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RESEARCH ARTICLE

Concentration of trace elements in arable soil after long-term application of organic and inorganic fertilizers

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Abstract The Ruzyně Fertilizer Experiment (RFE, the Czech Republic) was established on a permanent arable field (illimerized Luvisol) in 1955. The effects of long-term application of several organic fertilizers (dung water, farmyard manure, poultry litter) and mineral N, P and K fertilizers on plant-available (extracted by $CaCl_2$), easily mobilizable (extracted by EDTA), potentially mobilizable (extracted by HNO_3) and total concentrations of trace elements were investigated in 2008. Concentrations of all analyzed trace elements in the applied fertilizers did not exceed the limits permitted by Czech national legislation. Concentrations of As, Cd and Cr were highest in single superphosphate, those of Cu, Mn and Ni were highest in poultry litter and those of Pb and Zn were highest in dung water. Poultry litter had the second highest concentration of As and Zn. Poultry litter supplied the soils with considerable amounts of Cu, Mn and Zn and increased their concentrations in the soil. There was also a significant increase in plant availability of Mn, Ni and Zn and a decrease in soil pH. Although all fertilizers were applied for five decades, total concentrations of As, Cr, Cu, Ni, Pb and Zn in soil remained far below Czech legislation limits. For Cu and Zn this was probably due to the relatively low mean annual application rates of poultry litter. Total Cd concentrations in soil exceeded the legislative limit even in the control (without any fertilizer inputs) and the effect of treatment was not significant. This indicates that fertilizers were not the main source of Cd in the experimental area. Therefore, common cropping practices do not induce soil contamination by trace elements even if they have been applied for more than 50 years.

Keywords Chicken slurry · Long-term field experiment · Heavy metals · Gray-brown soil · Zinc and Copper

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Introduction

The presence of trace elements in fertilizers implies that their long-term application has the potential to induce soil contamination (Németh et al. 2002; Chen et al. 2007; Otero et al. 2005). Many mineral P fertilizers possess considerable amounts of trace elements, especially in those fertilizers that are produced from North African phosphates (Oyedelel et al. 2006; Malak and Emad 2007; Ramadan and Ashkar 2007). In addition to mineral fertilizers and atmospheric deposition (Sucharová and Suchara 2004; Fernandez et al. 2007; Schröder et al. 2008), organic fertilizers may also be significant sources of trace elements in agro-ecosystems.

Animals require metals as a part of their diet, so elements like Cu and Zn are added to feedstuffs as growth promoters or probiotics that prevent diarrhea (Carlson et al. 2004, 2008). Up to 250 mg kg^{-1} of zinc is allowed in a complete feedstuff in the European Union (Directive 70/524/EEC). Depending on the source, Zn bioavailability to livestock is around 20% of total Zn content in feed and the rest is directly excreted, especially in the feces, and thus appears in organic fertilizers (Poulsen and Larsen 1995).

According to recent studies, repeated application of poultry litter (Pederson et al. 2002; Adeli et al. 2007; Schomberg et al. 2008), pig slurry (De la Torre et al. 2000; Novak et al. 2004; Berenguer et al. 2008) or cattle manure (Lipoth and Schoenau 2007; Benke et al. 2008) can also substantially increase the Cu and Zn contents in the upper soil layer.

Although decades-long fertilizer experiments are still being conducted throughout the world (see Shiel 1995; Blair et al. 2006; Körschens 2006; Silvertown et al. 2006; Girma et al. 2007; Hejcman et al. 2007; Honsová et al. 2007; Merbach and Deubel 2008; Kunzová and Hejcman 2009), the effects of decades-long fertilizer application on soil contamination by trace elements has seldom been studied. Gray et al. (1999) reported an increase in Cd content in grassland soil due to decades-long P fertilizer application. In the Woburn Market Garden experiment (UK), soil content of Cd remained high even though application of Cd-contaminated organic fertilizer had been terminated 30 years prior to soil sampling (Abaye et al. 2005). In the Rengen Grassland Experiment (Germany), where basic slag has been applied for 65 years, a substantial increase in As and Cr content in the upper soil layer was reported by Hejcman et al. (2009).

Models and simulations are not a substitute for real data obtained by decades-long experimentation. That is why data obtained from long-term studies are essential for setting legislative limits for concentrations of trace elements in agricultural soils and fertilizers. Knowledge of the total concentrations of trace elements in the soil is not sufficient. Chemical fractionation into plant-available, easily and potentially mobilizable concentrations is also necessary to characterize their behavior (Sinaj et al. 2004; Kashem et al. 2007).

