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Boron availability and uptake under increasing phosphorus rates in a pot experiment

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ABSTRACT

Mühlbachová G., Čermák P., Vavera R., Káš M., Pechová M., Marková K., Kusá H., Růžek P., Hlušek J., Lošák T. (2017): Boron availability and uptake under increasing phosphorus rates in a pot experiment. Plant Soil Environ., 63: 483–490.

The boron (B) availability in soils, B uptake and possible B interactions with phosphorus (P) were studied in a pot experiment with increasing P rates. Three soil types of different characteristics were used for the experiment that lasted two years. The two soil tests were used in the experiment – Mehlich 3 and NH_4-acetate. Significant positive correlations were found between barley dry matter yield, B content in plants and B uptake under increasing P treatments ($P \leq 0.001$). The significant relationship for B content ($P \leq 0.001$) was obtained between the used soil tests for all tested soils. A decrease of soil B content in soils under increasing P doses was determined. NH_4-acetate soil test showed a significant relationship between B and P contents ($P \leq 0.001$) within the studied soils in comparison with the Mehlich 3 method. Considering individual soils, the NH_4-acetate test showed more often higher significance in comparison with the Mehlich 3 test. The interactions between P and B in soils should be taken in consideration when deciding about the phosphorus fertilization.

Keywords: *Hordeum vulgare* L.; deficiency; micronutrient; bioavailability; toxicity limits; nutrition

Boron (B) represents one of the essential micronutrients necessary for the proper plant growth, which is limited both when deficient or at elevated level (Davies et al. 2011). B is absorbed from soil solution by roots mainly as the undissociated boric acid which is well soluble in water (Hu and Brown 1997). Another possible mechanism is the formation of borate-diol complexes with a variety of organic molecules (Hu and Brown 1997, Hu et al. 1997). The boron concentration in soil solution decisive for plant availability is buffered by the adsorbed B in the oxyanion form (Goldberg 1997) on mineral surfaces through adsorption and desorption reactions. They are important for the determination of boron bioavailability for plants (Majidi et al. 2010). As the range between deficiency and toxicity limits of B for plants is very narrow, any change in B equilibrium concentration may turn to considerable influence on plant growth and this aspect should be taken into account when B nutrition of plants is adjusted by fertilization (Keren and Bingham 1985, Matula 2009).

The excess B is not a problem in the Czech Republic because the majority of soils are naturally deficient in boron there (Matula 2009). The availability of B to plants is affected by a variety of soil

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factors including: concentration of soil solution, pH, soil texture, soil moisture, temperature, oxide, carbonate, organic matter, aluminium and iron hydroxides, as well as clay mineralogy (Goldberg et al. 2000, Zhu et al. 2007, Matula 2009).

B supply strongly promoted water and nutrient uptake as well as biomass formation (Eggert and Wirén 2016). The available P and B contents can determine plant dry matter production (Davies et al. 2011). It was already found that high B content can reduce P content in plant leaves (Blamey and Chapman 1979, Kaya et al. 2009). Yamanouchi (1980) showed that increasing P supply led to the decrease in B concentrations in plant tissues. These results were confirmed also by Kaya et al. (2009) who showed that supplementary P can mitigate the adverse effects of high B content on fruit yield and growth of tomato plants.

The aim of the research was to evaluate the effects of increased P rates on: (1) the B content and B uptake by barley and the relationships between these characteristics; (2) changes in B content determined by two soil tests (Mehlich 3 and NH_4-acetate); (3) possible relationships between P and B contents in soils.

