The fluctuation of copper content in oilseed rape plants (*Brassica napus* L.) after the application of nitrogen and sulphur fertilizers

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ABSTRACT

The influence of N-S fertilizers on the copper content in the inter oilseed rape plants was studied in field experiments. The evaluation involved two treatments of a single rate for the first spring fertilizer application with 100 kg N/ha in the AN treatment (nitrochalk) and 100 kg N/ha + 50 kg S/ha in the ANS treatment (ammonium nitrate and ammonium sulphate). A positive influence of the ANS fertilizer on the copper contents in different parts of plants was determined. The highest Cu concentrations were determined in the leaves and inflorescences, the lowest ones occurred in the stem. The concentration of Cu ranged within the interval of 1.56–8.75 mg Cu/kg of dry matter depending on the growth period and the part of the plant. No differences in copper content were determined in the seeds of individual treatment. The highest uptake in the above-ground parts of the plants was recorded in the green pod period and amounted to 57.4 g Cu/ha for the ANS treatment.

Keywords: copper; sulphur; winter oilseed rape; N-S fertilizers

The overall copper content on non-contaminated soils is usually within 2–40 mg Cu/kg. On contaminated soils more than 1000 mg Cu/kg was determined. Copper is predominantly absorbed by organic matter and also into iron and manganese hydrooxides. As stated by Sims (1986) and Zeien (1995), the proportion of individual sorbents differs according to soil type and soil texture; in the A soil horizon of humid areas it was determined as follows: 25–75% Cu was fixed in the organic fraction, 15–70% was fixed in Mn and Fe oxides, and 1–10% was fixed in silicates. At the value of pH < 6 the organic fraction forms the largest proportion; however if the soil pH reaction is neutral the fixation to oxides usually prevails. In the subsoil, copper is absorbed mainly into oxides (as much as 80% of the total content). The importance of iron oxides for the sorption of Cu is also indirectly shown in the analyses of the concretions from the gley and pseudogley soils, in which the Cu content is 8–10 times higher than in the surrounding soil (Sims 1986, Zeien 1995). Copper compounds of the $Cu_2(OH)_2CO_3$ type (malachite) (McBridge and Bouldin 1984) can occur in carbonate soils that are strongly contaminated with copper. Under anaerobic conditions sulphides (CuS, CuS_2) can form in the soil. The bond of copper fixed to organic compounds or to Mn and Fe oxides is very strong and an overwhelming proportion of Cu can be desorbed with difficulties. Therefore, the proportion of exchanged copper in the soils with pH > 5 is usually less than 1% of Cu_T.

The concentration of copper in the soil solution of agricultural soils is usually less than 0.03 to 0.3 mg/l. In the presence of HCO_3^- ions and organic complex-forming substances at pH > 6, the proportion of carbonate complexes ($CuCO_3^0$) and organic complexes of Cu (Cu_{org}) is more than 80% of the total copper content in the soil solution (McBridge and Bouldin 1984). Low molecular organic matter being formed as a product of the microbial decomposition of the postharvest crop residues or as a product of the root exudates can particularly contribute to the increase of Cu concentration in

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the soil solution (Herms and Brümmer 1984). At pH < 5 the proportion of Cu_{org} can reach 95% of the copper content of the soil solution. The presence or addition of high molecular insoluble organic compounds (e.g. peat) can markedly increase the sorption of copper and cause its deficit in the plant nutrition. If there is an absence or low content of soluble organic complex-forming substances (e.g. in the subsoil) while an acid soil reaction is present, there is a higher proportion of inorganic species of Cu, particularly Cu^{2+} or $CuSO_4^0$. Cu^+ ions can form only under strong reduction conditions. Apart from $CuCO_3^0$, at weakly acid to neutral pH values the soil solution also contains hydroxo-Cu-complexes [$CuOH^+$, $Cu(OH)_2^0$] (Ritchie and Jarvis 1986). On the soil that is intensively fertilized with phosphorus or has a high phosphorus content in the soil solution, Cu-phosphate complexes can form, resulting thus in a decrease of Cu content in the soil solution.

