

Differences in available phosphorus evaluated by soil tests in relation to detection by colorimetric and ICP-AES techniques

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ABSTRACT

Differences in the evaluation of soil phosphorus status by three soil tests (Mehlich 3, extraction with NH_4 -acetate and water extraction) were tested on 63 agricultural soils with different agrochemical characteristics from the territory of the Czech Republic. Differences between the colorimetric determination of phosphorus and ICP technique were studied. The median of the values of phosphorus supply in soils determined by soil tests was considerably different. Compared to the colorimetric detection of water extraction of soils the median of the NH_4 -acetate test showed 2.2 times higher values and in Mehlich 3 test the values were 34.8 times higher. The largest difference between the end-point analytical techniques of phosphorus determination, colorimetry and ICP-AES, was observed in the soil test of water extraction. The median of the values determined by ICP-AES was higher by 47%. In NH_4 -acetate extraction of soils the median of the measured values of phosphorus was higher by 12% and in Mehlich 3 extraction by 7%. Differences in phosphorus concentrations determined by colorimetry and by ICP-AES increased as the phosphorus supply in soils decreased. When the supply of 'available' phosphorus in soil is given, it is always necessary to specify the used soil test including the end-point analytical technique of phosphorus determination to avoid the misleading interpretation of results. The problem of phosphorus in agriculture and in the environment requires thorough revision and methodical standardization.

Keywords: Mehlich 3; NH_4 -acetate extraction; water extraction; phosphorus; colorimetry; ICP-AES technique

To evaluate the phosphorus supply in soils, different soil tests have been used for a long time; however they differ markedly in extractants and extraction methods. Nevertheless, the result of the test is generally reported as phosphorus available to plants. In the beginnings of soil tests, i.e. approximately after World War I (Matula 2009a), it was phosphorus extraction from soils with aggressive single-nutrient agents. The use of strong extractants was motivated by a low content of extractable phosphorus forms in the beginnings of more intensive fertilization of agricultural soils with commercial fertilizers and by the analytical phosphorus detection limits.

With developments of chemical analytical techniques and their automations, a requirement appeared for multi-nutrient extractants of soil tests to determine as many as possible nutrients in one extract so that modern analytical instruments will

be utilized as well as possible (Houba et al. 1994, Jones 1998).

Colorimetry by means of the phosphomolybdenum blue complex has always been the most widespread analytical method of phosphorus determination; it assumes that mainly inorganic phosphorus, i.e. orthophosphate ions, is detected. Phosphorus determination on ICP instruments where total phosphorus, including inorganic and organic phosphorus, should be detected has been used more and more frequently in recent years.

The history of phosphorus determination by help of molybdenum blue chemistry has underwent developments in the use of various reductants from e.g. hydrazine sulphate through tin (II) chloride to the most frequent use of ascorbic acid and antimony catalyst that was standardized by Murphy and Riley (1962). The formation of the blue-coloured complex of the reduced phos-

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phomolybdenum blue is related with pH of the medium, time of the reaction of developing the blue colour and temperature. The automation of determination by the techniques of continuous flow analysis (CFA) and flow injection analysis (FIA) contributed to standardization of the blue colour development and to more accurate results of determination compared to manual determination because in the manual procedure of determination it is not possible to observe in a standard way the time from the reagent addition to the time of absorbance measurement on a colorimeter, especially in larger series of samples. The acid pH value ($\text{pH} < 2$) is a condition of the blue colour development. However, the hydrolysis of less stable esters of phosphorus may take place at this acidity of the medium. It may cause an augmentation of the assumed determination of inorganic P by phosphorus from the organic component of soil. Baldwin (1998) reported such a case. Kowalenko and Babuin (2007) described further interferential components influencing the formation of the blue-coloured complex. They demonstrated the influence of Al, Mn, Fe, K, Ca, Mg, Na, NO_3^- and EDTA, i.e. of common components in soil extracts and plant material mineralizates.

In agronomic interpretation, it will be necessary to respect potential differences in the determination of extracted phosphorus by the classical colorimetric method and by modern ICP detection of phosphorus because the preceding calibration of soil test P was based on colorimetric determination of phosphorus. Mallarino (2003) accentuated the need of different interpretation in Mehlich 3 soil test according to the method of analytical detection of phosphorus although he observed a very good correlation between colorimetry and ICP technique.

