Determination of Micro Nutrients in Substrates by Water Extraction and Interpretation of the Analytical Data

C. Sonneveld
Tolhuislaan 35
3862 WK Nijkerk
The Netherlands

W. Voogt
Wageningen UR Greenhouse Horticulture
PO Box 20, 2665 ZG Bleiswijk
The Netherlands

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Abstract

In 1974 the 1:1½ volume extract was published (Sonneveld et al., 1974) as a water extraction method for the determination of available nutrient elements and of the salinity status of peaty substrates. The analytical data of this extract were related to the data of the "substrate" solution extracted from the substrates with a moisture condition of -3.2 kPa. The method has been widely used and offers a suitable basis for fertilization of peaty substrates. In the years after publication, the composition and application of substrates has undergone much change and the irrigation methods have also been thoroughly adjusted. The latter was responsible for increased water contents in the substrate during crop growth. Therefore, another study was carried out in which the analytical data of the 1:1½ volume extract was compared with the analytical data of the "substrate" solution, where the "substrate" solution was defined at the moisture condition of -1.0 kPa. There was a good agreement between the results of both studies. However, the regression coefficients for the relationships between the data of the extracts differ, as expected, because of the higher moisture contents on which the substrate solution was defined. In addition to the data of major elements published, in the second study, micro nutrients were determined in the extracts, but not published. Therefore in this paper the relationships between the concentrations of micro nutrient as determined in the 1:1½ extract and in the "substrate" solution are given. The relationships presented support the interpretation of analytical data of micro nutrients by means of water extraction.

INTRODUCTION

In 1974 the 1:1½ volume extraction method was published (Sonneveld et al., 1974) as being a water extraction method for the determination of available nutrient elements and of the salinity status of peaty substrates. The extract was prepared of a standardised volume of substrate mixed with 1½ volume of water. The analytical data of this extract were related with those of the "substrate" solution at a pressure head of -3.2 kPa. The analytical data of the 1:1½ volume extract were closely related with the data of the substrate solution; the correlation coefficients varied between 0.957 and 0.986. The method has been widely used and offers a suitable basis for fertilization management of peaty substrates.

In the years after the introduction of the method, the composition and application of substrates has been changed considerably. Apart from the traditional applications for potted plants and propagation purposes, substrates have become increasingly used for growing vegetables and cut flowers in the production phase, whilst the composition of the substrates were changed, and the irrigation methods were thoroughly adjusted. The last item was responsible for increased water contents in the substrate during crop growth. Thus, the suction of -3.2 kPa on which the comparison with the substrate solution was made was no longer applicable to the situation in practice. Therefore, a second study was carried out in which the analytical data of the 1:1½ volume extract was compared with the analytical data of the "substrate" solution at a pressure head of -1.0 kPa (Sonneveld and Van Elderen, 1994). The correlation coefficient for the relationship between the analytical data of the 1:1½ volume extract and that of the "substrate" solution varied between 0.912 and 0.992. There was a good agreement between the results of the previous and the latter

study. However, the regression coefficients for the relationship between the data of the different extracts differed according to the higher moisture contents on which the substrate solution was based.

In both studies results of major ions were published. In the second study also the micro nutrients were determined in the extracts, but the results were not published. The main reason for this omission was the development of the 1:5 volume extraction methods by CEN (2001) as being a universal method for every type of substrate. The 1:1½ volume extract has some advantages in comparison with the 1:5 volume method and therefore is still widely used. On the contrary it has the drawback that its use is restricted to natural organic mixtures. A problem that arises with the 1:5 method is the shortage of interpretation information. The interpretation given by Sonneveld and Voogt (2001) has a sufficient basis for major elements, but has been sparingly studied for micro nutrients. In this paper some principles of the 1:1½ volume extract will be discussed, with which it is possible to compare different water extraction methods applied with substrates on an equal basis. In this paper the data of the micro nutrients will be presented and used to develop a universal basis for interpretation of these elements.

