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THE DOSE-DEPENDENT INFLUENCE OF ZINC AND CADMIUM CONTAMINATION OF SOIL ON THEIR UPTAKE AND GLUCOSINOLATE CONTENT IN WHITE CABBAGE (*BRASSICA OLERACEA* VAR. *CAPITATA* F. *ALBA*)

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Abstract—The relationship between the ability to accumulate heavy metals (represented by Cd and Zn) and to synthesize bioactive compounds (represented by glucosinolates [GLS]) was investigated in two cabbage cultivars. Plants were grown in the greenhouse of a phytotron under controlled conditions in soils spiked with two different Zn or Cd concentrations. The measurements of Cd and Zn contents in soil and cabbage (leaf) samples were performed by atomic absorption spectroscopy, whereas GLS levels in cabbage were determined by high-performance liquid chromatography. The ranges of metal contents in soil were 80 to 450 mg/kg dry weight for Zn and 0.3 to 30 mg/kg dry weight for Cd, whereas the levels of accumulated Zn and Cd in cabbage amounted to 15 to 130 and 0.02 to 3 mg/kg dry weight, respectively. After initial symptoms of toxicity, during a later stage of growth, the plants exhibited very good tolerance to both metals. Enhanced biosynthesis of GLS was observed in a dose-dependent manner following exposure to the heavy metals. The GLS content in Zn-exposed cabbage rose from 3.2 to 12 μmol/g dry weight, and the corresponding values for Cd-treated plants were 3.5 to 10 μmol/g dry weight. Thus, the increased soil contamination by metals caused greater accumulation in cabbage, as well as stimulation of GLS biosynthesis. The results obtained point to the high phytoremediation and biofumigation potential of white cabbage. Environ. Toxicol. Chem. 2012;31:2482–2489. © 2012 SETAC

Keywords—Cabbage Heavy metals Glucosinolates Phytoremediation Biofumigation

INTRODUCTION

Contemporary agriculture has reached the stage where it faces challenges whose resolution seems mutually exclusive. The growing human population requires intensification of food production on shrinking arable land area. At the same time, consumers' demands are now oriented toward high-quality food crops, with the emphasis on products of environmentally friendly, sustainable agriculture. In reality, farmland has been exposed for the past decades to devastating anthropogenic pressure. As a result, water as well as soil has become a source of pollution entering the food production chain. Further intensification of agricultural production may only deepen the problem. The introduction of genetically modified crops does not address this issue and is far from being well received by consumers. Therefore, other biotechnological approaches are sought that will assist in soil recultivation and allow large–scale, sustainable crop growth. Those most promising include phytoremediation as a means of removing pollutants from the environment and biofumigation for plant protection against phytophages. Both approaches take advantage of the natural capacities of certain plants and may gain consumers' acceptance.

Phytoremediation is defined as the process by which plants and their associated microorganisms detoxify soils, sediments, and aquatic sites contaminated with organic and inorganic pollutants, including toxic metals [1,2]. Phytoremediation is believed to represent an effective, inexpensive, and safe treatment to remove, contain, or render the contaminants harmless [3]. The efficient clean-up of soil relies on plants known as hyperaccumulators, which are capable of taking up exceptionally large amounts of metals without visible symptoms of toxicity [4]. Several Brassicaceae species display such a property. The best recognized is mustard (*Sinapis alba*) for its ability to accumulate substantial amounts of heavy metals and at the same time to produce biomass ensuring a reasonable level of soil clean-up [5,6]. Once used for phytoremediation, harvested plants become toxic waste and must be disposed of accordingly. However, some plant species, including Brassicaceae, exhibit another feature that makes them candidates of choice for green agriculture. The Brassicaceae synthesize secondary metabolites, glucosinolates (GLS), which are the best-known examples of preformed defense compounds [7,8]. Products of enzymatic GLS degradation yield isothiocyanates (ITC) and indoles with strong biocidal activity. This antibiological activity has given GLS agricultural significance for the use of biofumigation to suppress phytophages and weeds, usually by incorporating harvested material into the soil. Biofumigation refers to the suppression of soil-borne pests and pathogens by biocidal compounds released in soil, when GLS, produced by Brassicaceae applied as green manure or rotation crops, are hydrolyzed. In particular, numerous studies have demonstrated broad biocidal activity of ITC, including insecticidal, nematocidal, fungicidal, antibiotic, and phytotoxic effects [9–13].

