# Influence of elemental sulphur and nitrogen fertilisation on the concentration of essential micro-nutrients and heavy metals in *Tropaeolum majus* L.

**Silvia Haneklaus[1], Elke Bloem[1], Sawsan Hayfa[2] und Ewald Schnug[1]**

[1]Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Centre (FAL), Bundesallee 50, D-38116 Braunschweig
[2]Tishreen University, P.O. Box 2250, Lattakia, Syria

**Summary**

Elemental S fertilisation increased the total S and glucotropaeolin content of *Tropaeolum majus* L. leaves from on average 4.8 to 9.8 mg $g^{-1}$ (d.w.), and 22.0 to 37.9 µmol $g^{-1}$ (d.w.), respectively on a sandy loam soil. Similar results were found on a loam soil. N fertilisation caused a significant reduction of the glucotropaeolin content. Leaves showed about three times higher glucotropaeolin contents than stems. Elemental S fertilisation significantly increased the Mn and Cu content of leaves on both soils. Due to an antagonism with S the Mo, B, Se and As content of *T. majus* leaves was significantly reduced, but this effect was not consistent on both sites. On the loam soil, where the actual soil pH was distinctly below the recommended value, the micro-nutrient content and the heavy metal concentrations were distinctly higher than on the loamy sand soil, which had an optimum soil reaction. The Cd concentrations of *T. majus* leaves exceeded the upper recommended value for phytopharmaceuticals of 0.2 µg $g^{-1}$ (d.w.) on the loam soil and the Cd uptake of *T. majus* leaves was significantly increased by N applications on both soils. The lime requirement and plant available concentration of micro-nutrients and heavy metals in soils should be determined routinely in order to select appropriate cultivation sites and to adapt the fertiliser management to site-specific conditions.

**Key words:** *elemental sulphur, glucotropaeolin, heavy metals, soil acidifying fertilisation, sulphur*

**Zusammenfassung**

Die Düngung mit Elementarschwefel erhöhte auf einem lehmig sandigem Standort den Gesamtschwefelgehalt der Kapuzinerkresse (*Tropaeolum majus* L.) von im Mittel 4.8 auf 9.8 mg $g^{-1}$ (TM) und den Glucotropaeolingehalt von 22.0 auf 37.9 µmol $g^{-1}$ (TM). Vergleichbare Ergebnisse wurden auf einem Lehmboden gefunden. Die Zufuhr von N führte zu einer signifikanten Abnahme der Glucotropaeolingehalte. Die Glucotropaeolingehalte in den Blättern von *T. majus* waren ungefähr drei Mal höher als die in den Stengeln. Die Zufuhr von Elementarschwefel erhöhte auf beiden Standorten den Mn- und Cu-Gehalt in Blättern, wobei diese Änderungen signifikant waren. Aufgrund antagonistischer Wirkungen mit S kam es zu einer signifikanten Abnahme der Mo-, B-, Se- und As-Gehalte in den Blättern von *T. majus*, wobei dieser Effekt allerdings nicht konsistent auf beiden Versuchsstandorten zu beobachten war. Der aktuelle pH-Wert lag auf dem Lehmboden weit unterhalb des Sollwertes. Hier wurden dann auch im Vergleich zum sandigen Lehmboden, der eine optimale Bodenreaktion aufwies, für alle Mikronährstoffe und Schwermetalle die deutlich höheren Gehalte gefunden. Der Cd-Gehalt in den Blättern von *T. majus* lag auf dem Lehmboden deutlich über dem empfohlenen Höchstwert von 0.2 µg $g^{-1}$ (TM). Die N-Düngung führte auf beiden Standorten zu einem signifikanten Anstieg der Cd-Gehalte in den Blättern von *T. majus*. Bei der Auswahl geeigneter Standorte für den Anbau von *T. majus* sollte in jedem Fall der Kalkbedarf sowie die Gehalte an pflanzenverfügbaren Mikronährstoffen und Schwermetallen prophylaktisch bestimmt werden, um Düngungspraktiken den standortspezifischen Bedingungen anzupassen.

**Schlüsselworte:** *bodenversauernde Düngung, Elementarschwefel, Glucotropaeolin, Schwefel, Schwermetalle*

## Introduction

Phytopharmaceuticals are old, and nowadays rediscovered remedies for many medical disorders. The knowledge of the action of medicinal plants goes back to the $10^{th}$ century, when *Hildegard von Bingen* (1098-1183) described the curative effect of 213 trees and plants. The increasing interest in herbal medicine is supposedly associated with side-effects of conventional chemical substances, increasing resistance against antibiotics, carry-over of chemicals such as antibiotics into the food chain and increasing costs for health care.

The method which is used for producing a drug mainly depends on characteristics of the active agent and the form in which the drug is used. Direct use of the plant material for producing tablets without prior extraction has the following advantages: it reduces production costs and avoids losses of intact active agents during extraction (Keller, 1991). Sulphur (S) fertilisation proved to have a strong and significant influence on the formation of S containing secondary metabolites in vegetables and agricultural crops (Schnug et al., 1995; Hoppe et al., 1996; Schnug, 1997; Haneklaus et al., 1999). S fertilisation is also an important measure to increase the glucotropaeolin content of *Tropaeolum majus* (*T. majus*). Under optimum climatic conditions as to be found for instance in

Egypt the concentration of the main bio-active compound is regularly so high that the plant material may be directly used for preparing a phytopharmaceutical (Bloem et al., 2001a, 2001b, 2002). Special attention to plant quality needs to be paid when a fertiliser is not only supplying the nutrient S, but also shows relevant side-effects as for instance elemental S does. Elemental S is approved as a fertiliser in the German decree on fertilisers (Anon, 2003), which is better known for its fungicidal effect and has been widely used for this purpose in agricultural production since the end of the nineteenth century (Hoy, 1987). In the guidelines for the cultivation of medicinal plants it is recommended to "encourage natural pest-control mechanism" and to "limit the use of pesticides to the minimum effective level" (WHO, 2003). Thus, elemental S will not only improve the quality of the plant product, but may also contribute to the natural resistance of *T. majus* against fungal diseases. Besides this, elemental S acidifies the soil and thus increases the availability of essential micro-nutrients. The following principal chemical reaction takes place in the soil:

$$2\,S^0 + 2\,H_2O + 3O_2 \xrightarrow{\textit{Thiobacillus ssp.}} 2\,H_2SO_4$$

This acidifying effect can be beneficial, particularly on light soils, which have a too high soil pH as it improves the mobility and uptake of essential heavy metals such as manganese (Mn) and zinc (Zn). But it may also increase the availability of undesired contaminants such as cadmium (Cd), lead (Pb) and nickel (Ni). And a higher uptake of Ni for instance proved to change the composition of relevant secondary compounds in the herbal plant St. John's wort (Murch et al., 2003). Another factor that influenced the concentration of hypericin and pseudohypericin in leaves of St. John's wort proved to be the nitrogen (N) supply (Briskin et al., 2000). With respect to micro-nutrients, acidifying products will promote their uptake (Schnug, 1982). In comparison, the growth promoting effect of N may yield a reduced concentration of minerals.

