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Seventy-five years of fertiliser application: insights from the Steinach grassland experiment

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Abstract

Purpose Soil contamination by potentially toxic elements, especially heavy metals, poses risks to food security and human health. The long-term use of mineral and organic fertilisers may lead to the accumulation and mobilisation of such elements. This study investigates how different fertilisation regimes influence the total content, mobility, and bioaccessibility of risk elements in soils of a historical grassland trial.

Methods The research was conducted within the Steinach Grassland Experiment (SGE), initiated in 1933 on an alluvial meadow featuring Alopecurus pratensis in Southeast Germany, which is one of the longest-running grassland fertiliser experiments in Continental Europe. Soil samples were collected from 75 plots treated with various nitrogen (N), phosphorus (P), and potassium (K) fertilisers. Element concentrations were analysed using four extraction methods: aqua regia (total), $CaCl_2$ (plant-accessible), EDTA (easily mobilisable), and diluted HNO_3 (potentially mobilisable).

Results Phosphorus fertilisation led to a slight increase in Fe, Zn, Mn, As, Cr, and Cd concentrations. The modest enrichment of arsenic and chromium was likely related to the historical application of Thomas slag. Despite decades of fertiliser application, concentrations of risk elements did not exceed national regulatory thresholds.

Conclusion Long-term fertilisation at the SGE site has not resulted in excessive accumulation of potentially toxic elements beyond regulatory limits established for agricultural soils. Nevertheless, elements such as As, Cd, and Cr require regular monitoring to prevent their potential accumulation beyond safe thresholds.

Keywords Arsenic · Cadmium · Fertilisation with N, P, k · Mobility · Accumulation

1 1 Introduction

Soil contamination with trace elements such as Fe, Zn, Cu, Mn, As, Cr, Ni, Pb, and Cd has become a significant global environmental concern (Molina et al. 2009; Huang et al. 2015; Khan et al. 2021). These elements pose serious threats to crop production, food safety, and human health due to their toxicity, bioaccumulation potential, and long-term persistence in the environment (Lavado et al. 2007; Wang et al. 2019; Guo et al. 2021). Soil degradation resulting from such contamination has far-reaching impacts, including damage to natural ecosystems, alterations in climate dynamics, and economic losses (Tóth et al. 2016; Palansooriya et al. 2020).

Trace elements occur naturally in soils (Bradl 2005, Jungová et al. 2022), where, at appropriate levels, they serve as essential micronutrients for plants and animals. However, elevated concentrations, especially of elements like As and Cd, pose risks to the food chain and are highly toxic to humans and animals. Zn, Cu, and Ni are also known for their phytotoxic effects (Tóth et al. 2016; Wang et al. 2021). While these elevated levels may stem from specific geological or pedogenic conditions (Kabata-Pendias and Pendias 2001; Pavlović et al. 2021; Jungová et al. 2022), contamination is often linked to anthropogenic sources. Following

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the approach adopted in previous long-term grassland studies (Hejcman et al. 2009; Uprety et al. 2009; Jungová et al. 2022), this study jointly evaluates microelements (Cu, Fe, Mn, Ni, and Zn) and risk elements (As, Cd, Cr, Pb). Although these groups differ in biological function, they exhibit comparable geochemical behaviour in fertilised soils, and several (e.g., Zn, Cu) may shift from essential to potentially toxic concentrations depending on soil pH, organic matter, and redox conditions.

Key contributors to soil contamination include mining, industrial emissions, waste disposal, and airborne deposition. However, in agroecosystems, a major pathway is the long-term application of both mineral and organic fertilisers (Sucharová and Suchara 2004; Roberts 2014; Kidd et al. 2017; Wang et al. 2021). Many phosphate (P) fertilisers derived from North African phosphorites contain trace elements such as Cd, As, Cr, and Pb (Oyedelel et al. 2006; Bergen et al. 2022; Yu et al. 2022), while others—originating as by-products from leather or coke industries—may exhibit particularly high Cr contents (Ciavatta et al. 2012; Duan et al. 2024). Organic amendments such as slurry and manure can also introduce elevated levels of trace elements (e.g., Cu, Zn), mainly due to veterinary pharmaceuticals and feed additives used in livestock production (Carlson et al. 2004, 2008; Berenguer et al. 2008; Uprety et al. 2009).

Although fertilisation is essential for sustaining soil fertility and crop yields, the long-term application of such amendments can lead to the gradual accumulation of risk elements in soils (Ahlberg et al. 2006; Komárek et al. 2008; Ray and Das 2023). Their mobility and plant availability depend not only on their total concentration but also on soil biogeochemical properties such as pH, redox potential, organic matter content, and clay mineralogy (Sungur et al. 2021). Under natural conditions, only a small fraction of trace elements is usually bioavailable (Kabata-Pendias and Pendias 2001; Jungová et al. 2022). Yet in soils developed from metal-rich parent materials, available forms may account for up to 60% of the total metal content (Karczewska et al. 1998; Shakoor et al. 2015). Moreover, even naturally occurring contamination can pose significant health risks, as evidenced by arsenic poisoning from groundwater in alluvial aquifers of the Ganges River (Nickson et al. 1998; Palansooriya et al. 2020).

To better understand how long-term fertilisation practices influence soil chemistry and the accumulation of trace elements, researchers have turned to historical field experiments. Decades-long grassland fertilisation experiments provide valuable insights into the interaction between fertilisation regimes, soil chemical properties, plant species composition, productivity, and forage quality (Silvertown et al. 2006; Semelová et al. 2008; Hejcman et al. 2009; Uprety et al. 2009; Pierik et al. 2011; Pavlů et al. 2012; Titěra et al. 2020). To date, only six experiments have been ongoing for at least 40 years, generating extensive research outputs. Among them, the Park Grass Experiment, established in 1856 on *Lolium perenne* grassland, is particularly notable (Crawley et al. 2005; Silvertown et al. 2006). Each of these long-term experiments is unique, covering a wide range of environmental conditions, including climate and soil management, as well as variations in fertilisation regimes and defoliation frequency, which influence productivity and forage quality (Hrevušová et al. 2009).

The Steinach Grassland Experiment (SGE), located in Steinach near Straubing, Bavaria, is one of the oldest ongoing grassland fertilisation trials in Germany. Originally established in 1933, it has been expanded several times, most notably in the early 1970 s, and remains a valuable long-term study on the effects of fertilisation on soil chemistry and plant composition (Rieder 1989; Diepolder and Schröpel 2003).

Historically, Steinach holds significance in the development of grassland management in Germany. After World War I, leading scientists and agricultural experts sought to improve pasture productivity, leading to the formation of the Bavarian Grassland Association in Steinach (Rieder 1989). This initiative contributed to the broader German Grassland Society, which was instrumental in advancing grassland research and management practices (Tallowin and Jefferson 1999; Hejcman et al. 2013, 2014).

The SGE was initially designed with five fertilisation treatments, each covering 300 m², but over time, the number of treatments increased, leading to 22 fertilisation variants by 1971. These include both balanced fertilisation strategies and nutrient-deficiency treatments. In 1986, an additional liming treatment was applied to certain plots to investigate its long-term effects on soil pH and nutrient availability (Rieder 1989; Diepolder et al. 2005).

The experiment is located on an alluvial meadow within the *Alopecurus pratensis - Deschampsion caespitosae* alliance (Botta-Dukát et al. 2005). This vegetation type is found in river floodplains, influenced by periodic spring flooding and summer drought, and is characterised by species with a broad moisture tolerance that regenerate well after disturbance. Tall grasses, such as meadow foxtail (*Alopecurus pratensis*) and tufted hairgrass (*Deschampsia caespitosa*), typically dominate. The *Deschampsion caespitosae* association has been extensively documented in Central Europe (Botta-Dukát et al. 2005).

Traditional floodplain meadows were integral to local farming systems, with agricultural advancements primarily involving increased fertiliser application and higher cutting frequencies (Isselstein et al. 2005; Hejcman et al. 2013). Due to its unique history and long duration, the SGE serves as an important reference for studying the impact of

fertilisation on soil properties, trace element accumulation, and grassland productivity. This study builds upon previous research, with the aim of evaluating how decades of different fertilisation regimes have affected the distribution and mobility of risk elements in the soil.

The SGE comprises 75 plots subjected fertilisation treatments, including both mineral and organic fertilisers differing in composition and quantity. This experiment is particularly valuable due to its 75-year duration, offering critical baseline data for future research on element uptake by vascular plants, soil chemistry, and risk element accumulation.

Given the global awareness of potential soil contamination resulting from fertiliser use (Bloem et al. 2017), this study aimed to evaluate whether 75 years of experimental fertilisation led to the accumulation of risk elements in the soil. Unfortunately, despite the long-term application of various fertiliser types in this experiment, their precise composition was never systematically recorded, making it impossible to retrospectively determine their exact content.

Unfortunately, despite the long-term application of various fertiliser types in this experiment, their precise composition was never systematically recorded, making it impossible to retrospectively determine their exact content. Nevertheless, the possible presence of certain elements can be inferred based on the known composition of commonly used fertilisers. The presence of trace elements in fertilisers suggests that long-term application may contribute to soil contamination (Németh et al. 2002). However, unlike more recent long-term trials, the SGE lacks the extensive replication of treatments commonly seen in comparable fertilisation experiments initiated before the 1940s. Nonetheless, its distinctive contribution lies in the broad range of fertilisation treatments (46 in total), which create significant variability in soil chemistry, plant composition, and herbage production (Hejcman et al. 2014). A comparable diversity of treatments is observed only in the Park Grass Experiment (Silvertown et al. 2006).

During a phytocoenological survey focused on assessing the effects of fertilisation on plant communities, Hejcman et al. (2014) also collected soil samples for chemical analyses. Although the original study did not process these samples in detail, four types of chemical extractions were subsequently carried out on them. These extraction data, which remained unpublished, were used in the present study. Here, we evaluated and interpreted them in the context of long-term fertilisation effects on soil chemistry and trace element mobility, thus filling an important knowledge gap and providing new insights into element dynamics in fertilised grassland soils.

Accordingly, the main objective of this study is to assess the long-term effects of varied fertilisation regimes on soil chemical properties, with special emphasis on the accumulation and mobility of risk elements. The study specifically addresses the following research questions:

1. How does long-term application of N, P, and K fertilisers influence soil contamination with risk elements?

   i. Plant-accessible fractions
   ii. Easily mobilisable fractions
   iii. Potentially mobilisable fractions
   iv. Total concentrations of Fe, Zn, Cu, Mn, As, Cr, Ni, Pb, and Cd in the topsoil (0–100 mm)?

2 Materials and methods

2.1 Study site

The Steinach grassland experiment (SGE), initiated in 1933, is situated on an alluvial meadow near the village of Steinach (Straubing-Bogen, Bavaria, Germany, 48°57'N, 12°36'E. The study site is positioned at an altitude of 344 m above sea level, with an average annual precipitation of 840 mm, and maintains a mean annual temperature of 7.3 °C (as recorded at the Steinach meteorological station). According to the German soil classification system (KA5, Ad-hoc-AG Boden, 2005), the soil is classified as pseudogley, and as of 2008, the pH (H_2O) measured 5.4 in the upper 100 mm layer of the control plot without fertiliser application and liming. The underground water table depth fluctuated between 1 and 2 m. Following the phytosociological nomenclature by Botta-Dukát et al. (2005), the vegetation at the experimental meadow aligns with the classification of *Alopecurion pratensis* (syn. *Deschampsion cespitosae*) as reported by Hejcman et al. (2014).

2.2 Experimental design

In 1933, five fertiliser treatments plus control (Hejcman et al. 2014; Table S1 in the Supplementary Material) were established without any replication on plots 300 m^2 in size (Rieder 1989; Hejcman et al. 2014). Subsequently, the experimental plots were divided, thereby increasing the number of fertiliser treatments. The first division was conducted in 1954, with 10 fertiliser treatments, the second in 1965, with 20 treatments, and the last in 1986, with 46 treatments. In 1971, several treatments underwent modifications to the fertiliser regime, including an increase in N application rates. The evolution of the fertiliser regimes in each plot is presented in Table S1 (Hejcman et al. 2014).