METHODS AND MATERIALS

Substrate Samples

Fifty samples of substrates were gathered with widely varying chemical and physical characteristics. The composition of the substrates was based on different types of peat and contained generally at least 75% by volume of this material. A number of the mixtures contained materials like pine leaf mould, wood fibre, rice hulls, sand, perlite, vermiculite, volcanic material, sand, clay, composted bark, rock wool fibres and polystyrene granules. Most of the substrates were fertilized according to common practice.

Extraction Methods

The “substrate solutions” were prepared from substrates with moisture contents in agreement with a pressure head of -1.0 kPa. This moisture condition was found to largely coincide with the moisture contents under growing conditions (Wever, 1995). The adjustments to bring the substrates on the required moisture condition were based on measurements on the sand box. The 1:1½ volume extract was prepared with substrate adjusted to the water content at a pressure head of -3.2 kPa. The extract was prepared from 1 volume of substrate and 1½ volumes of water. The 1:1½ volume extraction was carried out with adjustment of the water contents exactly based on measurements of the sandbox method and by visual estimation, as is common for routine laboratories. The volume of the substrate was measured in a ring at a pressure of 10 kPa. For detailed information about the preparation of the extracts reference see Sonneveld and Van Elderen (1994).

Analytical Methods

Water contents at a pressure head of -1 kPa and of - 3.2 kPa were determined by drying at 105°C and expressed in g g^{-1} of dry material. The following elements were determined:

- Fe by flame atomic absorption at λ=248.3 nm
- Mn by flame atomic absorption at λ=279.4 nm
- Zn by flame atomic absorption at λ=324.7 nm
- Cu by flame atomic absorption at λ=324.7 nm
- Mo by heating in graphite oven at λ=313.3 nm
- B spectrophotometrically as azomethine complex at λ=410 nm, with correction on the colour of the extract when necessary

RESULTS

The average and extreme values of the analytical data of the substrate solutions and the 1:1½ extracts are shown in Table 1. The regression equations and correlation coefficients for the relation between the analytical data of the micro nutrients in the substrate solution and in the 1:1½ extract are listed in Table 2. The regression coefficients vary between 0.23 and 0.31, which is somewhat lower than the regression coefficients found for the major elements, where these coefficients varied between 0.34 and 0.39 (Sonneveld and Van Elderen, 1994). For cations like Fe, Mn, Zn and Cu this will be explained by adsorption following the dilution and valence effects, which will be expected with ions of a valence higher than 1. This has been found also for Ca and Mg, though less evidently. An example of the relationships is shown for Mn in Figure 1. B and Mo elements that occur as anions have in relation to the average, relatively high intercepts, which will suppress the regression coefficient. This is also the case with Cu and moreover, the regression coefficient of this element is also affected by the mentioned dilution and valence effect. These effects together resulted in the lowest regression coefficient.

The correlation coefficients for B, Cu and Mo are low in comparison with those found for the other elements. This merely will be explained by the precision of the determinations and not by an inaccurate preparation of the extract. This conclusion is supported by the close correlations found for the analytical data of the major elements and the precise and quick extraction preparation of the substrate solution as well the 1:1½ extract (Sonneveld and Van Elderen, 1994). The poor correlation found with the micro elements mentioned is caused by an insufficient sensitivity of the determination method on the concentrations in the low range, especially by the low concentration in the 1:1½ extract. In this extract many concentrations decreased below the determination limit. This is shown by the data in Table 3, where the correlation coefficients are listed for relationships between the analytical data obtained with the same extraction method, being the precise and quick extract preparation. The determinations with low correlation coefficients for the relationships substrate solution and 1:1½ extract (Table 2), showed also low correlation coefficients for the determinations when the determination were carried out with the same extraction method. This especially is the case for the data of the 1:1½ extracts, which is in agreement with the low concentrations in these extracts. With low concentrations relatively big errors will be expected (Sonneveld, 1979). This, for example, has been found also for the analytical deviations of micro nutrients in substrate solutions of rock wool slabs (Sonneveld and Voorthuizen, 1988), as shown in Table 4. For all determinations, except Cu, the analytical error increases with the concentrations. For Cu no regression was found between concentration and standard deviation and thus, one standard deviation was calculated for all values, which was 0.0952. However, the relative error strongly increases in the low range of concentrations. This is shown in Figure 2, where the absolute and relative errors are compared for the element B. The coefficient of variation increases strongly with values of the determination below a value of 20 and the data in Table 1 informs that for the 1:1½ extract all values are below this limit. The coefficient of variation will be calculated following formula (1).