Copper is taken up by plants mainly in form of the Cu^{2+} ion and also probably in the form of low molecular organic complexes. The copper content in plants usually ranges within 2–20 Cu/kg of dry matter. A toxic effect of copper was recorded in plants (in leaves) at the level of 20–35 Cu/kg in dry matter (Lidon and Henriques 1992). However, Reuter and Robinson (1997) indicated the toxicity level for oilseed rape plants as low as 15 mg Cu/kg.

Copper shows similar properties as iron, i.e. 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 (Graham 1979). The same applies for cytoplasm and the cell organelles in which the concentration of Cu^{2+} a 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 for 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 shown by Thiel and Finck (1973) the plants with higher nitrate nutrition also have a higher requirement for copper. Decreased lignification of cell walls is a typical indication of insufficient copper nutrition in higher plants. It is known that copper deficiency affects the yield and seed formation more than the vegetative growth. Deficient copper nutrition causes pollen sterility. Copper content in the vegetative parts of plants at the level of 1–5 mg Cu/kg of dry matter is critical, but it also depends on the plant species, plant organ, developmental period and nitrate nutrition (Thiel and Finck 1973).

Sulphur is nowadays beginning to be a limiting element in the nutrition of winter oilseed rape. Application of sulphate fertilizers before rape sowing and N-S fertilizer application during growing period has become a common part of cultivation technology. The aim of the published experiments was to determine the influence of N-S fertilizers on the copper content and Cu removal during vegetation period of oilseed rape.

MATERIAL AND METHODS

A field experiment was established in Prague-Uhříněves, at the experimental station of the Faculty of Agrobiology, Food and Natural Resources. The following treatments were followed in the experiment:

(1) 100 kg N/ha (a single application of AN: nitrochalk, 27% N) – sidedress the first spring application;
(2) 100 kg N/ha + 50 kg S/ha (a single application of ANS: ammonium nitrate + ammonium sulphate, 26% N and 13% S) – sidedress the first spring application.

The area of the trial plot was 20 m^2. There were 4 replicates of each variant. The soil used in the experiment was Luvisol. The sorption complex was saturated. Within the agricultural chemical analyses of soils the following contents of available nutrients were determined (Mehlich III): 220 mg/kg of potassium, 119 mg/kg of phosphorus and 123 mg/kg of magnesium. The total sulphur content was 850 mg/kg, mineral S content was 4–7 mg/kg (before fertilizers application); the total copper content was 26 mg/kg; pH/$CaCl_2$ value was 6.2.

Winter oilseed rape (Bristol cultivar – 00 variety) was used as the experimental crop.

The total contents of chemical elements in plant samples were determined in mineral extracts, which were obtained with the dry decomposition method. The content of copper was determined by the optical emission spectrometry with induction fixed plasma (ICP-OES, Varian VistaPro, Australia).

More information is available in the paper by Balík et al. (2006).

RESULTS AND DISCUSSION

The Uhříněves site is very fertile, with the higher average yields achieved over a period of three years. In the unfertilized control treatment the seed yield was 3.7 t/ha, while in the AN treatment it was 49% higher and in the ANS treatment it was 60% higher. After the application of ANS fertilizer particularly the concentrations of S, Mn, and Zn in the plants were conclusively increased (Balík et al. 2005a, b), while the concentration of B was not significantly changed. The decrease in the molybdenum content was statistically significant (Balík et al. 2006).

The plants that are sensitive to Cu deficiency include wheat, oats and spinach. The relatively tolerant plants include peas, rye and oilseed rape (Alloway and Tills 1984). Data on the Cu concentrations in the plants of winter oilseed rape for the current period in the Czech Republic are not available. Neuberg (1990) report less than 3 mg Cu/kg of dry matter of leaves (with the vegetation height of 30–40 cm) as a very low content, 3–5 mg Cu/kg as low, 5–20 mg Cu/kg as medium, and above 20 mg Cu/kg as high. Similarly, Bergmann (1993) considers 5–12 mg/kg in the dry matter of the oilseed rape leaves (with the vegetation height of 30–50 cm) as an adequate content.

Khurana et al. (2006) carried out vegetation experiments with oilseed rape in sand cultures. They consider 3.8 mg/kg of the dry matter as a deficient content in young leaves. Finck (1979) published the values of an optimum copper content in fully developed leaves at the budding period at the level of 3–5 mg Cu/kg of dry matter. Subsequently, Finck (1997) amended these values to 5–10 mg Cu/kg of dry matter.