Due to the treatment reorganization, the SGE currently comprises three adjacent strips, subject to regular triannual mowing (Fig. 1). Each strip is subdivided into 25 plots, with

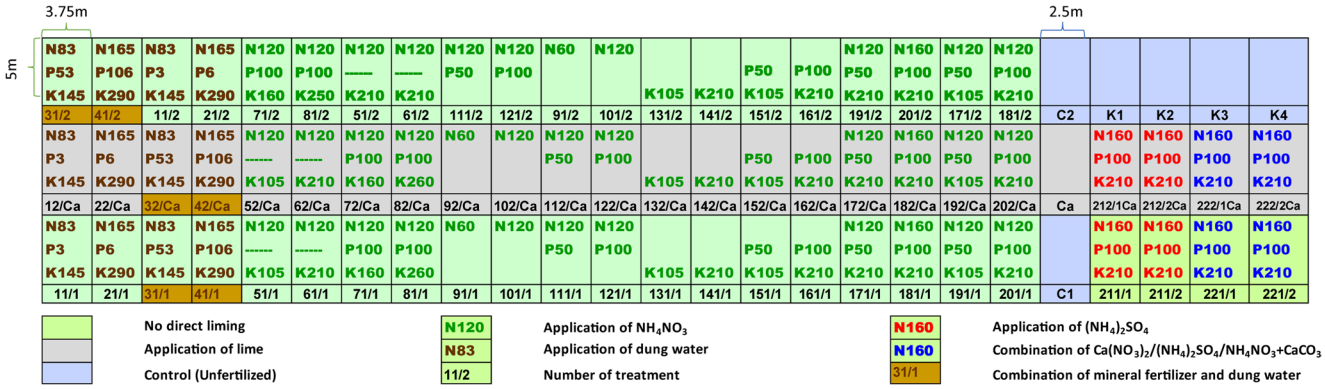


Fig. 1 The spatial organisation of treatments in the SGE is presented in the form of annual application rates of N, P, and K (kg ha^{-1}). Original markings for monitoring plots are represented by black numbers and letters at the bottom of each plot. Plots that have been limed and fertilised with N, P, and K fertilisers are highlighted in a light green shade. Grey indicates plots that were directly limed in 1986, in conjunction with the application of N, P, and K fertilisers. Control plots without fertiliser input are represented in a more prominent blue colour. Dung water application is denoted by brown letters and numbers, while mineral N, P, and K fertiliser application is indicated by green letters. The application of mineral nitrogen in the form of $(NH_4)_2SO_4$ is represented in red, while the application of nitrogen as a combination of three different mineral fertilisers is highlighted in blue

a standard individual plot area of 18.75 m^2 (3.75 × 5 m) in 60 plots. The remaining 15 plots have an area of 12.5 m^2 (2.5 × 5 m) (Fig. 1).

Between 1985 and 2001, aboveground biomass production in the SGE ranged from 4.1 t ha^{-1} in the treatment receiving 174 kg K ha^{-1} to 10.8 t ha^{-1} in a treatment with dung water application (66 m^3 ha^{-1}) supplemented with 44 kg P ha^{-1}in the form of Thomas slag, or in the treatment with a mineral fertilisation of 120 kg N, 44 kg P, and 174 kg K ha^{-1} (Diepolder et al. 2005). Another distinctive aspect of the SGE is the incorporation of organic fertilisers (a mixture of dung water, urine, and water with nutrients leached from feces) together with mineral fertilisers (Thomas slag and lime) in certain treatments, a practice seldom employed in long-term experiments (Hejcman et al. 2014).

Although the exact P-fertiliser batches applied at SGE were not archived, published datasets show that Thomas phosphate historically used in Germany, Austria, and Switzerland often contained comparatively high Cr concentrations, and pre-1948 phosphate fertilisers frequently had elevated As levels (Hartley et al. 2013; Smolders and Six 2013). To account for this uncertainty, the interpretation of P–Cr relationships qualitatively considered both low- and high-Cr fertiliser scenarios reported in the literature. This approach ensured that the variability in historical fertiliser composition was reflected in the discussion of potential element sources. There are presently six control plots without fertiliser and lime input (depicted in a more prominent green colour in Fig. 1), along with 69 fertilised plots. Among the fertilised plots, 25 were limed in 1986 (depicted in grey) with 8 t ha^{-1} of dolomite lime (Diepolder et al. 2005; Hejcman et al. 2014), while 44 plots remain without direct liming (depicted in the light shade of green). The treatments included limed plots with and without dung water, a combination of mineral P and dung water, liming only, $(NH_4)_2SO_4$, a combination of Ca $(NO_3)_2$, $(NH_4)_2SO_4$, and NH_4NO_3 (with the amount of applied N set at 60, 50, and 50 kg N ha^{-1}), and a combination of mineral N, P, and K fertilisers (Hejcman et al. 2014). The fertilised plots without any direct liming comprise plots treated with only dung water, a combination of P and dung water, and a combination of mineral N, P, and K fertilisers. Some plots were treated with $(NH_4)_2SO_4$, or a combination of $Ca(NO_3)_2$, $(NH_4)_2SO_4$, and NH_4NO_3 (Fig. 1, Table S1) (Hejcman et al. 2014). Annual N application rates were 60, 120, and 160 kg ha^{-1} in mineral fertiliser treatments and 116 and 232 kg ha^{-1} in organic fertiliser treatments (dung water–a mixture of urine and water with nutrients leached from faeces, together with mineral fertilisers, Thomas slag, and lime) (Hejcman et al. 2014). Annual P application rates were 22 and 44 kg ha^{-1} in mineral fertiliser treatments applied as Thomas slag and 4 and 8 kg ha^{-1} in organic fertiliser treatments. Thomas slag also supplied the soil with Ca at an annual rate of 107 and 214 kg Ca ha^{-1} in treatments with an annual application of 22 and 44 kg P ha^{-1}, respectively. Annual K application rates were 87, 133, 174, and 216 kg ha^{-1} in mineral fertiliser treatments applied as a mixture of KCl and $MgSO_4$ and 232 and 465 kg K ha^{-1} in organic fertiliser treatments (dung water). Mineral P and K fertilisers were applied in the autumn, and all N fertilisers were applied during the vegetation season. The amount of Ca and Mg applied in the form of dolomite lime (53% CaO and 25% MgO) in 1986 was 3032 and 1206 kg ha^{-1}, respectively, in limed treatments (Table S1; Diepolder et al. 2005; Hejcman et al. 2014).

Abbreviations used include: PA and KA for mineral phosphorus and potassium fertilisers, respectively, and PO

and KO for organic phosphorus and potassium fertilisers. Nitrogen fertilisers are distinguished by their form: NO_3^- (nitrate) and NH_4^+ (ammonium) (Hejcman et al. 2014).

Each of the 75 experimental plots received a unique combination of nitrogen (N), phosphorus (P), and potassium (K), applied in varying dosages and fertiliser forms (e.g. ammonium sulphate, Thomas slag). For clarity and consistency, treatment codes such as N160P44K174 are used throughout the text, where the numbers indicate annual nutrient application rates in kg ha^{-1}. Additional annotations, such as an asterisk (*) for ammonium sulphate, highlight treatments influencing soil acidification.

2.3 Soil extraction procedures

In mid-April 2008, ten separate soil samples were collected from each experimental plot, following the methods of Hejcman et al. (2014). Samples were obtained from the 0 to 100 mm layer and thoroughly mixed to obtain one representative soil sample per plot. After removing living roots, the soil samples were air-dried, ground in a mortar, and sieved to 2 mm. The soil pH (H_2O) was measured in a suspension of 10 g dry soil mixed with 50 ml distilled H_2O at Eko-Lab, Žamberk (http://www.ekolab.zamberk.cz), an accredited Czech national laboratory.

The decomposition and extraction of microelements Fe, Zn, Cu, Mn, As, Cr, Ni, Pb, and Cd were performed according to the methodology described by Uprety et al. (2009) and Hejcman et al. (2009). The digests obtained by the following two-step decomposition procedure determined the real total contents of elements in the soils as follows: Exactly 0.5 g of a sample was decomposed by dry ashing in an Apion Dry Mode Mineralizer (Tessek, Czech Republic). The ash was then dissolved in a mixture of HNO_3 and HF, evaporated to dryness at 160 °C, and the retaining dry residue was then 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.

To determine the plant-accessible fraction of the elements, 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) as described by Novozamsky et al. (1993). To determine the easily mobilisable content of microelements in the soil, the soil samples (0.5 g each) were extracted with a 0.05 mol L^{-1} ethylene diamine tetraacetic acid (EDTA) aqueous solution at pH 7, using a ratio of 1:10 (w/v) for 1 hour (Quevauviller et al. 1993). The potentially mobilisable content of microelements was determined by extracting 0.5 g soil samples with a 2 mol L^{-1} aqueous solution of HNO_3, using a ratio of 1:10 (w/v) at 20 °C for 6 h (Borůvka et al. 1996).

The reaction mixtures were centrifuged at 3,000 rpm for 10 min, and supernatants were stored at 6 °C before measurement. Blank extracts, constituting 5% of the total number of extracts, were prepared using the same batch of reagents and apparatus and analysed concurrently with soil extracts. All reagents used were of electronic-grade purity (Analytika, Ltd, Czech Republic).

All the procedures described here (air-drying, decomposition, and extraction) were carried out immediately in 2008.

Abbreviations for individual elements in the 'Results' section are accompanied by the method of extraction: Ac—for plant-accessible content ($CaCl_2$), E—for easily mobilisable content (EDTA), M—for potentially mobilisable content (HNO_3), and T—for real total content. N120P22K87 - digits denote the amount of applied fertiliser N, P, and K in kg ha^{-1}.

2.4 Determination of elements

The microelements Fe, Zn, Cu, Mn, As, Cr, Ni, Pb, and Cd in the soil in soil digests and extracts were determined by optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration, (Varian, VistaPro, Australia) equipped with an autosampler SPS-5. 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-OES 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 contents of As in 0.01 mol l^{-1} $CaCl_2$ and 0.05 mol l^{-1} EDTA extracts were determined by a continuous hydride generation technique (HGAAS) using a Varian AA280Z (Varian, Australia) atomic absorption spectrometer equipped with a hydride generator VGA-77. A mixture of potassium iodide and ascorbic acid was used for the prereduction of the sample, and the extract was acidified with HCl before measurement. The methodology described followed those applied by Hejcman et al. (2009).

2.5 Permissible limits for risk elements according to decree of the ministry of the environment CZ No. 153/2016 Coll

Decree No. 153/2016 Coll. of the Ministry of the Environment on the determination of details for the protection of the quality of agricultural land (DME CZ No. 153/2016 Coll. (n.d.)) provides the following information:

Indicative values represent concentration levels (mg·kg^{-1} dry matter) above which the safety of food or feed may be at

risk, and plant growth or the soil's production function may be adversely affected.

Preventive values serve as upper threshold concentrations. Exceeding these values does not automatically indicate an environmental risk, but may signal the need for more detailed monitoring or assessment to evaluate potential impacts.

These values, determined by Aqua regia extraction ($mg \cdot kg^{-1}$ dry matter), are listed in Table 1.

2.6 Data analysis

To assess the impact of long-term fertilisation on soil chemical properties and the accumulation of risk elements, both multivariate and univariate statistical approaches were employed. The multivariate approach was used to evaluate the relationships between fertilisation treatments and the distribution of different chemical forms of risk elements in the soil, while regression analysis was applied to quantify the influence of fertilisation and soil pH on element concentrations across different extractions.

