PHYSIOLOGICAL AND AGROECOLOGICAL ASPECTS OF CADMIUM INTERACTIONS WITH BARLEY PLANTS: AN OVERVIEW
ФИЗИОЛОГИЧНИ И АГРОЕКОЛОГИЧНИ АСПЕКТИ НА ВЗАИМОДЕЙСТВИЕТО НА КАДМИЯ С ЕЧЕМИЧНИТЕ РАСТЕНИЯ: ОБЗОР

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РЕЗЮМЕ

Тази работа е обзор върху публикации и непубликувани резултати на автора, както и данни от достъпната литература върху реакцията на ечемика към замърсяване с Cd. Кратко са описани физиологичните основи на острата (акутна) Cd токсичност при ечемични растения. Приведени са и данни характеризиращи хроничната Cd токсичност при ечемика във връзка с възможното му използването за семепроизводство и Cd фитоекстракция на замърсени с тежкия метал почви. Представена е информация за основните физиологични фактори, лимитиращи растежа на третирани с Cd ечемични растения, както и за добива на зърно, качествата на семената и капацитета на ечемика за екстракция Cd при отглеждане върху замърсени с Cd почви.

КЛЮЧОВИ ДУМИ: кадмий, ечемик, фитотоксичност, семепроизводство, фитоекстракция

ABSTRACT

This work is a review of author's previous publications, unpublished results as well as available literature on barley responses to Cd contamination. The physiological backgrounds of the acute Cd toxicity in barley plants are briefly described. Some data characterizing the chronic Cd toxicity in barley have been also provided in relation to its possible use for seed production and Cd phytoextraction on Cd-contaminated agricultural soils. Information about the main physiological factors limiting growth of Cd-exposed barley plants and grain yield, seedling quality as well as Cd phytoextraction capacity of barley grown in Cd-contaminated soils is presented.

KEY WORDS: cadmium, barley, phytotoxicity, seed production, phytoextraction

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DETAILED ABSTRACT

A part of agricultural soils all over the world is slightly to moderately contaminated by cadmium (Cd) creating risk for human and environmental health. Consequently, several strategies have been proposed for the management of Cd-contaminated agricultural soils. Some of them recommend cereals for seed production as a profitable option for heavy metal-contaminated soils as well as Cd phytoextraction as an environmentally friendly approach for soil remediation. Barley is a cereal crop attracting attention in both mentioned directions. Nevertheless, its successful use for those purposes needs better understanding of many questions related to its behavior under Cd contamination.

To obtain this information complex research experiments have been conducted during the last decade at the Agricultural University of Plovdiv, Bulgaria. One part of experiments was focused on the main physiological disorders of Cd-exposed barley plants grown in hydroponics, sand and soil conditions. Another part of experiments aimed to study barley productivity and seedling characteristics has been conducted with plants grown in soils differing in Cd contamination and soil properties. The generalised information from these research efforts as well as available literature sources that could be of help to a wide spectrum of agricultural specialists is presented in this review.

The main conclusions drawn from the conducted studies are the following:

- The reductive analysis of factors limiting growth of Cd-exposed barley plants revealed photosynthesis retardation as one of the most important factors. Cd negatively affects barley photosynthesis directly at different structural-functional levels and indirectly by the metal induced disturbances in the other physiological processes.
- The use of barley for seed production on Cd-contaminated agricultural soils seems a rationale option, as (1) barley seedling qualities are high; (2) its productivity is not significantly affected (except at some very high soil Cd contamination levels); (3) barley plants in the next generation, grown on non-contaminated soils have grain Cd levels within the norm and normal development and productivity.
- Shoot Cd accumulation of barley plants is not high enough to meet the requirements for short-term phytoextraction perspective. If higher shoot Cd accumulation is achieved by means of the induced phytoextraction approach, barley Cd phytoextraction will be limited by phytotoxicity problems.