Although phytoremediation and biofumigation employ plants belonging to the same or related plant families, they are not typically considered together. In the present study, we

selected white cabbage (*Brassica oleracea* subsp. *capitata* f. *alba*) as a plant species that could combine the two biotechnologies into one environmentally oriented process with possible application in sustainable crop production. In the proposed approach, cabbage is cultivated in soil contaminated with heavy metals. It is assumed that relatively quick growth to substantial biomass will result in the efficient extraction of polluting elements by these plants. In the next stage, the harvested cabbage becomes a raw material for production of biofumigant preparation. The technology of refinement of biofumigant substances from plant material serves two purposes: the isolation of contaminant-free bioactive fraction and simultaneous reduction of the bulk volume of contaminated plant waste requiring costly, specialized utilization. It is important to note that cabbage is particularly appropriate for pest control purposes, because its major GLS is sinigrin, which releases allyl isothiocyanate (AITC), the most biologically active compound among natural ITCs [14]. Moreover, it may be assumed that, similar to other Brassicaceae species, prior exposure to chemical stress will stimulate GLS biosynthesis [15,16].

The research described here was aimed at verifying some assumptions underlying the soundness of the proposed biotechnology. The present study examined the following points: the ability of cabbage to accumulate heavy metals; ensuring optimal biomass gain from contaminated soil; the plant's tolerance to heavy metals; and the influence of chemical stress on GLS content and composition. The first two issues are expected to assess the cabbage's usability for phytoremediation; the last addresses its potential in the case of biofumigation. The proposed investigations also have more general implications. The relationship between the metals in soil and organic defenses against biotic stress has rarely been studied, although these defenses influence the nutritional quality of *Brassica* crops. To date, only our preliminary investigations have been reported [17] for cabbage, despite its extensive worldwide cultivation.

MATERIALS AND METHODS

Chemicals

Acetonitrile (high-performance liquid chromatography [HPLC] grade), imidazole ACS (acetic acid; glacial), nitric acid 65% (suprapure grade), and hydrogen peroxide 30% (suprapure grade) were purchased from Merck. Glucotropaeolin was from AppliChem and sulfatase from *Helix pomatia* H1 (22,400 U/g solid) and DEA-Sephadex A-25 anion-exchange resin from Sigma. Desulfo-glucoiberin (GIB), desulfo-progoitrin (PRO), desulfo-glucoraphanin (GRA), desulfo-sinigrin (SIN), desulfo-gluconapin (GNA), and desulfo-glucobrassicin (GBS) were obtained courtesy of Renato Iori. Standard Cd and Zn stock solutions (atomic absorption spectroscopy/inductively coupled plasma [AAS/ICP] grade) were from J.T. Baker. Matrix modifier 1% solution of $NH_4H_2PO_4$ in 1% HNO_3 was purchased from CPI International. Water was purified using a QPLUS185 system from Millipore.

Plant material and growth conditions

Two cabbage (*Brassica oleracea* subsp. *capitata* f. *alba*) cultivars were used: early cv. Ditmarska Najwcześniejsza (DN) and late cv. Kamienna Głowa (KG). Plants were grown in the greenhouse of a phytotron of the University of Agriculture in Kraków in pots (10 L) filled with the local soil taken from the arable layer (0–20 cm). The granulometric composition of the soil was 19% sand, 5% coarse silt, 41% fine silt, 24% coarse silt clay, 6% clay fine silt, and 5% colloidal clay (pH 7.0). The initial heavy metal contents in the soil were 0.38 mg Cd, 117.5 mg Zn, 21.3 mg Cr, 30.8 mg Pb, 16.2 mg Cu, 19.4 mg Ni per kg dry weight. Seedlings were transplanted into the soil at the stage of six to eight leaves. The soil and plants were fertilized according to agricultural standards, but at the minimal level, just ensuring proper growth and development: N (NO_3-N + NH_4-N) – 105 (DN) and 120 (KG) mg/L dry weight of soil, P ($Ca[HPO_4]_2$) – 50 and 60 mg/L and K (KCl, 60% potassium salt) – 160 and 180 mg/L in the case of DN and KG, respectively. The duration of plant growth in the greenhouse depended on the cultivar and lasted for 113 and 134 d for DN and KG, respectively. The air temperature was 20 to 25/17 to 20°C (day/night), photoperiod was 14/10 h (additional lighting during short and cloudy days), and relative humidity was 30 to 50%.

Cadmium and zinc treatment

The soil used for the experiments was derived from the humus horizon. It contained Cd, Pb, Cu, Cr, and Ni at the natural level and elevated content of Zn. Ten days before planting the seedlings, the soil was mixed with solutions of $ZnSO_4$ and $CdSO_4$. The volume of added salt solutions was calculated to give the metal content in milligrams per kilogram dry weight of soil, equal in the case of Zn to 50 (Zn1) or 200 (Zn2) and in the case of Cd to 10 (Cd1) or 40 (Cd2) [18]. Control pots contained the same soil, but without addition of either Cd or Zn. Each experimental group of plants consisted of four replicates.

Morphometric analyses of young leaves

For fresh and dry weight quantitation, leaves taken on the third week of vegetation were weighed immediately after cutting, dried to a stable mass (80°C, 24 h), kept in the presence of silica gel for 24 h, then weighed again. Leaf area measurements were performed photometrically as described previously [19].

