Mazetti Fernandes, Adalton; Peres Soratto, Rogério; Andriani dos Santos, Letícia

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NUTRIENT EXTRACTION AND EXPORTATION BY COMMON BEAN CULTIVARS UNDER DIFFERENT FERTILIZATION LEVELS: II - MICRONUTRIENTS[1]

Adalton Mazetti Fernandes[2], Rogério Peres Soratto[3] & Letícia Andriani dos Santos[4]

SUMMARY

Where the level of agricultural technology is higher, common bean cultivars with a higher yield potential possibly require greater amounts of micronutrients. In Brazil however, there is a lack of information about the micronutrient extraction and exportation by the main grown cultivars. The objective of this study was to evaluate micronutrient (B, Cu, Fe, Mn, and Zn) extraction and exportation by common bean cultivars Pérola and IAC Alvorada, under different levels of NPK fertilization, on a dystroferric Red Nitosol, in Botucatu, São Paulo State, Brazil. The experiment was arranged in a randomized complete block (split plot) design with four replications. The plots consisted of six treatments based on a 2 x 3 factorial model, represented by two cultivars and three NPK levels (PD0 - 'Pérola' without fertilization, PD1 - 'Pérola' with 50 % of recommended fertilization, PD2 - 'Pérola' with 100 % of recommended fertilization, AD0 - 'IAC Alvorada' without fertilization, AD1 - 'IAC Alvorada' with 50 % of recommended fertilization, and AD2 - 'IAC Alvorada' with 100 % of recommended fertilization) and subplots sampled seven times during the cycle. Higher levels of NPK fertilization increased micronutrient extraction by both cultivars, and treatments with 100 % of recommended NPK fertilization extracted on average 167 g B, 58 g Cu, 1,405 g Fe, 1,213 g Mn and 211 g Zn per hectare. Regardless of the treatment, the highest demand period for B, Cu, Fe, Mn and Zn in both cultivars occurred at the R_7 stage (pod formation), i.e. 42 to 55 days after emergence (DAE). The amount of B, Cu, Fe, Mn and Zn exported depended mainly on the level of NPK fertilization used, with values per hectare ranging from 38 to 90 g of B, 12 to 26 g of Cu, 222 to 568 g of Fe 234 to 467 g of Mn, and 40 to 96 g of Zn.

Index terms: ***Phaseolus vulgaris*****, mineral nutrition, absorption curves, absorption rates, nutrients accumulation.**

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(2) Doctorate in Agronomy (Agriculture), College of Agricultural Sciences, São Paulo State University - FCA/UNESP, P.O. Box 37. CEP 18610-307 Botucatu (SP), Brazil. E-mail: adaltonfernandes@hotmail.com

(3) Associate Professor at Department of Crop Science, FCA/UNESP. Research award from CNPq. E-mail: soratto@fca.unesp.br

(4) Graduate in Agronomy, FCA/UNESP. E-mail: leticiaandriani@hotmail.com

RESUMO: ***EXTRAÇÃO E EXPORTAÇÃO DE NUTRIENTES EM CULTIVARES DE FEIJOEIRO, SOB NÍVEIS DE ADUBAÇÃO: II - MICRONUTRIENTES***

Sob maior nível tecnológico, cultivares de feijão com maior potencial produtivo possivelmente exigem maior quantidade de micronutrientes. Porém, no Brasil há carência de informações sobre extração e exportação de micronutrientes pelos principais cultivares utilizados. Neste estudo, objetivou-se avaliar a extração e a exportação de micronutrientes (B, Cu, Fe, Mn e Zn) pelos cultivares de feijão Pérola e IAC Alvorada, sob diferentes níveis de adubação NPK, em um Nitossolo Vermelho distroférrico, no município de Botucatu, SP. O delineamento experimental utilizado foi de blocos casualizados, em esquema de parcela subdividida, com quatro repetições. As parcelas foram constituídas por seis tratamentos referentes a um fatorial 2 x 3, sendo dois cultivares e três níveis de adubação NPK (PD0 - 'Pérola' sem adubação NPK, PD1 - 'Pérola' com 50 % da adubação recomendada, PD2 - 'Pérola' com 100 % da adubação recomendada, AD0 - 'IAC Alvorada' sem adubação, AD1 - 'IAC Alvorada' com 50 % da adubação recomendada e AD2 - 'IAC Alvorada' com 100 % da adubação recomendada) e as subparcelas, por sete épocas de coletas no decorrer do ciclo. Maiores níveis de adubação NPK aumentaram a extração de micronutrientes pelos dois cultivares; no entanto, 100 % adubação NPK recomendada proporcionou extração média de 167 g de B, 58 g de Cu, 1.405 g de Fe, 1.213 g de Mn e 211 g de Zn por hectare. Independentemente do tratamento, a época de maior demanda por B, Cu, Fe, Mn e Zn, em ambos os cultivares, ocorreu no estádio R_7 (formação das vagens), ou seja, de 42 a 55 dias, após a emergência (DAE). A quantidade de B, Cu, Fe, Mn e Zn exportada foi dependente, principalmente, do nível de adubação NPK utilizado, com valores por hectare variando de 38 a 90 g de B, 12 a 26 g de Cu, 222 a 568 g de Fe, 234 a 467 g de Mn e 40 a 96 g de Zn.

Termos de indexação: Phaseolus vulgaris*, nutrição mineral, curvas de absorção, taxas de absorção, acúmulo de nutrientes.*

INTRODUCTION

In Brazil, common bean (*Phaseolus vulgaris* L.) is no longer considered only a subsistence crop. Currently, this crop is also grown by farmers in medium and large areas, using advanced technology such as irrigation, higher levels of fertilization and cultivars with a high yield potential, resulting in grain yields above 3,000 kg ha^{-1} (Vieira, 2006; Fernandes et al., 2007). The nutrient demand and exportation under these conditions are possibly different from those of low-yielding crops (Gallo & Miyasaka, 1961; Haag et al., 1967; Cobra Netto et al., 1971; Rosolem, 1987).

Despite the increase in technology used by farmers for common bean cultivation, mineral nutrition is often overlooked, especially of micronutrients (Teixeira et al., 2004; Fernández et al., 2007; Ascoli et al., 2008). However, micronutrients are essential for crop growth and development (Kirkby & Römheld, 2007), and a low availability of any micronutrient in the soil leads to a decrease in the common bean yield (Oliveira et al., 1996).

To date, studies about nutrient absorption by common bean were restricted to macronutrients only, although they were developed in the 1960's and 1970's, when grain yield was low (Gallo & Miyasaka, 1961; Haag et al., 1967; Cobra Netto et al., 1971). Absorption and accumulation of micronutrients were investigated with common bean, but these studies were conducted in pots, or only the micronutrient extraction at flowering and the exportation of these elements by harvested grains were determined (Fageria, 1989; Fageria & Souza, 1995; Barbosa Filho & Silva, 2000). Therefore, information on the demand for micronutrients during the crop cycle under field conditions and with high levels of grain yield is scarce.

Thus, current studies are needed about the mineral nutrition of common bean, especially with regard to micronutrients, since these become increasingly important as grain yield levels are increased (Barbosa & Gonzaga, 2012). Studying micronutrient absorption during the development cycle of the current common bean cultivars, grown at different levels of fertilization, is essential, underlying the definition of the absorbed amounts, the times of greatest demand and the distribution of nutrients in the different plant structures. This information will help establish more rational fertilization programs for specific growth conditions, to increase fertilizer use efficiency, reduce costs and to maintain soil fertility.

