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## SULPHUR AND COPPER CONTENT IN OILSEED RAPE PLANTS AFTER THE APPLICATION OF AMMONIUM NITRATE-SULPHATE

### ZAWARTOŚĆ SIARKI I MIEDZI W ROŚLINACH RZEPAKU PO ZASTOSOWANIU SALETROSIARCZANU AMONU

**Abstract:** Sulphur is an important nutrient for winter rape because its large requirement for this element. The influence of N-S fertilizer on the copper content in the plants of winter oilseed rape plants was studied in precise field experiment. The evaluation involved two treatments of a single rate for the first spring fertilizer application with 100 kg N · ha<sup>-1</sup> in the AN treatment (nitro-chalk) and 100 kg N + 50 kg S · ha<sup>-1</sup> in the ANS treatment (ammonium nitrate-sulphate). Its application to soil affected the content of total extractable and mineral sulphur in soil. Common application of N-S nutrients in ANS fertilizer had a positive effect on sulphur content in winter rape plants. Sulphur content in root ranged from 0.23 to 0.52 % and aboveground biomass from 0.33 to 0.79 %. The highest content was found in leaves (to 1.76 % of S in head leaves). A positive influence of the ANS fertilizer on the copper contents in different part of plants was determined. The highest Cu concentrations were determined in the leaves and in florescences, the lowest ones occurred in the stem. The concentration of Cu ranged within the interval of 1.56–8.75 mg Cu · kg<sup>-1</sup> of dry matter depending on the growth period and the part of the plant. No differences in the copper content have been determined in the seeds of individual treatment. The highest uptake in the aboveground parts of the plants was recorded in the green pod period and amounted to 57.4 g Cu · ha<sup>-1</sup> for the ANS treatment.

**Keywords:** copper, N-S fertilizer, sulphur, oilseed rape

Copper is taken up by plants mainly as the Cu<sup>2+</sup> ion and also probably in the form of low molecular organic complexes. The copper content in plants usually lies in the range of 2–20 mg Cu · kg<sup>-1</sup> of dry matter. A toxic effect of copper has been recorded in plants (in leaves) at the level of 20–35 mg Cu · kg<sup>-1</sup> in dry matter [1]. However, Reuter and

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Robinson [2] indicated the toxicity level for oilseed rape plants as low as  $15 \text{ mg Cu} \cdot \text{kg}^{-1}$ .

Copper shows similar properties as iron, ie it has highly stable complexes and the ability to transfer electrons. The copper activity is mainly involved in the enzyme redox reactions in plants. Copper has a high affinity to -SH groups, particularly to the proteins rich in cysteine and also to the carboxylic and phenolic groups. Therefore, in a xylem solution more than 98–99 % of copper is fixed in the form of complexes [3]. The same applies to cytoplasm and the cell organelles in which the concentration of  $\text{Cu}^{2+}$  and  $\text{Cu}^+$  ions is exceptionally low. As a rule, more than 50 % of copper in chloroplasts is fixed in plastocyanin. Copper is also a component of superoxide dismutase (CuZnSOD) and is very important in the activity of ascorbate oxidase. Furthermore, copper is a component of diamine oxidase and phenol oxidase. Phenol oxidase takes part in biosynthesis of lignin and alkaloids.

As Thiel and Finck show [4], the plants with higher nitrate nutrition also have a higher requirement for copper. Decreased lignification of cell walls is also a typical indication of insufficient copper nutrition in higher plants. It is known that copper deficiency affects the yield and seed formation more than vegetative growth. Deficient copper nutrition causes pollen sterility. Copper content in the vegetative parts of plants which occurs at the level of  $1\text{--}5 \text{ mg Cu} \cdot \text{kg}^{-1}$  in dry matter is critical, but this depends on the plant species, plant organ, developmental period and nitrate nutrition [4].