Leaf membrane status

Leaf membrane status from leaves sampled on the third and eighth weeks of vegetation was measured conductometrically as electrolyte leakage (EL) according to a procedure described previously [20].

Determination of Zn and Cd in soil and cabbage

For metal determinations, the air-dried soil samples and lyophilized cabbage samples were mineralized in a microwave-assisted Anton Paar mineralizator. Mineralization was performed at 240°C and 60 bar for 0.5 g portions of cabbage or soil mixed with 5 ml 65% HNO_3 + 2 ml H_2O_2 or 7 ml HNO_3 as a mineralizing solution, respectively. After digestion, the acidic solutions were transferred into 25 ml polymethylpentene volumetric flasks and brought to volume with deionized water. A GBC SensAA atomic absorption spectrometer furnished with deuterium lamp background correction, single element hollow cathode lamps, and air–acetylene flame was used for Zn and Cd determination. In the event of particularly low Cd content, a GBC SavantAA Z atomic absorption spectrometer with graphite furnace atomization was applied. The sample volume injected was 10 μl, and the matrix modifier (1% solution of $NH_4H_2PO_4$) volume was 5 μl. Zinc and cadmium hollow cathode lamps were used as the radiation sources at wavelengths of 213.9 and 228.8 nm, respectively. The calibration curve prepared with the use of AAS/ICP-grade standard stock solutions (1,000 μg/ml of Cd or Zn) was applied for quantitative analysis. For all cabbage and soil samples, four independent determinations were performed. Precision of measurements,

calculated as a coefficient of variation (CV) for both metals determined, was below 10%. The recovery of the metals in the case of cabbage samples was calculated by using standard addition methods. The obtained values were $98.6 \pm 4.5\%$ and $93.7 \pm 7.2\%$ for Zn and Cd, respectively. For soil samples, the laboratory reference material (soil 1) [21] with Zn content of $1{,}722 \pm 30$ mg/kg and Cd content of 1.251 ± 0.030 mg/kg was used for recovery determination. The obtained values were $101.6 \pm 3.3\%$ and $94.1 \pm 6.7\%$ for Zn and Cd, respectively.

Determination of glucosinolates in cabbage

Glucosinolate content in the samples was assessed in triplicate by using the EU official method [22], based on the HPLC analysis of desulfo-GLS obtained through the removal of the sulfate group of GLS via sulfatase-catalyzed hydrolysis. Briefly, 200-mg samples of freeze-dried material were extracted twice with boiling methanol (70%, 3 ml). A known amount of glucotropaeolin (5 mM, 0.2 ml) was added to each sample just before the first extraction as an internal standard for HPLC analysis. The extracted glucosinolates were purified on a 1-ml column filled with 0.5 ml DEA-Sephadex A-25 anion-exchange resin. The column was prewashed with 2 ml imidazol formate (6 M) and twice with 1 ml water and then loaded with 6 ml of each extract. Purified sulfatase enzyme was added to the column, and desulfatation was carried out overnight at room temperature. The next day, the desulfo-glucosinolates were eluted with water (2×1 ml). Desulfo-glucosinolates were analyzed by using an HPLC Agilent 1200 series system with a Grace Altima HP AQ RP-C18 column (150×4.6 mm, 3 μm). Chromatography was performed with a 1 ml/min flow rate at 30°C and a gradient consisting of water (A) and acetonitrile/water (20:80, v/v; B) as follows: linear gradient from 100% A to 100% B for 20 min and isocratically 100% B for 5 min. Desulfo-glucosinolates were detected by diode array detection (DAD) with monitoring of the absorbance at 229 nm. The analytes were identified based on the sequence of elution [23] and retention times of the available standards.

RESULTS AND DISCUSSION

Metal accumulation

The application of cabbage as a phytoremediator depends on its ability to take up metals from the soil. Therefore, the accumulation of Zn, the metal pollutant most abundant in the environment [24], and Cd, regarded as a particularly toxic contaminant [25,26], by cabbage was examined as the first stage of the project. Two cultivars of cabbage that potentially could be used in two different technological schemes were selected for these tests: Ditmarska (DN), the early, quickly growing cultivar that may be harvested twice over agricultural season, thereby accelerating the phytoremediation process, and Kamienna Głowa (KG), a medium-late cultivar grown only once over a season but achieving a large size, ensuring substantial uptake of metals from soil. The contents of Cd and Zn, determined for plants of both cultivars exposed to different concentrations of the studied metals, are presented in Figure 1.

