

THE IMPACT OF THE LONG – TERM FERTILISATION WITH MINERAL FERTILISERS ON THE CHEMICAL PROPERTIES OF VERTISOL (CENTRAL SERBIA)

GUDŽIĆ, N.¹ – ŠEKULARAC, G.^{2*} – DJIKIĆ, A.¹ – DJEKIĆ, V.³ – AKSIĆ, M.¹ – GUDŽIĆ, S.¹

¹*Faculty of Agriculture, Lešak, University of Priština, Serbia*

²*Faculty of Agronomy, Čačak, University of Kragujevac, Serbia*

³*Centre of Small Grains, Kragujevac, Serbia*

**Corresponding author
e-mail: gordasek@kg.ac.rs*

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Abstract. The study is based on establishing soil acidity and fertility of Vertisols after the mineral fertilisers had continuously been applied from 1984 to 2016. The two phosphorus (35 and 70 kg P ha⁻¹), the invariable nitrogen (120 kg N ha⁻¹) and potassium (66.4 kg K ha⁻¹) doses were used in Kragujevac location (central Serbia) for over 33 study years. The impact of fertilisation with mineral fertilisers on soil acidity, organic carbon content, the total N, P and K as well as on P, K forms balance and on some microelements (Fe, Mn, Cu and Zn) availability was studied at soil depths of 0-20 and 20-40 cm. After 33 years, the highest changes were noticed in the surface layer (0-20 cm), with an increase in the acidity level and a decrease in organic carbon content and in the total N. Simultaneously, a higher P and K content and a higher Fe and Mn mobility were established in the same soil layer whereas mineral fertilisers had no significant effect on the changes in chemical properties at 20-40 cm deep soils.

Keywords: *plant production, soil properties, nutrient, acidification, farming sustainability*

Introduction

Constantly used mineral fertilisers in conventional plant production were found to affect numerous soil properties. In this regard, continuously used nitrogen, phosphorus and potassium in mineral form were found to somewhat influence soil physical characteristics (Herencia et al., 2011; Suwara et al., 2016), and to a larger extent the soil chemical and biological properties (Belay et al., 2002; Zhong et al., 2010) as well as the soil enzyme activity (Piotrowska and Wilczewski, 2012; Chen et al., 2018). Soil physical and chemical properties and fungi diversity and abundance were also found to be significantly affected by long-term mineral fertilisation, especially in terms of fungi diversity reduction and soil acidification (Yan et al., 2019).

Soil and water contamination most often bring about soil acidification (Herencia et al., 2007; Undurraga et al., 2009). One of the solutions made to the potential harmful effect of the long-term and one-sided use of fertilisers is considered to be fertilisation management system managing the nutrients put into and taken out of the soil within the crop rotation system (Hirzel et al., 2011). Therefore, a reasonable use of the mineral fertilisers over a longer period of time may keep up or even upgrade the soil quality and its production capacities, too (Belay et al., 2002).

One of the best ways to assess how fertilisation affects fertility and other soil properties is through the long-term experiments (Mitchell et al., 1991). Such experiments may provide useful information about farming systems sustainability,

particularly when fertilisers are invariably used and for quite a long time. That is why the current study was aimed at establishing the changes in soil chemical properties caused by these fertilisers on Vertisols in the central Serbia.

Materials and methods

Experimental location

The investigation was performed in a long-term trial field of the Centre of Small Grains, in Kragujevac (44°02' N and 20°56' E, altitude: 185 m asl), central Serbia (*Fig. 1*), on Vertisols (IUSS Working Group WRB, 2014). The study location of Kragujevac is approximately 113 km away from Belgrade.



Figure 1. Study location map (Kragujevac, central Serbia)

Climatic conditions of the study location

Based on the climatic data outlined in Walter climate-diagram (Walter et al., 1975) (*Fig. 2*), the annual air temperature of Kragujevac location averaged 12.2°C and the total annual rainfalls averaged 673 mm over the last twenty years (Republic Hydrometeorological Service of Serbia, 1998-2017). July and August were found to be the hottest (22.9°C and 22.4°C, respectively) and January and December the coldest months (1.3°C and 2.3°C, respectively). On average, May (74 mm) and June (70 mm) were the rainiest and February (42 mm) and January (45 mm) the driest months.