A redundancy analysis (RDA) in the CANOCO 5 software (Ter Braak and Šmilauer 2002) was used to evaluate multivariate data because the datasets were sufficiently homogeneous, and environmental variables (applied fertilisation) were in the form of categorical predictors. A Monte Carlo permutation test with 999 random permutations was used to determine whether the tested explanatory environmental variables—application of ammonium (NH_4), nitrate (NO_3), mineral phosphorus (PA), potassium (KA), and organic phosphorus (PO)—had a significant effect on the response variables: elements in plant-accessible (Ac), easily mobilisable (E), potentially mobilisable (M), and total element content (T). The results of the multivariate analysis were visualised using ordination diagrams created in CanoDraw© software.

To further explore the relationships between fertilisation treatments and soil risk element concentrations, a simple linear regression analysis was performed in STATISTICA software 13.3 (StatSoft, Tulsa, AZ, USA). The analysis assessed the relationship between the amounts of applied fertilisers (N, P, and K—both organic and mineral forms combined) and soil pH (H_2O) with all extractions ($CaCl_2$, EDTA, HNO_3, and Total) of risk elements. Dependent variables included extractions of Fe, Zn, Cu, Mn, As, Cr, Ni, Pb, and Cd, while independent variables were fertiliser applications and pH. The data followed a normal distribution, and significance was tested at a 0.01–0.05 level. This approach was chosen due to the absence of replication for individual fertilisation treatments, resulting in a high gradient of soil chemical properties, which was better suited for a regression-based analysis.

3 Results

The total content of Fe, Zn, Cu, Mn, As, Cr, Ni, Pb, and Cd in the soil was determined using a two-step decomposition method (hydrofluoric acid + aqua regia, Száková et al. 1999), providing a conservative estimate of potential contamination (Table 2). In most cases, microelement contents remained below the Czech legislative thresholds (Decree of the Ministry of the Environment No. 153/2016 Coll., (n.d) Table 1). However, CrT concentrations exceeded the indicative value of 90 mg kg^{-1} (based on Czech preventive limits) in several plots, particularly 41/1, 161/1, 162/Ca, 72/Ca, 181/1, 211/2, 212/1Ca, 212/2/Ca, and 221/2 (Table 3). Elevated FeT, MnT, AsT, CrT, NiT, PbT, and CdT values were observed in mineral-fertilised plots (N120/P44/K174 and N160/P44/K174) and in the organically fertilised plot 41/1 (N232/P52/K465), which also exceeded the indicative limit for Cr and the preventive limit for Cd (Table 3).

Table 1 The table is based on the Decree of the Ministry of Agriculture on the Protection of Agricultural Soil Quality, which establishes indicative and preventive threshold values for risk element content in agricultural soil. The first two rows represent indicative values (mg kg^{-1} dry matter) determined by aqua regia extraction. Exceeding these values may pose a potential risk to human and animal health. The grey rows represent preventive values (mg kg^{-1} dry matter) determined by Aqua regia extraction. They represent upper limits for the concentration of risk elements in the soil. Exceeding these values does not imply an immediate risk, but signals a potential deterioration of certain soil functions, as defined in the Decree of the Ministry of the Environment No. 153/2016 Coll (n.d.)

Type of soil	Cu	Zn	As	Cr	Ni	Pb	Cd
Indicative value	150–200	400	40	90	150	300	1.5
Other soils[1]	300*	400	40	90	200*	300	2*[2]
Preventive value	60	120	20	90	50	60	0.5
Other soils[1]							
Sandy soils[2]	45	105	15	55	45	55	0.4

[1]Other soils: sandy-loam, loam, clay-loam, and clay soils, which constitute the majority of agriculturally utilized soils. These are soils with normal element variability and normal soil development under various geomorphological conditions, including soils on carbonate rocks

[2]Sandy soils: soils formed on very light and poor parent rocks such as sands and gravelly sands. When defining these soils, the focus is on representing fine particles (up to 0.01 mm), constituting a maximum of 20%. These soils are characterised by very low absorption capacity

*Values marked with an asterisk indicate the upper limit for soils with pH > 6.5. For other soils, the applicable limit is the value in the row above. However, for Cu, Ni, and Cd, the permissible limit increases when pH > 6.5

Table 2 The table presents the minimum and maximum values (mg kg^{-1}) of individual microelements across the experimental plots, along with a comparison to the three control plots. Additionally, it indicates the number of plots that exceed the permitted limits. The color scale in the table represents increasing values within each microelement (column), using a gradient from white to green. The lightest shade (white) indicates the lowest values, while progressively darker shades of green represent increasing values, with the darkest green indicating the highest content

Elements (mg kg^{-1})	FeAc	ZnAc	CuAc	MnAc	AsAc	CrAc	NiAc	PbAc	CdAc	FeE	ZnE	CuE	MnE	AsE	CrE	NiE	PbE	CdE
Min	0.06	0.01	_	1.3	0.07	0.00	0.02	_	0.00	13	0.07	0.37	10.5	0.01	0.00	0.06	0.44	0.01
Max	3.75	0.74	_	25.0	0.35	0.05	0.16	_	0.05	240	5.35	1.63	90.0	0.63	0.79	0.47	2.63	0.12
Control 1	0.41	0.16	_	14.3	0.22	0.03	0.10	_	0.01	43	0.26	1.14	38.2	0.26	0.07	0.16	1.34	0.07
Control 2/Ca	0.20	0.10	_	8.6	0.10	0.01	0.05	_	0.01	86	1.14	1.22	41.0	0.18	0.07	0.46	2.10	0.04
Control 3	0.22	0.33	_	6.7	0.26	0.02	0.06	_	0.05	39	3.80	0.56	13.1	0.17	0.05	0.11	1.23	0.08
Elements (mg kg^{-1})	**FeN**	**ZnN**	**CuN**	**MnN**	**AsN**	**CrN**	**NiN**	**PbN**	**CdN**	**FeT**	**ZnT**	**CuT**	**MnT**	**AsT**	**CrT**	**NiT**	**PbT**	**CdT**
Min	645	4.25	1.58	69	0.33	0.74	0.47	2.89	0.10	13030	38	6	436	5	34	10	23	0.4
Max	1330	7.75	3.96	189	1.11	11.86	1.00	4.51	0.17	26984	69	19	1413	32	173	29	54	1.0
Control 1	837	6.70	3.17	109	0.70	1.33	0.67	3.57	0.12	14737	65	10	618	10	44	16	28	0.6
Control 2/Ca	872	6.28	3.13	105	0.50	1.47	0.69	3.36	0.13	14332	60	9	553	12	41	13	24	0.6
Control 3	949	5.14	2.05	128	0.92	5.83	0.67	3.56	0.15	14180	45	7	468	15	43	18	35	0.5
No. of plots exceeded indicative value	_	_	_	_	_	_	_	_	_	_	0	0	_	0	22	0	0	0
No. of plots exceeded preventive value	_	_	_	_	_	_	_	_	_	_	0	0	_	10	22	0	0	60

No. Exceed plots: This represents the number of plots where the total content (determined by aqua regia extraction) exceeded the indicative values specified in the Decree of the Ministry of the Environment No. 153/2016 Col (n.d.)

The number of plots exceeding preventive and indicative values (Table 1) is summarised in Table 2. However, it is essential to note that the „real total" content determined in this study by HF digestion is not directly comparable to the aqua regia-extrable fraction used as the regulátory reference basis. The HF method generally yields higher values, particularly for Cr bound in silicate minerals, and therefore the apparent exceedances should be regarded as conservative upper estimates rather than evidence of actual regulatory non-compliance. For Cd, the difference between HF and aqua regia is minor, and the measured concentrations remain within the indicative range. Accordingly, all comparisons with national limits are expressed on a real-total basis, and true exceedances relative to aqua regia–extractable thresholds are unlikely.

AsT content exceeded the indicative value in plot 91/2 (N60 - pH 5.1), reaching 32.0 mg kg^{-1} (Table 2). Notable variability was observed even within identical fertilisation variants: 41/1 (N232P52K465 - pH 6.6) had 7.0 mg kg^{-1}, whereas 41/2 (N232P52K465 - pH 6.7) had 24.3 mg kg^{-1} (Table 3). The lowest AsT value 4.9 mg kg^{-1} was recorded in plot 132/Ca (K87 – pH 5.7; Table S2).

For CdT, slightly elevated contents were found in several plots, particularly in the organically fertilised plot 41/1 (N232P52K465 - pH 6.6; 0.91 mg kg^{-1}). and in mineral-fertilised plots: 201/1 (N160P44K174 - $(NH_4)_2SO_4$ - pH 6.3; 0.94 mg kg^{-1}), 212/1Ca (N160P44K174/Ca - $(NH_4)_2SO_4$ - pH 5.0; 1.0 mg kg^{-1}), and 221 (N160P44K174 - $(NH_4)_2SO_4$ - pH 6.0; 0.98 mg kg^{-1}; Table 3).

These concentrations are close to the preventive limit but remain below the indicative thresholds for agricultural soils when expressed on an aqua regia basis.

3.1 Redundancy analysis (RDA) of soil element extractions

The RDA analysis was conducted separately for each soil extraction method ($CaCl_2$, EDTA, HNO_3, and Total), allowing for a more detailed evaluation of the relationship between applied fertilisers and elemental composition in the soil (Fig. 2a, b,c, d).

For the $CaCl_2$ extraction fertilisation explained 19.6% of the total variability (adjusted explained variation: 11.8%). The permutation test ($F = 2.5$, $p = 0.001$) indicated a statistically significant effect of fertiliser application on the composition of elements extracted with $CaCl_2$. The ordination plot showed a weak separation of treatments, suggesting that fertiliser application had only a minor impact on the mobilisation of elements extracted by $CaCl_2$. Most elements were negatively correlated with all fertilisation treatments, except for AsAc, which showed a positive correlation with PO fertilisation (Fig. 2a).

In the case of the EDTA extraction, fertilisation explained 13.4% of variability (adjusted explained variation: 5.0%). However, the permutation test ($F = 1.6$, $p = 0.054$) suggested that the effect of fertilisation on element composition was not statistically significant at the 5% level. The ordination plot indicated that CdE was positively correlated with NO_3 and KA, while CrE and FeE were positively correlated with NH_4 and PA (Fig. 2b).