MATERIAL AND METHODS

Design of the pot experiment. The pot experiment with different P rates was carried out in 2015 and 2016. Three soil types were chosen for the experiment – Chernozem (Experimental station of the Central Institute for Supervising and Soil Testing in Žatec, GPS 50.335766, 13.434389), Cambisol (Kámen near Havlíčkův Brod, GPS 49.732600, 15.565858), Haplic Luvisol (Crop Research Institute – Prague 6, GPS 50.088944, 14.297057). The soils for the experiment were taken from the same sampling localities in 2015 and in 2016, homogenized and sieved through 2 mm sieve before the start of experiment. The basic characteristics of the experimental soils are given in Table 1. The phosphorus used for the experiment was applied as triple superphosphate. The experimental design was: (1) C – control; (2) P 0.5 (0.3 g P/pot); (3) P 1 (0.6 g P/pot); P 2 (1.2 g P/pot). Each treatment including the control received also 1 g of nitrogen (calcium ammonium nitrate). Each treatment had four replicates. Each pot contained 5 kg of soil calculated on an oven-dry basis. The experimental crop was the spring barley – cv. KWS Irina (KWS Saat SE, Klein Wanzleben, Germany). 30 seeds were sown at the beginning of the experiment. After germination, the number was reduced to 10 plants remaining till the end of experiment. The experiment in both years started on the 1st April and the plants above-ground biomass was harvested on the 22nd June (2015) and 24th June (2016) at the milky ripeness stage. After the end of experiment, the soils were sieved immediately after harvest through 2 mm sieve and dried at room temperature before analysis.

Laboratory analysis. Exchangeable nutrient contents and cation exchange capacity (CEC) were determined by NH_4-acetate method (Matula 2007). Briefly, the 5 g of soil was extracted in 100 mL of 0.5 mol/L ammonium acetate and 0.005 mol/L ammonium fluoride solution adjusted to pH = 7. The suspensions of soil with the NH_4-acetate solution were left for 16 h, thereafter they were shaken manually 4 times before filtration. For CEC determination,

Table 1. Basic soil characteristics

Year	Soil type	CEC (mmol NH_4^+/kg)	pH_{CaCl_2}	Mehlich 3					Criteria for P supply (Regulation of CR No. 275, 1998)
				Mg	Ca	K	P	B	
				(mg/kg)					
2015	Chernozem	203.19	5.33	256.4	2967.0	230.6	53.1	0.74	sufficient
	Cambisol	143.14	5.74	80.0	1805.6	118.0	39.3	2.09	low
	Luvisol	203.8	6.08	193.5	3899.1	194.8	77.5	2.71	sufficient
2016	Chernozem	233.7	6.21	255.5	3904.7	179.0	56.8	1.69	sufficient
	Cambisol	129.9	5.12	82.6	1293.2	109.5	61.8	0.48	sufficient
	Luvisol	205.2	6.67	189.3	3492.5	196.9	52.5	1.46	sufficient

CEC – cation exchange capacity

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the soils after filtration were washed by ethanol to remove the excess of NH_4^+ ions and thereafter 10% KCl (pH 2.5) was used to displace NH_4^+ by K^+.

Total bioavailable nutrients were extracted by the Mehlich 3 method (Mehlich 1984) (0.2 mol/L CH_3COOH, 0.015 mol/L NH_4F, 0.013 mol/L HNO_3, 0.25 mol/L NH_4NO_3, 0.001 mol/L EDTA) in the ratio 1:10 (w:V – 10 g of soil and 100 mL of extractant), the extraction time (10 min) was slightly modified by Trávník et al. (1999). Subsequently, the samples were filtered.

Concentrations of B in the samples of spring barley above-ground biomass were determined after digestion in concentrated HNO_3, 30% H_2O_2 using microwave Milestone 1200 (Connecticut, USA). In all soil and plant extracts, B was determined by the ICP-OES Thermo Jarrel Ash (Nebraska, USA). The memory effect of boron was avoided by regular rinsing with deionised water and recalibration of instrument after each 10 individual measurements. The overall uptake of nutrients was calculated from the yields and the B contents in plants.

pH determination. The soil pH was determined in the 0.01 mol/L $CaCl_2$ using the ratio 1:2.5 (w:V – 10 g soil/25 mL 0.01 mol/L $CaCl_2$). The soil samples were shaken on an overhead shaker for 1 h and then left to equilibrate for 20 h. The suspension was then agitated for 10 min and the pH value was measured immediately using a pH meter WTW 315i/SET (Weilheim, Germany).

