Stowasser, W. F. 1991. Analysis of the phosphate rock situation in the United States: 1990-2040. Engineering and Mining Journal 192/10:16.

Ståhlberg, S. 1980. A new extraction method for estimation of plant-available P, K and Mg. Acta Agric. Scand. 30:93-107.

Technicon Scandinavia. 1976. Product Labeling for Technicon Autoanalyzer II Continuous-flow Analytical Instrument. Technical Publication No UA4-0170-01. Technicon Instruments Corporation, Tarrytown, NY.

COMMUN. SOIL SCI. PLANT ANAL., 27(5-8), 1513-1526 (1996)

# INFLUENCE OF NITROGEN, PHOSPHORUS, AND POTASSIUM ON PIGMENTS CONCENTRATIONS IN CUCUMBER LEAVES

**Z. Lamrani, A. Belakbir, J. M. Ruiz, L. Ragala, I. López-Cantarero, and L. Romero**

*Department of Plant Biology, Faculty of Sciences, University of Granada. 18071-Granada, Spain*

***ABSTRACT***: Cucumber plants (*Cucumis sativus* cv. Brunex) were grown under controlled conditions and submitted to an individual fertilization with different doses of N ($N_1$= 5g/m$^2$; $N_2$= 10g/m$^2$; $N_3$= 20g/m$^2$; $N_4$=40g/m$^2$) in $NO_3NH_4$ form; P (P1= 7 g/m$^2$; P2= 14 g/m$^2$) in $H_2PO_4$ form and K (K1= 20 g/m$^2$; K2= 40 g/m$^2$) in $SO_4K_2$ form. This fertilization was complemented with organic matter and micronutrients solution, these doses were standard throughout the plant biological cycle. Control plants were included in the experimental design. Cucumber leaves were picked every 15 days all along the plant cycle and they were subjected to the determination of chlorophyll a and b (Chl a, Chl b), carotenes and licopenes. Our results showed that N fertilization have induced an increase of 15% in Chl a respect to control plants in the case of $N_3$ treatment, whereas $N_4$ resulted in a decrease of Chl a. Chlorophyll b presented similar behavior than Chl a, 10% of increase was obtained in $N_3$. Carotenes behaved with the same manner than chlorophyll, while licopenes did not show any significant variation among treatments. Inversely, P fertilization induced a decrease of both chlorophyll in $P_2$ treatment, whereas the P did not affect accessory pigments concentration. $K_2$ treatment acted positively on chl a and carotenes levels, with 11% and 7% of increase respect to control plants respectively. Chl b did not present any significant differences within K doses. In summary, our results lead us to think that

N affected pigments contents, specially photosynthetic pigments, and high doses of P have acted negatively on all the pigments studied.

## INTRODUCTION

Chlorophyll are important for energy capture during the photosynthesis process. Differences in chlorophyll activity, could be considered as a current symptom of an inappropriate fertilization, or a nutritional imbalance, however other environmental factors such as temperature, salinity or drought can decrease chlorophyll activity (Zekri, 1991)

In many horticultural crops grown under greenhouse conditions, it's common to use fertilization in order to increase production and fruit quality, improving many physiological processes such as nutrient absorption, photosynthesis activity and therefore increase plant growth (Bauler and Fricker, 1970).

Puritch and Barker (1967) found that as $NH_4$ toxicity symptoms progressed, there were gross changes in chloroplast morphology, plastid degradation, and loss of chorophyll. An adequate N supply is considered to be essential in the establishment of maximum sink capacity and in maintaining photosynthetic capacity in rice (Murata and Matsushima, 1975). However, Murata and Matsushima suggested that excessive use of N is undesirable since it can result in excessive leaf area expansion, which is inversely correlated with photosynthetic capacity.

Numerous studies have demonstrated that K-deficiency plants have reduced rates of photosynthesis and translocation (Jackson and Volk, 1968). It is generally recognized that K-deficiency results in decreased plant growth and perturbations in many aspects of leaf metabolism, such as altered carbohydrate concentrations and decreased rates of photosynthesis and translocation (Huber, 1984). Inversely a high level of K nutrition promotes formation of chlorophyll and better utilization of light energy (Forster, 1982). Collins and Duke (1981) concluded that providing K increased the chlorophyll content of alfalfa leaves.