It was the aim of the presented investigations to determine the influence of elemental S and N applications on the heavy metal concentration in vegetative tissues of *T. majus* in relation to soil characteristics.

**Materials and Methods**

In 2002, two field experiments with *T. majus* were conducted on a loamy sand soil in Braunschweig (E 10° 27′, N 52° 18′ E) and a loam soil in Hessisch-Oldendorff (E 9° 16′, N 52° 10′). According to the German standards, the actual soil pH was in the optimum range on the loamy sand with a mean value of 5.5, while on the loam the soil pH was distinctly below the recommended value of 5.9 with a mean value of 5.2.

A commercially available seed mixture "Niedrige Mischung" of *T. majus* was used. In each seed hole five seeds were placed. The intra and inter row distance was on each site 60 cm. Seeds were sown on 22 May 2002.

S and N fertilisation was split into two equal rates of 0, 25 and 50 kg $ha^{-1}$ with the first dose being applied before sowing; the second rate was fertilised 4 weeks after sowing. S was soil-applied as wettable elemental S (Kumulus®) and N as nitrochalk. Each treatment had four replicates and plots were arranged in a completely randomised block design (Schuster & Lochow, 1979).

Leaf and stem material was collected at the start of flower setting on August 16 and 20, 2002 in Braunschweig and Hessisch-Oldendorff, respectively. The plant material was immediately shock-frozen in liquid N and then freeze-dried. The glucotropaeolin content was determined by HPLC according to the EU method L170/28 for desulphoglucosinolates (Anon, 1990) with minor modifications (Bloem et al., 2001b).

For the determination of N, S and heavy metals the plant material was dried in a ventilated oven at 60° C until constancy of weight. Then the material was fine-ground to a particle size of < 0.12 mm employing an ultra-centrifugal mill (Retsch ZM1). The total N content (d.w.) was determined by using the *Kjeldahl* method. The total S content (d.w.) was determined by X-ray fluorescence spectroscopy according to Schnug & Haneklaus (1999). For the determination of the heavy metal content (d.w.) 0.5g plant material was digested with 4ml $HNO_3$ + 1ml $H_2O_2$ in a microwave (CEM/Mars) at 600 Watt for in total 27 minutes. The temperature was raised to 120° C within five minutes, was kept for another two minutes, and then was further raised to 200° C within five minutes and kept at this temperature for another 15 minutes. Then the samples were allowed to cool down for 30 minutes. Afterwards the digest was filled up to 50ml. The iron (Fe), Mn, Zn, copper (Cu) and boron (B) concentration was determined by ICP-OES; molybdenum (Mo), Cd, Pb, chromium (Cr), cobalt (Co), Ni, arsenic (As), selenium (Se) and titanium (Ti) were analysed by ICP-MS.

For data processing the ANOVA procedure was employed; the Tukey-Kramer test of the Cohort software, version 6.0 was used for the comparison between means (Simons, 1995).

**Results and discussion**

*T. majus* is used in combination products as a herbal medicine against urinary tract infections because of its anti-microbial activity. Glucotropaeolin is the characteristic, aromatic glucosinolate found in *T. majus*, which has been indicated in the treatment of scurvy, bronchitis, cystitis, pyelitis and as a general tonic and stimulant (Cartheuser, 1765; Cazin, 1868; Dragendorf, 1976). In the pharmaceutical industry it has long been recognised for its antimicrobial action against urethral infections (Braun, 1981; Dannenberg et al., 1956). The active component is benzylisothiocyanate, which is released after enzymatic cleavage of glucotropaeolin by myrosinase. Benzylisothiocyanate derived from *T. majus* may also have the ability to induce the synthesis of protective enzymes which reduce the effects of chemical carcinogens (Fahey et al., 1997) and this gives it a possible role in cancer chemoprevention (Patten & DeLong, 1999). A consistently high glucotropaeolin content in *T. majus* would enable the preparation of a mono drug directly from the plant material so that no extraction procedure would be required. The natural glucotropaeolin content of *T. majus* is usually, however, not high enough to accomplish this target.

So far extracts of *T. majus* have been used in combination products, but a mono drug from the original plant material would be advantageous as it reduces production costs and avoids losses of intact glucosinolate during extraction (Keller, 1991). The problem using *T. majus* in this form is that a minimum content of glucotropaeolin is required in order to provide the prescribed daily intake of glucotropaeolin by an acceptable number of pills (Bloem et al., 2001a). The strongest exogenous factor influencing the glucosinolate content in both, vegetative and generative tissues, proved to be the S supply (Schnug, 1987; Walker & Booth, 1994; Bloem et al., 2001a and 2001b). From all commercially available S fertiliser products only elemental S has a proven fungicidal effect (Hoy, 1987). This makes it a favourable product for the cultivation of medicinal plants such as *T. majus* as it enhances the glucotropaeolin content and it possibly promotes the natural resistance against certain pathogens (Fig. 1). Fungal pathogens infecting *T. majus* are for instance *Colletotrichum* spp. (Kriz, 1998), *Verticillium dahliae* (Anon, 2004) and *Plasmodiophora brassicae* (Ludwig-Müller et al., 1999), but so far no information is available about the possible health promoting effect of elemental S against these or other diseases. The application of elemental S will reduce the soil pH, which may be relevant with view to infections by soil-borne pathogens, for instance *Streptomyces scabies* infestations of potatoes (Klikocka et al., 2005), and which significantly promotes the availability of essential heavy metals in soils such as Mn and Zn, but also that of undesired contaminants such as Cd and Ni. The upper recommended thresholds for the contamination of phytopharmaceuticals with Pb, Cd and Hg are 5, 0.2 and 0.1 mg $kg^{-1}$ (Anon, 1991). The acidifying effect of elemental S will be more pronounced on soils with a pH that is sub-optimum than on soils with a soil reaction in the optimum range. These pH dependent mobilisation processes of cations need to be distinguished from antagonistic effects between S and heavy metals, for example for B, Se, As and Mo (Schnug, 1983; Alhendawi et al., 2005; Haneklaus et al., 2006). In case of Mo and Se, this influence may have been further enforced by a reduced availability with decreasing soil pH. A measurement of changes in soil pH after fertilisation was waived as a decrease of the soil pH in mixed sample cores is unlikely (Burns, 1968) and thus was not regularly found in previous experiments (Schnug & Finck, 1980). Schnug & Finck (1980) point out that the acidification concentrates in the nearest vicinity of fertiliser particles.