For the HNO_3 extraction, the applied fertilisation explained 37.6% of the total variability (adjusted explained variation: 31.6%). The permutation test ($F = 6.2$, $p = 0.001$) confirmed a highly significant effect of fertilisation on element composition. Mineral phosphorus (PA) application

Table 3 Selected experimental plots based on Table S2, where distinct concentrations of risk elements were recorded compared to other plots. The table presents the content of trace elements such as Fe, Zn, Cu, Mn, as, cr, Ni, Pb, and cd in the 0–100 mm soil layer, extracted using different methods: $CaCl_2$, EDTA, HNO_3, and total extraction. Concentrations of Cu and Pb were so low that they were not detected in $CaCl_2$ extraction. Abbreviation C = control plots without fertilisation, Ca = limed in 1986. Plots with mineral phosphorus fertilisation (P 44) were indirectly limed with Thomas slag. Plots marked with an asterisk (*) were fertilised with $(NH_4)_2SO_4$. Values exceeding the permissible indicative limits for agricultural soils (Decree of the ministry of the environment No. 153/2016 Coll. (n.d.)) are highlighted in bold: Cu 200; Zn 400; as 40; cr 90; Ni 150; Pb 300; cd 1.5–2 mg kg^{-1}

		Experimental plots											
Element	Extraction	C/1	C/2/Ca	C3	21/1	22/Ca	31/2	41/1	41/2	52/Ca	91/2	102/Ca	112/Ca
N (kg ha^{-1})					232	232	116	232	232	120	60	120	120
P (kg ha^{-1})					8	8	26	52	52	0	0	0	22
K (kg ha^{-1})					465	465	232	465	465	87	0	0	0
pH (H_2O)		5.1	5.8	5.1	5.9	6.2	5.9	6.6	6.7	5.5	5.1	6.2	6.7
Fe	$CaCl_2$	0.41	0.20	0.22	0.13	0.07	0.10	0.21	0.07	0.09	0.85	0.07	0.09
	EDTA	43.0	86.4	38.9	20.2	51.8	50.8	29.7	22.7	29.0	33.0	34.1	59.4
	HNO_3	837	872	949	854	829	867	901	830	747	848	730	1034
	Total	14,737	14,332	14,180	14,998	15,196	16,695	22,085	15,933	15,527	15,336	15,003	15,752
Zn	$CaCl_2$	0.16	0.10	0.33	0.11	0.09	0.13	0.22	0.02	0.07	0.39	0.10	0.09
	EDTA.	0.26	1.14	3.80	0.62	0.24	0.73	0.42	0.35	5.35	0.28	0.31	0.43
	HNO_3	6.70	6.28	5.14	6.19	5.58	5.30	4.79	4.97	4.82	6.46	4.25	7.04
	Total	64.7	59.7	45.3	63.9	44.8	44.7	63.6	55.9	53.8	69.0	41.7	44.7
Cu	EDTA	1.14	1.22	0.56	0.43	1.26	1.01	0.60	0.50	0.76	1.28	1.02	1.52
	HNO_3	3.17	3.13	2.05	2.67	2.90	2.05	2.03	2.88	2.65	2.84	1.86	3.47
	Total	9.54	9.01	7.06	9.55	7.86	8.07	11.19	10.79	9.52	18.00	7.56	8.14
Mn	$CaCl_2$	14.25	8.64	6.71	5.52	2.78	3.89	1.51	2.48	7.75	22.53	3.14	1.71
	EDTA	38.24	40.97	13.10	13.88	38.45	49.93	20.47	21.86	22.85	36.00	35.56	51.53
	HNO_3	109	105	128	112	109	116	123	135	103	108	84	139
	Total	618	553	468	522	585	801	1266	821	891	637	570	812
As	$CaCl_2$	0.22	0.10	0.26	0.19	0.08	0.09	0.19	0.15	0.14	0.19	0.14	0.24
	EDTA	0.26	0.18	0.17	0.01	0.40	0.08	0.07	0.03	0.09	0.22	0.12	0.49
	HNO_3	0.70	0.50	0.92	0.48	0.57	0.61	0.59	0.66	0.56	0.55	0.45	0.61
	Total	10.2	12.0	15.4	7.6	19.0	19.8	7.0	24.3	23.5	32.0	16.0	21.6
Cr	$CaCl_2$	0.03	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.03	0.04	0.02
	EDTA	0.07	0.07	0.05	0.04	0.10	0.12	0.08	0.08	0.07	0.02	0.03	0.14
	HNO_3	1.33	1.47	5.83	0.93	1.07	3.34	4.62	3.61	0.92	1.08	1.06	4.21
	Total	44.2	41.0	42.5	39.1	48.7	74.4	**140.9**	68.1	71.8	40.0	48.7	76.2
Ni	$CaCl_2$	0.10	0.05	0.06	0.03	0.03	0.02	0.03	0.05	0.05	0.12	0.03	0.04
	EDTA	0.16	0.46	0.11	0.19	0.24	0.28	0.17	0.16	0.12	0.21	0.18	0.25
	HNO_3	0.67	0.69	0.67	0.69	0.66	0.70	0.66	0.61	0.55	0.65	0.53	0.76
	Total	15.98	12.68	18.42	13.13	20.37	19.20	29.20	14.46	12.72	14.00	20.39	20.17
Pb	EDTA	1.34	2.10	1.23	0.68	1.43	1.82	0.58	0.88	1.73	1.53	1.39	2.07
	HNO_3	3.57	3.36	3.56	3.72	3.80	3.68	3.65	3.65	3.13	3.44	4.11	4.08
	Total	28.02	24.18	34.96	25.25	31.71	43.08	38.86	33.22	26.08	25.00	41.39	37.91
Cd	$CaCl_2$	0.01	0.01	0.05	0.00	0.05	0.04	0.02	0.01	0.01	0.02	0.04	0.05
	EDTA	0.07	0.04	0.08	0.06	0.02	0.03	0.02	0.06	0.11	0.02	0.02	0.03
	HNO_3	0.12	0.13	0.15	0.11	0.12	0.14	0.14	0.12	0.11	0.13	0.12	0.15
	Total	0.65	0.60	0.50	0.59	0.57	0.59	0.91	0.72	0.63	0.60	0.74	0.55

Table 3 (continued)

	Experimental plots											
Element	122/Ca	141/1	161/1	162/Ca	172/Ca	181/1	201/1	211/2*	212/1Ca*	212/2/Ca*	221/2	222/Ca
N (kg ha^{-1})	120	0	0	0	120	120	160	160	160	160	160	160
P (kg ha^{-1})	44	0	44	44	22	44	44	44	44	44	44	44
K (kg ha^{-1})	0	174	174	174	87	174	174	174	174	174	174	174
pH (H_2O)	7.0	5.2	6.0	6.7	6.4	6.5	6.3	4.7	5.0	5.0	6.0	6.4
Fe	0.26	0.28	0.09	0.08	0.08	0.33	0.89	2.10	0.54	0.61	0.15	0.14
	55.8	25.7	41.4	39.8	21.1	21.0	33.2	239.8	13.3	203.2	30.3	46.5
	1140	645	992	971	894	911	1255	1174	1135	1253	1116	1093
	15,965	15,068	15,882	16,753	13,496	20,090	20,160	17,021	26,984	14,743	24,558	14,747
Zn	0.06	0.42	0.12	0.11	0.02	0.06	0.39	0.19	0.15	0.15	0.02	0.04
	1.04	0.36	0.35	1.39	0.60	3.04	0.16	0.47	0.11	0.44	0.23	1.18
	7.75	4.52	5.18	6.62	5.03	6.13	6.97	6.27	5.37	5.68	6.35	6.63
	60.5	43.3	38.4	44.1	48.1	57.0	54.4	41.8	60.1	50.3	64.5	57.5
Cu	0.71	0.78	0.59	0.75	0.52	0.58	0.58	1.63	0.37	0.99	0.40	0.67
	3.96	1.88	2.16	2.30	2.90	2.23	3.50	3.22	2.90	3.30	3.09	3.34
	10.51	8.21	7.48	7.82	8.71	11.00	9.99	7.76	16.78	9.57	15.18	10.14
Mn	3.62	7.91	3.35	1.37	3.33	5.58	3.63	4.26	9.15	11.38	4.17	5.44
	21.35	24.98	24.54	30.29	16.63	16.00	26.97	38.42	13.79	34.11	19.08	35.23
	172	79	144	153	128	135	189	85	108	117	150	150
	916	529	944	958	690	1413	1278	603	1091	522	1306	761
As	0.14	0.17	0.12	0.14	0.12	0.13	0.11	0.25	0.22	0.20	0.35	0.09
	0.16	0.09	0.09	0.28	0.09	0.21	0.03	0.64	0.06	0.19	0.02	0.29
	0.76	0.33	0.76	0.85	0.59	0.60	0.94	0.94	1.02	1.11	0.99	0.81
	12.7	12.4	19.9	24.3	17.6	24.0	19.1	0.6	20.9	8.2	11.8	10.2
Cr	0.04	0.01	0.01	0.01	0.01	0.04	0.01	0.02	0.02	0.01	0.02	0.01
	0.06	0.06	0.09	0.09	0.05	0.03	0.05	0.66	0.01	0.57	0.08	0.11
	7.51	0.90	6.30	6.49	3.91	6.36	7.29	8.13	7.28	8.15	5.75	5.50
	84.2	53.8	**102.2**	**100.7**	**147.9**	**130.0**	**134.9**	**126.8**	**172.6**	**171.2**	**148.5**	90.4
Ni	0.05	0.06	0.05	0.02	0.05	0.04	0.03	0.03	0.06	0.06	0.05	0.04
	0.19	0.09	0.16	0.13	0.07	0.14	0.11	0.30	0.06	0.20	0.07	0.26
	0.88	0.55	0.77	0.79	0.52	0.79	0.87	0.58	0.62	0.66	0.78	1.00
	14.44	19.20	17.63	19.52	11.04	25.00	24.66	18.12	22.65	13.34	25.66	13.15
Pb	1.03	0.96	0.60	1.01	0.44	1.66	0.79	2.07	0.48	1.46	0.55	1.74
	4.14	3.59	3.60	4.00	3.30	3.58	4.36	3.73	3.86	4.18	3.61	3.49
	28.11	35.53	35.69	43.15	23.43	49.00	46.82	35.70	51.05	24.12	45.00	23.10
Cd	0.01	0.03	0.05	0.04	0.01	0.01	0.02	0.01	0.02	0.00	0.00	0.00
	0.02	0.08	0.02	0.08	0.02	0.08	0.02	0.04	0.01	0.03	0.06	0.05
	0.16	0.10	0.15	0.16	0.12	0.15	0.17	0.14	0.14	0.16	0.16	0.15
	0.67	0.59	0.40	0.54	0.61	0.82	0.94	0.58	1.01	0.61	0.98	0.58

was strongly correlated with MnM, ZnM, NiM, CrM, and CdM, suggesting that these elements may become more available in response to phosphorus inputs. However, AsM and FeM were positively correlated with KA (Fig. 2c).

The total element content extraction (aqua regia) showed that fertilisation accounted for 20.9% of the total variability (adjusted explained variation: 13.2%). The permutation test ($F = 2.7$, $p = 0.002$) indicated a statistically significant effect of fertiliser application on the total elemental composition in the soil. NO_3 and mineral potassium (KA) fertilisation were positively associated with FeT, CrT, and CdT, while mineral P was positively correlated with CrT, NiT, and MnT. In contrast, organic phosphorus (PO) application was negatively correlated with AsT and PbT, but positively correlated with CuT and ZnT (Fig. 2d).

These results indicate that fertilisation has a significant effect on element composition in soil, particularly for potentially mobilisable elements (HNO_3 extraction) and for total