With respect to P nutrition, it was reported that P deficiency may reduce photosynthesis activity (Fredeen et al. 1990). This decrease seems to be related to an accumulation of polysaccharides (Sheu-Hwa et al., 1975). A supply of inorganic P increases photosynthesis (Bouma, 1975); however, few workers studied the relationship between P nutrition and chlorophyll content in plants.

Due to the excessive and combined use of N, P, and K fertilizers in the zone under study, the objective of the current work was to determine the effect of the individual fertilization of N, P, and K separately on the behavior of chlorophyll, carotenes, and licopenes in cucumber leaves to see if they could indicate the nutritional status of plants (López-Cantarero et al., 1994).

## MATERIAL AND METHODS

### Crop Design

Cucumber plants (*Cucumis sativus* L. cv. Brunex), were grown under controlled conditions of greenhouse in southeast of Spain, with fertigation system. The experiment was conducted under the following conditions: 25°C/18°C day/night temperature and 60/80% day/night relative humidity. The experimental design consisted in 27 plots, which corresponded to eight N, P, K treatments with three replications each one. Three plots were used for control plants, 14 plants per plot were used for this experience. N, P and K treatments were applied as follows: four doses of N ($N_1$= 5g/m$^2$, $N_2$= 10g/m$^2$, $N_3$= 20g/m$^2$ and $N_4$= 40g/m$^2$) in $NH_4NO_3$ form, two levels of P ($P_1$= 8g/m$^2$, $P_2$= 16g/m$^2$) supplied as $H_2PO_4$ and finally two levels of K ($K_1$= 20g/m$^2$, and $K_2$= 40g/m$^2$) administered as $K_2SO_4$. All fertilizers were applied throughout the biological cycle of plants, the experimental plots were covered with organic matter of 2 cm height, applied as manure, the fertilization was complemented with a macro and micronutrients solution administered as sulphates except for Fe applied as Fe-EDDHA (5 ppm), and B as $H_3BO_3$ (0.5 ppm). Manganese (2 ppm), Zn (1 ppm), Cu (0.25 ppm), and Mo (0.05 ppm) were also used. Control plants were fertilized only with organic matter.

**Plant Analysis**

Leaves free of damage and at the same physiological stage, were picked every 15 days from the middle part of the plants throughout the vegetative cycle, the material was washed three times with distilled water after disinfecting with non ionic detergent at 1% (Wolf, 1982).

**Chlorophyll Analysis**

Chlorophyll a and b were extracted following the procedure described by Hiscox and Israelstam (1979). Forty-mg leaf disks were submerged into dimethyl sulfoxide solution (DMSO) then incubated at 65 °C during 90'. Chlorophyll were determined by colorimetric spectrophotometry at the different wavelengths proposed by Bruinsma (1963). Chlorophyll were expressed as mg/100g f.w. using McKinney equations (1941).

**Carotene Analysis**

For carotene determination we followed the procedure proposed by Jaspar (1965) by spectrophotometry at 502 nm. Carotenes were expressed as mg/100 g f.w.

**Licopene Analysis**

Leaf (1g) disks were incubated during 24h in methanol HCl solution, licopenes were determined by spectrophotometry at 530 nm wavelength (Bajaj et al., 1981), The results were expressed as percentages.

## RESULTS AND DISCUSSION

Generally, the different treatments showed an increase in chlorophyll a concentrations respect to control plants. In the case of N fertilization (Fig. 1), the higher increase expressed as percentage was found in $N_3$ (15%) while in $N_1$ and $N_4$ we observed 5% and 6% increase respectively. It seems that an excess of N doses, as it was the case of $N_4$, was involved in a decrease in the concentration of chlorophyll a, by an inhibition in the synthesis of this pigment. Thus, the determination of chlorophyll a could be useful in the diagnosis of N levels in plants, in order to avoid the excessive use of N fertilizer and therefore soil contamination.

As shown in Figure 1, the effect of P on chlorophyll a was partially similar to that of N, since the low doses of P ($P_1$) induced a 15% increase respect to control plants, whereas the higher P dose produced a decrease in the concentration of chlorophyll a, presenting only 8% over control plants. We can deduce, that although P fertilization conditioned the content of chlorophyll a, the level of this pigment was not directly dependent on P, phosphorus application did not improve the concentration of chlorophyll a, this behavior probably, was due to the small range of P levels applied. Inversely and in the case of N fertilization, the relationship between N and chlorophyll a was evident (Fig. 1).

