

# Relationship between phosphorus concentration in soil solution and phosphorus in shoots of barley

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## ABSTRACT

Phosphorus concentration in the soil solution of agricultural soils should be a consensus of the agronomic and environmental aspect. Data from literary sources are inconsistent if the method of soil solution extraction from the soil and the method of phosphorus detection are not indicated. In the present paper a simplified procedure of soil solution extraction is used that is derived from the need of water to attain saturated soil paste. Based on barley cultivation in a plant growth chamber on 72 different soils the relationship between P concentration in simulated soil solution and the response of test plant (spring barley) was evaluated. Three approaches were used to derive an adequate P concentration in soil solution. Based on the diagnostics of P content in barley the following adequate P concentrations in soil solution were derived: 0.23–0.86 ppm P for colorimetry and 0.9–1.75 ppm P for ICP-AES. Using the concept of the boundary line of yield the critical P concentration in soil solutions was 0.8 ppm P for colorimetry and 1.3 ppm P for ICP-AES. The concept of the boundary line of P efficiency index enabled to define P concentrations in soil solution that can be considered as the lower limits of suitability from the agronomic aspect: 0.15 ppm P in simulated soil solution for colorimetry and 0.7 ppm P for ICP-AES.

**Keywords:** soil testing; plant growth chamber; colorimetric detection; ICP-AES detection

An adequate concentration of phosphorus in soil solution and its refilling from the solid phase of soil are necessary for the required production of agricultural crops (Tisdale and Nelson 1975, Denison and Kiers 2005, Reid and Scholas 2005). The concentration of soluble phosphorus fractions in the aqueous phase of the surface layer of soil is also important from the environmental aspect of potential phosphorus runoff into surface waters (Matula 2009).

The knowledge of composition of the phosphorus concentration in soil solution could underlie a reasonable consensus between the agronomic and environmental approach. The equilibrium P-concentration in the soil solution is also a good parameter for improving P-soil testing. It agrees with the opinion (Sims 2000, Fixen 2005, White and Brown 2010) that only an unconditionally necessary level of soluble forms of phosphorus should be maintained in agriculturally productive soils to ensure the necessary yield of agricultural crops.

Data on an adequate optimum concentration of phosphorus in the soil solution are scarce and

inconsistent; in fertile arable soils they range from 0.03 to 0.3 ppm P (Tisdale and Nelson 1975, Sanchez 1976, Sims 2000, Mengel and Kirkby 2001). It is generally agreed that the soil solution is a centre of all chemical and biological activities in soil. The cause of the inconsistency of data on phosphorus concentration in soil solution may also be a very difficult methodical procedure and labour-consuming isolation of soil solution from the soil. Sampling methodology of soil solution has a profound influence on the composition of the soluble constituents, so one cannot study the soil solution without altering it (Schwab 2000). The specification of the method of soil solution extraction from the solid phase of soil and also the end-point analytical procedure of phosphorus determination will influence the obtained values of phosphorus concentration. All older data on phosphorus concentration in the aqueous phase were mostly determined colorimetrically using the phosphomolybdenum blue complex that basically detects only inorganic phosphorus, orthophosphate

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ions. Recently, an ICP-AES technique has been introduced for phosphorus detection on a larger scale that detects all soluble phosphorus, i.e. inorganic phosphorus including organic phosphorus. The determination of total soluble phosphorus in the aqueous phase is important from the environmental aspect because all forms of soluble phosphorus are detected that may be transported to surface and subsurface runoff waters. Matula (2011) found that differences between the phosphorus values detected by ICP and colorimetry in the water extracts were uneven, they relatively increased with a decrease in the phosphorus reserve in soils.

The objective of our study was to contribute to the knowledge of an adequate phosphorus concentration in soil solution for the needs of innovation and correction of criteria of phosphorus reserve in soils both from agronomic and environmental aspects. Another objective was to simplify and standardise the method of soil solution extraction from the solid phase of soils that would be applicable also in routine testing of a larger number of soil samples. In the present study this problem is solved by so called simulated soil solution. Vegetation trials of the cultivation of a test plant in a plant growth chamber were used to find the response of plants to P concentration in a simulated soil solution of the set of 72 different soils. The advantage of growth chamber studies is the acquisition of cheaper and more expeditious information on the relationship between biological availability of nutrients in a larger set of soils with different characteristics of soil reserve under standardised conditions of cultivation of test plants (Dahnke and Olson 1990).