The objective of this study was to evaluate the micronutrient (B, Cu, Fe, Mn, and Zn) extraction and exportation by the common bean cultivars Pérola and IAC Alvorada, under different levels of NPK fertilization.

MATERIALS AND METHODS

A field experiment was conducted on the Experimental Farm Lageado of the College of Agricultural Sciences - São Paulo State University, in Botucatu, São Paulo State, Brazil (48º 26' W, 22º 51' S, 740 m asl), in a dystroferric Red Nitosol (Embrapa, 2006). According to the Köppen classification, the climate is Cwa (tropical highland), with a dry winter and a hot, rainy summer. The experiment was carried out in an area under no-tillage cultivation for several years. Before the installation of this experiment, wheat/maize/pearl millet crops were grown in the area.

Prior to the experiment, a soil sample consisting of 10 subsamples was taken in the 0-20 cm layer to determine the chemical properties (Raij et al., 2001): organic matter = 28 g dm^{-3}; pH ($CaCl_2$ 0.01 mol L^{-1}) = 5.0; P (resin) = 33 mg dm^{-3}; K^+ = 3.0 $mmol_c$ dm^{-3}; Ca^{2+} = 25 $mmol_c$ dm^{-3}; Mg^{2+} = 11 $mmol_c$ dm^{-3}; H+Al = 32 $mmol_c$ dm^{-3}; base saturation = 55 %; S = 6.4 mg dm^{-3}; B = 0.40 mg dm^{-3}; Cu = 12.4 mg dm^{-3}; Fe = 23 mg dm^{-3}; Mn = 5.1 mg dm^{-3}; and Zn = 1.6 mg dm^{-3}.

Fertilization at sowing was 20 kg ha^{-1} N (urea), 40 kg ha^{-1} P_2O_5 (triple superphosphate), and 40 kg ha^{-1} K_2O (potassium chloride), according to soil analysis and following the recommendation of Ambrosano et al. (1997), for an expected grain yield of 3,500-4,500 kg ha^{-1}. The recommendation for the topdressing fertilizer was 90 kg ha^{-1} N (urea), considering the area as a high expected response to N application (Ambrosano et al., 1997). No S or micronutrient fertilization was applied.

The experiment was arranged in a randomized complete block design with split-plots and four replications. Plots consisted of six treatments in a 2 x 3 factorial arrangement, consisting of two cultivars and three NPK levels (PD0 - Pérola without NPK fertilization, PD1 - Pérola with 50 % of recommended NPK fertilization; PD2 - Pérola with 100 % of recommended NPK fertilization; AD0 - IAC Alvorada without NPK fertilization, AD1 - IAC Alvorada with 50 % of recommended NPK fertilization, and AD2 - IAC Alvorada with 100 % of recommended NPK fertilization). Subplots consisted of seven plant sampling (evaluations) times, (Table 1). Each plot consisted of five 12 m-long rows, spaced 0.45 m apart (27 m^2); the three central rows of each plot were evaluated, without the borders of 0.5 m at either end of each row (14.8 m). Each subplot was represented by six plants sampled per plot. The plants from the surroundings of the sampled plants were not used.

Sowing was performed mechanically on 02/15/2011 (15 seeds per meter). In all treatments, the seeds were treated with the fungicide carbendazim + thiram (45 + 105 g a.i. per 100 kg of seed) and the insecticide thiamethoxam (75 g a.i. per 100 kg of seed). Seedling emergence occurred on 02/22/2011. A topdressing fertilization of nitrogen was split in two applications, one 14 days after emergence (DAE) and the second 24 DAE (V4 stage). Irrigation and phytossanitary management during the crop cycle were performed as needed and according to the technical recommendations.

At common bean flowering (37 DAE), leaves were collected as described by Ambrosano et al. (1997), and concentrations of B, Cu, Fe, Mn, and Zn were determined according to Malavolta et al. (1997).

At each sampling time, the shoots of six plants - without symptoms of damage caused by pests and diseases and surrounded by healthy plants on all sides - were collected per plot. Stems, leaves and reproductive structures (pods + grains) of the sampled plants were separated. The plant parts were dried separately in a forced-air oven at 65 ºC for 72 h and weighed. The data regarding dry matter (DM) associated with the times of plant sampling were used to obtain the curves of DM accumulation (Soratto et al., 2013).

The samples were ground in a Willey mill and the micronutrient concentrations (B, Cu, Fe, Mn, and Zn) were determined according to Malavolta et al. (1997). The amounts of accumulated micronutrients were estimated for all compartments of the aboveground part of the plant (shoot) separately and together, using data from the micronutrient concentration and amounts of DM accumulated. Accumulation rates of micronutrients in the reproductive structures and shoot were obtained by the first derivative of the adjustment equations.

At the end of the cycle (90 DAE), the grain yield was determined in two 3-m-long rows of each plot (Soratto et al., 2013). A sample of grains from each plot was dried in a forced-air oven at 65 ºC for 72 h. Afterwards, these grains were ground and micronutrient (B, Cu, Fe, Mn, and Zn) concentration was determined according to Malavolta et al. (1997). Micronutrient exportation was obtained using the data of grain yield DM (Soratto et al., 2013) and micronutrient concentration in grains.

Data were subjected to analysis of variance. The means of the treatments at each sampling time were separated by the LSD test at 0.05 probability, using the Sisvar software. The effects of plant sampling times were evaluated by regression analysis, using SigmaPlot 10.0 software.

RESULTS AND DISCUSSION

Boron concentration in plant leaves from treatment AD0 (32 mg kg^{-1}) was higher than in PD1 (24 mg kg^{-1}), PD2 (28 mg kg^{-1}), and AD1 (27 mg kg^{-1}), but did not differ significantly from the other treatments (Table 2). Despite the differences among treatments,

Table 1. Description of the common bean growth stages in each time of evaluation

Time of evaluation	Growth stage[(1)]	Plant characteristics in each time of evaluation
DAE[(2)]		
14	V_4	Third trifoliate leaf expanded
28	R_5	Pre-flowering (flower buds)
35	End of R_5	Pre-flowering (a little before to full flowering)
42	Early R_7	Beginning of pod formation
55	End of R_7	End of pod formation
70	R_8	Grain filling
90	R_9	Maturation

(1) Fernández et al. (1986). (2) Days after emergence.

Table 2. Concentration of micronutrients (B, Cu, Fe, Mn and Zn) in leaf diagnosis, collected at R6 stage (37 DAE), of common bean cultivars under different levels of fertilization

Treatment[(1)]	B	Cu	Fe	Mn	Zn
	mg kg^{-1}				
PD0	29 abc	8 a	224 ab	86 b	31 b
PD1	24 d	10 a	144 c	124 a	34 ab
PD2	28 bc	9 a	171 bc	116 a	35 a
AD0	32 a	10 a	231 a	90 b	31 b
AD1	27 c	10 a	182 abc	123 a	30 b
AD2	30 ab	10 a	134 c	118 a	32 ab
CV (%)	6.8	7.8	21.8	11.4	8.3

Values followed by same letter in columns, are not significantly different at $p \leq 0.05$ according to LSD test. (1) PD0 = Pérola without NPK fertilization, PD1 = Pérola with 50 % of recommended NPK fertilization; Pérola PD2 = Pérola with 100 % of recommended NPK fertilization; AD0 = IAC Alvorada without NPK fertilization, AD1 = IAC Alvorada with 50 % of recommended NPK fertilization; AD2 = IAC Alvorada with 100 % of recommended NPK fertilization.

in treatment PD1, the B concentration in leaves was within the range of 15-26 mg kg^{-1}, which is considered appropriate by Ambrosano et al. (1997). In the other treatments, the B concentration in the leaves was above this range. The high B concentrations, especially in the treatments without NPK fertilization, may be due to a concentration effect, once the DM production in these treatments was lower (Soratto et al., 2013). The copper concentration in leaves was not affected by treatments, but in all treatments the B concentration was within the range considered appropriate for common bean (4-20 mg kg^{-1}) (Ambrosano et al., 1997).

