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The Dynamics of Molybdenum, Boron, and Iron Uptake, Translocation and Accumulation by Pea (*Pisum sativum* L.)

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Abstract: Molybdenum (Mo), boron (B), and iron (Fe) play an important role in symbiotic nitrogen fixation by legume plants. The intensity of this process varies in different growth stages of legumes, and the changes are accompanied by changes in the content and translocation of these micronutrients in the plant. A two-year field experiment was conducted to investigate the dynamics of molybdenum, boron, and iron content, translocation, and accumulation in pea plants. Two pea cultivars were studied in six stages of growth, from the four-leaf stage to full maturity. The content of Mo, B, and Fe in the roots of pea was highest from the four-leaf stage to the full flowering stage, i.e., the period of establishment of symbiosis and the most intensive atmospheric nitrogen fixation. The bioaccumulation factors of Mo and Fe were generally highest in the initial stages of pea growth and decreased during generative development, while the reverse pattern was observed for boron. The bioaccumulation factors also indicate high bioaccumulation of Mo and B and low bioaccumulation of Fe in the biomass of pea. The translocation factor indicated a high potential for allocation of Mo from the roots to the aerial parts, increasing during growth; high and stable potential for allocation of boron; and very minor allocation of iron to the aerial parts. The values of all parameters tested were usually dependent on the conditions in which the experiment was conducted (the year), but not on the cultivar of a pea.

Keywords: bioaccumulation coefficient; legume plant; micronutrient; translocation factor

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1. Introduction

The green matter and seeds of legume plants have high value as food and feed, as they contain large amounts of nutrients: protein, carbohydrates with a low glycemic index, dietary fiber, minerals, and vitamins [1–4]. Moreover, the cultivation of legume crops is beneficial for the succeeding crops [5–7]. One of the most important assets of legume crops is their symbiosis with rhizobia, owing to which an estimated 40 to 170 Tg of nitrogen enters the atmosphere every year [8–12]. By exploiting the process of biological nitrogen fixation (BNF), or more precisely symbiotic nitrogen fixation (SNF), some crop plants take up from several dozen to even several hundred kg of nitrogen per hectare from the atmosphere per year [13–19]. In the case of crop plants, some of the nitrogen taken up from the atmosphere is removed with the harvested crops, and the rest, together with the crop residues, enters the soil. The amount of symbiotically fixed nitrogen depends on numerous factors and has been the subject of many studies [13,20–23]. These factors include the properties of symbiotic microbes and the plants living with them in symbiosis, as well as environmental factors. Among the latter, we can distinguish independent factors (e.g., soil type) and factors dependent on human activity (e.g., the content of plant nutrients in the soil). The course of symbiotic nitrogen fixation is influenced not only by macroelements but also by microelements regulating enzymatic processes [11,13,24,25].

All organisms that fix atmospheric nitrogen owe this ability to a complex enzymatic system in which the enzyme nitrogenase catalyzes the reduction of nitrogen molecules to ammonium ions. The reaction also requires reducing power in the form of electrons and protons (H^+) [26]. The enzyme nitrogenase is a complex consisting of two components containing iron and molybdenum. One of these, dinitrogenase reductase, which contains iron (the Fe protein), supplies electrons for the reduction reaction, while the other, dinitrogenase, which contains molybdenum and iron (the MoFe protein), reduces the N_2 molecule [26,27].

An adequate supply of the microelements molybdenum, boron, and iron to plants plays an important role in the symbiosis of legume plants and rhizobia [24] by increasing the efficiency of nitrogen fixation and nodulation. It promotes nodule maturation and increases yields [24,28].

Iron deficiencies can affect nodule initiation and development and reduce nitrogenase activity, nodule mass, leghemoglobin content, and the number of bacteroids [24,29]. Boron deficiency limits the formation of root nodules, reduces the number of *Rhizobium* bacteria and infection threads, and restricts the development of the O_2 barrier preventing the inactivation of nitrogenase [24,30]. In the case of molybdenum deficiency, impairment of enzymatic reactions leads to pronounced symptoms of nitrogen deficiency in legume plants [24].

The nitrogen fixation process is most intensive up to the end of the flowering stage and the start of the pod-forming stage in legume plants. In subsequent development stages, the nodules disintegrate and the process is inhibited [19,31]. It is important to determine not only the accumulation and translocation of symbiotically fixated nitrogen but also the content, allocation, and accumulation of micronutrients of importance for the nitrogen fixation process (molybdenum, iron, and boron) in different parts of the plant throughout the growing period.

The study aimed to determine the dynamics of molybdenum, boron, and iron, content, translocation, and accumulation in pea during six stages of growth, from the four-leaf stage to full maturity.

Determining the amount of uptake and accumulation of these micronutrients in different pea's parts at different stages of their growth and development will indicate their amount, which can be removed with the yield (as green matter or with seeds), causing soil depletion, and the rest returning to the soil with postharvest residues.

2. Materials and Methods

2.1. Field Experiment

In a field experiment conducted in Siedlce, Poland (52°10′12′′ N, 22°17′15′′ E) in 2015 and 2016, pea (*Pisum sativum* L.) was grown in a traditional soil cultivation system. The soil was classified among Luvisols (LV), consisting of 81% sand, 17% silt, and 2% clay. The soil used for the study, with pH 6.6 in 2015 and 6.5 in 2016, contained respectively: 2.10 and 1.45 $g \cdot kg^{-1}$ total nitrogen; 34.2 and 23.5 $g \cdot kg^{-1}$ total carbon; 309 and 301 $mg \cdot kg^{-1}$ available phosphorus, as well as 86.0 and 111.0 $mg \cdot kg^{-1}$ available potassium determined by the Egner-Riehm method; 4781 and 4448 $mg \cdot kg^{-1}$ total iron; 0.065 and 0.067 $mg \cdot kg^{-1}$ total molybdenum; 1.047 and 0.313 $mg \cdot kg^{-1}$ total boron. The experiment was two-factorial, set up in a randomized block design in three replications. The first factor was two pea cultivars: 'Milwa', a fodder cultivar, and 'Batuta', a multi-purpose cultivar. The second factor was the growth stage of pea (6 harvest dates according to the BBCH scale [32]): (1) 4-leaf stage, BBCH 14; (2) 3-internodestage, 33 BBCH; (3) stage of first visible single buds outside the leaves, BBCH 55; (4) full flowering stage, 65 BBCH; (5) stage when 50% of pods are of typical length, BBCH 75; and (6) full maturity, BBCH 90. The experiment included 36 microplots, each with an area of 1 m^2.

Pea was fertilized with nitrogen at 30 kg $N \cdot ha^{-1}$ in the form of $(NH_4)_2SO_4$. Potassium was applied in an amount corresponding to 100 kg $K \cdot ha^{-1}$. The plots were fertilized before every season of pea cultivation. Phosphorus was not applied, because the soil was shown to have a very high content of this macronutrient in forms available to plants.