In this study, fractionation of trace elements in the arable layer after 54 years of poultry litter, dung water, farmyard manure and mineral N, P and K fertilizer application were investigated in the Ruzyně Fertilizer Experiment (RFE), which was established on illimerized Luvisol in 1955. The aim of the study was to answer the following questions: (1) Is there any effect of long-term fertilizer applications on plant-available, easily mobilizable, potentially mobilizable and total concentrations of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the arable soil layer? (2) We also considered whether the trace element concentrations could reach a level that might necessitate regulation of long-term fertilizer use. To our knowledge, there is no previous study of such a long-term effect of fertilizer treatments on trace element fractionations of illimerized Luvisols.

Materials and methods

Site description

The RFE was established on a permanent arable field in 1955. The RFE is situated 3 km east of the Prague-Ruzyně international airport on the western edge of Prague, the capital of the Czech Republic. At the study site, the mean annual temperature is 8.2°C (ranging from 6.4 to 9.7°C) and mean annual precipitation is 422 mm (ranging from 255 to 701 mm; Prague-Ruzyně meteorological station, 1955–2007).

According to the FAO and Czech taxonomic soil classification system (Němeček et al. 2001), the soil type was classified as illimerized luvisol (syn. illimerized gray-brown soil or illimerized brown earth). The parent material was loess mixed with highly weathered chalk. The ground water level was 20 m below the field surface. The upper 30 cm (arable layer) contained 27% clay, increasing to 40% clay in the soil layer at 30–40 cm (subsoil) and to 49% clay in the soil layer at 40–50 cm. The organic C content was 1.17% and pH (H_2O) was 6.5 in the top-soil layer (0–20 cm) before establishment of the experiment in 1955.

Experimental design

The RFE is a large-scale experiment consisting of five field strips "144 m × 96 m". Each field strip consists of 24 fertilizer treatments replicated four times and arranged in a complete randomized design (96 individual monitoring plots). Individual plot size is 12 m × 12 m and only the central 5 m × 5 m plot is used for experimental purposes. Analysis of trace element concentrations in the arable layer was performed only in strip number 2. A 9-year crop rotation system (45% cereals, 33% root crops and 22% legumes) was used in field strip 2: alfalfa, alfalfa, winter wheat, sugar beet, spring barley, potatoes, winter wheat, sugar beet and spring barley with alfalfa underseeding.

Organic fertilizers were applied each autumn before planting of beet or potatoes in particular treatments. Calcium ammonium nitrate (27% N, LAV 27), single superphosphate (8.3% P) and potassium chloride (49.8% K) were applied as mineral fertilizers. The most contrasting treatments were selected in this study: unfertilized control, dung water (DW), farmyard manure (FM), poultry litter (PL), mineral N(Np), mineral N, P and K fertilizer application at two rates ($N_1P_1K_1$ and $N_4P_2K_2$), and combinations of organic fertilizers with two rates of mineral N, P and K fertilizers ($DWN_1P_1K_1$, $DWN_4P_2K_2$, $FMN_1P_1K_1$, $FMN_4P_2K_2$, $PLN_1P_1K_1$ and $PLN_4P_2K_2$ treatments).

Mean amounts of N, P and K were applied annually in individual treatments as given in Table 1. Basic soil chemical properties in investigated treatments are given in Table 2. These analyses were performed in 2007 using the Mehlich III methodology to determine plant-available P and K concentrations. Total N content was determined by the Kjehdal method, and organic C content by the NIRS method in an accredited national laboratory.

Soil and fertilizer sampling

Sampling of the arable layer was done in March 2008. The depth of soil sampling was 0–20 cm and five subsamples were mixed into one representative sample per individual monitoring plot. Plant residues were removed immediately and then the samples were air-dried, ground in a mortar, and sieved to 2 mm.

Three samples of each mineral fertilizer were collected in the fertilizer store. To minimize variability

Table 1 Amounts of nutrients supplied annually to the investigated treatments since 1955

Treatments	Amounts of nutrients supplied (kg ha^{-1}) by organic fertilizers			Amounts of nutrients supplied (kg ha^{-1}) by mineral fertilizers			Total amounts of applied nutrients (kg ha^{-1})		
	N	P	K	N	P	K	N	P	K
Control	–	–	–	–	–	–	–	–	–
FM	22	5	53	–	–	–	22	5	53
DW	3	1	3	–	–	–	3	1	3
PL	27	39	118	–	–	–	27	39	118
Np	–	–	–	24	–	–	24	–	–
$N_1P_1K_1$	–	–	–	39	24	109	39	24	109
$N_4P_2K_2$	–	–	–	91	31	146	91	31	146
$DWN_1P_1K_1$	3	1	3	39	24	109	42	25	13
$DWN_4P_2K_2$	3	1	3	91	31	146	94	32	149
$FMN_1P_1K_1$	22	5	53	39	24	109	61	29	162
$FMN_4P_2K_2$	22	5	53	91	31	146	113	36	199
$PLN_1P_1K_1$	27	39	118	39	24	109	66	63	227
$PLN_4P_2K_2$	27	39	118	91	31	146	118	70	264

