From Forest Nursery Notes, Summer 2008

155. Micronutrient determination in water extracts of peat incubated with mineral fertilizers. Abreu, C. A. , Abreu, M. F., Bataglia, O. C., Furlani, A. M. C., Furlani, P. R., and Paz-Gonzalez, A. Acta Horticulturae 779:375-383. 2008.

Micronutrient Determination in Water Extracts of Peat Incubated with Mineral Fertilizers

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Keywords: soilless, substrates, boron, copper, iron, manganese, zinc, analysis

Abstract

Substrates for plants are of varied composition and the non-standardized water extraction methods for nutrient chemical analysis have raised difficulties to adequately interpret results and manage substrate fertilization and/or correction. In the United States (USA), the saturation extract procedure is widely adopted, while in Europe, the water extraction using fixed volumes (1:1.5 v/v; 1:2 v/v; 1:5 v/v; 1:10 m/v) is used. This research aimed at evaluating the current water extraction methods for the chemical analysis of peat incubated with conventional (NPK) and slow-release fertilizers (SRF). The treatments were applied as follows: (1) control; (2) lime; (3) lime + NPK + S; (4) lime + NPK + S + micronutrients; (5) lime + slow-release fertilizer (SRF) + S and; (6) lime + SRF + S + micronutrients. Both, NPK and SRF (formula N - P_2O_5 - K_2O = 14–14–14) were applied at a rate of 6 g L^{-1} of substrate. Substrate samples were collected at 20, 60 and 120 days after incubation for the determination of micronutrient concentrations, using the following water extraction methods: 1:1.5 v/v; 1:2 v/v; 1:5 v/v; 1:10 m/v; and saturation extract. The micronutrient concentrations were influenced by fertilization treatments (NPK or SRF with micronutrients), especially B. No effect was observed on Cu, Fe and Zn concentrations. Manganese was the most affected by liming, since significant decrease on Mn concentration was observed with the pH increase. The incubation period had little influence on B and Fe concentrations, but affected Cu, Mn and Zn, which concentrations tended to increase with time. Such effect was more evident in the SRF treatments. On the overall, positive and high correlations were found between the several extraction procedures (correlation coefficients > 0.92) for B, Mn and Zn data. There was no correlation between methods for Cu and Fe. The saturation extract best discriminated the fertilization treatments, followed by the 1:1.5 extract.

INTRODUCTION

The chemical characterization of substrates for plants is necessary for fertilizer monitoring and recommendation in production systems under semi- or protected cultivation. However, there are little information and many controversies about the adequate extraction procedures for substrate nutrient analysis that turned the nutrient management difficult (Abreu et al., 2002b). In the United States (USA), the saturation extract is the adopted procedure (Warncke, 1986), while in Europe, the water extraction using fixed volumes (1:1.5 v/v; 1:2 v/v; 1:5 v/v; 1:10 m/v) (Sonneveld et al., 1974; Sonneveld, 1988; Sonneveld and Elderen, 1994; CEN, 2001) is used. Different laboratory procedures (water volume, shaking, extraction period) have affected the results unequally, what make comparisons difficult and lead to misinterpretations. On the other hand, considering the great variability of substrates in water retention capacity, dilutions higher than 1:6 (m/v) would be expected to minimize the initial substrate moisture effect (Johnson, 1980). In the Netherlands, the low dilution (1:1.5 water extract) has been adopted with previous standardization of substrate initial moisture by submitting samples to a constant pressure of 0.1 kg cm^{-2} (Sonneveld et al., 1974; Sonneveld, 1988). Abreu et al. (2002a) compared the results obtained for the several dilution procedures (saturation

extract - SE, 1:1.5 v/v; 1:2 v/v; 1:5 v/v and 1:10 m/v) applied to samples of *Pinus* bark substrate and observed that dilution strongly affected the analysis precision: in the 1:5 and 1:10 water extracts, no differences in Cu concentrations were found among treatments with and without added Cu; and for some other micronutrients (B, Mn and Zn) the treatment results were significantly different only for the SE and 1:1.5 extract. The authors suggested that more research on this subject might be required.

