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## Long-term effects of organic and mineral fertilisation on soil manganese dynamics and agricultural sustainability

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**Abstract:** Manganese (Mn) is often underestimated in plant nutrition. Its availability to plants is influenced by several factors, which can lead to Mn deficiency or toxicity. The objective was to evaluate the transformation of soil Mn over 21 years in a long-term field experiment. Fertilising with (i) sewage sludge 1 (SS<sub>1</sub>); (ii) sewage sludge 3 (3 times higher nitrogen (N) dose, SS<sub>3</sub>); (iii) farmyard manure (FYM); (iv) mineral nitrogen, phosphorus and potassium (NPK) and (v) mineral nitrogen in addition to straw (Nst) was studied to evaluate the transformations of Mn in soil using different extraction methods at the 5 locations. There was a general reduction in the pH during the experiment. Soil acidification caused by mineral N fertiliser increased the bioavailable Mn forms under NPK treatment. This Mn was mobilised from soil reserves, leading to depletion of Mn sources. Application of SS and FYM led to an increase in non-bioavailable Mn fractions, while the expected increase in biologically available Mn was not observed. As the high pH of soil limits Mn availability, foliar Mn application can be recommended for agricultural practice in high-pH soils. On the contrary, liming can be recommended for low-pH soil with high bioavailable Mn content to mitigate the risk of Mn toxicity.

**Keywords:** soil manganese bioavailability; soil acidification; nutrient immobilisation; nonexchangeable and pseudo-total manganese

Micronutrient deficiency is a major regional and global problem affecting more than two billion people (Bailey et al. 2015). Fluctuating soil micronutrient levels and insufficient replenishment of nutrients removed by crops negatively affect productivity (Monreal et al. 2016). Although mineral fertilisers contribute substantially to nutrient supply, their unbalanced use can aggravate deficiencies, whereas combined organic and mineral fertilisation increases micronutrient availability and improves soil properties (Aulakh and Grant 2008).

Manganese (Mn), the eleventh most abundant element in the Earth's crust, occurs in soils at 20–3 000 mg/kg (average 600 mg/kg). Mn<sup>2+</sup> is the key form for plant

uptake, largely associated with clays and organic matter (Malakouti and Tehrani 2001). In soils, Mn appears as exchangeable Mn, Mn oxides, organically bound Mn, and Mn in silicates, and its availability is strongly controlled by pH, organic matter, moisture, and aeration (Schulte and Kelling 1999). Because Mn<sup>2+</sup> is readily oxidised to Mn<sup>3+</sup> or Mn<sup>4+</sup>, it plays a central role in redox reactions, photosynthetic electron transport, and enzyme activation (Millaleo et al. 2010).

Mn deficiency reduces non-structural carbohydrates, particularly in roots (Zewide and Sherefu 2021), and lowers crop yield and quality due to impaired pollen formation and reduced carbohydrate

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supply during grain filling. Symptoms resemble magnesium deficiency but appear first on young leaves because Mn mobility in plants is limited (Alejandro et al. 2020, Rengel et al. 2022). Conversely, Mn toxicity, common in acidic soils, restricts growth by inhibiting photosynthesis (Obeng et al. 2024, Zhu et al. 2025). Manganese toxicity in plants varies according to plant species and environmental factors (Obeng et al. 2024). It is a primary factor restricting development in acidic soils, where excessive amounts of manganese in the leaves inhibit photosynthesis and thus limiting growth (Zhu et al. 2025).

Long-term use of farmyard manure (FYM) generally increases the availability of Zn, Cu, Fe, and Mn. Significant increases in DTPA-extractable micronutrients were observed under FYM in a maize-wheat system (Mann et al. 1978). Understanding micronutrient uptake is essential for assessing both deficiency and toxicity risks (Šimić et al. 2009). However, instead of monitoring the plant, it is much easier to monitor soil systems, from where the plant is taking up the nutrients. The two main approaches for explaining soil nutrient bioavailability are single-extraction and sequential-extraction procedures. However, sequential extraction has been criticised for its lack of selectivity (Peijnenburg et al. 2007). Chelating agents such as DTPA and EDTA estimate metal availability, but both may extract more aggressively than natural processes (Meers et al. 2007). EDTA correlates well in acidic soils (Kovačević et al. 2002) but poorly in neutral to alkaline soils, while DTPA performs better in neutral and alkaline conditions but correlates poorly in acidic soils (Feng et al. 2005). Strong-acid extractions provide pseudo-total metal contents but are poor indicators of toxicity, although still widely used in regulations.

Mn deficiency is widespread, particularly in calcareous, high-pH soils and poorly aerated environments; Mn solubility decreases 100-fold with each unit increase in pH (Mousavi et al. 2011). While pH effects are well documented, the influence of fertilisation systems, especially sewage sludge, on bioavailable and pseudototal Mn remains insufficiently understood. This study therefore tested the hypotheses that: (i) organic fertilisers (FYM, sewage sludge) increase soil bioavailable Mn; (ii) mineral N fertilisation exacerbates Mn deficiency relative to the control; and (iii) soil pH predominates over fertilisation effects in determining Mn availability.

## MATERIAL AND METHODS

Long-term field trials were established in 1996 at five experimental stations of the Czech University of Life Sciences (Červený Újezd, Hněvčeves, Humpolec, Lukavec, Prague-Suchdol) under contrasting soil and climatic conditions (Table 1). In 2017, after finishing the 7<sup>th</sup> crop rotation, four replications per treatment were evaluated. A three-crop rotation (potatoes, winter wheat, spring barley) is used, except at Červený Újezd, where silage maize replaces potatoes. Six treatments were studied: unfertilised control (CON), two sewage sludge doses (SS<sub>1</sub>, SS<sub>3</sub>; where the SS<sub>3</sub> treatment received a 3 times higher N dose as compared to SS<sub>1</sub>), farmyard manure (FYM), mineral NPK fertilisation (NPK), and mineral N combined with straw (Nst). Organic fertilisers are applied in autumn after barley harvest to potatoes or maize. The same sewage sludge was used across sites; FYM and straw originated locally.