Abbreviations used in treatment codes: DW—dung water, FM—farmyard manure, PL—poultry litter, N_1 and N_4—application of ammonium nitrate at the rate of 39 and 91 kg N ha^{-1}, P_1 and P_2—application of super phosphate at the rate of 24 and 31 kg P ha^{-1}, K_1 and K_2—application of potassium chloride at the rate of 109 and 146 kg K ha^{-1}

Table 2 Results of basic soil chemical properties analyzed in 2007

Treatments	pH/$CaCl_2$	C org. (%)	N total (%)	P (mg kg^{-1})	K (mg kg^{-1})
Control	6.0	2.6	0.13	17	112
DW	5.9	2.7	0.15	32	191
FM	6.7	3.8	0.16	37	159
PL	6.0	3.3	0.14	85	125
Np	6.3	3.6	0.14	13	114
$N_1P_1K_1$	–	–	–	–	–
$N_4P_2K_2$	–	–	–	–	–
$DWN_1P_1K_1$	5.7	2.9	0.14	26	169
$DWN_4P_2K_2$	5.5	3.4	0.15	79	240
$FMN_1P_1K_1$	6.2	3.5	0.16	83	215
$FMN_4P_2K_2$	5.8	3.9	0.25	97	360
$PLN_1P_1K_1$	5.9	3.3	0.14	135	203
$PLN_4P_2K_2$	5.4	3.4	0.15	135	309

Treatment abbreviations are given in Table 1. Numbers represent the mean values per treatment. Data for $N_1P_1K_1$ and $N_4P_2K_2$ treatments were not available

in chemical composition, each sample of mineral fertilizer was a mixture of four sub-samples taken from four different bags.

Three samples of organic fertilizers were collected directly from farms supplying the fertilizers for this experiment. Each sample was a mixture of four sub-samples taken from different parts of the fertilizer stores. Organic fertilizers were dried at 60°C to total desiccation and the percentage of dry matter (DM) was determined.

Soil and fertilizer analyses

The total concentrations of trace elements in the soils were determined in the digests obtained by the following two-step decomposition procedure. Exactly 0.5 g of a sample was decomposed by dry ashing in an Apion Dry Mode Mineralizer. The ash was then decomposed in a mixture of HNO_3 and HF, evaporated to dryness at 160°C and dissolved in diluted *aqua regia* (Száková et al. 1999). A certified reference material (RM 7001 light sandy soil) was used for the quality assurance of analytical data. Subsequently, 0.5 g soil samples were extracted with a 0.01 mol l^{-1} $CaCl_2$ aqueous solution in a ratio of 1:10 (w/v) for 6 h to determine plant-available portions of the elements in the soil (Novozamsky et al. 1993). To determine easily mobilizable concentrations, additional 0.5 g soil samples were extracted with a 0.05 mol l^{-1} EDTA aqueous solution at pH 7 at a ratio of 1:10 (w/v) for 1 h (Quevauviller et al. 1993). Potentially mobilizable concentrations of elements were determined by extraction of separate 0.5 g soil samples with a 2 mol l^{-1} aqueous solution of HNO_3 at a ratio of 1:10 (w/v) at 20°C for 6 h (Borůvka et al. 1996).

Abbreviations for individual elements in the “Results” section are supplemented by the method of extraction: Ca—for $CaCl_2$, E—for EDTA, N—for HNO_3 and T—for *aqua regia* (total concentration). The reaction mixtures were centrifuged at 3,000 rpm for 10 min and supernatants were kept at 6°C before measurement. Blank extracts representing 5% of the total number of extracts were prepared using the same batch of reagents and the same apparatus analyzed at the same time and in the same way as soil extracts. All reagents used were of electronic grade purity (Analytika, Ltd, Czech Republic).

Two 2-g samples of each fertilizer were dissolved in 10 ml of *aqua regia* for 30 min at 110°C in 50 ml Teflon beakers. The digests were transferred to glass test tubes and diluted with deionized water to 25 ml. The elements in the solutions were determined as described below.

Determination of trace elements

The trace elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in soil digests and extracts were determined by optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration (Varian, VistaPro, equipped with an autosampler SPS-5, Australia). Calibration solutions were prepared in corresponding extraction agents as follows: 5–50 μg l^{-1} for Cd, 50–500 μg l^{-1} for As, Cr, Cu, and Ni, and 1–10 mg l^{-1} for Fe, Mn, Pb and Zn.