Thus, in order to give a better support to potted plant production in substrates, this research aimed at evaluating the current water extraction methods for the chemical analysis of peat substrate incubated with conventional (NPK) and slow-release fertilizers (SRF) with and without addition of micronutrients.

MATERIALS AND METHODS

Peat substrate (original peat without mixture, pH_{H2O} 4.1) was incubated with conventional and slow-release fertilizers and treatments were applied as follows: (1) control; (2) lime; (3) lime + NPK + S; (3) lime + NPK + S + micronutrients; (4) lime + slow-release fertilizer (SRF) + S and; (5) lime + SRF + S + micronutrients. Lime was applied as $CaCO_3/MgCO_3$ (Ca:Mg = 4:1) at the rate of 5.0 g L^{-1} to reach pH 5.5. The SRF (formula N - P_2O_5 - K_2O = 14–14–14) presents a releasing period of three to four months and it was applied at a rate of 6 g L^{-1} of substrate. The conventional fertilizer was applied at the same rate (6 g L^{-1} of substrate) using the sources: urea, potassium chloride and triple superphosphate. Sulfur (S) was added as $CaSO_4.2H_2O$ (also source of Ca) at the rate of 40 mg L^{-1} of substrate. All macronutrients were applied in the solid form and thoroughly mixed with the substrate. The micronutrients were applied as follows (mg L^{-1} of substrate): B - 0.6; Cu - 1.5; Zn - 2.1; Mn - 2.1; and Fe - 2.1, using solutions of H_3BO_3, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.H_2O$ and $[Fe(NH_4)_2(SO_4)_2.6H_2O$, respectively. Each substrate treatment was again thoroughly homogenized and transferred to 50 L-plastic containers, where stayed incubated for 120 days, during summer, under greenhouse conditions (average day-time temperature = 35 ±5ºC and night-time temperature = 20 ±2ºC. During the incubation period (120 days), the substrate humidity was maintained close to the "container capacity" through daily pot weighing. The "container capacity" was determined using an empiric method consisting of thoroughly adding water to the substrate until the point that, when lightly hand-squeezed, water starts to flow out between fingers.

After 20, 60 and 120 days of incubation, each substrate treatment (in the 50 L-containers) was again revolved to guarantee homogenization, and then, a 6 L-substrate sample was collected from each container. The 6 L- sample was divided in three (2 L) subsamples and submitted to chemical analysis by the following water extraction methods: 1:1.5 v/v (Sonneveld et al., 1974); 1:2 v/v (Sonneveld et al., 1990); 1:5 v/v (CEN, 2001); 1:10 m/v; and saturation extract (Warncke, 1986), as described below: (1) 1:1.5 v/v water extract (Sonneveld et al., 1974) - this method is currently used in The Netherlands for pH, EC, macro and micronutrients determination in substrates. The procedure consisted as follows: to 200 ml of substrate sample, deionized water was thoroughly added until the point of "substrate capacity" (when lightly hand-squeezed, water starts to flow out between fingers). The sample was then submitted to 10 kPa in a pressure ring; thereafter, 100 ml previously treated sample was added to 150 ml deionized water, shaken during 15 minutes at 220 rpm and filtered through paper filter; (2) 1:2 v/v water extract (Sonneveld et al., 1990) - method used by some Brazilian substrate producing companies for pH and EC evaluation according the following procedure: 100 ml of substrate sample (without previous prepare) is added to 200 ml of deionized water, shaken for 20 minutes at 220 rpm and filtered through paper filter; (3) 1:5 v/v water extract (CEN, 2001) – method adopted by the Comité Européen de Normalization (CEN) - 250 ml of water is added to 50 ml of substrate sample (without previous prepare), shaken for 20 minutes at 220 rpm and filtered through paper filter; (4) 1:10 m/v water extract – method originally used in Germany and adopted by the research group of the "Universidade Federal do Rio Grande do Sul", Brazil, for EC, pH and macronutrient

