BULG. J. PLANT PHYSIOL., 1997, 23(3–4), 114–133

REDUCTIVE ANALYSIS OF FACTORS LIMITING GROWTH OF CADMIUM-TREATED PLANTS: A REVIEW

***Andon Vassilev*[1]*, Ivan Yordanov**[2]**

[1]Agricultural University of Plovdiv, 12 Mendeleev Str., 4000 Plovdiv, Bulgaria
[2]Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

Received 20 November 1997

Summary. A reductive analysis of factors limiting growth of plants subjected to Cd treatment is made in this review. The non-specific character of Cd phytotoxicity and its negative effects on the basic growth components: RGR, NAR, LAR, SLA, etc. are presented. An attempt to connect the changes in plant growth with some effects of Cd on water relations, dark respiration, and photosynthesis was made. It was established that growth inhibition is mainly due to disorders both in dark respiration and photosynthesis. Decreased photosynthetic rate is a consequence of the negative Cd effects on a number of different sites of this process and mainly on the biochemical reactions of Calvin's cycle. Some unclear sides of the problem of Cd phytotoxicity that could be the object of further investigations are pointed out.

Key words: Cd, growth analysis, photosynthesis, dark respiration, water relations

Abbreviations: RGR – relative growth rate; LAR – leaf area ratio; NAR – net assimilation rate; LMR – leaf mass ratio; RMR – root mass ratio; SMR – stem mass ratio; SLA – specific leaf area; DM/FM – dry mass/fresh mass ratio; Ψ – water potential; RWC – relative water content; NADP – nicotinamide adenine dinucleotide phosphate; RNA – ribonucleic acid; ABA – abscisic acid; PC – phosphatidilcholine; MGDG – monogalactosyldiacylglicerol; DGDG – digalactosyldiacylglicerol; LHCII – light-harvesting chlorophyll *a/b* protein complex II; GL – galactolipase activity; OEC – oxygen evolving complex; CP1a and CP1-Chl *a* – chlorophyll–protein complexes of PS1;

* Corresponding author, E-mail: yordanov@obzor.bio21.bas.bg

CPa-Chl *a* – chlorophyll–protein complex of PS2; A – photosynthetic rate; E – transpiration rate; PS1 – photosystem 1; PS2 – photosystem 2; F_0 – initial chlorophyll fluorescence; F_v – variable chlorophyll fluorescence; F_m – maximal chlorophyll fluorescence; $t_{1/2}$ – half-rise time of fluorescence signal from F_0 to F_m; $PS2_\alpha$ – active centers of photosystem 2; q_p – photochemical quenching; q_N – non-photochemical quenching; Q_A – primary electron acceptor of PS2; R_{fd} – vitality index; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP – ribulose-1,5-bisphosphate; PGA – 3-phosphoglyceric acid; DHAP – dihydroxyacetate phosphate.

Introduction

Cadmium belongs to the group of so-called "heavy metals". It is a relatively rare metal, has no biological function, and is highly toxic to plants and animals (Alloway, 1990). The maximum tolerable intake of Cd for humans, recommended by FAO/WHO is 70 µg/day.

In Bulgaria about of 19500 ha arable lands are contaminated with heavy metals, including Cd (Tassev, 1995). The oxidizing conditions of weathering in soils release Cd as the soluble and mobile Cd^{2+} ion. The main sources of Cd pollution are metal mining, manufacture and disposal as well as some agricultural practices as applying of Cd-containing phosphate fertilizers, sewage sludge and pesticides (Van Bruwaene et al., 1984).

Cd pollution is of increasing scientific interest since Cd^{2+} is readily taken up by the roots of many plants species and its toxicity is generaly considered to be 2–20 times higher than that of other heavy metals (Jagodin at al., 1995). Most investigations of Cd pollution focus on the processes involved in Cd accumulation in crop plants and on the consequences of this accumulation on human health (Wagner, 1993). Cd phytotoxicity is a minor, but also important problem, especially in some highly heavy metal polluted regions, where a decrease in agricultural crop productivity has been observed (Bingham et al., 1976; Vassilev et al., 1996; Zheljazkov and Nielsen, 1996).

It was well established that in high internal concentrations Cd disturbed almost all physiological processes in plants (see reviews: Barcelo and Poschenrieder, 1990; Van Asshe and Clijsters, 1990; Krupa and Baszynski, 1995; Siedlecka, 1995). Despite the achievements in elucidating Cd phytotoxicity, its physiological nature is not fully understood. Among the discussed questions are those about the relationship between plant growth inhibition and disorders in the cardinal physiological processes. The main goal of this review is to outline our current understanding of the factors limiting the growth of plants exposed to Cd treatment.

I. Methodological approach

The present review was conducted after the scheme of Hall and Long (1993) for reductive analysis of factors limiting plant growth (Fig. 1). These authors noted that "a common mistake in the scientific approach is to look at the isolated parts of the plant first, rather than to study the whole". Growth on the whole plant level is an integral physiological process with a higher degree of organization and regulation compared to the other cardinal physiological process. Thus it is a good approach to follow a logical sequence of steps in investigating plant growth limitations.

Some parameters of growth analysis are presented in Fig.1. Relative growth rate (RGR) is an integral parameter, which depends on two basic components: leaf area ratio (LAR) and net assimilation rate (NAR) (Lambers et al., 1989). They are accepted as "morphological" and "physiological" components of RGR, respectively. On the other hand, LAR depends on leaf mass ratio (LMR) and specific leaf area (SLA), while NAR – on the photosynthetic rate, the rate of dark respiration and on the relative part of non-photosynthetic plant organs.

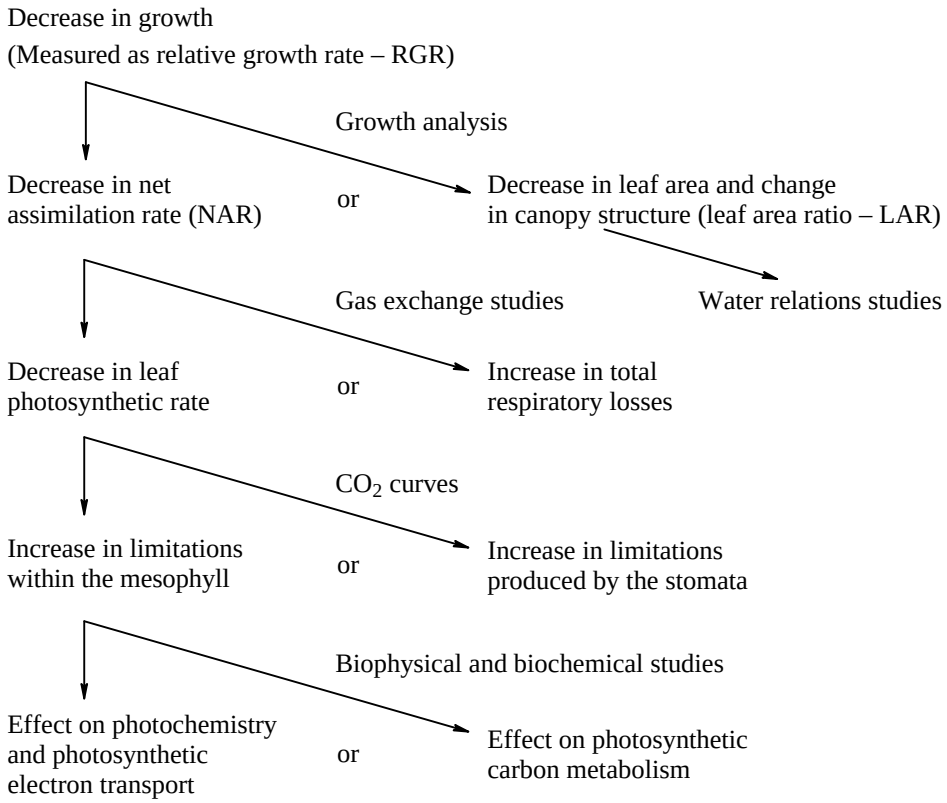

Fig. 1. A reductive analysis of factors limiting plant growth (after Hall and Long, 1993, with slight modifications)