K fertilization induced an opposite effect to that of P one, the results of chlorophyll a were proportional to K doses (Fig. 1). Increases of 8% and 12% were observed within $K_1$ and $K_2$ respectively. This result leads us to think that a direct relationship exists between K and chlorophyll a; however, it would be interesting to increase the doses of K fertilization in order to further investigate this phenomenon. These findings could be explained by the role of K in the process of photosynthesis (Steven, 1985), in protein synthesis and growth (Marshner, 1995). Our results suggest that a positive relationship exists between K and chlorophyll a.

The effect of fertilization on chlorophyll b levels is represented in Figure 2. As expected the behavior of chlorophyll b was similar to that of chlorophyll a, thus presenting a 2%, 6%, and 10% increase with respect to control plants, within $N_1$, $N_2$ and $N_3$ treatments, respectively. The higher dose of N fertilizer showed an evident decrease of chlorophyll b, obtaining the same level than control plants. This result indicates that $T_0$ free of N, or $N_4$ plants with high N dose, produced a decrease of chlorophyll b, which could consequently reduce photosynthesis rate, chlorophyll b levels in non-treated plants or in those supplied with high N dose, showed the same behavior.

Phosphorus fertilization in the current experience exerted a negative effect on foliar chlorophyll b (Fig. 2), since $P_1$ presented a 6% increase while $P_2$ showed only 2% increase with respect to control plants. This phenomenon was similar but more

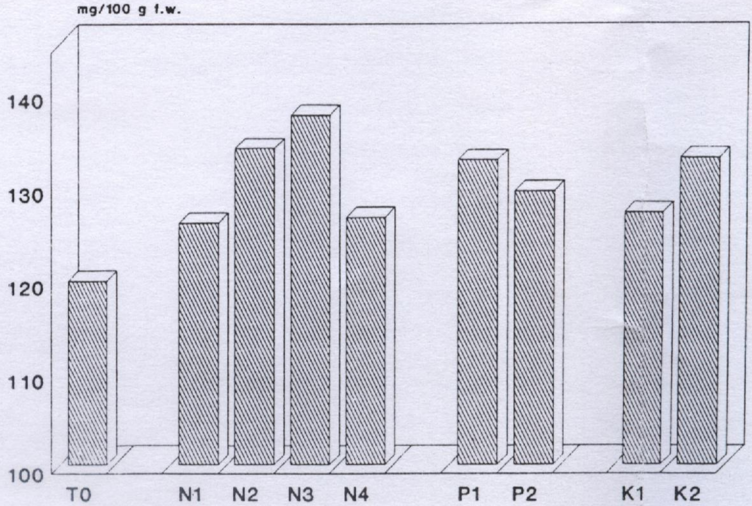


Fig.1.- The effect of N, P and K treatments on chlorophyll a concentration in cucumber leaves.

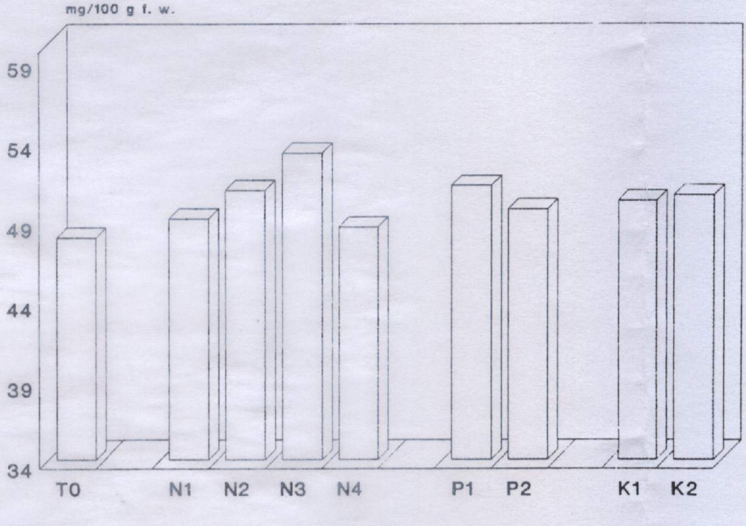


Fig.2.- The effect of N, P and K treatments on chlorophyll b concentration in cucumber leaves.

pronounced to that observed with chlorophyll a. Our results lead us to think that an excess of P fertilization could inhibit the synthesis of chlorophyll pigments.