Walter climate-diagram denotes that, on average, humid periods over the year were present from January to June and from September to December whereas dry periods prevailed in July and in August. According to Köppen classification (1936) and based on the previously outlined data, the climate of Kragujevac location could be determined as moderately warm and humid with hot summers and dry winters.

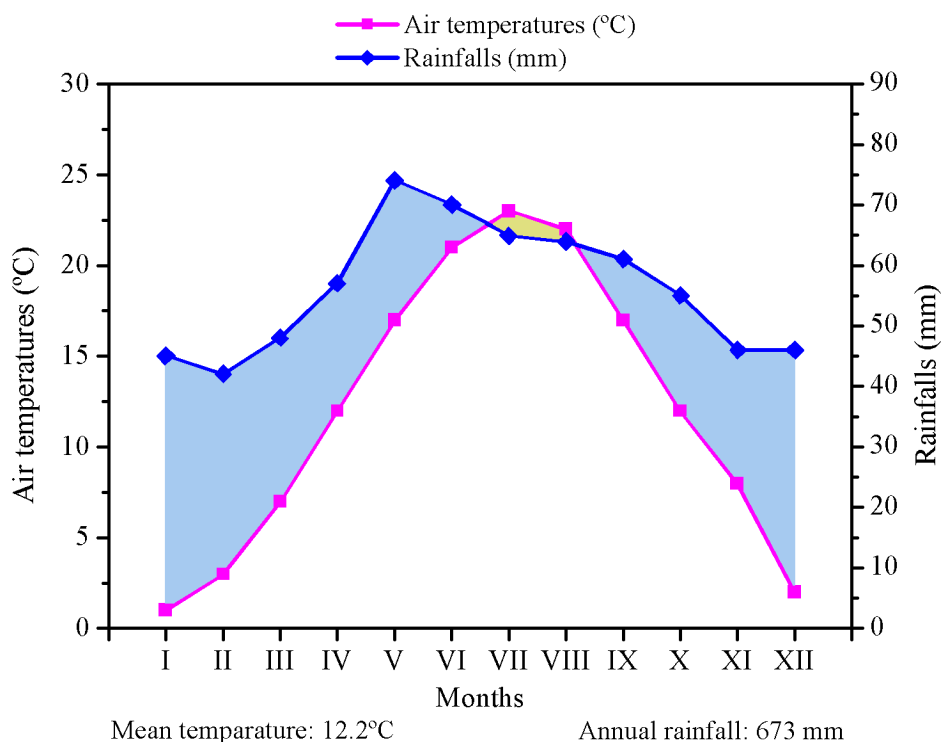


Figure 2. Walter climate-diagram of research area (1998-2017)

Agrochemical soil properties

The soil studied is characterised by leaching process. Prior to the experiment, the soil had an excessive acid reaction, a low phosphorus content, moderate organic carbon (OC) and potassium content (Table 1). Soil acidity and the total and available K content were found to increase at 20-40 cm depth whereas OC content, total N and total and available P contents were found to decrease. The available Fe, Mn and Cu contents also decreased whereas Zn one increased.

Table 1. Agrochemical properties before setting up of the experiment

Depth cm	pH		Hh cmol kg ⁻¹	OC %	Total			Available					
	H ₂ O	KCl			N %	P	K	P	K	Fe	Mn	Cu	Zn
0-20	5.90	4.60	13.29	1.56	0.17	977.4	15000	260	186	71	118	2.4	1.7
20-40	5.90	4.47	11.11	0.96	0.11	763.6	15700	190	215	64	110	2.2	2.8

Experimental design

Having started in 1984, the experiment was continuously carried out till 2016 within the two-field crop rotation of the winter wheat (*Triticum aestivum*) and of maize (*Zea mays* L.). Soil cultivation was typical of the existing environmental conditions with standard agronomical measures suiting maize and wheat used and plant remains from the previous vegetation removed.