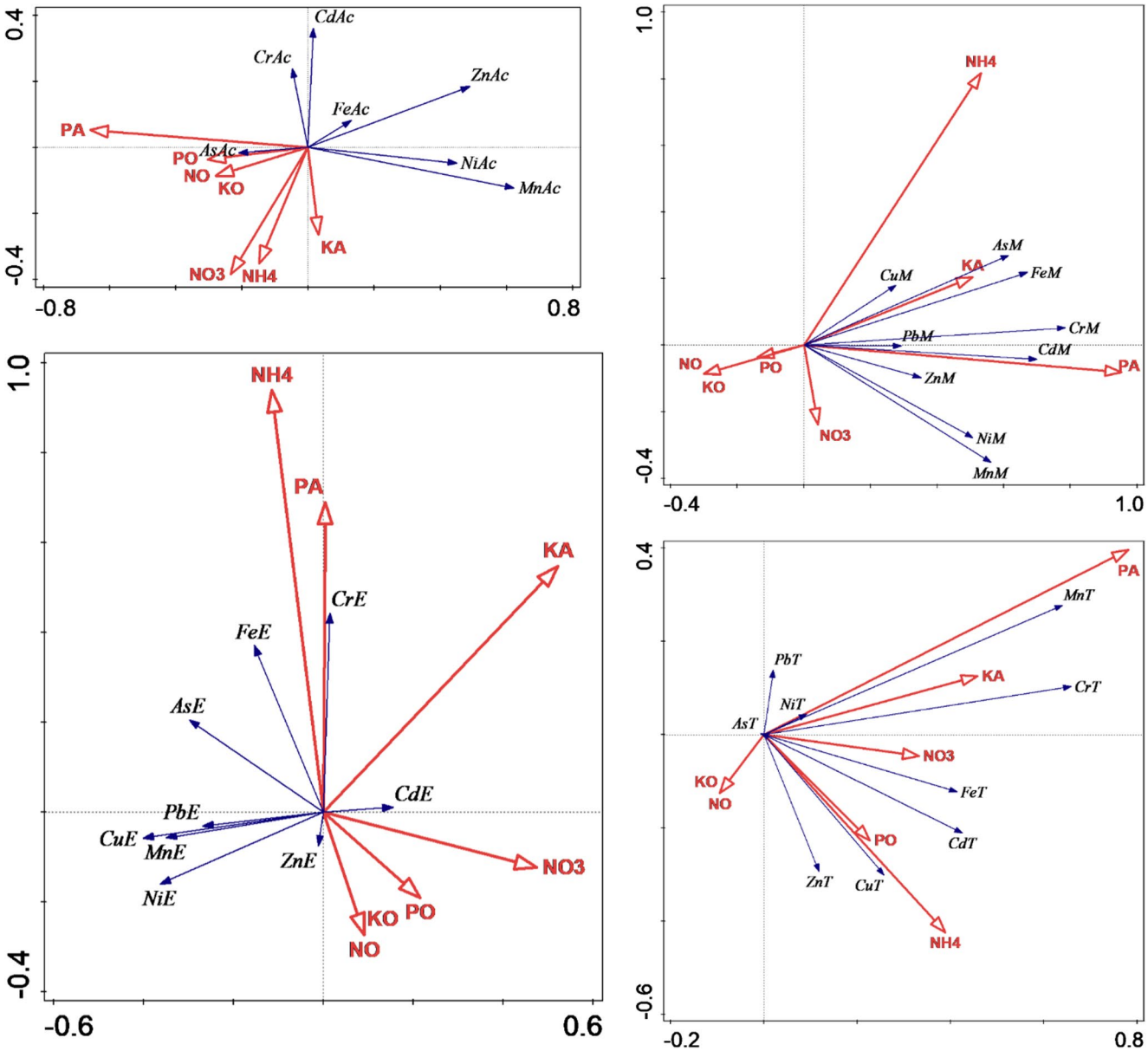


Fig. 2 Ordination diagram showing the result of RDA analysis of different extractions composition data concerning the applied fertilisers. The four panels are arranged as follows: **a** top left for $CaCl_2$ extraction (Ac), **b** bottom left - EDTA extraction (E), **c** top right - HNO_3 extraction (M), and **d** bottom right - Total (aqua regia) extraction (T). Abbreviations for fertiliser applications are as follows: PO - organic phosphorus (4,8,26 kg ha^{-1}); PA - mineral phosphorus (22, 44 kg ha^{-1}); KO - organic potassium (232, 465 kg ha^{-1}) KA - mineral potassium (84, 133, 174, 216 kg ha^{-1}); NO_3 - nitrate (60, 120 kg ha^{-1}); and NH_4 - ammonium (160 kg ha^{-1}) *We are providing the graphs exported from Canoco one by one to ensure better quality (unfortunately, this is the highest quality that Canoco allows). If* Fig. 3, *where we merged the individual graphs, is usable, please keep that one instead. Canoco can generate graphs of different sizes, and cannot be made the same size*

content, whereas its impact on plant-accessible elements ($CaCl_2$ extraction) is less pronounced.

3.2 Regression analysis results

Calculated by linear regression, organic and mineral P fertilisers had a significant positive effect on the potentially mobilisable and total content of several elements, including Fe, Mn, As, Cr, Pb, and Cd (Table 4, analyses 1–2, 6–8, 9, 11–14; Fig. 3a, c, e, f). Conversely, P fertilisation was negatively correlated with ZnAc and MnAc (Table 4, analyses 3, 5). Regarding nitrogen, a significant positive correlation was observed between N fertilisation and plant-accessible contents of AsAc and CdAc (Table 4, analyses 18, 19), and total contents of ZnT and CdT (Table 4, analyses 16, 20). In contrast, ZnAc and MnAc showed a significant negative response to N fertilisation (Table 4, analyses 15, 17; see Fig. 3d for CdT).

Soil pH (H_2O) exhibited a significant negative correlation with the plant-accessible content of Fe, Zn, Mn, and Ni (Table 4, analyses 21–23, 26). However, pH was positively correlated with MnM, CdM, and MnT (Table 4, analyses 24, 25, 27; Fig. 3b), highlighting the influence of pH on micro-element availability.

Table 4 Results of regression analyses of potentially trace elements content in the soil as a function of elements applied with fertilisers and pH (H_2O)

Analysis	Ind. var.	Dep. Var.	Equations	*r*	*p*-value
1	P	FeM	y = 811 + 4.70*x	0.58	0.01
2	P	FeT	y = 15641 + 42*x	0.34	0.01
3	P	ZnAc	y = 0.23–0.00*x	−0.38	0.00
4	P	ZnM	y = 5.40 + 0.01*x	0.28	0.02
5	P	MnAc	y = 10.7 − 0.17*x	−0.60	0.01
6	P	MnM	y = 102 + 0.92*x	0.61	0.01
7	P	MnT	y = 636 + 7.25*x	0.62	0.01
8	P	AsM	y = 0.55 + 0.01*x	0.57	0.01
9	P	PbM	y = 3.56 + 0.01*x	0.26	0.04
10	P	CrE	y = 0.04 + 0.00*x	0.29	0.05
11	P	CrM	y = 1.56 + 0.10*x	0.73	0.01
12	P	CrT	y = 53.4 + 1.13*x	0.65	0.01
13	P	CdM	y = 0.12 + 0.01*x	0.65	0.01
14	P	CdT	y = 0.61 + 0.00*x	0.30	0.01
15	N	ZnAc	y = 0.263- 0.00*x	−0.44	0.01
16	N	ZnT	y = 48.3 + 0.03*x	0.27	0.05
17	N	MnAc	y = 8.99 − 0.02*x	−0.25	0.05
18	N	AsAc	y = 0.12 + 0.00*x	0.27	0.05
19	N	CdAc	y = 0.03–0.00*x	0.27	0.05
20	N	CdT	y = 0.58 + 0.00*x	0.32	0.01
21	pH	FeAc	y = 2.16–0.31*x	−0.36	0.01
22	pH	ZnAc	y = 0.88 − 0.12*x	−0.55	0.01
23	pH	MnAc	y = 41.98–5.98*x	−0.67	0.01
24	pH	MnM	y = −35.4 + 26.9*x	0.57	0.01
25	pH	MnT	y = −303 + 188*x	0.52	0.01
26	pH	NiAc	y = 0.19 − 0.02*x	−0.51	0.01
27	pH	CdM	y = 0.08 + 0.01*x	0.32	0.01

Ind. var.–independent variable, *Dep.* var.–dependent variable, *Equation*–regression equation, *r*–coefficient of determination, *p*-value–corresponding probability value

Abbreviation for independent variables of organic and mineral fertilisers: Phosphorus (*P*), Nitrogen (*N*), and soil pH (*H_2O*). Abbreviations for individual extractions used in the data analysis are as follows: *Ac* for $CaCl_2$ extraction, *E* for EDTA extraction, *M* for HNO_3 extraction, and *T* for Total (HF aqua regia) extraction

3.3 Correlation patterns between extraction methods

The Pearson correlation matrix (Table 5) showed distinct patterns among the four extraction methods for trace elements.

Strong positive correlations ($r > 0.80$) were consistently observed between the HNO_3-extractable (M) and total (T) fractions, particularly for Fe ($r = 0.95$), Mn ($r = 0.96$), and Zn ($r = 0.90$), indicating that a large proportion of mobilisable content contributes to the total pool.

The EDTA-extractable (E) fractions were moderately correlated ($r = 0.50$–0.75) with HNO_3 (M) fractions for Fe ($r = 0.72$), Mn ($r = 0.72$), and As ($r = 0.53$), suggesting partial overlap of extractable pools.

In contrast, the $CaCl_2$-extractable (Ac) fractions generally showed weak or no correlation ($r < 0.4$) with the other methods, especially for less mobile elements like Cr ($r = 0.15$ with M) and Pb ($r = 0.13$ with T), reflecting their low solubility and strong adsorption in the soil matrix.

Some elements, such as Fe and Mn, displayed consistently high correlations across all extraction methods (e.g., FeE–FeM $r = 0.72$; MnM–MnT $r = 0.96$), whereas others, like Cd and Cr, exhibited extraction-specific variability.

4 Discussion

This study evaluated the long-term effects of different fertilisation regimes on the accumulation and mobility of potentially toxic elements in grassland soils at the Steinach Grassland Experiment (SGE). Despite the absence of replicated treatments in the early SGE design, the long-term consistency of fixed plots and stable management history ensures sufficient robustness for interpretation of nutrient–element relationships, consistent with other long-term fixed-plot grassland experiments (Crawley et al. 2005; Silvertown et al. 2006).

4.1 Long-term fertilisation and risk element accumulation

The main objective of this study was to analyse experimental data from the long-term fertilisation of permanent SGE

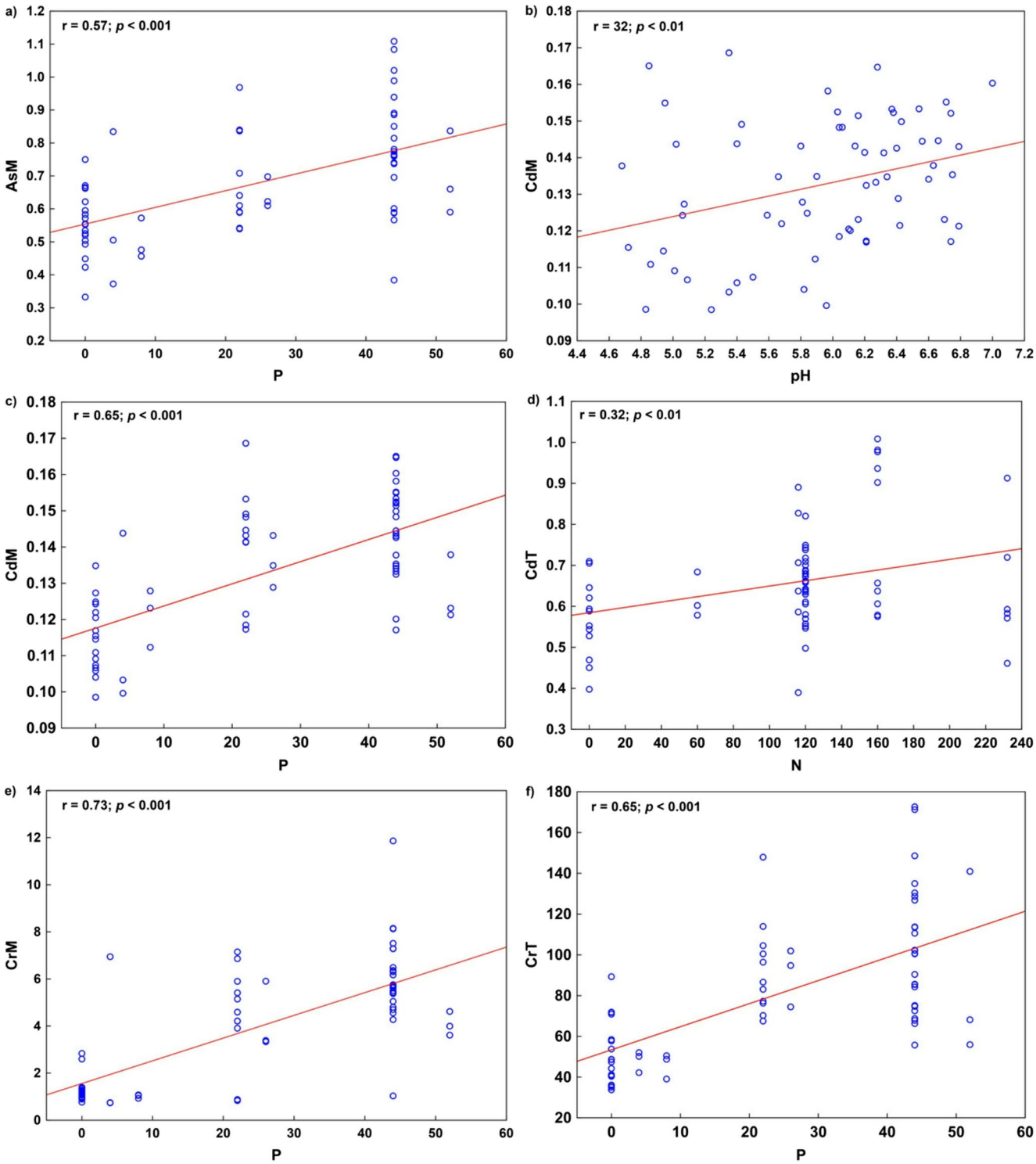


Fig. 3 Graphical representation of statistically significant regression analyses of risk element content in the soil as a function of N, P fertilisation, and pH (H_2O). The abbreviations for the different extraction methods used in the graphical analysis are as follows: Ac for $CaCl_2$ extraction, E for EDTA extraction, M for HNO_3 extraction, and T for total extraction (aqua regia). The abbreviations N and K refer to undifferentiated organic and mineral fertilisers containing phosphorus (P), nitrogen (N), and potassium (K). The values for microelement extractions are presented in mg kg^{-1}, while the amounts of fertilisers applied (N, P) are given in kg ha^{-1}

