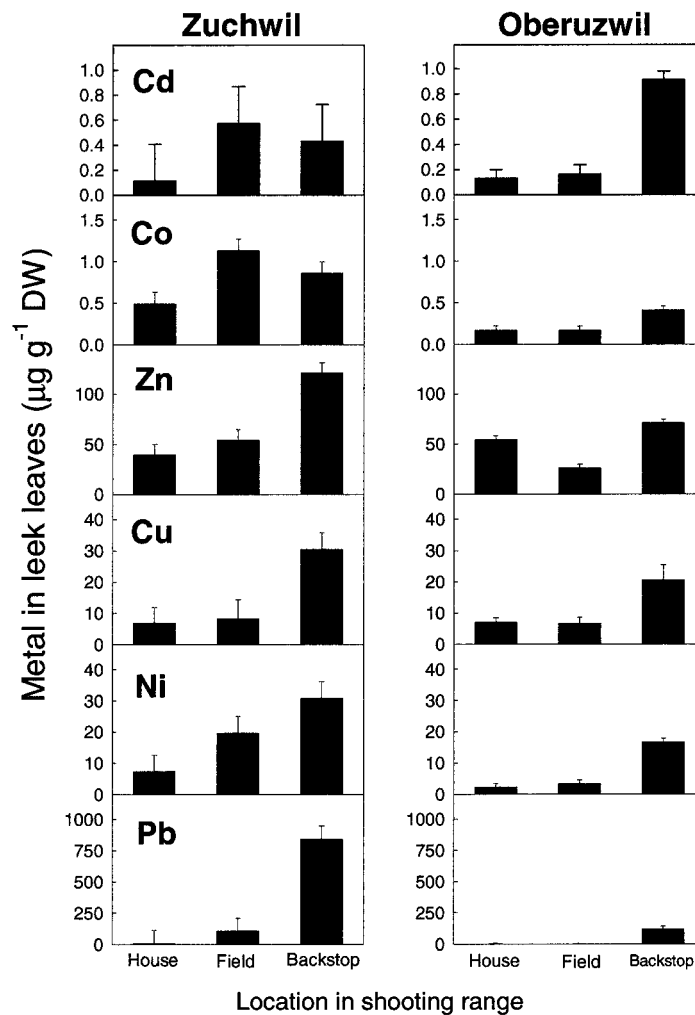


Figure 5. Metal concentrations in the leek tops grown in soils from locations HOUSE, FIELD and BACKSTOP in the shooting ranges Zuchwil and Oberuzwil. Bars denote standard errors.

mycorrhiza was always close together in PCA graphs (Figures 6 and 7). Several negative relationships were found between the metal concentration in the leek leaves and the mycorrhizal colonization of the leek roots. For example, percentage of root lengths having arbuscules was negatively correlated with Co, Cu, Ni, Pb and Zn in plants grown in soils from Zuchwil (Table IV) and with Cd, Cr, Cu, Ni, and Pb in plants grown in soils from Oberuzwil (Table IV). Mn was the only element whose concentration in the leaves did not show any relationships with mycorrhizal structures (Tables V and VI). Dry weight of leek tops was negatively related with only one element (Cr) in plants grown in Zuchwil soils but with seven elements



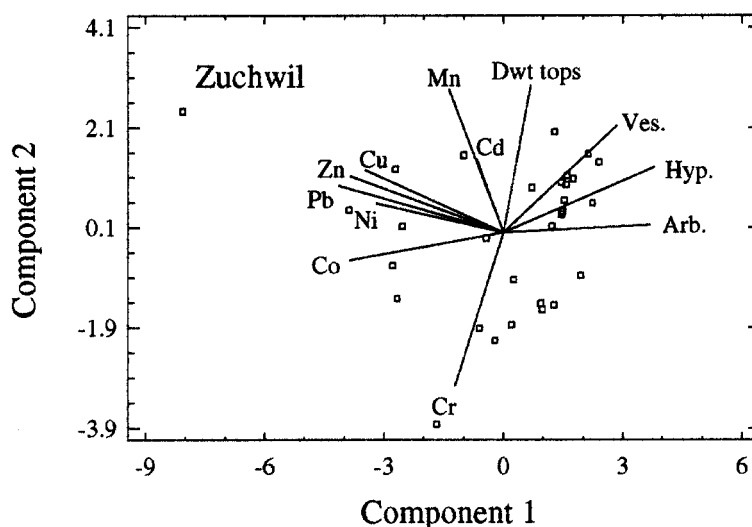


Figure 6. Plot of the occurrence of the variables analysed against the first two axes of the Principal Component Analysis of data from location Zuchwil.