Operating measurement wavelengths for ICP-AES were 189.0 nm for As, 214.4 nm for Cd, 324.7 nm for Cu, 238.2 nm for Fe, 205.6 nm for Cr, 259.4 nm for Mn, 231.6 nm for Ni, 220.4 nm for Pb and 206.2 nm for Zn. Measurement conditions were as follows: power 1.2 kW, plasma flow 15.0 l min^{-1}, auxillary flow 0.75 l min^{-1}, nebulizer flow 0.9 l min^{-1}.

Low concentrations of As in 0.01 mol l^{-1} $CaCl_2$, and 0.05 mol l^{-1} EDTA extracts were determined by a continual hydride generation technique (HGAAS) using a Varian AA280Z (Varian, Australia) atomic absorption spectrometer equipped with a hydride generator VGA-77. A mixture of KI and ascorbic acid was used for prereduction of the sample and the extract was acidified with HCl before measurement. Two measurements were taken for each sample.

Data analysis

A redundancy analysis (RDA) in the CANOCO 4.5 program (ter Braak and Šmilauer 2002) was used to evaluate multivariate data. The RDA was used because the datasets were sufficiently homogeneous and environmental variables (e.g., treatments) were in the form of categorical predictors. A Monte Carlo permutation test with 999 permutations was used to reveal whether the tested explanatory variables (environmental variables in the CANOCO terminology) had a significant effect on the plant-available, easily mobilizable, potentially mobilizable and total concentrations of trace elements. Results of the multivariate analysis were visualized in the form of a bi-plot ordination diagram created by CanoDraw© software. The percentage of the explained variability in soil data induced by fertilizer treatments was used as a measure of explanatory power.

All univariate analyses were performed using STATISTICA 5.0 software (StatSoft© 1995). When RDA analysis demonstrated significance, a one-way ANOVA followed by post-hoc comparison using Tukey's test was applied to identify significant differences between treatments for individual elements. A regression analysis was used to evaluate the relationship between the amount of the applied trace elements in fertilizers and their concentrations in soil samples.

Results

The mean concentrations of trace elements in the fertilizers applied are given in Table 3. Concentrations of As, Cd and Cr were the highest in single superphosphate. Concentrations of Cu, Mn and Ni were the highest in poultry litter and concentrations of Pb and Zn were the highest in dung water. Poultry litter contained the second highest concentration of As and Zn.

The amounts of trace elements applied annually to the investigated treatments are given in Table 4; approximately the same amounts have been used since 1955. The highest application rates for all trace elements, Cu and Zn especially, were recorded in the $PLN_4P_2K_2$ treatment followed by all treatments involving poultry litter application.

According to the RDA, the effect of treatments on the concentrations of trace elements in the soil was significant ($F = 2$; $P = 0.001$) and explained 31% variability of the data. The ordination diagram (Fig. 1) clearly shows the effects of individual fertilizer treatments on concentrations of all elements. According to the first ordination axis, the treatments can be generally divided into two groups, with increased concentrations of elements (on the right side of the ordination diagram) and those more similar to the control treatment (on the left side of the ordination diagram). With the exception of AsCa, CuCa, NiCa and PbCa, the concentrations of all elements were higher in treatments on the right side of the diagram, because arrows for these elements point to this part of the diagram.

Poultry litter applications had a highly positive effect on the concentrations of ZnCa, ZnE, ZnN and ZnT according to the long arrows for these elements pointing towards the PL, $PLN_1P_1K_1$ and $PLN_4P_2K_2$ treatments. In addition, there was a positive effect of poultry litter application on concentrations of CuN, CuT, MnN, MnT and NiN, although the effect was not as strong as in the case of ZnN. Highly positive effects of the $DWN_4P_2K_2$, $FMN_4P_2K_2$, $PLN_1P_1K_1$

Table 3 Concentration of trace elements in applied fertilizers and dry matter (DM) content in organic fertilizers

Treatment	DM (%)	As (mg kg^{-1} in DM)	Cd (mg kg^{-1} in DM)	Cr (mg kg^{-1} in DM)	Cu (mg kg^{-1} in DM)	Mn (mg kg^{-1} in DM)	Ni (mg kg^{-1} in DM)	Pb (mg kg^{-1} in DM)	Zn (mg kg^{-1} in DM)
DW	2.7	0.50	0.23	7.76	19.84	60.03	4.47	5.34	340.84
FM	18.6	0.31	0.21	7.42	19.87	124.80	3.05	1.06	112.06
PL	21.4	1.02	0.15	7.06	36.03	243.25	6.88	0.62	268.88
Ammonium nitrate	–	0.35	0.02	0.22	1.46	44.27	0.71	0.54	5.84
Super phosphate	–	1.62	1.37	40.44	7.82	2.99	6.01	0.73	105.69
Potassium chloride	–	0.09	0.02	0.02	0.49	1.72	0.16	0.63	4.44

Abbreviations of organic fertilizers: PL—poultry litter, DW—dung water, FM—farmyard manure. All fertilizers of the same chemical composition were used for at least the last 5 years

and $PLN_4P_2K_2$ treatments on MnCa concentration were recorded. Similar results were detected even for AsN, NiCa, CdN, CrE, NiE, CdE, CdCa, CrN and PbE, although the effects of these treatments were not as strong as for MnCa.