Table 5 Table of correlations for trace elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) across four different extractions (Ac—for plant-accessible content ($CaCl_2$), E—for easily mobilisable content (EDTA), M—for potentially mobilisable content (HNO_3), and T—for total content. The color scale shows increasing values for each allele within a variety. Blue indicates lower values (light to dark), white is medium, and red shows the highest values (light red to dark red)

	AsAc	CdAc	CrAc	FeAc	MnAc	NiAc	ZnAc	AsE	CdE	CrE	CuE	FeE	MnE	NiE	PbE	ZnE	AsM	CdM	CrM	CuM	FeM	MnM	MiM	PbM	ZnM	AsT	CdT	CrT	CuT	FeT	MnT	NiT	PbT	ZnT	pHH$_2$O
AsAc	1.000	0.004	0.105	-0.038	0.014	0.051	-0.076	0.070	-0.109	-0.040	0.013	-0.050	-0.114	-0.192	-0.145	-0.150	0.100	0.132	0.063	0.020	0.102	0.005	-0.025	0.176	0.015	0.067	0.027	0.167	0.087	0.169	0.083	0.186	0.149	-0.070	0.017
CdAc	0.004	1.000	0.124	-0.170	-0.335	-0.081	0.143	0.165	-0.469	-0.003	0.191	-0.015	0.173	-0.001	-0.012	-0.129	-0.104	0.064	0.053	-0.461	-0.130	-0.097	-0.004	0.178	-0.316	0.270	-0.163	0.032	-0.482	-0.024	0.049	0.411	0.433	-0.685	0.039
CrAc	0.105	0.124	1.000	0.167	0.019	0.169	0.109	-0.012	-0.146	-0.089	0.191	-0.039	0.165	0.075	0.085	0.072	0.049	0.277	0.197	0.098	0.169	0.083	0.157	0.208	0.174	0.072	0.041	0.040	0.119	0.058	0.067	0.085	0.171	-0.007	0.011
FeAc	-0.038	-0.170	0.167	1.000	0.360	0.256	0.683	0.145	-0.049	0.294	0.260	0.374	0.171	0.252	0.219	0.011	0.166	0.108	0.167	0.081	0.142	-0.045	0.065	0.083	0.175	-0.046	0.031	0.176	0.086	0.147	0.037	0.117	0.165	-0.009	-0.350
MnAc	0.014	-0.335	0.019	0.360	1.000	0.728	0.583	-0.061	0.022	-0.158	-0.028	-0.071	-0.109	0.117	0.155	0.021	-0.248	-0.363	-0.447	-0.006	-0.265	-0.436	-0.327	-0.107	0.026	0.061	-0.174	-0.343	0.200	-0.113	-0.393	-0.107	0.022	0.219	-0.674
NiAc	0.051	-0.081	0.169	0.256	0.728	1.000	0.517	0.036	-0.110	-0.148	-0.095	-0.109	-0.187	-0.032	-0.025	-0.111	-0.107	-0.242	-0.307	-0.003	-0.190	-0.356	-0.260	0.063	-0.007	0.033	-0.243	-0.279	0.123	-0.160	-0.331	-0.120	0.028	0.086	-0.522
ZnAc	-0.076	0.143	0.109	0.683	0.583	0.517	1.000	-0.062	-0.220	0.044	0.069	0.111	0.091	0.172	0.123	-0.026	-0.119	-0.121	-0.115	-0.236	-0.157	-0.257	-0.160	0.071	-0.087	0.001	-0.053	-0.071	-0.038	0.067	-0.124	0.282	0.323	-0.115	-0.553
AsE	0.070	0.165	-0.012	0.145	-0.061	0.036	-0.062	1.000	-0.084	0.330	0.629	0.416	0.289	0.300	0.502	0.007	0.203	0.166	0.177	0.058	0.146	-0.043	0.034	0.122	0.151	0.162	-0.208	0.018	-0.206	-0.199	-0.127	-0.022	0.040	-0.284	-0.068
CdE	-0.109	-0.469	-0.146	-0.049	0.022	-0.110	-0.220	-0.084	1.000	-0.036	-0.172	-0.094	-0.139	-0.095	0.069	0.532	0.111	-0.003	-0.010	0.284	0.053	0.277	0.176	-0.159	0.314	-0.132	-0.043	-0.202	0.093	-0.115	0.025	-0.310	-0.323	0.335	0.118
CrE	-0.040	-0.003	-0.089	0.294	-0.158	-0.148	0.044	0.330	-0.036	1.000	0.281	0.933	0.166	0.120	0.286	-0.009	0.288	0.248	0.388	0.015	0.302	-0.085	-0.091	0.210	-0.030	-0.108	0.115	0.385	-0.079	0.091	0.085	0.055	0.046	-0.152	-0.105
CuE	0.013	0.191	0.191	0.260	-0.028	-0.095	0.069	0.629	-0.172	0.281	1.000	0.478	0.722	0.633	0.733	-0.027	-0.062	0.035	0.013	-0.093	-0.082	-0.102	0.035	-0.045	0.040	0.186	-0.093	-0.017	-0.129	-0.154	-0.098	0.084	0.112	-0.242	-0.021
FeE	-0.050	-0.015	-0.039	0.374	-0.071	-0.109	0.111	0.416	-0.094	0.933	0.478	1.000	0.349	0.378	0.472	0.016	0.234	0.200	0.323	-0.011	0.233	-0.124	-0.050	0.116	0.004	-0.119	0.023	0.328	-0.131	0.025	-0.013	0.056	0.066	-0.189	-0.172
MnE	-0.114	0.173	0.165	0.171	-0.109	-0.187	0.091	0.289	-0.139	0.166	0.722	0.349	1.000	0.747	0.694	0.024	-0.054	0.123	0.087	-0.187	-0.047	0.045	0.232	-0.096	0.041	0.145	0.009	0.095	-0.153	-0.020	0.064	0.148	0.222	-0.246	0.094
NiE	-0.192	-0.001	0.075	0.252	0.117	-0.032	0.172	0.300	-0.095	0.120	0.633	0.378	0.747	1.000	0.742	0.064	-0.118	-0.027	-0.046	-0.096	-0.109	-0.076	0.157	-0.185	0.085	0.005	-0.142	-0.144	-0.185	-0.166	-0.165	0.013	0.085	-0.117	-0.084
PbE	-0.145	-0.012	0.085	0.219	0.155	-0.025	0.123	0.502	0.069	0.286	0.733	0.472	0.694	0.742	1.000	0.402	-0.039	0.031	0.029	-0.166	-0.088	-0.100	0.064	-0.057	0.057	0.207	-0.025	-0.047	-0.114	-0.080	-0.050	0.078	0.233	-0.139	-0.136
ZnE	-0.150	-0.129	0.072	0.011	0.021	-0.111	-0.026	0.007	0.532	-0.009	-0.027	0.016	0.024	0.064	0.402	1.000	0.030	0.065	0.057	-0.073	-0.042	0.104	0.060	-0.083	0.067	0.171	-0.080	-0.032	-0.059	-0.064	0.085	-0.097	-0.001	0.041	0.006
AsM	0.100	-0.104	0.049	0.166	-0.248	-0.107	-0.119	0.203	0.111	0.288	-0.062	0.234	-0.054	-0.118	-0.039	0.030	1.000	0.679	0.804	0.433	0.807	0.541	0.383	0.346	0.329	-0.040	0.242	0.421	0.216	0.231	0.205	-0.091	-0.007	0.040	-0.043
CdM	0.132	0.064	0.277	0.108	-0.363	-0.242	-0.121	0.166	-0.003	0.248	0.035	0.200	0.123	-0.027	0.031	0.065	0.679	1.000	0.854	0.285	0.827	0.683	0.604	0.481	0.402	0.077	0.273	0.491	0.121	0.298	0.472	0.130	0.127	-0.017	0.301
CrM	0.063	0.053	0.197	0.167	-0.447	-0.307	-0.115	0.177	-0.010	0.388	0.013	0.323	0.087	-0.046	0.029	0.057	0.804	0.854	1.000	0.275	0.835	0.608	0.431	0.392	0.273	0.043	0.286	0.579	0.083	0.296	0.409	0.067	0.139	-0.121	0.175
CuM	0.020	-0.461	0.098	0.081	-0.006	-0.003	-0.236	0.058	0.284	0.015	-0.093	-0.011	-0.187	-0.096	-0.166	-0.073	0.433	0.285	0.275	1.000	0.640	0.435	0.391	0.160	0.773	-0.159	-0.048	-0.042	0.291	-0.151	-0.107	-0.501	-0.575	0.402	0.085
FeM	0.102	-0.130	0.169	0.142	-0.265	-0.190	-0.157	0.146	0.053	0.302	-0.082	0.233	-0.047	-0.109	-0.088	-0.042	0.807	0.827	0.835	0.640	1.000	0.643	0.535	0.453	0.569	-0.043	0.222	0.433	0.194	0.204	0.246	-0.113	-0.137	0.107	0.089
MnM	0.005	-0.097	0.083	-0.045	-0.436	-0.356	-0.257	-0.043	0.277	-0.085	-0.102	-0.124	0.045	-0.076	-0.100	0.104	0.541	0.683	0.608	0.435	0.643	1.000	0.743	0.303	0.496	-0.078	0.243	0.350	0.113	0.140	0.527	-0.022	-0.060	0.143	0.560
MiM	-0.025	-0.004	0.157	0.065	-0.327	-0.260	-0.160	0.034	0.176	-0.091	0.035	-0.050	0.232	0.157	0.064	0.060	0.383	0.604	0.431	0.391	0.535	0.743	1.000	0.170	0.669	-0.056	0.051	0.237	0.063	0.125	0.392	0.057	-0.064	0.095	0.429
PbM	0.176	0.178	0.208	0.083	-0.107	0.063	0.071	0.122	-0.159	0.210	-0.045	0.116	-0.096	-0.185	-0.057	-0.083	0.346	0.481	0.392	0.160	0.453	0.303	0.170	1.000	0.126	0.017	0.148	0.256	-0.072	0.093	0.196	0.107	0.254	-0.171	0.139
ZnM	0.015	-0.316	0.174	0.175	0.026	-0.007	-0.087	0.151	0.314	-0.030	0.040	0.004	0.041	0.085	0.057	0.067	0.329	0.402	0.273	0.773	0.569	0.496	0.669	0.126	1.000	-0.047	-0.084	-0.015	0.245	-0.041	0.053	-0.245	-0.329	0.370	0.151
AsT	0.067	0.270	0.072	-0.046	0.061	0.033	0.001	0.162	-0.132	-0.108	0.186	-0.119	0.145	0.005	0.207	0.171	-0.040	0.077	0.043	-0.159	-0.043	-0.078	-0.056	0.017	-0.047	1.000	0.038	0.112	0.172	0.207	0.152	0.280	0.358	-0.212	0.003
CdT	0.027	-0.163	0.041	0.031	-0.174	-0.243	-0.053	-0.208	-0.043	0.115	-0.093	0.023	0.009	-0.142	-0.025	-0.080	0.242	0.273	0.286	-0.048	0.222	0.243	0.051	0.148	-0.084	0.038	1.000	0.495	0.484	0.727	0.610	0.450	0.376	0.361	0.144
CrT	0.167	0.032	0.040	0.176	-0.343	-0.279	-0.071	0.018	-0.202	0.385	-0.017	0.328	0.095	-0.144	-0.047	-0.032	0.421	0.491	0.579	-0.042	0.433	0.350	0.237	0.256	-0.015	0.112	0.495	1.000	0.210	0.641	0.725	0.452	0.404	-0.102	0.244
CuT	0.087	-0.482	0.119	0.086	0.200	0.123	-0.038	-0.206	0.093	-0.079	-0.129	-0.131	-0.153	-0.185	-0.114	-0.059	0.216	0.121	0.083	0.291	0.194	0.113	0.063	-0.072	0.245	0.172	0.484	0.210	1.000	0.519	0.275	0.014	-0.089	0.741	-0.049
FeT	0.169	-0.024	0.058	0.147	-0.113	-0.160	0.067	-0.199	-0.115	0.091	-0.154	0.025	-0.020	-0.166	-0.080	-0.064	0.231	0.298	0.296	-0.151	0.204	0.140	0.125	0.093	-0.041	0.207	0.727	0.641	0.519	1.000	0.724	0.725	0.571	0.247	0.070
MnT	0.083	0.049	0.067	0.037	-0.393	-0.331	-0.124	-0.127	0.025	0.085	-0.098	-0.013	0.064	-0.165	-0.050	0.085	0.205	0.472	0.409	-0.107	0.246	0.527	0.392	0.196	0.053	0.152	0.610	0.725	0.275	0.724	1.000	0.565	0.465	0.094	0.539
NiT	0.186	0.411	0.085	0.117	-0.107	-0.120	0.282	-0.022	-0.310	0.055	0.084	0.056	0.148	0.013	0.078	-0.097	-0.091	0.130	0.067	-0.501	-0.113	-0.022	0.057	0.107	-0.245	0.280	0.450	0.452	0.014	0.725	0.565	1.000	0.763	-0.195	0.094
PbT	0.149	0.433	0.171	0.165	0.022	0.028	0.323	0.040	-0.323	0.046	0.112	0.066	0.222	0.085	0.233	-0.001	-0.007	0.127	0.139	-0.575	-0.137	-0.060	-0.064	0.254	-0.329	0.358	0.376	0.404	-0.089	0.571	0.465	0.763	1.000	-0.409	-0.042
ZnT	-0.070	-0.685	-0.007	-0.009	0.219	0.086	-0.115	-0.284	0.335	-0.152	-0.242	-0.189	-0.246	-0.117	-0.139	0.041	0.040	-0.017	-0.121	0.402	0.107	0.143	0.095	-0.171	0.370	-0.212	0.361	-0.102	0.741	0.247	0.094	-0.195	-0.409	1.000	-0.003
pHH$_2$O	0.017	0.039	0.011	-0.350	-0.674	-0.522	-0.553	-0.068	0.118	-0.105	-0.021	-0.172	0.094	-0.084	-0.136	0.006	-0.043	0.301	0.175	0.085	0.089	0.560	0.429	0.139	0.151	0.003	0.144	0.244	-0.049	0.070	0.539	0.094	-0.042	-0.003	1.000