In the course of the experiment, the phosphorus doses were continuously used, the lower dose of 35 kg P ha⁻¹ (P1) and the higher one of 70 kg P ha⁻¹ (P2). The two P doses were combined with the constant N dose (120 kg N ha⁻¹) in the variants NP1 and NP2 with the constant N (120 kg N ha⁻¹) and K (66.4 kg K ha⁻¹) doses in the variants NP1K and NP2K. Nitrogen and potassium were also used in the same rates in the variant – NK. Fertilisation treatments were compared with the control treatment in which no fertilisers were used (0). Super-phosphate – Ca(H₂PO₄)₂ · CaSO₄, urea – CO(NH₂)₂ and potassium chloride – KCl were used for fertilisation. Fertilisation technology was standard for each vegetation season, in which the total amounts of super-phosphate and potassium chloride as well as 1/3 of urea were always applied before sowing. The remaining urea amount was used during vegetation depending on the climatic circumstances so that for wheat, it was used in the period from the end of March to the beginning of April and for maize during the second half of May.

The experiment proceeded according to the random system block design (RSBD) with four replications. Each plot was 15 m² in size. The average (composite) soil sample was taken for each treatment and formed from the five sub-samples. In every treatment, firstly at the depth 0-20 cm, the probe (Eijkelkamp) was used to take five individual samples (sub-samples) being mixed together and an average or composite sample was singled out from the total mass. The procedure was repeated for each treatment (0-20 cm) as well as for that of 20-40 cm. Composite samples (24 for 0-20 cm and 24 for 20-40 cm) were chemically analysed after the corresponding treatment. Soil samples were collected in the period from the 10th to the 12th October, 2016 after the maize harvest.

Soil laboratory analysis

Soil pH was determined on the pH meter (MA 5730 ISKRA) in 1 : 2.5 suspension with water (AA) and 1 M KCl (SA). Hydrolytic acidity (Hh) was determined following the method of Kappen while the availability of phosphorus (AP) and potassium (AK) was determined following that of Egner-Riehm with ammonium-lactate (pH = 3.7) used as the extraction means. Upon the extraction, potassium was determined through the atomic absorption spectrometry (AAS) and phosphorus through spectrophotometry UV/Vis S2000 (WPA, England) after the colour had been developed with NH₄MoO₄ and SnCl₂. The total phosphorus (TP) was determined through spectrophotometry at the wave length of 400-490 nm after digestion with HClO₄ and treatment with ammonium paramolybdate-vanadate reagent (Olsen and Sommers, 1982) and the total potassium (TK) AAS after degrading the sample with mixture HF and H₂SO₄. Further, the content of soil microelements available forms (Fe, Mn, Cu and Zn) was determined through atomic absorption spectrophotometry (AAS) using the apparatus AAS N-1 (Carl Zeiss Jena, Germany). The available Fe (AFe) was determined after being extracted in the solution 1 M CH₃COONH₄ (pH 7). Determining the available Mn (AMn) content was done using the extraction means of 0.1 M H₂SO₄ and that of Cu (ACu) and Zn (AZn) using 0.1 M HCl.

Statistical analysis

The data obtained from the parameters were subjected to statistical analysis using the WinSTAT program being the statistics Add-In for Microsoft Excel (Copyright © 2018 Robert K. Fitch). The effects of treatment with fertilisers were tested using ANOVA and the differences between the treatments were registered using t-test for the levels of

95 and 99%, whereas the balance of available microelements dependant on the substantial acidity and available P, was tested using the square regression.

Results and discussion

The experiment conducted for 33 years was characterised by monitoring acidifying of both layers tested. AA and SA were found to have significantly increased in the surface layer whereas Hh was found to have had just a mild increase. Thus, AA of 5.90 fell in the control variant after 33 years amounting to 5.57. In addition, SA pH in KCl decreased from 4.60 to 4.18 whereas Hh mildly increased from 13.29 cmol kg⁻¹ to 14.10 cmol kg⁻¹ for the same period. The process of somewhat milder acidification also prevailed in the 20-40 cm deep layer.