Before sowing, the pea seeds were inoculated with symbiotic *Rhizobium leguminosarum* bacteria. Pea was sown by hand on April 8 of both years in the amount of 110 germinating seeds per m^2. No herbicides were used, and weeds were removed manually.

Whole pea plants were harvested by hand at the specified growth stages by digging them out of the soil with a spade to a depth of 0.25 m, separately from each plot.

2.2. Laboratory Analyses

All pea plants harvested at BBCH stages 14–75 were separated into the roots and aerial parts, and at BBCH stage 90 the seeds were separated as well. The aerial parts included stems, leaves, flowers, and pods, respectively in the growth phases, except the seeds, which were separated at full maturity. Then the separated parts were weighed, and representative samples were taken from each plot. The plant samples were dried at 70 °C, ground, and subjected to dry mineralization (ashing) at 450 °C. The soil was also mineralized in the same way. The ash was dissolved in 6 mol·dm^{-3} HCl, which was evaporated to dryness. The resulting chlorides were transferred to volumetric flasks in a 1% HCl solution. Total Mo, B, and Fe content in these solutions were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Optima 3200 RL, Perkin-Elmer, Waltham, MA, USA).

2.3. Weather Conditions

The temperature and moisture conditions during the growing season of pea were varied during the years of the study (Tables 1 and 2).

Table 1. Rainfall and air temperatures in 2015–2016, (Institute of Meteorology and Water Management, National Research Institute in Warsaw).

Month	Total Monthly Rainfall, mm			Average Monthly Temperatures, °C		
	Year		Long-Term Mean 1981–2014	Year		Long-Term Mean 1981–2014
	2015	2016		2015	2016	
III	53.1	46.4	29.6	4.8	3.3	2.0
IV	30.0	50.2	33.4	8.2	8.9	8.1
V	100.2	35.5	6.3	12.3	14.6	13.6
VI	43.3	55.6	72.9	16.5	18.1	16.3
VII	62.6	126.8	67.6	18.7	19.0	18.5
Sum/Means IV–VII	236.1	268.1	234.2	13.9	15.1	14.1

Table 2. Values of the Selyaninov hydrothermal index (*k*) during the vegetation periods of pea and spring barley and moisture characteristics (*wm*) of individual months.

Month	Year			
	2015		2016	
	k	***wm***	***k***	***wm***
IV	1.2	md	1.9	mw
V	2.6	vw	0.8	d
VI	0.9	d	1.0	d
VII	1.1	md	2.2	w
VIII	0.2	ed	1.1	md

$k \leq 0.4$—extremely dry (ed); $0.4 < k \leq 0.7$—very dry (vd); $0.7 < k \leq 1.0$—dry (d); $1.0 < k \leq 1.3$—moderately dry (md); $1.3 < k \leq 1.6$—optimum (o); $1.6 < k \leq 2.0$—moderately wet (mw); $2.0 < k \leq 2.5$—wet (w); $2.5 < k \leq 3.0$—very wet (vw); $k > 3.0$—extremely wet (ew).

Selyaninov's hydrothermal index indicates that April and July of 2015 were moderately dry, May was very wet, and June was dry (Table 2). In 2016, April was moderately wet, May and June were dry, and July was wet.

2.4. Calculations

Selyaninov's hydrothermal index (k) was calculated by the formula:

$$k = P \cdot 0.1\Sigma t \quad (1)$$

where:

P—the monthly sum of precipitation in mm

Σt—the monthly sum of air temperatures >0 °C

This index is the measure of pluviometric conditions—it considers atmospheric precipitation and air temperature.

Obtained results in the experiment were used to calculate the uptake, the coefficient of bioaccumulation, and translocation factor of Mo, B, and Fe [33–35], according to the following formulas:

(a) Element (Mo, B, Fe) accumulation in pea's dry mass (uptake by pea) [33], E_{up}

$$E_{up} = Y \cdot C_{plant} \quad (2)$$

where:

Y—weight (Yield) of pea (part of a pea, respectively)

C_{plant}—total element content (concentration of Mo, B, or Fe) in pea's dry mass (in separated parts, respectively)

(b) Bioaccumulation Factor of the selected element (Mo, Fe, B), BAF_E

BAF index values were calculated and presented in the paper because it represents the plant's potential to take up and accumulate elements from the soil. It defines their allocation from the environment (soil) to the plants. In the literature, it is recommended to calculate it according to the following formula [34–37]:

$$BAF_E = C_{plant}/C_{soil} \quad (3)$$

where:

C_{plant}—total element content (concentration of Mo, B, or Fe) in pea's dry mass (in separated parts and as averages in the plant, respectively)

C_{soil}—total element content (concentration of Mo, B, or Fe) in soil

(c) Translocation Factor of selected element [34,35], TF_E

$$TF_E = C_{agp}/C_r \quad (4)$$

where:

C_{agp}—total element content (concentration of Mo, B, or Fe) in pea's aerial parts (in separated aerial parts and as averages, respectively)

C_r—total element content (concentration of Mo, B, or Fe) in the roots

2.5. Statistical Analyses

The results obtained in the experiment were analyzed by ANOVA with the Fisher–Snedecor distribution. Least Significant Difference (LSD) values at a significance level of $\alpha = 0.05$ were calculated by the Tukey test. The Statistica 13.1 PL statistics package (StatSoft Inc., Tulsa, OK, USA) was used for the calculations. In addition, Pearson's linear correlation coefficient was calculated for selected pea's features

3. Results

The content of molybdenum, iron, and boron in the separated parts and its average in the whole plants changed significantly in the development stages of pea and between the years of the study (Tables 3 and 4). The exception was boron content in the aerial parts, which did not vary significantly in successive stages of development.

Table 3. Mo, B, and Fe content in dry mass of pea in successive growth stages, mg·kg^{-1} DW.

Micronutrients	**Part of Pea**	**Growth Stages (BBCH)**						**LSD$_{0.05}$**
		14	**33**	**55**	**65**	**75**	**90**	
Mo	Seed						5.75	
	Aerial part	3.83 b	3.66 b	3.27 ab	2.73 a	2.80 a	2.59 a	0.79
	Root	3.75 c	4.98 d	3.30 bc	2.70 bc	2.54 b	0.72 a	1.05
	Weighted average	3.72 b	3.93 b	3.27 ab	2.71 a	2.79 a	3.72 b	0.70
B	Seed						22.2	
	Aerial part	25.0	24.9	26.6	26.0	26.8	26.3	n.s.
	Root	22.1 ab	23.8 b	22.8 ab	21.7 ab	21.8 ab	20.2 a	2.8
	Weighted average	23.7 a	24.7 ab	26.2 cb	25.8 cb	26.6 c	24.7 ab	1.9
Fe	Seed						105.8	
	Aerial part	312.1 d	229.6 c	152.1 b	112.2 a	119.0 ab	131.4 ab	38.0
	Root	3639.0 d	2226.2 c	1707.5 b	1437.4 ab	1373.8 a	1284.2 a	369.2
	Weighted average	1819.1 d	674.6 c	283.9 b	182.3 ab	165.5 a	144.7 a	115.9

a,b,c,d—Means for investigated factors with different letters in the rows are significantly different; n.s.—Not significantly differ at $p \leq 0.05$.