In treatment AD0, the Fe concentration in leaves was higher than in PD1, PD2, and AD2, but similar to the other treatments (Table 2). Only in AD2, the Fe leaf concentration was within the range proposed by Ambrosano et al. (1997) (40-140 mg kg^{-1}), while in all other treatments, the Fe concentrations were higher than the values reported by these authors. Soratto & Crusciol (2008) also observed higher the Fe leaf concentrations of common bean cultivars than the appropriate range proposed by Ambrosano et al. (1997). This result may be due to the high Fe availability in the soil (23 mg dm^{-3}). In treatments without NPK fertilization, the Mn concentration in leaves of both cultivars (PD0 and AD0) was similar, but lower than in the fertilized treatments, indicating no significant difference in Mn concentrations (Table 2). Treatments without NPK fertilization showed Mn leaf concentrations in the range of 15 to 100 mg kg^{-1}, considered appropriate by Ambrosano et al. (1997), however, in treatments with NPK fertilization, the Mn concentration was higher than the appropriate range. The zinc leaf concentrations in treatment PD2 were significantly different from PD0, AD0, and AD1 (Table 2), although the Zn concentrations in all treatments were within the range considered appropriate by Ambrosano et al. (1997) (18 - 50 mg kg^{-1}).

In general, micronutrient concentrations in leaves in all treatments were similar and/or higher than the values considered adequate for common bean, which result from the availability of these micronutrients in the soil, considered medium (B) to high (Cu, Fe, Mn, and Zn) (Raij et al., 1997). These results indicate that the availability of micronutrients was adequate in the soil and there was no limitation to adequate absorption of these micronutrients by common bean.

With regard to micronutrient absorption, it was noted that regardless of the treatment, the amount accumulated in the stem increased up to 70-75 DAE (R8) and decreased slightly in the following stages (Figures 1a, 2a, 3a, 4a, and 5a). Treatments showed no significant difference in the amounts of micronutrients accumulated in the stem during the first 28 DAE, but from the beginning of flowering, i.e., 35 DAE, the amounts accumulated in stems in the case of the treatments with 100 % of recommended NPK fertilization were higher than the amount obtained in treatments without fertilization. In treatments with half the recommended NPK fertilization, the accumulated amounts of micronutrients in the stem were intermediate.

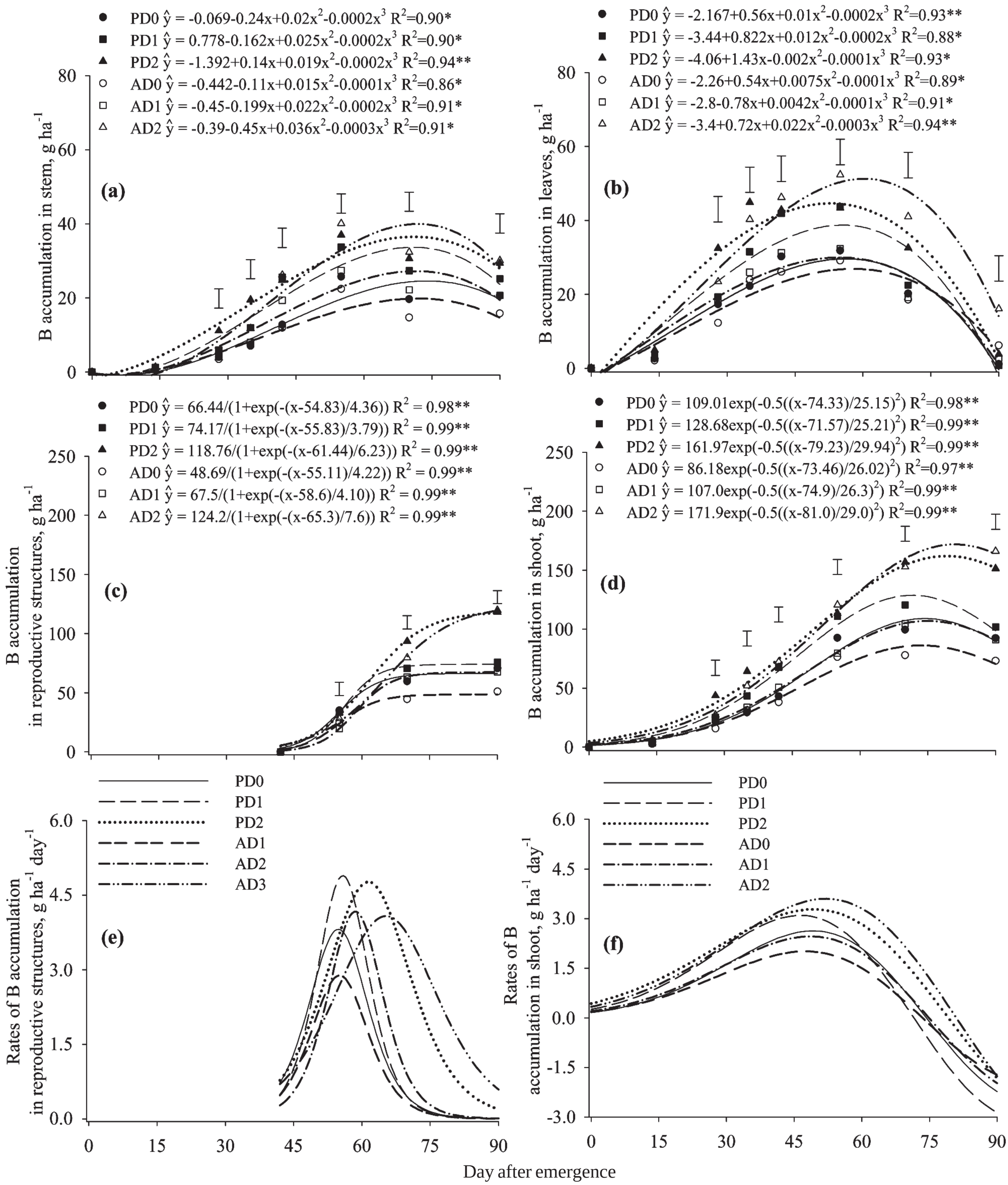

Figure 1. Boron accumulationin stem (a), leaves (b), reproductive structures (c), and shoot (d) and rates of accumulation of B in reproductive structures (e) and shoot (f) of common bean cultivars, under different levels of fertilization. ** and * are: significant at $p \leq 0.01$ and $p \leq 0.05$ by the F test. Vertical bars represent the Least Significant Difference (LSD) at $p \leq 0.05$. PD0 = Pérola without NPK fertilization, PD1 = Pérola with 50 % of recommended NPK fertilization; PD2 = Pérola with 100 % of recommended NPK fertilization; AD0 = IAC Alvorada without NPK fertilization, AD1 = IAC Alvorada with 50 % of recommended NPK fertilization; AD2 = IAC Alvorada with 100 % of recommended NPK fertilization.