Phosphate and potassium mineral fertilisers are applied to all crops in autumn. Mineral nitrogen fertilisers

Table 1. Characteristics of department experimental fields

	Červený Újezd	Hněvčeves	Humpolec	Lukavec	Suchdol
GPS location	50°4'22"N, 14°10'19"E	50°18'46"N, 15°43'3"E	49°33'16"N, 15°21'2"E	49°33'23"N, 14°58'39"E	50°7'40"N, 14°22'33"E
Latitude (m a.s.l.)	410	265	525	610	286
Average yearly temperature (°C)	7.7	8.2	7.0	7.7	9.1
Average yearly precipitation (mm)	493	573	665	666	495
Soil type <sup>1</sup>	Luvisol	Luvisol	Cambisol	Cambisol	Chernozem
Soil texture <sup>1</sup>	Loamy	Clayey-loam	Sandy-loam	Sandy-loam	Loamy
pH <sub>CaCl<sub>2</sub></sub> <sup>2</sup>	6.5	5.9	5.1	4.3	7.5
Total organic carbon (%)	1.7	1.6	2.3	1.7	2.6
Cation exchange capacity (mmol <sub>+</sub> /kg)	145	179	159	128	230

<sup>1</sup>NRCS USDA; <sup>2</sup>0.01 mol/L CaCl<sub>2</sub> extraction

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are applied to potatoes and barley before sowing. In the case of wheat, the nitrogen dose is divided into two halves. The first is applied as a regenerative fertiliser, the second as a production fertiliser. The nutrient rates of the fertilisers applied are given in Table 2. The size of the experimental plot is 60 m<sup>2</sup> at the Humpolec, Lukavec, and Hněvčeves sites, 60.5 m<sup>2</sup> at the Suchdol site, and 80 m<sup>2</sup> at the Červený Újezd site.

Topsoil samples (0–30 cm) were air-dried, sieved (2 mm), and analysed for the following Mn fractions: readily available forms (Mn<sub>CaCl<sub>2</sub></sub>, Mn<sub>H<sub>2</sub>O</sub>), exchangeable Mn (Mn<sub>Ac</sub>), potentially available Mn (Mn<sub>M3</sub>), nonexchangeable Mn (Mn<sub>NE</sub>), and pseudo-total Mn (Mn<sub>AR</sub>).

Mn<sub>H<sub>2</sub>O</sub> was determined following Luscombe et al. (1979) by shaking soil with distilled water (1:10 w/v, 60 min, 120 rpm), centrifugation (8 000 g). Mn<sub>CaCl<sub>2</sub></sub> was extracted with 0.01 mol/L CaCl<sub>2</sub> (1:10 w/v, 60 min), used first for pH determination, then centrifuged (5 000 g).

Mehlich 3 Mn (Mn<sub>M3</sub>) followed Mehlich (1984): soils were extracted (1:10, w/v) with the standard solution, shaken for 5 min (120 rpm), and filtered. Exchangeable Mn was determined using ammonium acetate (Warncke and Brown 1998): 3 g soil shaken with 30 mL extractant (120 min), centrifuged (8 000 g).

Mn<sub>NE</sub> was obtained by boiling 2.5 g of soil with 25 mL of 1 mol/L HNO<sub>3</sub> for 10 min, filtering to 100 mL, and analysing the filtrate by ICP-OES. Pseudo-total Mn followed modified ISO 11466:1995 using *aqua regia* with microwave digestion and evaporation to wet residue, then diluted to 25 mL.

All Mn contents in the extracts were measured using an optical emission spectrometer with inductive coupled plasma (ICP-OES, Varian Vista-Pro, Mulgrave, Australia).

For better orientation in the following text, here is a list of individual forms of manganese in relation to their availability to plants: Mn<sub>H<sub>2</sub>O</sub> and Mn<sub>CaCl<sub>2</sub></sub> are readily available Mn forms. Both of these forms can be immediately taken up with plants. Mn<sub>Ac</sub> represents the exchangeable fraction weakly sorbed on the soil exchangeable sorption complex. Mn<sub>M3</sub> is the potentially available fraction, where the strong Mehlich 3 extractant can dissolve even a small ratio of hardly available Mn forms. Mn<sub>NE</sub> and Mn<sub>AR</sub> both represent non-bioavailable Mn, with Mn<sub>AR</sub> considered a residual (close to total) Mn content. Additionally, Mn<sub>H<sub>2</sub>O</sub>, Mn<sub>CaCl<sub>2</sub></sub>, Mn<sub>Ac</sub> and Mn<sub>M3</sub> belong to the bioavailable Mn forms as well.

Statistical evaluation included one-way ANOVA, Tukey's *HSD* (honestly significant difference) test ( $P < 0.05$ ), Pearson correlation, and linear regression ( $y = ax + b$ ).

## RESULTS

Across the five experimental sites, several consistent trends in soil Mn dynamics emerged despite contrasting soil types and climatic conditions. In general, fertilisation systems primarily affected Mn fractions indirectly through changes in soil pH rather than through Mn inputs themselves. Mineral NPK fertilisation was consistently associated with higher contents of bioavailable Mn forms, whereas organic fertilisation (FYM, SS<sub>1</sub>, SS<sub>3</sub>) tended to increase non-bioavailable Mn pools without a corresponding increase in readily or exchangeable Mn. Local deviations from these general patterns were mainly attributable to site-specific soil properties, particularly initial pH and buffering capacity.

Soil pH strongly controls Mn behaviour; pH trends were therefore evaluated (Figure 1). Across sites and

Table 2. Applied nutrient doses (kg/ha for 3-year crop rotation)

Treatment	Potatoes/maize				Wheat			Barley		
	N	P	K	Mn	N	P	K	N	P	K
CON	–	–	–	–	–	–	–	–	–	–
SS <sub>1</sub>	330 <sup>1</sup>	201 <sup>2</sup>	55 <sup>2</sup>	3.60 <sup>2</sup>	0	0	0	0	0	0
SS <sub>3</sub>	990 <sup>1</sup>	603 <sup>2</sup>	165 <sup>2</sup>	10.8 <sup>2</sup>	0	0	0	0	0	0
FYM	330 <sup>1</sup>	118 <sup>2</sup>	374 <sup>2</sup>	6.70 <sup>2</sup>	0	0	0	0	0	0
NPK <sup>3</sup>	120	30	100	0	140	30	100	70	30	100
Nst <sup>5</sup>	138	62	47 <sup>2</sup>	0.14	140	0	0	70	0	0

<sup>1</sup>Total nitrogen applied with organic fertilisers; <sup>2</sup>average rate by nutrient content of fertiliser – organic fertilisers are applied only to potatoes/maize; <sup>3</sup>Mineral fertilisers: N – CAN (27% N); P – triple superphosphate (21% P); K – potassium muriate (50% K); The doses of P, K and Mn differed only slightly according to different FYM (farmyard manure) applied to the locations; <sup>5</sup>5 t/ha of barley straw applied before potatoes; CON – unfertilised control