Statistical analysis. The results from two years 2015 and 2016 were statistically analysed using the Statistica 12.0 software (Palo Alto, USA). The one-way ANOVA and the Tukey's test were used

Table 2. Spring barley dry matter yield, boron (B) plant content and uptake, and B soil content (Mehlich 3 and NH_4 acetate) under increasing phosphorus (P) rates

Year	Soil type	Treatment	Dry matter yield (g/pot)	B content barley (mg/kg)	B uptake (mg/pot)	B-Mehlich 3 (mg/kg)	B-NH_4 acetate (mg/kg)
2015	Chernozem	control	63.3^{a}	7.16^{a}	0.45^{a}	1.03^{b}	0.16^{c}
		P 0.5	66.7^{a}	8.05^{a}	0.53^{a}	1.08^{b}	0.11^{bc}
		P 1	68.6^{a}	9.92^{a}	0.53^{a}	0.42^{a}	0.09^{ab}
		P 2	70.7^{a}	7.48^{a}	0.59^{a}	0.55^{a}	0.05^{a}
	Cambisol	control	41.3^{a}	3.02^{a}	0.12^{a}	2.16^{c}	0.35^{b}
		P 0.5	61.1^{b}	2.81^{a}	0.17^{a}	2.01^{a}	0.31^{ab}
		P 1	63.0^{b}	3.27^{a}	0.18^{a}	2.05^{b}	0.30^{ab}
		P 2	63.1^{b}	3.98^{a}	0.25^{a}	2.02^{a}	0.27^{a}
	Luvisol	control	45.1^{a}	5.37^{a}	0.24^{a}	2.74^{b}	0.81^{b}
		P 0.5	55.3^{b}	5.36^{a}	0.30^{a}	2.71^{ab}	0.69^{a}
		P 1	55.7^{b}	5.53^{a}	0.30^{a}	2.70^{a}	0.67^{a}
		P 2	55.9^{b}	4.99^{a}	0.28^{a}	2.71^{ab}	0.66^{a}
2016	Chernozem	control	72.9^{b}	8.00^{a}	0.58^{a}	1.68^{a}	0.65^{b}
		P 0.5	68.9^{ab}	15.84^{b}	1.09^{b}	1.65^{a}	0.47^{a}
		P 1	71.4^{ab}	15.16^{b}	1.08^{b}	1.68^{a}	0.45^{a}
		P 2	67.7^{a}	12.95^{ab}	0.87^{ab}	1.71^{a}	0.47^{a}
	Cambisol	control	68.6^{ab}	10.36^{a}	0.81^{a}	0.47^{a}	0.58^{b}
		P 0.5	61.1^{a}	13.95^{a}	0.81^{a}	0.48^{a}	0.48^{a}
		P 1	72.0^{b}	13.96^{a}	1.06^{a}	0.48^{a}	0.42^{a}
		P 2	76.4^{b}	16.76^{b}	1.27^{b}	0.48^{a}	0.43^{a}
	Luvisol	control	67.5^{a}	13.88^{a}	0.94^{a}	1.51^{c}	0.23^{b}
		P 0.5	71.0^{a}	15.73^{a}	1.12^{a}	1.48^{bc}	0.21^{b}
		P 1	66.7^{a}	13.53^{a}	0.90^{a}	1.45^{ab}	0.16^{ab}
		P 2	70.1^{a}	13.21^{a}	0.92^{a}	1.42^{a}	0.10^{a}

The same characters represent statistically identical values of the examined treatments. C – control; P 0.5 – 0.3 g P/pot; P 1 – 0.6 g P/pot; P 2 – 1.2 g P/pot

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to determine the significant differences among the treatments. The letters represent statistical differences. The correlation coefficients (*r*) based on the Spearman's equation were calculated and their significance at levels at $P \leq 0.05$, 0.01 and 0.001 was determined.

RESULTS AND DISCUSSION

Spring barley dry matter yield, B content in plants and B uptake. A significant increase of dry matter production of barley biomass following the P treatments was observed in 2015 in Cambisol and Luvisol (Table 2). In 2016, a significant increase of barley biomass was observed only after higher P doses in Cambisol. Positive effects of P on the plant growth have already been numerously reported (i.e. Usherwood and Segars 2001, Davies et al. 2011). P deficiencies may reduce the yields of barley (Rowe and Johnson 1995, Hoppo et al. 1999). McKenzie et al. (1998) described that phosphate fertilizer significantly increased barley silage yields at 25 of the 32 site-year locations. Applied P generally increased yields by about 25%, but occasionally the response was much higher. Nyborg et al. (1999) conducted field experiments at 60 sites to determine the yield response of barley to phosphorus fertilizer and showed that barley yields increased with increasing concentration of extractable P in the soil.