## MATERIAL AND METHODS

Research was conducted on 72 soil samples taken from the surface profile (20 cm) of agricultural lands in different localities of the Czech Republic. The soil samples were homogenized by screening through a 2-mm sieve, and finished by air-drying.

The following important basic agrochemical soil characteristics were determined in soil samples: pH, CEC value and content of oxidisable soil organic matter. The pH value was determined in a soil suspension with 0.2 mol KCl/L solution at a 1:1 ratio (w/v). The CEC value was determined according to Matula (1996). The content of oxidizable soil organic matter was determined according to Sims and Haby (1971).

Soil solution was extracted from the soil samples to determine the concentration of colorimetrically

detected phosphorus, so called dissolved reactive phosphorus (Matula 2011), and dissolved total phosphorus by means of ICP-AES technique. Biological availability of phosphorus was determined in these soils using a test plant in growth chamber studies.

**Procedure of extraction of simulated soil solution.** It is a modification of the laboratory procedure of water extraction that consists of three steps: (a) addition of water to obtain saturated water paste or beyond; (b) equilibrating; (c) removing the solution. The advantages of these techniques include ease of preparation, simple separation of soil and solution, and the ability to control many experimental parameters (Schwab 2000).

The simulation of soil solution is based on the need of water for attaining so called saturated soil paste using a friction test of 25 g of soil. Determination of the water need for attaining so called saturated water paste is a very labour- and time-consuming procedure especially if standardised technological equipment is lacking. For this reason we focused our efforts on an experimental solution of the relationship between water need for the creation of saturated soil paste and cation exchange capacity of soil (CEC).

Based on our research into a large set of soils from topsoil of the Czech Republic a close regression relation was found out between the CEC value of soil and water need for the creation of saturated soil paste:  $y$  (mL of water)/25 g of soil) =  $0.4616 \times \text{CEC}^{0.552}$

**Procedure of water addition.** An amount of 50 g of the soil sample is weighed into a centrifugation cuvette with lid. Shake the soil in a cuvette to make its surface slant as far as the edge of the cuvette neck. Add the calculated volume of distilled water to the soil with a graduated pipette to create saturated paste in relation to the CEC value of soil that will be increased by a half. The calculation of water addition to 50 g of soil:  $\text{mL H}_2\text{O}/50 \text{ g} = ((0.4616 \times \text{CEC}^{0.552}) \times 2) \times (1.5)$ . After water is added to the soil, close the cuvette with lid and leave it in laboratory overnight (equilibrating step).

**Procedure of removing the solution.** On the next day the cuvettes are centrifuged in a high speed refrigerated centrifuge with cooling at 20°C. The time of centrifugation is 10 min at 9000 rpm, which is equivalent to the gravity of 12 680 g. Take carefully the cuvettes out of the rotor, remove the lids and suck gently the clear solution with a graduated pipette (10 mL to 20 mL) equipped with a suction balloon. Record the sucked volume of the solution and immediately refill the pipette

volume with distilled water to the full volume of the pipette. Deduce the dilution of simulated soil solution from data. Empty the pipette contents through the folded filter paper cone (7–9 cm in diameter, Filtrak 389 or Whatman 40) in order to potentially capture floating organic impurities into sample tubes. The samples of solutions are analysed without delay for phosphorus content by ICP-AES technique and colorimetry.

The phosphorus concentration in simulated soil solution was determined by means of the reduced phosphomolybdenum blue complex on a SKALAR auto-analyser (Skalar 1992) and by the ICP-AES technique on a Thermo Jarrell Ash Trace Scan analyser (Franklin, USA).