determinations (this procedure requires humidity standardization, by oven drying the substrate sample at 105°C, or adding water, when sample humidity is different from 50%): 20 g of substrate material with 50% humidity is added to 200 ml of deionized water, shaken during 3 hours and filtered after a 24-hour rest period; and (5) saturation extract (SE) (Warncke, 1986) - distilled water is added to about 400 ml of substrate and stirred with spatula, until the mixture shows a shining surface or until a spatula-made ridge vanishes fast. This amount of water is registered. After one-hour rest, the mixture is again adjusted to saturation and thereafter transferred to a Buchner funnel with paper filter connected to a vacuum flask to which suction is applied to obtain 25 ml extract.

The micronutrients B, Cu, Fe, Mn and Zn were determined by ICP-OES (spectrometer model Jobin Yvon JY50P). The data was submitted to analysis of variance for a completely randomized design with three replications, arranged in a 6x3x5 factorial (fertilization, incubation period and extraction method) and the means were compared by Tukey test (0.05). The extraction efficacy of each method was evaluated by comparison with the considered standard method (saturation extract – SE) through the angular coefficient of the linear regression equation obtained between SE and other method data.

RESULTS AND DISCUSSION

In general, the micronutrient concentration in the water extracts was affected by the applied fertilizer treatment (Table 1). Boron was easily detected in treatments with micronutrient addition (NPK + micro and SRF + micro). This fact was not observed for Cu, Fe and Zn probably due to their strong adsorption to insoluble organic compounds. It is well known the strong association between copper and the organic matter negative charges. Copper forms stable complex molecules with fulvic and humic acids which are rich in carboxylic and phenolic groups (Stevenson, 1991). Iron also tightly binds to organic matter forming compounds with high stability constants, higher than Mn^{2+} does; however, even Fe- and Mn-complexes are less stable than Zn and Cu-complexes (Irving and Williams, 1948).

Among the tested micronutrients, Mn extraction was the most affected by lime application. The control showed 6.31 μmol L^{-1} of Mn (in average), which decreased to 0.29 μmol L^{-1} after liming. The control (original peat material without addition) average pH was 3.97 and the peat+lime average pH was 4.80. Manganese solubility is strongly dependent on pH: for each pH unit increase, there is a 100-fold decrease in ion activity (Lindsay, 1991). It also should be noticed that NPK and SRF applied as fertilizers presented Mn as contaminant, once these treatments showed higher manganese concentration than the control and lime treatments.

Sampling period had little effect upon B and Fe determination. However, Cu, Mn and Zn showed increasing concentrations as incubation period was extended (Table 1). This effect was much more evident in treatments with SRF, irrespectively of micronutrient addition what reinforces the evidence that the used SRF contained Cu, Mn and Zn as contaminants in its composition, which was made available with time. Zn concentration in SRF treatment after 20 and 120 days of incubation was 0.64 and 6.25 μmol L^{-1}, respectively; and, when the micronutrient was added, these values rose to 0.70 and 7.77 μmol L^{-1} (Table 1).

Considering the average micronutrient concentrations over the three sampling periods, it was observed the 1:10 m/v water extraction method (adopted in Germany) was not effective in differentiating the fertilization treatments (Table 2). Similar results were observed in the 1:5 v/v water extracts: no differences were found among treatments for B, Cu, Mn and Zn concentrations. The SE was the method that best discriminated treatments (Table 2). This is an overwhelming issue, since substrate fertilization is a largely used practice in protected cultivation and the result of substrate analysis is supposed to express nutrient availability and it is important for nutrient management purposes.

It was observed that copper concentration was always low in all extracts, despite the method used (Table 2). Under the analytical stand point of view this is an important issue, once low concentrations in the extract, close to the equipment detection limits, may

lead to error and misinterpretation, thence the use of these solutions might not be adequate to evaluate copper availability and should be carefully undertaken.

