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Fractionation of soil phosphorus in a long-term phosphate fertilization

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Abstract: The changes in inorganic and organic phosphorus (P) fractions of soil resulting from long-term fertilization (40 years) were investigated. In order to improve understanding of the sink and sources of phosphorus, P-fractions were extracted from soil samples from depths of 0–30 and 30–60 cm with different amounts of monoammonium-phosphate (MAP) and then determined. The studied soil was of the Stagnosol type. Phosphate fertilizer was applied in 26, 39 and 52 kg P ha⁻¹ amounts during a period of 40 years. Samples were subjected to sequential extraction according to a modified Chang and Jackson method and the Community Bureau of Reference (BCR) sequential extraction procedure in order to extract different forms of phosphorus. The certified reference material CRM 684 (river sediment extractable phosphorus) was used to confirm the accuracy of the instrument and of both employed methods. Furthermore, the association of phosphorus with substrates was provided by comparison of the results of sequential methods of phosphorus species with the sequential extraction of metals (Fe, Al, Mn and Ca). The results of continuous fertilization during 40 years indicated increases of all forms phosphorus in the soil except of phosphorus bound to calcium and organic phosphorus. Application of higher amounts of P-fertilizer resulted in the dominance of the Al–P fraction in the studied soil, which indicated that this fraction was the most responsible for the migration of phosphorus along the soil profile.

Keywords: phosphorus; soil fertilization; sequential extraction; CRM 684.

INTRODUCTION

Phosphates, the most abundant form of phosphorus in the environment, are available for assimilation; thus, they have been traditionally used as fertilizers.¹ For long-term sustainability of cropping, adequate amounts of P through fertilization are needed. It was reported that the addition of inorganic P fertilizers increases the concentration of inorganic P in soil.² Phosphorus fertilization given in

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the form of manure, on the other hand, was found to increase the concentration of organic phosphorus.³ More than 90 % of the total phosphorus in soils is present as insoluble and fixed forms, including the primary phosphate minerals, humus P, insoluble Ca-, Fe-, and Al-phosphates and phosphorus fixed by hydrous oxides and silicate minerals.⁴ Guggenberger *et al.*⁵ found that fertilization increased the labile organic P pool, but the type of fertilizer did not seem to be of any particular importance. The cumulative accumulation of available P in agricultural soils may partially saturate the soil capacity for P sorption, resulting in an increase of P leaching into the subsoil layers⁶ or may sometimes reach a depth of more than 90 cm,⁷ suggesting that erosion, rather than leaching, could cause a threat to water bodies.⁸ Such a leaching process is especially effective in a Stagnosol type of soil, with a clear E horizon due to its lower adsorption capacity, with relatively shallow ground water.^{9,10} Fertilization is an essential part of soil management as plants absorb P from the soil solution or soluble P is lost in runoff, hence, mobile P dissolves or desorbs from the solid phase to the solution.¹¹ In soil where fertilizers were not used, the concentration of phosphorus was gradually decreased to the level where the addition of P is a necessity for plants. Fertilization with mineral P and the inorganic pools explain 96 % of the variation in the level of available phosphorus.¹²

To characterize soil inorganic P in detail, Chang and Jackson¹³ developed a sequential fractionation procedure based on the ability of selected extractants to distinguish between phosphorus in different pools. Originally, their procedure was thought to solubilize separately P in discrete minerals. The discovery of ligand exchange by Hingston *et al.*¹⁴ created a new theoretical basis for this approach. In the light of current knowledge, it is clear that the procedure that Chang and Jackson developed separates different P pools according to the surface the P is bound too. The first extraction step aims at removing the loosely bound P and exchangeable Ca²⁺, possibly interfering with the subsequent extraction steps. The use of NH₄F in the second extraction step is based on the ability of fluoride to form a stable complex with Al and to extract selectively Al-bounded P. Several modifications of the fractionation procedure had been published.¹⁵⁻¹⁷ It was discovered that the Chang and Jackson fractionation procedure was not applicable to calcareous soils or sediments. Based on their work, a fractionation procedure for calcareous soils was further developed. Another widely used fractionation procedure was developed by Hedley *et al.*¹⁸ The BCR extraction method according to the European Program, Standards, Measurements and Testing (SMT) standard protocol was applied for the determination of total P in soil samples. The BCR method is a non-specific extraction procedure developed for the determination of P in freshwater sediments. It was developed within the SMT protocol and used for a certification campaign for reference materials. A detailed description of the SMT protocol was given by Ruban.¹⁹