**Growth chamber trials.** Short-term (21-day) vegetation trials were established on each soil with spring barley cv. Jersey as a test plant. Each variant had three replications. Vegetation pot of 6 cm in diameter was filled with 150 g of soil. Fifteen barley seeds (after their washing and one-hour soaking in distilled water) were planted onto the soil surface in the vegetation pots and covered with 25 mL of coarse-grained quartz sand. Moistening of the vegetation pots was differentiated on the basis of an experimentally determined relationship between the field water capacity of soil and the value of its cation exchange capacity (CEC) (Matula et al. 2000). The moisture content was regularly renewed according to the weight loss of the vegetation pot. Cultivation took place in a plant growth chamber with the light and temperature regime: daylight 16 h, 20°C, dark 8 h, 15°C; photosynthetically active radiation 500  $\mu\text{E}/\text{m}^2/\text{s}$ . Nitrogen dose of 6 mg N/pot, as  $\text{NH}_4\text{NO}_3$  solution, was applied continuously ten times during the trial. Harvested barley shoots were instantly dried at 65°C. A Milestone microwave device was used for mineralisation of barley dry matter in the medium

of nitric acid and hydrogen peroxide; the analysis was carried out on an ICP-AES Trace SCAN apparatus (Thermo Jarrell Ash, Franklin, USA).

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

## RESULTS AND DISCUSSION

The set of soils with a large range of agrochemical characteristics was chosen intentionally for these experiments so that obtained results would have a more universal application for the exact specification of adequate phosphorus reserve in agricultural soils. The feature of universal functionality in different soil conditions is one of the fundamental prerequisites of sophisticated soil testing in future (Skogley 1994).

Tables 1 and 2 show statistical parameters of soil characteristics. Minimum values of oxidisable carbon ( $\text{C}_{\text{ox}}$ ) content, values of cation exchange capacity (CEC) and pH values were as follows: 0.85%  $\text{C}_{\text{ox}}$ , 59  $\text{mmol}_+/\text{kg}$  and 3.55  $\text{pH}_{0.2 \text{ mol/L KCl}}$  respectively. On the contrary, maximum values of oxidisable carbon ( $\text{C}_{\text{ox}}$ ) content, values of cation exchange capacity (CEC) and pH values were substantially higher: 5.13%  $\text{C}_{\text{ox}}$ , 305  $\text{mmol}_+/\text{kg}$  and 7.21  $\text{pH}_{0.2 \text{ mol/L KCl}}$  respectively. The determined phosphorus concentration in soil solution at colorimetric detection ranged from the minimum 0.016 mg P/1000 mL to 4.333 mg P/1000 mL. When phosphorus was determined in simulated soil solution by ICP-AES technique, the concentration ranged from the minimum 0.258 mg P/1000 mL to 5.259 mg P/1000 mL. Differences in the concentration of phosphorus in simulated soil solution detected by colorimetry and ICP were uneven in the range of determined concentrations (Table 2). Similarly like in Matula (2010, 2011) the

Table 1. Column statistics of basic soil characteristics (72 analysed soils)

Statistic parameters	Oxidised organic matter (% C)	CEC ( $\text{mmol}_+/\text{kg}$ )	pH (0.2 mol/L KCl)
Minimum	0.85	59	3.55
25% percentile	1.60	128	5.27
Median	1.97	139	5.70
75% percentile	2.46	154	6.28
Maximum	5.13	305	7.21
Mean	2.15	146	5.77
Coefficient of variation (%)	37.4	27.9	12.9
Geometric mean	2.03	141	5.71

Table 2. Column statistics of the phosphorus concentration in simulated soil solution of 72 soils and P-content in barley shoots

Statistic parameters	Phosphorus concentration in simulated soil solution (mg P/1000 ml)		Percentage of colorimetric P from ICP-AES phosphorus (%)	Phosphorus content in dry matter of barley shoots
	Colorimetry determination	ICP-AES determination		
Minimum	0.016	0.258	2.8	1.83
25% percentile	0.246	0.935	26.3	3.56
Median	0.632	1.389	43.2	4.48
75% percentile	1.498	2.458	62.0	5.35
Maximum	4.333	5.259	86.5	6.45
Mean	0.946	1.801	43.45	4.37
Standard deviation	0.940	1.169	22.1	1.09
Standard error	0.111	0.138	2.6	0.129
Coefficient of variation (%)	99.4	64.9	50.9	25
Geometric mean	0.538	1.494	36.0	4.22

