INTERACTION BETWEEN CADMIUM AND IRON. ACCUMULATION AND DISTRIBUTION OF METALS AND CHANGES IN GROWTH PARAMETERS OF *PHASEOLUS VULGARIS* L. SEEDLINGS

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ABSTRACT

The interaction between cadmium, one of the most toxic heavy metals, and iron, an essential plant nutritional element, was investigated in *Phaseolus vulgaris* L. (cv. Słowianka) seedlings. The interaction was externally induced by changing the content of both metals in the nutrient medium. Under iron deficiency conditions (0 and 0.5 of normal dose of this element), the toxic effects of cadmium on plant growth parameters, like fresh and dry weight accumulation, primary leaves area, *etc.*, were generally much more pronounced than under normal iron supply. At normal and excess iron supply (1, 2 and 4 doses) cadmium diminished iron accumulation in roots and primary leaves, but on the other hand excess iron decreased cadmium level, preventing plants from extreme toxicity of very high cadmium concentrations in the growth environment. It is to be noted that iron is classified also as a heavy metal, and its excess may become toxic, *e.g.* decreasing root dry weight or diminishing leaf area, especially at the highest dose. The detoxication role of iron against cadmium, and possibly other toxic metals is, however, limited to concentrations of this element in the nutrient solution which themselves are not toxic for the organism.

KEY WORDS: Cadmium/iron interaction, *Phaseolus vulgaris* L., metal accumulation, plant growth.

INTRODUCTION

It was suggested over forty years ago that the excess supply of certain heavy metals like Cu, Zn or Mn, interfering with Fe metabolism may induce changes which resemble the Fe deficiency syndrome (Smith and Specht 1953; DeKock 1956). Some of these effects appeared to be reversible by application of extra Fe doses to the nutrient solution (Smith and Specht 1953). Up to then there were many other reports giving reasonable evidence to support the hypothesis that heavy metals supplied to plants in excess may cause physiological and metabolic responses characteristic of Fe deficiency (Hunter and Vergnano 1953; Khan and Khan 1983; Misra and Ramani 1991). However, Fe is also classified as a heavy metal and its excess may be toxic for plants (Kabata-Pendias and Pendias 1992).

Cd as heavy metal not essential to the metabolism of plants has become a major environmental pollutant being a byproduct of many industrial processes. It is mostly absorbed by plants from the soil, although in some cases it may enter the plant organism from the air. Although easily taken up by plants its uptake and accumulation depend on the species and plant organs involved. The multiplicity and complexity of the toxic influence of Cd on plants makes it impossible to distinguish between direct and indirect mechanisms of its action (Baszyński 1986; Barceló and Poschenrieder 1990; Van Assche and Clijsters 1990; Krupa and Baszyński 1995). One of the most commonly observed external symptoms of Cd toxicity is leaf chlorosis. The most important reason for that is an observed interaction between Cd and Fe (Bjerre and Schierup 1985; Burzyński 1987, Greger and Lindberg 1987; Burzyński and Buczek 1989, Thys et al. 1991, Siedlecka and Baszyński 1993; Jalil et al. 1994). Cd induces Fe deficiency which leads to serious disturbances of the metabolism and of physiological processes, for instance inhibition of chlorophyll synthesis, diminution in the pools of Fe-containing electron carriers in the photosynthetic apparatus, retarded growth of roots and leaves, *etc.* (Miller et al. 1984; Terry and Abadia 1986; Burzyński 1987; Siedlecka and Baszyński 1993; Krupa and Baszyński 1995).

In this paper we have studied the effects of interaction between excessive Cd content in the nutrient medium and Fe at

Abbreviations:
PPFD – photosynthetic photon flux density

different levels (deficit, normal and excess) on accumulation and distribution of metals and plant growth parameters.

MATERIAL AND METHODS

Plant cultivation conditions.