The total copper content in non-contaminated soils usually fluctuates from 2 to 40  $\text{mg Cu} \cdot \text{kg}^{-1}$ . In contaminated soils more than  $1000 \text{ mg Cu} \cdot \text{kg}^{-1}$  has been determined. Copper is predominantly absorbed by organic matter and also into iron and manganese hydroxides. As Zeien states [5], the proportion of individual sorbents differs according to soil types and soil texture. The copper content in the soil solution of agricultural soils is usually less than  $0.03$  to  $0.3 \text{ mg} \cdot \text{dm}^{-3}$ .

Sulphur is nowadays beginning to be a limiting element in the nutrition of winter oilseed rape. Application of sulphate fertilizer before rape sowing and N-S fertilizer application during growing period has become a common part of cultivation technology.

The aim of the presented experiment was to determine the influence of N-S fertilizer on the copper content and Cu removal during vegetation period of oilseed rape.

## Material and methods

A precision field experiment was established in Prague – Uhřetěves, at the experimental station of the Faculty of Agrobiolgy, Food and Natural Resources. The following treatments were followed in experiment: 1)  $100 \text{ kg N} \cdot \text{ha}^{-1}$  (a single application of AN: nitro-chalk, 27 % N) – sidedress the first spring application, 2)  $100 \text{ kg N} + 50 \text{ kg S} \cdot \text{ha}^{-1}$  (a single application of ANS: ammonium nitrate-sulphate, 26 % N and 13 % S) – sidedress the first spring application.

The area of the trial plot was  $20 \text{ m}^2$ . There were 4 replicates of each variant. The experiment was established on Luvisol with saturated sorption complex. Within the framework of agricultural chemical analyses of soil the following contents of available nutrients were determined (Mehlich III): 220 mg of potassium, 119 mg of phosphorus

and 123 mg of magnesium per 1 kg of soil. The total sulphur content before fertilizers application was  $850 \text{ mg} \cdot \text{kg}^{-1}$ , mineral S content was  $4\text{--}7 \text{ mg} \cdot \text{kg}^{-1}$ , and the total copper content amounted to  $26 \text{ mg} \cdot \text{kg}^{-1}$ . Value of pH/CaCl<sub>2</sub> equaled 6.2. Winter oilseed rape ('Bristol' cv. – two zero variety) was used as the experimental crop.

The total contents of chemical elements in plant samples were determined in mineral extracts, which were obtained by the dry decomposition method. The content of copper was determined by the optical emission spectrometry with inductively coupled plasma (ICP-OES, Varian VistaPro, Australia). More information is available in the previous paper [6].

## Results and discussion

The Uhřetín site is very fertile, with the appropriate higher average yields achieved over a period of three years. The seed yield in the unfertilized control object was  $3.7 \text{ Mg} \cdot \text{ha}^{-1}$ , while in the AN treatment it was 49 % higher and in the ANS treatment was 60 % higher. After the application of ANS fertilizer the microelement concentrations, particularly Mn and Zn in the plants were conclusively increased [7]. The decrease in the molybdenum content was statistically significant [8].

There is no data on the Cu contents in the plants of winter oilseed rape for the current period in the Czech Republic. Neuberg et al [9] cite less than  $3 \text{ mg Cu} \cdot \text{kg}^{-1}$  of the dry matter of leaves (with the vegetation height of 30–40 cm) as a very low content,  $3\text{--}5 \text{ mg Cu} \cdot \text{kg}^{-1}$  as low,  $5\text{--}20 \text{ mg Cu} \cdot \text{kg}^{-1}$  as medium, and above  $20 \text{ mg Cu} \cdot \text{kg}^{-1}$  as high. Similarly, Bergmann [10] considers  $5\text{--}12 \text{ mg Cu} \cdot \text{kg}^{-1}$  in the dry matter of the oilseed rape leaves (with the vegetation height of 30–50 cm) as an adequate content.