The objective of our study was to verify differences in the determination of so-called available phosphorus by three soil tests (Mehlich 3, extraction with NH_4 -acetate and water extraction) on a manifold set of soils with large differences in the agrochemical characteristics. We also aimed to determine the impact of classical colorimetric determination of phosphorus and currently expanding ICP-technique on the results of extracted phosphorus determination by soil tests.

MATERIAL AND METHODS

Research was conducted on 63 soil samples taken from the surface profile of agricultural lands from

different localities of the Czech Republic. After air-drying the soil samples were homogenized by screening through a 2-mm sieve.

The basic agrochemical characteristics of soils were determined in soil samples: pH, CEC value and content of oxidizable soil organic matter. The pH value was determined in a soil suspension with 0.2M KCl solution at a 1:1 ratio (w/v) with a microprocessor pH-mV meter with pH combined electrode. The CEC value was determined according to Matula (1996). The content of oxidizable soil organic matter was determined according to Sims and Haby (1971).

To evaluate the phosphorus supply in soil, three soil tests were used similarly like in Matula (2009b): Mehlich 3 (Zbiral 2002), water extraction at 1:5 w/v (SPAC 1999) and ammonium acetate extraction with the addition of ammonium fluoride (Matula 1996). The phosphorus concentration in soil extracts was determined by means of the reduced phosphomolybdenum blue on a SKALAR auto-analyzer (Skalar 1992) and by the ICP-AES technique on a Thermo Jarrell Ash Trace Scan analyser. The Skalar automated procedure of phosphate determination is based on the following reaction: ammonium heptamolybdate and potassium antimony (III) oxide tartrate react in an acid medium with diluted solutions of phosphate to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-coloured complex by L(+)-ascorbic acid. The complex is measured at 880 nm.

In addition, in soil extraction with water the suspension was centrifuged at relative centrifugal force 12 680 g for 10 min before filtration. The cooling of ultracentrifuge was kept at 20°C. The filter paper Filtrak, grade 389, was used for the filtration of all extracts from soil tests.

Experimental results were evaluated by statistical programmes GraphPad PRISM, Ca., USA, version 3 and Microsoft Excel 2007.

RESULTS AND DISCUSSION

Tables 1 and 2 show data on the agrochemical characteristics of the set of soils used for research. The tables document that it was a manifold set of soils with a large range of the characteristics of soil chemistry, especially of phosphorus concentrations in the extracts of soil tests. Figures 1–3 illustrate the frequency of occurrence of soil organic matter content, values of cation exchange capacity of soils, and values of soil acidity in the used set

Table 1. Column statistics of basic soil characteristics (63 analyzed soils)

Statistic parameters	Oxidized organic matter (% C)	CEC (mmol(+)/kg)	pH (0.2M KCl)
Minimum	0.850	59	3.55
25% percentile	1.63	124	5.08
Median	2.02	137	5.47
75% percentile	2.57	153	5.92
Maximum	5.13	305	6.97
Mean	2.24	140	5.50
Standard deviation	0.886	39.9	0.606
Standard error	0.112	5.02	0.076
Lower 95% (confidence interval)	2.01	130	5.35
Upper 95% (confidence interval)	2.46	150	5.65
Coefficient of variation (%)	39.61	28.41	11.01
Geometric mean	2.09	136	5.47

of soils. The highest frequency of the content of oxidizable soil organic matter was in the range of 1.5–2.5% C. The most frequent value of soil acidity in the set of soils was in the range of 5–6 pH (0.2M KCl). In the set of soils, medium-textured

soils were represented the most frequently with the CEC value 120–160 mmol(+)/kg.