II. Plant growth response to cadmium treatment

The inhibiting effect of Cd on fresh and dry mass accumulation, height, root length, leaf area, and other biometric parameters of plants are reported in almost all investigations. The following phytotoxic symptoms were observed: root browning (Arduini et al., 1994), leaf red-brownish discolouration (Malone et al., 1977), leaf epinasty (Vazquez et al., 1989), and leaf chlorosis (Foy et al., 1978).

Differences in the degree of expressed phytotoxicity due to various Cd-concentrations applied to the root medium, the duration of treatment, as well as the characteristics of species and cultivars were established. Increasing the duration of treatment and/or the Cd-concentrations led to transition of leaf chlorosis into yellowing and necrosis of leaf tips. The symptoms of phytotoxicity were expressed more clearly in roots because of the significantly higher heavy metal accumulation in them (Breckle, 1991). The above pointed symptoms are not specific for Cd-treatment only, they have been observed in response to other heavy metals, too (Moustakas et al., 1994; Prasad, 1995).

Growth analysis of Cd-treated plants was made in a limited number of investigations. Abo-Kassem et al. (1995) established 20% inhibition of RGR in wheat plants subjected to a 15-day treatment with Cd in concentration 10 μM. The inhibition of RGR was mainly due to decreased NAR, while the changes in LAR, LMR, and SLA were insignificant. Inhibition of RGR in Cd-treated sugar beat plants was also reported by Greger et al. (1991). In our investigations with young barley plants we established that Cd in concentration 54 μM inhibits RGR by 85% (Table 1). The effect of Cd on NAR was identical to that on RGR, while on LAR it was expressed by only 8 % inhibition. Dry mass allocation in plant organs was changed only in roots – RMR decreased by 12 %.

The negative effect of Cd on plant growth was accompanied by an increase in dry to fresh mass (DM/FM) ratio in all organs (Greger and Lindberg, 1986; Becceril et al., 1989; Moya et al., 1993). Despite that DM/FM ratio was changed during ontogenesis, its sharp increase in young plants is a criterion for stress response, which is indicative on whole plant level (Baker et al., 1993).

Table 1. Effect of Cd on some growth parameters of young barley plants from cv. Hemus

Parameters	Control plants	Cd-treated plants	% of control
RGR [$g.kg^{-1}.day^{-1}$]	34.0 ± 0.2	5.1 ± 0.1**	15
NAR [$g.m^{-2}.day^{-1}$]	0.96 ± 0.010	0.15 ± 0.004**	16
LAR [$m^2.kg^{-1}$ (plant)]	35.4 ± 1.4	32.6 ± 1.2	92
LMR [kg leaf.kg^{-1} (plant)]	0.567 ± 0.011	0.586 ± 0.015	103
SMR [kg stem.kg^{-1} (plant)]	0.260 ± 0.005	0.262 ± 0.007	101
RMR [kg roots.kg^{-1} (plant)]	0.173 ± 0.007	0.152 ± 0.007*	88
SLA [$m^2.kg^{-1}$ (leaf)]	62.4 ± 2.5	55.6 ± 1.9	89

Values are means of 3 separate experiments ± S.E. (n=15); * $p<0.05$; ** $p<0.001$.

III. Analysis of changes in leaf area ratio

The reduction of LAR could be a consequence from lowered LMR, as well as from SLA. In Cd-treated plants, usually LMR was not changed significantly as pointed above, while RMR decreased because of the much higher Cd accumulation and toxicity in roots. That is why Barcelo et al. (1988a) considered that the main reason for LAR reduction in Cd-treated bean plants is the lower SLA. This statement was confirmed in our investigations (Table 1), where the inhibitions of LAR and SLA were of the same degree. Barcelo et al. (1988a) related the negative effect of Cd on SLA with disorders in water supply. These authors suggested that reduced cell turgor potential and cell-wall elasticity led to formation of small cells and intercellular space area in Cd-treated plants.

Lower turgor potential was due to disturbed water balance in these plants. It is well known that Cd affects water uptake, transport and transpiration (Barcelo et al., 1986; Costa et al., 1994; Vassilev et al., 1997). The reduced water uptake in Cd-treated plants can be easily explained with root growth inhibition. Lamoreaux and Chaney (1977), Bacelo et al. (1988b) and Marchiol et al. (1996) reported that root hydraulic conductivity into xylem vessels decreased from two to four times depending on the applied Cd stress and characteristics species. Barcelo et al. (1988b) considered that the reasons for reduced water movement were the decreased vessel radius and number of vessels due to Cd-induced inhibition of division, elongation and differentiation of cambium cells. These authors hypothesized that this is a consequence from disturbed hormonal balance, but until now, there are no data supporting the suggestion. According to Fuhrer (1982) another reason for decreased water movement could be the structural disorders in some vessels because of the accumulation of lignin-like insoluble phenols and depositions of calcium oxalate (Van Balen et al., 1980).

In result of the disorders pointed above the relative water content (RWC) as well as water potential (Ψ) and its component turgor potential of leaves of Cd-treated plants decreased. Reduction of turgor potential could also be due to absence of significant osmotic adjustment. This is a mechanism for maintenance of a stable water balance in shortage of water (Yancey et al.,1982). Alia and Saradhi (1991) and Chen and Kao (1995) reported for some proline accumulation in Cd-treated plants. In our investigations this effect was also registered (Vassilev et al., 1998), but to a much lesser degree than the data known about proline accumulation under high water stress (Paleg and Aspinall, 1981). This gave us reasons to accept the opinion of Alia and Saradhi (1991) that proline accumulation in heavy metal treated plants is not so much related to osmotic adjustment than to the mechanism for regulation of cytosolic acidity or it is a possible enzyme protectant as suggested Shah and Dubey (1997/98).

Barcelo et al. (1986, 1989) established that turgor loss point in the leaves of Cd-treated bush bean plants is reached at higher RWC and Ψ than in the control plants. The authors explain this phenomenon with the lowered elasticity of cell walls. They

try to find the reasons for disturbed elasticity in phenols and callose deposition, as well as partially Ca substitution from Cd in the middle lamella of cell walls. According to Vazquez (personal opinion, according to Barcelo and Poschenrieder, 1990) the lowered elasticity could be due to decreased synthesis of cell wall components because of reduced functional activity of Golgi's apparatus.