Table 2. Characteristics of the acidity after 33 years of fertilisation

Variants	pH		Hh cmol kg ⁻¹
	H ₂ O	KCl	
0-20 cm			
0	5.58 ± 0.08	4.18 ± 0.14	14.10 ± 0.41
NK	5.30 ± 0.13	4.02 ± 0.10	17.80 ± 0.23
NP1	5.36 ± 0.15	4.10 ± 0.09	16.98 ± 0.22
NP2	5.40 ± 0.08	4.12 ± 0.07	16.37 ± 0.16
NP1K	5.42 ± 0.07	4.10 ± 0.12	16.43 ± 0.15
NP2K	5.52 ± 0.08	4.13 ± 0.11	16.18 ± 0.30
Lsd 0.05	0.066	0.069	0.174
Lsd 0.01	0.091	0.094	0.238
20-40 cm			
0	5.60 ± 0.10	4.40 ± 0.11	10.51 ± 1.44
NK	5.63 ± 0.11	4.16 ± 0.11	12.30 ± 0.11
NP1	5.50 ± 0.09	4.30 ± 0.11	10.92 ± 0.10
NP2	5.60 ± 0.09	4.33 ± 0.19	10.53 ± 0.17
NP1K	5.50 ± 0.09	4.23 ± 0.11	11.11 ± 0.11
NP2K	5.90 ± 0.13	4.26 ± 0.13	11.17 ± 0.20
Lsd 0.05	0.0679	0.084	0.408
Lsd 0.01	0.0929	0.116	0.559

In addition to the natural acidification, the long-term and continuous use of mineral fertilisers also favoured additional acidification of the Vertisol in both soil layers (*Table 2*) in that the acidification of the surface layer seemed to be more expressed. This may be accounted for by the fact that it was the zone of fertilisers used and, therefore, of their direct impact on this layer. In the 0-20 cm deep layer, the differences in AA, SA i Hh between the control variant and those with fertilisation appeared to be highly significant ($P \leq 0.01$). However, the highest acidification was established in NK variant, meaning that their long-term use increased acidity more significantly than their combination with phosphorus fertilisers did. Such a trend was expected considering that soil acidification over a long period of nitrogen fertilisation is well-known and already confirmed. However, the long-term and continuous application of the fertilisers, which, beside nitrogen, also contain phosphorus such as MAP, may often result in higher acidification (Belay et al., 2002; Saleque et al., 2004).

Therefore, at 20-40 cm depth, the differences in AA between the control and fertilisation treatments were lower, denoting at fertilisation to have a lower impact on this type of acidity in deeper soil layers even after 33 years of being applied. On the other hand, the variations in SA and Hh between fertilisation and control treatments seemed to be more pronounced. Identically with the changes in soil acidity in the surface layer, those in the deeper ones also suggested the highest acidity in NK variant.

When comparing the initial (1984) organic carbon (OC) content and the total nitrogen (TN) one in 2016 in the control variant, study period was not found to be profoundly reflected on their balance. However, more significant changes did happen in the experimental stage with fertilisers applied (*Table 3*). Namely, in all the fertilisation variants, the OC content and TN one were significantly reduced in the surface layer compared to the control variant ($P \leq 0.01$). In contrast to the significant impact of the long-term fertilisation with nitrogen on sustaining and improving OC content (Bundy et al., 2011), the total and nitrate nitrogen (Zhang et al., 2012), then that contained in the fertilisers used over the current research had, however, no positive effect. The content of these two components could be expected to drop with the depth (*Table 4*).