In plants grown in soil spiked with Zn, an increased metal content in leaves was observed only for the higher dose of added Zn (Zn2; Fig. 1). Compared to nontreated control plants, the concentration of Zn rose by approximately two- and threefold in the samples of DN and KG plants, respectively. The increase in uptake of Cd, despite lower initial concentration in soil, turned out to be more marked. Plants of the DN cultivar grown in soil spiked with a lower dose of Cd (Cd1) accumulated three times more of this metal than was determined for control plants. The four times higher Cd content in soil (Cd2) resulted in 18 times higher accumulation of this metal. In the case of the KG cultivar, these increases were much more prominent and amounted to 30 and 100 times, respectively.

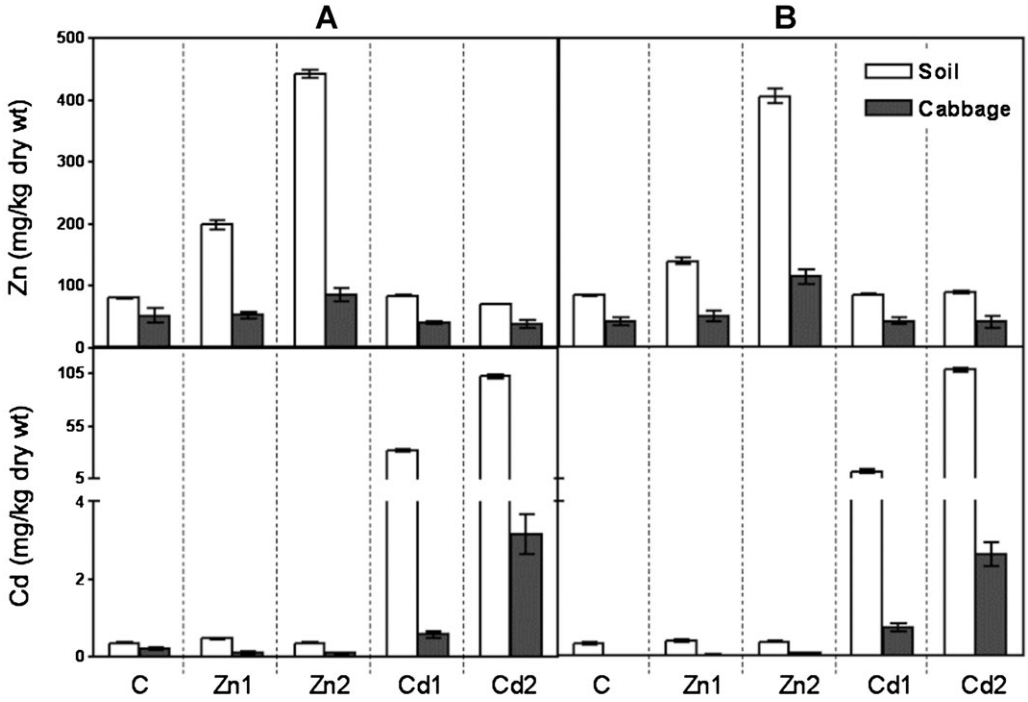

Fig. 1. Contents of Cd and Zn (mg/kg dry wt) in samples of two cabbage cultivars, early Ditmarska Najwcześniejsza (DN; **A**) and medium-late Kamienna Głowa (**B**) and in corresponding soil samples collected after harvest of plants. All samples were derived from phytotron experiments performed on untreated soil (C) or soil spiked with Zn (Zn1 50 mg/kg dry wt, Zn2 200 mg/kg dry wt) or Cd (Cd1 10 mg/kg dry wt, Cd2 40 mg/kg dry wt). Values are means of four determinations ± SD.

The efficient absorption of Cd and Zn was not unexpected because these elements form in soil compounds that are relatively soluble in water and, in comparison with other metals, are easily taken up by roots and stored both in roots and in aerial parts of the plant. As a result, both metals are characterized by high soil–plant transfer coefficients, which are also affected by physicochemical properties of soil, such as pH, and organic matter content [27,28]. These various influences promote the idea that the concentrations of Cd or Zn in plants do not always reflect their content in soil [29].

The values presented in Figure 1 suggest that cabbage leaves are capable of withdrawing a higher percentage of Zn from the contaminated soil (above 30%) than is possible in the case of Cd (less than 10%). It may be presumed that toxicity of Cd stimulates a stronger defense mechanism, limiting uptake of this metal. The proposed main mechanism affecting plant tolerance to Cd toxicity involves reduction of this metal's bioavailability as a result of its sequestration by organic acids secreted by plants to soil. It is also known that root exudates may modify the composition of the rhizosphere in such a way that Cd transfer in soil–plant systems is reduced [30].