Bean seeds (*Phaseolus vulgaris* L. cv. Słowianka) were germinated and grown on wet filter paper for 7 days in a darkened thermostated chamber at 25°C and 95% relative humidity. After that time etiolated seedlings were transferred to Hoagland full strength nutrient solution (Hoagland and Arnon 1950) containing 0.25 mM Fe in the form of ferric citrate (iron[III]citrate) as a basal source of Fe (1 dose). Plants (5 seedlings per 1 pot containing 1000 ml of nutrient solution) were grown for the next 7 days at 20°C and PPFD of 150 $\mu mol\ m^{-2}s^{-1}$ at a 16/8 h day/night regime. After this growth period plants were transferred to fresh nutrient solution containing variable Fe supplies and Cd concentrations. Fe doses were of 0, 0.5 (deficiency), 1 (normal supply), 2 and 4 (excess Fe). One Fe dose was equivalent to 0.25 mmol Fe in the form of ferric citrate. Cadmium (in the form of $3CdSO_4 \times 8H_2O$) was added to final concentrations of 0, 10, 20 and 50 µM. After 7 days of growth in these conditions plants were subjected to all subsequent analyses.

Metal content and growth analyses.

Fe and Cd content in roots and primary leaves was estimated after wet ashing of the dry plant material in a $HNO_3/HClO_4$ mixture (4:1, v:v) by atomic absorption spectrophotometry (Unicam 939AA Spectrometer). Primary leaves areas were measured using a Geniscan GS-4500 scanner (Genius, Taiwan), and the area was calculated by dedicated computer software manufactured by Witra (Warsaw, Poland). Dry weights and water content were determined after drying the plant material at 105°C until constant weight of samples was reached.

All results represent the mean ± SE obtained from at least 3 independent series of experiments (4-8 measurements each).

RESULTS AND DISCUSSION

Cd and Fe accumulation in bean plants

It is well known that Cd is strongly accumulated in plants when present in growth medium. According to Greger (1989) this heavy metal, when present in the nutrient medium at low concentrations, is taken up metabolically, but non-metabolically when in high ones. Cd affects the uptake and distribution of various nutrient elements in plants (Jastrow and Koeppe 1980). One of the most striking visible symptoms of such interference is chlorosis resulting mostly from Cd-induced Fe deficiency both in roots and shoots of treated plants (Terry 1981; Siedlecka and Baszyński 1993; Jalil et al., 1994). On the other hand, Cd uptake can also be influenced by other cations, including Fe (Thys et al. 1991; Siedlecka and Baszyński 1993; Siedlecka 1995).

In order to examine the effects of different combinations of Cd concentrations and Fe supplies in the nutrient solution, the metal accumulation both in roots and primary leaves of bean seedlings was analyzed. As seen from Fig. 1A Cd concentration in the nutrient solution had a substantial impact on Fe content in the plants grown on normal Fe supply. In this case Cd visibly altered the level of Fe in roots, diminishing its content at 50 µM Cd to 75% of control values. The inhibitory effect of Cd on Fe accumulation in roots was to a high extent reduced by additional Fe supply (2 and 4 doses) to the medium solution. Therefore, even at high concentrations Cd showed a small effect on Fe level in roots. On the other hand, at insufficient Fe supply (0 and 0.5 dose), Cd had also a negligible effect on Fe accumulation (Fig. 1A). It may be related to general Fe deficiency status of these plants.

Considering the possibility of Cd/Fe interaction the question arises about the role of Fe as a detoxification factor in Cd-treated plants. Fig. 1B shows that excessive Fe doses (2 and 4 times the normal one) had some effect on Cd accumulation at high concentrations of this heavy metal in the nutrient solution. The decrease of Cd accumulation in the roots of bean plants reached 15 to over 20% of the levels observed for plants grown at normal Fe supply. Since, when present in