As it is clear from the Figure 1a, 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 our experiments was sufficient. The Figures 1a–1c 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 than in the AN treatment. This tendency towards the Cu increase (although no significant differences have been determined) has to be subjected to a critical evaluation. The results show clearly that the highest contents were deter-

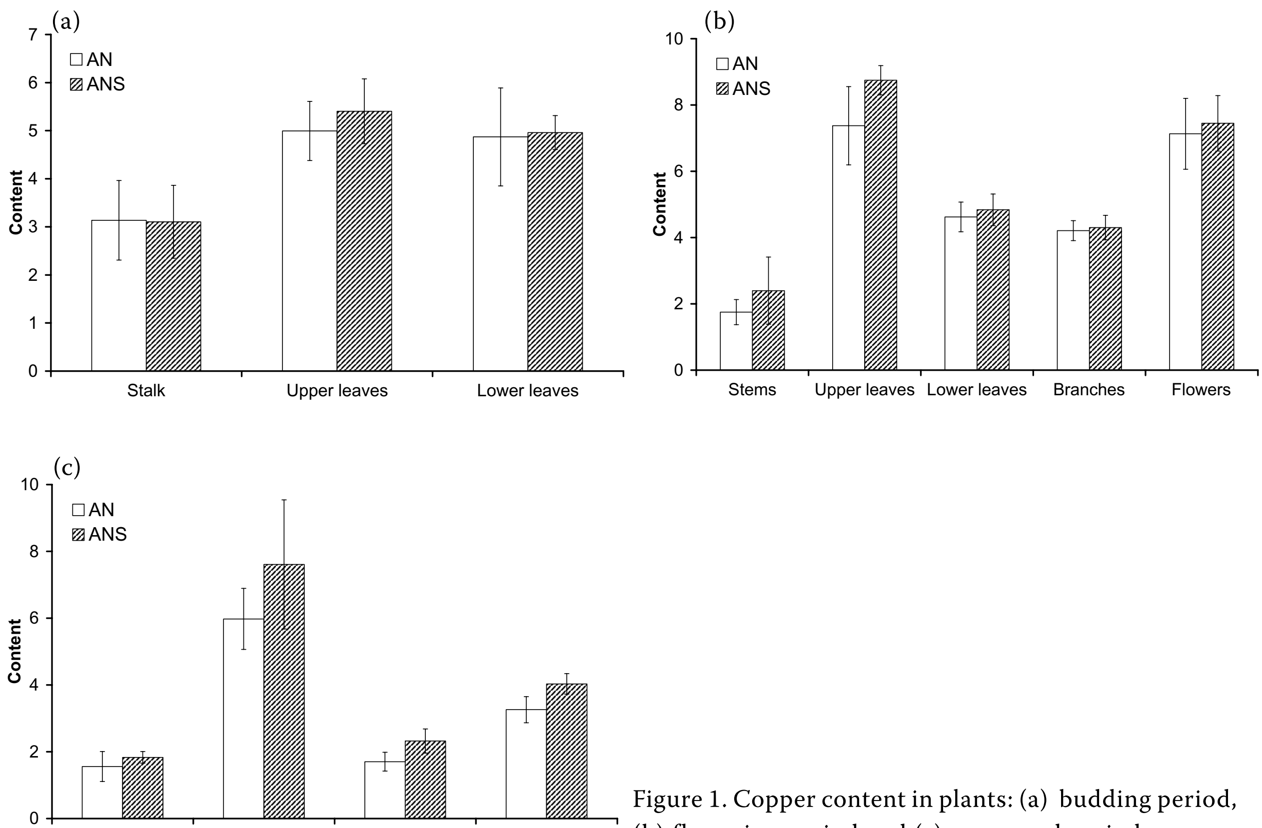

Figure 1. Copper content in plants: (a) budding period, (b) flowering period and (c) green pod period

mined in the leaves, particularly in the young ones. Inflorescence analysis also showed high contents. At all collection dates the lowest concentrations were determined in the stems, or branches. The values obtained in our experiment are in good correlation with the results of Rossi et al. (2002), who showed the concentration in the leaves of oilseed rape at 10.5 mg/kg of dry matter in the leaves, and 7.6 mg/kg of dry matter in the stems. Similarly, Angelova et al. (2004) also found higher concentrations in the leaves than in the stems.