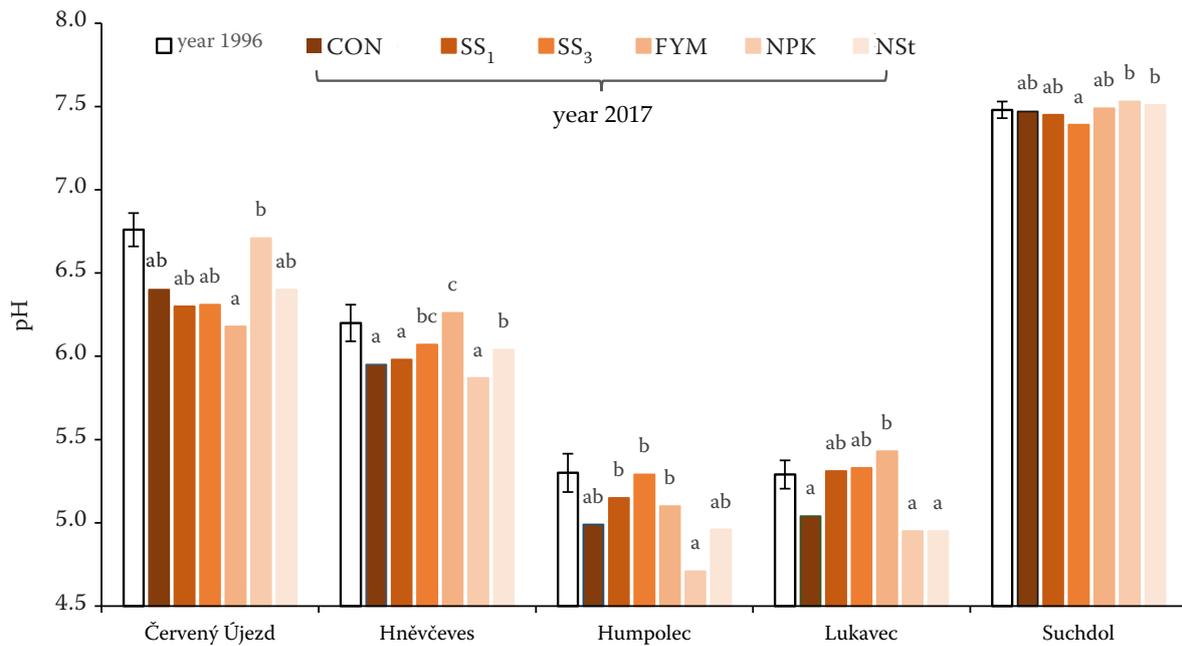


Figure 1. Soil pH changes at various locations in 1996 and 2017. Different letters in the frame of locations are meaning significant differences among treatments in the year 2017 (Tukey's test,  $P < 0.05$ ). CON – unfertilised control;  $SS_1$ ,  $SS_3$  – sewage sludge doses, where the  $SS_3$  treatment received a 3 times higher nitrogen (N) dose as compared to  $SS_1$ ; FYM – farmyard manure; NPK – mineral NPK fertilisation; Nst – mineral N combined with straw

treatments, pH generally decreased, with the lowest values typically under NPK. Even the CON treatment showed pH decline at all sites, most pronounced at Červený Újezd. The alkaline Suchdol site exhibited only slight decreases due to buffering by the calcareous parent material. Treatments with organic fertilisers (SS, FYM) often showed the smallest pH changes or even a slight increase.

Table 3 summarises Mn levels in 1996, before fertilisation.

Readily available Mn extracted with  $CaCl_2$  was approximately tenfold higher than  $Mn_{H_2O}$ .  $Mn_{Ac}$  ranged from 8.63 to 69.8 mg/kg, while  $Mn_{M3}$  values were higher (105–179 mg/kg). Bioavailable Mn fractions were generally higher in low-pH soils.  $Mn_{NE}$  contents

ranged from 450 to 902 mg/kg and  $Mn_{AR}$  from 586 to 1 215 mg/kg. The lowest Mn content was observed at Suchdol, whereas Humpolec showed the highest Mn content, except for  $Mn_{M3}$ .

Despite differences in soil-climate conditions, similar trends emerged after 21 years of fertilisation (Table 4). All sites showed the highest  $Mn_{H_2O}$  values under NPK or CON; the same applied to  $Mn_{CaCl_2}$ , except at Suchdol. Comparable patterns appeared for  $Mn_{Ac}$  and  $Mn_{M3}$ , though differences were mainly insignificant. Bioavailable Mn did not increase with SS or FYM. Three explanations are likely: (i) low yields in CON reduced Mn uptake, leaving more Mn in soil; (ii) high yields under NPK enhanced soil acidification and mobilised Mn from

Table 3. Average manganese (Mn) contents (mg Mn/kg) at the experimental stations in the year 1996, before experiment establishment

Location	$Mn_{H_2O}$	$Mn_{CaCl_2}$	$Mn_{Ac}$	$Mn_{M3}$	$Mn_{NE}$	$Mn_{AR}$
Červený Újezd	$2.88 \pm 0.55$	$19.5 \pm 7.24$	$41.9 \pm 6.76$	$179 \pm 13.8$	$578 \pm 45.3$	$746 \pm 48.6$
Hněvčeves	$3.20 \pm 0.51$	$40.6 \pm 8.82$	$63.0 \pm 10.1$	$139 \pm 14.3$	$466 \pm 35.4$	$586 \pm 16.3$
Humpolec	$4.49 \pm 1.16$	$55.6 \pm 14.3$	$69.8 \pm 8.72$	$126 \pm 14.2$	$902 \pm 44.4$	$1\ 215 \pm 48.2$
Lukavec	$3.35 \pm 0.81$	$44.2 \pm 12.1$	$47.0 \pm 11.4$	$105 \pm 21.0$	$730 \pm 74.1$	$1\ 116 \pm 73.8$
Suchdol	$0.35 \pm 0.18$	$2.06 \pm 0.86$	$8.63 \pm 2.59$	$155 \pm 25.3$	$450 \pm 51.7$	$677 \pm 25.5$

Manganese fractions:  $Mn_{CaCl_2}$ ,  $Mn_{H_2O}$  – readily available forms;  $Mn_{Ac}$  – exchangeable Mn;  $Mn_{M3}$  – potentially available Mn;  $Mn_{NE}$  – nonexchangeable Mn;  $Mn_{AR}$  – pseudo-total Mn

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Table 4. Average manganese (Mn) contents (mg/kg) in all 5 locations in the year 2017, after the 7<sup>th</sup> crop rotation harvest