Table 4. Mo, B, and Fe content in dry mass of pea, mg·kg^{-1} DW.

Micronutrients	**Part of Pea**	**Source of Variation**					
		Pea Cultivar			**Year of Research**		
		'Milwa'	**'Batuta'**	**LSD$_{0.05}$**	**2015**	**2016**	**LSD$_{0.05}$**
Mo	Seed	5.92 ^{1}	5.59 ^{1}	n.s.	4.78 ^{1}a	6.73 ^{1}b	0.62
	Aerial part	3.23 ^{2}	3.06 ^{2}	n.s.	3.34 ^{2}b	2.95 ^{2}a	0.31
	Root	3.29 ^{2}b	2.70 ^{2}a	0.41	3.75 ^{2}b	2.25 ^{2}a	0.41
	Weighted average	3.45 ^{2}	3.26 ^{2}	n.s.	3.50 ^{2}b	3.21 ^{2}a	0.27
B	Seed	22.2 ^{1}	22.3 ^{1}	n.s.	16.5 ^{1}a	28.0 ^{1}b	2.1
	Aerial part	26.2 ^{2}	25.7 ^{2}	n.s.	20.1 ^{2}a	31.7 ^{2}b	0.9
	Root	22.2 ^{2}	21.9 ^{2}	n.s.	16.5 ^{2}a	27.7 ^{2}b	1.1
	Weighted average	25.5 ^{2}	25.1 ^{2}	n.s.	19.6 ^{2}a	31.0 ^{2}b	0.8
Fe	Seed	105.3 ^{1}	106.3 ^{1}	n.s.	110.9 ^{1}b	100.7 ^{1}a	7.8
	Aerial part	179.6 ^{2}	172.5 ^{2}	n.s.	185.4 ^{2}b	166.7 ^{2}a	14.9
	Root	2046.8 ^{2}b	1842.6 ^{2}a	144.5	2144.9 ^{2}b	1744.4 ^{2}a	144.5
	Weighted average	572.5 ^{2}b	517.5 ^{2}a	49.3	644.8 ^{2}b	445.2 ^{2}a	49.3

^{1}—Only for BBCH 90 growth phase, ^{2}—Meanly during the growing season–for all growth phases; a,b—Means for investigated factors with different letters in the rows are significantly different; n.s.—Not significantly differ at $p \leq 0.05$.

The molybdenum content in the pea roots was highest at the three-internode stage and lowest at full maturity. There were no significant differences in Mo content in the roots collected at BBCH stages 55–75 or at stages 14, 55, and 65. The aerial parts contained the most molybdenum at BBCH stages 14 and 33, and the least at BBCH 65–90. The average content of this microelement in the entire pea biomass was highest at BBCH 14, 33, and 90, and lowest at stages 65 and 75. Molybdenum content in the roots and aerial parts and averaged for the entire plants was highest in the conditions prevailing in 2015 than in 2016, while the reverse pattern was noted for the seeds.

Iron content in the roots, aerial parts, and entire biomass of pea was highest at the four-leaf stage and decreased up to the stage when the first single flower buds were visible outside the leaves. In the separated parts and on average in the whole biomass of the pea plants, the iron content did not differ significantly in the last three development stages (BBCH 65, 75, and 90). The iron content in the roots, aerial parts, seeds, and whole plants was higher in 2015 than in 2016.

Boron content in the roots was highest at the three-internode stage and lowest at full maturity. Its content in the roots did not differ significantly at BBCH stages 14, 33, 55, 65, and 75. On average in the whole pea plants, boron content was lower at BBCH stage 14 than at stages 55–75. At BBCH stages 33, 55, 65, and 90 the average boron content in the whole plant did not differ significantly. Boron content in all separated parts and the average for the whole pea plants were lower in pea grown in the conditions of 2015 than in 2016.

Boron content in all of the separated parts and the average for the whole plants were not significantly dependent on the cultivar of pea (Table 4). In the case of molybdenum as well, the cultivar did not significantly affect the content of this microelement in the aerial parts or seeds or the average in the whole plants. Only in the roots was higher molybdenum content noted in the 'Milwa' cultivar than in 'Batuta'. Iron content was higher in the roots and whole plants of the 'Milwa' cultivar than in 'Batuta'. The cultivar did not significantly affect the content of iron in the aerial parts or the seeds.

Like the content of micronutrients, their uptake also varied significantly between development stages and years of pea cultivation (Tables 5 and 6). A significant increase in the amount of molybdenum and boron accumulated in the roots was obtained between the four-leaf stage and the three-internode stage of a pea. Uptake of these micronutrients by the roots from the three-internode stage to the stage when 50% of pods are of typical length was generally similar. Uptake of iron by the roots decreased from the four-leaf stage to full maturity of a pea, but the significance of the differences was confirmed only for the values obtained between the stage when 50% of pods are of a typical length and full maturity. Among all development stages, the accumulation of all micronutrients in the roots was lowest at full maturity. The amount of accumulated molybdenum, boron, and iron in the aerial parts of pea (together with the seeds at the full maturity stage) and the total uptake by the whole plant generally increased from the four-leaf stage to full maturity, but the changes were not statistically significant between all stages. At full maturity, the percentage shares of accumulated molybdenum, boron, and iron in the separate parts in the total uptake by the whole plant were 0.4%, 1.6,% and 18.0%, respectively for the roots; 42.4%, 64.7% and 53.5% for the aerial parts; and 57.2%, 33.7% and 28.5% for the seeds.

Peas grown in the 2015 conditions accumulated more molybdenum and iron in the separate parts of the plants and the whole plants than in 2016 (Table 6). Accumulation of boron in the roots and seeds was similar in the two years of the study, while its accumulation in the aerial parts and its total uptake by the whole plant were greater in 2016 than in 2015.

Table 5. Mo, B, Fe uptake by pea in successive growth stages, g·ha^{-1}.

Micronutrients	Part of Pea	Growth Stages (BBCH)						$LSD_{0.05}$
		14	33	55	65	75	90	
Mo	Seed						14.21	
	Aerial part	0.70 a	2.66 b	9.22 c	13.27 d	15.78 e	10.54 c	1.84
	Root	0.54 b	1.04 d	0.87 cd	0.73 bc	0.62 bc	0.09 a	0.30
	Sum	1.24 a	3.70 b	10.09 c	14.00 d	16.40 d	24.85 e	2.44
B	Seed						53.5	
	Aerial part	4.8 a	18.8 a	69.6 b	119.6 c	145.3 d	102.9 c	19.5
	Root	3.1 a	4.9 bc	5.5 c	5.4b c	4.4 b	2.5 a	1.1
	Sum	7.9 a	23.7 a	75.1 b	125.0 c	149.7 d	158.9 d	19.6
Fe	Seed						278.9	
	Aerial part	55.9 a	169.2 a	404.6 b	556.4 c	667.9 c	522.9 bc	124.1
	Root	548.9 d	466.0 cd	425.3 c	377.1 bc	303.3 b	176.3 a	104.3
	Sum	604.8 a	635.2 a	829.9 b	933.5 b	971.2 b	978.1 b	176.0

a,b,c,d,e—Means for investigated factors with different letters in the rows are significantly different.