The term ‘available phosphorus’ is generally used for extracted phosphorus by all tests. Differences among the soil tests (Table 2) indicate that this

Table 2. Statistics of P-soil test of 63 soils measured by phosphoantimonyl blue colorimetry and by inductively coupled argon plasma atomic emission spectrophotometry (ICP-AES)

Statistic parameters	Soil test (mg P/kg)					
	Mehlich 3		NH ₄ -acetate		water extraction	
	colorimetry	ICP-AES	colorimetry	ICP-AES	colorimetry	ICP-AES
Minimum	9.7	19.6	3.6	5.8	0.25	1.12
25% percentile	50.4	61.9	7.50	10.6	1.80	4.84
Median	86.3	90.7	13.2	16.3	3.50	6.34
75% percentile	144	167	28.5	30.2	6.48	10.90
Maximum	3040	3110	163	174	77.70	98.60
Mean	336	361	21.4	24.00	9.65	14.20
Standard deviation	709	742	24.6	24.9	16.90	20.90
Standard error	89.3	93.4	3.10	3.14	2.13	2.64
Lower 95% (confidence interval)	158	174	15.2	17.7	5.38	8.95
Upper 95% (confidence interval)	515	548	27.6	30.3	13.90	19.50
Coefficient of variation (%)	210.81	205.33	115.3	103.7	175.63	147.23
Geometric mean	108	127	14.4	18.0	3.88	8.20
Correlation coefficient (<i>r</i>)	0.9995		0.9966		0.9934	
Two-tailed paired <i>t</i> -test	<i>P</i> < 0.0001		<i>P</i> < 0.0001		<i>P</i> < 0.0001	
Mean of differences	–25.04		–2.665		–4.571	
<i>R</i> squared	0.2769		0.6264		0.5072	

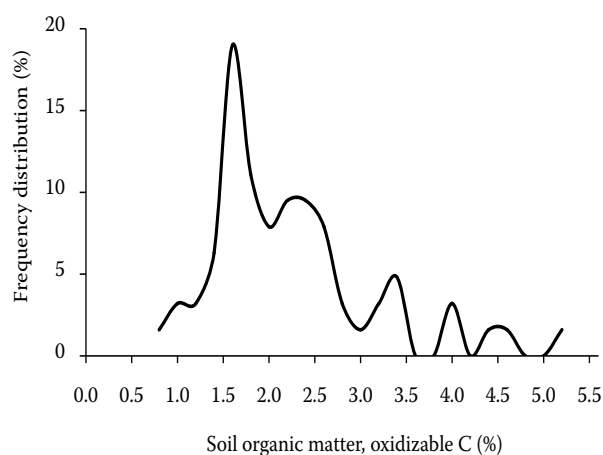


Figure 1. Frequency distribution of soil organic matter in the set of 63 soils

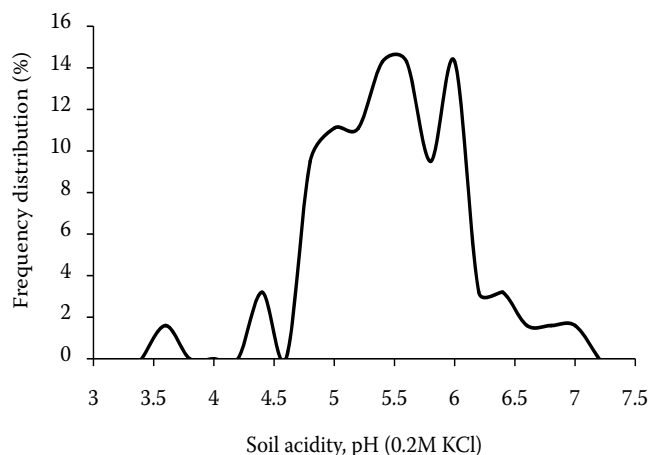


Figure 2. Frequency distribution of pH (0.2M KCl) value in the set of 63 soils

term is not correct and can be misleading for farmers. Table 2 also documents the impact of the used end-point analytical technique on the result of the soil test even though a close correlation was observed within one soil test. However, the pair test demonstrated a highly significant difference between the sets of both end-point analytical techniques in all three soil tests.

If we make a comparison of the used soil tests and if we take the soil test of water extraction with colorimetric detection of phosphorus as the basic test, the median of the values obtained by NH_4 -acetate extraction reached 2.2 times higher values of phosphorus. In Mehlich 3 extraction even 34.8 times higher values of extracted phosphorus were reached. Apparently, it is a result of the strength of Mehlich 3 extractant given by the pH value below 2.9.