On the overall, there was high correlation (significant correlation coefficients > 0.92) between micronutrients B, Mn, Zn concentrations in the several water extraction methods, except for Zn in the saturation extract (Table 3). The high correlations mean the water extracts presented similar behavior for these elements. Probably, the weak B and Mn binding to the organic matter, compared to the strong Cu and Fe binding, might have contributed to the higher B and Mn availability in the solution, and consequently, be more easily extractable in water. No correlations between methods were found for Cu and Fe concentrations, except for one negative correlation between Fe in the SE and Fe in the 1:10 extract (significant correlation coefficient, Table 3), what indicated opposite results for these two water extracts.

The angular coefficient of a linear regression equation obtained from data of two methods allows interpreting their extraction efficacy: an angular coefficient equal to 1 means both methods present the same extraction efficacy. Fig. 1 shows the relationships between the micronutrient concentrations obtained by SE (X axis) and the ones obtained by the other methods (Y axis). The angular coefficients of the linear regression equations indicated the following methods ranked according to their decreasing extraction efficacies, compared to the SE method: SE > 1:1.5 > 1:2 > 1:5 ~ 1:10 for B and Mn; SE > 1:1.5 > 1:2 for Cu; and SE > 1:10 for Fe. No relationship was found between SE and the other water extracts for Zn concentrations (Fig. 1).

These results are in accordance with those of Abreu et al. (2002b) that noticed the lesser diluted the extract, the closer were the results with those obtained with the SE. However, it should also be noticed that micronutrient concentrations were not linearly affected by the applied dilution. As for example, it was observed that 5.0 μmol L^{-1} of Mn in the SE, corresponded to 1.82 (1:1.5); 1.28 (1:2); 0.46 (1:5) and 1.53 (1:10) μmol L^{-1}, that is, there was not a proportional relation among the water extracts (Fig. 1), probably because of other factors, such as shaking period and initial substrate humidity.

CONCLUSIONS

Boron and manganese were easily detected in water extracts of original peat (without mixture) with added micronutrients, what was not verified with copper, iron and zinc. Manganese concentrations in the extracts were pH dependent, since they decreased drastically with liming. Copper, manganese and zinc concentrations increased with the period of incubation, up to 120 days. The saturation extract and the 1:1.5 v/v water extract were the only procedures that allowed discriminating micronutrient concentrations among treatments with and without added fertilizers.

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Tables

Table 1. Effect of incubation period on B, Cu, Fe, Mn and Zn concentrations in water extracts of peat substrate incubated with fertilizers (average over water extracts).