IV. Analysis of changes in net assimilation rate

IV. 1. Changes in dark respiration

Physiological response of plants to various stresses is connected largely with dark respiration. As a basic source of energy resources, dark respiration plays the leading role in the regulation of cell metabolism. It is well known that approximately half of all the photosynthates produced per day are respired in the same period (Lambers, 1985), so the intensity of dark respiration has a significant importance for dry mass accumulation.

Data about effect of Cd upon dark respiration are often controversial. We think that is possible to differentiate two basic effects of Cd: *early* and *relatively late*. This differentiation is connected with the reach of a definite "critical" Cd concentration in plant organs.

The *early* Cd effect is expressed by accelerated dark respiration and enzyme induction. In this physiological state, which Van Assche et al. (1988) define as stress, despite of Cd induced disturbances, the cell control of metabolism is stable. Lee at al. (1976) established that Cd increased dark respiration and activity of the enzymes isocitrate dehydrogenase (E. C. 1.1.1.42), glutamate dehydrogenase (E. C. 1.4.1.2), and malate dehydrogenase (E. C. 1.1.1.37). Van Assche et al. (1988) reported that the activity of malic enzyme (E. C. 1.1.1.40) and the key enzyme of the oxidative pentose-phosphate pathway – glucose-6-phosphate dehydrogenase (E. C. 1.1.1.49) in Cd-treated bean plants was enhanced. As both enzymes catalyze NADPH producing reactions, these authors suggested that their induction might partially compensate NADPH deficit originating from the limited electron transport in the chloroplasts. Ernst (1980) hypothesized that accelerated dark respiration is a compensatory mechanism supplying ATP through oxidative phosphorylation.

It is considered that under stress the balance between production and quenching of reactive oxygen species as superoxide radical, singlet oxygen and hydrogen peroxide is disturbed (Okuda et al., 1991). This results in generation of HO^- radicals causing extensive damages of membranes by peroxidation of their constituent lipids. In this state, the activity of the so-called antioxidative enzymes peroxidase (E. C. 1.11.1.7), catalase (E. C. 1.1.1.7), and superoxide dismutase (E. C. 1.15.1.1), that are also terminal oxidases of dark respiration, is increased. Shaw (1995) established that

in Cd-treated *Phaseolus aureus* plants the activity of these three enzymes was enhanced. Higher peroxidase activity, as well as changes in its iso-enzyme pattern in plants subjected to Cd treatment is found by Lee et al. (1976), Van Assche and Clijsters (1987), Van Assche et al. (1988).

The *relatively late* Cd effect is expressed on the inhibition both of dark respiration and of the activity of a number of enzymes connected with that process. Inhibition of dark respiration rate in Cd-treated soybean plants is established by Oliveira et al. (1994). According to Van Assche et al. (1988) when heavy metal concentrations exceed the toxic level, the physiological state of the cell is irreversibly changed. Cd inhibits enzyme activity directly – by interactions with SH-groups, or indirectly – by disturbing cation balance at the subcellular level (Van Assche and Clijsters, 1990). Lindberg and Wingstrand (1985), and Fodor et al. (1995) established inhibition of ATP-ase activity, while Chugh and Sowhney (1996) reported of a retarded activity of hydrolytic enzymes α- and β-amylase (E. C. 3.2.1.2).

On the base of the increase in activity of peroxidase, some hydrolytic enzymes, as well as in the rate of the dark respiration, Lee et al. (1976) supposed that Cd causes an anticipated premature senescence response. Other facts established later supporting this hypothesis are: increased ethylene production (Fuhrer, 1982), lowered soluble protein content (Stiborova et al., 1986b; Sheoran et al., 1990a; Vassilev et al., 1997), disturbed turnover of ribonucleic acid (RNA) (Shah and Dubey, 1995), etc.

IV. 2. Changes in photosynthetic rate

It was established that Cd inhibits the photosynthetic rate, and that the toxic Cd effect depends on the applied concentration, the species and cultivar characteristics, the age of leaves and the phenological development of plants (Baszynski et al., 1980; Becerril et al., 1989; Costa and Morel, 1994; Vassilev et al., 1995; Lang et al., 1995). It is considered that the toxic Cd effect is stronger in the leaves functioning actively and during the earlier phenophases of vegetation (Lang et al., 1995; Sheoran et al., 1990a, b; Vassilev et al., 1998).

Changes in the rate of leaf photosynthesis are not always analogous to those of canopy photosynthesis. Landberg and Greger (1994) established increased leaf photosynthesis but decreased canopy photosynthesis due to the smaller leaf area in Cd-treated wheat plants. Merakchijska and Yordanov (1983) observed the same fact in Cd-treated bean plants.

Photosynthesis is an integral process with a high degree of self-regulation. Inhibition of photosynthetic rate could be due to structural and functional disorders in many different sites of this process. It is accepted that the factors limiting photosynthesis have stomatal and non-stomatal nature.

IV. 2. 1. Stomatal limitation of photosynthesis

In one of the first reports about the negative effect of Cd on the photosynthetic rate it was suggested that inhibition is due to limited access of CO_2 (Bazzaz et al., 1974). The potential possibility of such a limitation in *in vivo* Cd-treated plants was based on inhibition of the transpiration established later in many studies (Poschenrieder et al., 1989; Costa et al., 1994; Marchiol et al., 1996; Vassilev et al., 1997).

Barcelo and Poschenrieder (1990) consider that in the dynamics of transpiration rate could be differentiated three phases that were confirmed by Leita et al. (1995) in Cd-treated soybean plants. Stomatal limitation of photosynthesis is possible only during the first and second phase when transpiration rate is decreased by ABA-mediated closing of stomata. To what extent the decreased transpiration rate limits the photosynthetic rate could be explained after an analysis of the dependence of CO_2 assimilation on the intercellular CO_2 concentration. At present, there is one report establishing equal stomatal limitation of photosynthesis in control and Cd-treated soybean plants (Marchiol et al., 1996). Most of the investigations on photosynthesis response to *in vivo* Cd stress are focused on non-stomatal limitations.

IV. 2. 2. Non-stomatal limitations of photosynthesis

IV. 2. 2. 1. Changes in chloroplast ultrastructure and composition of thylakoid membranes

Baszynski et al. (1980) established disorders in chloroplast ultrastructure in Cd-treated tomato plants expressed in disorganization of grana and increased both number and size of plastoglobuli due to a loss of lipids in thylakoid membranes. Krupa (1988) reported that Cd induced delay both in the formation of thylakoid membranes and in the organization of grana stacks in chloroplasts of radish. Chloroplasts in these plants are smaller and the number of both grana and thylakoids is reduced by 25%. According to Skorzinska and Baszynski (1993) the degradation of stroma thylakoids is more severe than the degradation of grana ones. Barcelo et al. (1988a) noted the fact that the negative Cd effect is not expressed to the same degree in all chloroplasts of bean plants. In the most damaged chloroplasts the envelope is partially disturbed and grana are severely disorganized.