The largest difference between the end-point analytical techniques of colorimetry and ICP-AES was observed in the soil test with water extraction (Table 2). The values of the median determined by ICP-AES were higher by 47%. In soil extraction with NH_4 -acetate the median of the measured phosphorus values was higher by 12%, and in Mehlich 3 extraction by 7%.

From the environmental point of view, in aquatic systems, phosphorus is determined on the colorimetric reaction of phosphate with an acidified molybdate reagent to yield phosphomolybdate heteropolyacid, which is then reduced to an intensely coloured blue compound and is specified as 'dissolved reactive phosphorus' (Robards et al. 1994, Worsfold et al. 2005, Soldat et al. 2009, Regan et al. 2010) or as 'dissolved molybdate-reactive phosphorus' (Styles and Coxon 2006). It is supposed that the molybdate-reactive phosphorus should

be mainly equal to the inorganic *orthophosphate*. Phosphorus detected by ICP-technique is defined as 'total phosphorus'. The difference between total phosphorus and reactive phosphorus is termed 'unreactive phosphorus'. The dissolved unreactive phosphorus represents mainly organic form of phosphorus and P fixed into an organic and an inorganic particles ('particulate phosphorus') which passed procedure of filtration (Worsfold et al. 2005, Styles and Coxon 2006, Blackwell et al. 2009). In our case of water extract of soils, the great difference between the colorimetric and ICP phosphorus in comparison with Mehlich 3 and NH_4 -acetate extracts indicates a possibility of a significant part of 'particulate phosphorus' due to limited aggregation of soil particles in the low ionic strength of water extract (Ebeling and Davis 2009).

Differences between phosphorus concentrations revealed by colorimetry and ICP-AES increased as

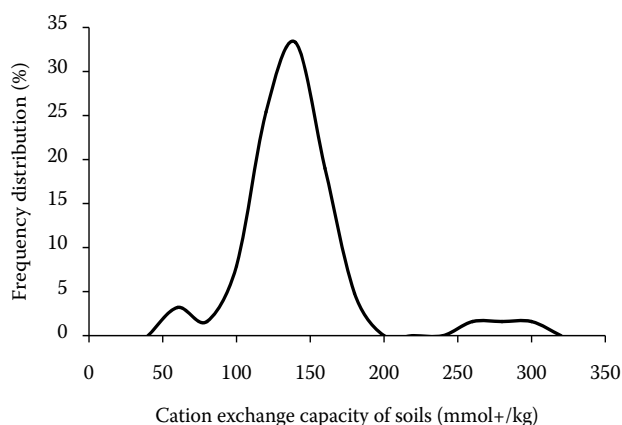


Figure 3. Frequency distribution of cation exchange capacity in the set of 63 soils

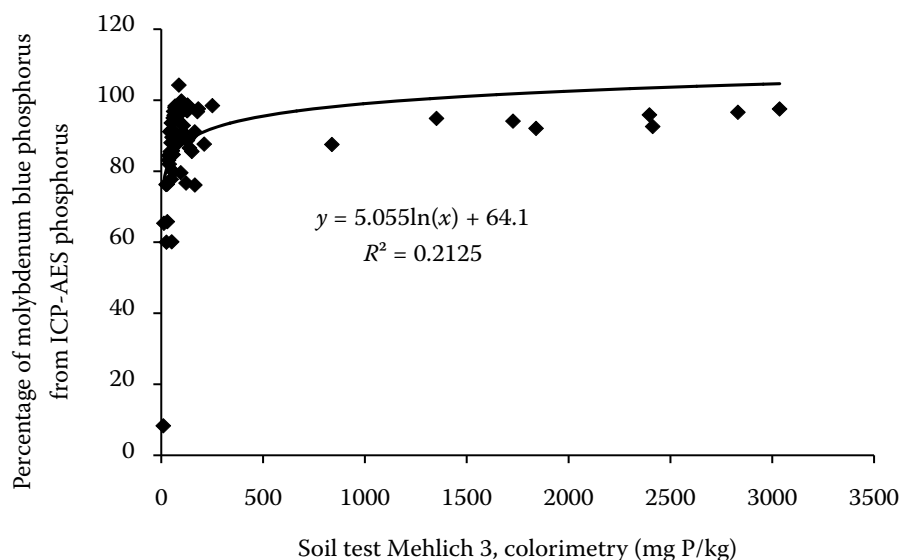


Figure 4. Percentage of spectrophotometrically determined phosphorus from ICP-AES P-detection in the case of Mehlich 3 soil test by the colorimetric detection of phosphorus