Treatments	Incubation period (days)			
	20	60	120	Means[2]
	Boron (B), µmol L^{-1}			
Control	0.91b A	1.00 dA	1.52 bA	1.15 d
Lime	1.09 bA	1.15 cdA	1.38 bA	1.21 cd
NPK	2.24 bA	1.01 dA	1.30 bA	1.51 cd
NPK + micro	6.38 aA	3.32 bcB	5.97 aA	2.46 c
SRF[1]	1.20 bB	4.75 bA	1.44 bB	5.22 b
SRF + micro	7.89 aA	8.81 aA	7.90 aA	8.20 a
Means[2]	3.28 A	3.34 A	3.25 A	
	Copper (Cu), µmol L^{-1}			
Control	0.14 aA	0.21 bA	0.17 cA	0.17 c
Lime	0.17 aA	0.20 bA	0.17 ca	0.18 c
NPK	0.23 aA	0.22 bA	0.22 cA	0.22 bc
NPK + micro	0.25 aA	0.37 aA	0.27 bcA	0.30 a
SRF	0.22 aB	0.34 aA	0.38 aA	0.31 a
SRF + micro	0.19 aB	0.28 abAB	0.35 abA	0.27 ab
Means	0.20 B	0.27 A	0.26 A	0.24
	Iron (Fe), µmol L^{-1}			
Control	0.99 aA	0.80 bA	0.70 bA	0.83 b
Lime	0.94 aB	1.40 aA	1.29 aA	1.11 a
NPK	1.11 aA	1.02 bA	1.34 aA	1.16 a
NPK + micro	1.04 aA	1.35 bA	1.32 aA	1.24 a
SRF	0.96 a A	1.06 bA	1.28 aA	1.10 ab
SRF + micro	0.84 aA	1.02 bA	1.29 aA	1.05 a
Means	0.98 B	1.05 A	1.20 A	
	Manganese (Mn), µmol L^{-1}			
Control	2.91 abA	7.89 bcA	8.14 cA	6.31 c
Lime	0.35 bA	0.26 cA	0.26 cA	0.29 d
NPK	8.14 abB	10.55 abB	21.49 bA	13.40 b
NPK + micro	10.76 aB	18.81 aA	21.23 bA	16.66 ab
SRF	4.07 abC	17.09 aB	28.83 bA	16.93 ab
SRF + micro	5.06 abB	10.38 abB	46.08 a A	20.51 a
Means	5.21 C	10.83 B	21.00 A	
	Zinc (Zn), µmol L^{-1}			
Control	0.74 aA	1.14 aA	0.96 bA	0.95 b
Lime	0.35 aA	0.60 aA	0.56 bA	0.50 b
NPK	1.13 aA	0.76 aA	1.03 bA	0.98 b
NPK + micro	0.87 aa	0.92 aA	1.18 bA	0.99 b
SRF	0.64 aB	1.10 aB	6.25 aA	2.66 a
SRF + micro	0.70 aB	1.61 aB	7.77 aA	3.36 a
Means	0.74 B	1.02 B	2.96 A	1.57

[1] SRF = slow release fertilizer; [2] Means followed by the same letters, small in the columns and capital in the lines do not differ by Tukey test (0.05).

Table 2. Effect
concentration
(average over

Treatments
Control
Lime
NPK
NPK+micro
SRF [2]
SRF+micro
Means [3]
Control
Lime
NPK
NPK+micro
SRF
SRF+micro
Means
Control
Lime
NPK
NPK+micro
SRF [3]
SRF+micro
Means [2]
Control
Lime
NPK
NPK+micro
SRF
SRF+micro
Means
Control
Lime
NPK
NPK+micro
SRF
SRF+micro
Means

[1] SE= saturation extr
the columns and cap

Table 2. Effect of different fertilizer types and additions on B, Cu, Fe, Mn and Zn concentrations in water extracts (of peat substrate) obtained by several methods (average over incubation periods).