The process above described was not observed with K treatments (Fig. 2). The effect of K doses on chlorophyll b was very similar to that observed for chlorophyll a, a 4% increase in chlorophyll b was found in $K_1$ and 6% in $K_2$, both with respect to control plants. Thus, K could be involved in the formation of photosynthetic pigments, specially chlorophyll b.

In many plant species chlorophyll a levels are greater than those of chlorophyll b so, as expected, total chlorophyll (Chl a + Chl b) presented analogous behavior to that of chlorophyll a. In the current study, the higher content of total chlorophyll was observed in $N_3$ followed by $N_2$, 14% and 11% increases were found in the two treatments, respectively, over control plants (Fig. 3, whereas only a 5% increase was found in both $N_1$ and $N_4$ treatments. These results showed that low ($N_1$) or high N ($N_4$) doses could limit, by deficiency or toxicity, the formation of chlorophyll.

Total chlorophyll showed similar responses to P doses (Fig. 3). Thus, in $P_1$ total chlorophyll reached 10% increase, while in $P_2$, 6% difference was observed over control. This result demonstrates that high P doses could be toxic for total chlorophyll accumulation in cucumber leaves.

On other hand, K had an opposite effect on total chlorophyll levels (Fig. 3), K doses were proportional to total chlorophyll concentrations. Thus $K_1$ induced a 7% and $K_2$ a 10% increase, both over control plants. Therefore K acted positively on chlorophyll pigments and this favored the assimilation capacity of leaves.

The ratio chl a/chl b (Fig. 4) revealed the dominance of chlorophyll a. Among the treatments applied there was a relationship between the degree of chlorophyll a dominance and the dose of fertilizers. The results showed 4%, 5%, 6%, and 7% increases within $N_1$, $N_4$, $N_3$ and $N_2$, respectively, demonstrating that low or high N doses produced similar effects on chlorophyll and consequently on the balance chl a/chl b of the treated plants.

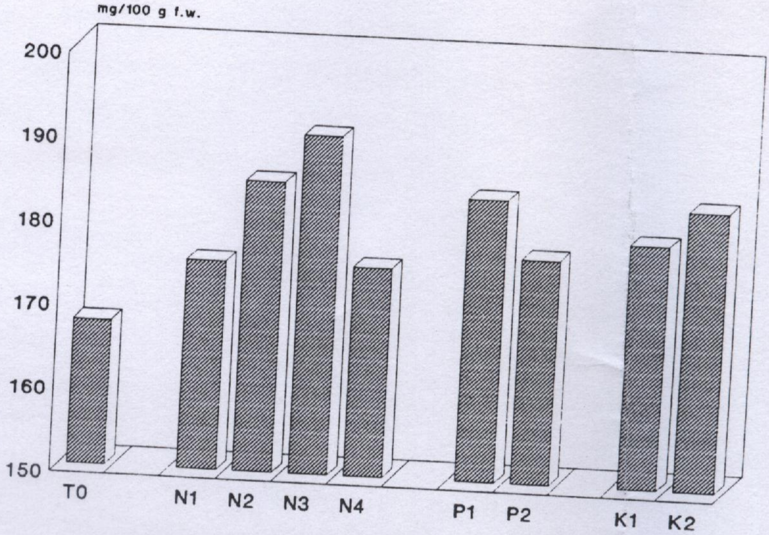


Fig.3.- The effect of N, P and K treatments on total chlorophyll in cucumber leaves.

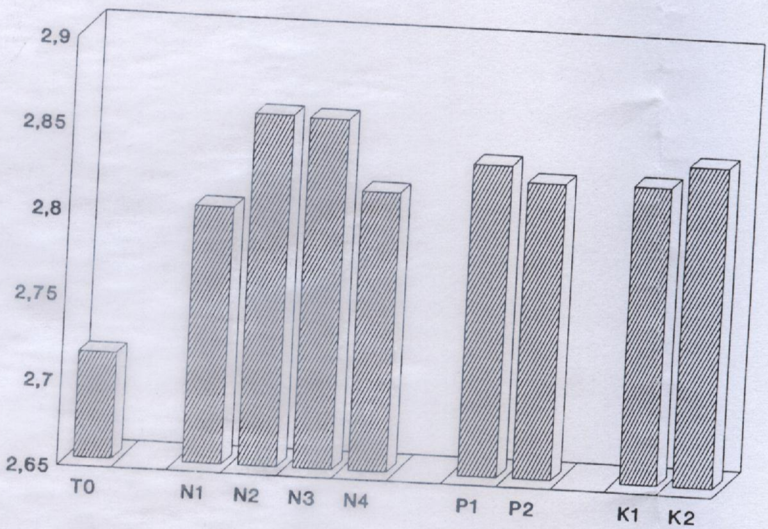


Fig.4.- The effect of N, P and K treatments on Chlorophyll ratio in cucumber leaves.