INTRODUCTION

A part of agricultural soils all over the world are slightly to moderately contaminated by cadmium (Cd) due to extended use of superphosphate fertilisers, sewage sludge application as well as smelters dust spreading [2, 8]. Due to high Cd mobility in the soil-plant system it can easily enter into food chain and can create risk for human and environmental health [15, 25]. Increasing international concern about the risks associated with long-term consumption of crops with Cd concentrations has led the international food standards organisation, Codex Alimentarius Commission, to propose a 0.1 mg Cd kg^{-1} limit for cereals, pulses and legumes [16]. Consequently, several strategies have been proposed for the successful management of the Cd-contaminated agricultural soils. One approach, applicable on slightly contaminated soils, is aiming to screen and use low Cd-accumulating genotypes of crops, known to accumulate unacceptable high Cd levels in grain [3]. The second approach recommends profitable use of non-food crops [49]. The third option is directed towards phytoextraction, representing use of plants for metal (including Cd) removal from contaminated soils [9, 23].

Besides non-food crops, there are some expectations that seed production of cereals could be another possibility for rationale use of Cd-contaminated soils. This idea is motivated by several facts and presumptions: (1) it is known that cereals are semi-resistant to Cd [19]; (2) it is well documented that Cd accumulation in above-ground organs of many crops decreases towards generative organs [1]; (3) a presumption exists that when Cd-enriched seeds are sown in non-contaminated soil, grain Cd content of plants from the next generation will be bellow guideline values for combustion [17].

Cd phytoextraction from contaminated agricultural soils has been proposed by Robinson et al. [23, 24] pointing at the high mobility of this metal in soil-plant system and its relatively low contamination levels as compared, for example, with Pb and Zn. According to Ebbs and Kochian [13] barley is a promising crop for Zn and Cd phytoextraction as it could resist (to some extend) these metals as well as accumulate elevated concentrations in shoots.

The use of barley for both above-mentioned directions needs better understanding of its behavior at Cd contamination. From the plant physiological point of view there are many questions that are still not fully understood. For example, how this element, that is non-essential for the plant metabolism and has a low redox potential, is able to participate in biological redox reactions is attracting now a significant research attention [11]. In more applied aspects it is very important to describe the capacity of barely to resist and accumulate Cd in the grain, effects of Cd on seed quality, etc. To obtain this information complex research experiments have been conducted during the last decade at the Agricultural University of Plovdiv, Bulgaria. One part of experiments was focused on the main physiological disorders of Cd-exposed barley plants grown in hydroponics, sand and soil conditions. Another part of experiments aimed to study barley productivity and seedling characteristics has been conducted with plants grown in soils differing in Cd contamination and soil properties. The generalised information from these research efforts as well as available literature sources that could be of help to a wide spectrum of agricultural specialists is presented in this review.

PHYSIOLOGICAL RESPONSES OF BARLEY PLANTS TO CD CONTAMINATION

The studies on Cd interactions with plants have been conducted during the last three decades but there still remain some aspects unclear enough. Partly, it is due to differences in the experimental designs used concerning the applied metal concentrations, kind of medium, age of plant exposure to metal treatment, etc. Generally, each plant is able to withstand Cd loading into the plant tissue until the metal reaches the toxic threshold level, suffering toxicity at higher levels. If it takes long enough to achieve this "critical" Cd concentration plant can respond to the treatment by different Cd detoxifying mechanisms. In this situation plants are able to resist Cd to some extend and grow continuously at the presence of Cd but with lower growth rate (chronic Cd phytotoxicity). In a case of exposure of plants to very strong Cd concentrations, they are not able to express their protective mechanisms and as a result of this suffer Cd toxicity and generally die within couple of weeks (acute Cd phytotoxicity). In our experimentation we studied the physiological backgrounds of both chronic and acute Cd toxicity in barley plants.

ACUTE CD PHYTOTOXICITY

The studies on the acute Cd toxicity in barley seedlings and young plants have been realized in both hydroponics and sand culture experiments. In the hydroponics experiments the Bulgarian cultivars Obzor and Hemus were used. The applied Cd concentrations varied between 9 and 54 μmol/L, given at 3-day-old seedlings and maintained for 12 days [32]. The Portuguese cultivars Ribeka and CE 9704 were used in the sand experiments, where Cd concentrations varied from 14 to 56 mg kg^{-1} sand, given at 20-day-old plants for 10 days [46, 47]. At the highest Cd treatments in both experimental designs the acute Cd phytotoxicity was well expressed. The visual phytotoxicity symptoms observed were chlorosis and necrosis of leaf tips as well as necrosis and reduction of site root formation in the root system, all having nonspecific nature. The basic physiological disorders connected with the acute Cd toxicity in barley plants are described below and listed in Table 1.