The B content in barley biomass increased up to the P 1 or P 2 treatments nearly in all the studied soils (Table 2). Despite the clear trend of increased B contents in barley plants and B uptake, no significant differences according to the ANOVA Tukey's test were found among P treatments in 2015, possibly due to relatively low B contents in plants. Significant differences between boron contents and B uptake were found for Chernozem ($P < 0.05$) and Cambisol ($P = 0.001$) but only at the highest P rate in 2016 suggesting that phosphorus can affect also the boron uptake by plants. The positive correlation was found also between the dry matter yield and B content in harvested plants ($R^2 = 0.3273$, $P \leq 0.001$, Figure 1) and highly significant relationship was found between B content in barley and B uptake ($R^2 = 0.7102$, $P < 0.001$, Figure 2) within the studied soils, indirectly indicating a positive effect of P on the plant growth and B uptake by plants. In addition, high positive correlations were found between B content in plants and B uptake in all soil types. These results are in a discrepancy with Kaya et al. (2009) who reported that the additional P applications can mitigate the boron toxicity to tomatoes. However, Kaya et al. (2009) showed the situation of excess boron concentrations in soils and its adverse effects on tomatoes, whereas soils in the Czech Republic are generally low in B contents (Matula 2009). Anyway, it is possible to assume that the noted increase of B content in barley plants was induced by increasing P treatments of soils. Both elements P and B can be available to plants as anions $H_2PO_4^-$ and $B(OH)_4^-$ (Hu and Brown 1997, Smith 2002, Matula 2009) and therefore a possible competition between P and B is not excluded. Experiments conducted on cotton also demonstrated that boron concen-

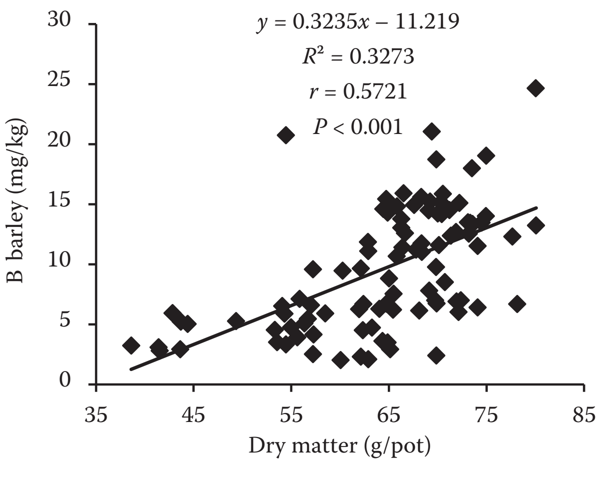

Figure 1. Relationship between dry matter yield and boron (B) content in spring barley

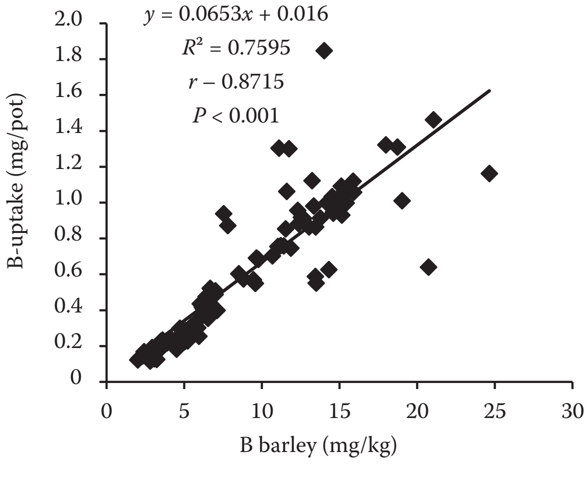

Figure 2. Relationship between boron (B) content in barley and B uptake by plants