The impact of P on the ratio chl a/chl b (Fig. 4) was similar to that observed in the case of chlorophyll a and b separately. Thus in $P_1$, the increase was slightly greater (6%) than the rise in $P_2$ (5%) respect to $T_0$ plants. Moreover the effect of P could be involved in a distortion of the ratio chl a/chl b, and therefore of the photosynthetical balance between pigments.

K acted positively on the chl a/chl b ratio (Fig. 4), since an increase of K doses produced an increase from 4% ($K_1$) to 5% ($K_2$), respect control plants. From this finding, we could suppose that K was not only related to chlorophyll determined individually, but also to their ratios, leading to a good photosynthetical balance.

Another aspect of this study was to determine the effect of N, P and K fertilizer on accessory pigments such as licopenes and carotenes. The levels of licopenes were not affected by the different doses of the treatments applied, among N, P and K levels the concentration of licopenes remained practically unchanged respect to the result obtained in control plants (Fig. 5). Inversely, carotenes levels showed similar responses to N treatments than the observed for chlorophyll a and b (Fig. 1 and 2), since $N_3$ induced the higher increase (11%) respect control plants, followed by $N_4$ (5%), $N_2$ (4%,) and finally by $N_1$ (2%). This phenomenon could demonstrate clearly that $N_3$ represented the optimal dose of the nitrogenous fertilization applied.

Concerning P treatments in Figure 5, we observed analogous results to those obtained in the case of chlorophyll. In $P_1$ the increase was slightly greater (6%) than in $P_2$ (5%). We thus conclude that high doses of P fertilization induced a reduction of carotene level in cucumber leaves as well as chlorophyll a and b one.

Similarly to chlorophyll, the response of carotene to K treatments, was proportional to K doses applied, thus 4% and 7% increases over control plants was found within $K_1$ and $K_2$ respectively. Therefore K acted positively on carotene accumulation in leaves.

Independently of the treatment applied, the levels of chlorophyll a in cucumber leaves were approximated to those obtained in previous experiences with

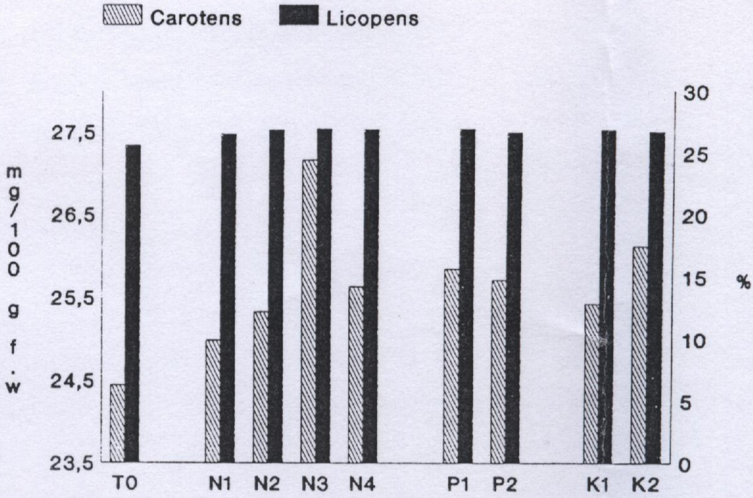


**Fig.5.- The effect of N, P and K treatments on Accessory pigments concentration in cucumber leaves.**

melon (Valenzuela et al., 1993), eggplants (López-Cantarero et al., 1994) and with pepper plants (Sánchez and Romero, 1995). In all the mentioned experiences, there was a direct relationship between the N applied and the increase of chlorophyll a levels. Moreover, in the same experiments, no evident effects of P fertilizer was observed on pigments (López-Cantarero and Romero, 1993), inversely to K which was closely related to pigments (Sánchez and Romero, 1995).