Hydroponics studies

It was established that Cd concentrations above 4.5 μM Cd/L inhibited dry mass accumulation in barley plants [35]. In general, Cd accumulation and distribution in barley plants followed a similar pattern to that reported for other gramineae species [20, 21]. The observed root Cd accumulations were several fold higher than in the leaves. For example, Cd concentrations in the roots and the leaves of plants from *cv.* Obzor reached at 54 μmol Cd/L 289 and 94 mg kg^{-1}, respectively [32].

The relative growth rate (RGR_{DM}) of Cd-exposed barley plants (*cv.* Obzor) was retarded due mainly to net assimilation rate (NAR) inhibition, while leaf area ratio (LAR) was less influenced [43]. Plants from *cv.* Hemus grown at 54 μmol/L Cd treatment showed 85% inhibition of RGR_{DM} and accumulated about 188 mg Cd kg^{-1} DW in their leaves [41].

NAR depends on photosynthetic rate (P_N), cell respiration (R_D) and the relative ratio of non-photosynthesising plant organs, mainly root mass ratio (RMR) in young plants. RMR in Cd-exposed barley plants decreased due to greater Cd accumulation and subsequently stronger toxicity as compared with leaves [32]. The inhibition of root growth and functioning provoked changes in plant cytokinins levels that could further affect the growth rate (4). It also induced some mineral imbalances that might have impact on barley physiology. For example, the levels of K, Cu and Zn in barley plants were significantly diminished [33]. A tendency towards acceleration of R_D of roots and leaves was observed [34], which according to Ernst [14] could be explained as a compensatory mechanism supplying ATP through oxidative phosphorylation.

The negative effects of Cd on photosynthesis were also studied. Generally, Cd inhibited P_N; in the highest treatments P_N averaged 80-85% of control values [36]. The established negative Cd effect was mainly due to mesophyll constrains. In barley plants at 54 μmol Cd/L destructive changes were observed in chloroplast ultrastructure, namely reduction in the number of grana and their disorganisation, swelling of thylakoids, thinning and partial tearing of the chloroplast envelope, etc. [36]. Cd decreased photosynthetic pigments content too, on average by 20% at 54 μmol/L. All mentioned disturbances leaded to decreased photosynthetic functioning, shown also by weaker incorporation of ^{14}C in the early photoproducts [40]. Furthermore, Cd changed the pattern of ^{14}C partitioning towards that characteristic for aging leaves. The fluorescence analysis showed down regulation of PSII as the light dependence of the maximum apparent electron transport rate of these plants were reached under lower light intensities and earlier than in control plants [44]. Additionally, we established that susceptibility of the photosynthetic apparatus of Cd-exposed plants to other stresses (low and high temperature stresses) was higher than that of controls [35, 39].

Although to a lesser extend, the RGR_{DM} inhibition of Cd-exposed barley plants was also related to changes of LAR, mainly due to decreased in specific leaf area (SLA), indicating that Cd induced water relation problems. In fact, we established lower values of leaf water potential (Ψ_w) and transpiration rate (E) as well as an increase in leaf proline content of Cd-exposed plants [43].

Sand culture studies

The exposure of barley plants to Cd in the sand experiments has been done on 20-day-old plants having well developed root system and photosynthetic apparatus and thus, being able to withstand better the influence of the metal. Consequently, all observed physiological disorders were similar, but weaker expressed as compared with the plants grown at hydroponics conditions.

RGRDM of plants from cv. CE 9704 was significantly lower as compared to control plants, being retarded at 42 mg Cd kg-1 sand by 41% and having leaf Cd concentration of 128 mg kg-1 DW [47]. However, well-expressed cultivar differences in growth responses and Cd accumulation have been also detected. For example, plants from cv. Ribeka grown at the same treatments as cv. CE 9704 accumulated less Cd and their growth rate was less affected [48].