Stoyanova and Chakalova (1990) reported that Cd disturbs not only chloroplasts, but cellular envelopes, plasmalemma, mitochondria and other organelles, too. They noted that in cells of Cd-treated *Elodea canadensis* Rich there is a structure-functional association between energy producing organelles (chloroplasts and mitochondria), and interpreted it as an element of the compensatory energetic mechanism. This effect was also observed in our investigations with Cd-treated young barley plants (Vassilev et al., 1995).

Analysis of the reasons for weaker development and degradation of lamellar structure in chloroplasts of Cd-treated plants directs the next investigations towards studies of the changes in composition of the thylakoid membranes.

Krupa and Baszynski (1989) reported that 14-day treatment with 20 μM Cd decreased 25% the total content of acyl lipids in thylakoid membranes of tomato plants. The content of phosphatidilcholine (PC) was reduced to the greatest extent, followed by mono- and digalactosyldiacylglicerol (MGDG; DGDG). Fatty acid composition of thylakoid membranes was also changed – the content of the major unsaturated fatty acid decreased, as the negative Cd effect was highest on trans-Δ3-hexadecenoic acid. Staehelin (1986) suggested that due to the specific location of this acid in PC molecules, it has some influence on the association of protein subunits into oligomere forms of light-harvesting chlorophyll *a/b* protein complex II (LHCII). As this complex plays role in the formation of the grana structure of chloroplasts, Krupa (1988) admits that the delayed formation and weaker development of grana in Cd-treated plants is due to disorders in LHCII.

Skorzinska et al. (1991) established that both galactolipase activity (E.C. 3.1.1.26, GL) and the content of extracted free fatty acids in chloroplasts of Cd-treated bean plants are significantly higher. The content of 18:3 acid was increased to the greatest extent. Despite changes in the lipid matrix, changes in polypeptide composition of photosystem 2 (PS2), particularly in oxygen evolving complex (OEC), were also observed (Skorzinska and Baszynski**,** 1993). The content of polypeptides with M_r 17, 23 and 33 kDa was reduced.

Krupa and Baszynski (1995) noted that the above mentioned changes in thylakoid membranes of Cd-treated plants are identical to a great degree with those in senescent leaves: decrese of MGDG in thylakoids (Fong and Heath, 1977), high activity of galactolipase and release of large amounts of 18:3 fatty acid (O'Sullivan, 1987). According to Siegenthaler et al. (1987) one of the major reasons for the degradation of thylakoid membranes in senescence leaves is the higher activity of GL. This enzyme is localized in the outer monolayer of the membrane, and it is important in maintaining the native configuration of the thylakoid membrane.

From the observed resemblance, Krupa and Baszynski (1995) deduced that Cd stress induces premature senescence of the photosynthetic apparatus. Their opinion updates the hypothesis of Lee et al. (1976) about the accelerated senescence of plants in response to Cd treatment.

IV. 2. 2. 2. Changes in plastid pigments and chlorophyll-protein complexes

It was established that Cd applied *in vivo* decreased plastid pigment concentrations. Concentration of Chl *a* is redused more than that of Chl *b* and carotenoids. Baszynski et al. (1980) reported first about this negative Cd effect in investigations with tomato plants. Lately inhibiting effect of Cd was established in other species too: wheat and cucumber (Buszek, 1984; Malik et al., 1992), maize (Stiborova et al., 1986b; El-Enany, 1995), bean (Barcelo et al., 1988a; Siedlecka and Krupa, 1996), etc. Cd effect on plastid pigments depends on leaf age and plant development: young cucumber leaves and true bean leaves are more susceptible than old leaves and cotyledons (Buszek, 1984; Barcelo et al., 1988a).

Most researchers connect the reduction of chlorophyll in Cd-treated plants with inhibition of its biosynthesis, regarding the investigations of Stobart et al. (1985). These investigations were made *in vitro* with plant segments incubated with Cd for a short period. The authors established that Cd inhibits chlorophyll biosynthesis on two levels: in the synthesis of 5-aminolaevulinic acid and in the formation of photoactive protochlorophyllide reductase complex. In a recent investigation made also *in vitro*, Horvath et al. (1996) reported that the photoconversion of protochlorophyllide was not inhibited, but Cd disturbed chlorophyll molecules integration in stable complexes.

Based on the expressed symptoms and the established lower concentrations of Mg and Fe in the leaves of Cd-treated sugar beet plants, Greger and Lindberg (1986) suggested that the lower chlorophyll concentrations in these plants were a result of the deficiency of these nutrients. The decrease in Mg and Fe content in leaves as a response to Cd treatment was established in other plant species also (Breckle and Kahle, 1992; Rubio et al., 1994). More convincing data about the interactions between Fe deficiency (this element is a co-factor of an enzyme taking a part in chlorophyll biosynthesis) and chlorophyll concentrations were reported recently by Krupa et al. (1995). They showed that 50 µM Cd induced Fe deficiency and decreased by 55% chlorophyll level in bean plants. Lang et al. (1995) confirmed these statements establishing that translocation of the labeled Fe to the overground organs of Cd-treated cucumber plants is inhibited.

Chlorophyll concentrations in *in vivo* Cd-treated plants could be lowered by the activation of its enzyme degradation. Somashekaraiah (1992) established that after a 6-day treatment with 100 µM Cd in *Phaseolus vulgaris* plants, the lipoxygenase (E.C. 1.13.11.12) activity increased, while chlorophyll concentrations and activity of the so-called antioxidative enzymes such as superoxide dismutase (E.C.1.15.1.1.) and catalase (E.C.1.11.1.6.) significantly decreased.

Thylakoid membranes contain several types of chlorophyll-protein complexes: CP1a and CP1-Chl *a* protein complexes of PS1, CPa-Chl *a*-protein complex of PS2 and LHCII *a/b* connected mainly with PS2. Krupa et al. (1987) established that Cd does not influence significantly CP1a and CP1-Chl *a* complexes but it changes the ratio between monomeric and oligomeric forms of LHCII *a/b* in greening seedlings of *Raphanus sativus*. Lang et al. (1995) found the contrary – the content of all chlorophyll-protein complexes in Cd-treated cucumber plants was reduced.

IV. 2. 2. 3. Changes in photochemical processes

Investigations on the photochemical processes in Cd-treated plants apply two methods: destructive and non-destructive. In the first case electron transport is studied *in vitro*, as chloroplasts extracted from Cd-treated plants are incubated in a medium with different electron donors and acceptors with participation of suitable substances blocking electron transport. In non-destructive analysis as criteria for the state of the photo-

synthetic apparatus are used the main parameters of chlorophyll fluorescence: initial (F_0), variable (F_v), maximal (F_m) fluorescence, ratio F_v/F_m, $t_{1/2}$ – half-rise time of fluorescence signal from F_0 to F_m, photochemical (q_P) and non-photochemical (q_N) quenching.