Concerning chlorophyll b, the amounts found were similar to those obtained by other workers (Valenzuela et al., 1993; López-Cantarero and Romero, 1993; Sánchez and Romero, 1995). In the current study, N and K acted positively on this pigment, whereas high P doses affected negatively chlorophyll b levels (Fig. 2).

Total chlorophyll or chl a/chl b ratio were conditioned by the content of chlorophyll a in cucumber leaves, N and K doses enhanced both ratios, In contrast, P may produce an imbalance between chlorophyll a and b, moreover the ratio chl a/chl b was more affected by N fertilizer than K as could be expected.

Carotenes could be used as indicators of an appropriate N or K fertilization, but not of P. Our results are supported by those of Sánchez and Romero (1995) who found that carotenes as well as chlorophyll a and b, were related with the amounts of the N administered as fertilizer.

## CONCLUSIONS

Generally, chlorophyll a and b were closely related with the concentration of N, P, and K fertilizers applied. The effect of N was more pronounced than the other fertilizers. The relationship between chlorophyll and N, could be explained by the fact that in chloroplasts, chlorophyll are linked to structural proteins. Roux (1982) and Sestak (1985) reported that the formation of this complex is indispensable in the process of chlorophyll biosynthesis (Haworth et al., 1983). Nitrogen deficiency could produce a reduction in proteins synthesis and therefore may induce the distortion of chlorophyll synthesis (López-Cantarero et al., 1994).

In the present work, assessment of N status in cucumber plants has been based on the determination of chlorophyll a and b. In our study or others, this approach is usually used for one season in order to correct the potential nutrient stress. Some workers use the SPAD chlorophyll meter to assess the N status in trees, especially apple trees (Neilsen et al., 1995). This technique was also used in a variety of annual crops such as maize (Follet et al., 1992) and wheat (Piekielek and Fox, 1992). In the current study, the determination of accessory pigments, and specially carotenes was interesting, since it can be used to assess N and less K status, whereas no relationships were observed between licopenes and N, P, and K fertilizers.

## REFERENCES:

Bajaj, K. L., G. Kour, M. L. Chadha, and B. P. Singh. 1981. Polyphenol oxidase and other chemical constituents varieties. Vegetable Science 8:37-44.

Bauler, H. and C. Fricker. 1970. The Fertilizer Treatment of Forest Trees. Transl. by C.L. Whittles, BIV. Verlag, Munich, Germany.

Bouma, D. 1975. Effects of some metabolic P compounds on rates of photosynthesis of detached P deficient subterranean clover leaves. J. Exp. Bot. 26:52-59.

Bruinsma, J. 1963. The quantitative analysis of chlorophylls a and b in plant extracts. Photochem. Photobiol. 2:241-249.

Collins, M. and S. H. Duke. 1981. Influence of potassium fertilization rate and form on photosynthesis and $N_2$ fixation of alfalfa. Crop Sci. 21:481-485.

Follet, R. H., R. F. Follet, and A. D. Halvorson. 1992. Use of a chlorophyll meter to evaluate the nitrogen status of dryland winter wheat. Commun. Soil Sci. Plant Anal. 23:687-697.

Forster, H. 1982. Capacity of various plant species for the uptake of potassium in pot experiments. Potash Rev. Subject 16, 90th Suite, no. 9, Bern, Switzerland.

Fredeen, A. L., T. K. Raab, I. M. Rao, and N. Terry. 1990. Effects of phosphorus nutrition on photosynthesis in glycine max. L. Mer. Planta. 181:399-405.

Haworth, P., J. L. Watson, and C. J. Armtzen. 1983. The detection, insolation and characterization of a light harvesting complex which is specifically associated with photosystem I. Biochem. Biophys. Acta. 724:151-158.

Hiscox, J. D. and G. F. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57:1332-1334.

Huber, S. C. 1984. Biochemical basis for effects of K-deficiency on assimilate export rate and accumulation of soluble sugars in soybean leaves. Plant Physiol. 76:424-430.

Jackson, W. A. and R. J. Volk. 1968. Role of potassium in photosynthesis and respiration, pp. 109-145. IN: The Role of Potassium in Agriculture. American Society of Agronomy, Madison, WI.

Jaspar, E. M. J. 1965. Pigmentation of tobacco Crown-Gall tissues cultured in vitro in dependence of the composition of the medium. Physiol. Plant. 18:933-940.