(Cd, Co, Cr, Cu, Ni, Pb and Zn) in plants grown in soils Oberuzwil (Tables IV and V; Figures 6 and 7).

#### 4. Discussion

Very few spores of mycorrhizal fungi could be found in the heavily polluted soils taken from the vicinity of FIELD and BACKSTOP as compared with the soil taken from the vicinity of the shooting HOUSE in Zuchwil. Although it is hard to ascribe the effect observed to any one or combination of metals, since Ni is more toxic than Cr for fungal growth (Aggangan *et al.*, 1998) and Cu and Ni are reported to be more toxic than Pb and Zn to fungi (Ross and Kaye, 1994), one may speculate that the strong reduction in spore counts in the FIELD and BACKSTOP soils in Zuchwil might have been due to the high concentrations of Cu and Ni in these soils. Some mycorrhizal fungi produce little or no spores. It is conceivable that the low spore counts in the BACKSTOP soil of Zuchwil might also be due to changes in the species diversity of mycorrhizal fungi in these metal-contaminated soils as observed by others (Val *et al.*, 1999).

VA mycorrhiza may adapt to metals such as Zn and Cd (Gildon and Tinker, 1983b). High colonization of roots of *Agrostis capillaris* L. with VA mycorrhizas in sites with a natural high Zn concentration was taken as evidence for a co-evolution of *Agrostis* and *Glomus* towards a high degree of Zn tolerance (Ietswaart *et al.*, 1992). Equal colonization of the Ribwort's plantain's roots collected in different locations in the shooting range Zuchwil irrespective of the degree of soil con-

TABLE IV

Pearson correlation coefficients ( $r$ ) among heavy metal concentrations in the leek leaves, dry weight of plant tops, and the colonization of roots with mycorrhizal structures (arbuscule, vesicle and hyphae) across the three locations in plants grown in soils taken from shooting range in Zuchwil<sup>a</sup>

	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn	Top	Arb	Ves
Cd											
Co	0.10 NS <sup>b</sup>										
Cr	-0.10 NS	0.52 **									
Cu	0.12 NS	0.83 ** *	0.05 NS								
Mn	0.19 NS	0.29 NS	-0.18 NS	0.48 **							
Ni	0.11 NS	0.57 **	0.14 NS	0.41 *	0.13 NS						
Pb	0.14 NS	0.77 ** *	0.04 NS	0.84 ** *	0.36 *	0.65 ** *					
Zn	0.19 NS	0.61 **	0.04 NS	0.62 ** *	0.37 *	0.72 ** *	0.84 ** *				
Top	0.05 NS	-0.32 NS	-0.52 **	-0.01 NS	0.22 NS	0.07 NS	-0.05 NS	0.02 NS			
Arb	-0.13 NS	-0.53 **	-0.05 NS	-0.48 **	-0.19 NS	-0.58 **	-0.75 ** *	-0.72 ** *	-0.04 NS		
Ves	0.04 NS	-0.60 ** *	-0.43 *	-0.39 *	0.09 NS	-0.39 *	-0.55 **	-0.48 **	0.22 NS	0.76 ** *	
Hyph	-0.04 NS	-0.61 ** *	-0.21 NS	-0.50 **	-0.07 NS	-0.50 **	-0.73 ** *	-0.66 ** *	0.31 NS	0.93 ** *	0.73 ** *

<sup>a</sup> \*, \*\*, \* \* Significant at the 0.05, 0.01, 0.001 levels of probability, respectively.

<sup>b</sup> NS = Not significant.

TABLE V

Pearson correlation coefficients ( $r$ ) among heavy metal concentrations in the leek leaves, dry weight of plant tops, and the colonization of roots with mycorrhizal structures (arbuscule, vesicle and hyphae) across the three locations in plants grown in soils taken from shooting range in Oberuzwil<sup>a</sup>

	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn	Top	Arb	Ves
Cd											
Co	0.47 NS <sup>b</sup>										
Cr	0.58 **	0.91 ***									
Cu	0.82 ***	0.77 ***	0.81 ***								
Mn	0.36 NS	0.12 NS	0.18 NS	0.14 NS							
Ni	0.68 ***	0.77 ***	0.92 ***	0.88 ***	0.17 NS						
Pb	0.82 ***	0.56 ***	0.68 ***	0.91 ***	0.28 NS	0.85 ***					
Zn	0.58 *	0.48 **	0.50 NS	0.63 **	0.01 NS	0.54 *	0.60 **				
Top	-0.82 ***	-0.61 **	-0.70 ***	-0.92 ***	-0.19 NS	-0.80 ***	-0.89 ***	-0.67 ***			
Arb	-0.79 ***	-0.48 NS	-0.61 **	-0.77 ***	-0.39 NS	-0.75 ***	-0.85 ***	-0.47 NS	0.70 ***		
Ves	-0.26 NS	-0.15 NS	-0.16 NS	-0.29 NS	0.03 NS	-0.22 NS	-0.32 NS	-0.33 NS	0.24 NS	0.44 NS	
Hyph	-0.78 ***	-0.49 NS	-0.63 **	-0.76 ***	-0.40 NS	-0.76 ***	0.86 ***	-0.46 NS	0.70 ***	0.89 ***	0.43 NS