Khurana et al [11] carried out vegetation experiment with oilseed rape in sand cultures. They consider  $3.8 \text{ mg Cu} \cdot \text{kg}^{-1}$  of the dry matter as a deficient content in young leaves. Finck [12] published the values of an optimum copper content in fully developed leaves at the budding period at the level of  $3\text{--}5 \text{ mg Cu} \cdot \text{kg}^{-1}$  of dry matter. Subsequently, Finck [13] amended these values to  $5\text{--}10 \text{ mg Cu} \cdot \text{kg}^{-1}$  of dry matter.

As is clear from the Fig. 1, the Cu contents in the leaves were higher than the critical values indicated in the literature. It is obvious that the Cu nutrition of the plants in presented experiment was sufficient. The Figs. 1–3 indicate that there is a noticeable tendency towards the higher copper contents in the plants of the ANS treatment. It must be emphasised that in the ANS treatment there was a steadily higher growth of the above-ground biomass and the influence of the diluting effect was higher here than in the AN treatment. This tendency towards the Cu increase (even though no significant differences have been determined) has to be subjected to a critical evaluation. The results show clearly that the highest contents were determined in the leaves, particularly the young ones. Inflorescence analysis also showed high copper contents. At all collection dates the lowest concentrations were determined in the stems, or branches. These results are in good correlation with those of Rossi et al [14], who showed the content in the oilseed rape at  $10.5 \text{ mg Cu} \cdot \text{kg}^{-1}$  of dry matter in the leaves and  $7.6 \text{ mg} \cdot \text{kg}^{-1}$  of dry matter in the stems. Similarly, Angelova et al [15] also found higher copper contents in the leaves than in the stems.