Table 3. The content of OC, TN, TP, TK, AP and AK in the 0-20 cm layer after 33 years

	OC	TN	TP	TK	AP	AK
Variants	%		mg kg ⁻¹			
0	1.57 ± 0.06	0.157 ± 0.007	924 ± 79	13200 ± 600	18.9 ± 4.5	188.0 ± 4.0
NK	1.25 ± 0.07	0.125 ± 0.007	967 ± 166	16500 ± 1200	20.2 ± 2.9	258.3 ± 4.4
NP1	1.38 ± 0.10	0.138 ± 0.010	1202 ± 86	14100 ± 700	104.4 ± 7.6	168.9 ± 9.5
NP2	1.39 ± 0.10	0.140 ± 0.011	1381 ± 213	14000 ± 600	115.5 ± 10.5	154.8 ± 5.3
NP1K	1.37 ± 0.10	0.137 ± 0.010	1283 ± 96	15000 ± 600	125.5 ± 4.8	288.3 ± 3.8
NP2K	1.44 ± 0.14	0.144 ± 0.020	1605 ± 107	16000 ± 800	168.2 ± 5.8	255.5 ± 7.4
Lsd 0.05	0.078	0.0078	86.56	530.33	4.37	4.08
Lsd 0.01	0.107	0.0107	118.57	726.46	5.99	5.58

OC: organic carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium; AP: available phosphorus; AK: available potassium

When phosphorus and potassium content in Vertisols are concerned with, the fertilisers containing these two elements were reported to directly influence their concentration in relation to the initial level in 1984. So, after 33 years, in all the variants with P fertilisers, the TP and AP content increased with variations in relation to the control and NK treatment was found to be statistically highly significant ($P \leq 0.01$) proportionally to the applied fertilisers rates. The highest changes were noticed in the surface layer while the long-term use of fertilisers strongly favoured the accumulation of the available forms in the zone of their input (Otto and Kilian, 2001; Cakmak et al., 2010).

Such a balance of phosphorus was influenced by both, phosphorus doses used on a year basis (35 and 70 kg ha⁻¹) and by its being incompletely utilised. Thus, in this layer, the same phosphorus rates were found to favour the accumulation of this element considerably more in NP1K and NP2K than in NP1 and NP2 variants. The long-term use of lower P rates with NP1K treatment favoured the increase in AP highly significantly ($P \leq 0.01$) compared with the higher rate used in NP2 treatment. Similar trend was reported for the fertilisers impact on TK and AK.

Table 4. The content of OC, TN, TP, TK, AP and AK in the 20-40 cm layer after 33 years

Variants	OC	TN	TP	TK	AP	AK
	%		mg kg ⁻¹			
0	0.90 ± 0.11	0.089 ± 0.011	924 ± 79	13400 ± 450	19.3 ± 1.7	204.5 ± 8.8
NK	1.06 ± 0.09	0.105 ± 0.011	704 ± 129	16500 ± 300	18.7 ± 2.7	266.0 ± 7.1
NP1	1.11 ± 0.03	0.111 ± 0.005	984 ± 86	12500 ± 450	52.0 ± 7.2	208.0 ± 8.0
NP2	1.09 ± 0.06	0.109 ± 0.007	1158 ± 93	13100 ± 400	54.0 ± 5.3	181.0 ± 5.8
NP1K	1.15 ± 0.07	0.116 ± 0.007	994 ± 108	14800 ± 600	51.0 ± 4.0	237.4 ± 3.6
NP2K	1.13 ± 0.05	0.112 ± 0.005	1093 ± 155	14800 ± 350	63.3 ± 2.2	237.0 ± 7.4
Lsd 0.05	0.049	0.0053	75.13	284.48	2.85	6.26
Lsd 0.01	0.067	0.0072	102.92	389.68	3.90	8.58

P content in 20-40 cm deep layer unambiguously suggested its barely slight mobility compared to its input zone (Table 4) whereas a much better mobility applied to potassium, with its content in the 20-40 cm layer considerably matching that in the surface layer. Therefore, the potassium introduced through fertilisation over 33 years of the experiment was continuously leached and more or less evenly distributed in the 40 cm layer being analysed.