The metal contents determined during the phytotron experiments were subsequently used to assess the efficiency of phytoremediation as a means of farmland clean-up of Zn and Cd by white cabbage. The measurements of accumulated amounts of these metals in plant biomass and the yields of cabbage crops declared by registered producers of seedlings to range from 150 to 300 tons per hectare were the basis for calculating the output of pollutants from the environment. Table 1 presents the results of these calculations for the bottom limit of crop yields. In the case of Zn, the output from soil with a single crop of white cabbage (assuming a range 150–300 tons/ha) was estimated to amount to 300 to 600 g/ha for plants grown in normal soils (equivalent to nonspiked, C samples). The plants grown in heavily Zn-contaminated soils (such as Zn-spiked soil, Zn2 samples) might be capable of removing 1,800 to 3,600 g/ha of this metal depending on the level of contamination and the cabbage variety. According to Poniedziałek et al. [31], white cabbage accumulates Zn mainly from the surface horizon, reducing its concentration in soils containing approximately 50 mg/kg of this metal by more than 20%. Cabbage turned out also to be the best Zn phytoremediator among nine crops tested [31]. The efficiency of Brassicaceae, namely, rapeseed (*Brassica napus*), was also shown in field experiments carried out for Se, another biogenic element that, similarly to Zn, at higher doses poses an environmental threat. The ability of this crop to remove Se from seleniferous regions of India varied from 716 to 1,374 g/ha/year at peak flowering, decreasing to 736 to 949 g/ha/year at maturity [32]. These results suggest that a substantial portion of accumulated Se was reintroduced to soil with rapeseed pollen and flower remnants. It is important to point out that during the first year of vegetation of cabbage, such a route of secondary release of pollution does not exist.

Table 1. Output of Zn and Cd from farmland with cabbage crop (field) predicted based on pot experiments (cabbage)

		C	Zn1	Zn2	Cd1	Cd2
Early cultivar DN						
Zn	Cabbage[a]	1.997	3.131	7.129	2.073	2.598
	Field[b]	0.300	0.467	1.069	0.311	0.390
Cd	Cabbage[a]	0.018	0.023	0.027	0.295	0.505
	Field[b]	0.003	0.003	0.004	0.044	0.076
Medium-late cultivar KG						
Zn	Cabbage[a]	3.411	4.305	12.09	3.00	3.400
	Field[b]	0.512	0.646	1.813	0.450	0.510
Cd	Cabbage[a]	0.03	0.015	0.049	0.287	1.198
	Field[b]	0.005	0.002	0.007	0.043	0.180

[a] Metal accumulation (mg/kg fresh wt) calculated for cabbage grown in the greenhouse of a phytotron in pots with soil spiked with Zn or Cd.

[b] Metal uptake from soil (g/ha) assuming the lowest limit of crop yield expected for cabbage by registered producers of seedling, that is, 150 tons/ha.

C = control sample (untreated soil); Zn1 = soil spiked with Zn (50 mg/kg dry wt); Zn2 = soil spiked with Zn (200 mg/kg dry wt); Cd1= soil spiked with Cd (10 mg/kg dry wt); Cd2 = soil spiked with Cd (40 mg/kg dry wt); DN = Ditmarska Najwcześniejsza; KG = Kamienna Głowa.

When it comes to Cd, according to the results from the phytotron experiment, the estimated output of this pollutant from noncontaminated (C samples) and strongly Cd-contaminated (Cd2 samples) soils may vary from 3 to 6 g/ha to 180 to 360 g/ha, respectively, assuming the low to high cabbage crop yield. As shown in field experiments by other authors [31], in the case of soil containing approximately 50 mg/kg of this element, cabbage takes up Cd mainly from the 20- to 40-cm horizon with an efficiency of up to 40%. Interestingly, our estimates for cabbage significantly exceed the phytoremediation efficiency most recently reported for Cd-contaminated wastewater irrigation area in China cleaned up with *Beta vulgaris* L. var. *cicla* [33]. In this field study, the application of Cd hyperaccumulator plant species resulted in removal of maximally 144.6 mg/ha of this metal over one growing season (two months).

The output estimates presented and comparisons with the actual field clean-up efficiency offered by other plants suggest that white cabbage may represent a very promising crop for phytoremediation of farmlands polluted with either biogenic or toxic heavy metals.

Plant morphology and growth

Effective phytoremediation is possible only when the proper growth of a phytoremediator is not jeopardized. Therefore, the influence of Zn and Cd on cabbage condition was evaluated. Visual examination of leaves revealed no injuries, such as wilting or necrosis, although the shade of green and the shape (in the case of DN cultivar only) differed from those of control plants (Fig. 2). These alterations of color and size of the leaves (thinner petioles and smaller blades) were more apparent in plants grown in Cd-spiked soils and seemed concentration dependent.