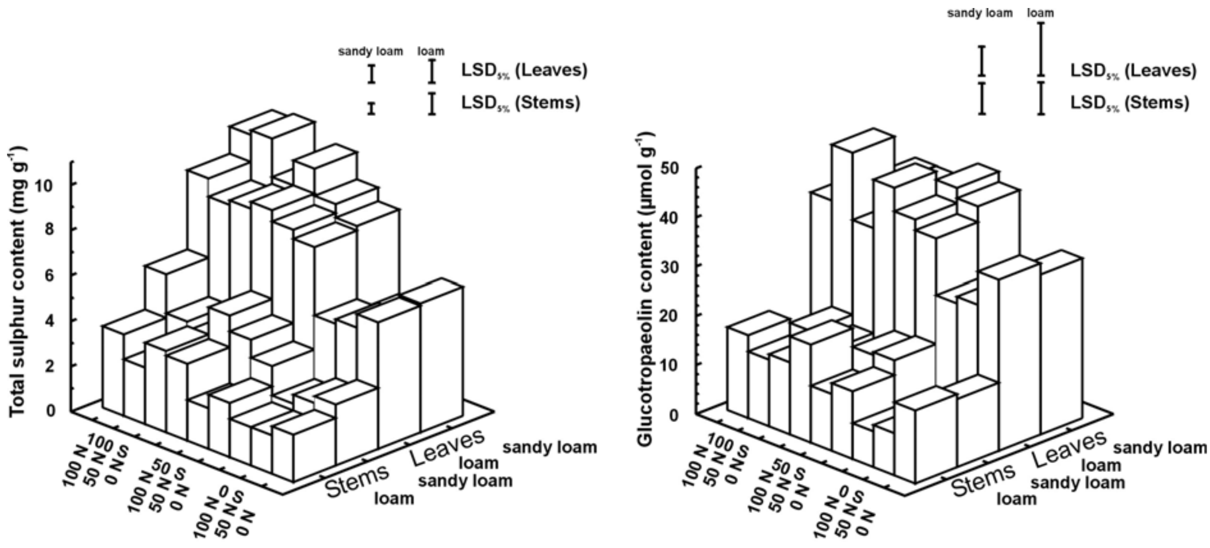


**Fig. 1:** Influence of increasing S and N rates on the total S and glucotropaeolin content of *T. majus* leaves and stems at the start of flowering on two experimental sites

Yet, changes in the S and glucotropaeolin content of leaves and stems in relation to increasing S and N doses enable an indirect evaluation of the efficacy of the conversion rate of elemental S (Fig.1). Elemental S fertilisation increased significantly the total S content of leaves and stems from on average 4.8 to 9.8 mg $g^{-1}$ and 2.4 to 4.2 mg $g^{-1}$, respectively on the sandy loam site. Similar results were obtained on the loam, though the increase was not all that strong. Here, the S concentration increased by 41% in leaves and 47% in stems (Fig. 1). The N rate had no significant influence on the S content at both sites. In contrast, a significantly lower glucotropaeolin content was found in leaves and stems on the sandy loam. In those treatments, which received no S, the total S and glucotropaeolin content decreased from 5.7 to 4.3 mg S $g^{-1}$ and 32.5 to 13.3 µmol $g^{-1}$ in leaves, and 2.7 to 2.0 mg S $g^{-1}$ and 13.9 to 4.2 µmol $g^{-1}$ in stems after the application of 100 kg $ha^{-1}$ N. At the same time the N content increased from on average 36 to 54 mg N $g^{-1}$ in the leaves. These results fit to the findings of Rosa et al. (1997) that N fertilisation decreases the glucosinolate content.

Accordingly, a positive and significant relationship between total S and glucotropaeolin content was found (Fig. 2). For N and the glucotropaeolin content the relationship was negative, however, not significant (Fig. 2).
In total, 43% of the variability of the glucotropaeolin content could be explained by variations in the S nutritional status, which makes the S supply a prominent factor for the cultivation of *T. majus* containing a high level of glucotropaeolin. These results corroborate those from earlier studies with *T. majus* (Bloem et al., 2001a and 2001b).

Recapitulating it can be stated that elemental S applications significantly enhanced the S nutritional status of *T. majus* so that it may be concluded that elemental S was rapidly oxidised and thus plant available on both sites. Particle size and specific surface are besides the count of thiobacilli in the soil, the most important criteria for the oxidation rate (Fox et al., 1964; Schnug & Eckhardt, 1981). Wettable elemental S, which was used for fertilising had a particle size of < 10 µm and thus provides optimum conditions for a rapid conversion (Fox et al., 1964). Additionally, on both soils elemental S has been applied previously so that it may be assumed that the count of thiobacilli was correspondingly high (Schnug & Eckhardt, 1981).

The primary effect of elemental viz to substantially enhance the S nutritional status and concentration of the phytopharmaceutical relevant compound glucotropaeolin was shown above (Fig. 1). Secondary effects viz the mobilisation of heavy metals by acidifying the soil, which yields a correspondingly higher uptake by the plant and antagonistic effects between S and anionic elements such as B, Mo, Se and As will be presented next. The influence of acidifying S and N fertiliser products on the plant availability of heavy metals in soils and their uptake by plants was intensely studied by Schnug (1982), Schnug and Finck (1981), Schnug & Schnier (1982) and Schnug & Schnier (1986).

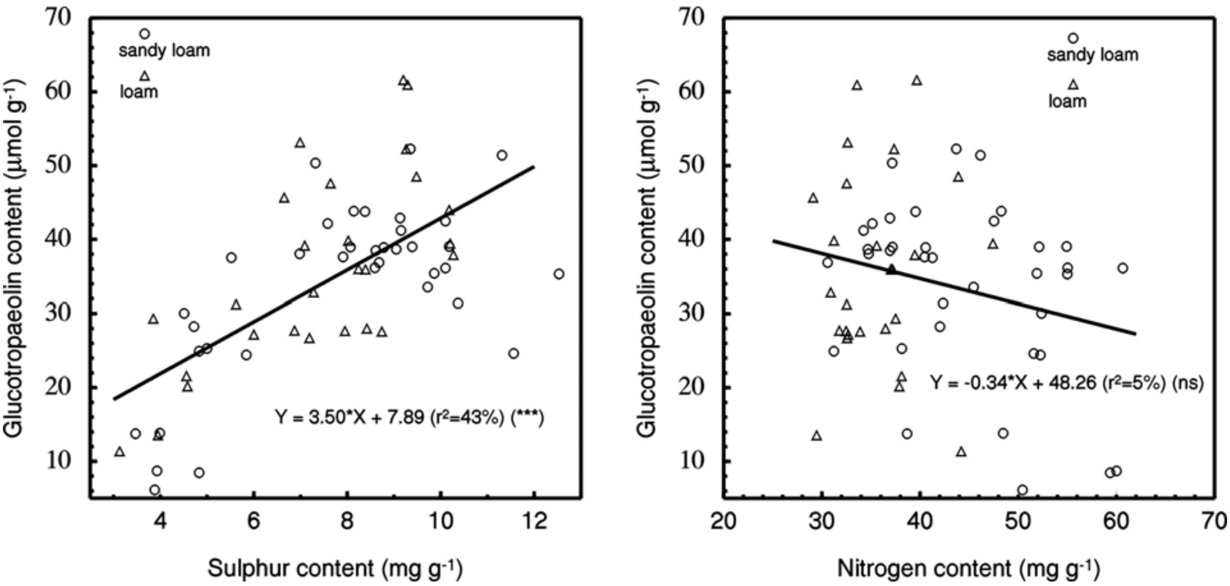


**Fig. 2:** Relationship between total S (left) and N (right) and glucotropaeolin content of *T. majus* leaves at the start of flowering on two experimental sites

A higher mobility can be beneficial for improving the supply with essential micro-nutrients, particularly on heavy soils with a pH of > 7, as for instance the supply of winter wheat with Mn is often marginal (Schnug & Finck, 1981). In the presented investigation elemental S acted acidifying, while nitrochalk is rated as neutral in its soil reaction (Finck, 1979). The influence of increasing S and N rates on the essential micro-nutrient content in leaves and stems of *T. majus* is shown in Tab. 1.