Table 6. Mo, B, and Fe uptake by pea at BBCH stage 90, g·ha^{-1} dm.

Micronutrients	Part of Pea	Source of Variation					
		Pea Cultivar			Year of Research		
		'Milwa'	'Batuta'	$LSD_{0.05}$	2015	2016	$LSD_{0.05}$
Mo	Seed	14.38	14.04	n.s.	15.96 b	12.46 a	2.0
	Aerial part	10.58	10.50	n.s.	11.45 b	9.63 a	1.39
	Root	0.10	0.09	n.s.	0.11 b	0.08 a	0.02
	Sum	25.06	24.63	n.s.	27.52 b	22.17 a	3.48
B	Seed	52.2	54.8	n.s.	55.0	52.0	n.s.
	Aerial part	102.2	103.7	n.s.	85.7 a	120.3 b	26.3
	Root	2.5	2.4	n.s.	2.2	2.7	n.s.
	Sum	157.0	160.9	n.s.	142.8 a	175.0 b	25.2
Fe	Seed	275.5	282.3	n.s.	370.4 b	187.3 a	58.2
	Aerial part	549.3	496.5	n.s.	547.6	498.3	n.s.
	Root	188.3	164.3	n.s.	244.4 b	108.3 a	59.1
	Sum	1013.1	943.1	n.s.	1162.3 b	793.9 a	98.8

a,b—Means for investigated factors with different letters in the rows are significantly different; n.s.—Not significantly differ at $p \leq 0.05$.

The uptake of all tested micronutrients, in all separated parts as well as the total uptake by the whole plant, did not vary significantly depending on the pea cultivar (Table 6).

The bioaccumulation factors of molybdenum and iron calculated for the roots and aerial parts and on average for the whole plant were generally highest in the first two stages of vegetative growth of pea and decreased during its generative development (Table 7). In the case of boron, the pattern was reversed. The bioaccumulation factor of this element in the aerial parts and on average in the whole biomass was lower in the initial stages of development than in the final stages. The values calculated for the roots were not significantly varied between development stages.

Table 7. Bioaccumulation factors of Mo, B, and Fe in successive growth stages of a pea.

Micronutrients	Part of Pea	Growth Stages (BBCH)						$LSD_{0.05}$
		14	33	55	65	75	90	
Mo	Seed						87.1	
	Aerial part	58.1 b	55.5 b	49.7 ab	41.3 a	42.4 a	39.2 a	12.1
	Root	56.8 c	75.7 d	50.4 bc	41.3 bc	38.8 b	10.9 a	16.1
	Averages in the whole plant	57.4 bc	65.6 c	50.0 ab	41.3 a	40.6 a	45.7 a	10.7
B	Seed						52.6	
	Aerial part	56.8 a	56.2 a	61.1 ab	60.7 ab	63.6 b	63.4 b	6.2
	Root	50.2	53.1	53.4	52.1	53.8	49.6	n.s.
	Averages in the whole plant	54.1 a	55.6 a	60.5 b	60.3 b	63.2 b	59.3 b	4.5
Fe	Seed						0.02	
	Aerial part	0.07 d	0.05 c	0.03 b	0.02 a	0.03 b	0.03 b	0.01
	Root	0.79 c	0.48 b	0.37 a	0.31 a	0.30 a	0.28 a	0.10
	Averages in the whole plant	0.39 c	0.15 b	0.06 a	0.04 a	0.04 a	0.03 a	0.08

a,b,c,d—Means for investigated factors with different letters in the rows are significantly different; n.s.—Not significantly differ at $p \leq 0.05$.

The bioaccumulation factor of molybdenum calculated for the roots and aerial parts was higher in the conditions of the experiment conducted in 2015 than in 2016, while in the case of the seeds the pattern was reversed (Table 8). The average value of this factor for the entire plant was similar in the two years of the experiment. A lack of significant variation between years was also noted in the case of the iron bioaccumulation factor in all separate parts and on average for the whole plants. The bioaccumulation factor of boron for all separate parts and on average for the whole plants was higher in 2016 than in 2015.

Table 8. Bioaccumulation factors of Mo, B, and Fe in pea.

Micronutrients	**Part of Pea**	**Source of Variation**					
		Pea Cultivar			**Year of Research**		
		'Milwa'	**'Batuta'**	**$LSD_{0.05}$**	**2015**	**2016**	**$LSD_{0.05}$**
Mo	Seed	89.4 [1]	84.5 [1]	n.s.	73.5 [1]a	100.5 [1]b	9.3
	Aerial part	49.0 [2]	46.4 [2]	n.s.	51.4 [2]b	44.0 [2]a	4.7
	Root	50.2 [2]b	41.1 [2]a	6.3	57.7 [2]b	33.6 [2]a	6.3
	Averages in the whole plant	52.3 [2]	49.5 [2]	n.s.	60.9 [2]	59.4 [2]	n.s.
B	Seed	52.9 [1]	52.3 [1]	n.s.	15.7 [1]a	89.5 [1]b	5.8
	Aerial part	61.3 [2]	59.3 [2]	n.s.	19.2 [2]a	101.4 [2]b	2.4
	Root	52.1 [2]	52.0 [2]	n.s.	15.7 [2]a	88.4 [2]b	3.1
	Averages in the whole plant	59.7 [2]	58.0 [2]	n.s.	18.7 [2]a	98.9 [2]b	1.9
Fe	Seed	0.02 [1]	0.02 [1]	n.s.	0.02 [1]	0.02 [1]	n.s.
	Aerial part	0.04 [2]	0.04 [2]	n.s.	0.04 [2]	0.04 [2]	n.s.
	Root	0.44 [2]	0.40 [2]	n.s.	0.45 [2]	0.39 [2]	n.s.
	Averages in the whole plant	0.12 [2]	0.11 [2]	n.s.	0.13 [2]	0.10 [2]	n.s.

[1]—Only for BBCH 90 growth phase; [2]—Meanly during the growing season–for all growth phases; a,b—Means for investigated factors with different letters in the rows are significantly different; n.s.—Not significantly differ at $p \leq 0.05$.

The bioaccumulation factors of all tested micronutrients were similar for the two pea cultivars. Only the bioaccumulation factor of molybdenum in the roots was higher for 'Milwa' than for 'Batuta'.