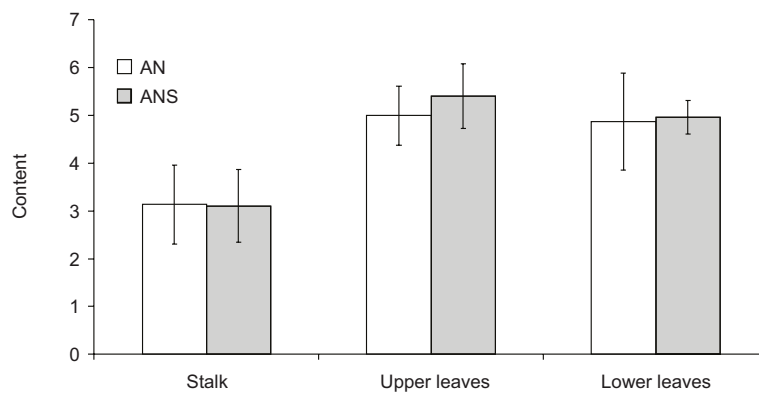


Fig. 1. Copper content in plants [mg Cu · kg<sup>-1</sup>] – budding period

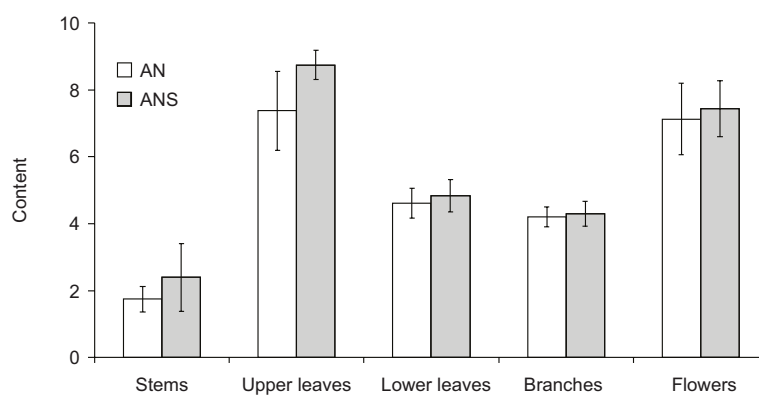


Fig. 2. Copper content in plants [mg Cu · kg<sup>-1</sup>] – flowering period

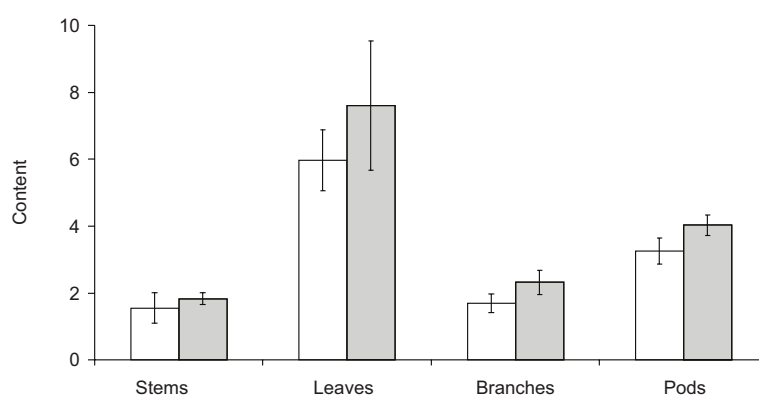


Fig. 3. Copper content in plants [mg Cu · kg<sup>-1</sup>] – green pod period

The cause of the increased Cu content in the ANS treatment plants is related to the change of copper availability in the soil. Copper mobility in the soil depends on the pH value, redox status, the CEC value, the content and quality of organic matter, as well as on the content of clay minerals and Fe and Mn oxides [16]. It is well known that the copper mobility increases with the growing acidity of the soil environment, which is particularly obvious in the soils that are contaminated with copper [17]. Acidification effect can be expected for the ANS fertilizer as well as an associated increase in the Cu mobility. Indirect evidence is also provided by the results of the mineral sulphur content in the topsoil. The S content in the elongation growth period was  $3.9 \text{ mg} \cdot \text{kg}^{-1}$  in the AN treatment, and  $9.2 \text{ mg} \cdot \text{kg}^{-1}$  in the ANS treatment, in the flowering period it amounted to  $6.3 \text{ mg S} \cdot \text{kg}^{-1}$  in the AN treatment and  $15.2 \text{ mg} \cdot \text{kg}^{-1}$  in the ANS treatment, while in the green pod period it equaled  $6.1 \text{ mg S} \cdot \text{kg}^{-1}$  in the AN treatment and  $14.3 \text{ mg} \cdot \text{kg}^{-1}$  in the ANS treatment.

A number of authors [18, 19] emphasise, however, that the plant itself significantly influences the mobility of copper due to the changes in the rhizosphere (changes in ions concentrations, values of the redox potential, concentrations of the root exudates, etc). The changes in the rhizosphere pH values are also dependent on the form of nitrogen nutrition. The plants responded to nitrate nitrogen uptake by a relative increase in the rhizosphere pH value. In discussed experiment the different forms of nitrogen did not, apparently, have any influence on the uptake of copper. As determined by soil analyses, no significant differences in the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  contents in the topsoil and subsoil were found after 35 days of fertilizers application. It was also in good correspondence with an intensive microbiological activity of the Luvisol at the Uhříněves site.

As far as the maintenance of the cation-anion balance is concerned, the increased uptake of anions (particularly  $\text{SO}_4^{2-}$ ) by the plants in the ANS treatment was equalised by an increased uptake of cations. It was, evidently, a very significant factor influencing the increased Cu concentration in these variants. Significantly higher sulphur content in individual parts of ANS treatment plants was determined during practically all growing period (Figs. 4–6). For example, during flowering the sulphur content in the AN treatment was 1.03 % (upper leaves) and 0.67 % (lower leaves), while the ANS treatment reached the values up to 1.18 % (upper leaves) and 0.97 % (lower leaves). The seed uptake of this element was  $19 \text{ kg S} \cdot \text{ha}^{-1}$  (AN treatment) and  $24 \text{ kg S} \cdot \text{ha}^{-1}$  (ANS treatment).

During its entire vegetation period the ANS treatment showed an increased formation of the biomass. It is known, that plants excrete a considerable proportion of the products of photosynthesis in the form of root exudates. It can therefore be expected that there was a greater quantity of exudates particularly in the ANS treatment. As Herms and Brümmer stated [20], these low molecular organic substances contribute to the increased Cu concentration in the soil solution.