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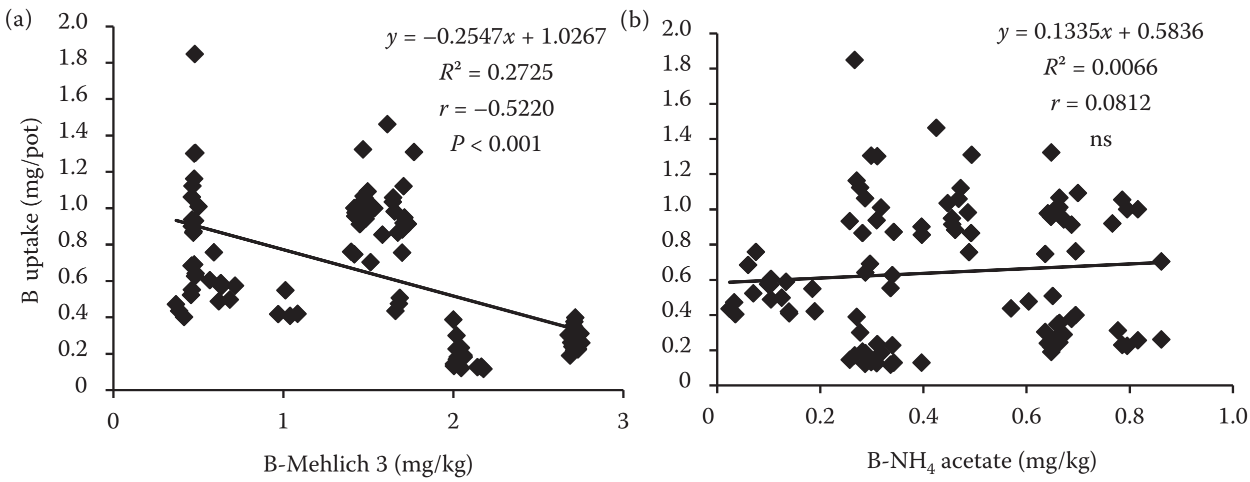

Figure 3. The relationships between boron (B) uptake, (a) B-Mehlich 3 and (b) B-NH_4 acetate. ns – non signifcant

tration in leaves was greatest with phosphorus fertilization (Snyder et al. 1993).

The negative relationship was found between B uptake and boron determined by the Mehlich 3

Table 3. Values of linear correlation coefficients between phosphorus (P) and boron (B) in plants and soils

Soil type		2015			2016		
		B uptake	B-Mehlich 3	B-NH_4 acetate	B uptake	B-Mehlich 3	B-NH_4 acetate
Chernozem	dry matter yield	ns	ns	ns	ns	ns	ns
	P barley	ns	−0.821***	−0.868***	ns	ns	−0.573*
	P uptake	ns	−0.771**	−0.765**	ns	ns	−0.530*
	P-Mehlich 3	ns	−0.890***	−0.716**	ns	ns	−0.604*
	P-NH_4 acetate	ns	−0.886***	−0.823***	ns	ns	−0.546*
	B barley	0.794**	ns	ns	0.992***	ns	−0.512*
	B uptake	–	ns	ns	–	ns	ns
	B-Mehlich 3		–	0.776**		–	ns
Cambisol	Dry matter yield	ns	−0.875***	−0.578*	ns	ns	ns
	P barley	0.620*	−0.664**	−0.753**	ns	−0.892***	−0.783***
	P uptake	0.628**	−0.774**	−0.756**	ns	−0.885***	−0.788***
	P-Mehlich 3	ns	−0.577*	−0.752**	ns	−0.830***	−0.607*
	P-NH_4 acetate	0.554*	−0.534*	−0.656**	ns	−0.853***	−0.549*
	B barley	0.873***	ns	ns	0.939***	ns	ns
	B uptake	–	−0.497*	ns	–	ns	ns
	B-Mehlich 3		–	0.724**		–	0.789***
Luvisol	dry matter yield	ns	ns	−0.732**	ns	ns	ns
	P barley	ns	−0.571*	−0.746**	ns	−0.892***	−0.783***
	P uptake	ns	−0.564*	−0.765**	ns	−0.885***	−0.788***
	P-Mehlich 3	ns	ns	−0.619*	ns	−0.830***	−0.607*
	P-NH_4 acetate	ns	ns	−0.586*	ns	−0.853***	−0.549*
	B barley	0.891***	ns	ns	0.939***	ns	ns
	B uptake	–	ns	ns	–	ns	ns
	B-Mehlich 3		–	0.893***		–	0.854***