Morphometric studies confirmed visual observations and revealed that leaves of DN plants were not affected by Zn but responded negatively to Cd (Table 2). Decreases in both leaf fresh (dependent on water content) and leaf dry weight was seen. This drop was more than twofold in the case of DN plants exposed to higher Cd concentration (Cd2). The results, however, suggest that Cd did not affect water management of leaves. On the contrary, the response of leaves of KG plants to Zn was surprisingly more dramatic than the response to Cd, which is regarded as the more toxic metal (Table 2). Such results together with higher accumulation of Cd (Fig. 1 and Table 1) point to the KG cultivar as a particularly appropriate phytoremediator for Cd-polluted areas.

The evaluation of membrane status of both cultivars grown in metal-spiked soils (Fig. 3) paralleled the results of morphometric measurements. The toxic impact of Cd to DN plants was mirrored by stronger electrolyte leakage (EL) but stood in contrast to the more detrimental effect of Zn than Cd even at higher dose (Cd2) to KG plants. All values of EL, whether determined for Cd- or Zn-treated plants, seemed to be dose dependent (two- or threefold EL increase with increased Zn or

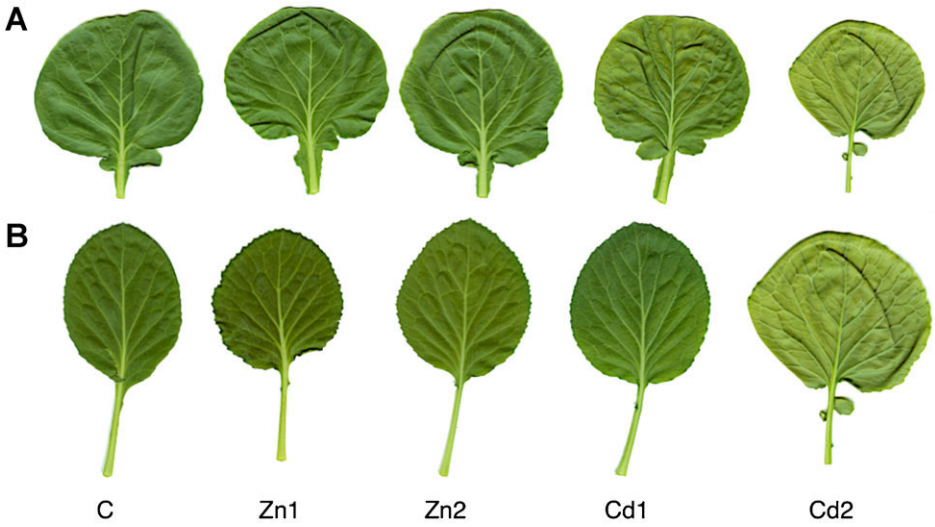

Fig. 2. Morphology of leaves of cabbage plants, early Ditmarska Najwcześniejsza (DN; **A**) and medium-late Kamienna Głowa (KG; **B**) cultivars. Leaves were collected during phytotron experiments performed on untreated soil (C) or soil spiked with Zn (Zn1 50 mg/kg dry wt, Zn2 200 mg/kg dry wt) or Cd (Cd1 10 mg/kg dry wt, Cd2 40 mg/kg dry wt). [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com.]

Cd dose in relation to control). Most interestingly, no injuries were detected on the eighth week of vegetation. This could mean that newly formed leaves were devoid of Cd and contained supraoptimal Zn levels, whereas the accumulated metals remained in the older leaves. Hence, the restriction of transport from roots to shoots is not the only mechanism of the tolerance of cabbage to Zn and Cd.

Glucosinolate content

The composition of GLS is determined genetically and thus characteristic for a given plant species. More than 80% of the identified GLS occur in the Brassicaceae plant family, which consists of nearly 3,000 species [34]. The content of these secondary metabolites in plants is highly variable and can range from less than 1% of the dry weight in some tissues of Brassica vegetables [35] to 10% in the seeds of some species, where GLS may represent half of the sulfur content of the seeds. Glucosinolates can be divided into three groups depending on the nature of the side chain: aromatic, aliphatic, and indolyl.

In the case of white cabbage, major GLS belong to aliphatic and indolic metabolites and include glucoiberin, progoitrin, epiprogoitrin, sinigrin, glucoraphanin, glucobrassicin, neoglucobrassicin, and 4-hydroxyglucobrassicin [36,37]. In Figure 4, a typical profile of desulfo-glucosinolates in the KG cabbage sample is presented. As can be seen, all GLS described in the literature as typical for white cabbage were detected in plants of this cultivar. The same composition was determined also for DN specimens (Table 3).

In general terms, although the types of GLS are relatively constant for a given species and are unaffected by environmental factors, the total concentration and the abundance of individual compounds can be affected by several such factors. For example, it has long been recognized that exposure to heavy metals in soil induces changes in the profiles of GLS synthesized by Brassicaceae [38–41].