$$\%cv_x = 100(a + \frac{b}{x}) \qquad (1)$$

where cv_x = coefficient of variation at a concentration of x
a = regression coefficient from the equations presented in Table 4
b = intercept of the equations presented in Table 4
x = determined concentration

For Cu, where no regression was found for the standard deviation, the coefficient of variation will be calculated following formula (2).

$$\%cv = \frac{100 s_a}{x} \qquad (2)$$

where s_a = standard deviation of the analytical data
and the other values are as mentioned under formula (1)

Extended investigations with soil testing showed that for most analytical determinations of major elements a standard deviation could be realised below 5% (Sonneveld, 1979). When this value is claimed as a limit for the present situation for micro nutrients, the analytical methods applied meet this limit for Fe, Mn, Zn and Cu at values above 18, 4 and 16 and 2 respectively. For B the limit of 5% is reached for more or less all values. From this it will be concluded that for the 1:1½ extract more or less all values are too low for the analytical methods used, especially those for B and Cu. For the latter elements even the values in the substrate solution will be estimated as being too low for the analytical methods used. Mo was not yet included in the research of Sonneveld and Voorthuizen (1988). Calculations from the results of the present research offers a standard deviation of 0.12 at an average concentration of 0.2 µmol L^{-1}, which results in a coefficient of variation of 60%. This accentuates the problem for Mo already mentioned for B and Cu.

In the present samples the relative water volume in the substrate at a pressure head of -3.2 kPa was on average 0.52, and ranged overall between 0.43 and 0.62, like shown in Figure 3. Thus, the ratio water:substrate v/v in the 1:1½ suspension on average was 2.02, with a range of 1.93 and 2.12, being the water volume in the substrate together with the 1½ volume water added. With this information it is possible to express the analytical data of the 1:1½ extract also on the substrate volume, when multiplied by 2. The maximum error made by this calculation will be 6%, with exception of substrate Nr 47 which is outside the range mentioned. This sample showed a relative water volume of 0.33 and consisted of 85% white peat and 15% of rock wool fibres. The mineral fibres possibly promote the drainage of the substrate and thus, mixtures containing mineral fibres are less suitable for the 1:1½ extraction method. In this way it is possible to express for more or less all peaty substrates the data of the 1:1½ extract on the substrate volume, as required for the 1:5 v/v method of CEN (2001). Another difference between both methods, beside the ratio water:substrate, is the different bulk density realised with the measurements of the substrate volume, caused by the different pressure applied. However, these bulk densities are closely related as shown in formula (3) (Verhagen, 2007).

$$\rho_{1:5} = 0.851\rho_{1:1.5} + 16.1 \qquad r=0.951 \qquad (3)$$

where $\rho_{1:5}$ = bulk density at the preparation of the 1:5 v/v extract g kg^{-1}
$\rho_{1:1.5}$ = bulk density at the preparation of the 1:1½ v/v extract g kg^{-1}

The bulk densities of the samples under investigation ranged between values of about 150 and 500 g kg^{-1}. Thus, the ratios between the bulk densities for the extreme values varied between 0.96 and 0.88, respectively. The ratio between the average densities was 0.90. Data with which analytical results of the 1:1½ v/v extract can be roughly converted to values for the 1:5 v/v extract by formula (4).

$$x_{1:5} = x_{1:1.5} \times 2 \times 0.90 \qquad (4)$$

where $x_{1:5}$ = analytical result of the 1:5 v/v extract
$x_{1:1.5}$ = analytical result of the 1:1½ v/v extract

DISCUSSION

It is clear from the results presented that water soluble micro nutrients can be expressed either on the substrate solution, or on the volume of the substrate. The first method is related to the sampling of the circulating solution of hydroponics, the sampling

of the substrate solution from rock wool slabs and the development of the 1:1½ extract; and the second method is linked to the development of the 1:5 v/v method of CEN (2001).