largest differences were observed in the area of low phosphorus concentrations in soil extracts. It is assumed that colorimetrically determined phosphorus using the phosphomolybdenum blue complex represents inorganic phosphorus, i.e. orthophosphate ions. Phosphorus detection by ICP-AES technique shows total phosphorus. The difference between ICP determination and colorimetry can be considered as organic phosphorus including particulate phosphorus (Baldwin 1998, Worsfold et al. 2005, Kowalenko and Babuin 2007). The scatter plot in Figure 1 illustrates a relation-

ship between the total phosphorus concentration in simulated soil solution and the percentage of inorganic phosphorus detected colorimetrically in simulated soil solution. These results document a possibility of great differences between inorganic phosphorus (detected colorimetrically) and total phosphorus detected by ICP-AES. In other words, data on the phosphorus concentration in soil solution may be misleading if the method of phosphorus detection is not indicated, especially in the area of low phosphorus concentrations in soil solution. It also may elucidate the reason for a

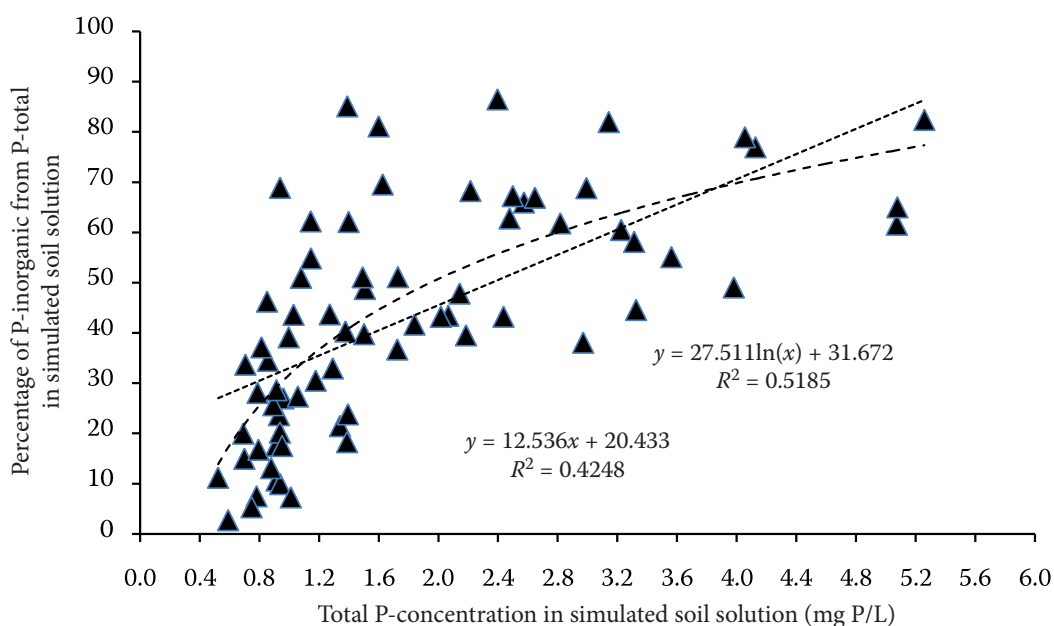


Figure 1. Scatter diagram of percentage of inorganic phosphorus from total phosphorus of simulated soil solution in relation to the total phosphorus concentration in simulated soil solution of 72 soils