On the low-pH loam soil the Fe, Mn, Zn and B content of *T. majus* leaves was 1.2, 2.2, 1.8 and 1.5 times higher than on the loamy sand soil. For Cu similar values were found on both sites and in case of Mo the concentrations were 4.7 times higher on the sandy loam (Tab. 1). This conspicuous increase is most likely the combined effect of an antagonism with S and a limited availability due to the reduction of the soil pH.

Elemental S fertilisation significantly increased the Mn and Cu content and decreased the B concentration in the leaves of *T. majus* on the sandy loam. On the loam soil the Zn content of leaves and stems decreased significantly from on average 107.3 to 81.6 µg $g^{-1}$ and 56.4 to 39.4 µg $g^{-1}$, respectively. A decrease of the Fe and Zn contents on the loam soil after elemental S applications was also found for corn by Soliman et al. (1992). The increase of the Zn and Cu concentrations on the loamy sand is putatively caused by an antagonistic relationship between Mo and these two elements. Additionally, in case of Zn the acidifying effect of elemental S yielded a higher/lower plant availability (see below) and correspondingly higher/lower Zn/Mo concentrations in *T. majus* leaves. Similar effects were found for spring wheat, tobacco and maize (Cui & Wang, 2005; Wenger et al., 2002).

In case of B the concentration in leaves and stems increased from 18.8 to 23.8 µg $g^{-1}$ and from 13.4 to 14.9 µg $g^{-1}$, respectively on the loam soil (Tab. 1). In comparison, on the loamy sand soil, the B and Mo concentration decreased significantly in leaves and stems of *T. majus*. The decrease of the Fe concentrations in *T. majus* leaves on the loam soil has been observed for maize after application of acidifying S-containing N fertilisers, too (Schnug, 1983). Either a direct effect of sulphate or the stimulation of *T. ferrooxidans* might have yielded this result (Schnug, 1983; Schnug & Eckhardt, 1981). An increasing N rate yielded significantly higher Mn and lower Mo contents in *T. majus* leaves on both sites. Berard et al. (1990) report about similar effects on midribs of stored cabbage.

**Tab. 1:** Influence of elemental S and N fertilisation (kg $ha^{-1}$) on the essential micro-element content (µg $g^{-1}$) in leaves and stems of *T. majus* at the start of flowering on two experimental sites

| | **Micro-nutrient content (µg $g^{-1}$)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Fe** | | **Mn** | | **Zn** | | **Cu** | | **B** | | **Mo** | |
| | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** |
| | **Soil texture class: loamy sand** | | | | | | | | | | | |
| **(kg $ha^{-1}$)** | | | | | | | | | | | | |
| **S** | | | | | | | | | | | | |
| 0 | 64.0 | 21.5 | 77.4 | 23.8 | 43.7 | 46.3 | 8.4 | 6.2 | 16.5 | 16.7 | 2.5 | 0.8 |
| 50 | 64.5 | 20.4 | 103.3 | 31.5 | 45.6 | 42.8 | 10.0 | 6.3 | 15.3 | 13.7 | 1.1 | 0.4 |
| 100 | 65.9 | 21.5 | 151.0 | 40.5 | 56.6 | 42.4 | 9.6 | 6.8 | 15.3 | 13.4 | 0.8 | 0.3 |
| **N** | | | | | | | | | | | | |
| 0 | 55.2 | 21.0 | 84.7 | 23.0 | 42.2 | 41.6 | 8.1 | 6.5 | 15.4 | 14.4 | 1.8 | 0.6 |
| 50 | 63.1 | 20.1 | 113.0 | 32.5 | 48.6 | 42.5 | 8.5 | 5.4 | 15.5 | 14.1 | 1.3 | 0.5 |
| 100 | 76.0 | 22.3 | 134.0 | 40.3 | 55.1 | 47.4 | 11.4 | 7.3 | 16.2 | 15.0 | 1.3 | 0.5 |
| $LSD_{5\%}$ | 4.1 | 4.0 | 15.2 | 5.9 | 16.3 | 9.2 | 0.9 | 1.7 | 1.0 | 1.1 | 0.5 | 0.1 |
| | **Soil texture class: loam** | | | | | | | | | | | |
| **S** | | | | | | | | | | | | |
| 0 | 85.5 | 26.8 | 190.4 | 23.4 | 107.3 | 56.4 | 8.0 | 5.5 | 18.8 | 13.4 | 0.3 | 0.1 |
| 50 | 73.0 | 23.1 | 215.2 | 27.3 | 79.0 | 47.5 | 9.9 | 5.0 | 24.8 | 14.2 | 0.3 | 0.2 |
| 100 | 79.4 | 18.4 | 299.2 | 28.8 | 81.6 | 39.4 | 11.5 | 4.8 | 23.8 | 14.9 | 0.3 | 0.1 |
| **N** | | | | | | | | | | | | |
| 0 | 76.8 | 24.1 | 149.6 | 23.0 | 72.1 | 43.3 | 10.1 | 5.4 | 25.9 | 14.8 | 0.6 | 0.3 |
| 50 | 80.8 | 21.3 | 240.4 | 27.0 | 91.0 | 46.4 | 10.3 | 5.0 | 21.8 | 14.5 | 0.2 | 0.1 |
| 100 | 78.3 | 19.6 | 310.9 | 34.0 | 94.5 | 48.2 | 9.8 | 4.5 | 21.8 | 13.3 | 0.1 | 0.1 |
| $LSD_{5\%}$ | 14.8 | 11.2 | 91.2 | 10.9 | 9.8 | 8.7 | 0.8 | 1.2 | 3.5 | 1.0 | 0.1 | 0.1 |

On the sandy loam the Cu concentration was significantly higher, too (Tab. 1). Striking was that the Mn concentration of the leaves increased on average by 74 µg $g^{-1}$ because of increasing S rates and 49 µg $g^{-1}$ because of increasing N rates on the sandy loam, while on the loam the corresponding values were 109 and 161 µg $g^{-1}$, respectively. These findings stress indirectly the strong dependence of the plant available Mn content on soil pH.

A higher concentration of these essential micronutrients contributes positively to the mineral human nutrition and thus is beneficial for the quality of the herbal plant product. However, besides the desired heavy metals, the concentration of the undesired and toxic heavy metals such as Cd, Pb, Cr, Ni and As may be elevated. Co and Se are not essential though beneficial for plants and animals (Palit et al., 1994; Aery & Jagetiya, 2000; Rosbrook et al., 1992; Xue et al., 2001) so that an increasing content may be positive by all means. Ti is rated as a beneficial element for plants (Alcaraz-Lopez et al., 2004; Carvajal & Alcaraz, 1998), while the inhalation of Ti compounds may cause stenosis in humans (Zumkley & Kisters, 1990).