The modifications imposed by chemical stress affecting the total content of GLS, as well as abundance of individual compounds, will influence the final biofumigatory effect. Therefore, it was important to examine whether and in what way the cultivation of cabbage in metal-spiked soils impacts GLS profile of this plant species. Table 3 presents the contents of major GLS determined for plants of the two studied cabbage cultivars exposed to different concentrations of Zn and Cd. Although the composition of GLS in control and treated cabbage was the same, the determined contents of individual components varied depending on growing conditions. This means that biosynthesis of GLS must have been affected by metal stress, in most cases stimulated in a dose-dependent manner. In the case of the samples studied, total GLS concentrations varied from 3.5 to 10 μmol/g dry weight for the early cabbage cultivar DN and from 6 to 12 μmol/g dry weight for the medium-late cultivar KG and thus were in the range expected

Table 2. Morphometric parameters of leaves of two cabbage cultivars grown on soil spiked with Zn or Cd

Morphometric parameters[a]	C	Zn1	Zn2	Cd1	Cd2
Early cultivar DN					
Fresh weight of leaves (g)	5.53 ± 0.99	5.47 ± 0.70	5.13 ± 0.62	3.24 ± 0.26	2.47 ± 0.17
Dry weight of leaves (g)	0.471 ± 0.068	0.418 ± 0.118	0.419 ± 0.035	0.248 ± 0.031	0.177 ± 0.011
Leaf area (cm^2)	146.4 ± 13.6	141.9 ± 14.5	127.1 ± 13.4	91.3 ± 8.36	73.6 ± 3.17
Medium-late cultivar KG					
Fresh weight of leaves (g)	4.99 ± 0.49	3.25 ± 0.64	2.81 ± 0.45	3.98 ± 0.45	3.44 ± 1.22
Dry weight of leaves (g)	0.334 ± 0.086	0.209 ± 0.070	0.170 ± 0.030	0.274 ± 0.044	0.248 ± 0.093
Leaf area (cm^2)	89.7 ± 16.5	57.8 ± 6.39	61.5 ± 8.50	80.1 ± 17.5	67.7 ± 17.7

[a] Values are means of four determinations ± SD, assayed on the third week of cabbage vegetation.
C = control sample (untreated soil); Zn1 = soil spiked with Zn (50 mg/kg dry wt); Zn2 = soil spiked with Zn (200 mg/kg dry wt); Cd1 = soil spiked with Cd (10 mg/kg dry wt); Cd2 = soil spiked with Cd (40 mg/kg dry wt); DN = Ditmarska Najwcześniejsza; KG = Kamienna Głowa.

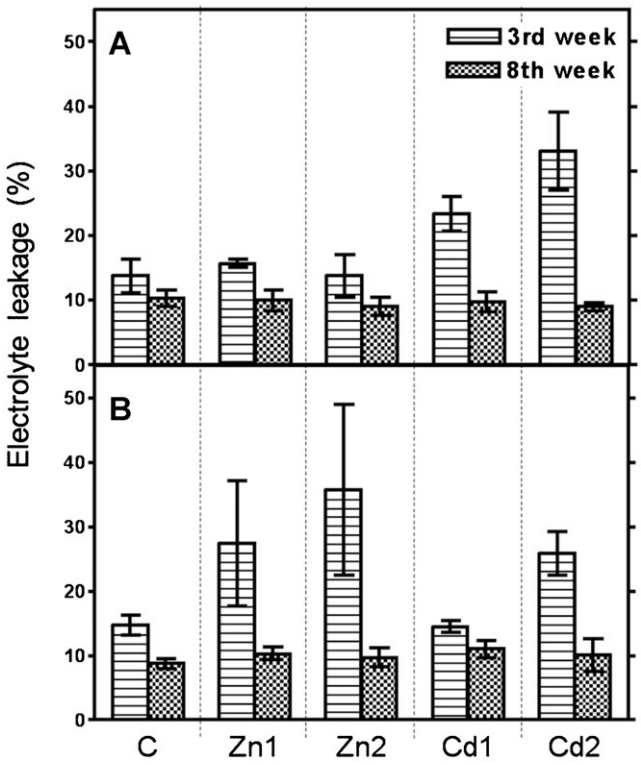

Fig. 3. Influence of Zn and Cd on cabbage leaf membrane status assayed by electrolyte leakage: early Ditmarska Najwcześniejsza (DN; **A**) and medium-late Kamienna Głowa (KG; **B**) cultivars. Leaves were collected during phytotron experiments performed on untreated soil (C) or soil spiked with Zn (Zn1 50 mg/kg dry wt, Zn2 200 mg/kg dry wt) or Cd (Cd1 10 mg/kg dry wt, Cd2 40 mg/kg dry wt). The values are means of four determinations ± SD.

(total GLS contents ranging 3–27 μmol/g dry wt have been reported [36,37]). From a biofumigation point of view, the most important finding is that in plants of both studied cultivars Zn as well as Cd enhanced production of sinigrin. As mentioned above, the AITC formed as result of sinigrin degradation exhibits the strongest biocidal activity among natural ITCs [14].