The choice which of both methods of expression will be preferred depends on the reaction of the plant. Several studies suggest that plants mainly react with their uptake of micro nutrients in relation to the substrate solution concentration of the element involved (De Kreij et al., 1993; Sonneveld and Voogt, 1975; Sonneveld and De Bes, 1984). This argues for an interpretation based on concentrations in the substrate solution and not for concentrations in the substrate volume. In view of this argument, it is more logical to express the analytical data of micro nutrients on the substrate solution than on the substrate volume. For a direct interpretation on this basis the substrate solution will be sampled, as is done with hydroponics and with rock wool cultivation, or the determinations will be carried out in an extract with a stable dilution in relation to the substrate solution, as with the 1:1½ extract used for peaty substrates. When the analytical data are expressed on the substrate volume, as with the 1:5 v/v, the data will be expressed on the relative volume of water under growing conditions following formula (5) and interpreted as substrate solution.

$$X_{ss} = \frac{x_v}{w_v} \tag{5}$$

where X_{ss} = estimated concentration in the substrate solution under growing conditions (mmol L^{-1})
x_v = the analytical data expressed on the substrate volume (mmol L^{-1})
w_v = the relative water volume under growing conditions

The water volume under growing conditions for peaty substrates is best reflected by the water content at a pressure head of -1 kPa and for mineral substrates by the water content at saturation (Kipp et al., 2000). For very coarse mineral substrates placed in a water layer, the supernatant water at the bottom of the basins will be used for analysis and will be interpreted in the same manner as the substrate solution.

For interpretations of substrate solution concentrations with rock wool substrates, recommendations are already available (IKC, 1994). In Table 5 the average guide values for the nutrient solution of rock wool slabs as used for vegetables and for flower crops are listed (Sonneveld and Straver, 1994; Bloemhard and Van der Lugt, 1995). With the aid of the regression equations given in Table 2 these values can be used to calculate guide values for the 1:1½ extract, the results of which are also listed in Table 5. The guide values presented for the nutrient solutions in the rock wool slabs are average values for crops derived from experience (Sonneveld and Straver, 1994) and the current guide values presented for the 1:1½ extract are derived from IKC (1994) and from Sonneveld and Boertje (1981). The guide values as calculated from the mineral substrates show a good agreement with the current guide values (IKC, 1994), except for the Cu with which about half of the current values are calculated. For Mo insufficient data for the 1:1½ extract are available to make a comparison. The higher current Cu values can be explained by complex formation of this element with soluble organic matter, which occurs in peaty substrates (Verloo, 1980). Such complex formation reduces the availability of Cu to plants in the substrate solution: however the Cu in the complex is extractable and thus included in the determination by AAS.

In Table 6 rough universal guide values are given for micro nutrient concentrations in substrate solution. The data in this table presented for the 1:1½ v/v and 1:5 v/v extractions are just for peaty substrates. The data are summarized from the values presented in Table 5. The values for the 1:5 v/v extract can be roughly calculated with the aid of formula (4), but can be more precisely determined with the factors given for the relationship between analytical data of the 1:1½ extract and the 1:5 v/v extract (Wever et al., 2005). In this way both dilution and valence effects are included. Such effects are well known with soil extraction (Deist and Talibudeen, 1967; Van den Ende, 1991), but occur

also with substrates (Sonneveld and Van Elderen, 1994). The guide values listed in Table 6 are not specified for crops and may be adjusted to the requirements of crops within the limits given. For Cu in peaty substrates at least the high side of the range will be maintained, but for this substrate even these values will be too low as will be discussed later.