The plant availability of micro-nutrients and heavy metals increases distinctly in relation to the soil pH when values are lower than 6.5 (Cd, Mn), 6.0 (Zn), 5.5 (Ni, Mo), 4.5 (Cu, As, Cr) and 4.0 (Pb) (Hintermaier-Erhard & Zech, 1997). In contrast the availability of Se and Mo increases when the soil pH exceeds values of 7 and 5, respectively (Hintermaier-Erhard & Zech, 1997). On the acid loam soil the mean heavy metal content of *T. majus* leaves was 2.6, 1.2, 1.2, 2, 3.9, 2.3 and 1.5 times higher for Cd, Pb, Cr, As, Ni, Co and Se than on the loamy sand. Only the Ti concentration was 1.1 times higher on the sandy loam soil (Tab. 2).

On the sandy loam soil, fertilisation with elemental S significantly increased the Cd, Co and Ni content of *T. majus* leaves; in stems the Cd and Co was significantly elevated, while that of Ti was reduced (Tab. 2). In contrast, on the loam soil the Cd content decreased with the S dose, while variations in the Co and Ni content of *T. majus* leaves proved to be statistically not different. N and S fertilisation yielded inverse effects with view to the Cd concentration of *T. majus* leaves on the loam soil with the first increasing the content by on average 40% and the latter decreasing it by 20% (Tab. 2). N fertilisation resulted also in increased Cd concentration of durum wheat (Mitchell et al., 2000). In any case the Cd concentration of *T. majus* leaves on the loam soil proved to be above the upper recommended concentration of 0.2 µg $g^{-1}$ in phytopharmaceuticals so that this site is not suitable for cultivating herbal plants irrespective of the fertiliser products used. Interesting in this context is that from nine traditional Chinese medicines six exceeded the upper recommended threshold for Cd (Sun et al., 2002). The tested products in this investigation showed elevated levels of other heavy metals, too with maximum values of 17.2 µg $g^{-1}$ (Ni), 5.0 µg $g^{-1}$ (Co), 9.6 µg $g^{-1}$ (Cr) and 4.4 µg $g^{-1}$ (Pb). Even higher concentrations of heavy metals were found in medicinal plants grown in the Negev desert with 22 µg $g^{-1}$ Cd in *Achillea fragantissima*, 33 µg $g^{-1}$ Pb in *Asparagus aphyllus*, 19 µg $g^{-1}$ Ni in *Paronychia argentea* (Sathiyamoorthy et al., 1997). Kabelitz (1998) provides an overview of variations in the Pb and Cd concentrations in more than 100 different medicinal and herbal plants.

N fertilisation significantly decreased the Se concentration of *T. majus* leaves on both sites. On the loam soil this effect was even stronger for the N than S treatments (Tab. 2). Such reductions can be explained by the dilution effect caused by an over-proportional increase in biomass.

Usually the heavy metal concentration was equal or higher in leaves than stems of *T. majus* (Tab. 1 and 2). On the sandy loam soil, however, the Pb, Cr, As and Se concentration was on average 47%, 278%, 150% and 218% higher in stems than leaves (Tab. 2). Higher Pb and Cr concentrations in stems than leaves were also found in *Polygonum salicifolium* and wheat, respectively (Dogan & Saygideger, 2004; Sharma et al., 1995). Dwivedi & Dey (2002) found usually higher Pb and Cd concentrations in leaves than in stems of in total 28 medicinal plants, whereby the differentiation was not made for individual plants, but rather with view to the used plant parts.

Further on, the Cr, Ni, and Ti concentration in *T. majus* stems increased significantly in relation to the treatment and experimental site (Tab. 2). Both acidification of elemental S and antagonistic effects between S and anionic trace elements should be reflected in correlative relationships between the total S and individual heavy metal concentrations. Ancillary, the relationships between N and heavy metal concentrations in leaves of *T. majus* were determined (Tab. 3). Close correlations with a regression coefficient of ≥ 50% were found between S and Mn, Mo, Cd, Co, and Ni and between N and Fe and Cu (Tab. 3). So, elemental S increased obviously not only the plant available sulphate concentration, but also available Mn, Cd and Ni concentration in the soil and finally uptake of these elements by *T. majus*. With increasing S supply the uptake of Mo was reduced most explicitly (Tab. 3). A significant inverse relationship between S and B content was found only on the loam soil. Not significant were the relationships between S and As or Se, respectively. As for N, slightly negative correlations were found for Pb, As and Se (Tab. 3).

**Tab. 2:** Influence of elemental S and N fertilisation (kg $ha^{-1}$) on the heavy metal content (µg $g^{-1}$) in leaves and stems of *T. majus* at the start of flowering on two experimental sites

| | Heavy metal content (µg $g^{-1}$) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Cd** | | **Pb** | | **Cr** | | **As** | | **Ni** | | **Co** | | **Se** | | **Ti** | |
| | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** | **Leaves** | **Stems** |
| | **Soil texture class: loamy sand** | | | | | | | | | | | | | | | |
| **(kg $ha^{-1}$)** | | | | | | | | | | | | | | | | |
| S | | | | | | | | | | | | | | | | |
| 0 | 0.13 | 0.13 | 0.17 | 0.27 | 0.22 | 0.49 | 0.04 | 0.09 | 0.70 | 0.68 | 0.14 | 0.07 | 0.11 | 0.26 | 10.2 | 9.1 |
| 50 | 0.13 | 0.11 | 0.18 | 0.22 | 0.18 | 0.48 | 0.04 | 0.04 | 0.87 | 0.63 | 0.16 | 0.09 | 0.12 | 0.24 | 9.8 | 8.2 |
| 100 | 0.16 | 0.19 | 0.17 | 0.27 | 0.12 | 0.52 | 0.04 | 0.06 | 0.80 | 0.60 | 0.20 | 0.10 | 0.10 | 0.23 | 8.7 | 7.4 |
| N | | | | | | | | | | | | | | | | |
| 0 | 0.12 | 0.13 | 0.18 | 0.30 | 0.19 | 0.51 | 0.05 | 0.08 | 0.68 | 0.62 | 0.16 | 0.08 | 0.15 | 0.26 | 9.3 | 8.5 |
| 50 | 0.14 | 0.15 | 0.17 | 0.22 | 0.15 | 0.44 | 0.04 | 0.06 | 0.79 | 0.63 | 0.17 | 0.09 | 0.10 | 0.23 | 9.0 | 7.7 |
| 100 | 0.16 | 0.15 | 0.16 | 0.24 | 0.19 | 0.55 | 0.04 | 0.06 | 0.90 | 0.64 | 0.17 | 0.09 | 0.08 | 0.24 | 10.4 | 8.6 |
| $LSD_{5\%}$ | 0.03 | 0.04 | 0.04 | 0.10 | 0.06 | 0.20 | 0.01 | 0.04 | 0.12 | 0.14 | 0.03 | 0.01 | 0.03 | 0.05 | 1.5 | 1.0 |
| | **Soil texture class: loam** | | | | | | | | | | | | | | | |
| S | | | | | | | | | | | | | | | | |
| 0 | 0.46 | 0.23 | 0.22 | 0.19 | 0.29 | 0.13 | 0.11 | 0.08 | 2.76 | 1.13 | 0.32 | 0.08 | 0.20 | 0.19 | 9.9 | 9.1 |
| 50 | 0.30 | 0.26 | 0.21 | 0.18 | 0.18 | 0.16 | 0.08 | 0.05 | 2.85 | 1.26 | 0.39 | 0.08 | 0.17 | 0.09 | 8.0 | 6.3 |
| 100 | 0.37 | 0.23 | 0.19 | 0.15 | 0.21 | 0.22 | 0.07 | 0.05 | 3.57 | 1.59 | 0.45 | 0.12 | 0.14 | 0.12 | 7.8 | 6.5 |
| N | | | | | | | | | | | | | | | | |
| 0 | 0.26 | 0.19 | 0.20 | 0.17 | 0.18 | 0.20 | 0.09 | 0.05 | 2.38 | 1.34 | 0.28 | 0.08 | 0.19 | 0.12 | 8.6 | 6.0 |
| 50 | 0.39 | 0.26 | 0.22 | 0.16 | 0.22 | 0.17 | 0.09 | 0.06 | 3.18 | 1.40 | 0.38 | 0.11 | 0.18 | 0.11 | 8.5 | 7.5 |
| 100 | 0.43 | 0.29 | 0.19 | 0.18 | 0.26 | 0.16 | 0.07 | 0.06 | 3.58 | 1.39 | 0.49 | 0.10 | 0.13 | 0.15 | 8.1 | 7.1 |
| $LSD_{5\%}$ | 0.11 | 0.11 | 0.07 | 0.07 | 0.1 | 0.06 | 0.02 | 0.02 | 1.3 | 0.33 | 0.13 | 0.04 | 0.04 | 0.05 | 0.8 | 1.3 |