The results of Hinsinger [18, 19] show that the root respiration and exudation of organic acids contribute to the acidification of the rhizosphere. An increased of exudates formation in the plants with ANS fertilization evidently contributed towards a higher mobility of copper in the soil environment.

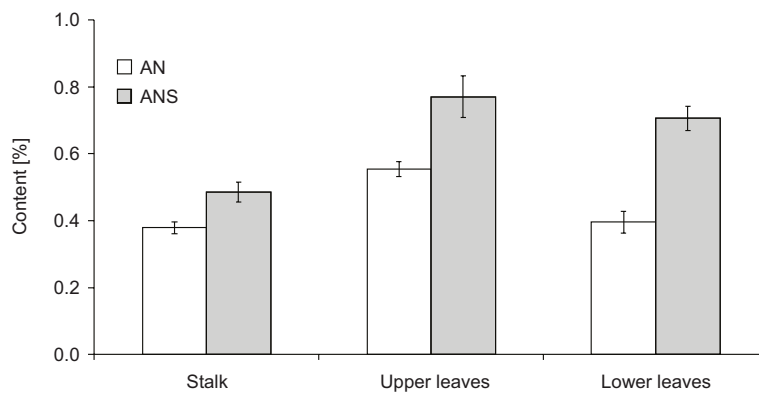


Fig. 4. Sulphur content in plants [% S] – budding period

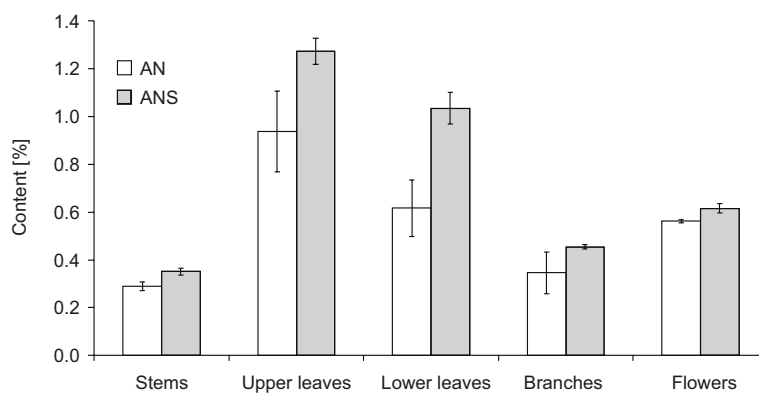


Fig. 5. Sulphur content in plants [% S] – flowering period

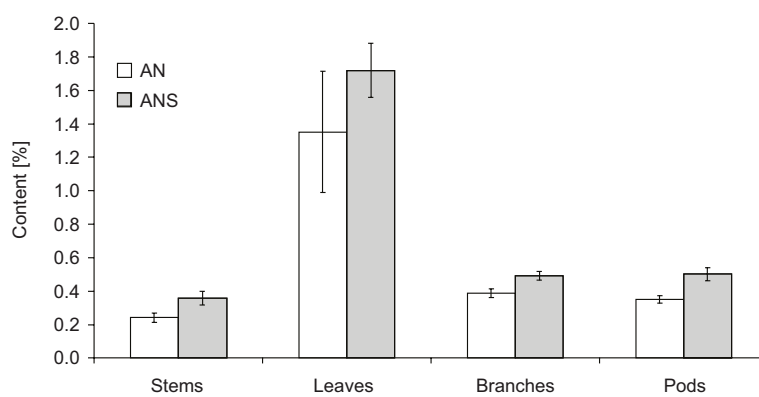


Fig. 6. Sulphur content in plants [% S] – green pod period

It is interesting that the differences in the Cu contents in the vegetative organs did not transform into the differences in the Cu contents in the seeds. An average content was  $2.54 \text{ mg Cu} \cdot \text{kg}^{-1}$  of the seed dry matter in the AN treatment, and  $2.55 \text{ mg Cu} \cdot \text{kg}^{-1}$  of the seed dry matter in the ANS treatment. The values which were determined are higher than the critical content of  $2.2 \text{ mg Cu} \cdot \text{kg}^{-1}$ , by Khurana et al [11]. This, again, proves the fact that the oilseed rape plants in discussed experiment were adequately supplied with copper.