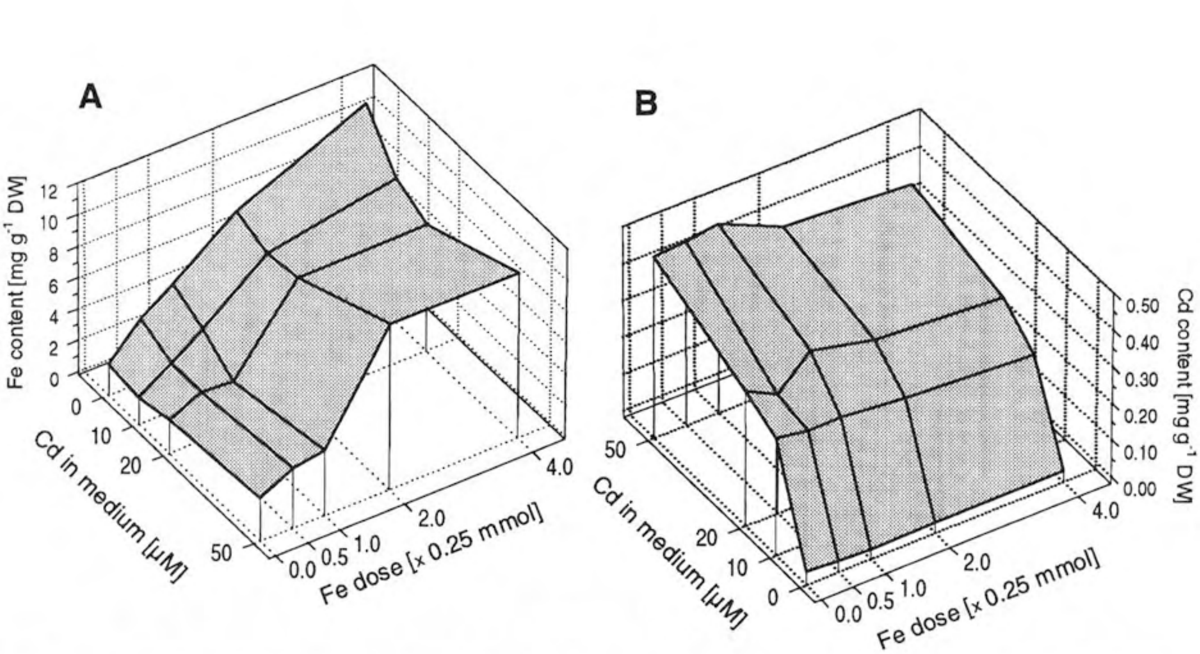

Fig. 1. The accumulation of Fe (A) and Cd (B) in roots of bean seedlings grown under Cd/Fe interaction conditions. Doses 0 and 0.5 – Fe deficiency; dose 1 – normal Fe supply; doses 2 and 4 – Fe excess in the nutrient solution (0.6 ≤ SE ≤ 2.7 and 0.01 ≤ SE ≤ 0.13, respectively).

the nutrient medium at high concentrations, Cd is believed to be taken up non-metabolically (Greger 1989), we may speculate that excess Fe influences one of three or all non-metabolic ways of Cd uptake by roots postulated by Cutler and Rains (1974):

1° – exchange adsorption – reversible binding of Cd to the root surface;

2° – non-metabolic binding which was postulated to be the primary Cd accumulation mechanism or most probably:

3° – diffusion, non-metabolic process responsible for Cd entering into plant and its translocation from roots to shoots.

On the other hand the protective role of Fe against excessive Cd accumulation has not been observed in plants grown under insufficient Fe supply.