The aim of this study was to compare different forms of phosphorus in soil after 40 years of fertilization strategies. In the period of 40 years, phosphate fertilizer was applied in amounts of 26, 39 and 52 kg P ha⁻¹, but the added amount of urea was limited. Speciation of phosphorus was performed using two sequential extraction procedures: a modified Chang and Jackson²⁰ and the BCR procedure.¹⁹ The goal of this study was to establish the most active form of P in a long-term mineral fertilization experiment, as well as to monitor the movement of different phosphorus forms along the soil profile.

EXPERIMENTAL

Site description

The investigation was conducted at the Varna experimental station, 44°41'38" and 19°39'10" (near Belgrade, Serbia) where a wide range of different fertilization treatments has been undertaken since 1968. The soil type was Stagnosol, a loam textured Pseudogley developed on Pliocene loam and clay materials under aquatic conditions at 109 m above sea level. Detailed descriptions of the studied site and the soil characteristics were given in a previous paper.²¹ The experiment was arranged as a randomized block design, with each treatment randomized in three blocks for a total of 12 plots (5 by 11 m). Composite samples of five soil subsamples were taken from two depths: surface (0–30 cm) and subsurface (30–60 cm) layers in the spring of 2008. Three different amounts of NH₄H₂PO₄ fertilizer (MAP) were applied during the 40-year experiment (26, 39 and 52 kg P ha⁻¹) in combination with a constant amount of N (urea, 60 kg ha⁻¹) and K (KCl, 50 kg ha⁻¹). The mineralogical composition of the studied soil was illite (50–70 %), vermiculite (10–30 %), and other clay minerals (kaolinite, chlorite, feldspar, quartz, and amphibolites). The cultivated crops were winter wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.) with crop residues removed. The soil cultivation was performed by a standard plowing to a depth of 25 cm. The results were compared with those obtained from the soil samples where only N and K fertilizer was used.

Sequential extraction procedures

A portion of each soil sample (air-dried) was subjected to sequential extraction. Speciation of phosphorus was realized using two sequential extraction procedures: a modified Chang and Jackson²⁰ and the BCR procedure.¹⁹ Details are given in Table I. Furthermore, the soil was subjected to a 5-phase sequential extraction²² in order to study the substrates (Fe, Al, Mn and Ca) of phosphorus and metals. Details are given in Table II.

TABLE I. Reagents and corresponding P fractions for the two sequential extraction procedures used in this study

Procedure	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
Manojlovic <i>et al.</i> 2007	1 M NH ₄ Cl, water soluble P	0.5 M NH ₄ F, Al bound P	0.1 M NaOH, Fe bound P	0.3 M Na dithionite, Na citrate, reducible P	0.1 M NaOH, occluded P	0.25 M H ₂ SO ₄ , Ca bound P
BCR (Ruban <i>et al.</i> , 2001)	1 M NaOH, NaOH-P	3.5 M HCl, HCl-P	1 M NaOH, inorganic P	1M HCl + calcination, Organic P	3.5 M HCl + calcinations, conc. HCl-P	–

The certified reference material CRM 684 (river sediment extractable phosphorus, from the Po River, Italy) was used.