Treatments	SE [1]	1:1.5	1:2	1:10	1:5	Means[3]
			Boron (B), µmol L^{-1}			
Control	1.11 dA	1.60 bA	1.15 bA	0.73 aA	1.15 aA	1.15 d
Lime	1.50 dA	1.57 bA	1.14 bA	0.85 aA	0.97 aA	1.21 cd
NPK	2.78 dA	1.55 bA	0.98 bA	0.69 aA	1.57 aA	1.51 cd
NPK+micro	11.88 ba	6.24 aB	3.58 abBC	2.02 aC	2.39 aC	2.46 c
SRF [2]	7.73 cA	2.06 bB	0.70 bB	0.82 aB	1.01 ab	5.22 b
SRF+micro	20.82 aA	7.95 aB	5.48 aBC	3.36 aC	3.38 aC	8.20 a
Means [3]	7.64 A	3.49 B	2.17 C	1.75 C	1.41 C	3.29
			Copper (Cu), µmol L^{-1}			
Control	0.27 cA	0.24 bAB	0.10 bAB	0.15 aB	0.11 aB	0.17 c
Lime	0.23 cAB	0.25 bA	0.14 abAB	0.17 aAB	0.11 aB	0.18c
NPK	0.37 bcA	0.36 abA	0.18 abB	0.11 aB	0.10 aB	0.22 bc
NPK+micro	0.51 aA	0.43 aA	0.23 abB	0.18 ab	0.12 aB	0.30 a
SRF	0.52 aA	0.40 aA	0.24 aB	0.23 aB	0.18 aB	0.31 a
SRF+micro	0.48 abA	0.41 aA	0.26 aB	0.10 aC	0.12 aC	0.27ab
Means	0.40 A	0.35 A	0.19 B	0.16BC	0.12 C	0.24
			Iron (Fe), µmol L^{-1}			
Control	1.20 abA	0.68 aA	0.55 aA	0.95 aA	0.77ab A	0.83 a
Lime	0.89 bA	0.81 aA	1.09 aA	1.14 aA	1.64 aA	1.11 a
NPK	2.06 aA	1.04 aAB	1.03 a Ab	0.74 aB	0.91 abB	1.16 a
NPK+micro	2.22 a A	1.31 aAB	1.21 aAb	0.76 aB	0.68 abB	1.24 a
SRF [3]	2.16 aA	1.13 aAB	0.97 aB	0.74 aB	0.51 bB	1.10 a
SRF+micro	1.99 abA	1.04 aAb	0.95 aAB	0.62 aB	0.66 abB	1.05 a
Means [2]	1.75 A	1.00 B	0.97 B	0.82 B	0.86 B	
			Manganese (Mn), µmol L^{-1}			
Control	19.61 cA	5.54 abB	3.54 aB	1.59 aB	1.29 aB	6.31 c
Lime	0.88 bA	0.23 bA	0.15 aA	0.09 aA	0.10 aA	0.29 d
NPK	43.09 bA	10.67abB	7.40 aB	2.98 aB	2.85 aB	13.40 b
NPK+micro	58.94 aA	11.13 abB	8.06 aB	3.33 aB	3.20 aB	16.66 ab
SRF	61.96 aA	9.91 abB	5.98 aB	2.90 aB	2.57 aB	16.93 ab
SRF+micro	67.37 aA	16.62 aB	10.10 aBC	4.38 aC	4.06 aC	20.51 a
Means	41.97 A	9.02 B	5.87 BC	2.54 C	2.34 C	12.35
			Zinc (Zn), µmol L^{-1}			
Control	2.77 bA	0.81 bcAB	0.61 bAB	0.25 aB	0.30 aB	0.95 b
Lime	1.42 bA	0.38 cA	0.28 bA	0.26 aA	0.17 aA	0.50 b
NPK	3.32 abA	0.60 bcB	0.50 bB	0.12 aB	0.33 aB	0.98 b
NPK+micro	2.86 abA	0.87 bcAB	0.61 bAB	0.21 aB	0.40 a	0.99 b
SRF	5.83 aA	3.07 bB	2.24 abB	0.80 aB	1.36 aB	2.66 a
SRF+micro	3.79 abAC	5.68 aA	3.96 aAB	1.38 aC	2.00 aBC	3.36 a
Means	3.33 A	1.90 B	1.37 BC	0.76 C	0.50 C	1.57

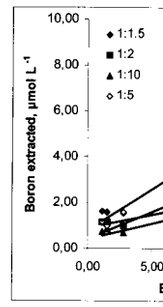
[1] SE= saturation extract; [2] SRF = slow release fertilizer; [3] Means followed by the same letters, small in the columns and capital in the lines do not differ by Tukey test (0.05).

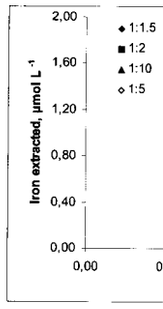
Table 3. Correlation between micronutrient concentrations in the water extracts (of peat substrate) obtained by different methods (average over 20, 60 and 120 days of incubation)[1].