In the present study it was found that the concentrations of micro nutrients in the different extracts often decrease to values below the limit where the determinations offer significant results. This was already the case for the data in the 1:1½ extract as used in the present study, but it is likely to occur more readily for the 1:5 v/v extract introduced by CEN, because the concentrations in this extract will be about 50% of those in the 1:1½ extract. Thus, the development of analytical methods sufficiently suited to the purpose is a first requirement. To this purpose beside the AAS technique the use of ICP.MS technique will be considered. In a personal communication with Ing P.R. Nobels (2007) of Wageningen University, it was concluded that with this technique the determinations of Fe, Mn, Zn, B, Cu and Mo are significant from levels of 0.095, 0.0008, 0.070, 0.063, 0.0013 and 0.015 µmol L^{-1}, respectively. However, for this determination the total salt concentration in the extracts should be below <0.02%, which is comparable with an EC value of 0.3 dS m^{-1}. The EC values of the different extracts roughly can rise to 4, 2 and 1 dS m^{-1} for substrate solutions, 1:1½ extracts and 1:5 v/v extracts, respectively. Thus, for the determinations in the extracts dilutions of 15, 7 and 3, of the respective extracts can be necessary. The lowest significant determination levels increase with the dilution factor. Comparison with the guide values listed in Table 6 leads to the conclusion that the technique at such will offer suitable results, despite the dilutions. Possible high analytical errors may occur through possible adsorption and contamination with elements of interest in the materials used with sampling, storage and handling in the laboratory.

The effect of the pH on the uptake of micro nutrients is generally substantial (Lucas and Davies, 1961; Peterson, 1982), but roughly incorporated in the extraction with water. This is shown for example with the availability and uptake of Mn by gerbera grown in rock wool with different pH regimes (Sonneveld and Voogt, 1997). However, such relations can be disturbed by complex formation of micronutrients with organic compounds. It is possible that micro nutrients are extracted and analysed as water soluble, but that they are not available for uptake, because of too strong a binding on an organic compound, as has been found for Zn with the DTPA compound at pH values higher than 6.5 (Sonneveld and Voogt, 2001). Comparable effects will occur with Cu in surroundings with natural humus substances, as mentioned before. Under such conditions even a decreased concentration in the plant tissue can occur with an increased concentration in the root environment, as shown for Zn in Figure 4. This can be explained by accumulation of non-plant absorbed Zn. The element in such cases is bound on an organic complex and not absorbed by plants and accumulates in the root environment. This may be especially evident in systems with reuse of the drainage water. Such anomalous results up till now only have been found for Zn and Cu in nutrient solution with DTPA as chelating agent and for Cu in peaty substrates. In view of the complex formation with chelates the interpretation system is valid under condition of a pH between 5 and 6. The effect of the pH on the complex formation of Cu with soluble organic matter requires further study.

Some aspects of micro nutrient applications in substrates obviously need to be studied further. Special attention is necessary for Cu with respect to the complex formation with natural organic compounds. Boertje (1982) concluded that the Cu concentration in the 1:1½ extract for tomato grown in peat substrate will be 1-2 µmol L^{-1}, which is comparable with 3.5-7.0 µmol L^{-1} in the substrate solution, which is substantially higher than the values given in Table 6. The data of De Kreij et al. (1993) indicates that the required concentration in the substrate solution for cucumber grown in peaty substrates will be 10-15 µmol L^{-1}. Verhagen (1992) concluded, on basis of an experiment with chrysanthemum in peat substrate, that the Cu concentration in the circulating solution will be 1-2 µmol L^{-1}. Great differences in the recommendations occur and as noted above need further study. Special attention should be given on the specific

determination of Cu ions in the extracts. Donnan membrane filtration is a suitable option to this purpose. Removal of the organic matter from the extracts by precipitation and filtration is also an option, but has restrictions (Nobels, 2007).

The application of Mo also needs further study. In particular, the required application of this element is insufficiently studied for crops in full production. The range between deficiency and toxicity is very wide and covers in crops a factor of 10^4 (Marschner, 1995) and therefore, a concentration of 0.5 µmol L^{-1} is applied as a standard. Marschner mentioned that critical concentrations for Mo vary between 1 and 10 µmol kg^{-1} dry matter. Thus, in substrates a heavy overdosing of Mo is not uncommon, possibly by a factor of 50-100. The desirability of such an exuberant dosing might be considered not only from environmental viewpoint, but also from the viewpoint of human health.

Finally, the role of Ni as an essential nutrient has been debated for many years. The concentration required in plant tissues is of the same order as Mo (Marschner, 1995). Up till now little attention is paid to the requirements for Ni in substrate grown crops. In a study with tomatoes in acid washed volcanic gravel slight positive effects on the growth were noticed with the application of 85 µmol L^{-1} Ni in the nutrient solution (Balaguer et al., 1998). However, the additions of Ni in this experiment were principally focussed on toxic effects.