Average concentrations of individual elements in the arable layer are given in Table 5 (As, Cd, Cr and Cu) and Table 6 (Mn, Ni, Pb and Zn). Calculated individually by one-way ANOVA, significant effects were recorded on concentrations of AsN, CdN, CrN, CrT, CuN, CuT, MnCa, MnN, NiCa, NiN, ZnCa, ZnE, ZnN and ZnT.

The concentration of AsN was significantly correlated with the amount of As applied in all fertilizers (Table 7). Similar results were recorded for CdE, CdN, CrE, CuN, CuT, MnN, MnT, ZnCa, ZnE, ZnN and ZnT.

Discussion

Concentrations of trace elements in mineral fertilizers did not exceed the limits permitted by Czech National legislation. These limits are 10, 50 and 10 mg of As, Cr and Pb kg^{-1} of mineral N or K fertilizers, respectively (Budňáková et al. 2004). The concentration of Cd in superphosphate (16 mg Cd kg^{-1} of P) was below the Czech Cd limit for mineral P fertilizers (22 mg Cd kg^{-1} of P). Similarly, the concentration of Cr in superphosphate was below the permitted limit in fertilizer (150 mg of Cr kg^{-1} of P). The highest concentration of Cd in superphosphate from all applied fertilizers is consistent with previously published results, showing that P fertilizers are the major source of Cd in agroecosystems (Németh et al. 2002; Otero et al. 2005; Cesur and Kartal 2007; Chen et al. 2007). Concentrations of trace elements in organic fertilizers also did not exceed Czech limits, which are 10, 2, 100, 100, 50, 100 and 400 mg of As, Cd, Cr, Cu, Ni, Pb and Zn kg^{-1} of dry matter, respectively (Budňáková et al. 2004).

Although concentrations of all elements were within the permitted limits, the most significant increases followed application of poultry litter, which supplied a considerable amount of Cu, Mn and Zn. High concentrations of Cu and Zn in poultry litter and their increased concentration in the upper soil layers are consistent with previous studies (Pederson et al. 2002; Adeli et al. 2007; Schomberg et al. 2008). Although all fertilizers were applied for five decades,

Table 4 Doses of trace elements supplied annually to the investigated treatments

Treatment	As (g ha^{-1})	Cd (g ha^{-1})	Cr (g ha^{-1})	Cu (g ha^{-1})	Mn (g ha^{-1})	Ni (g ha^{-1})	Pb (g ha^{-1})	Zn (g ha^{-1})
Control	–	–	–	–	–	–	–	–
DW	0.2	0.1	2.6	6.6	19.9	1.5	1.8	113.1
FM	0.4	0.2	8.7	23.4	147.0	3.6	1.3	132.0
PL	2.9	0.4	20.4	104.3	703.9	19.9	1.8	778.1
Np	0.0	0.0	0.0	0.1	3.9	0.1	0.0	0.5
$N_1P_1K_1$	0.5	0.4	11.8	2.6	7.6	1.9	0.4	32.4
$N_4P_2K_2$	0.7	0.5	15.2	3.6	16.5	2.5	0.6	42.8
$DWN_1P_1K_1$	3.5	0.8	32.2	106.9	711.6	21.8	2.2	810.5
$DWN_4P_2K_2$	3.7	1.0	35.6	107.8	720.5	22.4	2.4	820.9
$FMN_1P_1K_1$	0.7	0.5	14.3	9.2	27.5	3.4	2.2	145.5
$FMN_4P_2K_2$	0.9	0.6	17.8	10.1	36.5	4.0	2.4	155.9
$PLN_1P_1K_1$	0.9	0.7	20.5	26.0	154.6	5.5	1.7	164.4
$PLN_4P_2K_2$	1.1	0.8	24.0	27.0	163.5	6.1	1.9	174.8