Table 5. The content of the available microelements in the Vertisol after 33 years

Variants	Fe	Mn	Cu	Zn
	mg kg ⁻¹			
	0 - 20 cm			
0	88 ± 20	134 ± 18	2.9 ± 0.7	1.4 ± 0.5
NK	94 ± 15	125 ± 20	2.5 ± 0.5	0.8 ± 0.4
NP1	81 ± 15	105 ± 18	2.6 ± 0.9	1.3 ± 0.3
NP2	88 ± 24	110 ± 25	2.0 ± 0.4	1.2 ± 0.3
NP1K	93 ± 32	121 ± 19	2.8 ± 1.1	1.6 ± 0.2
NP2K	84 ± 19	118 ± 30	2.4 ± 0.5	1.5 ± 0.7
Lsd 0.05	14.77	14.90	0.496	0.300
Lsd 0.01	20.23	20.41	0.678	0.412
	20 - 40 cm			
0	83 ± 26	122 ± 15	2.5 ± 0.6	1.4 ± 0.5
NK	89 ± 24	121 ± 17	2.0 ± 0.7	0.8 ± 0.5
NP1	81 ± 12	103 ± 10	2.2 ± 0.5	1.2 ± 0.6
NP2	86 ± 18	100 ± 11	2.1 ± 0.4	1.4 ± 0.6
NP1K	81 ± 14	118 ± 16	2.4 ± 1.0	1.5 ± 0.7
NP2K	78 ± 14	115 ± 18	2.1 ± 0.4	1.5 ± 1.1
Lsd 0.05	12.48	10.15	0.449	0.460
Lsd 0.01	17.10	13.90	0.616	0.630

Fertilisation lasting thirty-three years exhibited its effect on the available microelements forms content (Table 5) in that the highest available Fe content was established with NK and the lowest one with NP1 in 0-20 cm and with NP2K in 20-40 cm, respectively. Neither statistically significant differences between the variants with the highest and lowest Fe content were revealed nor those between the other variants, were. Contrary to Fe content, the lowest Zn content was recorded in both studied layers in NK variant in that in 0-20 cm layer the differences between NK and NP2 variants were statistically significant ($P \leq 0.05$) and those between NK and remaining variants

highly statistically significant ($P \leq 0.01$). As for 20-40 cm layer, highly significant differences could be recorded only between NK with the lowest Zn content and NP1K and NP2K in which the highest Zn content was established.

Thus, the lowest Mn content was recorded in NP1 and NP2 variants but with no clearly established effect of the phosphorus rate. On the other hand, Cu content decreased with fertiliser rate increased, with Cu lowest content established in the NP2 and NP2K variants.

The decreasing trend of active Mn and Cu forms with NP1 and NP2 variants in relation to the control and Zn with NK, NP1 and NP2 variants, was observed at the 20-40 cm depth, at which the lower content of all the four microelements analysed in relation to the surface layer, was also observed.