The translocation factors of molybdenum, boron, and iron were significantly dependent on the growth stage of a pea, and in the case of molybdenum also on the year (Tables 9 and 10). The highest translocation factors for boron and iron were obtained at full maturity for the aerial parts (Ap), and in the case of molybdenum for the seeds. The lowest translocation factors for molybdenum and boron were generally obtained in the initial stages of growth. The molybdenum translocation factor for the aerial parts and seeds was lower in the conditions of 2015 than in 2016.

Table 9. Translocation factors of Mo, B, and Fe to aerial parts (Ap) and seeds (S) of a pea.

Micronutrients	**Growth Stages (BBCH)**								
	14 (Ap)	**33 (Ap)**	**55 (Ap)**	**65 (Ap)**	**75 (Ap)**	**90 (Ap)**	**90 (S)**	**Meanly to Ap and S at 90 BBCH**	**$LSD_{0.05}$**
Mo	1.1 a	0.8 a	1.3 a	1.5 a	1.8 a	3.7 b	8.3 d	5.4 c	1.6
B	1.1 ab	1.0 a	1.2 bc	1.2 bc	1.3 c	1.3 c	1.1 ab	1.2 bc	0.2
Fe	0.09 ab	0.10 bc	0.09 ab	0.08 a	0.09 ab	0.11 c	0.08 a	0.10 bc	0.02

a,b,c,d—Means for investigated factors with different letters in the rows are significantly different.

Table 10. Translocation factors of Mo, B, and Fe in aerial parts of pea (on average during the growing season.

Micronutrients	**Part of Pea**	**Source of Variation**					
		Pea Cultivar			**Year of Research**		
		'Milwa'	**'Batuta'**	**$LSD_{0.05}$**	**2015**	**2016**	**$LSD_{0.05}$**
Mo	Seed	8.9 [1]	7.6 [1]	n.s.	6.9 [1]a	9.6 [1]b	2.2
	Aerial part	1.7 [2]	1.6 [2]	n.s.	1.3 [2]a	2.0 [2]b	0.3
	Averages	5.8 [2]	5.1 [2]	n.s.	5.0 [2]a	5.9 [2]b	0.7

Table 10. *Cont.*

Micronutrients	Part of Pea	Source of Variation					
		Pea Cultivar			Year of Research		
		'Milwa'	'Batuta'	$LSD_{0.05}$	2015	2016	$LSD_{0.05}$
B	Seed	1.1 [1]	1.2 [1]	n.s.	1.3 [1]	1.0 [1]	n.s.
	Aerial part	1.2 [2]	1.2 [2]	n.s.	1.3 [2]	1.2 [2]	n.s.
	Averages	1.2 [2]	1.3 [2]	n.s.	1.3 [2]	1.1 [2]	n.s.
Fe	Seed	0.08 [1]	0.09 [1]a	n.s.	0.08 [1]	0.09 [1]	n.s.
	Aerial part	0.09 [2]	0.09 [2]	n.s.	0.08 [2]	0.09 [2]	n.s.
	Averages	0.09 [2]	0.09 [2]	n.s.	0.08 [2]	0.09 [2]	n.s.

[1]—Only for BBCH 90 growth phase, [2]—meanly during the growing season-for all growth phases; a,b—Means for investigated factors with different letters in the rows are significantly different; n.s.—Not significantly differ at $p \leq 0.05$.

The translocation factors were not significantly affected by the conditions of the experiment (year of the study) in the case of boron and iron, or by the cultivar in the case of any of the micronutrients (Table 10).

4. Discussion

The authors cited in the 'Introduction' section indicate the important role of the micronutrients analyzed in this study, i.e., molybdenum, boron, and iron, in the process of symbiotic nitrogen fixation and the self-sufficiency of plants in terms of nitrogen nutrition. Molybdenum plays an important role in iron metabolism, in part as a component of enzymes catalyzing nitrogen reduction and the assimilation and reduction of nitrates [38–40]. Iron is essential to bacteroids for the synthesis of the enzyme that fixes atmospheric nitrogen–nitrogenase, as well as cytochromes, ferredoxins, and hydrogenases [29,41–44]. Nitrogenase activity is dependent on boron, and both a deficiency and a surplus of boron are undesirable [45,46]. Furthermore, boron is essential for the development of root nodules, in which the reduction of N_2 to available forms for plants takes place [47].

The content of molybdenum in most plants ranges from 0.1 to 3.0 mg Mo·kg^{-1} DW [48,49]. Symanowicz and Kalembasa [50] report that the increased content of this element in plants may be due to a neutral soil reaction, which increases its solubility and availability. Our experiment was conducted on the soil of pH 6.5–6.6, which indicates that the test plant was well supplied with molybdenum. The average molybdenum content in the pea roots in the entire growing period was 3.00 mg·kg^{-1}, ranging from 0.72 mg·kg^{-1} at full maturity to 4.98 mg·kg^{-1} at the three-internode stage. Similarly, the highest content of this element in the aerial parts (3.94 mg Mo·kg^{-1}) was noted at the three-internode stage. At this stage, in which the molybdenum content in the roots and aerial parts was highest, there was a marked increase in the rate of atmospheric nitrogen fixation [19]. At full maturity, the highest molybdenum content was noted in the seeds (on average 5.76 mg Mo·kg^{-1}), which is in agreement with reports by Hamlin [49] and Butnariu et al. [51]. According to these researchers, among all parts of the pea, the seeds have the highest content of this element. In addition, the amount of molybdenum accumulated in the seeds was 57.2% of the total uptake by the whole plant.

The iron content in legume plants usually ranges from 75 to 400 mg Fe kg^{-1} DW [52]. According to the authors cited, the content of iron changes considerably during the growing period, in varying degrees in different parts of the plant. Burton et al. [53] report that in the early growth period of soybean, the root nodules have the highest iron content (about 44% of Fe). In our study, the average iron content in pea for the entire growing period was 1944.7 mg·kg^{-1}, ranging from 1284.2 mg·kg^{-1} at full maturity to 3639.0 mg·kg^{-1} at the four-leaf stage. According to Römheld and Nikolic [54], after a pea attains full maturity, the iron content in the seeds is lower than in the vegetative organs, which was confirmed in the present study. The iron content in the total biomass of pea averaged 544.9 mg·kg^{-1}. The highest iron content, as in the case of the roots, was noted at the four-leaf stage (1820.9 mg·kg^{-1}), and the lowest at full maturity (144.7 mg·kg^{-1}). At this stage, 105.8 mg Fe·kg^{-1} was recorded in the seeds, and the amount accumulated in them was 28.5% of the total uptake by the whole plant.