with various organic and mineral N, P, and K fertilisers. The main question was to determine whether 75 years of fertilisation of the permanent grassland had an impact on higher levels of micro and potentially toxic elements in the soil through extractions using $CaCl_2$, EDTA, HNO_3, and total extractions.

To assess the risks of contamination, it was necessary to compare the results with other long-term experiments and the legislative limits for agricultural soils. This process is quite complex because obtaining a single value is not entirely straightforward. For instance, European limits (EEA 2023) establish specific thresholds for certain elements in soil: Germany 2–20, Czechia 1–1.5.5, Austria 1–40, and Denmark 5 mg kg^{-1} for Cd, most commonly based on aqua regia extraction. The values can vary by one or two levels of magnitude elements, such as Cu. It should be noted that the applicable legislation varies from one country to another. For purposes of comparison, the Czech legislative (DME CZ No. 153/2016 Coll. (n.d.)), which regulates specific details of agricultural soil protection, has been utilised. This legislation lays down specific details of farmland protection and precisely defines preventive and informative values, making it one of the most stringent in the EU.

4.2 Influence of phosphorus fertilisation on arsenic mobility

The content of AsAc and AsE in the fertilised and control plots showed consistently low and comparable average values, as seen also in previous studies (Hejcman et al. 2009; Uprety et al. 2009), where the long-term effect of various fertilisers was assessed, as well. All these results confirmed the limited mobility of As in the soil (Kumpiene et al. 2008; Resongles et al. 2014; Száková et al. 2016; Palansooriya et al. 2020). As is not very mobile because it is easily precipitated as sulfates and carbonates and is strongly adsorbed by clays, oxides, and hydroxides; however, it is relatively mobile in acidic soil (Palansooriya et al. 2020).

The contents of AsT slightly exceeded the preventive limits (DME CZ No. 153/2016 Coll. (n.d.)), but as already mentioned, the real total contents of elements were determined in this study. Száková et al. (2000) reported 85% as an average mobile proportion of As extractable with aqua regia compared to the real total. Therefore, the overall total As contents remained low across all plots after 75 years of fertilisation, as also observed at other long-term fertilised localities (Hejcman et al. 2009; Uprety et al. 2009; Jungová et al. 2022). The RDA analysis revealed only a weak impact of PA fertilisation on AsAc and AsE in soil.

Similarly, Sisr et al. (2007) observed no significant effect in the mobile fraction of As in soil with P fertilisation in laboratory settings. However, there was a positive effect of PA fertilisation on AsM content in the soil, which is consistent with other studies Dissanayake and Chandrajith 2009; Zhao and Wang 2020), where the application of P fertilisers has led to a significant increase of As accumulation and mobility in some agricultural soils. Moreover, this is consistent with the results of Hejcman et al. (2009), in which the phosphate fertiliser Thomas' slag was also used, suggesting that fertilisers may be a source of As in the soil. This interpretation is consistent with findings from the Park Grass Experiment, where long-term application of

phosphate fertilisers before 1948 led to a near doubling of total soil As concentrations (from ca. 10 to 20 mg kg^{-1}) (Hartley et al. 2013). The authors demonstrated that historic superphosphate contained exceptionally high As levels (401–1575 mg kg^{-1}), which fully accounted for the observed accumulation through fertiliser inputs. In contrast, post-1948 phosphate fertilisers contained < 20 mg As kg^{-1}, resulting in stable soil As concentrations thereafter. These results highlight that As accumulation patterns in long-term grassland experiments are primarily controlled by the composition of the applied phosphate sources and their historical variability. Despite this, variations in AsM content across the experimental plots may be attributed to water leaching or plant uptake (Wang et al. 2016), the different soil pH levels (Newton et al. 2006; Niazi et al. 2018), or variable soil sorption (Mehmood et al. 2017).

4.3 Cadmium accumulation and potential sources

Cadmium is of particular interest since its elevated content in many phosphate fertilisers, which, were applied continuously, can result in cadmium accumulation in soils and subsequent increase of Cd content in plant biomass (Taylor and Percival 2001; Németh et al. 2002; Chen et al. 2007; Bolan et al. 2013). In this experiment, Cd exhibited a different trend than As, with the preventive limit (DME CZ No. 153/2016 Coll. (n.d.)) being exceeded on 60 plots.

According to Száková et al. (2000), the aqua regia-soluble fractions of Cd represent approximately 80% of the real Cd total content, which should be which should be considered when interpreting the results. The elevated Cd levels may be partially explained by natural origins, as reported in the Rengen Grassland Experiment (Hejcman et al. 2009). Furthermore, Cd could have been present before the experiment due to anthropogenic or geogenic sources in the area, as it can persist in ecosystems for long periods (Abaye et al. 2005; Shaheen and Tsadilas 2010; Alloway 2013; Palansooriya et al. 2020). Occasional flooding may have also contributed to the elevated Cd levels (Dennis et al. 2009; Delgado et al. 2010; Middelkoop et al. 2010; Magesh et al. 2011; Aleksander-Kwaterczak and Ciszewski 2012).

Despite this, the CdT values ranged from 0.01 to 1.22 mg kg^{-1}, and CdM ranged from 0.01 to 0.84 mg kg^{-1}, regardless of the type or amount of fertiliser applied; thus the indicative limits were not exceeded (DME CZ No. 153/2016 Coll. (n.d.)). Consequently, the determined Cd levels can be considered low, in comparison with the global soil Cd range reported by Kabata-Pendias and Pendias (2001).

Nevertheless, multivariate analysis showed a strong positive correlation between PA fertilisation and the CdM content in the soil. Although Cd concentrations in Thomas slag, which is predominantly used as phosphate fertiliser, are generally low due to the steel process (which involves temperatures that cause Cd volatilisation) (Hejcman et al. 2009), its potential contribution as a source of soil Cd cannot be ruled out.

Regression analysis further revealed a positive correlation between soil pH and CdM content. Higher pH values corresponded to increased CdM levels, consistent with findings by Jiao et al. (2012) and Vaněk et al. (2012), who observed Cd precipitation in alkaline environments, leading to its retention in the solid phase of the soil. Similar outcomes were reported in experiments conducted by Hejcman et al. (2009) and Uprety et al. (2009).

4.4 Chromium enrichment and its connection to fertiliser application

In contrast to other long-term experiments (Hejcman et al. 2009; Uprety et al. 2009), the average CrM and CrT content were significantly higher and exceeded the limits allowed by Czech legislation (DME CZ No. 153/2016 Coll. (n.d.)) in several plots. Chromium speciation was not determined; however, under the slightly acidic to neutral pH at the SGE, trivalent chromium (Cr^{3+}) is expected to dominate, while historical phosphate fertilisers were known to contain relatively high total Cr concentrations (Kabata-Pendias and Pendias 2001; Violante et al. 2010; Hejcman et al. 2009; Uprety et al. 2009; Zhang et al. 2025). Moreover, the regression analysis results indicated a positive correlation between CrM and CrT contents and phosphorus application. This trend was supported by the RDA analysis, which showed the PA application's positive effect on soil CrE, and CrM content. A likely proximate source is the historical use of Thomas phosphate (Thomasmehl/basic slag) in Central Europe. Although the precise Cr oxidation state or chemical form was not analysed in this study, independent monitoring reports have shown that historical Thomas phosphate products frequently contained elevated total Cr concentrations — up to ~ 1,800 mg kg^{-1} — compared to other phosphate fertilisers (Kühnen and Goldbach 2004). Therefore, it is reasonable to infer that long-term P inputs, particularly from basic slag, could have contributed to the observed enrichment of total Cr in SGE soils. In addition, pre-1950 phosphate fertilisers often contained As impurities (Hartley et al. 2013), which provides a plausible pathway for concurrent As and Cr accumulation in P-amended plots (Vogel et al. 2020; Mehmood et al. 2021). At the same time, it was observed that lower Cr levels were recorded in areas where no fertiliser application was applied. Nevertheless, the CrM content remained low in most experimental plots with no clear trend. However, the higher CdT content was recorded in plots where N160P44K174 $(NH_4)_2SO_4$ was used, suggesting that more acidic fertilisers may be a source of Cr

in the soil. The higher accumulation of total Cr in the soil could probably be related to the past application of Thomas slag, which is among the phosphate fertilisers historically reported to contain relatively high total chromium concentrations (Hejcman et al. 2009; Uprety et al. 2009). It should be emphasised that, because no archived fertiliser samples from the early SGE period are available, the chromium speciation and precise concentrations in historical inputs cannot be verified. The interpretations presented here therefore refer to total soil Cr patterns rather than specific oxidation states.