ns – non significant; *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$

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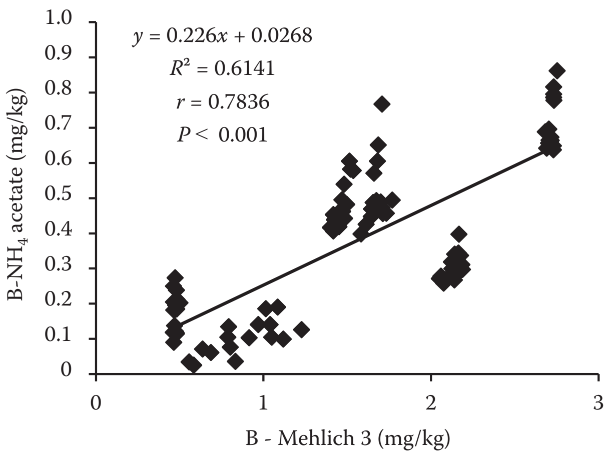


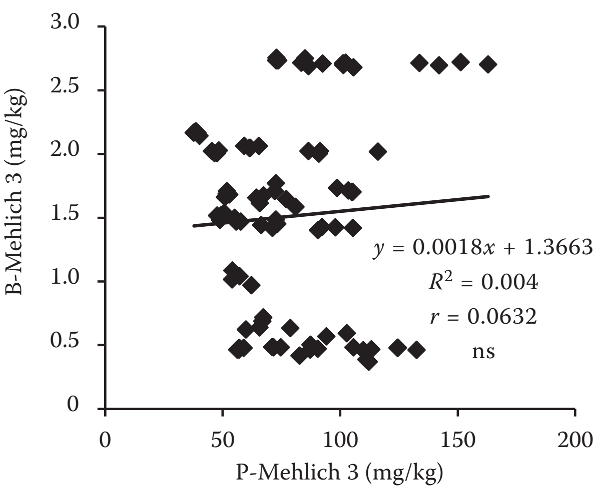
Figure 4. Relationship between B-Mehlich 3 and B-NH_4 acetate in all studied soils

soil test (B-Mehlich 3: R^2 = 0.2725, $P \leq 0.001$) indicating that increased B uptake by plants affected negatively B content in soils. No significant relationship was found between B uptake and B-NH_4 acetate if all studied soils were considered (in 2015 – R^2 = 0.0066 and in 2016 – R^2 = 0.0132; not significant) (Figure 3). The relationships between B uptake and B content in plants and B-Mehlich 3 or B-NH_4 acetate determined in individual soils did not show any significant correlations (Table 3) indicating that neither Mehlich 3 nor NH_4-acetate soil tests responded sufficiently to real B uptake by plants.

B in soils. A significant positive relationship between B concentrations determined by the Mehlich 3 and NH_4-acetate soil tests was obtained within the studied soils (R^2 = 0.3401, $P < 0.001$) (Figure 4). The P treatments mostly caused a significant decrease of B contents in soils determined by Mehlich 3 and NH_4 acetate (Table 3). Despite the highly significant correlation found between B-Mehlich 3 and B-NH_4 acetate, the groups of data available in the figure indicate that the boron availability of each soil was related to the particular soil characteristics. Matula (2009) suggested that whereas NH_4 acetate can be useful also for the estimation of B availability, the Mehlich 3 soil test did not give a sufficient response under increasing B rates. The results considering also the B uptake by barley did not confirm the NH_4-acetate soil test to predict well the B availability for plants.