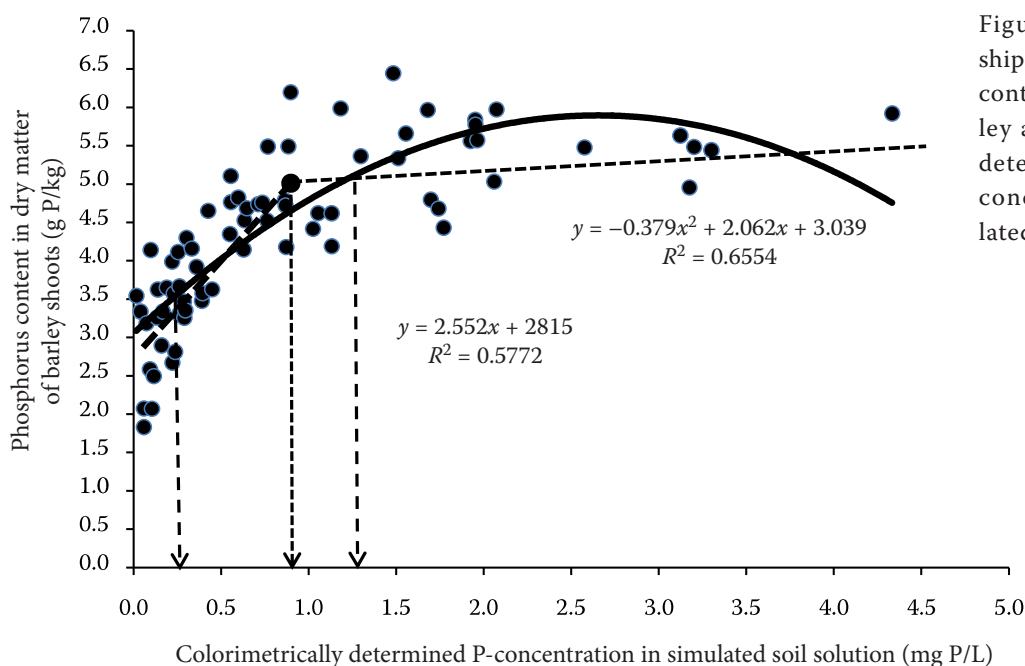


Figure 2. The relationship between phosphorus content in shoots of barley and colorimetrically determined phosphorus concentration in simulated soil solution

high inconsistency in literary data on the adequate phosphorus concentration in soil solution.

Table 2 shows the column statistics of determination of phosphorus content in barley shoots. Variability of the range of phosphorus content in plants was substantially lower than that of phosphorus concentration in simulated soil solution, which is consistent with general knowledge. The range between 2 and 5 mg P/g dry matter is considered as sufficient phosphorus content in plant leaves while toxicity occurs when the content of 10 mg P/g dry matter is exceeded (White and Brown 2010). The sufficient phosphorus content in dry weight of shoots for young barley plants is reported to be 3.5–5 mg P/g (Bergmann and Neubert 1976, Jones et al. 1991).

Figures 2 and 3 illustrate the determined relationship between phosphorus concentration in simulated soil solution and the response of phosphorus content in barley. Using a second-order polynomial regression, the phosphorus concentration of 0.23 mg P/L detected colorimetrically in simulated soil solution corresponded to the content of 3.5 g P/kg of barley dry matter and it was substantially higher, 0.9 mg P/L, at ICP-AES detection. The following values of phosphorus concentration in simulated soil solution corresponded to the content of 5 g P/kg dry matter: 1.25 mg P/L at colorimetric detection and 2.2 mg P/L at ICP-AES detection. The scatter plots of the relationship in Figures 2 and 3 suggest that the use of continuous regression of the second-order

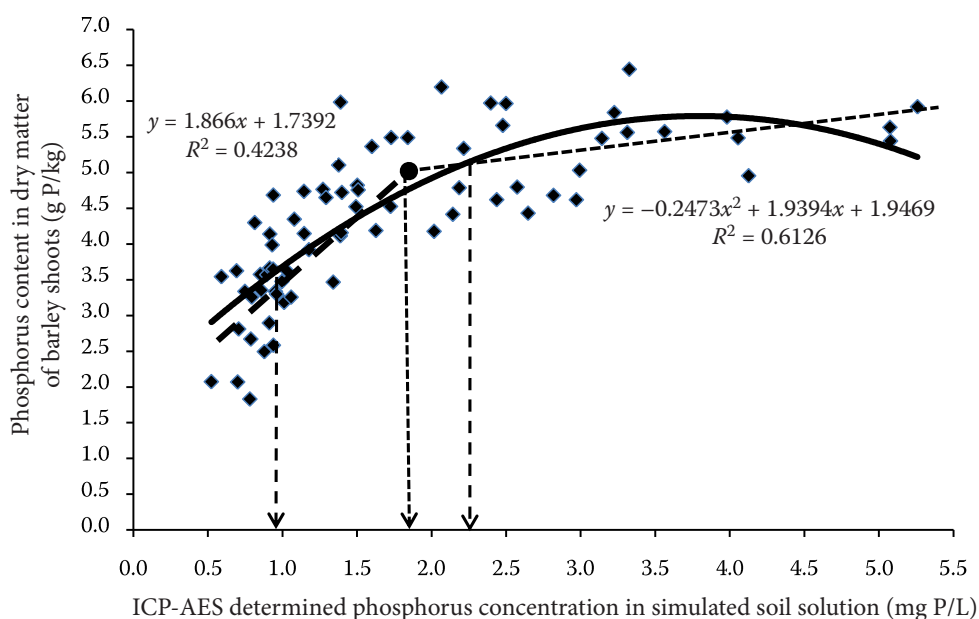


Figure 3. The relationship between phosphorus content in shoots of barley and ICP-AES determined phosphorus concentration in simulated soil solution