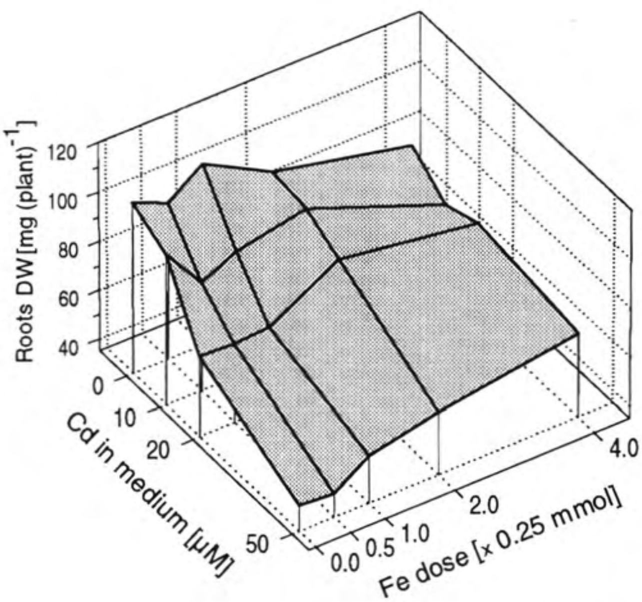

Fig. 2. The effect of Cd/Fe interaction on root dry weight of bean plants ($3.1 \le SE \le 15.5$).

However, the additional Fe doses had visible effects on root dry weight per plant (Fig. 2). Fe deficiency enhanced the toxic Cd influence on root growth. At 0 and 0.5 Fe doses and highest Cd concentration (50 µM) root dry weight of individual plant dropped to about 44-45% of control. The excess Fe doses clearly diminished the inhibitory effect of Cd on root growth. In control plants with no Cd added to the nutrient medium or at lowest metal concentration of 10 µM, deficient or excess Fe had a clear negative influence on root dry weight (Fig. 2).

Fe accumulation in primary leaves of bean plants strictly depended on Cd concentration in the nutrient solution, but the detoxificatory abilities of extra Fe doses were much more pronounced here than in the roots (Fig. 3A). Therefore, at quadrupled Fe dose Cd had no negative effect on the accumulation of this essential nutrient in primary leaves. There was also a very small effect of Cd on Fe accumulation in primary leaves of bean seedlings cultivated under Fe deficiency conditions (Fig. 3A). Excess Fe clearly decreased Cd accumulation in primary leaves (Fig. 3B). At 50 µM Cd the accumulation of this heavy metal decreased by about 50% in comparison with primary leaves from plants cultivated at 0 and 4 doses of Fe (Fig. 3A). Moreover, the ratio of Cd accumulated in roots to Cd located in primary leaves showed very distinct changes with increased Fe supply (data not shown). Excess doses of Fe visibly diminished Cd accumulation in primary leaves, especially in plants grown at 20 and 50 µM Cd in the medium. A relatively high root/leaf Cd ratio at high Fe doses was related to 50% decrease in Cd accumulation in primary leaves in comparison with normal Fe supply. On the other hand, observed low root/primary leaves Cd ratio at low Fe supply resulted from increased accumulation of this heavy metal in leaves of plants grown in Fe deficiency conditions – 22-26% more Cd in leaves in comparison with plants grown at normal Fe supply.

We also observed very characteristic external symptoms of Cd/Fe interaction in the studied plants. Primary leaves showed reddish-brown discoloration of petioles and nerves. In plants grown at deficient or normal Fe supply and at highest Cd concentration these symptoms were observed on both sides of primary leaf lamina, whereas at 2 and 4 doses of Fe