TABLE II. Reagents used in the sequential extraction procedure employed to study the substrate metals (Fe, Al, Mn and Ca) and phosphorus

Procedure	Step 1	Step 2	Step 3	Step 4	Step 5
Petrovic <i>et al.</i> 2009	1 M CH ₃ COONH ₄ , exchangeable	1 M NH ₂ OH·HCl, bound to car- bonates and easily reducible	0.2 M (NH ₄) ₂ C ₂ O ₄ and 0.2 M H ₂ C ₂ O ₄ , mo- derately re- ducible	30 % H ₂ O ₂ + 3.2 M CH ₃ COONH ₄ , organic sulfide	6 M HCl, residual

Reagents and instruments

All chemicals were of analytical reagent grade and were supplied by Merck (Darmstadt, Germany). A stock standard solution of orthophosphate (100 mg P⁻¹) was prepared from KH₂PO₄. Various solutions were prepared by dilution of the stock phosphorus solution. Calibration standards were prepared from multi-element standard stock solutions. These multi-element standards and blanks were prepared in the same matrix as the extracting reagents in order to minimize matrix effects.

The content of phosphorus and metals in all extracts were determined by inductively coupled plasma atomic emission spectrometry, iCAP 6500 Duo (Thermoscientific, United Kingdom).

Statistical analysis

Statistical analyses were performed using SPSS (version) 16 software. The effects of treatments on all variables were tested by ANOVA. The significance of their correlations was analyzed via the Pearson correlation matrix (SPSS, 2007).

RESULTS AND DISCUSSIONS

Modified Chang and Jakson method

The obtained results are presented in Table III.

Phosphorus extractable by NH₄Cl (water-soluble P). The content of phosphorus soluble in ammonium chloride solution (NH₄Cl) was low, about 1 % of the total content of mineral phosphorus. Fertilization with mineral phosphorus increased the content of water-soluble phosphorus in comparison with the control sample (6 times for sample treated by 52 kg P ha⁻¹, 0–30 cm depth). This significant increase was directly related to the accumulation of P²³ and was influenced by the processes of saturation of free spaces for adsorption of P in soil.²⁴

The increase of water soluble P was lower for samples of 30–60 cm depth, which could be explained by P fixation during through-soil leaching.

Phosphorus bound to aluminum (Al-P). Phosphorus bound to aluminum (Al-P) represented a significant part of the mineral phosphorus. The recorded relative increase of Al-P versus the other mineral forms of P (from 16.08 % in the control to 34.51 % in the treatment with 52 kg P ha⁻¹) indicated the strong effect of fer-

TABLE III. Sequential analysis by a modified Chang and Jackson method. Forms of phosphorous in Stagnosol in a long-term phosphate fertilization experiment; m in mg kg^{-1} ; w - ratio to control; w_{\min} - content of mineral P in %; a, b, c, d - the identical letters show no significant difference between the different fertilization treatments

kg P ha ⁻¹	Treatment																		
	NH ₄ Cl-P			Al-P			Fe-P			Reducible P			Occluded P			Ca-P			
	m	w	w_{\min}	m	w	w_{\min}	m	w	w_{\min}	m	w	w_{\min}	m	w	w_{\min}	m	w	w_{\min}	
	Depth: 0-30 cm																		
0	3.08a	1.0	0.8	63.77a	16.1	1.0	0.30a	0.07	1.0	143.08a	36.1	1.0	46.94ab	11.8	1.0	139.35a	35.1	1.0	
26	5.35a	1.7	1.2	100.80b	23.4	1.6	0.54b	0.13	1.8	125.04a	29.1	0.9	32.57a	7.6	0.8	165.75a	38.5	1.2	
39	9.96b	3.2	0.6	130.16c	23.8	2.0	0.44ab	0.08	1.5	195.57b	35.7	1.4	67.37c	12.3	1.4	144.50a	26.4	1.0	
52	19.38c	6.3	1.1	194.88d	34.5	3.1	0.46b	0.08	1.5	176.07b	31.2	1.2	60.70bc	10.7	1.3	126.33a	22.4	0.9	
P	< 0.01			< 0.001			< 0.001			< 0.01			< 0.05						
	Depth: 30-60 cm																		
0	2.69a	1.0	0.8	48.50a	14.4	1.0	0.30a	0.09	1.0	120.27a	35.6	1.0	33.93a	10.0	1.0	132.15a	39.11	1.00	
26	3.71a	1.4	0.9	63.33ab	15.9	1.3	0.41a	0.10	1.4	130.44a	32.6	1.1	44.73a	11.2	1.3	157.02a	39.29	1.19	
39	5.38a	2.0	1.4	78.96b	19.8	1.6	0.31a	0.08	1.0	172.10b	43.1	1.4	56.67b	14.2	1.7	137.91a	34.51	1.04	
52	4.67a	1.7	1.1	83.78b	19.2	1.7	0.28a	0.06	0.9	116.37a	26.6	1.0	47.47a	10.9	1.4	184.15a	42.17	1.39	
P				< 0.01			< 0.01			< 0.01			< 0.05						
Correlation coefficient between two depths	0.518 ($P < 0.05$)			0.876 ($P < 0.01$)			0.405			0.405			0.254						0.385