In the presented study vegetative plant material was analysed. Usually, the heavy metal concentration of generative plant material is distinctly lower than in vegetative tissues. Thus it may be favourable on soils, which have geogenically conditioned high concentrations of heavy metals to use seed instead of leaf material for the production of phytopharmaceuticals. Other advantages of using seeds are the easy and loss-free sampling and storage, which warrants a uniform product with view to the phytopharmaceutical quality. In contrast, losses of glucotropaeolin by the degradation of glucotropaeolin of the leaf material may be as high as 85% in relation to the drying technique (Bloem et al., 2001b).

**Tab. 3:** Correlations between total S and N concentrations and essential micro-nutrients, and heavy metals in leaves of *T. majus* in relation to the cultivation location (only those correlations are listed that proved to be significant on at least one experimental site)

| | Soil texture class | Regression equation (Y = aX + b) | Coeff. of determination ($r^2$) | Significance level |
|---|---|---|---|---|
| | | **X = Sulphur (mg g$^{-1}$)** | | |
| **Y = (µg g$^{-1}$)** | | | | |
| Fe | lS | Y = 11.01*X + 56.17 | $r^2$ = 7 % | ns |
| | L | Y = 2.83*X + 57.99 | $r^2$ = 15 % | * |
| Mn | lS | Y = 12.00*X + 16.87 | $r^2$ = 49 % | *** |
| | L | Y = 44.16*X – 82.52 | $r^2$ = 59 % | *** |
| Zn | lS | Y = 2.65*X + 27.92 | $r^2$ = 12 % | * |
| | L | Y = 3.49*X + 61.69 | $r^2$ = 18 % | * |
| B | lS | Y = -0.18*X + 17.14 | $r^2$ = 10 % | ns |
| | L | Y = -1.37*X + 32.97 | $r^2$ = 44 % | *** |
| Mo | lS | Y = -0.30*X + 3.79 | $r^2$ = 60 % | *** |
| | L | Y = -0.11*X + 1.07 | $r^2$ = 75 % | *** |
| Cd | lS | Y = 0.006*X + 0.09 | $r^2$ = 13 % | * |
| | L | Y = 0.05*X – 0.01 | $r^2$ = 60 % | *** |
| Cr | lS | Y = -0.01*X + 0.27 | $r^2$ = 12 % | * |
| | L | Y = 0.03*X + 0.03 | $r^2$ = 14 % | ns |
| Co | lS | Y = 0.01*X + 0.09 | $r^2$ = 31 % | *** |
| | L | Y = 0.05*X + 0.002 | $r^2$ = 50 % | *** |
| Ni | lS | Y = 0.03*X + 0.59 | $r^2$ = 13 % | * |
| | L | Y = 0.46*X – 0.30 | $r^2$ = 54 % | *** |
| | | **X =Nitrogen (mg g$^{-1}$)** | | |
| Fe | lS | Y = 1.07*X + 16.86 | $r^2$ = 82 % | *** |
| | L | Y = 0.97*X + 44.23 | $r^2$ = 9 % | ns |
| Mn | lS | Y = 1.84*X + 28.51 | $r^2$ = 14 % | * |
| | L | Y = 12.83*X - 216.99 | $r^2$ = 25 % | ** |
| Cu | lS | Y = 0.16*X + 2.02 | $r^2$ = 50 % | *** |
| | L | Y = 0.24*X + 1.53 | $r^2$ = 44 % | *** |
| Cd | lS | Y = 0.002*X + 0.05 | $r^2$ = 18 % | ** |
| | L | Y = 0.01*X - 0.01 | $r^2$ = 13 % | ns |
| Pb | lS | Y = -0.002*X + 0.27 | $r^2$ = 15 % | * |
| | L | Y = -0.006*X + 0.43 | $r^2$ = 20 % | * |
| Ni | lS | Y = 0.009*X + 0.37 | $r^2$ = 20 % | ** |
| | L | Y = 0.17*X - 2.83 | $r^2$ = 34 % | ** |
| As | lS | Y = -7.55e-4*X +0.08 | $r^2$ = 22 % | ** |
| | L | Y = -0.002*X + 0.16 | $r^2$ = 16 % | * |
| Se | lS | Y = -0.003*X + 0.25 | $r^2$ = 34 % | *** |
| | L | Y = -0.002*X + 0.21 | $r^2$ = 2 % | ns |
| Ti | lS | Y = 0.09*X +5.59 | $r^2$ = 15 % | * |
| | L | Y = -0.11*X + 12.24 | $r^2$ = 18 % | * |

lS = loamy sand; L = Loam

## Conclusions

The selection of a cultivation site for medicinal plants should imply the determination of soil characteristics such as pH and the plant available content of essential micro-nutrients and undesired heavy metals in order to evaluate the transfer into the plant material. Particularly in areas with geogenically conditioned high background values the cultivation of medicinal plants may yield insufficient qualities. This may also be a problem on soils, where the soil reaction is distinctly below the optimum and can be enforced drastically by applying acidifying fertiliser products. The maintenance of an optimum lime status is therefore a major contribution to limit the uptake of contaminants. A favourable method for the precise determination of the lime requirement is that by assessing site-specific buffer curves (Haneklaus & Schnug, 2000). Acidifying fertiliser products should only be used on sites where the supply with essential micro-nutrients is critical because of a limited availability. The advantage of soil-applied acidifying fertilisers proved to be that they were more efficient and independent of climatic conditions than foliar applications (Schnug & Finck, 1980). Additionally, this procedure increases the micro-nutrient content stronger and more constant than soil-applied micro-nutrients (Jungermann, 1962). The harvesting procedure might also contribute to an improved phytopharmaceutical quality: the higher is the proportion of leaves, the higher will be the glucotropaeolin content (Fig. 1) and particularly on light soils the contamination with Pb, Cr, As and Se will be lower (Tab. 2).