Baszynski et al. (1980) established that PS2 activity in Cd-treated tomato plants was greately inhibited, while changes in PS1 were not observed. Using diphenylcarbazide – an electron donor alternative to water, these researchers located the disturbances in OEC of PS2. The authors observed that adding Mn in the nutrient media entirely restored electron transport in the system water–methylviologen. This fact made them assume that Cd blocks the function of Mn or decreases its content in OEC. This hypothesis is shared by Clijsters et al. (1991), who consider that normal photolysis of water requires a ratio Mn/chlorophyll (ion/molecules) in thylakoid membranes of 5-8 to 400. Data about changes in the mineral content of thylakoid membranes (extracted from Cd-treated plants) are not available yet, but a tendency towards a decrease in Mn concentration in leaves of those plants was established by Breckle and Kahle (1992), Rubio et al. (1994) and Abo-Kassem et al. (1995).

Becerril et al. (1989) and Tukendorf and Baszynski (1991) confirmed that electron transport in PS2 in Cd-treated lucerne, clover, and bean plants is disturbed. But at the same time, these authors showed that in high concentrations (50–75 μM) Cd inhibits the activity of PS1 also. According to Siedlecka and Baszynski (1993), the disturbed functional activity of PS1 is due to Cd-induced iron deficiency that limits the level of ferredoxin and $NADP^+$-oxydoreduction. Siedlecka and Krupa (1996) confirm this suggestion in their investigations on the interactions between Cd and Fe in bean plants.

The above mentioned results concerning the high susceptibility of PS2 to Cd stress are not confirmed when judging its photochemical activity by the ratio F_v/F_m. Greger and Ogren (1991), Krupa et al. (1992, 1993), Vassilev et al. (1995), and Siedlecka and Krupa (1996) established that F_v/F_m in Cd-treated plants does not significantly deviate from the rate typical for the normal functioning photosynthetic apparatus: 0.80–0.85 (Bjorkman and Demming, 1987). However, there is a decrease in the values of another parameter – $t_{1/2}$, the so-called half-rise time of fluorescence signal from the minimal (F_0) to the maximum (F_m) (Siedlecka and Krupa, 1996). It is a very complex parameter since its values depend on the effective PS2 antenna size, a diminished pool of plastoquinone or even, although very indirectly, on the number of $PS2_\alpha$ centers (Oquist and Waiss, 1988). Siedlecka and Krupa (1996) admit that the decreased $t_{1/2}$ is related with the reduced number of $PS2_\alpha$ centers due to the lower number of granal thylakoids and their ultrastructural disturbances.

Krupa et al. (1993) established that Cd reduces the values of the photochemical quenching (q_P) while those of the non-photochemical quenching (q_N) are increased significantly. This research connects the increased q_N with its energy dependant component q_{Ne} that is indicative of an increase in the transmembrane pH gradient. They

also consider that in Cd-treated plants the utilisation of stored photosynthetic energy is disturbed. As its major sink are the enzyme reactions from Calvin's cycle, these authors suppose that the inhibition of enzyme reactions results in an excess of reducing equivalents and feedback inhibition of PS2. The decrease of q_P is discussed as a mechanism to avoid over-reduction of Q_A, which is the primary electron acceptor of PS2. The above hypothesis was recently confirmed by Siedlecka et al. (1997), who assessed that the adenilate pool (ATP and ADP) in the leaves of Cd-treated bean plants is increased. In accordance with this hypothesis there are data about the reduced values of the so-called "vitality index" (R_{fd}) in those plants (Siedlecka and Krupa, 1996). According to Lichtenthaler and Rinderle (1988) R_{fd} values indicate the potential photosynthetic activity of a leaf, especially the CO_2 fixation rates of Calvin's cycle.

IV. 2. 2. 4. Changes in biochemical reactions of Calvin's cycle

The investigations on the dark reactions of photosynthesis in Cd-treated higher plants are few and are focused mainly on the changes of some key enzymes. Sheoran et al. (1990b) and Malik et al. (1992) established that Cd inhibits to a great extent the activity of almost all enzymes from Calvin's cycle in pigeon pea and wheat plants. The mechanism of the inhibition of enzyme activity and, especially that of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), in Cd-treated plants is under discussion. Considered are two possibilities: inhibition of *de novo* synthesis and/or activity of the already functioning enzyme. Inhibition of *de novo* synthesis, but not of the activity of the functioning enzyme was established in Cd-treated plants of *Laminarina saccharina* (Kremer and Markham, 1982). Stiborova et al. (1988) consider that Rubisco activity is decreased due to two negative effects of Cd: a) interaction between this metal and two SH-groups from the active enzyme center (Cys_{173} and Cys_{456}); and b) changes in the quaternary structure, followed by disassociation due to the irreversible binding with the heavy metal. The second effect was determined *in vitro* only.

Information about the functioning of primary carbon metabolism in Cd-treated bean plants was recently presented by Siedlecka et al. (1997). On the basis of the increased ratios: ATP/ADP, RuBP/PGA and DHAP/RuBP as well as on the decreased PGA/DHAP ratio the authors concluded that the main limitation caused by the onset of Cd stress is at the Rubisco step and not in the following steps of Calvin's cycle including the regeneration phase. That statement was confirmed in our investigations with Cd-treated barley plants (Vassilev et al., 1997). We established that the rate of ^{14}C-incorporation in the fraction of organic phosphates was lower while in the fraction of sugars it was higher than in the controls. The explanation was that the relatively smaller pool of the labile phosphorylated compounds was metabolised faster to the final compounds, such as sugars. We also determined that the relative share of glucose and fructose in total sugar radioactivity increased by extending the duration of dark metabolism for the control plants, while this tendency was not so clearly expressed in the Cd-treated plants. Partitioning of radioactivity in individual sugars in

the control plants was characteristic for young, active leaves, while in Cd-treated plants it was more similar to ageing leaves.

Conclusion

As a result of the reductive analysis of the factors limiting growth of Cd-treated plants, the following conclusions could be made:

Cadmium suppresses RCR through inhibiting mainly NAR and to a lesser extend LAR. Suppression of NAR is caused by the disturbances in the processes of dark respiration and photosynthesis. Dark respiration rate increases till reaching the "critical" concentrations of Cd in the organs, after which it is being inhibited. Suppression of dark respiration is a result of a set of negative effects of Cd on different structural and functional units of the process, led by the mesophyll limitations and mainly biochemical processes in Calvin's cycle.

Decreased LAR in Cd-treated plants is caused by reduced SLA. The main reason for that is the decreased turgor potential and cell wall elastisity, resulting in smaller size of leaf cells formed with smaller intercellular space area.

The analysed facts clarify to a great extend the factors limiting the growth of the Cd-treated plants. Nevertheless some incompletely explained points should be mentioned:

- The discrimination between the *early* and *relatively late* effect of Cd on dark respiration is relative and could be further specified if more data on the usage of ATP in the so called "growth respiration", "maintenance respiration" and "anion supply respiration" (in the roots) would be available. Models exist that permit for the quantitative discrimination of the respiration components (Van der Werf et al., 1988), but they still have not been used for the analysis of ATP usage in heavy metal treated plants.
- Changes in water relations of Cd-treated plants have been mainly studied using water cultures. Under different conditions the water relations disorders and its effect on other physiological processes could be even more significant. Increasing the effect of stomatal limitation of photosynthesis could be expected.
- For a more complete description of the inhibition of growth processes in Cd-treated plants more data on the phytohormonal status of such plants is essentially needed. At present such information is scarce, except for the few data on the ABA content changes (Poschenreider et al., 1989) and endogenous cytokinin levels (Atanassova et al., unpublished data).