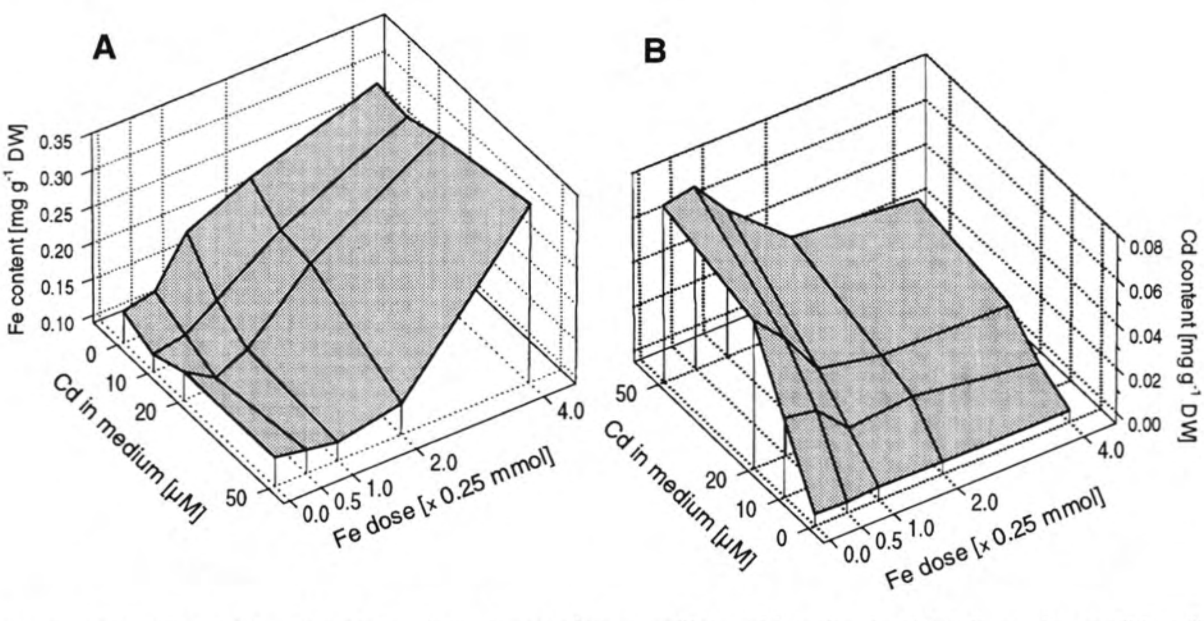

Fig. 3. The accumulation of Fe (A) and Cd (B) in primary leaves of bean seedlings cultivated under Cd/Fe interaction conditions ($0.02 \le SE \le 0.11$ and $0.003 \le SE \le 0.010$, respectively).

and 50 μM Cd discoloration became weaker and were located only on the lower side of lamina. Necrotic spots were also observed among plants grown at deficient Fe supply at higher Cd concentrations.

Growth of primary leaves of bean plants as affected by Cd/Fe interaction

It was observed by Van Volkenburgh and Cleland (1979) as well as by Barceló et al. (1989) that in bean plants older than 9 days from sowing the lamina expansion of primary leaves involved only cell expansion but not division. Therefore, while analyzing the growth changes in primary leaves of bean plants at that age or older, as in our experimental pattern, we deal only with the cell enlargement, which among other things depends on water relations in the plant as a whole and on water availability and content in leaves as such.

From Fig. 4 it appears that the primary leaf area was affected to a high extent by Cd presence in the nutrient solution as well as by Fe deficiency or excess. Fe supply had distinctly the greatest influence on leaf growth, which was more pronounced than that of Cd. One may ask about the reason for such drastic changes – was it cell enlargement as a general process inhibited by Cd or Fe in excess or a combination of both, or was it imbalance in leaf water relations caused by these metals? If we consider leaf water content expressed by the water amount per leaf area unit (so-called succulence) it appears that relative water content had no effect on primary leaf expansion abilities (Fig. 5). Succulence of primary leaves was significantly decreased only by the highest Cd concentrations from deficient to moderate excess Fe (2 doses). The highest Fe dose markedly increased leaf water content, especially at 50 μM Cd (Fig. 5). The ratio of fresh to dry weight of primary leaves did not show drastic changes, either. Except Fe deficiency growth conditions (0 doses of Fe) and control plants at 0 μM Cd, where we observed a high accumulation of dry matter, this ratio remained quite stable with only a small inhibitory effect of high Cd concentrations (Fig. 6). The calculation of the specific leaf area (m^2 per g^{-1} DW, data not shown) showed that the primary leaf area was more affected than the dry weight. It was postulated earlier that in plants under heavy metal stress the level of IAA growth hormone is considerably decreased (Barceló et al. 1989). Therefore, the reason for such drastic leaf area decrease might be related to IAA level in the treated plants. This might explain the inhibition of primary leaf growth observed in our experiments. It must also be noted that excess Fe may show toxicity symptoms typical for all heavy metals, inhibiting largely plant growth, which confirms earlier studies (Kirsch et al. 1960; Tanaka and Navasero 1966; Tanaka et al. 1966). Plant responses to combinations of metals in the nutrient solutions seem to be very dynamic and, according to Symeonidis and Karataglis (1992), might be divided into three basic groups:

(i) – additive, *i.e.* plant growth parameters under conditions of multiple metal stress are equal to those observed in the presence of the investigated metals supplied separately;

(ii) – antagonistic, *i.e.* plant growth parameters under multiple metal stress are bigger than those in the presence of metals supplied separately;

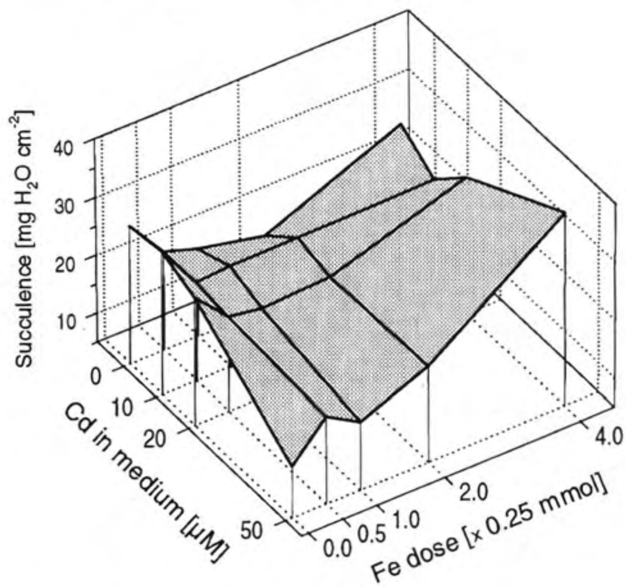

Fig. 5. The changes of primary leaves succulence expressed as mg H_2O per area unit in plants grown under Cd/Fe interaction conditions ($2.9 \leq SE \leq 5.7$).

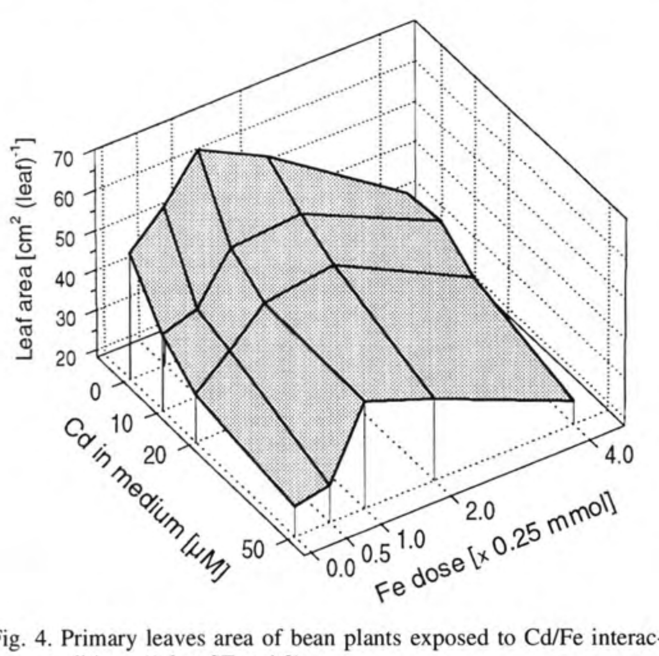

Fig. 4. Primary leaves area of bean plants exposed to Cd/Fe interaction conditions ($1.2 \leq SE \leq 4.0$).