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, CEC value, content and quality of organic matter, content of clay minerals and Fe and Mn oxides (McLaren and Crawford 1973, Alloway 1995). 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 (Kabata-Pendias and Pendias 1992, Tyler and Olsson 2001). Increase in the pH value lowers the availability of copper for plants (McLaren and Crawford 1973). An acidifying 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 mg/kg in the AN treatment and 9.2 mg/kg in the ANS treatment; in the flowering period it was 6.3 mg/kg in the AN treatment and 15.2 mg/kg in the ANS treatment; in the green pod period it was 6.1 mg/kg in the AN treatment and 14.3 mg/kg in the ANS treatment.

A number of authors (Hinsinger 2001a, b) however emphasise 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). This corresponds very well with the findings of Chaignon et al. (2002) who determined a significantly higher sensitivity to toxic copper contents in the soil in tomato plants than in the plants of oilseed rape. In extremely acid soils the plants of oilseed rape were more able to increase the pH value of the rhizosphere than the tomato plants. The changes in the rhizosphere pH values were 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 our experiments the different forms of nitrogen apparently did not have any influence on the uptake of copper. As determined by soil analyses, no significant differences in the NH_4^+ and NO_3^- contents in the topsoil and subsoil were found after 35 days of fertilizers application. This is also in a good agreement with the 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 SO_4^{2-}) by the plants in the ANS treatment was equalised by an increased uptake of cations. This was evidently a very significant factor influencing the increased Cu concentration in these variants. 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 of 1.18% (upper leaves) and 0.97% (lower leaves).

During the entire vegetation period the ANS treatment showed an increased formation of the biomass. It is known that plants excrete a considerable proportion of the photosynthesis production in the form of root exudates. It can be therefore expected that there was a greater quantity of exudates particularly in the ANS treatment. As stated by Herms and Brümmer (1984), it is the low molecular organic substances that contribute to the increased Cu concentration in the soil solution. The results of Hinsinger (2001a, b) show that the root respiration and exudation of organic acids contribute to the acidification of the rhizosphere. According to the results of Nye (1986) and Hinsinger (2001b), the CO_2 respiration can significantly contribute to the acidification of the rhizosphere only on calcareous soils, not acid, since H_2CO_3 dissociates significantly only in the area of neutral and alkaline pH (the pK value is 6.36). At the Uhříněves site the value of pH/$CaCl_2$ was 6.2 and a more significant influence of the root respiration and microorganisms on the change in pH cannot therefore be considered. By contrast, an increased formation of exudates in the plants with ANS fertilization evidently contributed towards a higher mobility of copper in the soil environment.

It is of interest that the differences in the Cu contents in the vegetative organs did not correspond to the differences in the Cu contents in the seeds. An average concentration was 2.54 mg Cu/kg of the seed dry matter in the AN treatment, and 2.55 mg Cu/kg of the seed dry matter in the ANS treatment. Our values are higher than the critical content of 2.2 mg Cu/kg, stated by Khurana et al. (2006). This again documents the fact that