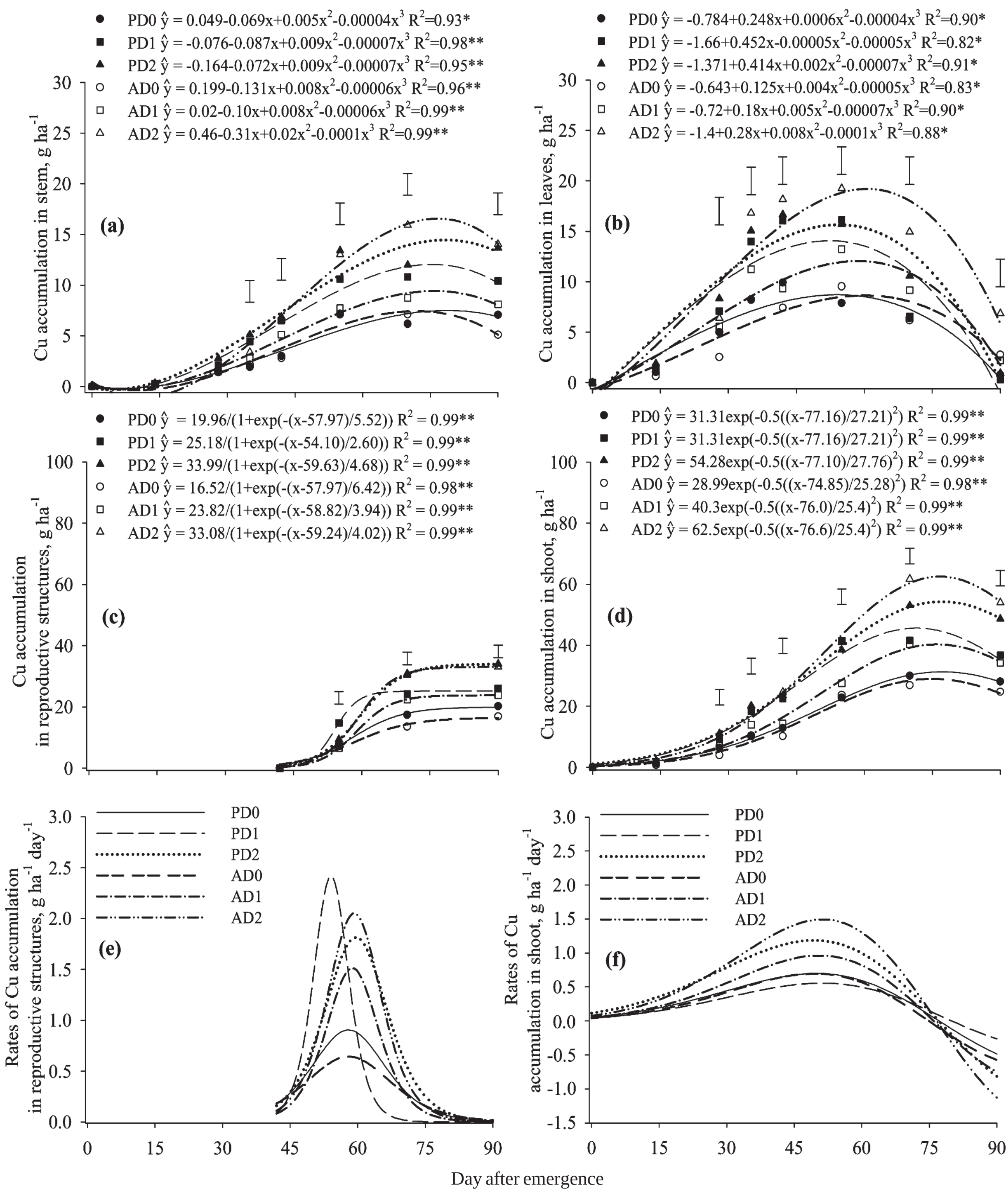

Figure 2. Copper accumulationin stem (a), leaves (b), reproductive structures (c), and shoot (d) and rates of accumulation of Cu in reproductive structures (e) and shoot (f) of common bean cultivars, under different levels of fertilization. ** and * are: significant at $p \leq 0.01$ and $p \leq 0.05$ by the F test. Vertical bars represent the Least Significant Difference (LSD) at $p \leq 0.05$. PD0 = Pérola without NPK fertilization, PD1 = Pérola with 50 % of recommended NPK fertilization; PD2 = Pérola with 100 % of recommended NPK fertilization; AD0 = IAC Alvorada without NPK fertilization, AD1 = IAC Alvorada with 50 % of recommended NPK fertilization; AD2 = IAC Alvorada with 100 % of recommended NPK fertilization.