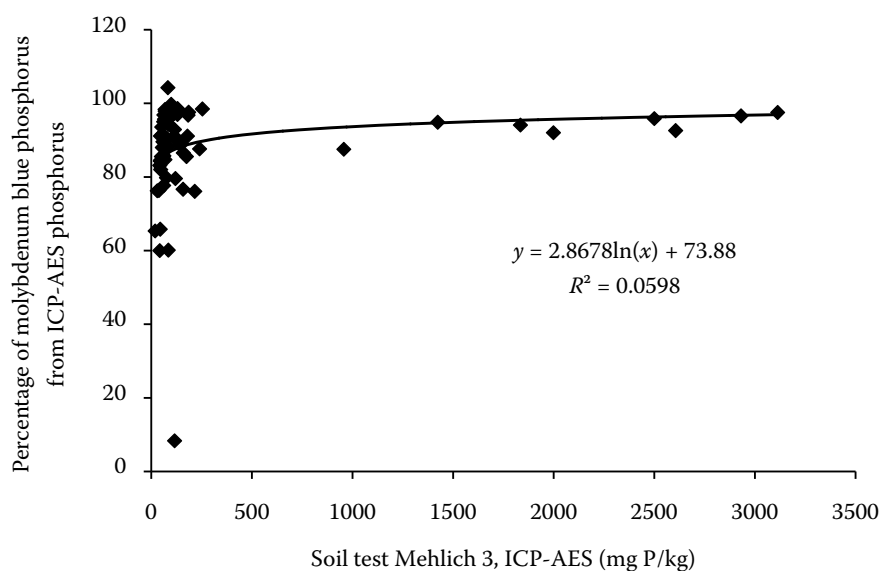


Figure 5. Percentage of spectrophotometrically determined phosphorus from ICP-AES P-detection in the case of Mehlich 3 soil test by the ICP-AES detection of phosphorus

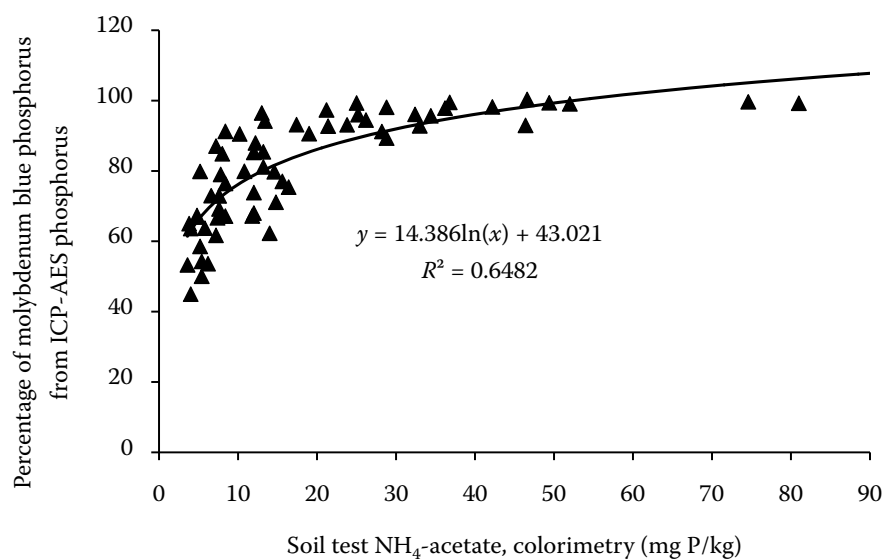


Figure 6. Percentage of spectrophotometrically determined phosphorus from ICP-AES P-detection in the case of NH₄-acetate soil test by the colorimetric detection of phosphorus