Boron content higher than 5 mg B·kg^{-1} in plant tissues is sufficient for the growth and development of plants [52]. The optimum content of this element for the growth of legume plants ranges from 20 to 50 mg·kg^{-1} DW. For pea, the optimum boron content in the leaves is 23 mg B·kg^{-1} [55]. According to literature data, the content of this microelement in the pea plants grown in our study can be considered sufficient. In the aerial parts, all values exceeded 23 mg B·kg^{-1}. The boron content in the roots of a pea for the entire growing period averaged 22.1 mg·kg^{-1}, ranging from 20.2 mg·kg^{-1} at full maturity to 23.8 mg·kg^{-1} at the three-internode stage. On average in the pea biomass, the content of this element was 25.3 mg·kg^{-1}, with the highest content recorded in the stage when 50% of pods were of typical length (26.6 mg·kg^{-1}), and the lowest at the four-leaf stage (23.8 mg·kg^{-1}). The increasing content of boron in successive development stages of a pea may be due to a greater demand for it in growing organs than in mature ones [56]. Apart from soil factors, boron uptake by plants is influenced by the transpiration rate, which depends on relative humidity and air temperature. Low humidity and higher temperatures increase the uptake of boron by plants [57]. This dependency was noted in our study as well. In 2016, when the average air temperature during the growing season was higher than in 2015, the content of boron was higher in all separate parts of the plants and on average in the whole biomass of pea, and its uptake by the whole plant was greater as well.

In the current cultivation system, as soon as the seeds are removed from the field and the remainder of the plant becomes crop residue (in our study consisting of the roots and aerial parts—stems, leaves, and threshed pods), less than half (42.8%) of the molybdenum, about two thirds (66.3%) of the boron, and less than three quarters (71.5%) of the iron accumulated in the biomass of the pea plants were returned to the soil.

The literature data indicate a positive correlation between the rate of symbiotic nitrogen fixation and the iron concentration in the root nodules [58]. Our study showed no significant relationship between iron content in the pea roots and the amount of nitrogen taken up by the plant from all sources (Table 11). The correlation coefficients indicated that the amount of nitrogen taken up by pea from the atmosphere and mineral fertilizer increased with the content of molybdenum in the roots, while in the case of boron content in this part of the plant the reverse relationship was observed. A study on yellow lupine also showed a negative correlation between boron content in the roots and the amount of nitrogen taken up from the atmosphere [59].

The bioaccumulation factor (BAF) represents the plant's potential to take up and accumulate elements from the soil. It defines their allocation from the environment (soil) to the plants [36,37]. A bioaccumulation factor from 1 to 10 indicates a hyperaccumulator plant, a BAF value of 0.1–1 indicates a moderate accumulator plant, a BAF value of 0.01–0.1 indicates a low accumulator plant, and a BAF value of <0.01 indicates a non-accumulator plant [60]. In our study, the bioaccumulation factors of molybdenum and boron were well over 10, which indicates the active accumulation of these elements in pea. The BAF for iron from 0.02 to 0.79 indicates low accumulation. Accumulation of this element was greater in the roots (BAF 0.28–0.79) than in the aerial parts (BAF 0.02–0.07). These differences prompted us to calculate the translocation factor (TF) for the elements. The TF shows the susceptibility of elements to translocation from the roots of plants to the aerial parts [61]. Among the elements tested, molybdenum and boron translocated more easily in the plant than iron, as indicated by the higher TF values obtained in both years of the study. A TF above 1 indicates the plant's potential for a hyperaccumulation of an element. Our study confirmed this potential in pea for molybdenum and boron. In the case of boron, the value was similar throughout the growing period, while in the case of molybdenum it increased markedly from the flowering stage until full maturity. TF calculated for iron did not exceed 0.12 during the growing period, which indicates a very minor allocation of this element in pea.

Table 11. Linear correlation coefficients between pea seed yield, the whole mass, and N uptake from different sources (presented by Wysokinski and Lozak [19]) and Mo, B, and Fe content in the roots and whole mass.

Specification	Pea's Parameter					
	Seeds Mass	Whole Mass	N Content in Whole Mass	N Uptake		
				From Air	From Fertilizer	From Soil
Mo content in roots	0.87 *	0.81 *	0.34	0.86 *	0.75 *	0.34
Mo content in whole mass	0.69 *	0.61 *	0.14	0.52	0.73 *	0.47
B content in roots	−0.88 *	−0.82 *	−0.30	−0.88 *	−0.71 *	−0.27
B content in whole mass	−0.91 *	−0.90 *	−0.22	−0.94 *	−0.70 *	−0.30
Fe content in roots	0.43	0.44	−0.09	0.37	0.40	0.01
Fe content in whole mass	−0.28	−0.38	−0.19	−0.37	−0.27	−0.36

*—The value of the correlation coefficient is significant, $p < 0.05$.

5. Conclusions

The highest content of Mo, B, and Fe in the pea roots was recorded from the four-leaf stage to the full flowering stage, i.e., in the period when symbiosis is established and atmospheric nitrogen fixation is most intensive. At full maturity, the pea plants accumulated the largest amount of molybdenum in the seeds (57.2% of total uptake) and the most boron and iron in the aerial parts (64.7% and 53.5%, respectively). The bioaccumulation factors of Mo and Fe were generally highest in the initial growth stages and decreased with the plant's generative development, while in the case of boron the dependency was reversed. The BAF values also indicate high bioaccumulation of Mo and B and low bioaccumulation of Fe in the pea mass. The translocation factors indicate that pea has a high potential for allocation of Mo and B from the roots to the aerial parts, while the allocation of Fe is very low. In 2016, when the average air temperature during the growing season was higher than in 2015, the content of boron was higher in the separate parts of the plant and on average in the whole plant, as was the uptake of this micronutrient by the whole plant. This pattern was reversed in the case of Mo and Fe. The values for all parameters tested in most cases did not differ between pea cultivars.

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References

1. De Ron, A.M.; Sparvoli, F.; Pueyo, J.J.; Bazile, D. Editorial: Protein crops: Food and feed for the future. *Front. Plant Sci.* **2017**, *8*, 105. [CrossRef] [PubMed]
2. Kulkarni, K.P.; Tayade, R.; Asekova, S.; Song, J.T.; Shannon, J.G.; Lee, J.D. Harnessing the potential of forage legumes, alfalfa, soybean, and cowpea for sustainable agriculture and global food security. *Front. Plant Sci.* **2018**, *9*, 1314. [CrossRef] [PubMed]
3. Çakir, Ö.; Uçarli, C.; Tarhan, Ç.; Pekmez, M.; Turgut-Kara, N. Nutritional and health benefits of legumes and their distinctive genomic properties. Review article. *Food Sci. Technol. Camp.* **2019**, *39*, 1–12. [CrossRef]
4. Didinger, C.; Thompson, H.J. Defining nutritional and functional niches of legumes: A call for clarity to distinguish a future role for pulses in the dietary guidelines for Americans. *Nutrients* **2021**, *13*, 1100. [CrossRef] [PubMed]