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Tables

Table 1. Average and extreme values of the analytical data as determined in both extracts compared. Data are expressed as μmol L^{-1} of the extract.

Determination	Substrate solution		1:1½ extract	
	Mean	Range	Mean	Range
Iron	15.54	1.5-41.9	5.72	0.5-14.2
Manganese	9.98	0.4-47.4	2.92	0.2-11.7
Zinc	6.36	0.5-21.1	2.10	0.2-6.6
Boron	17.90	4-46	7.74	1-19
Copper	0.83	0.11-2.17	0.33	0.00-0.93
Molybdenum	0.14	0.00-0.78	0.09	0.00-0.44

Table 2. Regression equations and correlation coefficients for the relationship between the analytical data of the substrate solution (x) and the 1:1½ extract (y).

Determination	Regression equation	r
Iron	y=0.27x + 1.51	0.84
Manganese	y=0.27x + 0.26	0.97
Zinc	y=0.31x + 0.16	0.98
Boron	y=0.31x + 2.26	0.66
Copper	y=0.23x + 0.13	0.57
Molybdenum	y=0.28x + 0.05	0.47

Table 3. Correlation coefficients for the analytical data of same extracts of the substrate solution and the 1:1½ extract, being the precise and quick extract preparation.

Determination	Substrate solution	1:1½ extract
Fe	0.98	0.83
Mn	0.99	0.91
Zn	0.98	0.94
B	0.71	0.28
Cu	0.78	0.29
Mo	0.65	nd

Table 4. Regression equations and correlation coefficients for the relationships between the concentrations in the extract (x) and the standard deviations brought by the performance of the determination on the laboratory (s_a). Data of Sonneveld and Voorthuizen (1988).

Determination	Regression equation	r	Range µmol L^{-1}
Fe	s_a=0.019x + 0.544	0.80	8-50
Mn	s_a=0.026x + 0.103	0.83	2-16
Zn	s_a=0.015x + 0.565	0.69	3-25
B	s_a=0.049x + 0.339	0.90	30-100
Cu	s_a=-0.004x + 0.099	-0.14	0.5-2.0

Table 5. Guide values for analytical data of the nutrient solution of rock wool slabs (Sonneveld and Straver, 1994; Bloemhard and Van der Lugt, 1995) and calculated guide values for the 1:1½ extract in comparison with current guide values (IKC, 1994; Sonneveld and Boertje, 1981).

Average guide values	Fe	Mn	Zn	B	Cu	Mo
Nutrient solution in rock wool slabs (substrate solution)						
Vegetables	17.5	6.5	7.0	60	0.9	0.5
Flowers	28.0	3.0	4.5	40	1.0	0.5
Calculated for 1:1½ extract						
Vegetables	6.2	2.0	2.3	20.8	0.38	0.2
Flowers	9.1	1.1	1.6	14.7	0.36	0.2
Current guide values 1:1½ extract						
Potted plants	8	2	2	15	0.7	-.-
Vegetables and flowers (average)	9.5	1.8	3.1	21	0.7	-.-

Table 6. Rough guide values for micro nutrients determined with water extraction. The data of the substrate solution can be universally applied, while those for the 1:1½ v/v and 1:5 v/v extraction only are suitable for peaty substrates.

Extraction method	Expressed	Fe	Mn	Zn	B	Cu	Mo
Substrate solution[1]	µmol L^{-1} extract	15-30	3-7	5-8	40-60	1-3	0.5
1:1½ v/v water extract[2]	µmol L^{-1} extract	6-10	1-2	2-3	15-20	0.4-0.7	0.2
1:5 v/v water extract[2]	µmol L^{-1} substrate	12-20	1.5-3.0	4-6	25-35	0.8-1.4	-.-
1:5 v/v water extract[2]	mg L^{-1} substrate	0.68-1.12	0.08-0.16	0.25-0.40	0.28-0.37	0.05-0.09	-.-

[1]For universal application; [2] Just for peaty substrates.