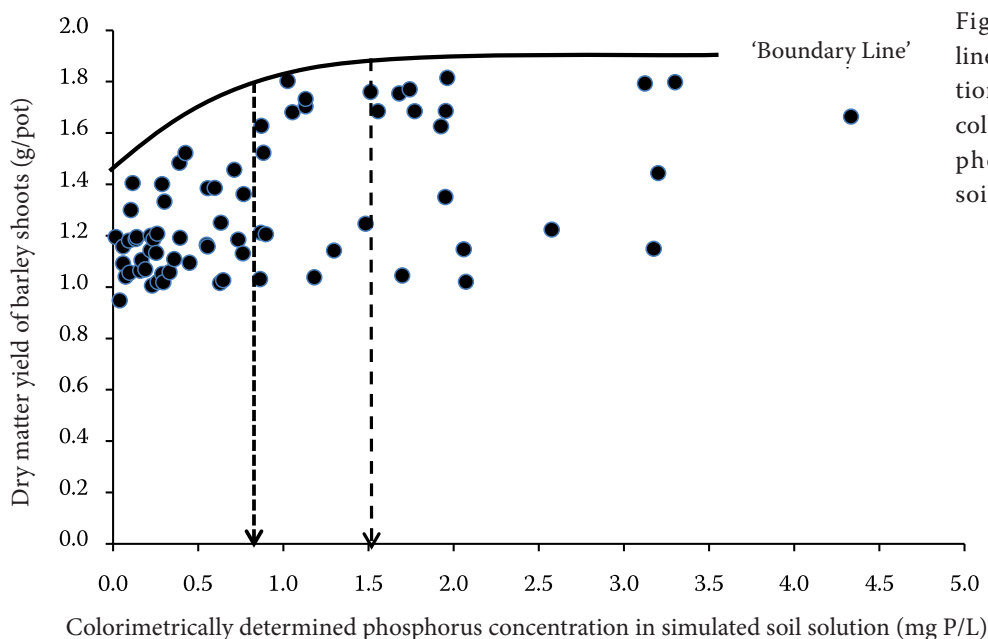


Figure 4. Yield boundary line of barley shoots in relation to the concentration of colorimetrically determined phosphorus in simulated soil solution

polynomial for the derivation of P concentration in simulated soil solution related to the content of 5 g P/kg barley dry matter overestimates the necessary concentration in simulated soil solution. The use of a linear approach (Dahnke and Olson 1990) will be more appropriate in this case. In the areas of low phosphorus concentrations in soil solution the scatter plots clearly indicate the steep relationship of an increase in phosphorus content in barley followed by the 'saturation level', i.e. with a minimum change in phosphorus content in barley. The point of intersection of the steep line and the 'saturation' line clearly defines the upper limit of an adequate phosphorus concentration in soil solution: 0.86 mg P/L for colorimetric detection and 1.75 mg P/L for ICP-AES detection,

i.e. substantially lower values than when the continuous curve of the second-order polynomial is used. Hussain et al. (2010) reported on the basis of research on three soils the concentration of soil solution colorimetrically detected 0.34–0.45 mg P to achieve the maximum wheat yield. In our case an adequate range between 0.23 and 0.86 mg P/L in soil solution was detected, which is similar to the findings of Hussain et al. (2010) if taking into account the average of the values.