5. Chen, L.S.; Figueredo, A.; Villani, H.; Michajluk, J.; Hungria, M. Diversity and symbiotic effectiveness of rhizobia isolated from field-grown soybean nodules in Paraguay. *Biol. Fertil. Soils* **2002**, *35*, 448–457. [CrossRef]
6. Faligowska, A.; Szymańska, G.; Panasiewicz, K.; Szukała, J.; Koziara, W.; Ratajczak, K. The long-term effect of legumes as forecrops on the productivity of rotation (winter rape-winter wheat-winter wheat) with nitrogen fertilization. *Plant Soil Environ.* **2019**, *65*, 138–144. [CrossRef]
7. Wysokinski, A.; Lozak, I.; Kuziemska, B. Sources of nitrogen for winter triticale (*Triticosecale Wittm.* ex A.Camus) succeeding pea (*Pisum sativum* L.). *Agronomy* **2021**, *11*, 527. [CrossRef]
8. Peoples, M.B.; Craswell, E.T. Biological nitrogen fixation: Investments, expectations and actual contribution to agriculture. *Plant Soil* **1992**, *141*, 13–39. [CrossRef]
9. Galloway, J.N.; Dentener, F.J.; Capone, D.G.; Boyer, E.W.; Howarth, R.W.; Seitzinger, S.P.; Asner, G.P.; Cleveland, C.C.; Green, P.A.; Holland, E.A.; et al. Nitrogen cycles: Past, present, and future. *Biogeochemistry* **2004**, *70*, 153–226. [CrossRef]
10. Cheng, Q. Perspectives in biological nitrogen fixation research. *J. Integr. Plant Biol.* **2008**, *50*, 786–798. [CrossRef]
11. Vitousek, P.M.; Menge, D.N.L.; Reed, S.C.; Cleveland, C.C. Biological nitrogen fixation: Rates, patterns and ecological controls in terrestrial ecosystems. *Phil. Trans. R. Soc. B* **2013**, *368*, 20130119. [CrossRef] [PubMed]
12. Yu, T.; Zhuang, Q. Modeling biological nitrogen fixation in global natural terrestrial ecosystems. *Biogeosciences* **2020**, *17*, 3643–3657. [CrossRef]
13. Zahran, H.H. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 968–989. [CrossRef] [PubMed]
14. Unkovich, M. Nitrogen fixation in Australian dairy systems: Review and prospect. *Crop Pasture Sci.* **2012**, *63*, 787–804. [CrossRef]
15. Mendonça, E.S.; Lima, P.C.; Guimarães, G.P.; Moura, W.M.; Andrade, F.V. Biological nitrogen fixation by legumes and N uptake by coffee plants. *Rev. Bras. Cienc. Solo* **2017**, *41*, e0160178. [CrossRef]
16. Hossain, Z.; Wang, X.; Hamel, C.; Knight, J.D.; Morrison, M.J.; Ga, Y. Biological nitrogen fixation by pulse crops on semiarid Canadian prairies. *Can. J. Plant Sci.* **2017**, *97*, 119–131. [CrossRef]
17. Reinprecht, Y.; Schram, L.; Marsolais, F.; Smith, T.H.; Hill, B.; Pauls, K.P. Effects of nitrogen application on nitrogen fixation in common bean production. *Front. Plant Sci.* **2020**, *11*, 1172. [CrossRef]
18. Rymuza, K.; Radzka, E.; Wysokinski, A. Nitrogen uptake from different sources by non-GMO soybean varieties. *Agronomy* **2020**, *10*, 1219. [CrossRef]
19. Wysokinski, A.; Lozak, I. The dynamic of nitrogen uptake from different sources by pea (*Pisum sativum* L.). *Agriculture* **2021**, *11*, 81. [CrossRef]
20. Hungria, M.; Vargas, M.A.T. Environmental factors affecting N_2 fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crops Res.* **2000**, *65*, 151–164. [CrossRef]
21. Zielke, M.; Ekker, A.S.; Olsen, R.A.; Spjelkavik, S.; Solheim, B. The influence of abiotic factors on biological nitrogen fixation in different types of vegetation in the high arctic, Svalbard. *Arct. Antarct. Alp. Res.* **2002**, *34*, 293–299. [CrossRef]
22. Mohammadi, K.; Sohrabi, Y.; Heidari, G.; Khalesro, S.; Majidi, M. Effective factors on biological nitrogen fixation. *Afr. J. Agric. Res.* **2012**, *7*, 1782–1788. [CrossRef]
23. De Borja Reis, A.F.; Rosso, M.L.; Purcell, L.C.; Naeve, S.; Casteel, S.N.; Kovács, P.; Archontoulis, S.; Davidson, D.; Ciampitti, I.A. Environmental factors associated with nitrogen fixation prediction in soybean. *Front. Plant Sci.* **2021**, *12*, 675410. [CrossRef] [PubMed]
24. Weisany, W.; Raey, Y.; Allahverdipoor, K.H. Role of some of mineral nutrients in biological nitrogen fixation. *Bull. Env. Pharmacol. Life Sci.* **2013**, *2*, 77–84.
25. Zheng, M.; Zhou, Z.; Luo, Y.; Zhao, P.; Mo, J. Global pattern and controls of biological nitrogen fixation under nutrient enrichment: A meta-analysis. *Glob. Chang. Biol.* **2019**, *25*, 3018–3030. [CrossRef]
26. Martyniuk, S. The importance of biological fixation of atmospheric nitrogen in ecological agriculture. *J. Res. Appl. Agric. Eng.* **2008**, *53*, 9–14. (In Polish)
27. Vance, C.P. Root bacteria interactions: Symbiotic nitrogen fixation. In *Plant Root. The Hidden Half*; Waisel, J., Eshel, A., Kafkafi, U., Eds.; Marcel Dekker Inc.: New York, NY, USA, 2002; 1136p.
28. Vieira, R.F.; Cardoso, E.J.B.N.; Vieira, C.; Cassini, S.T.A. Foliar application of molybdenum in common beans. I. Nitrogenase and reductase activities in a soil of high fertility. *J. Plant Nutr.* **1998**, *21*, 169–180. [CrossRef]
29. Brear, E.M.; Day, D.A.; Smith, P.M.C. Iron: An essential micronutrient for the legume–rhizobium symbiosis. *Front. Plant Sci.* **2013**, *4*, 359. [CrossRef]
30. Brown, P.H.; Bellaloui, N.; Wimmer, M.A.; Bassil, E.S.; Ruiz, J.; Hu, H.; Pfeffer, H.; Dannel, F.; Römheld, V. Boron in plant biology. *Plant Biol.* **2002**, *4*, 205–223. [CrossRef]
31. Ciampitti, I.A.; de Borja Reis, A.F.; Córdova, S.C.; Castellano, M.J.; Archontoulis, S.V.; Correndo, A.A.; Antunes De Almeida, L.F.; Moro Rosso, L.H. Revisiting biological nitrogen fixation dynamics in soybeans. *Front. Plant Sci.* **2021**, *12*, 727021. [CrossRef]
32. Bleinholder, H.; Weber, E.; Feller, C.; Hess, M.; Wicke, H.; Meier, U.; van den Boom, T.; Lancashire, P.D.; Buhr, L.; Hack, H.; et al. Growth stages of mono- and dicotyledonous plants. In *BBCH Monograph*; Meier, U., Ed.; Julius Kühn-Institut: Quedlinburg, Germany, 2001; pp. 1–160.
33. Kuziemska, B.; Trębicka, J.; Wysokinski, A. Uptake and utilization of nitrogen from organic fertilizers influenced by different doses of copper. *Agronomy* **2021**, *11*, 1219. [CrossRef]