It should also be noted that the apparent exceedances of Czech preventive or indicative values for Cr and As reflect the use of a complete HF digestion, which provides real-total element concentrations higher than those obtained by the aqua regia extraction applied in regulatory frameworks. Consequently, the reported Cr and As values represent upper-bound estimates rather than direct regulatory exceedances.

When adjusted to aqua regia–equivalent levels (typically 60–70% of the HF "real total"), the Cr concentrations observed in SGE soils would mostly fall within the indicative range of European reference values. This methodological distinction should be considered when comparing results across studies and evaluating potential environmental risks. Although the precise composition of the historical fertilisers used in the SGE was not systematically recorded, it is reasonable to assume that the phosphorus fertiliser applied during the early decades consisted of Thomas slag, as was common practice in Germany and neighbouring regions. Comparable materials analysed in the Rengen and Ruzyně Grassland Experiments contained elevated concentrations of chromium and arsenic (Kühnen and Goldbach 2004; Hejcman et al. 2009; Uprety et al. 2009; Hartley et al. 2013; Smolders and Six 2013), supporting the interpretation that Thomas slag represents a likely source of these elements in the SGE soils. In addition, $(NH_4)_2SO_4$, a commonly used fertiliser, can be generated as a by-product in the production of caprolactam, which is used in the synthesis of polyamide 6 (nylon). Due to its industrial origin, ammonium sulphate may be contaminated with heavy metals, including Cr, which could pose a potential risk to soil (Shukla and Jain 2022; Tasleem et al. 2023; Mehmood et al. 2021). Unlike Park Grass Experiment, which is situated on silty clay loam over chalk and experiences a mild oceanic climate, the SGE is located on a pseudogleyic alluvial soil under a more continental climate (Tilman et al. 1994; Silvertown et al. 2006). These differences likely contribute to the slower geochemical response and distinct accumulation pattern of potentially toxic elements such as As, Cd, and Cr. Moreover, while the Rothamsted plots rely exclusively on mineral fertilisers, the SGE uniquely combines mineral and organic sources (e.g., dung water and Thomas slag), introducing an additional complexity to element inputs and cycling. These inter-experimental contrasts highlight that while both sites experienced element enrichment through long-term fertilisation, the magnitude and pathways of accumulation at SGE are strongly mediated by soil type, hydrological regime, and the mixed mineral–organic fertilisation system. Similar historical contamination patterns were observed in the Park Grass Experiment, where the use of early superphosphates also led to marked As enrichment of the topsoil (Hartley et al. 2013), supporting the interpretation that trace-metal accumulation in SGE soils mainly reflects the long-term input of historically contaminated phosphate fertilisers rather than recent applications.

4.5 Impact of $(NH_4)_2SO_4$ and P fertilisation on microelemets availability

Plots fertilised with $(NH_4)_2SO_4$ and receiving annual P applications of 44 kg ha^{-1} exhibited the highest FeE, and CuM content, further supported in the RDA analysis. The utilisation of $(NH_4)_2SO_4$ fertiliser likely resulted in a greater degree of soil acidification, leading to an increase in the bioavailability of these microelements in the soil phase accessible to plants (Colombo et al. 2014).

The extractable contents of microelements Zn, Mn, Ni, and Pb in the experimental plots were similar and comparable to the unfertilised controls, regardless of the fertiliser applied. However, the FeM content was found to be higher than reported by Jungová et al. (2022), but lower than found by Hejcman et al. (2009), possibly due to differences in soil composition (Száková et al. 2016). Although RDA analysis showed a positive correlation of MnT with PA application, the availability of microelements was negligible (Kabata-Pendias and Pendias 2001; Alloway 2013; Jungová et al. 2022). Furthermore, the concentration of MnM and MnT in the soil tended to increase with higher P fertilisation rates and with higher soil pH, similar to the findings of Huang et al. (2015).

4.6 Soil acidification as a consequence of long-term fertilisation

Different types of fertilisation resulted in a wide soil pH gradient, ranging from slightly acidic to neutral. As studies have shown (Silvertown et al. 2006; Clark et al. 2007; Schroder et al. 2011; Hejcman et al. 2014), long-term fertilisation with $(NH_4)_2SO_4$ can lead to pronounced soil acidification. This effect results from the uptake of NH_4^+ by plant roots, which releases H^+ ions into the soil solution, whereas the uptake of NO_3^- leads to the release of OH^- ions, increasing soil pH (Hejcman et al. 2014; Tamir et al. 2021;

Naeem et al. 2023). The resulting decrease in pH enhances the solubility and mobility of metals such as Cd and Cr by promoting their desorption from exchange sites and carbonates. Although some collinearity between fertilisation treatments and soil pH cannot be completely excluded, partial correlation analyses confirmed that both factors significantly and independently affect metal mobility in SGE soils (Kabata-Pendias and Pendias 2001; Bolan et al. 2003; Violante et al. 2010; Hejcman et al. 2014). Comparable ecological trajectories have been reported from other European long-term grassland experiments. In the Rengen Grassland Experiment (Hejcman et al. 2009), continuous application of basic slag and phosphate fertilisers resulted in gradual enrichment of Cr and As in the topsoil, confirming the persistence of trace-element inputs from historical fertilisation. At the Ruzyně Fertiliser Experiment (Uprety et al. 2009), mineral and organic treatments influenced soil Cd, Cu, Mn, and Zn, with poultry litter and superphosphate identified as key sources, although concentrations remained below legal limits. These observations are consistent with the SGE findings, where historical fertiliser composition and soil pH jointly determined element accumulation and mobility.

Ecologically, the SGE also parallels alluvial meadow studies such as Černíkovice (Hrevušová et al. 2009), where long-term fertilisation and subsequent cessation produced sustained effects on soil P and K status and delayed recovery of plant species composition. Such parallels underscore that alluvial grasslands, in contrast to upland systems like Park Grass, retain the chemical and biological legacies of fertilisation for decades after treatment cessation. Similar long-term grassland experiments, such as the Park Grass Experiment at Rothamsted (Crawley et al. 2005; Silvertown et al. 2006), have demonstrated profound impacts of nitrogen and phosphorus fertilisation on soil acidification, nutrient cycling, and plant community composition. However, the extent of these changes in the SGE appears to be more moderate. While soil pH in Park Grass declined to values below 4.0 in some treatments, the minimum pH observed in Steinach rarely dropped below 5.0, likely due to the buffering capacity of the alluvial soils and the higher groundwater table. This indicates that soil type and hydrological regime play crucial roles in modulating acidification and metal mobility.

4.7 Elemental affinity and extraction methodology

The differences in correlation strength among extraction methods reflect the specific chemical affinities of trace elements to soil constituents and the varying aggressiveness of extractants. Each extraction method provides complementary ecological information by targeting element pools that differ in mobility, bioavailability, and potential environmental risk. $CaCl_2$-extractable (Ac) fractions, representing the most mobile and plant-available pool, exhibited generally weak correlations with other methods. This indicates that elements such Cr and Pb occur mainly in more strongly bound forms due to their low solubility and strong adsorption to mineral or organic surfaces (Rékási and Filep 2015). In contrast, elements such as Mn, Ni, Zn, and Fe showed slightly stronger $CaCl_2$ correlations, suggesting their higher mobility in the soil solution and greater potential for plant uptake under current soil conditions (Violante et al. 2010). Ecologically, this fraction reflects the short-term response of metals to changes in pH and ionic strength, thus providing an indicator of immediate bioavailability (Houba et al. 2000).

EDTA-extractable (E) fractions, which dissolve elements complexed with organic matter and Fe/Mn oxides, showed moderate correlations with the HNO_3-extractable (M) pool, particularly for Fe, Mn, and As ($r = 0.5–0.7$). These results support the notion that these elements form moderately labile complexes that can become mobile under changing redox or organic matter conditions (Rennert and Rinklebe 2017). In contrast, Cd exhibited weak correlations with EDTA, indicating that its solubility in SGE soils may be driven more by ionic equilibria than organic chelation (Rékási and Filep 2015). The high extractability of Fe in the EDTA fraction, along with strong correlations with other elements, likely reflects its abundance and high complexation capacity, which may allow Fe to outcompete other metals for chelation sites (Wang et al. 2013). From an ecological standpoint, this fraction approximates the pool potentially mobilisable by root exudates or microbial ligands, thus bridging the gap between bioavailable and geochemically stable forms.

The HNO_3-extractable (M) and „real total“ (T) extraction displayed the strongest mutual correlations across elements ($r > 0.8$), particularly for Fe, Mn, Ni, and Zn. This pattern indicates that a large proportion of the potentially mobilisable pool contributes directly to the total element stock. Since both extraction methods reflect elements associated with silicates and resistant oxides, their strong linkage suggests that long-term fertilisation and pH control the slow accumulation of metals in more recalcitrant soil fractions (Rao et al. 2008; Ivezić et al. 2011). Ecologically, these pools represent the long-term retention and potential legacy of metal enrichment in soils.

Overall, the correlation patterns confirm that each extraction method targets the ecological compartment of trace elements: $CaCl_2$ reflects the short-term, plant available pool; EDTA and HNO_3 represent intermediate, potentially mobilisable forms; and „ real total“ extraction indicates the stable lithogenic background. Consequently, relying solely on total concentrations may overestimate ecological risk (Borůvka et al. 1997), whereas integrating multiple extraction procedures provides a more realistic picture of metal mobility, bioavailability, and environmental behaviour in SGE soils.

5 Conclusion

Long-term fertilisation at the SGE caused moderate enrichment of several risk elements, particularly As, Cr, and Cd, mainly in plots receiving phosphorus fertilisers such as Thomas slag and ammonium sulphate. However, the overall element contents remained below European regulatory thresholds, confirming that sustained fertilisation did not lead to critical soil contamination.

Among the monitored elements, phosphorus input was the main driver of Cr and As accumulation, while nitrogen fertilisation enhanced the mobility of As and Cd. These findings highlight the importance of continued monitoring to detect gradual changes in element mobility under long-term management.

Based on the results from Steinach and comparable long-term grassland experiments such as Park Grass, we recommend that future monitoring prioritise both total and mobile fractions—specifically, $CaCl_2$-extractable As, HNO_3-extractable Cd, and EDTA-extractable Cr—as key indicators of bioavailability and environmental risk.

Monitoring should be conducted every five years under stable soil conditions and increased to every 2–3 years when soil pH (H_2O) falls below 5.5 or when fertilisation practices change. Thresholds should follow national soil protection limits (e.g., Czech Decree No. 153/2016 Sb.) but be interpreted in relation to soil pH and organic matter content. If mobile As, Cd, or Cr exceed indicative values, follow-up plant testing and replacement of slag-based phosphorus sources are advised.

Overall, the SGE demonstrates that even after 75 years, balanced fertilisation can sustain soil fertility without exceeding environmental safety limits. Its long history, diverse treatments, and alluvial soil context make it an essential complement to oceanic systems such as Park Grass, broadening our understanding of nutrient cycling and trace-element behaviour under contrasting climatic and edaphic conditions.

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Data availability The data sets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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