Figures

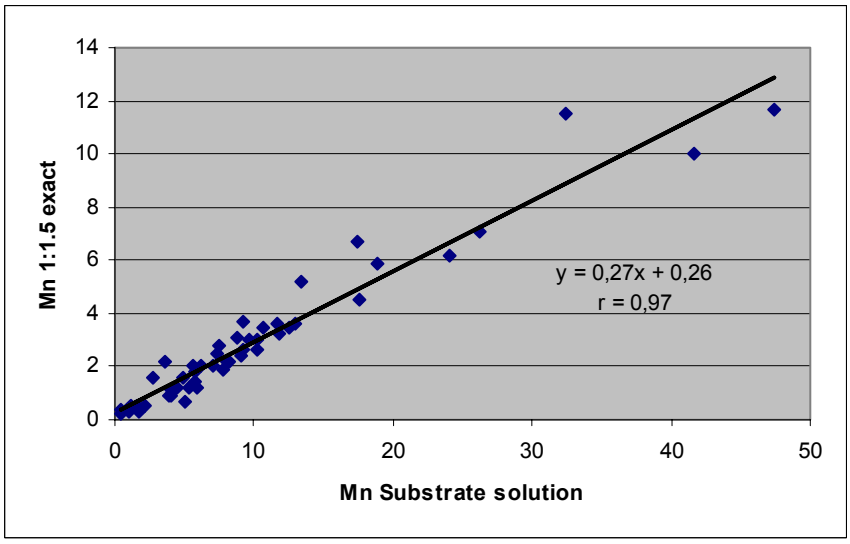

Fig. 1. The relationship between the Mn concentrations in the substrate solutions (x) and in the 1:1½ extracts (y). The concentrations are expressed as µmol L^{-1} extract.

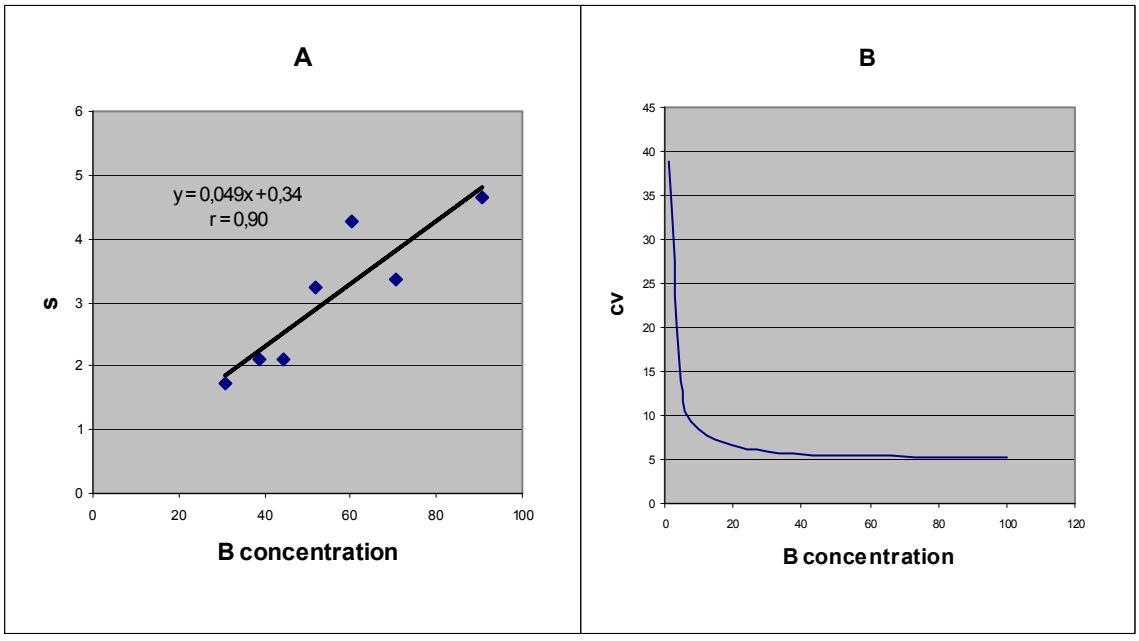

Fig. 2. The relationship between the concentration of B in rock wool solutions (µmol L^{-1}) and the absolute (s) in Figure A and relative standard deviation (cv) in Figure B caused by the determination on the laboratory.