The insufficient information available on the subjects mentioned, as well as on the interrelations of the Cd stress with other ecological factors like drought, low and high temperatures etc. motivates the need for further investigations.

References

Abo-Kassem, E., A. Sharaf-El-Din, J. Rozema, E. Foda, 1995. Synergistic effects of cadmium and NaCl on the growth, photosynthesis, and ion content in wheat plants. Biologia Plantarum, 37 (2), 241–249.

Alia, P. Saradhi, 1991. Prolin accumulation under heavy metal stress. J. Plant Physiol., 138, 554–558.

Alloway, B., 1990. Cadmium. In: Heavy Metals in Soil. Ed. B. Alloway, John Wiley & Sons, New Yersey, 100–124.

Arduini, I., D. Godbold, A. Onnis, 1994. Cadmium and copper change root growth and morphology of *Pinus pinea* and *Pinus piaster* seedlings. Physiol. Plantarum, 92, 675–680.

Baker, A., 1993. Cadmium sensitivity and constitutive resistance. In: Methods of Comparative Plant Ecology: A Laboratory Manual, Eds. G. Hendry and J. Grime, Chapman & Hall, London, 211–213.

Barcelo, J., Ch. Poschenrieder, 1990. Plant water relations as affected by heavy metal stress: a review. J. Plant Nutrition, 13(1), 1–37.

Barcelo, J., Ch. Pochenrieder, I. Andreu, 1986. Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. Contender). I. Effects of Cd on water potential, relative water content and cell wall elasticity. J. Plant Physiol., 125, 17–25.

Barcelo, J., Ch. Poschenrieder, B. Gunse, 1989. Water relations and cell wall elasticity in cadmium treated bush bean plants. In: Structural and Functional Responses to Environmental Stresses, Eds. K. Kreeb, H. Richter and T. Hinckley, SPB Academic Publishing bv, The Hague, The Netherlands, 55–65.

Barcelo, J., M. Vazquez, Ch. Poschenrieder, 1988a. Structural and ultrastructural disorders in cadmium-treated bush bean plants (*Phaseolus vulgaris* L.). New Phytol., 108, 37–49.

Barcelo, J., M. Vazquez, Ch. Poschenrieder, 1988b. Cadmium-indused structural and ultrastuctural changes in the vascural system of bush bean stems. Botanica Acta, 101, 254–261.

Baszynski, T., L. Wajda, M. Krol, D. Wolinska, Z. Krupa, A. Tukendorf, 1980. Photosynthetic activities of cadmium-treated tomato plants. Physiol. Plantarum, 48, 365–370.

Bazzaz, F., G. Rolfe, R. Carson, 1974. Effect of Cd on photosynthesis and transpiration of excised leaves of corn and sunflower. Physiol. Plant., 32, 373–376.

Becerril, J., A. Munoz-Rueda, P. Aparicio-Tejo, C. Gonzales-Murua, 1989. Changes induced by cadmium and lead in gas exchange and water relations of clover and lucerne. Plant Physiol. Biochem., 27(6), 913–918.

Bingham, F., A. Page, R. Mahler, T. Ganje, 1976. Yeld and cadmium accumulation of forage species in relation to cadmium content of sludge-amended soil. J. Envir. Qual., 5, 57–60.

Bjorkman, O., B. Deming, 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics among vascular plants of diverse origin. Planta, 170, 489 – 504.

Breckle, S., 1991. Growth under stress. Heavy metals. In: Plant Poots: The Hidden Half, Eds. Y. Waisel, A. Eshel and U. Kafkafi, M. Dekker, Inc., New York, 351–373.

Breckle, S., H. Kahle, 1992. Effects of toxic heavy metals (Cd, Pb) on growth and mineral nutrition of beech (*Fagus sylvatica* L.). Vegetatio, 101, 45–53.

Buszek, J., 1984. Effect of cadmium on chlorophyll content and dry matter yield in wheat and cucumber plants supplied either with nitrate or ammonium. Acta Univ. Bratislaviensis, 31, 11–21.

Chen, Sh., Ch. Kao, 1995. Cd induced changes in proline level and peroxidase activity in roots of rice seedlings. Plant Growth Regulation, 17, 67–71.

Chugh, L., S. Sowhney, 1996. Effect of cadmium on germination, amylases and rate of respiration of germinating pea seeds. Environmental Pollution, 92(1), 1–5.

Clijsters, H., F. Van Assche, L. Gora, 1991. Physiological responses of higher plants to soil contamination with metals. In: Ecological Responses to Environmental Stresses, Eds. J. Rozema and A.Verkleij, Kluwer Acadamic Publishers, 32–39.

Costa, G., J. Michaut, J. Morel, 1994. Influence of cadmium on water relations and gas exchanges, in phosphorus deficient *Lupinus albus* L.. Plant Physiol. Biochem., 32(1), 105–114.

Costa, G., J. Morel, 1994. Water relations, gas exchange and amino acid content in Cd-treated lettuce. Plant Physiol. Biochem., 32(4), 561–570.

El-Enany, A., 1995. Alleviation of cadmium toxicity on maize seedlings by calcium. Biologia Plantarum, 37(1), 93–99.

Ernst, W., 1980. Biochemical aspects of cadmium in plants. In: Cadmium in the Environment. Ed. J. Nriagu, Wiley and Sons, New York, 639–653.

Fodor, E., A. Szabo-Nagy, L. Erdei, 1995. The effects of cadmium on the fluidity and H^+–ATPase activity of plazma membrane from sunflower and wheat roots. J. Plant Physiol., 147(1), 87–92.

Fong, F., R. Heath, 1977. Age dependent changes in phospholipids and galactolipids in primary bean leaves (*Phaseolus vulgaris*). Phytochem., 16, 215–217.

Foy, C., R. Chaney, M. White, 1978. The physiology of metal toxicity in plants. Annu. Rev. Plant Physiol., 29, 511–566.

Fuhrer, J., 1982. Ethylene biosynthesis and cadmium toxicity in leaf tissue of beans (*Phaseolus vulgaris* L.). Plant Physiol., 70, 162–167.

Greger, M., E. Brammer, S. Lindberg, G. Larsson, J. Idestam–Almquist, 1991. Uptake and physiological effects of cadmium in sugar beet (*Beta vulgaris*) related to mineral provision. J. Exp. Botany, 42, 729–737.

Greger, M., S. Lindberg, 1986. Effects of Cd^{2+} and EDTA on young sugar beets (*Beta vulgaris*). I. Cd^{2+} uptake and sugar accumulation. Physiol. Plant., 66, 69–74.

Greger, M., E. Ogren, 1991. Direct and indirect effects of Cd^{2+} on photosynthesis in sugar beet (*Beta vulgaris*). Physiol. Plantarum, 83, 129–135.

Hall, D., S. Long, 1993. Photosynthesis and the changing environment. In: Photosynthesis and Production in a Changing Environment. A field and laboratory manual. Introduc-

tion. Eds. D. Hall, J. Scurlock, H. Bolhar-Nordenkampf, R. Leegood and S. Long, Chapman & Hall.