It is advantageous and popular to use the concept of boundary line, originally published by Webb (1972), for the analysis of biological data. In Figures 4 to 7 the concept of boundary line is used for the estimation of adequate phosphorus concentration in soil solution. In Figures 4 and 5

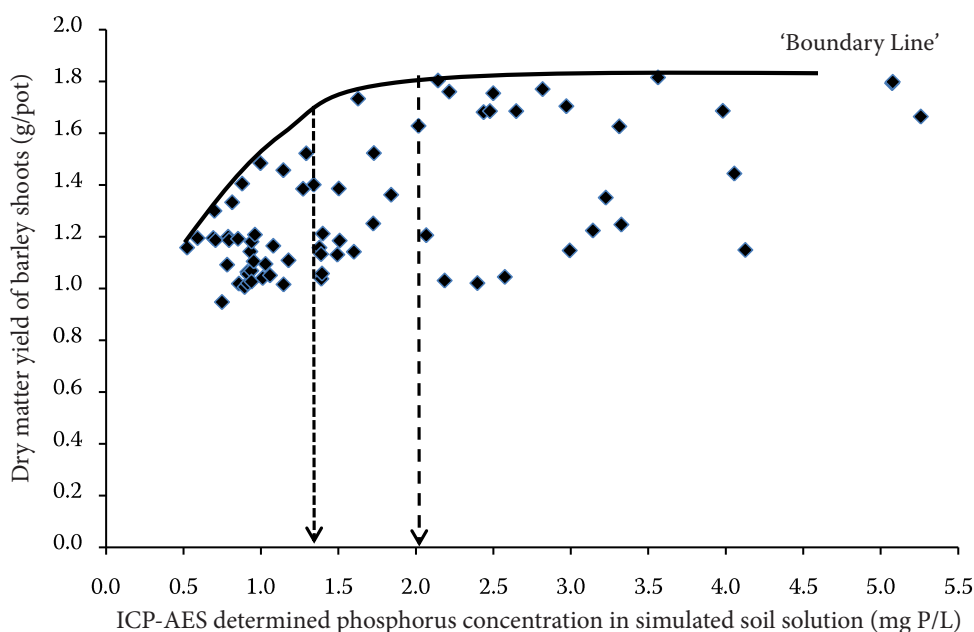


Figure 5. Yield boundary line of barley shoots in relation to the concentration of ICP-AES determined phosphorus in simulated soil solution

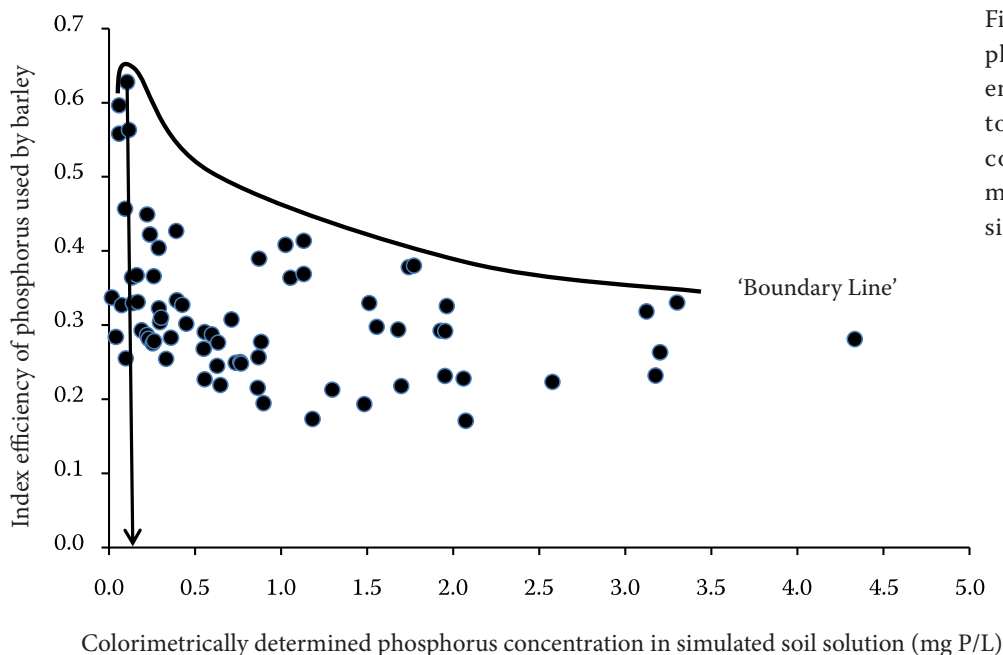


Figure 6. Boundary line of phosphorus index nutrient efficiency in relation to the concentration of colorimetrically determined phosphorus in simulated soil solution