Relationships between P and B content in soils. Despite the fact that soils were not amended by B, the significant differences between P treatments were often found. The soil tests in both studied years showed a decrease of B concentrations in correspondence to P concentrations in soils (Figure 5). The Mehlich 3 soil test did not show any clear relationship between P and B determined in the whole spectrum of the obtained data (Figure 5a) and despite the significant relationship ($P \leq 0.001$), a relatively large dispersion of data was observed also for P and B determined by the NH_4 acetate soil test (Figure 5b). The negative relationships were found, if the individual soils were evaluated by the NH_4 acetate soil test (Table 4) and ranged between R^2 = 0.6770 ($P \leq 0.001$) to R^2 = 0.0846 (not significant) which was the unique non-significant relationship from the six tested soils.

(a)



(b)

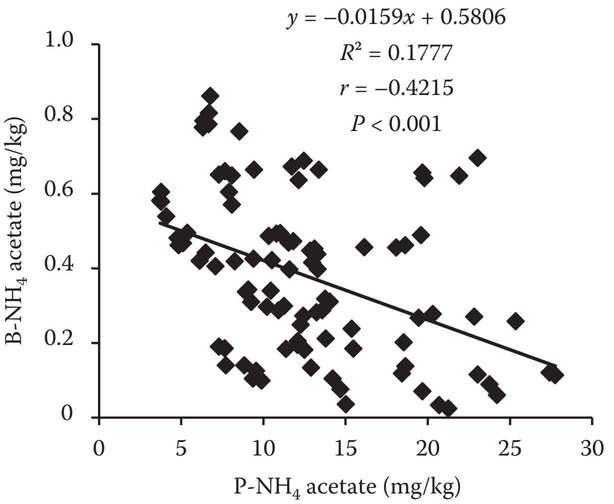


Figure 5. Relationship between phosphorus (P) and boron (B) determined by (a) Mehlich 3 and (b) NH_4 acetate soil test in all soils in the years 2015 and 2016. ns – non significant

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Table 4. The relationships between phosphorus and boron contents determined by Mehlich 3 and NH_4-acetate in individual soils in 2015 and 2016

Soil type	Year	Relation	Slope (*a*)	Intercept (*b*)	R^2	*r*
Chernozem	2015	P-Mehlich 3:B-Mehlich3	–0.0064	1.3878	0.7678	–0.8762***
	2016		0.0006	1.6355	0.066	0.2569[ns]
Cambisol	2015		–0.0016	2.1571	0.3324	–05465*
	2016		0.00005	0.4689	0.0379	0.1946[ns]
Luvisol	2015		–0.0003	2.7497	0.2196	0.4686*
	2016		–0.0018	1.5857	0.6891	–0.8301***
Chernozem	2015	P-NH_4 acetate:B-NH_4 acetate	–0.0075	2.011	0.6771	–0.8228***
	2016		–0.0131	0.6683	0.2985	–0.5463*
Cambisol	2015		–0.0023	0.3452	0.0846	–0.2908[ns]
	2016		–0.0077	0.3086	0.6226	–0.789***
Luvisol	2015		–0.0072	0.7938	0.344	–0.5865*
	2016		–0.0121	0.5622	0.4199	–0.648**

[ns]non significant; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

Individual B determination of soils by the Mehlich 3 test showed that relationships ranged between $R^2 = -0.7677$ ($P \leq 0.001$) and $R^2 = 0.0378$ (not significant) (Table 4). Non-significant data were obtained from analyses of Luvisol in the year 2015 and Chernozem and Cambisol in the year 2016. The obtained data of soil analysis showed the tendency to decrease the B contents in soils under the increasing P rates. The increase of B contents in barley tissues accompanied by an increase of B uptake under increasing P rates could suggest that also improvement of metabolic functioning of plants can play an important role. However, many other factors can play an important role in B availability. The adsorption of B on clay minerals, effects of soil pH, aluminium and iron hydroxides, clay minerals, calcium carbonates and organic components were numerously reported (i.e. Hu and Brown 1997, Chaudrary et al. 2005, Matula 2009) and therefore the soils should be evaluated individually according to their proper characteristics.

Considering all the obtained data together, the higher B uptake by plants induced by P rates led to a decrease of available B in the studied soils. Actually, the P fertilization of soils is low in the Czech Republic. Our results suggest that the phosphorus fertilization of soils could increase the B deficiency in soils. Therefore, the interactions between P and B should be taken into account when deciding about the fertilization of soils with phosphorus.

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