## References

**Aery NC, Jagetiya BL** (2000) Effect of cobalt treatments on dry matter production of wheat and DTPA extractable cobalt content in soils. Commun Soil Sci Plant Anal 31(9-10): 1275-1286

**Alhendawi RA, Kirkby EA, Pilbeam DJ** (2005) Evidence that sulfur deficiency enhances molybdenum transport in xylem sap of tomato plants. J Plant Nutr 28: 1347-1353

**Alcaraz-Lopez C, Botia M, Alcaraz CF, Riquelme F** (2004) Effect of foliar sprays containing calcium, magnesium and titanium on peach (*Prunus persica* L.) fruit quality. J Sci Food Agric 84 (9): 949-954

**Anon** (1990) EU method Nr. L170/28 Bestimmung des Glucosinolatgehaltes von Oelsaaten durch HPLC.

**Anon** (1991) Arzneimittel-Kontaminanten-Empfehlung-Schwermetalle vom 17. Oktober 1991 – AKEmpfS

**Anon** (2003) Verordnung über das Inverkehrbringen von Düngemitteln, Bodenhilfsstoffen, Kultursubstraten und Pflanzenhilfsmitteln. Düngemittelverordnung – DüMV vom 26. November 2003

**Anon** (2004) Verticllium wilt. In: Trees and shrubs – diseases and disorders. Missouri Botanical Garden, January 2004.

www.mobot.org/gardeninghelp/ipm/messages/225.pdf

**Berard LS, Senecal M, Vigier B** (1990) Effects of nitrogen fertilization on stored cabbage. II. Mineral composition in mid-rib and head tissues of two cultivars. J Hort Sci 65: 409-416

**Bloem E, Haneklaus S, Köhler T, Sator C, Schnug E** (2001a) Produktionstechnische Maßnahmen zur Erhöhung des Glucotropaeolingehaltes von Kapuzinerkresse (*Tropaeolum* majus L.). Proc. Narossa: 7th Int. Conf. for Renewable Resources, 11th-12th June 2001, Magdeburg, Germany, ÖHMI, 10S

**Bloem E, Haneklaus S, Peplow E, Sator C, Köhler T, Schnug E** (2001b). The effect of sulphur and nitrogen fertilisation on the glucotropaeolin content in *Tropaeolum majus* (L.). XXXVI. DGQ-Vortragstagung, Jena, 185-190

**Bloem E, Haneklaus S, Ahmed SS, Schnug E** (2002) Beitrag der Schwefel-Versorgung zur Qualitätssicherung beim Anbau von Arzneipflanzen in unterschiedlichen Klimaten. Zeitschrift für Arznei- & Gewürzpflanzen, 149-155, ISSN 1431-9292

**Braun H** (1981): Medicinal Herbs – a dictionary for physicians and pharmacists. Applications, effects and toxicology. Gustav Fischer Publ, Stuttgart

**Briskin DP, Leroy A, Gawienowski M** (2000) Influence of nitrogen on the production of hypericins by St. John's wort. Plant Physiol Biochem 38(5): 413-420

**Burns GR** (1968) Oxidation of sulphur in soils. The Sulphur Institute. Techn Bull 13, pp 41

**Cartheuser JFB** (1978) in Bardeau, F. (Hrsg.) Die Apotheke Gottes. Heilkräuter einst und jetzt, alphabetisch geordnet. Ullstein Verlag Publishers, Frankfurt

**Carvajal M, Alcaraz CF** (1998) Why titanium is a beneficial element for plants J Plant Nutr 21 (4): 655-664

**Cazin JF** (1978): In: Bardeau, F. (Hrsg.) Die Apotheke Gottes: Heilkräuter einst und jetzt. Ullstein Verlag publishers, Frankfurt

**Cui YS, Wang QR** (2005) Interaction effect of zinc and elemental sulfur on their uptake by spring wheat. J Plant Nutr 28: 639-649

**Dannenberg H, Stickl H, Wenzel F** (1956): About the antimicrobial component of *Tropaeolum majus*. J Physiol Chem 303: 248–256

**Dogan M, Saygideger S** (2004) Influence of pH on lead accumulation, chlorophyll and nitrogen content of *Polygonum salicifolium* Brouss. ex Willd. Fresenius Environm Bull 13(8): 777-782

**Dragendorf M** (1976): In: Madaus, G. (ed) Lehrbuch der biologischen Heilmittel, Band III. George Thieme Verlag, New York

**Dwivedi SK, Dey S** (2002) Medicinal herbs: a potential source of toxic metal exposure for man and animals in India. Arch. Environm Health 57: 229-231

**Fahey JW, Zhang Y, Talalay P** (1997): Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acad Sci 94: 10366–10372

**Finck A** (1979) Dünger und Düngung. Verlag Chemie, Weinheim

**Fox RL, Atesalp HML, Kampbell DH, Rhoades HF** (1964) Factors influencing the availability of sulfur fertilizers to alfalfa and corn. Soil Sci Soc Am Proc 28: 406-408

**Haneklaus S, Paulsen H M, Gupta AK, Bloem E, Schnug E** (1999) Influence of Sulfur Fertilization on Yield and Quality of Oilseed Rape and Mustard. Proc. 10th Int. Rapeseed Congress, Sept. 26-29, 1999, Canberra, (CD-ROM)

**Haneklaus S, Schnug E** (2000) Spatial Variability of Soil Acidity and its Impact on Production Technology. Proc. Int. Sci. Conf. Soil Acidification and Liming: Fundamental and Technological Researches, Klaipeda (Lithuania), 67-73

**Haneklaus S, De Kok LJ, Stulen I, Bloem E, Schnug E** (2006) Sulphur. In: Barker A V and Pilbeam D J (Eds) Handbook of Plant Nutrition, CRC Press, Boca Raton, Florida (2006)

**Hintermaier-Erhard G, Zech W** (1997) Wörterbuch der Bodenkunde. Ferdinand Enke Verlag, Stuttgart

**Hoppe L, Bahadir M, Haneklaus S, Schnug E** (1996) Sulphur supply and alliin content of allium species. XXXI. DGQ-Vortragstagung, Kiel 1996, 189-192