the boundary line of barley yield formation is used in relation to phosphorus concentration in simulated soil solution. The weak side of this approach is the indistinctly defined beginning of the maximum of yield formation, which has a very flat shape then. The flatness of the boundary line of yield curve complicates a more exact definition of the upper limit of adequate phosphorus concentration in soil solution. The beginning of the maximum yield of barley shoots corresponded with the following phosphorus concentrations in simulated soil solution in relation to the method of phosphorus detection: colorimetry 1.5 ppm P, ICP-AES 2 ppm P. The critical concentration of a nutrient in the soil is generally relative to the 90–95% level of achieved yield (Munson and Nelson

1990, Hussain et al. 2010). The critical concentration of phosphorus in simulated soil solution related to 95% yield level corresponded to 0.8 ppm P at colorimetric detection and 1.3 ppm at ICP-AES detection. In our previous studies (Matula 2004) the index of nutrient efficiency (IE) was used that expresses the relation of nutrient content to yield ( $IE = \text{yield}/\text{nutrient}$ ). An advantage of this approach is the sharply defined maximum that corresponds to the best utilisation of the nutrient for yield formation. Figures 6 and 7 show the relationship of the boundary line of phosphorus efficiency index in barley to phosphorus concentration in soil solution. The maximum of the boundary line corresponds to largely different values of phosphorus concentration in simulated soil solution

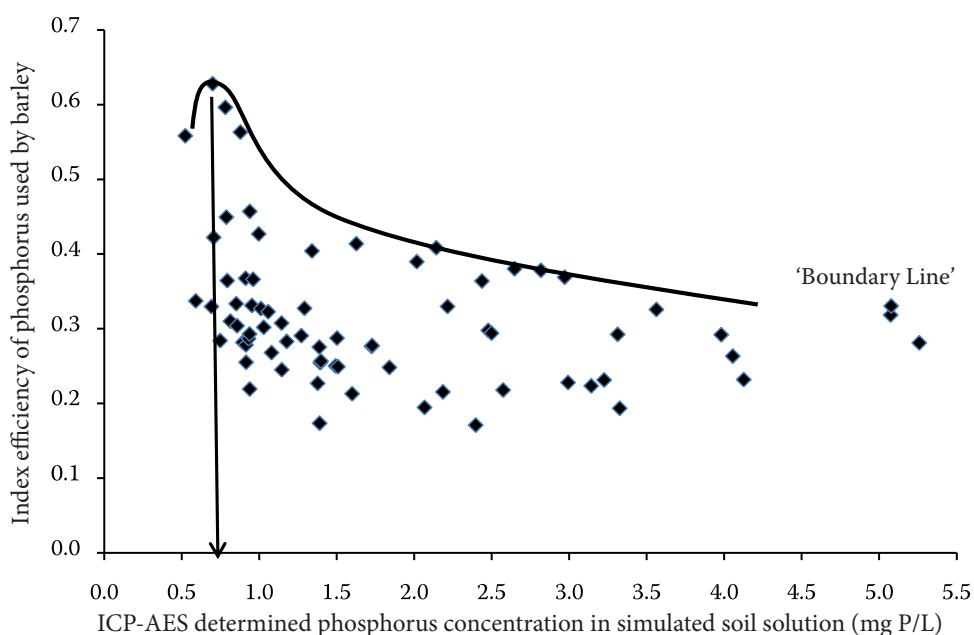


Figure 7. Boundary line of phosphorus index nutrient efficiency in relation to the concentration of ICP-AES determined phosphorus in simulated soil solution

according to the method of phosphorus detection: 0.15 ppm P for colorimetry and 0.7 ppm P for ICP. The difference may be connected with our previous findings about a marked relative decrease in the proportion of inorganic P in total phosphorus concentration in extracts from soils at low levels of P reserve in soil (Matula 2010, 2011). The derived concentrations in soil solution are close to the lower limit of adequate concentration in soil solution derived from phosphorus content in barley 3.5 g P/kg dry matter (Figures 2 and 3). From the aspect of agronomic production the defined phosphorus concentrations in soil solution can be considered as the lower limit of adequate P concentration in soil solution. These determined concentrations correspond to the content of 3 g P/kg in dry matter of barley shoots.

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