López-Cantarero, I., F. A. Lorente, and L. Romero. 1994. Are chlorophylls good indicators of nitrogen and phosphorus levels? J. Plant Nutri. 17:979-990.

López-Cantarero, I. and L. Romero. 1993. Influencia de la salinidad sobre indicadores bioquímicos en plantas de *Solanum melongena* cv. Bonica. Granada, Spain.

Marshner, H. 1995. Mineral Nutrition in Higher Plants, 2nd edition. Academic Press, London, England.

McKinney, G. 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. 40: 315-332.

Murata, Y. and S. Matsushima. 1975. Rice, pp. 73-99. IN: L. T. Evans (ed.) Crop Physiology. Cambridge University Press, London, England.

Neilsen, D., E. J. Hogue, G. H. Neilsen, and P. Parchomchuk. 1995. Using SPAD-502 values to assess the nitrogen status of apple trees. HortScience 30:508-512.

Piekielek, W. P. and R. H. Fox. 1992. Use of a chlorophyll meter to predict sidedress nitrogen requierements for maize. Agron. J. 84:59-65.

Puritch, G. S. and A. V. Barker. 1967. Structure and function of tomato leaf chloroplasts during ammonium toxicity. Plant Physiol. 42:1229-1238.

Roux, E. 1982. Le devenir de l'énergie au cours de la photosynthèse. C.R. Acad. Agr. Fr. 68:834-846.

Sánchez, A. and L. Romero. 1995. Efecto de la aplicación NK en condiciones de salinidad sobre algunos parámetros fisiológicos y bioquímicos en plantas de pimiento *Capsicum annuum* L. var. Lamuyo. Granada, Spain.

Sestak, Z. 1985. Chlorophylls and carotenoids during leaf antogony, pp. 76-106. IN: Photosynthesis During Leaf Development. Academia Praha, Prague, Czech Republic.

Sheu-Hwa, C., D. H. Lewis, and D. A. Walker. 1975. Stimulation of photosynthetic starch formation by sequestration of cytoplasmic orthophosphate. New Phytol. 74:383-392.

Steven, C. H. 1985. Role of potassium in photosynthesis and respiration, pp. 368-391. IN: D. M. Munson (ed.) Potassium in Agriculture. American Society of Agronomy, Madison, WI.

Valenzuela, J. L., A. Sánchez, and L. Romero. 1993. Influencia de la fertirrigación y el agua salobre sobre el metabolismo mineral y bioquímico en plantas de melon (*Cucumis melo* L. var. Galia). Granada, Spain.

Wolf, B. 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrients status. Comm. Soil Sci. Plant Anal. 13:1035-1059.

Zekri, M. 1991. Effects of NaCl on growth and physiology of sour orange and Cleopatra mandarin seedlings. Scientia Hortic. 47:305-315.

COMMUN. SOIL SCI. PLANT ANAL., 27(5-8), 1527-1536 (1996)

# DETERMINATION OF POTENTIALLY DANGEROUS ELEMENTS IN SOIL EXTRACTS BY ATOMIC EMISSION SPECTROPHOTOMETRY-ICP: CORRECTION OF INTERFERENCES

**Jiří Zbíral**

*State Institute for Agriculture Supervision and Testing, Hroznová 2, CZ-656 06, Brno, Czech Republic*

***ABSTRACT*:** Non-spectral interferences in AES-ICP are the main problem for the determination of some potentially dangerous elements in soil extracts. According to Czech law regulations content of As, Be, Cd, Co, Cr, Cu, Mo, Ni, Pb, V, Hg, and Zn have to be determined. Soils from the International Soil Exchange Programme (ISE) extracted in *aqua regia* were used for investigation. For all the elements and several spectral lines for most of them the influence of instrument conditions on the level of interferences was studied. The results were compared to the results found by FAAS, standard addition method and the results available from the ISE. No compromise interference-free instrument conditions for AES-ICP determination were found for this set of elements and therefore correction by comparative element was adopted. Scandium proved to be unsuitable because of its relatively high content in some soils. Lutetium added to the soil extracts in the concentration 5 mg $\cdot l^{-1}$ was finally chosen as a suitable comparative element. The agreement between the corrected results and the results obtained by interference-free procedures was very good. Correction is necessary only for ionic lines if measured in the height 6-8 mm above the apex of plasma where are practically no interferences on atomic lines.