Treatment abbreviations are given in Table 1

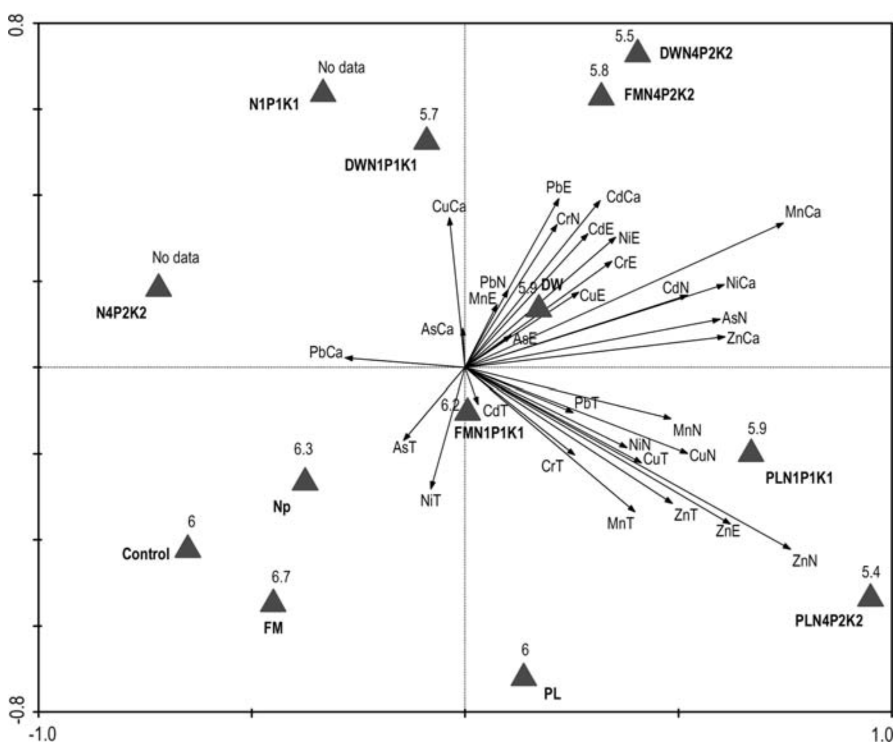

Fig. 1 Ordination diagram showing the result of RDA analysis of trace element concentrations in the arable layer of soil. Treatment abbreviations are given in Table 1. Abbreviations of trace elements are supplemented by method of extraction: Ca—$CaCl_2$ (plant-available), E—EDTA (easily mobilizable), N—HNO_3 (potentially mobilizable) and T—total concentration. Numbers above triangles indicate pH values in particular treatments. No data—pH value was not available

the total concentrations of As, Cr, Cu, Ni, Pb and Zn in the soil remained far below the Czech legislative limits. These total limits are 24, 105, 70, 59, 71 and 141 mg kg^{-1} for As, Cr, Cu, Ni, Pb and Zn, respectively (Anonymous 2001). The critical soil Cu and Zn concentrations were not achieved, probably due to the relatively low mean annual application rates of poultry litter. In the RFE, organic fertilizers were applied only in two of the 9 years of each crop cycle. This contrasted with soil contamination by Zn reported by Schomberg et al. (2008) in a study using a long-term annual application of poultry litter.

Table 5 Average concentrations of As, Cd, Cr, and Cu (mg kg^{-1}) in the arable soil layer extracted by different chemical reagents

Element	Extract	Treatment												
		Control	DW	FM	PL	Np	$N_1P_1K_1$	$N_4P_2K_2$	$DWN_1P_1K_1$	$DWN_4P_2K_2$	$FMN_1P_1K_1$	$FMN_4P_2K_2$	$PLN_1P_1K_1$	$PLN_4P_2K_2$
As	$CaCl_2$ n.s.	0.25	0.24	0.30	0.20	0.31	0.31	0.10	0.23	0.28	0.18	0.28	0.19	0.25
	EDTA n.s.	0.57	0.61	0.55	0.64	0.64	0.69	0.64	0.51	0.70	0.73	0.63	0.56	0.78
	HNO_3	1.98a	2.45ab	2.00ab	2.29ab	2.18ba	2.24ab	2.14ba	2.27ab	2.35ab	2.52ab	2.58ab	3.71a	2.44ab
	Total n.s.	13	11	11	11	15	11	10	12	10	14	12	11	12
Cd	$CaCl_2$ n.s.	<0.01	0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.01	0.01	<0.01	0.01	0.01	0.01
	EDTA n.s.	0.06	0.08	0.06	0.07	0.08	0.08	0.08	0.07	0.09	0.08	0.09	0.08	0.09
	HNO_3	0.16ab	0.17ab	0.16ab	0.16ab	0.17ab	0.17ab	0.15a	0.17ab	0.18ab	0.17ab	0.18b	0.18b	0.17ab
	Total n.s.	0.69	0.70	0.68	0.69	0.71	0.62	0.70	0.67	0.68	0.70	0.72	0.66	0.71
Cr	$CaCl_2$ n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	EDTA n.s.	0.02	0.04	0.02	0.03	0.05	0.04	0.04	0.03	0.06	0.04	0.05	0.04	0.07
	HNO_3	4.11ab	5.44c	4.02ab	4.10ab	3.78a	4.08ab	3.20a	5.85c	5.28bc	4.05ab	3.94ab	3.97ab	4.15ab
	Total	59ab	61ab	59ab	58ab	62ab	52a	59ab	60ab	60ab	60ab	59ab	67b	59ab
Cu	$CaCl_2$ n.s.	0.16	0.20	0.17	0.16	0.19	0.17	0.20	0.23	0.18	0.18	0.26	0.16	0.17
	EDTA n.s.	3.74	4.58	3.69	4.24	4.16	4.83	4.50	3.96	4.66	4.53	4.52	4.51	5.23
	HNO_3	8.88ab	8.95ab	9.27ab	9.69ab	8.84ab	9.21ab	7.56a	9.15ab	9.38ab	8.93ab	9.06ab	10.62b	9.85b
	Total	14ab	15ab	15ab	15ab	15ab	14a	14ab	15ab	15ab	15ab	15ab	16b	15ab