Treatment	Mn <sub>H<sub>2</sub>O</sub>	Mn <sub>CaCl<sub>2</sub></sub>	Mn <sub>Ac</sub>	Mn <sub>M3</sub>	Mn <sub>NE</sub>	Mn <sub>AR</sub>
<b>Červený Újezd</b>						
CON	1.28 <sup>c</sup>	9.57 <sup>d</sup>	16.7 <sup>b</sup>	166 <sup>c</sup>	619 <sup>ab</sup>	868 <sup>a</sup>
SS <sub>1</sub>	0.95 <sup>a</sup>	4.75 <sup>ab</sup>	11.4 <sup>a</sup>	142 <sup>b</sup>	622 <sup>ab</sup>	836 <sup>a</sup>
SS <sub>3</sub>	0.88 <sup>a</sup>	6.58 <sup>bc</sup>	12.7 <sup>a</sup>	104 <sup>a</sup>	627 <sup>ab</sup>	1 030 <sup>a</sup>
FYM	0.97 <sup>ab</sup>	5.60 <sup>b</sup>	13.2 <sup>a</sup>	166 <sup>c</sup>	682 <sup>b</sup>	935 <sup>a</sup>
NPK	1.18 <sup>bc</sup>	9.14 <sup>cd</sup>	17.0 <sup>b</sup>	181 <sup>cd</sup>	654 <sup>ab</sup>	871 <sup>a</sup>
NSt	0.78 <sup>a</sup>	2.89 <sup>a</sup>	11.0 <sup>a</sup>	197 <sup>d</sup>	581 <sup>a</sup>	856 <sup>a</sup>
<b>Humpolec</b>						
CON	1.38 <sup>b</sup>	18.2 <sup>b</sup>	26.4 <sup>c</sup>	111 <sup>c</sup>	389 <sup>a</sup>	571 <sup>a</sup>
SS <sub>1</sub>	1.37 <sup>b</sup>	14.9 <sup>ab</sup>	21.6 <sup>ab</sup>	87.8 <sup>b</sup>	423 <sup>ab</sup>	601 <sup>a</sup>
SS <sub>3</sub>	1.18 <sup>ab</sup>	9.77 <sup>a</sup>	18.9 <sup>a</sup>	67.6 <sup>a</sup>	407 <sup>ab</sup>	577 <sup>a</sup>
FYM	1.08 <sup>a</sup>	10.9 <sup>a</sup>	18.5 <sup>a</sup>	111 <sup>c</sup>	408 <sup>ab</sup>	589 <sup>a</sup>
NPK	1.39 <sup>b</sup>	18.6 <sup>b</sup>	24.1 <sup>bc</sup>	121 <sup>cd</sup>	429 <sup>b</sup>	567 <sup>a</sup>
NSt	1.16 <sup>ab</sup>	14.8 <sup>ab</sup>	19.7 <sup>ab</sup>	128 <sup>d</sup>	417 <sup>ab</sup>	600 <sup>a</sup>
<b>Hněvčeves</b>						
CON	2.32 <sup>b</sup>	32.9 <sup>ab</sup>	34.3 <sup>bc</sup>	106 <sup>c</sup>	935 <sup>b</sup>	1 249 <sup>a</sup>
SS <sub>1</sub>	2.10 <sup>b</sup>	29.4 <sup>ab</sup>	28.8 <sup>ab</sup>	90.3 <sup>b</sup>	872 <sup>ab</sup>	1 140 <sup>a</sup>
SS <sub>3</sub>	1.72 <sup>a</sup>	22.2 <sup>a</sup>	23.3 <sup>a</sup>	64.6 <sup>a</sup>	860 <sup>a</sup>	1 212 <sup>a</sup>
FYM	2.16 <sup>b</sup>	33.1 <sup>ab</sup>	31.3 <sup>b</sup>	110 <sup>c</sup>	940 <sup>b</sup>	1 327 <sup>a</sup>
NPK	2.96 <sup>c</sup>	32.6 <sup>ab</sup>	43.3 <sup>d</sup>	125 <sup>d</sup>	864 <sup>a</sup>	1 210 <sup>a</sup>
NSt	2.33 <sup>b</sup>	42.3 <sup>b</sup>	38.0 <sup>cd</sup>	116 <sup>cd</sup>	875 <sup>ab</sup>	1 268 <sup>a</sup>
<b>Lukavec</b>						
CON	1.70 <sup>b</sup>	20.6 <sup>ab</sup>	16.2 <sup>bc</sup>	70.5 <sup>b</sup>	800 <sup>ab</sup>	1 138 <sup>a</sup>
SS <sub>1</sub>	1.19 <sup>a</sup>	15.9 <sup>a</sup>	11.9 <sup>a</sup>	57.2 <sup>a</sup>	844 <sup>cd</sup>	1 140 <sup>a</sup>
SS <sub>3</sub>	1.30 <sup>a</sup>	15.1 <sup>a</sup>	12.8 <sup>ab</sup>	53.0 <sup>a</sup>	844 <sup>cd</sup>	1 195 <sup>a</sup>
FYM	1.31 <sup>a</sup>	21.8 <sup>abc</sup>	15.4 <sup>abc</sup>	95.4 <sup>d</sup>	877 <sup>d</sup>	1 198 <sup>a</sup>
NPK	2.10 <sup>c</sup>	29.8 <sup>c</sup>	21.3 <sup>d</sup>	79.1 <sup>c</sup>	836 <sup>bc</sup>	1 134 <sup>a</sup>
NSt	1.66 <sup>b</sup>	27.8 <sup>bc</sup>	18.9 <sup>cd</sup>	75.1 <sup>bc</sup>	776 <sup>a</sup>	1 127 <sup>a</sup>
<b>Suchdol</b>						
CON	0.30 <sup>a</sup>	0.11 <sup>ab</sup>	3.11 <sup>a</sup>	212 <sup>c</sup>	513 <sup>a</sup>	728 <sup>a</sup>
SS <sub>1</sub>	0.22 <sup>a</sup>	0.17 <sup>ab</sup>	3.71 <sup>a</sup>	157 <sup>b</sup>	498 <sup>a</sup>	761 <sup>a</sup>
SS <sub>3</sub>	0.23 <sup>a</sup>	0.29 <sup>b</sup>	3.73 <sup>a</sup>	121 <sup>a</sup>	498 <sup>a</sup>	727 <sup>a</sup>
FYM	0.26 <sup>a</sup>	0.26 <sup>ab</sup>	3.95 <sup>a</sup>	178 <sup>b</sup>	488 <sup>a</sup>	684 <sup>a</sup>
NPK	0.19 <sup>a</sup>	0.07 <sup>a</sup>	3.46 <sup>a</sup>	177 <sup>b</sup>	496 <sup>a</sup>	683 <sup>a</sup>
NSt	0.22 <sup>a</sup>	0.06 <sup>a</sup>	3.43 <sup>a</sup>	178 <sup>b</sup>	485 <sup>a</sup>	696 <sup>a</sup>

Different letters indicate a significant difference among treatments (Tukey's test,  $P < 0.05$ ). CON – unfertilised control; SS<sub>1</sub>, SS<sub>3</sub> – sewage sludge doses, where the SS<sub>3</sub> treatment received a 3 times higher nitrogen (N) dose as compared to SS<sub>1</sub>; FYM – farmyard manure; NPK – mineral NPK fertilisation; NSt – mineral N combined with straw; manganese fractions: Mn<sub>CaCl<sub>2</sub></sub>, Mn<sub>H<sub>2</sub>O</sub> – readily available forms; Mn<sub>Ac</sub> – exchangeable Mn; Mn<sub>M3</sub> – potentially available Mn; Mn<sub>NE</sub> – nonexchangeable Mn; Mn<sub>AR</sub> – pseudo-total Mn

non-bioavailable pools; (iii) Mn from organic fertilisers became incorporated into non-bioavailable forms, consistent with higher Mn<sub>AR</sub> values at SS<sub>1</sub>,

SS<sub>3</sub> and FYM. Mn<sub>NE</sub> showed significant treatment differences (except at Suchdol), but no consistent trend across locations.