As in the hydroponics experiments it was observed that Cd treatment induced plant mineral imbalances. The concentrations of K, Zn, Ca and Fe in both roots and leaves of plants from cv. Ribeka and cv. CE 9704 decreased at high Cd treatments probably due to break down of mineral regulatory functions [46, 47]. The most pronounced effect of Cd was established on PN and photosynthetic capacity (photosynthetic rate at non-limiting conditions), which were significantly retarded. In the highest treatments - 42 mg Cd kg-1 - PN averaged 50% of control values in both cultivars CE 9704 and Ribeka [47, 48]. There was some evidence for stomatal limitation in Cd-exposed plants because PN was stronger inhibited as compared to photosynthetic capacity, and stomatal conductance (gs) and intercellular CO2 concentration (ci) were strongly reduced. Besides the already mentioned disturbances, Cd-exposed plants showed mesophyll limitations as was shown by the lower efficiency of light utilisation (ϕe) and electron transport rates involving PSII and PSI (Vassilev and Lidon, unpublished data). After 10 days exposure of the plants from cv. Ribeka to 28 mg Cd kg-1, the decrease in PSII activity with OEC represented about 40% and without OEC 19%, whereas in PSI activity decreased ca. 30%. The similar inhibition of PSII activities with or without OEC at 42 mg Cd kg-1 treatment showed that Cd could interact with both the donor and the acceptor side of this photosystem. The lower photosynthetic electron transport in Cd-exposed plants from this cultivar was probably not due to Cd-induced lipid peroxidation at thylakoid level as the ethylene production associated with thylakoids was close to that in the control plants. On the contrary, the stronger inhibition of the photosynthetic electron transport in the more sensitive to Cd cultivar CE9704 was linked with increased ethylene production and diminished total fatty acids content in the thylakoids of Cd-exposed plants [Vassilev and Lidon, unpublished data].

CHRONIC CD PHYTOTOXICITY

The data on barley responses to Cd is mainly based on hydroponics experiments and more often represents an acute phytotoxicity [10, 7, 29]. Behaviour of barley on Cd-contaminated soils is rarely investigated. There is some information about metal uptake and productivity of barley grown on industrially polluted soil, but it is relevant just to mixed metal contamination [12]. In our experimentation the study on the chronic Cd toxicity in barley plants has been realized in pot-soil experiments where plants were grown continuously on Cd-contaminated soil. The Cd contamination was set up from 0.6 mg kg^{-1} soil (noncontaminated control soil) to 45 mg kg^{-1} soil (artificially spiked by cadmium sulphate), being up to 23-fold the limit value of Cd in Bulgarian soils - 2 mg kg^{-1} soil [17]. The experiments have been done with two soils, differing mainly in soil texture (clay loam and sandy loam) and two cultivars - Obzor and Hemus [31, 38]. The observed physiological disorders of barley plants related to the chronic Cd phytotoxicity are shortly described below and shown in Table 1.

Plants grown at up to 45 mg kg^{-1} clay loam soil did not exhibit any visual symptoms of toxicity in above-ground parts, but some browning of roots was observed [42] whereas plants grown in sandy loam soil at 25 mg Cd kg^{-1} suffered toxicity showing necrosis of leaf tips [31].

The most obvious effect of Cd at 45 mg kg^{-1} clay soil was found on the development of barley plants. It was retarded at tillering up to 10 days, but this effect became weaker during ontogenesis and at full maturity the development was partly compensated by shortening the duration of the following phases. The slower development could be partly attributed to later emergence of these plants, probably due to the negative effects of Cd on mobilisation of food reserves in the seeds as reported by Bishnoi *et al.* [5] for pea seeds. Dry mass accumulation of plants was diminished by 32-35% at tillering to 10-13% at full maturity compared to the values of control plants [42]. The relatively weaker effect of Cd at latter phases probably was due to an expression of efficient adaptation mechanisms leading to Cd binding in cell walls, complexation with phytochelatins, compartmentalisation in vacuoles, etc. [27].