$CaCl_2$ (plant-available), EDTA (easily mobilizable), HNO_3 (potentially mobilizable) and total concentration

n.s.—indicates non significant effect of treatment on concentration of element in one-way ANOVA. Using Tukey post-hoc test, treatments with the same letter are not significantly different. Treatment abbreviations are given in Table 1

Table 6 Average concentrations of Mn, Ni, Pb and Zn (mg kg^{-1}) in arable layer extracted by different chemical reagents

Element	Extract	Treatment												
		Control	DW	FM	PL	Np	$N_1P_1K_1$	$N_4P_2K_2$	$DWN_1P_1K_1$	$DWN_4P_2K_2$	$FMN_1P_1K_1$	$FMN_4P_2K_2$	$PLN_1P_1K_1$	$PLN_4P_2K_2$
Mn	$CaCl_2$	0.57ab	1.29abc	0.67ab	0.94abc	0.54a	1.3abc	0.43a	1.72bcd	2.62d	1.32abc	1.94 cd	1.72bcd	2.62d
	EDTA n.s.	136	164	149	155	145	159	161	149	155	160	150	166	142
	HNO_3	302ab	319ab	315ab	322ab	298ab	310ab	270a	303ab	320ab	315ab	316ab	332b	329ab
	Total n.s.	486	522	484	521	486	464	482	479	481	503	491	522	518
Ni	$CaCl_2$	0.08ab	0.11ab	0.08ab	0.11ab	0.09ab	0.10ab	0.06a	0.12ab	0.16ab	0.09ab	0.16ab	0.13ab	0.17b
	EDTA n.s.	2.03	2.59	2.19	2.38	2.54	2.60	2.61	2.37	2.73	2.57	2.60	2.86	2.51
	HNO_3	5.70ab	5.80ab	5.81ab	5.82ab	5.60ab	5.72ab	4.91a	5.54ab	5.71ab	5.73ab	5.73ab	5.85ab	5.98a
	Total n.s.	21	22	25	21	23	19	21	21	20	21	21	21	22
Pb	$CaCl_2$ n.s.	0.09	0.06	0.05	0.07	0.05	0.09	0.05	0.07	0.05	0.07	0.06	0.04	0.05
	EDTA n.s.	5.34	6.41	5.67	5.98	5.78	7.15	6.57	6.12	7.05	6.48	7.04	6.79	6.23
	HNO_3 n.s.	14	15	14	15	14	16	13	21	15	14	15	15	15
	Total n.s.	24	25	25	25	24	24	25	25	25	25	25	26	27
Zn	$CaCl_2$	0.06ab	0.09a	0.03a	0.06a	0.01a	0.03a	0.02a	0.12ab	0.12ab	0.08ab	0.13ab	0.12ab	0.20b
	EDTA	1.50a	2.15abc	1.75a	3.16 cd	1.68a	1.85a	2.07a	1.61a	1.85a	2.33abc	2.07ab	3.52d	2.99bcd
	HNO_3	13ab	14bc	14ab	18 cd	13ab	13ab	11a	13ab	14b	13ab	14b	18 cd	19d
	Total	53ab	55ab	54ab	58ab	57ab	49a	54ab	55ab	56ab	56ab	55ab	60b	62b

$CaCl_2$ (plant-available), EDTA (easily mobilizable), HNO_3 (potentially mobilizable) and total concentration

n.s.—indicates non significant effect of treatment on concentration of element in one-way ANOVA. Using Tukey post-hoc test, treatments with the same letter are not significantly different. Treatment abbreviations are given in Table 1

Table 7 Results of regression analyses of trace element concentrations in the soil as a function of elements applied in fertilizers