	Methods			
Methods	1:1.5	1:2	1:10	1:5
		Boron		
SE	0.95	0.92	0.96	0.91
1:1.5	1	0.98	0.97	0.96
1:2		1	0.99	0.97
1:10			1	0.96
		Copper		
SE	0.96	0.92	0.23	0.59
1:1.5	1	0.95	0.05	0.41
1:2		1	0.07	0.51
1:10			1	0.76
		Iron		
SE	0.88	0.40	-0.91	-0.77
1:1.5	1	0.70	-0.67	-0.50
1:2		1	-0.14	0.21
1:10				0.83
		Manganese		
SE	0.93	0.92	0.94	0.95
1:1.5	1	0.99	0.99	0.99
1:2		1	0.99	0.99
1:10			1	0.99
		Zinc		
SE	0.57	0.59	0.53	0.67
1:1.5	1	0.99	0.99	0.99
1:2		1	0.98	0.99
1:10			1	0.98

[1] r< 0.92 non-significant at P<0.05.

Figures





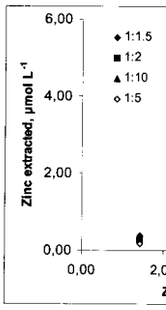


Fig. 1. Relatio
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Figures

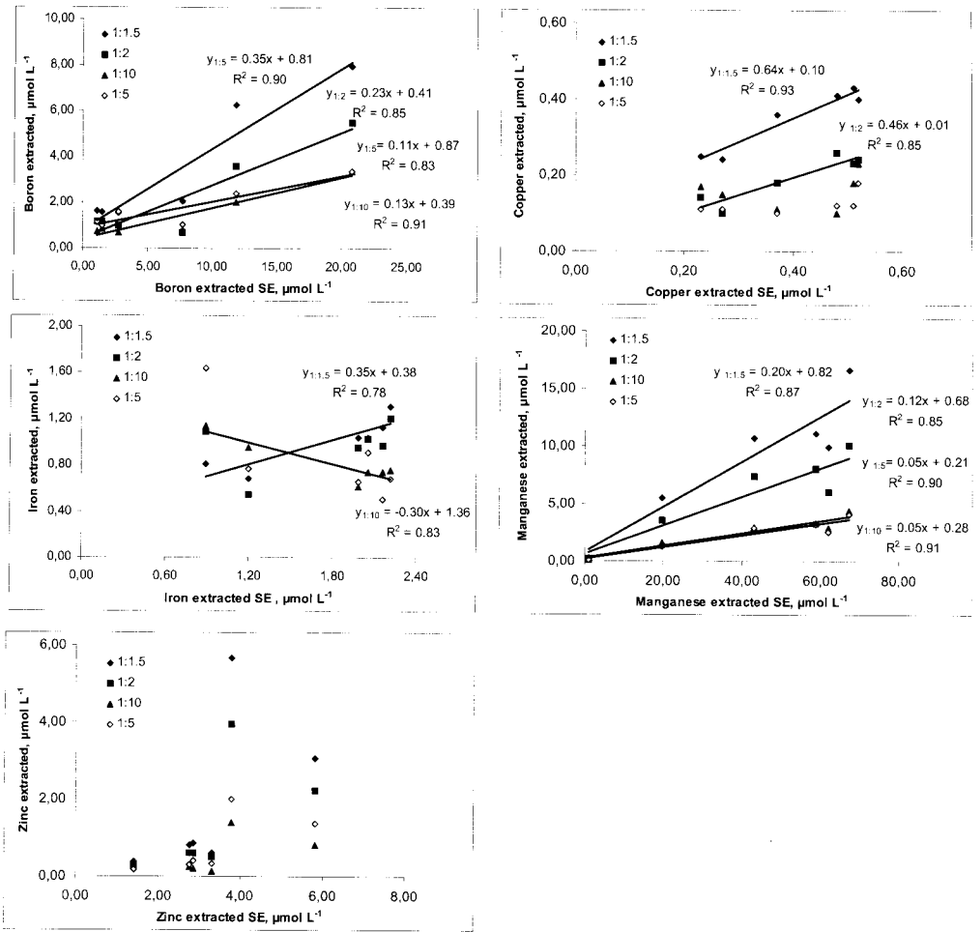

Fig. 1. Relationships between B, Cu, Fe, Mn and Zn concentrations in the saturation extract (SE) and in other water extracts, respectively, obtained by different methods ($R^2 < 0.86$ non-significant at $P < 0.05$).