tilization exactly on this form of phosphorus. This was confirmed by the significant absolute increase of Al-P according to the applied fertilizer (three times more in 52 kg P ha⁻¹ compared to the control). In acidic mineral soils, as in the present case, P is fixed mainly by aluminum and iron while in alkaline soils by calcium.²⁵

The phosphorus movement along the depth was very limited and slow due to its strong binding. However, the increase in the absolute and relative values (from 14.35 % in the control to 19.18 % in the treatment with 52 kg P ha⁻¹), as well as the high coefficient of correlation between the first and second depths, indicate its mobility.

Phosphorus bound to iron (Fe-P). Phosphate bound to iron represented a very small fraction (about 0.1 %) of the mineral Al forms, probably due to its binding to reducible and occluded forms of P, similarly as the labile fraction of Fe-P is included in the Al-P phase. However, in spite of the small amounts, application of P-fertilizer increased its content compared to the control for the first depth, 0–30 cm (Table III). The percentage of Fe-P compared to the other mineral forms changed very little.

Reducible phosphorus (R-P). The reducible-P was well represented in the fraction compared to other mineral forms of P (about 30 %). Noticeably, the portion of this P *versus* the other forms in the first depth was the greatest in the control treatment, while in the higher fertilizer treatments, it was similar to the values of Al-P (Table III). The absolute content of R-P in soil between treatments was different. However, no clear tendency of the increase in its content for each treatment was observed, especially for the second depth.

Occluded phosphorus (O-P). The occluded phosphorus fraction represented about 10 % of the mineral forms of P. Fertilization resulted in irregular increases in the content of occluded P, probably due to irregularities of the field conditions and the processes that influence the content of O-P. Changes in the content of O-P were not detected.

Phosphorus bound to calcium (Ca-P). Phosphorus bound to calcium was a significant fraction within the studied soil. For the second depth, its portion in the mineral forms of P was the greatest (42.17 % in the treatment with 52 kg P ha⁻¹). The absolute content of Ca-P did not show significant differences for both depths (Table III). The absence of the effect of P-fertilization in acidic soils was also recorded in another paper.²⁶ A possible explanation lies in the fact that Ca-P was localized within the mineral apatite and thus, weakly changeable. However, the relative increase of Ca-P for the second depth comparing to the other mineral P forms indicated a significantly reduced binding of phosphorous with Al in the second depth.

BCR sequential analysis

The BCR analysis is not a classic sequential analysis. Less specific means for isolation of the first two fractions are used in comparison with the Chang and Jackson method. The share of NaOH-P (Al-Fe-Mn bound P) and HCl-P (Ca-P) fractions increased for both depths and depended on the amount of added fertilizer (Table IV). The results obtained for the fertilized samples indicated the leaching of the NaOH fraction from the first to the second depth.

The fraction of organic phosphorous (Org-P) represented about 20–30 % of the sum of the inorganic and organic P. The relative amount of organic P decreased from 28.9 to 20.3 %, correspondingly to the application rate of the fertilizer in the first depth, while in the second depth, this decrease was not linear (Table IV). However, for the second depth, the two highest rates of treatment with fertilizer resulted in higher contents of Org-P in comparison with the first depth. The absolute content of Org-P was not changed, which indicates that phosphorus from the fertilizer did not transform to organic P.

The content of concHCl-P showed a linear increase with depth through both layers, with the increase in the greater depth indicating greater fixation of P.