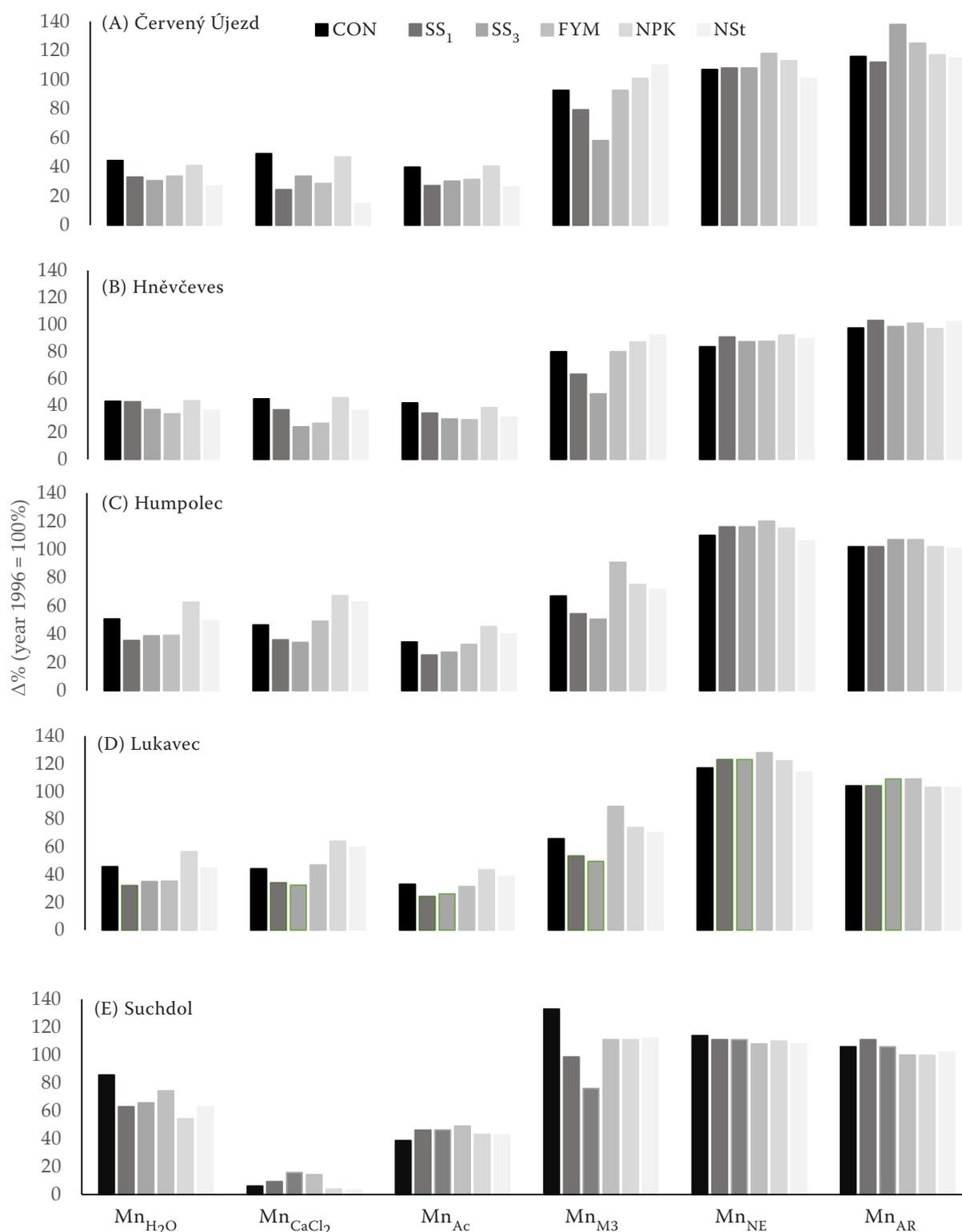


Figure 2. Changes in soil manganese (Mn) content in 2017 (%; year 1996 = 100%). The average from soil samples ( $n = 18$ ) for each location taken up in the year 1996, before fertilising, was considered as 100%. CON – unfertilised control;  $SS_1$ ,  $SS_3$  – sewage sludge doses, where the  $SS_3$  treatment received a 3 times higher nitrogen (N) dose as compared to  $SS_1$ ; FYM – farmyard manure; NPK – mineral NPK fertilisation; Nst – mineral N combined with straw; manganese fractions:  $Mn_{CaCl_2}$ ,  $Mn_{H_2O}$  – readily available forms;  $Mn_{Ac}$  – exchangeable Mn;  $Mn_{M3}$  – potentially available Mn;  $Mn_{NE}$  – nonexchangeable Mn;  $Mn_{AR}$  – pseudo-total Mn

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Differences in soil Mn between 1996 and 2017 are shown as relative Mn changes (1996 = 100%) in Figure 2. Because of the calculation from the average initial values, statistical analysis was not performed. Despite considerable site-specific variability, Figure 2 highlights several robust long-term trends common to all locations. Relative declines in readily available Mn forms occurred across treatments, indicating a general depletion of labile Mn pools over the 21-year period. This decline was not mitigated by organic fertilisation. Conversely, non-bioavailable Mn fractions remained stable or increased at most sites, particularly under FYM and sewage sludge treatments, suggesting progressive immobilisation of Mn into less reactive soil pools. Differences among these general trends were location-specific. For example, Suchdol exhibited comparatively stable  $Mn_{M3}$  values across several treatments, likely reflecting its high pH and strong buffering capacity, while Hněvčeves showed a unique decrease in  $Mn_{NE}$ . These responses underline the modifying role of inherent soil properties rather than that of the fertilisation regime alone.

The potentially available Mn forms also showed a decrease during the experiment, except  $Mn_{M3}$  in the CON, FYM, NPK, and NSt treatments at the Suchdol site. The  $Mn_{Ac}$  values were always lower than those for  $Mn_{M3}$ .