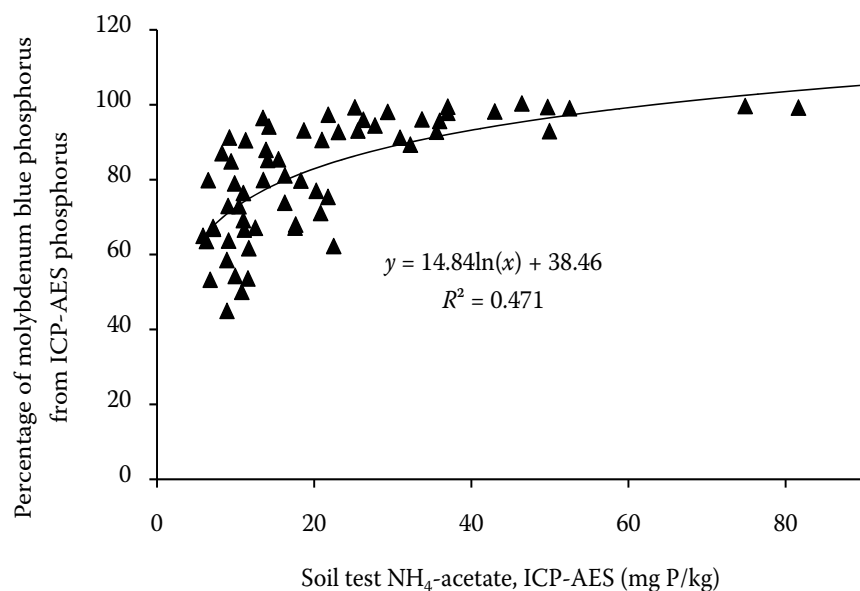


Figure 7. Percentage of spectrophotometrically determined phosphorus from ICP-AES P-detection in the case of NH_4 -acetate soil test by the ICP-AES detection of phosphorus

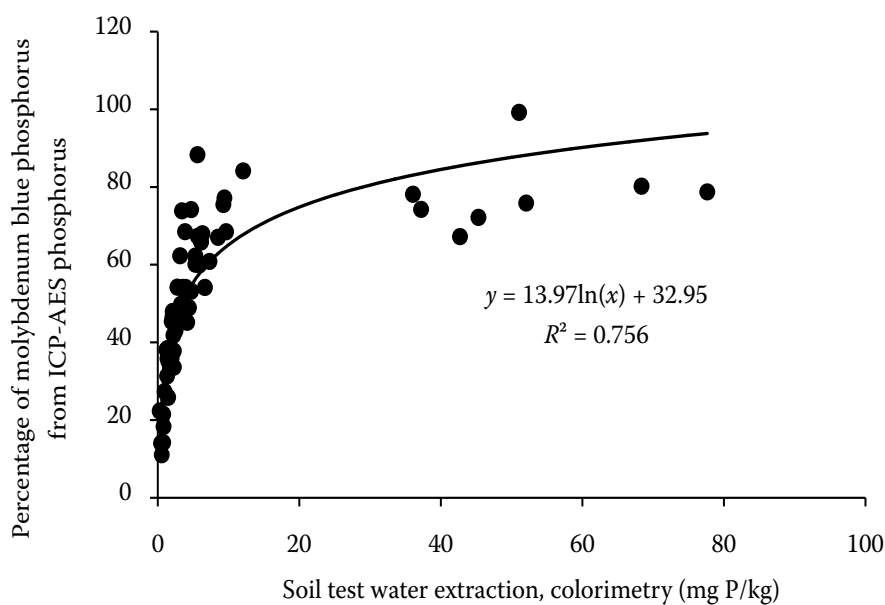


Figure 8. Percentage of spectrophotometrically determined phosphorus from ICP-AES P-detection in the case of water extraction soil test by the colorimetric detection of phosphorus