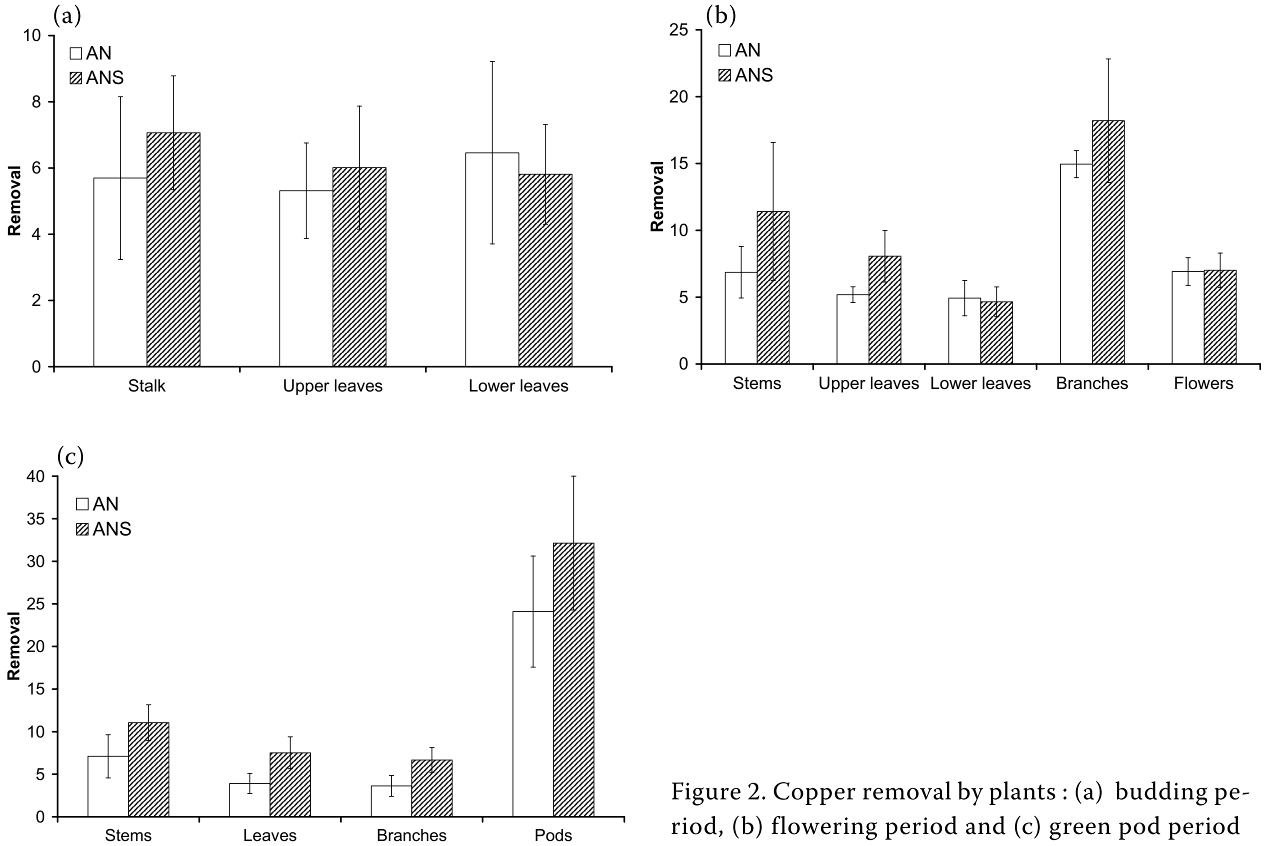

Figure 2. Copper removal by plants : (a) budding period, (b) flowering period and (c) green pod period

the oilseed rape plants in our experiments were adequately supplied with copper.

Higher Cu concentrations in the ANS treatment plants, together with the increase in biomass resulted in significant differences in the uptake of this element by the plants' biomass (Figures 2a–c). During the budding period the above ground biomass accumulated 17.5 g/ha in the AN treatment, and 18.9 g/ha in the ANS treatment. During flowering period this difference increased further. The extraction was 38.9 g/ha in the AN treatment and 49.4 g/ha in the ANS treatment. The values for the green pod period were determined as 38.9 g/ha (AN) and 57.4 g/ha (ANS). These values are several times higher than those determined by Kadar et al. (2001)on Chernozems. From the point of view of our results the CETION statement (Fábry 1992) is relevant. The authors mention a total removal of 37 g Cu/ha for the seed yield of 3.5 t/ha.

With respect to the values of the Cu removal dynamics during the vegetation of the ANS treatment it can be concluded that the accumulated Cu quantity in the plants was continuously increasing. This does not correspond to the Cramer removal curves (Vašák et al. 1997), which determined the peak of Cu removal in the flowering period. From the total amount of removed copper approximately 25–35% is related to the seed harvest, and the rest remains on the land in the postharvest crop residues. In the case of winter oilseed rape a similar situation can be observed for the majority of other nutrients. This factor also contributes to the evaluation of winter oilseed rape as an excellent preceding crop.

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