Nonavailable Mn forms behaved oppositely: only Hněvčeves showed declining  $Mn_{NE}$ . FYM produced the highest  $Mn_{NE}$  at three sites (Červený Újezd, Humpolec, Lukavec).  $Mn_{AR}$  increased at Červený Újezd, while other sites showed values similar to or slightly higher than those in 1996.

When evaluated across all locations, Table 4 reveals a consistent fertilisation response pattern. Readily available Mn fractions ( $Mn_{H_2O}$ ,  $Mn_{CaCl_2}$ ) and exchangeable Mn ( $Mn_{Ac}$ ) were generally highest under NPK

fertilisation or in the unfertilised control, whereas organic treatments rarely exceeded these variants. In contrast, non-bioavailable Mn fractions ( $Mn_{NE}$ ,  $Mn_{AR}$ ) were commonly higher under FYM and sewage sludge treatments across most sites, indicating preferential accumulation of Mn in stable soil pools. Although the magnitude of these effects varied among locations, the direction of response was largely consistent.

Pearson correlations (Table 5) indicated significant positive correlations between pH and  $Mn_{M3}$  and significant negative correlations between pH and the remaining extractants. Due to the strong acidity of Mehlich 3, it may extract non-bioavailable Mn. Strong correlations occurred between  $Mn_{H_2O}$  and  $Mn_{CaCl_2}$ , and  $Mn_{Ac}$  correlated with both. The positive correlation of  $Mn_{M3}$  with pH, together with weak correlations between  $Mn_{M3}$  and bioavailable Mn fractions, suggests that Mehlich 3 is less suitable for assessing bioavailable Mn. Additional research supported by plant analysis is needed. Very close relationships were also observed between  $Mn_{NE}$  and  $Mn_{AR}$ . The results of the correlation analysis were confirmed by regression analysis, which showed the best relationships were linear (data not shown).

Based on the obtained results, it is obvious that hypothesis (i), organic fertilisers (farmyard manure and sewage sludge) increase soil bioavailable Mn, was not confirmed under the conditions of this long-term experiment. Across all sites, neither FYM nor sewage sludge significantly increased readily available ( $Mn_{H_2O}$ ,  $Mn_{CaCl_2}$ ) or exchangeable Mn ( $Mn_{Ac}$ ) compared to the control. Instead, organic fertilisation was consistently associated with higher contents of non-bioavailable Mn fractions ( $Mn_{NE}$ ,  $Mn_{AR}$ ), indicating that Mn added with organic inputs was predominantly immobilised into stable soil pools rather than contributing to the plant-available Mn fraction.

Table 5. Correlation between different manganese (Mn) fractions and the relationship of these fractions with the soil pH value

	$Mn_{H_2O}$	$Mn_{CaCl_2}$	$Mn_{Ac}$	$Mn_{M3}$	$Mn_{NE}$	$Mn_{AR}$
pH	-0.620***	-0.719***	-0.512***	0.705***	-0.778***	-0.760***
$Mn_{H_2O}$		0.940***	0.950***	-0.225	0.481***	0.443***
$Mn_{CaCl_2}$			0.925***	-0.362**	0.545***	0.523***
$Mn_{Ac}$				-0.117	-0.302*	-0.257
$Mn_{M3}$					-0.468***	-0.493***
$Mn_{NE}$						0.937***

Significant correlation at \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Manganese fractions:  $Mn_{CaCl_2}$ ,  $Mn_{H_2O}$  – readily available forms;  $Mn_{Ac}$  – exchangeable Mn;  $Mn_{M3}$  – potentially available Mn;  $Mn_{NE}$  – nonexchangeable Mn;  $Mn_{AR}$  – pseudo-total Mn

The hypothesis (ii), that mineral N fertilisation exacerbates Mn deficiency relative to the control, was also not supported. On the contrary, mineral NPK fertilisation increased bioavailable Mn at most sites, primarily due to soil acidification. This increase was not due to external Mn inputs but rather to the mobilisation of Mn from existing soil reserves. It is therefore possible that the expected Mn deficiency will appear in the next decades of long-term monitoring due to depletion of the mentioned soil reserves.

The third hypothesis (iii) was clearly confirmed. Soil pH emerged as the dominant factor controlling Mn availability, as demonstrated by strong negative correlations between pH and all bioavailable Mn fractions (except  $Mn_{M3}$ ) and by the consistent increase of bioavailable Mn under acidifying treatments. Fertilisation effects on Mn availability were therefore largely indirect and mediated through pH changes rather than through Mn inputs.

## DISCUSSION

The pH values from 1996 to 2017 across the five sites usually declined. The results clearly demonstrate that soil pH is the primary driver of Mn availability, overriding direct effects of individual fertilisation systems. Across all locations, increases in bioavailable Mn fractions were consistently associated with pH decline rather than with Mn inputs from fertilisers. Mineral NPK fertilisation showed the strongest effect on Mn availability due to its acidifying influence, which enhanced Mn solubility and mobilised Mn from non-labile soil pools. Although nitrogen was applied as calcium ammonium nitrate, the acidifying effect of nitrification outweighed the calcium input (Padhi et al. 2020).

Organic fertilisation systems (FYM and SS), despite supplying Mn and organic matter, did not increase bioavailable Mn because their effects on soil pH were weak, neutral, or site-specific. Instead, organic inputs promoted stabilisation of Mn into non-bioavailable fractions, particularly under conditions where soil pH remained stable or increased. Differences among treatments reflect variation in buffering capacity (Yang et al. 2007). The decrease in pH in sewage sludge treatments can be attributed to organic matter decomposition and the production of organic acids, consistent with the findings of Arvas et al. (2011). Forsberg and Ledin (2006) also linked sludge-induced pH decline to reduced buffering capacity, with magnitude dependent on sludge dose (Navas et al. 1998).

These mechanisms align with the decreases observed at Červený Újezd, Hněvčeves, Humpolec and Suchdol.

Even in the control, pH declined at all sites, likely due to leaching and crop removal of basic ions. Similar effects were reported by Arvas et al. (2011). At the Chernozem site Suchdol, pH change was minimal owing to strong buffering by the limestone-derived parent rock. FYM typically enhances soil fertility (Repsiene and Skuodiene 2010) and may increase pH (Ondrášek and Čunderlík 2008), although effects depend on application rate and timing. Some studies report a decrease in pH with FYM due to the formation of organic acids and  $CO_2$  during decomposition (Hati et al. 2008, Meena et al. 2017). In this study, FYM effects were inconsistent: pH decreased at Humpolec and Červený Újezd, increased at Lukavec, and remained stable at Hněvčeves and Suchdol.