34. Rezvani, M.; Zaefarian, F. Bioaccumulation and translocation factors of cadmium and lead in Aeluropuslittoralis. *Aust. J. Agric. Res.* **2011**, *2*, 114–119.
35. Kuziemska, B.; Trębicka, J.; Wysokiński, A.; Jaremko, D. Supplementation of organic amendments improve yield and adaptability by reducing the toxic effect of copper in cocksfoot grass (*Dactylis glomerata* L. Cv Amera). *Agronomy* **2021**, *11*, 791. [CrossRef]
36. Gawryluk, A.; Wyłupek, T.; Wolański, P. Assessment of Cu, Pb and Zn content in selected species of grasses and in the soil of the roadside embankment. *Ecol. Evol.* **2020**, *10*, 9841–9852. [CrossRef]
37. Proc, K.; Bulak, P.; Kaczor, M.; Bieganowski, A. A new approach to quantifying bioaccumulation of elements in biological processes. *Biology* **2021**, *10*, 345. [CrossRef]
38. Kisker, C.; Schindelin, H.; Rees, D.C. Molybdenum–cofactor–containing enzymes: Structure and mechanism. *Ann. Rev. Biochem.* **1997**, *66*, 233–267. [CrossRef]
39. Zimmer, W.; Mendel, R. Molybdenum metabolism in plants. *Plant Biol.* **1998**, *1*, 160–168. [CrossRef]
40. Campbell, W.H. Structure and function of eukaryotic NAD(P)H: Nitrate reductase. *Cell. Mol. Life Sci.* **2001**, *58*, 194–204. [CrossRef]
41. Guerinot, M.L. Iron uptake and metabolism in the Rhizobia legume symbioses. *Plant Soil* **1991**, *130*, 199–209. [CrossRef]
42. O'Hara, G.W. Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: A review. *Aust. J. Exp. Agric.* **2001**, *41*, 417–433. [CrossRef]
43. Peters, J.W.; Szilagyi, R.K. Exploring new frontiers of nitrogenase structure and mechanism. *Curr. Opin. Chem. Biol.* **2006**, *10*, 101–108. [CrossRef]
44. Day, D.A.; Smith, P.M.C. Iron transport across symbiotic membranes of nitrogen-fixing legumes. *Int. J. Mol. Sci.* **2021**, *22*, 432. [CrossRef] [PubMed]
45. Bolaños, L.; Esteban, E.; Delorenzo, C.; Fernandez-Pascual, M.; Defelipe, M.R.; Garate, A.; Bonilla, I. Essentiality of boron for symbiotic dinitrogen fixation in pea (*Pisum sativum*) rhizobium nodules. *Plant Physiol.* **1994**, *104*, 85–90. [CrossRef]
46. Carpena, R.; Esteban, E.; Sarro, M.; Peñaloza, J.; Garate, A.; Lucena, J.; Zornoza, P. Boron and calcium distribution in nitrogen fixing pea plants. *Plant Sci.* **2000**, *151*, 163–170. [CrossRef]
47. Bolaños, L.; Brevin, N.J.; Bonilla, I. Effects of boron on rhizobium-legume cell-surface interactions and nodule development. *Plant Physiol.* **1996**, *110*, 1249–1256. [CrossRef]
48. Gupta, U.C.; Lipsett, J. Molybdenum in soils, plants and animals. *Adv. Agron.* **1981**, *34*, 73–115.
49. Hamlin, R.L. Molybdenum. In *Handbook of Plant Nutrition*; Barker, A.V., Philbeam, D.J., Eds.; Taylor and Francis Group: New York, NY, USA, 2007; pp. 375–394.
50. Symanowicz, B.; Kalembasa, S. Effect of iron, molybdenum and cobalt on the amount of nitrogen biologically reduced by *Rhizobium galegae*. *Ecol. Chem. Eng. A* **2012**, *19*, 1311–1320. [CrossRef]
51. Butnariu, M.; Robert, A.; Tonea, E. Quantity determination of molybdenum from *Pisum sativum* plants and the influence of heavy metal to chemical elements accumulation. *Lucr. Ştiinţifice–Zooteh. Şi Biotehnol.* **2008**, *41*, 735–743.
52. Kabata-Pendias, A.; Pendias, H. *Biogeochemistry of Trace Elements*; Wydawnictwo Naukowe PWN: Warszawa, Poland, 1999; ISBN 83-01128-23-2.
53. Burton, J.W.; Harlow, C.; Theil, E.C. Evidence for reutilization of nodule iron in soybean seed development. *J. Plant Nutr.* **1998**, *21*, 913–927. [CrossRef]
54. Römheld, V.; Nikolic, M. Iron. In *Handbook of Plant Nutrition*; Barker, A.V., Philbeam, D.J., Eds.; Taylor and Francis Group: New York, NY, USA, 2007; pp. 329–350.
55. Gupta, U.C. Boron. In *Handbook of Plant Nutrition*; Barker, A.V., Philbeam, D.J., Eds.; Taylor and Francis Group: New York, NY, USA, 2007; pp. 241–277.
56. Reguera, M.; Wimmer, M.; Bustos, P.; Goldbach, H.E.; Bolanos, L.; Bonilla, I. Ligands of boron in *Pisum sativum* nodules are involved in regulation of oxygen concentration and rhizobial infection. *Plant Cell Environ.* **2010**, *33*, 1039–1048. [CrossRef]
57. Hu, H.; Brown, P.H. Absorption of boron by plant roots. *Plant Soil* **1997**, *193*, 49–58. [CrossRef]
58. Slatni, T.; Krouma, A.; Aydi, S.; Chaiff, C.; Gouia, H.; Abdelly, C. Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris* L.) subjected to iron deficiency. *Plant Soil* **2008**, *312*, 49–57. [CrossRef]
59. Wysokiński, A.; Kuziemska, B.; Łozak, I.; Jaremko, D. Effect of various nitrogen doses on the accumulation of molybdenum, boron and iron in yellow lupine biomass. *J. Elem.* **2018**, *23*, 309–320. [CrossRef]
60. Netty, S.; Wardiyati, T.; Maghfoer, M.D.; Handayanto, E. Bioaccumulation of nickel by five wild plant species on nickel contaminated soil. *IOSR J. Eng.* **2013**, *3*, 1–6. [CrossRef]
61. Ociepa, E.; Pachura, P.; Ociepa-Kubicka, A. Effect of fertilization unconventional migration of heavy metals in the soil-plant system. *Eng. Prot. Environ.* **2014**, *17*, 325–338.