<sup>a</sup> \*, \*\*, \*\*\* Significant at the 0.05, 0.01, 0.001 levels of probability, respectively.

<sup>b</sup> NS = Not significant.

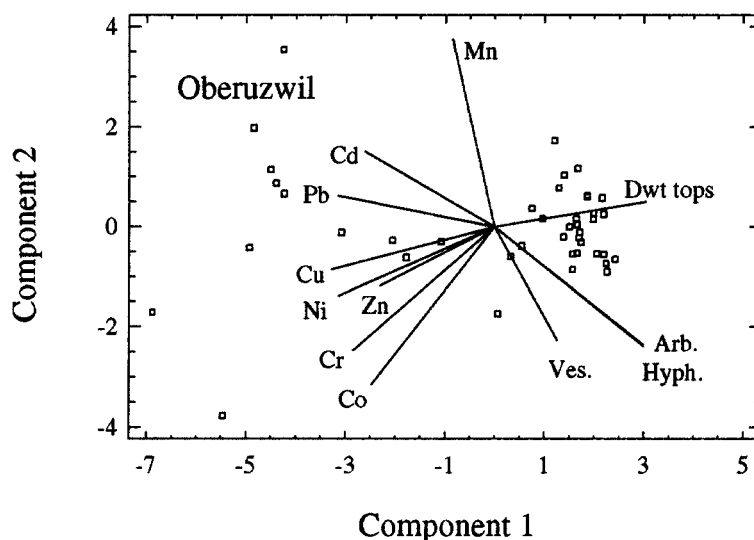


Figure 7. Plot of the occurrence of the variables analysed against the first two axes of the Principal Component Analysis of data from location Oberuzwil.

tamination with metals, and the relatively small effect of high soil pollution on colonization of roots of this plant in location Oberuzwil, may indicate that Plantain and mycorrhizal fungi in these shooting ranges may have also undergone a co-evolution (co-adaptation) process for metal tolerance similar to that reported for *Agrostis capillaris* (Ietswaart *et al.*, 1992). Although we did not measure the metal contents in the leaves of Ribwort plantain sampled for mycorrhizal measurements, investigation by others of the native and cultivated plants growing in the Zuchwil shooting range has shown high concentrations of Pb and Cu in the plants studied (AUKSO, 1995). If by inference we assume that Ribwort plantain sampled by us also contained high concentration of heavy metals, it means that uptake of heavy metals does not affect the degree of mycorrhizal colonization of native plants.

High absorption of several metals measured in leek plants grown in these soils, where, in cases the limit of toxicity tolerance for human nutrition was reached (Table III), indicates that metals measured were in bio-available forms in these soil and thus could be taken up by the plant roots themselves and/or by the hyphae of mycorrhizal fungi. The lower mycorrhizal activity in the heavily polluted BACKSTOP soils, on the one hand, and the high uptake of several elements by the leek plants grown in the BACKSTOP soils point to the ability of roots to absorb these elements alone and without the contribution of mycorrhizal fungi. On the other hand, it could also be assumed that the decreased mycorrhizal activity in the BACKSTOP soil was in fact the contributing factor for the higher uptake of elements by the roots growing in these soils. This interpretation of data is based on the view that mycorrhizal fungi, by accumulating the metals in their structures

(Kaldorf *et al.*, 1999; Brunner and Frey, 2000; Berreck and Haselwandter, 2001), may act as a filter (Marschner *et al.*, 1996, 1998) and thereby reduce the uptake of metals by the plant roots or lower their transport to the above ground parts of plants (Burke *et al.*, 2000).

## 5. Conclusion

This study indicates that despite extremely high total metal content in the soils of shooting ranges and their bio-availability, as measured by their uptake by leek, mycorrhizal fungi have not been eliminated from these soils and can effectively colonize native *Plantago* plants and test plant such as leek. Reduction in spore production in soils of BACKSTOP, however, points to possible changes in the composition of these fungi.

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