Initial Mn data showed the highest  $Mn_{H_2O}$  and  $Mn_{CaCl_2}$  levels at Humpolec and Lukavec, and the lowest at Suchdol, where values near 0.2 mg/kg indicate Mn deficiency for many crops (Havlin et al. 2014). Lower pH generally increases Mn availability (Kabata-Pendias 2010), which was consistent with trends seen in  $Mn_{NE}$  and  $Mn_{AR}$ , though not always for  $Mn_{Ac}$  and  $Mn_{M3}$ .

Table 4 shows higher bioavailable Mn under NPK, likely due to acidification (Moharana et al. 2016). Beyond pH, Mn availability depends on total Mn content, clay and oxide minerals, organic matter, and redox conditions (Murtaza et al. 2017). Moharana et al. (2016) found the highest Mn availability under FYM, partially confirmed at Suchdol, where Mn availability was slightly higher but not significant. Although organic fertilisers are often reported to enhance micronutrient availability (e.g., Rutkowska et al. 2014), their effect on Mn bioavailability appears to be context-dependent. In the present study, organic inputs probably increased soil organic matter and contributed Mn mainly to non-labile pools, likely through complexation with organic ligands, sorption to newly formed Mn oxides, or incorporation into organo-mineral associations. These processes can reduce short-term Mn bioavailability despite increased total Mn inputs, particularly under conditions where soil pH does not decline substantially.

According to Zbiral (2016),  $Mn_{M3} < 30$  mg/kg represents low, 30.1–200 mg/kg medium, and  $> 200$  mg/kg high or potentially toxic. Our highest  $Mn_{M3}$  values occurred at Suchdol under CON, likely an overestimation due to the strong acidity and EDTA chelation in Mehlich 3, which mobilises even nonavailable Mn. Other sites had

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medium  $Mn_{M3}$  levels. Overall, the results suggest that Mehlich 3 is more suitable for estimating Mn supply potential than for direct evaluation of bioavailable Mn, particularly in soils where pH strongly constrains Mn solubility. However, to confirm this fact, a study including the Mn uptake with plants, as well as an investigation of more soil-climatic conditions, is needed. For  $Mn_{Ac}$ , no single interpretive scale exists; evaluation must consider pH, texture, organic matter, and redox status.

Readily available Mn ( $Mn_{H_2O}$ ,  $Mn_{CaCl_2}$ ) decreased at all sites regardless of treatment, and organic fertilisers did not prevent this decline. Potentially available Mn also decreased except  $Mn_{M3}$  at Suchdol under CON, FYM, NPK and NSt. Nonavailable forms increased at most sites:  $Mn_{NE}$  rose except at Hněvčeves, and  $Mn_{AR}$  increased, mainly at Červený Újezd.

Pearson correlations showed a strongly positive relationship between pH and  $Mn_{M3}$  ( $R = 0.705$ ) and significantly negative correlations between pH and all other Mn fractions ( $Mn_{H_2O}$ ,  $Mn_{CaCl_2}$ ,  $Mn_{Ac}$ ,  $Mn_{NE}$ ,  $Mn_{AR}$ ). These results align with Xu et al. (2022) and earlier findings (Zhou et al. 2015). Regression analysis produced the strongest relationships for pH: $Mn_{NE}$ , pH: $Mn_{AR}$ ,  $Mn_{H_2O}$ : $Mn_{CaCl_2}$ ,  $Mn_{H_2O}$ : $Mn_{Ac}$ ,  $Mn_{CaCl_2}$ : $Mn_{Ac}$ , and  $Mn_{NE}$ : $Mn_{AR}$ , confirming the dominant role of pH in Mn bioavailability. Similar conclusions were presented by Adriano (2001) and Behera and Shukla (2014).

In summary, the long-term experiment demonstrated that soil Mn dynamics were governed by a limited number of consistent mechanisms across locations. Mineral fertilisation influenced Mn availability mainly *via* soil acidification, while organic fertilisation promoted Mn accumulation in non-labile fractions without increasing bioavailable Mn. Site-specific differences modified the magnitude but not the direction of these responses, indicating that fertilisation effects on Mn are largely predictable when soil pH and buffering capacity are considered. It is therefore important to integrate soil manganese diagnostics (especially bioavailable Mn) to get the relevant data about fertilisation needs. Furthermore, it is necessary to consider the soil pH value and its management. Generally, it is possible to encourage organic fertilising due to its positive influence on Mn balance. Used mineral fertilising led to a pH decrease, so it cannot be recommended for low pH soil. Advisory services should incorporate crop tissue analysis results into fertiliser recommendations, ensuring manganese supply aligns with crop demand.

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## REFERENCES

- Adriano D.C. (2001): Ecological and health risks of metals. Trace Elements in Terrestrial Environments, 4: 133–165.
- Alejandro S., Höller S., Meier B., Peiter E. (2020): Manganese in plants: from acquisition to subcellular allocation. *Frontiers in Plant Science*, 11: 1–23.
- Arvas O., Çelebi S.Z., Yilmaz I.H. (2011): Effect of sewage sludge and synthetic fertilizer on pH, available N and P in pasture soils in semi-arid area, Turkey. *African Journal of Biotechnology*, 10: 16508–16515.
- Aulakh M., Grant C.A. (2008): Integrated Nutrient Management for Sustainable Crop Production. New York, CRC Press, Taylor and Francis Group. ISBN-13: 978-1560223047
- Bailey R.L., West Jr. K.P., Black R.E. (2015): The epidemiology of global micronutrient deficiencies. *Annals of Nutrition and Metabolism*, 66: 22–33.
- Behera S., Shukla A. (2014): Total and extractable manganese and iron in some cultivated acid soils of India: status, distribution and relationship with some soil properties. *Pedosphere*, 24: 196–208.
- Feng M., Shan X., Zhang S., Wen B. (2005): A comparison of the rhizosphere-based method with DTPA, EDTA,  $CaCl_2$ , and  $NaNO_3$  extraction methods for prediction of bioavailability of metals in soil to barley. *Environmental Pollution*, 137: 231–240.
- Forsberg L.S., Ledin S. (2006): Effects of sewage sludge on pH and plant availability of metals in oxidising sulphide mine tailings. *Science of the Total Environment*, 358: 21–35.
- Hati K.M., Swarup A., Mishra B., Manna M., Wanjari R., Mandal K., Misra A. (2008): Impact of long-term application of fertilizer, manure and lime under intensive cropping on physical properties and organic carbon content of an Alfisol. *Geoderma*, 148: 173–179.
- Havlin J.L., Tisdale S.L., Nelson W.L., Beaton J.D. (2014): Soil Fertility and Fertilization: An Introduction to Nutrient Management. 8<sup>th</sup> Edition. North Carolina, Pearson Prentice Hall. ISBN-13: 978-0135033739
- Kabata-Pendias A. (2010): Trace Elements in Soils and Plants. Baton Rouge, Taylor and Francis Group. ISBN: 9780429192036
- Kovačević V., Kadar I., Koncz J., Brkić I., Banaj D. (2002): Cadmium and lead status in corn hybrids grown on acid soil of eastern Croatia. *Poljoprivreda*, 8: 10–14.
- Luscombe P.C., Syers J.K., Gregg P.E.H. (1979): Water extraction as a soil testing procedure for phosphate. *Communications in Soil Science and Plant Analysis*, 10: 1361–1369.
- Malakoti M.J., Tehrani M.M. (2001): Effects of micronutrients on the yield and quality of agricultural products. In: *Micro-Nutrients with Macro-Nutrients*. 2<sup>nd</sup> Edition, Tarbiat, Tehran, Tarbiat Modarres University Press.