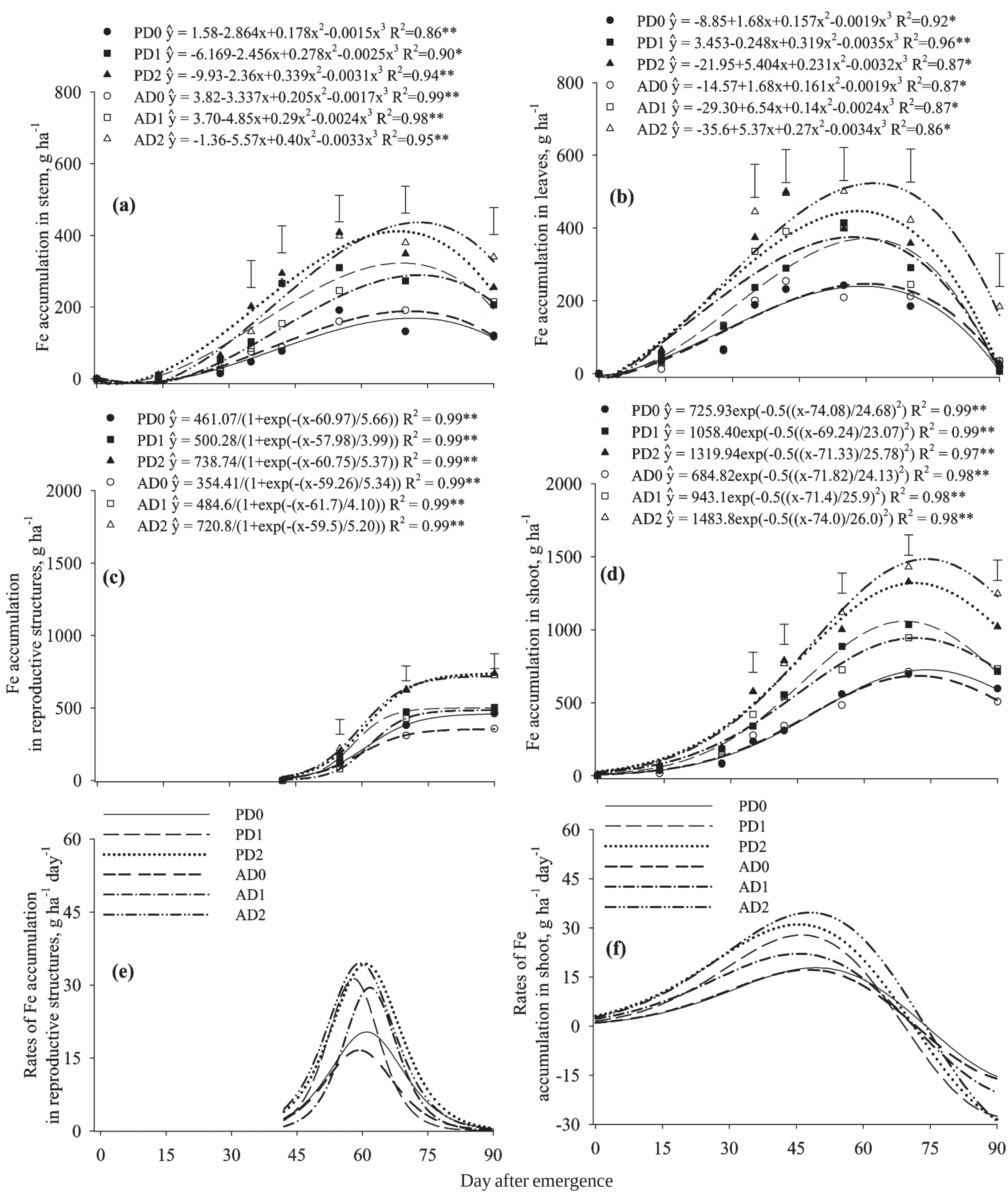

Figure 3. Iron accumulationin stem (a), leaves (b), reproductive structures (c), and shoot (d) and rates of accumulation of Fe in reproductive structures (e) and shoot (f) of common bean cultivars, under different levels of fertilization. ** and * are: significant at $p \leq 0.01$ and $p \leq 0.05$ by the F test. Vertical bars represent the Least Significant Difference (LSD) at $p \leq 0.05$. PD0 = Pérola without NPK fertilization, PD1 = Pérola with 50 % of recommended NPK fertilization; PD2 = Pérola with 100 % of recommended NPK fertilization; AD0 = IAC Alvorada without NPK fertilization, AD1 = IAC Alvorada with 50 % of recommended NPK fertilization; AD2 = IAC Alvorada with 100 % of recommended NPK fertilization.

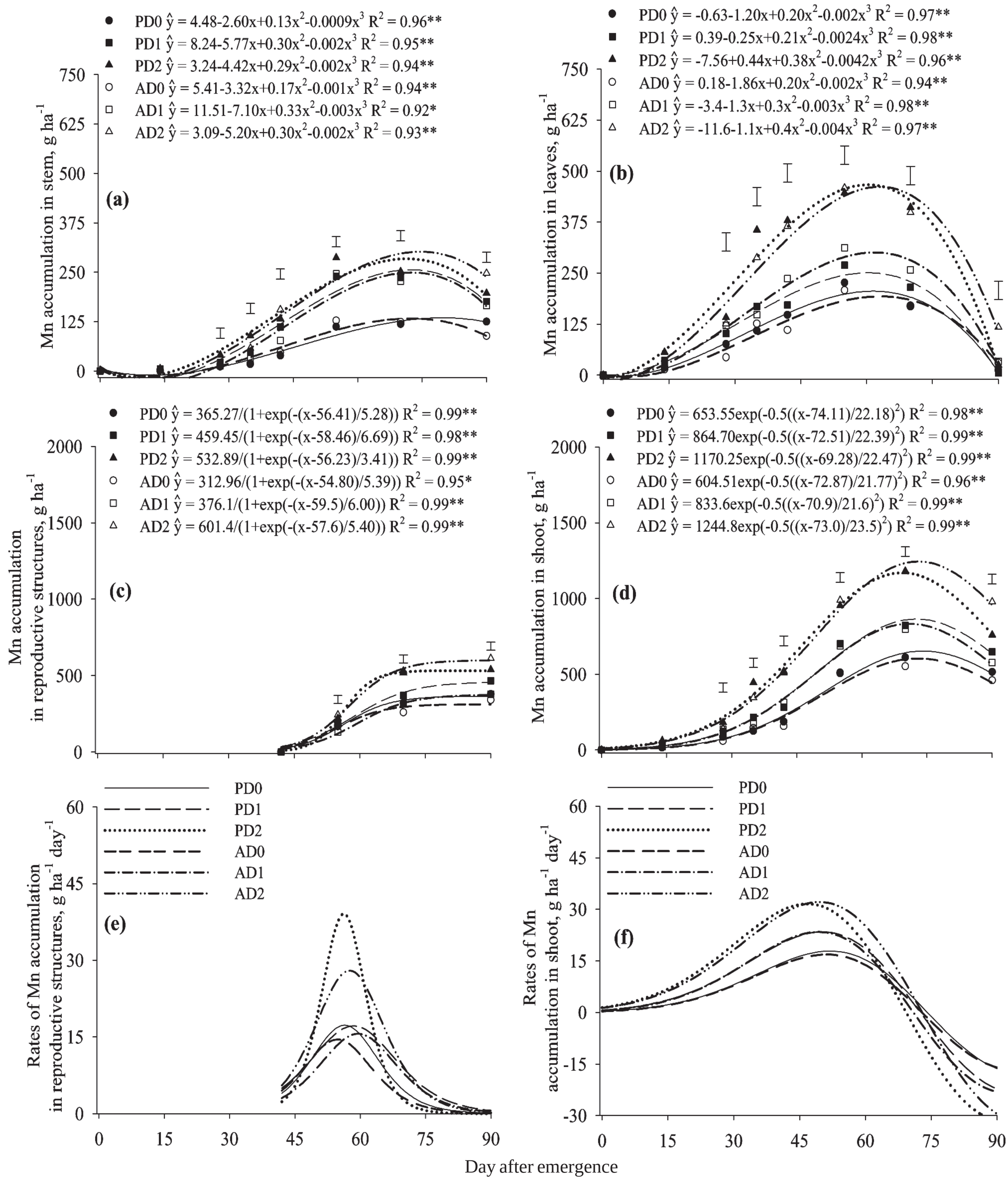

Figure 4. Manganese accumulationin stem (a), leaves (b), reproductive structures (c), and shoot (d) and rates of accumulation of Mn in reproductive structures (e) and shoot (f) of common bean cultivars, under different levels of fertilization. ** and * are: significant at $p \leq 0.01$ and $p \leq 0.05$ by the F test. Vertical bars represent the Least Significant Difference (LSD) at $p \leq 0.05$. PD0 = Pérola without NPK fertilization, PD1 = Pérola with 50 % of recommended NPK fertilization; PD2 = Pérola with 100 % of recommended NPK fertilization; AD0 = IAC Alvorada without NPK fertilization, AD1 = IAC Alvorada with 50 % of recommended NPK fertilization; AD2 = IAC Alvorada with 100 % of recommended NPK fertilization.