Horvath, G., M. Droppa, A. Oravecz, V. Raskin, J. Malder, 1996. Formation of the photosynthetic apparatus during greening of cadmium-poisoned barley leaves. Planta, 199(2), 238–243.

Jagodin, B., V. Govorina, S. Vinogradova, A. Zamaraev, G. Chapovskaja, 1995. Cadmium and lead accumulation in some agricultural crops, grown in podzolic soils. Izvestija TSHA, 2, 85–99 (in Russ.).

Kremer, B., J. Markham, 1982. Primary metabolic effects of cadmium in the brown alga *Laminaria saccharina*. Z. Pflanzenphysiol., 108, 125–130.

Krupa, Z., T. Baszynski, 1995. Some aspects of heavy metals toxicity towards photosynthetic apparatus – direct and indirect effects on light and dark reactions: a review. Acta Physiol. Plantarum., 17 (2), 177–190.

Krupa, Z., 1988. Cadmium-induced changes in the composition and structure of the light-harvesting chlorophyll *a*/*b* protein complex II in radish cotyledons. Physiol. Plantarum, 73, 518–524.

Krupa, Z., T. Baszynski, 1989. Acyl lipid composition of thylakoid membranes of cadmium–treated tomato plants. Acta Physiol. Plantarum, 11(2), 111–116.

Krupa, Z., A. Siedlecka, P. Mathis, 1995. Cd/Fe interaction and its effects on photosynthetic capacity of primary bean leaves. Proceed. X Intern. Photosynthesis Congress, Montpellier, France, 20–25.08.1995, 621–624.

Krupa, Z., G. Oquist, N. Huner, 1992. The influence of cadmium on primary photosystem II photochemistry in bean as revealed by chlorophyll *a* fluorescense – a preliminary study. Acta Physiol. Plant., 14, 71–76.

Krupa, Z., G. Oquist, N. Huner, 1993. The effects of cadmium on photosynthesis of *Phaseolus vulgaris* – a fluorescence analysis. Physiol. Plantarum, 88, 626–630.

Krupa, Z., E. Skorzynska, W. Maksymiec, T. Baszynski, 1987. Effect of cadmium treatment on the photosynthetic apparatus and its photochemical activities in greening radish seedlings. Photosynthetica, 21, 156–164.

Lambers, H., N. Freijsen, H. Poorter, T. Hirose, A. Van der Werf, 1989. Analyses of growth based on net assimilation rate and nitrogen productivity. Their physiological background. In: Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants. Ed. H. Lambers, SPB. Academic Publishing bv., The Hague, The Netherlands, 1–17.

Lambers, H., 1985. Respiration in intact plants and tissues: its regulation and dependance on environmental factors, metabolism and invaded organism. In: Encyclopedia of Plant Physiology, New Series. Eds. R. Douce and D. Day, Springer-Verlag, Berlin, 418–473.

Lamoreaux, R., W. Chaney, 1977. Growth and water movement in silver maple seedlings affected by cadmium. J. Environ. Qual., 6(2), 201–204.

Lang, F., E. Sarvari, Z. Szigeti, F. Fodor, E. Cseh., 1995. Effects of heavy metals on the photosynthetic apparatus in cucumber. In: Photosynthesis: from Light to Biosphere, IV, Kluwer Acad. Publishers, 533–536.

Landberg, T., M. Greger, 1994. Influence of selenium on uptake and toxicity of copper and cadmium in pea (*Pisum sativum*) and wheat (*Triticum sativum*). Physiol. Plantarum, 90, 637–644.

Lee, K., B. Gunningham, G. Paulsen, G. Liang, R. Moore, 1976. Effects of cadmium on respiration rate and activities of several enzymes in soybean seedlings. Physiol. Plantarum, 36, 4–6.

Leita, L., L. Marchiol, M. Martin, A. Peressotti, G. Delle Vedove, G. Zerbi, 1995. Transpiration dynamics in cadmium-treated soybean (*Glycine max* L.) plants. J. Agronomy & Crop Science, 175, 153–156.

Lichtenthaler, H., U. Rinderle, 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Crit. Rev. Anal. Chem., 19, S29–S85.

Lindberg, S., G. Wingstand, 1985. Mechanism for Cd^{2+} inhibition of (K^++Mg^{2+}) ATPase activity and K^+ ($86Rb^+$) uptake join roots of sugar beet (*Beta vulgaris*). Physiol. Plantarum, 63, 181–186.

Malik, D., I. Sheoran, R. Singh, 1992. Carbon metabolism in leaves of cadmium treated wheat seedlings. Plant Physiol. Biochem., 30, 223–229.

Malone, C., R. Miller, D. Koeppe, 1977. Root growth in corn and soybeans: effects of cadmium and lead on lateral root initiation. Can. J. Botany, 56, 277–281.

Marchiol, L., L. Leita, M. Martin, A. Peterssotti, G. Zerbi, 1996. Physiological responses of two soybean cultivars to cadmium. J. Environ. Qual., 25, 562–566.

Merakchiiska, M., I. Yordanov, 1983. The effect of some heavy metals upon growth, pigment content and photosynthetic activity of bean plants. In: III National Conference of Botany, Sofia, Bulg. Acad. Sci., 848–853 (in Bulg.).

Moustakas, M., T. Lanaras, L. Symeonidis, S. Karataglis, 1994. Growth and some photosynthetic characteristics on field grown *Avena sativa* under copper and lead stress. Photosynthetica, 30(3), 389–396.

Moya, J., R. Ros, I. Picazo, 1993. Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. Photosynthesis Research, 36, 75–80.

O'Sullivan, J., W. Warwick, M. Dalling, 1987. A galactolipase activity associated with the thylakoids of wheat leaves (*Triticum aestivum* L.). J. Plant Physiol., 131, 394–404.

Okuda, T., Y. Matsuda, A. Yamanaka, S. Sagisaka, 1991. Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat in caused by cold treatment. Plant Physiol., 97, 1265–1267.

Oliveira, J., M. Oliva, J. Cambraia, V. Vanegas, 1994. Absorption, accumulation and distribution of cadmium by two soybean cvs. Revista Brasiliera. Fisiologia Vegetal., 6(2), 91–95.

Oquist, G., R. Wass, 1988. A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. Physiol. Plant., 73, 211–217.

Paleg, L., D. Aspinall, 1981. The Physiology and Biochemistry of Drought Resistance in Plants. Academic Press, Sydney.

Poschenrieder, Ch., B. Gunse, J. Barcelo, 1989. Influence of cadmium on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. Plant Physiol., 90, 1365–1371.

Prasad, N., 1995. Cadmium toxicity and tolerance in vascular plants. Environ. Exper. Botany, 35(4), 535–545.

Rubio, M., I. Escrig, C. Martinez-Cortina, F. Lopez-Benet, A. Sanz, 1994. Cadmium and nickel accumulation in rice plants. Effects on mineral nutrition and possible interactions of abscisic and gibberellic acids. Plant Growth Regulation, 14, 151–157.

Shah, K., S. Dubey, 1995. Effect of cadmium on RNA level as well as activity and forms of ribonuclease in growing rice seedlings. Plant Physiol. Biochem., 33, 577–584.