<https://doi.org/10.17221/562/2025-PSE>

- Mann M.S., Takkar P.N., Bansal R.L., Randhawa N.S. (1978): Micro-nutrient status of soil and yield of maize and wheat as influenced by micronutrient and FYM application. *Journal of the Indian Society of Soil Science*, 26: 208–214.
- Meena H.M., Sharma R.P., Sankhyan N.K., Sepehya S. (2017): Effect of continuous application of fertilizers, farmyard manure and lime on soil fertility and productivity of the maize-wheat system in an acid Alfisol. *Communications in Soil Science and Plant Analysis*, 48: 1552–1563.
- Meers E., Du Laing G., Unamuno V., Ruttens A., Vangronsveld J., Tack F., Verloo M. (2007): Comparison of cadmium extractability from soils by commonly used single extraction protocols. *Geoderma*, 141: 247–259.
- Mehlich A. (1984): Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, 15: 1409–1416.
- Millaleo R., Reyes-Diaz M., Ivanov A., Mora M., Alberdi M. (2010): Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition*, 10: 470–481.
- Moharana P.C., Sharma B.M., Biswas D.R. (2016): Changes in the soil properties and availability of micronutrients after six-year application of organic and chemical fertilizers using STCR-based targeted yield equations under pearl millet-wheat cropping system. *Journal of Plant Nutrition*, 40: 165–176.
- Monreal C.M., DeRosa M., Mallubhotla S.C., Bindraban P.S., Dimkpa C. (2016): Nanotechnologies for increasing the crop use efficiency of fertilizer-micronutrients. *Biology and Fertility of Soils*, 52: 423–437.
- Mousavi S.R., Shahsavari M., Rezaei M. (2011): A general overview on manganese (Mn) importance for crops production. *Australian Journal of Basic and Applied Sciences*, 5: 1799–1803.
- Murtaza G., Usman Y., Niazi N.K., Usman M., Hussain T. (2017): Bioaccumulation of potentially toxic elements in cereal and legume crops: a review. *CLEAN – Soil, Air, Water*, 45: 1–15.
- Navas A., Bermúdez E., Machin J. (1998): Influence of sewage sludge application on physical and chemical properties of Gypsissols. *Geoderma*, 87: 123–135.
- Obeng S.K., Kulhánek M., Balík J., Černý J., Sedlár O. (2024): Manganese: from soil to human health – a comprehensive overview of its biological and environmental significance. *Nutrients*, 16: 3455.
- Ondrášek L., Čunderlík J. (2008): Effects of organic and mineral fertilisers on biological properties of soil under seminatural grassland. *Plant, Soil and Environment*, 54: 329–335.
- Padhi P.P., Chiranjeeb K., Das M., Behera T., Mishra A.P. (2020): Fertilizer use and soil acidity. *Biomolecule Reports – An International eNewsletter*, BR/02/19/17: 1-6.
- Peijnenburg W.J.G.M., Zablotskaja M., Vijver M.G. (2007): Monitoring metals in terrestrial environments within a bioavailability framework and a focus on soil extraction. *Ecotoxicology and Environmental Safety*, 67: 163–179.
- Rengel Z., Cakmak I., White J.P. (eds.) (2022): *Marschner's Mineral Nutrition of Plants*. Amsterdam, Elsevier Science Publishing.
- Repsiene R., Skuodiene R. (2010): The influence of liming and organic fertilisation on the changes of some agrochemical indicators and their relationship with crop weed incidence. *Zemdirbyste-Agriculture*, 97: 3–14.
- Rutkowska B., Szulc W., Sosulski T., Stępień W. (2014): Soil micro-nutrient availability to crops affected by long-term inorganic and organic fertilizer applications. *Plant, Soil and Environment*, 60: 198–203.
- Schulte E.E., Kelling K.A. (1999): Soil and applied manganese. *Understanding to Plant Nutrition*, A2526: 1–4.
- Šimić D., Sudar R., Ledencan T., Jambrović A., Zdunić Z., Brkić I., Kovačević V. (2009): Genetic variation of bioavailable iron and zinc in grain of a maize population. *Journal of Cereal Science*, 50: 392–397.
- Warncke D., Brown J.R. (1998): Potassium and other basic cations. In: Brown J.R. (ed.): *Recommended Chemical Soil Test Procedures for the North Central Region*. Columbia, NCR Publication No. 221, Missouri Agricultural Experiment Station.
- Xu D., Shen Z., Dou C., Dou Z., Li Y., Gao Y., Sun Q. (2022): Effects of soil properties on heavy metal bioavailability and accumulation in crop grains under different farmland use patterns. *Scientific Reports*, 12: 1–15.
- Yang S., Malhi S.S., Li F., Suo D., Xu M., Wang P., Xiao G., Jia Y., Guo T., Wang J. (2007): Long-term effects of manure and fertilization on soil organic matter and quality parameters of a calcareous soil in NW China. *Journal of Plant Nutrition and Soil Science*, 170: 234–243.
- Zbiral J. (2016): Determination of plant-available micronutrients by the Mehlich 3 soil extractant – a proposal of critical values. *Plant, Soil and Environment*, 62: 527–531.
- Zewide I., Sherefu A. (2021): Review paper on effect of micronutrients for crop production. *Nutrition and Food Processing*, 4: 1–8.
- Zhou S., Liu J., Xu M., Lv J., Sun N. (2015): Accumulation, availability, and uptake of heavy metals in a red soil after 22-year fertilization and cropping. *Environmental Science and Pollution Research*, 22: 15154–15163.
- Zhu H., Pan J., Wei Y., Lan H., Yang S., Li X., Tang X. (2025): Manganese toxicity suppressing nitrogen-fixing bacteria growth and impairing nitrogen uptake and utilization in sugarcane. *Frontiers in Microbiology*, 16: 1–13.

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