Higher Cu contents in the ANS treatment plants, together with the increase of biomass production resulted in significant differences in the uptake of this element by the plants' biomass. During the budding period the aboveground biomass accumulated  $17.5 \text{ g} \cdot \text{ha}^{-1}$  in the AN treatment, and  $18.9 \text{ g} \cdot \text{ha}^{-1}$  in the ANS treatment. During flowering period this difference increased further. The extraction was  $38.9 \text{ g Cu} \cdot \text{ha}^{-1}$  in the AN treatment and  $49.4 \text{ g} \cdot \text{ha}^{-1}$  in the ANS treatment. The values for the green pod period were determined as  $38.9 \text{ g} \cdot \text{ha}^{-1}$  (AN) and  $57.4 \text{ g} \cdot \text{ha}^{-1}$  (ANS).

## Conclusions

A positive effect of the ANS fertilizer on the copper contents in different part of plants was determined. The highest Cu contents were determined in the leaves and in florescences, the lowest ones occurred in the stems. The copper content ranged within the limits of  $1.56\text{--}8.75 \text{ mg Cu} \cdot \text{kg}^{-1}$  of dry matter depending on the growth period and the part of the plant. No differences in the copper content have been determined in the seeds of individual treatment. Significantly higher sulphur content in individual parts of ANS treatment plants was determined during practically all growing period.

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#### ZAWARTOŚĆ SIARKI I MIEDZI W ROŚLINACH RZEPAKU PO ZASTOSOWANIU SALETROSIARCZANU AMONU

**Abstrakt:** Siarka jest ważnym składnikiem dla rzepaku ozimego z powodu dużego zapotrzebowania na ten pierwiastek. W ścisłym doświadczeniu polowym badano wpływ nawozu azotowo-siarkowego (N-S) na zawartość miedzi w roślinach rzepaku ozimego. Ocena obejmowała dwie kombinacje pierwszego wiosennego nawożenia pojedynczą dawką:  $100 \text{ kg N} \cdot \text{ha}^{-1}$  w formie saletrzaku (AN) i  $100 \text{ kg N} \cdot \text{ha}^{-1} + 50 \text{ kg S} \cdot \text{ha}^{-1}$  w formie saletrosiarczanu amonu (ANS). Zastosowanie tego nawożenia do gleby oddziaływało na ogólną zawartość siarki oraz zawartość mineralnej siarki w glebie. Łączne stosowanie składników N-S w nawozie ANS miało dodatni wpływ na zawartość siarki w roślinach rzepaku. Zawartość siarki w korzeniach wahała się od 0,23 do 0,52 % a w nadziemnej biomacie od 0,33 do 0,79 % S. Najwięcej siarki zawierały liście (do 1,76 % S w liściach górnych). Stwierdzono dodatni wpływ nawozu ANS na zawartość miedzi w różnych częściach rośliny. Największą zawartość Cu zanotowano w liściach i kwiatostanach, a najmniejszą w łodygach. Zawartość miedzi mieściła się w zakresie  $1,56\text{--}8,75 \text{ mg Cu} \cdot \text{kg}^{-1}$  suchej masy zależnie od fazy rozwojowej i części rośliny. Nie stwierdzono różnic w zawartości miedzi w nasionach z poszczególnych obiektów. Największe pobranie przez części nadziemne roślin, wynoszące do  $57,4 \text{ g Cu} \cdot \text{ha}^{-1}$ , zanotowano w fazie zielonej luszczyny w kombinacji ANS.

**Słowa kluczowe:** miedź, nawóz N-S (saletrosiarczan amonu), siarka, rzepak