Shaw, B., 1995. Effects of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of *Phaseolus aureus*. Biologia Plantarum, 37(4), 587–596.

Sheoran, I., N. Aggarwal, R. Singh, 1990a. Effect of cadmium and nickel on *in vivo* carbon dioxide exchange rate of pigeon pea (*Cajanus cajan* L.). Plant and Soil, 129, 243–249.

Sheoran, I., H. Singal, R. Singh, 1990b. Effect of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeon pea (*Cajanus cajan* L.). Photosynthesis Research, 23, 345–351.

Siedlecka, A., 1995. Some aspects of interactions between heavy metals and plant mineral nutrients. Acta Soc. Bot. Pol., 64, 265–272.

Siedlecka, A., Z. Krupa, 1996. Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of *Phaseolus vulgaris*. Plant Physiol. Biochem., 34(6), 833–841.

Siedlecka, A., T. Baszynski, 1993. Inhibition of electron flow around photosystem I in chloroplasts of Cd-treated maize is due to Cd-induced iron deficiency. Physiol. Plantarum, 87, 199–202.

Siedlecka, A., Z. Krupa, G. Samuelsson, G. Oquist, P. Gardestrom, 1997. Primary carbon metabolizm in *Phaseolus vulgaris* plants under Cd/Fe interaction. Plant Physiol. Biochem., 35(12), 951–957.

Siegenthaler, P., A. Rawyler, Ch. Giroud, 1987. Spatial organization and functional roles of acyl lipids in thylakoid membranes. In: The Metabolism, Structure and Function of Plant Lipids. Eds. P. Stumpf, I. Mudd and W. Nes, Plenum Publishing Corporation, 161–168.

Shah, K., R. Dubey, 1997/98. Effect of cadmium on proline accumulation and ribonuclease activity in rice seedlings: role of proline as a possible enzyme protectant. Biologia Plantarum, 40(1), 121–130.

Skorzynska, E., T. Baszynski, 1993. The changes in PSII complex polypeptides under cadmium treatment – are they of direct or indirect nature? Acta Physiol. Plant., 15(4), 263–269.

Skorzynska, E., T. Urbanik-Sypniewska, R. Russa, T. Baszynski, 1991. Galactolipase activity of chloroplasts in cadmium-treated runner bean plants. J. Plant Physiol., 138, 454–459.

Somashekaraiah, B., K. Padmaja, A. Prasad, 1992. Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. Physiol. Plantarum, 85, 85–89.

Staehelin, L., 1986. Chloroplast structure and supramolecular organization of photosynthetic membranes. In: Encyclopedia of Plant Physiology, New Series, Eds. L. Staehelin and C. Arntzen, vol. 19, Springer Verlag, Berlin, 1–84.

Stiborova, M., R. Hromadkova, S. Leblova, 1986. Effect of ions of heavy metals on the photosynthetic characteristics of maize (*Zea mays* L.). Biologia Plantarum, 41(12), 1221–1228.

Stiborova, M., M. Doubravova, A. Bresinova, 1988. Mechanism of action of Cu, Cd and Zn on ribuloze 1,5-biphosphate carboxylase from barley (*H. vulgare* L.). Photosynthetica, 22, 161–167.

Stobart, A., W. Griffiths, I. Ameen-Bukhari, R. Sherwood, 1985. The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiol. Plantarum, 63, 293–298.

Stoyanova, D., E. Chakalova, 1990. The effect of cadmium on the structure of photosynthetic apparatus in *Elodea canadensis* Rich. Plant Physiology (Sofia), 16(3), 18–26 (in Bulg.).

Tassev, H., 1995. Ecological beneficial farming under conditions of soil contaminated with heavy metals. In: Agricultural Use of Polluted Lands, Ed. T. Dimitrova, ET "B. Hranov", Sofia, 32–38 (in Bulg.).

Tukendorf, A., T. Baszynski, 1991. The *in vivo* effect of cadmium on photochemical activities in chloroplasts of runner bean plants. Acta Physiol. Plantarum, 13(1), 51–57.

Van Assche, F., H. Clijsters, 1990. Effects of metals on enzyme activity in plants. Plant, Cell and Environment, 13, 195–206.

Van Assche, F., C. Cardinaels, H. Clijsters, 1988. Induction of enzyme capacity in plants as a result of heavy metal toxicity: dose-response relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. Environm. Pollution, 52, 103–115.

Van Assche, F., H. Clijsters, 1987. Enzyme capacity and isoenzyme pattern in plants as criteria for the phytotoxicity of heavy metal polluted soils. In: Heavy Metals in the Environment. Eds. S. Lindberg and T. Hutchinson, CEP Consultants, Edinburgh, 475–477.

Van Balen, E., Van de Geijn, G. Desmet, 1980. Autoradiographic evidence for the incorporation of cadmium into calcium oxalate crystals. Z. Pflanzenphysiol., 97, 123–133.

Van Bruwaene, R., R. Kichmann, R. Impens, 1984. Cadmium contamination in agriculture and zootechnology. Experientia, 40, 43–52.

Van der Werf, A., A. Kooijman, R. Welschen, H. Lambers, 1988. Respiratory costs for the maintenance of biomass, for growth and for ion uptake in roots of *Carex diandra* and *Carex acutiformis*. Physiol. Plant., 72, 483–491.

Vassilev, A., M. Berova, Z. Zlatev, 1998. Influence of Cd^{2+} on growth, chlorophyll content and water relations in young barley plants. Biologia Plantarum (in press).

Vassilev, A., V. Kerin, P. Atanassov, 1996. Effect of cadmium pollution of soil upon productivity and seedling qualities of two barley (*H. vulgare* L.) cultivars. Bulg. J. Agri. Sci., 2, 333–340.

Vassilev, A., I. Yordanov, E. Chakalova, V. Kerin, 1995. Effect of cadmium stress on growth and photosynthesis of young barley (*H. vulgare* L.) plants. 2. Structural and functional changes in photosynthetic apparatus. Bulg. J. Plant Physiol., 21(4), 12–21.

Vassilev, A., I. Yordanov, T. Tsonev, 1997. Effects of Cd^{2+} on the physiological state and photosynthetic activity of young barley plants. Photosynthetica, 34(2), 293–302.

Vassilev, A., I. Yordanov, T. Tsonev, 1998. Physiological responses of two barley cultivars to soil pollution with cadmium. Environmental Pollution, 100, 1–7.

Vazquez, M., Ch. Poschenrieder, J. Barcelo, 1989. Pulvinus structure and leaf abscission in cadmium-treated bean plants (*Phaseolus vulgaris*). Can. J. Botany, 67, 2756–2764.

Wagner, G., 1993. Accumulation of cadmium in crop plants and its consequences to human health. Advances in Agronomy, 51, 173–211.

Yancey, P., M. Clark, S. Hand, R. Bowlus, G. Somero, 1982. Living with water stress: evolution of osmolyte systems. Science, 217, 1212–1222.

Zheljazkov, V., N. Nielsen, 1996. Effect of heavy metals on peppermint and cornmint. Plant Soil, 178, 59–66.