Dep. Variable	Indep. Variable	*R*	*P*-value
As	AsCa ($CaCl_2$)	0.17	0.211
	AsE (EDTA)	0.04	0.755
	AsN (HNO_3)	0.22	**0.001**
	AsT (Total)	0.20	0.17
Cd	CdCa ($CaCl_2$)	0.23	0.101
	CdE (EDTA)	0.33	**0.016**
	CdN (HNO_3)	0.32	**0.019**
	CdT (Total)	0.04	0.77
Cr	CrCa ($CaCl_2$)	–	–
	CrE (EDTA)	0.28	**0.044**
	CrN (HNO_3)	0.12	0.366
	CrT (Total)	0.15	0.270
Cu	CuCa ($CaCl_2$)	0.20	0.150
	CuE (EDTA)	0.15	0.291
	CuN (HNO_3)	0.46	**<0.001**
	CuT (Total)	0.34	**0.013**
Mn	MnCa ($CaCl_2$)	0.30	**0.029**
	MnE (EDTA)	0.03	0.798
	MnN (HNO_3)	0.37	**0.007**
	MnT (Total)	0.47	**<0.001**
Ni	NiCa ($CaCl_2$)	0.35	**0.009**
	NiE (EDTA)	0.20	0.143
	NiN (HNO_3)	0.27	**0.049**
	NiT (Total)	0.06	0.641
Pb	PbCa ($CaCl_2$)	0.25	0.061
	PbE (EDTA)	0.20	0.147
	PbN (HNO_3)	0.19	0.106
	PbT (Total)	0.23	0.093
Zn	ZnCa ($CaCl_2$)	0.42	**0.002**
	ZnE (EDTA)	0.81	**<0.001**
	ZnN (HNO_3)	0.86	**<0.001**
	ZnT (Total)	0.52	**<0.001**

Abbreviations: Indep. var.—Independent variable: amount of trace elements applied by fertilizers (As, Cd, Cr, Cu,Fe, Mn, Ni, Pb, Zn); Depen. var.—Dependent variable: concentration of trace elements in the 0–20 cm soil layer extracted by $CaCl_2$ (plant-available), EDTA (easily mobilizable), HNO_3 (potentially mobilizable) and total concentration. Significant results are in bold

Total Cd concentration exceeded the legislative limit (0.59 mg kg^{-1}), even in the control treatment without any fertilizer input for at least the last 55 years. This indicates that fertilizers were not the main source of Cd contamination of soils in the experimental area. Atmospheric pollution was probably the main source of Cd because the experimental area is in close proximity to an industrial zone, the airport and city center where areal deposition of Cd can be substantially increased (Sucharová and Suchara 2004). Another explanation is that the contamination of the experimental area by Cd may have occurred before the start of the experiment, because Cd can persist in the ecosystem for a very long time (Abaye et al. 2005). Increased concentrations of total Cd in the control may also be of natural origin, as reported in the Rengen Grassland Experiment (Hejcman et al. 2009).

Although the concentrations of trace elements in applied fertilizers were determined only in the last year of the experiment, comparable rates of trace elements were probably used in other years as well. Treatments applied in the RFE represent a range of common cropping practices used in the Czech Republic. Therefore the main message of this paper is relatively optimistic, indicating that common cropping practices do not induce soil contamination by trace elements even if they are applied for more than 50 years. This conclusion is in accordance with a simulation model for As, constructed by Chen et al. (2007). However, it contradicts the simulation model for Cd, which predicted an increase in total soil Cd due to long-term fertilizer application. In the RFE, there was no effect of treatment on accumulation of total Cd in the soil. Only a slightly significant increase in the potentially mobilizable fraction occurred in those treatments with a combination of organic and mineral fertilizers. This was because the single superphosphate used for the last 50 years was manufactured from Kola volcanic rock phosphates of low Cd concentration.

Long-term fertilizer management probably affected reactivity in the soil. The concentrations of potentially mobilizable As, Cr, Cu, Mn and Ni were moderately, but significantly increased in those treatments with a combination of organic fertilizers (poultry litter especially) and mineral fertilizers. The ordination diagram clearly shows the positive effects of combined mineral and organic fertilizer applications on the concentrations of many forms of trace elements in the soil from plant-available to total concentrations. However, in many cases the increase was not significant and was only negligible. There was a significant increase in plant-availability of Mn, Ni and Zn according to application rates of fertilizer and soil pH. The soil pH decreased in treatments with high application rates of nutrients from the left bottom corner to the right upper corner of the ordination diagram (Fig. 1). Plant-availability of Cd, Mn, Ni and Zn increases with a decrease in soil pH and cannot be related to the total concentrations of elements (Gavi et al. 1997; Mench 1998; Tlustoš et al. 2006; Hejcman et al. 2009).

Conclusion

Concentrations of all analyzed trace elements in applied fertilizers did not exceed the limits permitted by Czech national legislation. So, the main message of this paper is that in the Czech Republic, normal cropping practices do not induce soil contamination by trace elements, even if they are applied for more than 50 years.

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