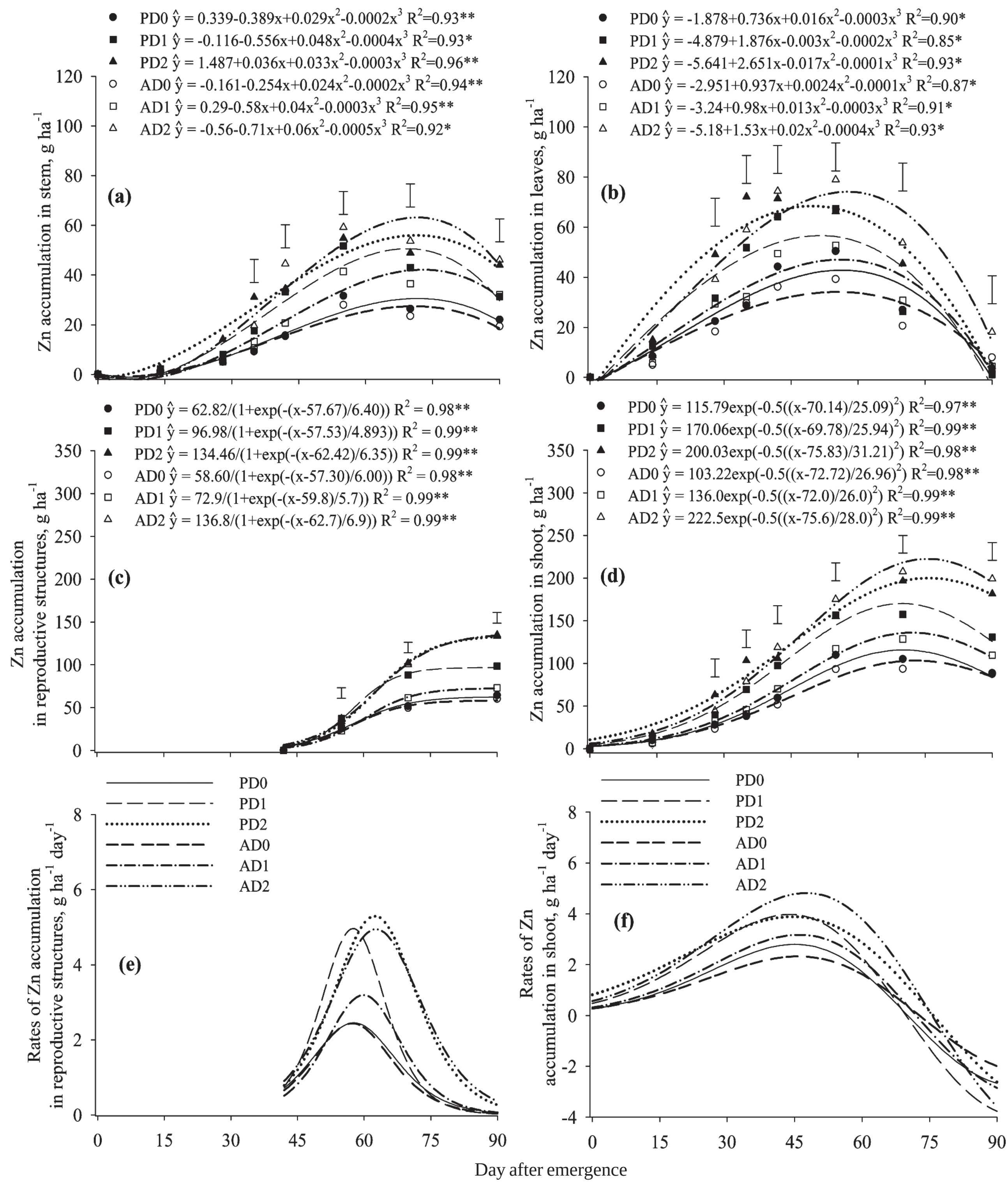

Figure 5. Zinc accumulationin stem (a), leaves (b), reproductive structures (c), and shoot (d) and rates of accumulation of Zn in reproductive structures (e) and shoot (f) of common bean cultivars, under different levels of fertilization. ** and * are: significant at p ≤ 0.01 and p ≤ 0.05 by the F test. Vertical bars represent the Least Significant Difference (LSD) at p ≤ 0.05. PD0 = Pérola without NPK fertilization, PD1 = Pérola with 50 % of recommended NPK fertilization; PD2 = Pérola with 100 % of recommended NPK fertilization; AD0 = IAC Alvorada without NPK fertilization, AD1 = IAC Alvorada with 50 % of recommended NPK fertilization; AD2 = IAC Alvorada with 100 % of recommended NPK fertilization.

In leaves, the accumulated amounts of Cu, Fe, and Zn increased up to 50 and 60 DAE (R7), while the amounts of Mn increased up to 60 and 65 DAE (Figures 1b, 2b, 3b, 4b, and 5b), i.e., the maximum leaf accumulations occurred between the stages R7 (pod formation) and R8 (grain filling). After these stages, the amounts of micronutrients accumulated in leaves decreased due to leaf senescence and abscission. In general, at each fertilization level studied there was no difference between cultivars concerning the amounts of micronutrients accumulated in leaves. However, treatments with 100 % of recommended NPK fertilization showed higher accumulations in leaves. Lower accumulations of micronutrients in leaves occurred mainly in treatments without fertilization. In treatments with 50 % of recommended NPK fertilization, the amounts of micronutrients accumulated in leaves were intermediate, but did not differ significantly from treatments without fertilization and/or with recommended NPK fertilization, during most of the cycle.

Regardless of the treatment, the micronutrients which accumulated highest amounts in the stems and leaves of common bean were Fe and Mn (Figures 1a,b; 2a,b; 3a,b; 4a,b; and 5a,b). Fageria (1989) also observed a higher accumulation of Fe and Mn in the shoot (represented by the leaves and stems) than in grains and pods of common bean plants grown at different P levels in a pot study. The amounts of these micronutrients accumulated in the vegetative part, i.e., in the leaves and stem, were higher than the amounts in the reproductive structures (Figures 1c, 2c, 3c, 4c, and 5c). This higher accumulation of Fe and Mn in leaves and stem is related to their functions in plant metabolism. Iron acts in the synthesis of chlorophyll, and participates in photosynthesis and respiration (Malavolta, 2006; Dechen & Nachtigall, 2006). After absorption, Fe is transported over long distances by the xylem and its mobilization into the phloem is decreased by the formation of insoluble compounds in the leaves (Dechen & Nachtigall, 2006). Therefore, Fe is accumulated mainly in leaves which are the site of the primary function of this nutrient (Kirkby & Römheld, 2007). Manganese acts as an enzyme activator in the respiration process and participates in the photosynthesis process (Malavolta, 2006). Thus, the higher amounts of Mn in the vegetative part, i.e., in leaves and stem, are due to its low redistribution in the plant. This micronutrient, after being absorbed, moves through the transpiration stream and accumulates in certain plant organs such as leaves (Malavolta, 2006). Moreover, the low quantity transported through the phloem is responsible for the low concentration of this nutrient in fruits, seeds and storage organs (Dechen & Nachtigall, 2006). Once Mn is incorporated or immobilized in the leaves it cannot be remobilized, not even under induced senescence (Wood et al., 1986).

In the reproductive structures, the amounts of Cu, Fe, and Mn accumulated in all treatments were increased from 42 to 70 DAE, i.e., from the beginning of R_7 until the beginning of the R_8 stage, but remained stable in the following stages (Figures 2c, 3c, and 4c). Only in the treatments without fertilization (PD0 and AD0) and with intermediate NPK fertilization (PD1 and AD1), the amounts of Zn and B accumulated in reproductive structures increased up to 70 DAE (Figures 1c and 5c). In treatments with 100 % of recommended NPK fertilization, the accumulation of B and Zn in reproductive structures increased up to the end of the crop cycle. In most treatments a well defined pattern of micronutrient accumulation was observed in the reproductive structures, with higher accumulation rates after flowering (42 DAE) and reaching the maximum rates of accumulation between 50 and 60 DAE, i.e., during the intense growth phase of the reproductive structures (Figures 1e, 2e, 3e, 4e, and 5e).