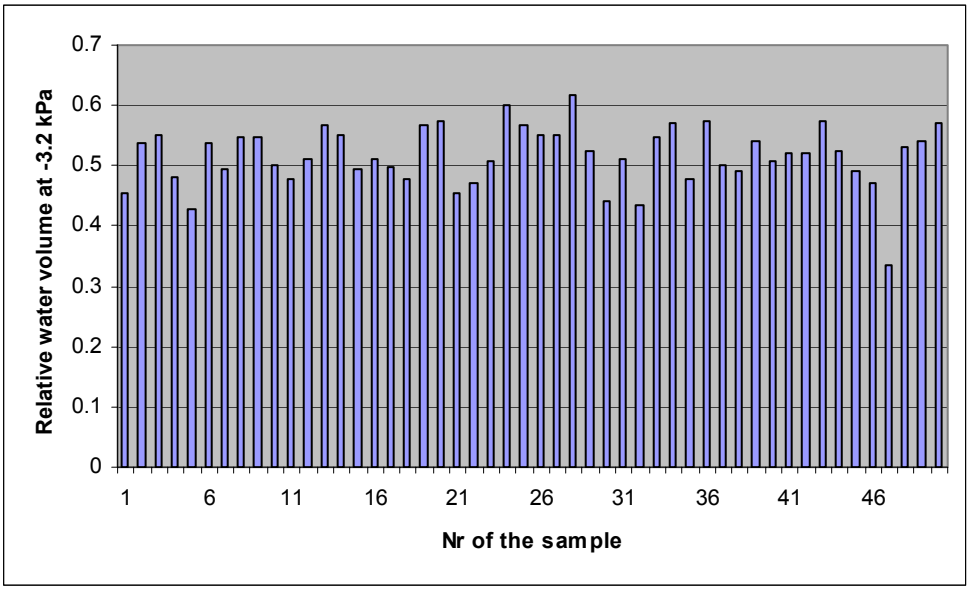

Fig. 3. The relative water volume of the substrates used in the present research at a pressure head of -3.2 kPa.

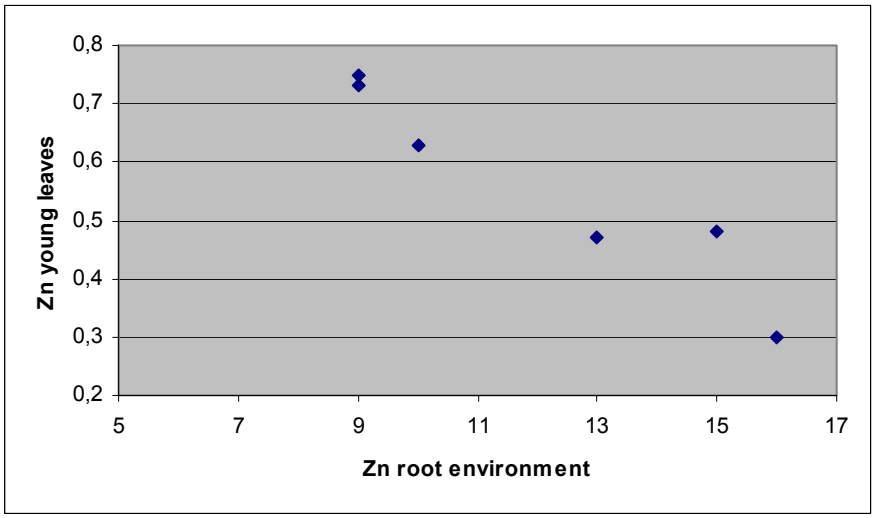

Fig. 4. A negative relationship between the Zn concentrations of the nutrient solution in rock wool slabs (µmol L^{-1}) and the Zn concentrations of young rose leaves (mmol kg^{-1} dry matter). For explanation see text. Data of Sonneveld and Voogt (2001).