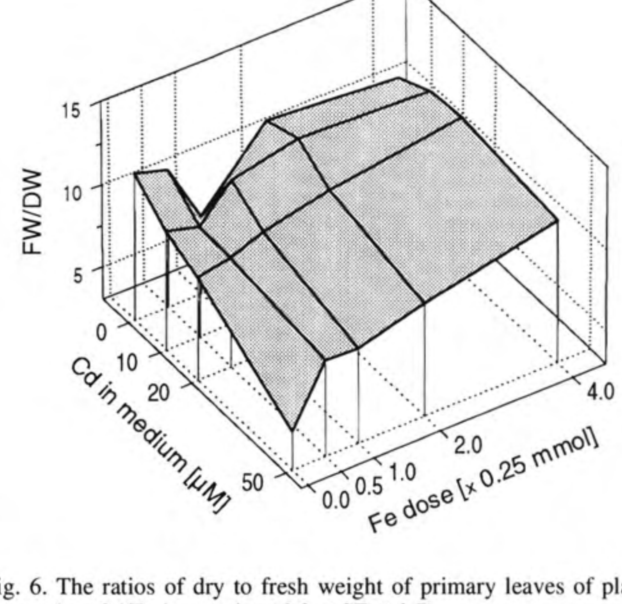

Fig. 6. The ratios of dry to fresh weight of primary leaves of plants exposed to Cd/Fe interaction ($0.9 \leq SE \leq 2.7$).

(iii) – synergistic, *i.e.* growth is smaller than those observed under separate metal supply.

In our experiments we observed much more complicated responses since we analyzed the effects of interactions between two heavy metals – one evidently toxic (Cd) and the other one being necessary nutritional element, though potentially toxic while in excess supply (Fe). It means that depending on mutual relations between Cd and Fe in the nutrient medium the final effects should be rather antagonistic or synergistic. In Fe deficiency conditions we generally observed the enhanced Cd toxicity towards plant growth, mostly related to facilitated Cd accumulation in roots and especially in primary leaves. On the other hand, excess Fe, although basically toxic, showed some detoxificatory effects limiting the toxicity of Cd. This was greatly attributed to decreased Cd accumulation in bean roots and primary leaves.

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INTERAKCJA MIĘDZY KADMEM I ŻELAZEM. AKUMULACJA I DYSTRYBUCJA METALI ORAZ ZMIANY PARAMETRÓW WZROSTOWYCH SIEWEK *PHASEOLUS VULGARIS* L.

STRESZCZENIE

Interakcję pomiędzy kadmem, jednym z najbardziej toksycznych metali ciężkich, a żelazem, niezbędnym dla roślin składnikiem mineralnym, badano na siewkach *Phaseolus vulgaris* L. odm. Słowianka. Interakcję indukowano poprzez zmiany zawartości obu metali w pożywce. W warunkach deficytu żelaza (0 i 0.5 normalnej dawki tego składnika) toksyczne oddziaływanie kadmu na wzrost roślin (zawartość świeżej i suchej masy czy powierzchnia liści pierwszorzędowych) było znacznie głębsze niż w przypadku normalnego zaopatrzenia w żelazo. Przy normalnym i nadmiernym zaopatrzeniu w żelazo (1, 2 i 4 dawki) kadm ograniczał akumulację żelaza w korzeniach i pierwszorzędowych liściach. Nadmiar żelaza zmniejszał jednak akumulację kadmu chroniąc rośliny przed nadmiernie szkodliwym wpływem wysokich stężeń kadmu w środowisku wzrostu. Należy zauważyć, że żelazo klasyfikowane także jako metal ciężki, w bardzo wysokich dawkach samo staje się toksyczne dla roślin. Tak więc detoksyfikacyjna rola żelaza w stosunku do kadmu, i prawdopodobnie także innych metali ciężkich, ogranicza się do takich stężeń tego pierwiastka w pożywce mineralnej, w jakich metal ten nie jest samoistnie toksyczny dla organizmu roślinnego.

SŁOWA KLUCZOWE: interakcja kadm/żelazo, *Phaseolus vulgaris* L., akumulacja metali, wzrost roślin.