The highest amounts of Fe and B accumulated in reproductive structures occurred in treatments with 100 % of recommended NPK fertilization; these amounts were smallest in treatment AD0 and intermediate and similar in the other treatments (Figures 1c and 3c). Higher amounts of Cu and Zn were accumulated in the reproductive structures in the treatments with 100 % of recommended NPK fertilization than in the others, but in treatments without and with intermediate NPK fertilization, the amounts of accumulated Cu and Zn were very similar, in spite of the higher Zn accumulations in treatment PD1 (Figures 2c and 5c). The amounts of Mn accumulated in reproductive structures in the treatment with recommended NPK fertilization were higher than in treatments PD0, AD0, and AD1 (Figure 4c). Possibly, NPK fertilization induced greater root growth, especially in depth, resulting in the exploitation of a greater volume of soil and, consequently, a greater absorption of micronutrients.

With regard to micronutrient accumulation in the shoot, it was observed that in the first 28 DAE the amounts of micronutrients accumulated were small and did not differ significantly between treatments (Figures 1d, 2d, 3d, 4d, and 5d), which is coincident with data of DM accumulation (Soratto et al., 2013). After flowering, the rates of micronutrient accumulation in shoots showed a great increase, reaching the maximum values during the R7 stage (42-55 DAE), but decreased in the following stages (Figures 1f, 2f, 3f, 4f, and 5f). Andrade et al. (2009) obtained similar results in a study with four common bean cultivars. At the end of the cycle, the rates of micronutrient accumulation in shoots were negative, due to leaf loss, i.e., a proportion of micronutrients accumulated by common bean during the cycle returned to the soil even before harvest. Thus, when micronutrients are recommended during the development of this crop, this must be supplied to the plants shortly before the beginning of the R7 stage, because in this period pods and grains start to grow rapidly (Soratto et al., 2013) and there is a greater demand for micronutrients.

In all treatments, Cu, Fe, Mn and Zn were accumulated in the shoots up to 70-75 DAE, i.e., up to the beginning of grain filling (Figures 2d, 3d, 4d, and 5d). Boron absorption occurred up to 80 DAE in the treatments PD2 and AD2 (100 % of recommended NPK fertilization), but in the other treatments this micronutrient was absorbed only up to 70 DAE (Figure 1d). When treated with the same levels of fertilization, both cultivars accumulated similar amounts of micronutrients in the shoot, but during the reproductive stage, the treatments with 100 % of recommended NPK fertilization always accumulated higher amounts than in the other treatments (on average 167 g ha^{-1} B, 58 g ha^{-1} Cu, 1.405 g ha^{-1} Fe, 1.213 g ha^{-1} Mn and 211 g ha^{-1} Zn) (Figures 1d, 2d, 3d, 4d, and 5d). In a study with four common bean cultivars, Andrade et al. (2009) found average values of B (146 g ha^{-1}) and Fe absorption (1.341 g ha^{-1}) similar to those obtained in this study, but much lower values of Cu (33 g ha^{-1}), Mn (68 g ha^{-1}), and Zn (68 g ha^{-1}) absorption. In the treatments with 50 % of recommended NPK fertilization, absorption values were intermediate (around 119, 43, 1,006, 849, and 153 g ha^{-1} B, Cu, Fe, Mn, and Zn, respectively) (Figures 1d, 2d, 3d, 4d, and 5d). In the unfertilized treatments however, the absorption values were lower (per hectare on average 96 g B, 30 g Cu, 706 g Fe, 630 g Mn, and 110 g Zn). Fageria & Souza (1995) determined the accumulation of micronutrients in common bean shoots during flowering and also found an increase in micronutrient extraction in response to fertilization with macro and micronutrients. These authors related these results to the increase in DM production.

The amounts of Cu and Mn accumulated in the shoots in all treatments were higher than the values observed by Fageria & Souza (1995) for common bean cv. Emgopa 202-Rubi under irrigation. These authors reported accumulated values of 16 g ha^{-1} Cu and 203 g ha^{-1} Mn up to the flowering stage. The amounts of Fe accumulated (1,094 g ha^{-1}) in common bean by these authors were similar to treatments with 50 % of recommended NPK fertilization, while the accumulation of Zn (109 g ha^{-1}) was similar to treatments without fertilization. In treatments with 100 % of recommended NPK fertilization, the amounts of all micronutrients accumulated in the shoot were higher than the values obtained by Fageria & Souza (1995), possibly, due to a higher yield level, since these authors obtained on average (three years) 2,162 kg ha^{-1} of grain yield, i.e., similar to the treatments without fertilization in this study (Table 3). In all treatments, only Fe absorption showed values lower than the 2,970 g ha^{-1} reported by Malavolta & Lima Filho (1997) for common bean. The values of B (138 g ha^{-1}) and Cu (46 g ha^{-1}) absorption reported by these authors were similar to values observed in treatments with half the recommended NPK fertilization, while the absorption of Mn (563 g ha^{-1}) was similar to that in the unfertilized treatments. In the treatments with 100 % of recommended NPK fertilization, Zn absorption (224 g ha^{-1}) was close to values reported by Malavolta & Lima Filho (1997) for common bean. In general, Barbosa Filho & Silva (2000) reported that for the production of 1,000 kg of grain, common bean absorbed about 17 g Cu, 500 g Fe, 94 g Mn, and 72 g Zn up to the flowering stage, i.e., lower amounts than those obtained in this study, in which the grain yield was higher.

Regardless of the treatment, it was observed that micronutrients were taken up in the following order: Fe > Mn > Zn > B > Cu. Except for B, the order of greater absorption obtained in all treatments was similar to that observed by other authors, which determined the micronutrient extraction by this crop only at the flowering stage (Fageria, 1989; Fageria & Souza, 1995; Barbosa Filho & Silva, 2000). Andrade et al. (2009) observed the following order, based on the average of four common bean cultivars: Fe > B > Mn > Zn > Cu. The greater Fe and Mn absorption was a result of higher accumulation rates compared with other micronutrients (Figures 1d, 1f, 2d, 2f, 3d, 3f, 4d, 4f, 5d, and 5f).

Highest B concentrations were observed in grains in treatment PD0, while treatments PD1 and AD1 showed lower and the other treatments intermediate concentrations (Table 3). Copper concentration in grains was not influenced by treatments, while Fe concentration showed a significant difference only between treatment AD2 (191.1 mg kg^{-1}) and PD0 (124.3 mg kg^{-1}). This indicates that NPK fertilization increased Fe concentration in grains of common bean. Andrade et al. (2004) found no differences between Cu and Fe concentrations in "Pérola" grain, of plants treated with three levels of NPK fertilization. Higher Fe concentrations in the grains could be an interesting aspect, since common bean is an important source of this nutrient in the human diet (Moura & Cinniatti-Brazaca, 2006).

The Mn grain concentration was lowest in treatment PD2 and highest in AD2, which differed only from treatments PD2 and AD1 (Table 3). Zinc concentration in grains differed only between treatment AD2 and PD0, AD0, and AD1, with highest concentrations in treatment AD2. Despite the variation among treatments, it was observed that Cu and Zn concentrations in grains agreed with the values observed by Fageria (1989) in common bean cultivars (Carioca, CNF 10, and CNF 4856) fertilized with different P levels. However, in this study Fe and Mn concentrations in grains were higher than the values observed by Fageria (1989). This author obtained Cu, Fe, Mn, and Zn concentrations ranging from 6 to 13, 30 to 70, 8 to 19, and 17 to 47 mg kg^{-1}, respectively. This difference between the results of this study and those of Fageria (1989) may be due to differences among the cultivars used. In a study on yield and nutritional quality of common bean cultivars grown under different levels of NPK fertilization, the Zn concentration in grains was not increased by NPK fertilization, while Cu, Fe, and Mn concentrations did increase (Andrade et al., 2004). According to these authors, it is important to know the concentration of micronutrients in common bean grains, once Zn, Fe, Cu, and Mn are considered essential to the diet of humans and animals, being required for many body functions and since they may be found at deficient, excessive or unbalanced levels in the diet (Andrade et al., 2004).