The certified reference material CRM 684 (river sediment extractable phosphorus, BCR, Community Bureau of Reference, Brussels, Belgium) was used to confirm the accuracy of the employed method. The results of phosphorous determination in the reference material showed very good agreement with the declared value (Table V).

Determination of substrates of metals and phosphorus

The results for the phosphorus sequential extraction methods were compared to the results for the sequential extraction of metals and a better assessment of substrates (Fe, Al, Mn and Ca) associated with phosphorus was thereby provided.

The most important correlations found between the contents of the different phosphorus forms and corresponding substrates are presented in Tables VI and VII.

The very high correlation between the applied P and aluminum from the second and the third phase confirms the importance of the aluminum present in soils for the chemodynamics of phosphorus. Regardless of the applied phosphorus extraction method, very high correlations between contents of aluminum and most phosphorus forms were obtained.

Aluminum from the second phase (aluminum II) is a typical representative of the clay minerals present in the examined samples and it could be extracted by an acid hydroxylamine solution, together with manganese oxides and the associated form of P. The role of aluminum II and manganese II confirmed the good correlation with different forms of P (Tables VI and VII).

Oxalate/oxalic acid (the extraction solution for phase III) is very efficient in the extraction of different forms of phosphorus associated with colloid amor-

TABLE IV. Results of the BCR method. Forms of phosphorous in Stagnosol in a long-term phosphate fertilization experiment; *m* in mg kg⁻¹; *w* – ratio to control; *w*_{IP+OP} – sum of IP + organic P, %; a, b, c – the identical letters show no significant difference between the different fertilization treatments

kg P ha ⁻¹	Treatment														
	NaOH-P			HCl-P			IP			Organic P			Conc. HCl-P		
	<i>m</i>	<i>w</i>		<i>m</i>	<i>w</i>		<i>m</i>	<i>w</i>	<i>w</i> _{IP+OP}	<i>m</i>	<i>w</i>	<i>w</i> _{IP+OP}	<i>m</i>	<i>w</i>	<i>w</i>
	Depth: 0–30 cm														
0	523.33a	1.00	366.7a	1.00	990.0a	1.00	71.0	403.3	1.0	28.9	892.0a	1.0			
26	806.67b	1.54	533.3b	1.45	1200.0ab	1.21	76.3	373.3a	0.9	23.7	1127.7ab	1.3			
39	1033.33b	1.97	683.3c	1.86	1473.3bc	1.49	79.6	376.7	0.9	20.6	1188.3b	1.3			
52	1003.33b	1.97	753.3c	2.05	1600.0c	1.61	79.7	406.7	1.1	20.3	1374.0c	1.5			
<i>P</i>	<0.001		<0.001		<0.05										<0.01
	Depth: 30–60 cm														
0	583.33a	1.00	340.0a	1.00	946.7a	1.00	71.0	386.7ab	1.0	29.0	813.7a	1.0			
26	890.00b	1.52	420.0a	1.23	1020.0a	1.08	74.5	350.0a	0.9	25.6	958.3ab	1.2			
39	1210.00c	2.07	546.7b	1.61	1093.3a	1.15	73.7	390.0ab	1.1	26.3	1048.7bc	1.3			
52	1196.67c	2.05	586.7b	1.64	1226.7a	1.30	72.0	476.7b	1.2	28.0	1068.0c	1.3			
<i>P</i>	<0.01		<0.05												
Correlation coefficient between two depths	0.901 (<i>P</i> < 0.01)		0.865 (<i>P</i> < 0.01)		0.483				-0.075						0.799 (<i>P</i> < 0.01)

phous aluminum oxides and iron oxides. This fraction in the examined samples represents a significant substrate of phosphorus and the highest increasing content of phosphorus due to fertilization. This is conformed by the very high correlation factor.