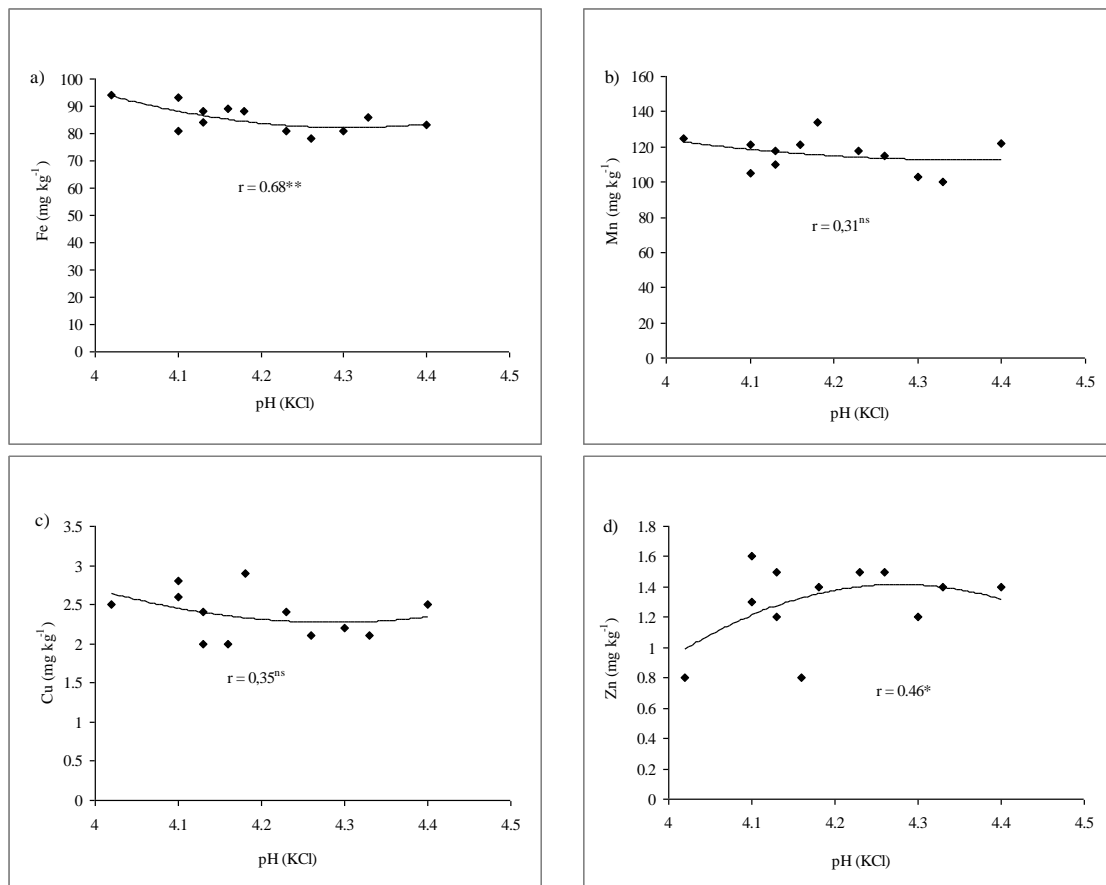


Figure 3. The ratio between the available microelements forms rates (Fe, Mn, Cu, Zn) and pH (KCl) in the surface layer

Therefore, the experience about fertilisers impact on the microelements available forms, some of which being Fe, Mn, Cu and Zn content in the soil, seemed to vary but mainly denying that mineral fertilisers had significantly altered their concentration (Rutkowska et al., 2009) and also stressing a more significant long-term use of organic fertilisers compared with the mineral ones (Li et al., 2010; Richards et al., 2011). As supported by Thakur et al. (2011), in addition to the organic, mineral fertilisers, of which particularly the phosphorus ones (Molina et al., 2009), mainly containing heavy metals (As, Cd, Cr) as well as numerous micronutrients, particularly Zn, may also

influence the microelements content. Thus, the input of P fertilisers may result in a higher Zn content, which was evident in NP1K and NP2K variants in both layers studied.

The availability of microelements in the soil may result from numerous factors, of which OC, soil response in the presence of the phosphorus soluble forms, take an important part (Wei et al., 2006; Asadu et al., 2014). Thus, micronutrients and toxic ions, cations availability, increased with the increase in soil acidity (Khabaz-Saberi and Rengel, 2010). When comparing the dependence between SA and Fe, Mn, Cu and Zn content in the surface layer, highly significant correlation between this trait of the studied soil and available Fe in it, could be found (Fig. 3). In addition, the correlation with the available Zn content seemed to be significant whereas no interdependence between Mn, Cu and SA was revealed.

Overall, significant correlation existed between the soluble forms of phosphorus and available Mn and Zn whereas it did not between phosphorus and available Fe and Cu content (Fig. 4).