Plants grown at 45 mg Cd kg^{-1} clay loam soil at early stages of the development had decreased photosynthetic rate (P_N) by 10 - 25% [42]. This negative effect varied in different leaves, being the strongest in the upper leaves, which have higher functional activity [45]. The retarded P_N could be related to disorders in many sites of this integral process. It clearly was not due to stomatal limitation, since transpiration rate was not significantly changed. Malik *et al*. [22] concluded the same for Cd-exposed wheat seedlings grown on sand culture. Cd-induced diminishing in P_N was neither linked to decreased chlorophyll content. Despite that Stobart *et al.* [28] stated Cd as strong inhibitor of chlorophyll biosynthesis in incubated barley leaf segments, obviously, when barley is grown from seed to seed on Cd-contaminated soil the situation is different. Probably, the observed slight negative effect on P_N at the early developmental stages was due to Cd-induced disorders at other sites of the process – enzymes activities, electron transport, etc. as well as other physiological processes having impact on the functioning of photosynthesis. On the other hand, the results obtained showed that whole barley plants were able to acclimatise to high Cd concentrations in the soil. This in turn has lead to a good performance of barley photosynthetic machinery on soils with increased Cd content.

Table 1: Physiological disorders of Cd-exposed barley plants

Parameter	Physiological disorders in barley plants
Disorders observed at Cd-exposed plants having 40 - 80% inhibition of RGR_{DW}	
Leaf water potential	↓ A depression due to a complex of negative effects of Cd on water relations
Root cytokinins	Changes in the free cytokinins levels that might reflect on the export of the hormone to the shoots
Transpiration rate	↓ An inhibition due to both indirect effects on leaf water content and direct effect on stomata functioning
Mineral status	Imbalances in some essential nutrients (K, Zn, Ca, Fe)
Cell respiration rate	↑↓ An initial increase (stress response) followed by decrease in a result of enzymes inhibition
Chloroplast ultrastructure	Disturbed envelope, thylakoid swelling, reduction in the number of grana and thylakoids therein, etc.
Net photosynthetic rate	↓ An inhibition due to both stomata and mesophyll limitations
^{14}C incorporation pattern	Pattern similar to that in aging leaves
Photosynthetic pigments content	↓ A decrease, probably due to biosynthesis inhibition as well as enzymatic degradation
Potential activity of PSII	↓ A slight inhibition
Quantum yield	↓ An inhibition, well expressed at light saturation level
Disorders observed at barley plants suffering chronic Cd toxicity	
Plant development	↓ A retardation at earlier stages, partly due to later emergence
Dry mass accumulation	↓ A decrease expressed better at earlier plant stages
Net photosynthetic rate	↓ A slight inhibition at earlier plant stages, no effect later
Transpiration rate	↓ No significant changes
Leaf respiration	↑ An increase, probably due to stress response

PRODUCTIVITY, SEEDLING CHARACTERISTICS AND CD PHYTOEXTRACTION CAPACITY OF BARLEY GROWN IN CD-CONTAMINATED SOILS

Seed production of barley on Cd-contaminated soil could be successful if this heavy metal does not remarkably reduce its productivity and seedling characteristics. We found that grain productivity of barley significantly decreased only at concentrations of 25 and 45 mg Cd kg^{-1} soil, which are far over the typical Cd contamination of agricultural soils [31, 38]. The decrease in grain productivity of *cvs*. Hemus and Obzor averaged 12 to 18% in Cd-contaminated clay loam soil, whereas in sandy loam soil it was higher. The regression equations describing dependence of grain yield of barley on soil Cd concentrations are shown in Table 2.

The yield formation in cereals involves processes of reduction and compensation in the major yield structural elements during ontogenesis. We found that Cd at 25 mg kg^{-1} and higher significantly decreased all yield structural elements. The inhibiting effect of the metal was first apparent through a decreased total tillering. During ontogenesis plants were characterised by weaker growth, the formation of less productive tillers with lower number of grains per ear and a lower 1000-seed weight [38]. On sandy loam soil, where the negative effect of Cd at tillering was stronger, the 1000-seed weight was slightly higher than in control plants due to an expression of compensation mechanisms based on sink-source interaction change [31].