TABLE V. Results of the BCR method. Forms of phosphorous in the certified reference material (CRM 684)

Phosphorous forms	NaOH-P	HCl-P	Inorganic P	Organic P	Conc. HCl-P
Declared, mg kg ⁻¹	550±21	536±26	1113±24	209±9	1373±35
Found, mg kg ⁻¹	587±11	530±11	1145±10	224±5	1401±10

TABLE VI. The correlation of extracted phosphorus by the modified Chang and Jackson method with various substrates

Parameter	Correlation coefficient, <i>r</i>	<i>P</i> value
Added P / Al II	0.9663	0.000
NH ₄ Cl-P / Al II	0.8502	0.000
Al-P / Al II	0.9037	0.000
Added P / Mn II	0.6911	0.013
NH ₄ Cl-P / Mn II	0.6599	0.020
Al-P / Mn II	0.7380	0.006
Al-P / Al III	0.8255	0.001
Red-P / Al III	0.5778	0.049
Added P / Al III	0.9546	0.000

TABLE VII. The correlation of extracted phosphorus by the BCR method with various substrates

Parameter	Correlation coefficient, <i>r</i>	<i>P</i> value
NaOH-P / Al II	0.8936	0.000
HCl-P / Al II	0.9120	0.000
IP / Al II	0.8931	0.000
concHCl-P / Al II	0.8879	0.000
NaOH-P / Mn II	0.6383	0.025
HCl-P / Mn II	0.7321	0.007
NaOH-P / Al III	0.9122	0.000
HCl-P / Al III	0.8945	0.000
IP / Al III	0.8709	0.000
concHCl-P / Al III	0.8721	0.000

Although most of iron oxides are present in phase III (there is no strong correlation with phosphorus), their role in the chemodynamics of phosphorus should not be ignored, especially because aluminum has a tendency to co-precipitate with iron oxide.

In addition, considering the soil type, the low correlations with calcium were expected.

CONCLUSIONS

Long-term fertilization with monoammonium-phosphate (MAP) influenced the content of all forms of P except P bound to Ca as determined by a modified Chang and Jackson method. The 40-year long fertilization period did not affect the content of organic P in soils. On the contrary, the results obtained by the BCR method showed that the contents of NaOH-P and HCl-P fractions were increased, as well as leaching along the soil profile.

Fertilization considerably increased the content of available P, especially of P bound to Al. Application of higher amounts of P-fertilizer resulted in the dominance of the Al-P fraction in the studied soil. A regular influence of fertilization on the second depth (30–60 cm) was established only for Al-P.

The application of mineral P fertilizer on the soil produced a significant increase in the bioavailable phosphorus. Most of this phosphorus was bound to clay minerals (hydrated oxides of aluminum and manganese from second and third phase) and remained in bioavailable forms.

ИЗВОД

ФРАКЦИОНИСАЊЕ ФОСФОРА У ЗЕМЉИШТУ НАКОН
ДУГОТРАЈНЕ ПРИМЕНЕ ФОСФОРНОГ ЂУБРИВА

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У овом раду испитиване су промене у фракцијама неорганског и органског фосфора (P) у земљишту које су изазване 40 годишњим ђубрењем амонијум-фосфатом (MAP). Ђубриво је додавано у количинама од 26, 39 и 52 kg P по хектару земљишта. У циљу праћења мобилности фосфора, екстраховане су фосфорне фракције са две различите дубине земљишта: површински слој од 0–30 cm и дубина од 30–60 cm. Тип испитиваног земљишта је Стагносол. Узорци су у циљу екстраховања различитих облика фосфора подвргнути секвенцијалној екстракцији и то на два начина: према модификованој *Chang* и *Jackson* методи и BCR методи. Сертификовани референтни материјал CRM 684 (речни седимент) је коришћен за праћење тачности обе коришћене методе. Осим тога је проучено везивање фосфора са супстратима (Fe, Al, Mn и Ca) који су екстраховани секвенцијалном екстракцијом метала. Резултати указују да континуално 40 годишње ђубрење доводи до повећања садржаја свих облика фосфора у земљишту осим фосфора који је везан за калцијум и органског фосфора. Применом веће количине фосфорног ђубрива долази до доминације фракције фосфора везаног за алуминијум у испитиваном земљишту што указује да је та фракција најодговорнија за миграцију фосфора кроз земљиште.

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