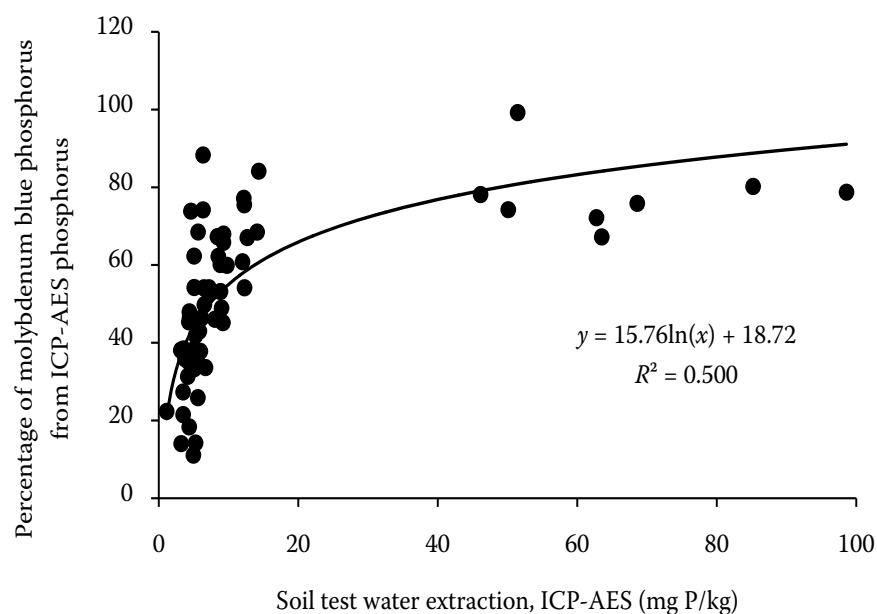


Figure 9. Percentage of spectrophotometrically determined phosphorus from ICP-AES P-detection in the case of water extraction soil test by the ICP-AES detection of phosphorus

the phosphorus level in soils decreased (Table 2). This relation is clearly obvious from the graphical representation of the relative proportion of colorimetrically determined phosphorus in ICP-AES phosphorus (y-axis) in relation to the soil test (x-axis) (Figures 4–9). In other words, at a decrease in the phosphorus level in soil an increase in the proportion of probably organic phosphorus that is detected by the ICP-AES technique compared to colorimetry can be assumed in the extracts of soil tests.

In the soil tests Mehlich 3 and NH_4 -acetate extraction it is possible to expect the precipitation of the fine colloidal fraction of 'particulate phosphorus' of both mineral and organic form by a high concentration of ions of these extractants (Ebeling and Davis 2009).

Meanwhile, in the water extraction of soils the fine colloidal fractions of unreactive phosphorus with phosphoantimonyl blue colorimetry may still be present after centrifugation and filtration of the solution. In our case of the soil test by water extraction in the area of a higher phosphorus level (above 10 mg P/kg) the undetected component of soluble phosphorus by colorimetry accounted for more than 20% of the determined soluble phosphorus by the ICP-AES technique (Figures 8–9). Obviously, at colorimetric detection of phosphorus when its soil level decreases, there arises a possibility of underestimating the content of mobile phosphorus in soil compared to ICP-AES detection (Figures 4–9). It is however questionable to what extent this soluble phosphorus detected by ICP-AES, probably organic phosphorus, is utilisable for plant nutrition. From the environmental aspect it is an easily mobile component. It may be a source of soluble phosphorus release to the environment.

Phosphorus supply in soils has been evaluated for a long time by means of different soil tests that are however hardly mutually comparable from the aspect of the control and formation of efficient P level in soil. Therefore it is always necessary to describe the method of determination of phosphorus extraction including the end-point analytical technique. For the above reason the general designation of phosphorus reserve in soil by the term 'available' is incorrect, misleading and confounding for farmers. The possibility of reached differences by using the same soil test in a different laboratories without exact specification of procedure can then lead to disappointing yields plus the possibility of creating a soil fertility imbalance (SPAC 1999).

As the previous background data for P of soil tests were based on colorimetric detection, it will be necessary to revise and redefine the criteria of agronomic interpretation of soil tests for the end-point analytical technique of determination in all soil tests, not only for Mehlich 3 soil test as reported by Mallarino (2003).

Our experimental results support the opinions that the problem of phosphorus in agriculture and in the environment requires thorough revision and methodical standardization (Baldwin 1998, Worsfold et al. 2005, Kowalenko and Babuin 2007).

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