The background levels of Cd in cereal grains range from 0.013 to 0.22 mg Cd kg^{-1} (DW). The highest Cd grain concentration has been reported for wheat (14.2 mg Cd kg^{-1}), but generally even in contaminated areas it is much lower [18]. In our studies Cd accumulation in grain exceeded the international food standard 0.1 mg Cd kg^{-1} DW [16] if plants were grown at 5 mg Cd kg^{-1} soil, thus, the grain produced may not be used as a foodstuff [31]. The observed mean Cd values in the straw varied between 2 and 15 mg kg^{-1} DW when barley was grown at 5 to 45 mg Cd kg^{-1} soil, respectively [31]. The value of Cd in the straw is not standardised and there is no generally shared opinion about its harmless amount in feeds. However, in view of variable and secondary effects of Cd in the food chain, it is desirable to minimize its concentrations in crops [18]. On the contrary, for phytoextraction purpose Cd concentration in the straw should be maximised, as after the harvest and post harvest treatments it may be disposed as a hazardous waste [6]. The regression equations representing the dependence of shoot and grain Cd concentrations on soil Cd concentrations and soil properties are given in Table 2.

Table 2: Dependence of grain yield, leaf and grain Cd concentrations of barley (*cv*. Obzor) on soil Cd concentrations (X) and soil properties

Parameter (Y)	Soil properties	Regression equation	R^2
Grain yield (g / pot)	sandy loam soil	Y = -0.15X+15.44	0.77
	clay loam soil	Y = -0.06X+12.71	0.84
Leaf Cd concentration(mg / kg DW)	sandy loam soil	Y = 0.35X+1.19	0.78
	clay loam soil	Y = 0.22X+1.07	0.69
Grain Cd concentration(mg / kg DW)	sandy loam soil	Y = 0.04X+0.21	0.77
	clay loam soil	Y = 0.03X+0.33	0.71

Soil Cd contamination of up to 45 mg kg^{-1} had no effect on the seedling characteristics of barley seeds. Germination energy, germination rate and ability of these seeds were within the norm [30, 38]. Barley plants originating from Cd-enriched seeds (up to 2 mg Cd kg^{-1} grain) sown in non-polluted soil grew well without any abnormalities. We did not observe any negative effects on growth, development and productivity of barley in the next generation and, on the other hand, Cd concentrations in grain were below 0.1 mg kg^{-1} [37]. However, if the contaminated soil had sandy loam texture and Cd concentration exceeds 20-25 mg kg^{-1}, a significant decrease (more than 10% of seed productivity) should be expected.

The maximum Cd concentration in the aerial parts of barley was about 22 – 25 mg kg^{-1} DW, found in the lower leaves of plants grown on soil containing 45 mg kg^{-1} soil [42]. We calculated that even in pot conditions, which are well known to increase metal transfer from soil to plants, the maximum Cd phytoextraction with barley would be estimated to not more than 100 g ha^{-1} yr^{-1} [45]. This value is 10 to 20 times lower than values reported for other crops proposed for Cd removal, for example willow and pannycress [24, 26]. It will take more than 100 consecutive croppings to decrease soil Cd concentration from 5 to 2 mg kg^{-1} soil by barley phytoextraction. Therefore, without additional enhancing of Cd uptake this option seems unrealistic for practical implementation.

CONCLUSIONS

Summing up, our studies showed that:

- The reductive analysis of factors limiting growth of Cd-exposed barley plants revealed photosynthesis retardation as one of the most important factors. Cd negatively affects barley photosynthesis directly at different structure-functional levels and indirectly by the metal induced disturbances in the other physiological processes.
- The use of barley for seed production on Cd-contaminated agricultural soils seems a rationale option, as (1) barley seedling qualities are high; (2) its productivity is not significantly affected (except at some very high soil Cd contamination levels); (3) barley plants in the next generation, grown on non-contaminated soils have grain Cd levels within the norm and normal development and productivity.
- Shoot Cd accumulation of barley plants is not high enough to meet the requirements for short-term phytoextraction perspective. If higher shoot Cd accumulation is achieved by means of the induced phytoextraction approach, barley Cd phytoextraction will be limited by phytotoxicity problems.

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