Table 3. Nutrient concentration in grain, nutrient exportation per area, nutrient exportation per ton of grain, and relative nutrient exportation by common bean cultivars, under different levels of fertilization

Treatment(1)	B	Cu	Fe	Mn	Zn
Grain concentration, mg kg^{-1}					
PD0	31.7 a	7.5 a	124.3 b	132.2 ab	22.5 b
PD1	22.8 c	7.5 a	152.9 ab	136.2 ab	25.7 ab
PD2	25.7 bc	7.4 a	164.5 ab	91.1 c	27.4 ab
AD0	25.3 bc	7.4 a	169.1 ab	150.5 ab	23.6 b
AD1	21.3 c	8.3 a	162.5 ab	127.8 b	23.5 b
AD2	29.0 ab	8.3 a	191.1 a	160.4 a	31.3 a
CV (%)	13.9	14.5	20.3	15.6	17.7
Exportation per area, g ha^{-1}					
PD0	55 b	14 c	222 c	234 d	40 d
PD1	58 b	20 b	388 b	354 b	68 b
PD2	89 a	26 a	549 a	313 c	92 a
AD0	38 c	12 c	267 c	245 d	41 d
AD1	50 b	18 b	381 b	294 c	52 c
AD2	90 a	26 a	568 a	467 a	96 a
CV (%)	10.0	10.3	13.5	7.3	10.9
Exportation per ton of grain, g ton^{-1}(2)					
PD0	27.6 a	6.5 a	108.1 b	115.0 ab	19.6 b
PD1	19.8 c	6.5 a	133.0 ab	118.5 ab	22.4 ab
PD2	22.4 bc	6.5 a	143.1 ab	79.2 c	23.9 ab
AD0	22.0 bc	6.5 a	147.1 ab	130.9 ab	20.6 b
AD1	18.6 c	7.2 a	141.3 ab	111.2 b	20.4 b
AD2	25.2 ab	7.3 a	166.2 a	139.6 a	27.3 a
CV (%)	14.0	15.2	19.0	14.6	19.8
Relative exportation, %(3)					
PD0	51	45	31	36	35
PD1	45	43	37	41	40
PD2	55	47	41	27	46
AD0	43	41	39	40	39
AD1	45	45	40	35	38
AD2	52	42	38	37	43
Média	49	44	38	36	40

Values followed by same letter in columns, are not significantly different at $p \le 0.05$ by the LSD test. (1) PD0: Pérola without NPK fertilization, PD1: Pérola with 50 % of recommended NPK fertilization; PD2: Pérola with 100 % of recommended NPK fertilization; AD0: IAC Alvorada without NPK fertilization, AD1: IAC Alvorada with 50 % of recommended NPK fertilization; AD2: IAC Alvorada with 100 % of recommended NPK fertilization. (2)Data based on grain yield (Soratto et al., 2013) and the values of maximum nutrient accumulation see figures 1, 2, 3, 4 and 5. (3)Relative nutrient exportation in relation to maximum amounts absorbed, see figures 1, 2, 3, 4 and 5.

Regardless of the cultivar used, the treatments with 100 % of recommended NPK fertilization showed higher values of exportation per area for all micronutrients (Table 3). According to Malavolta & Lima Filho (1997) in a grain production of 1,500 kg ha^{-1} of common bean, the exported amounts of micronutrients are around 54, 18, 120, 50, and 76 g ha^{-1} of Cu, Fe, Mn, and Zn, respectively. Although the values of exportation of B, Cu, and Zn reported by these authors agree with the values observed in this study, they are relatively high, since the grain yield obtained by Malavolta & Lima Filho (1997) was lower than in this experiment (Soratto et al., 2013). Pessoa et al. (2000), in a study with common bean, cv. Ouro Negro with a grain yield of 1,893 kg ha^{-1}, observed exported amounts per hectare of 25 g Cu, 102 g Fe, 23 g Mn, and 63 g Zn. Barbosa Filho & Silva (2000) obtained average values of exportation per hectare of 25 g of Cu, 175 g of Fe, 39 g of Mn, and 25 g Zn, for a grain yield around 2,500 kg ha^{-1}, i.e., with lower exportations of Fe, Mn, and Zn than found in this study.

The largest B exportation per ton of grain occurred in the PD0 treatment, followed by treatment AD2, which showed no significant difference from treatments PD2 and AD0 (Table 3). The lowest B exportation per ton was observed in treatments PD1 and AD1, i.e., B exportation had no relationship with grain yield (Soratto et al., 2013), since treatment PD0 with low yield showed higher exportations per ton than most treatments with a higher grain yield. Copper exportation per ton of grain was not affected by treatments, demonstrating that the exportation of this nutrient was greater with an increase in grain yield, as may be observed by the values of grain yield (Soratto et al., 2013) and exportation per area (Table 3). Except for treatment AD2, in the other treatments Fe and Zn exportations per ton of grain showed no significant differences, indicating that the exportation of these micronutrients is related to grain yield, i.e., when the yield level is increased, the exportation per area is increased. Manganese exportation per ton of grains differed only between treatment AD2 (139.6 g t^{-1}) and treatments AD1 (111.2 g t^{-1}) and PD2 (79.2 g t^{-1}), i.e., the amount of Mn exported was not related to grain yield (Soratto et al., 2013), since treatment PD2, one of those with highest grain yield did not show the greatest exportation, as a result of the low concentrations of this nutrient in grains (Table 3).

Despite the variations between treatments, on average approximately half of all B absorbed by common bean during the cycle is exported with the grains, while for the other micronutrients this proportion is lower, i.e., on average between 56 and 64 % of the amounts absorbed during the cycle return to the soil with crop residues (Table 3). This explains, in part, why even in the treatments without micronutrient application, and including those in which a higher level of NPK fertilization was used, the levels of micronutrient concentration in leaves were appropriate or even higher than recommended for this crop (Ambrosano et al., 1997). This provided a satisfactory grain yield (Soratto et al., 2013), i.e., above 1,800 kg ha^{-1}, even in the treatments without NPK fertilization.

CONCLUSIONS

1. Higher levels of NPK fertilization increased micronutrient extraction by the cultivars Pérola and IAC Alvorada, and the treatments with 100 % of the recommended NPK fertilization promoted an average extraction of 167 g B, 58 g Cu, 1,405 g Fe, 1,213 g Mn, and 211 g Zn per hectare.

2. Regardless of the treatment, the period of highest demand for B, Cu, Fe, Mn, and Zn of both cultivars occurred between 42 and 55 DAE, i.e., at pod formation (R_7), showing that in case of micronutrient deficiency, applications must be provided to ensure a higher availability to the plants in this period.

3. The amount of B, Cu, Fe, Mn, and Zn exported depended mainly on the level of NPK fertilization used, with values per hectare ranging from 38 to 90 g of B, 12 to 26 g of Cu, 222 to 568 g of Fe, 234 to 467 g of Mn, and 40 to 96 g of Zn.

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