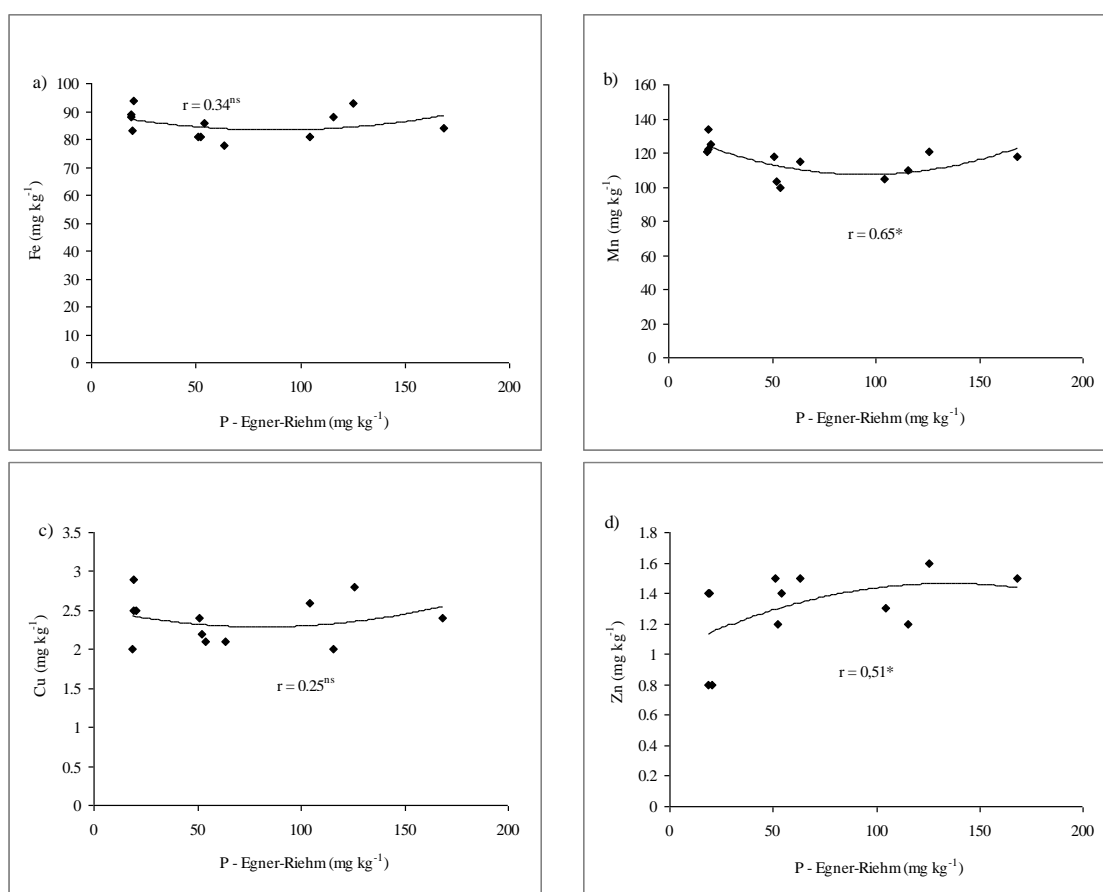


Figure 4. The ratio between the available microelements forms rates (Fe, Mn, Cu, Zn) and the available P in the surface layer

Conclusion

Mineral fertilisers, particularly nitrogen and potassium ones used over thirty-three years, were reported to have caused the acidification of Vertisol, reduced organic carbon content and the total nitrogen one in the surface layer. The changes in the same parameters seemed to be less expressed in the 20-40 cm deep layer. Simultaneously, the total and available phosphorus and potassium content increased in all the cases in which the fertilisers containing these two elements were used, being in proportion with their rates used. Due to low mobility, phosphorus remained in the input zone so that even thirty-three years of its continuous use exhibited a limited effect on its content in deeper horizons. To sum up, Fe, Mn, Cu and Zn available forms content was revealed to depend upon a fertiliser type and phosphorus amount introduced. While NK fertilisers exhibited a significant effect on Zn content, the NP and NPK ones had it on Mn and Cu content. Cu was also found to respond mostly to the amount of phosphorus input in that Cu availability forms decreased with increase in the phosphorus used.

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