**Hoy MA** (1987) Sulfur as a control agent for pest mites in agriculture. Proc. Int. Symp. Elemental Sulphur in Agriculture, Nice, 1: 51-61

**Jungermann K** (1962) Beiträge zur Mikronährstofffrage (III). Landw Forsch SH 16: 93-111

**Kabelitz L** (1998) Zur Schwermetallbelastung von Arznei- und Kräuterdrogen. Pharm Ind 60: 444-451

**Keller K** (1991) Legal requirements for the use of phytopharmaceutical drugs in the Federal Republic of Germany. J Ethnopharmacology 32: 225-229

**Klikocka H, Haneklaus S, Bloem E, Schnug E** (2005) Influence of sulfur fertilization on infections of potato tubers (*Solanum tuberosum*) with *Rhizoctonia solani* and *Streptomyces scabies*. J Plant Nutr 28:819-833

**Kriz RW** (1998) Plant disease and insect clinic. Report on whole year 1997 http://www.ces.ncsu.edu/depts/ent/clinic/Annual97/group4.htm

**Ludwig-Müller J, Bennett RN, Kiddle G, Ihmig S, Ruppel M, Hilgenberg W** (1999) The host range of *Plasmodiophora brassicae* and its relationship to endogenous glucosinolate content. New Phytologist 141(3): 443-458

**Mitchell LG, Grant CA, Racz GJ** (2000) Effect of nitrogen application on concentration of cadmium and nutrient ions in soil solution and in durum wheat. Can J Soil Sci 80(1): 107-115

**Murch SJ, Haq K, Rupasinghe HPV, Saxena PK** (2003) Nickel contamination affects growth and secondary metabolite composition of St. John's wort (*Hypericum perforatum* L.). Environm Exp Bot 49(3): 251-257

**Palit S, Sharma A, Talukder G** (1994) Effects of cobalt on plants. Bot Rev 60(2): 141-181

**Patten EJ., DeLong MJ** (1999): Temporal effects of the detoxification enzyme inducer, benzylisothiocyanate: Activation of C–Jun N–terminal kinase prior to the transcription factors AP–1 and NFκB. Biochem. and Biophys. Res. Commun. 257, 149–155

**Rosa EAS, Heaney RK, Fenwick GR, Portas CA M** (1997) Glucosinolates in crop plants. Hortic Rev 19: 99-215

**Rosbrook PA, Asher CJ, Bell LC** (1992) The cobalt status of Queensland soils in relation to pasture grwoth and cobalt accumulation. Tropical Grasslands 26(2): 130-136

**Sathiyamoorthy P, VanDamme P, Oven M, Golan-Goldhirsch A** (1997) Heavy metals in medicinal and fodder plants of the Negev desert. J Environm Sci Health A 32(8): 2111-2123

**Schnug E, Finck A** (1980) Einfluß unterschiedlicher Stickstoffdüngerformen auf die Mobilisierung von Spurennährstoffen. Landw Forsch SH 37: 243-253

**Schnug E, Finck A** (1981) Einfluß verschiedener N-Düngemittel auf die Spurennährstoffgehalte von Mais, Raps und Sellerie unter besonderer Berücksichtigung von Bor und Molybdän. Landw Forsch SH 38: 187-195

**Schnug E, Eckhardt FEW** (1981) Einfluß von Thiobacillus thiooxidans auf die Spurenelementversorgung von Lolium multiflorum bei Schwefeldüngung. Mitt Dt Bodenkd Ges 32: 491-498

**Schnug E (1982)** Untersuchungen zum Einfluß bodenversauernder Düngung auf die Spurennährstoff-Versorgung von Kulturpflanzen. Diss. Agrarwiss. Fak., Kiel

**Schnug E** (1983) Differenzierung von Primär- und Sekundärwirkung der N-Düngung auf die Spurennährstoff-Versorgung von Getreide und Mais. Landw Forsch SH 40: 231-240

**Schnug E, Schnier C** (1982) Influence of sulfur containing fertilizers on trace element concentrations of celery (*Apium grav.*) with special regard to As, B, Br, Mo, Sb and Se. Proc. 21st Intern. Hortic. Congr., Vol. I, Nr. 1495, Hamburg

**Schnug E, Schnier C** (1986) The effect of sulfur-containing nitrogen fertilizers on the elemental composition of celery (*Apium graveolens*) grown on a polluted marsh soil. Plant Soil 91: 273-278

**Schnug E** (1987): Pattern of nutritional elements as a genetic characteristic of species and cultivars of agricultural plants. Trans. XIV. Int. Botanical Congr., Berlin, 134-138

**Schnug E, Haneklaus S, Borchers A, Polle A** (1995) Relations between sulphur supply and glutathione and ascorbate concentrations in *Brassica* napus. Z Pflanzenernähr Bodenkde 158: 67-69

**Schnug E** (1997) Significance of sulphur for the quality of domesticated plants. In: Cram, W J, DeKok L J, Stulen I, Brunold C, Rennenberg H (Eds) Sulphur metabolism in higher plants. Backhuys Publishers, Leiden, The Netherlands, 109-130

**Schnug E, Haneklaus S** (1999) Diagnosis of the Nutritional Status and Quality Assessment of Oilseed Rape by X-Ray Spectroscopy. Proc. 10th Int. Rapeseed Congress, Sept. 26-29, 1999, Canberra, (CD-ROM)

**Schuster WH, von Lochow** J (1979) Anlage und Auswertung von Feldversuchen. DLG Verlag, Frankfurt (Main)

**Sharma DC, Chatterjee C, Sharma CP** (1995) Chromium accumulation and its effect on wheat (*Triticum aestivum* L. cv HD-2204) metabolism. Plant Sci 111(2): 145-151

**Simons, B** (1995) CoStat / CoPlot / CoDraw. CoHort Software, Minneapolis, USA

**Soliman M F, Kostandi SF, Vanbeusichem ML** (1992) Influence of sulfur and nitrogen-fertilizer on the uptake of iron, manganese , and zinc by corn plants grown in calcareous soils. Commun Soil Sci Plant Anal. 23(11-12): 1289-1300

**Sun RX, Zhou LM, Xue WG, Sun JH (2002)** Determination of trace elements in Chinese traditional medicines by atomic absorption spectrometry. Spectroscopy Spectral Analysis 22(5): 853-855

**Walker KC, Booth EJ** (1994): Sulphur deficiency in Scotland and the effects of sulphur supplementation on yield and quality of oilseed rape. Norwegian J Agric Sci 15: 97–104

**Wenger K, Kayser A, Gupta S K, Furrer G, Schulin R** (2002) Comparison of NTA and elemental sulfur as potential soil amendments in phytoremediation. Soil Sediment Contamination 11(5): 655-672

**WHO** (2003) WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. Geneva

**Xue TL, Hartikainen H, Piironen V** (2001) Antioxidative and growth-promoting effect of selenium on senescing lettuce. Plant Soil 237(1): 55-61

**Zumkley H, Kisters K** (1990) Spurenelemente – Geschichte, Grundlagen, Physiologie, Klinik. Wiss. Buchgesellschaft, Darmstadt