The effects observed here can be interpreted as induction of pathogen-resistance-related genes as a response to metal-stress-derived signal. Similar observations for a number of Brassica plants have been published. More than 30 years ago, Mathys [15] suggested that GLS have some roles in Zn tolerance mechanisms based on determinations of higher GLS levels in Zn-resistant than in Zn-sensitive *Thlaspi alpestre*. Later, total GLS concentrations in metal hyperaccumulator/tolerant species were reported to be higher than [16], lower than [15,42–44], or of the same order of magnitude as [45] those reported for Brassicaceae species. We have demonstrated that in cabbage, mechanisms of metal-induced fortification, recently reviewed by Poschenrieder et al. [46], are stimulated by chemical stress and probably involve genetic component.

To visualize better the relationship between exposure to metals and GLS accumulation in cabbage leaves, the total contents of indolic and aliphatic glucosinolates are given separately in Figure 5. In the case of both cultivars, the exposure to metals—especially Zn2 and Cd2 at higher doses—resulted in increased biosynthesis of GLS. Growth in Zn-spiked soil seemed to trigger strongly the jasmonate signal transduction pathway, which is known to stimulate indolic GLS biosynthesis [47]. The exposure to Cd resulted rather in the enhancement of production of aliphatic GLS. These data positively verified the working hypothesis that cabbage plants used for phytoremediation, thus exposed to chemical stress, may as a result become more efficient biofumigants.

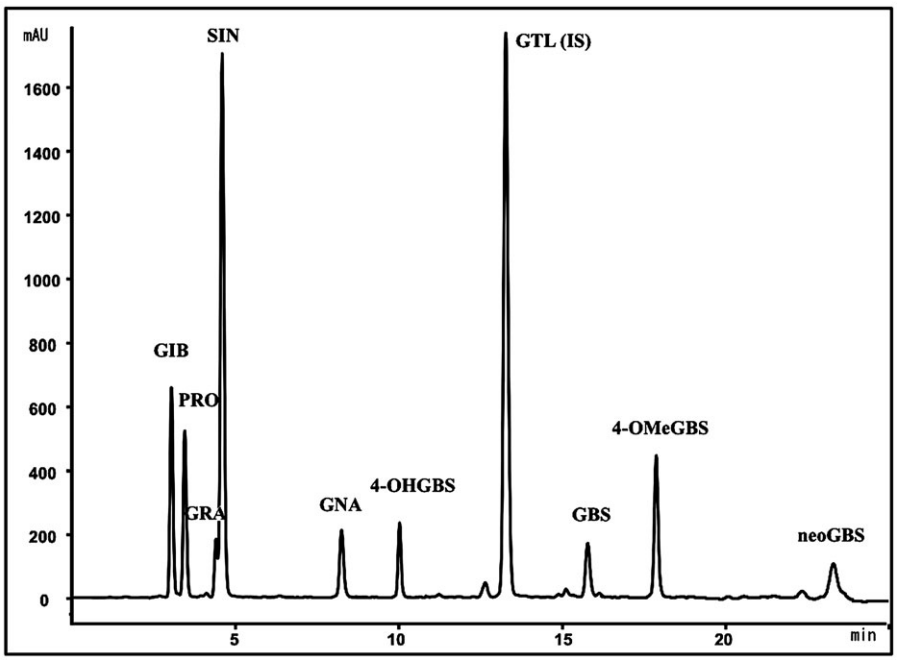

Fig. 4. Typical chromatographic profile of white cabbage (*Brassica oleracea* var. *capitata* f. *alba*) desulfo-glucosinolates (GLS) obtained during high-performance liquid chromatography (λ = 229 nm) analysis of control samples of medium-late cabbage cultivar Kamienna Głowa (KG). The GLS detected include glucoiberin (GIB), progoitrin (PRO), glucoraphanin (GRA), sinigrin (SIN), gluconapin (GNA), 4-hydroxyglucobrassicin (4-OHGBS), glucotropaeolin (GTL; internal standard), glucobrassicin (GBS), 4-metoxyglucobrassicin (4-OMeGBS), and neoglucobrassicin (neoGBS).

Table 3. Content of major glucosinolates in leaves of two cultivars of cabbage grown on soils spiked with Zn and Cd

Glucosinolate[a]	C	Zn1	Zn2	Cd1	Cd2
Early cultivar DN					
GIB	0.15 ± 0.01	0.15 ± 0.01	0.22 ± 0.03	0.34 ± 0.03	0.23 ± 0.01
PRO	0.10 ± 0.01	0.07 ± 0.01	0.17 ± 0.02	0.18 ± 0.02	0.21 ± 0.02
GRA	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.02 ± 0.01
SIN	0.86 ± 0.03	1.35 ± 0.09	3.89 ± 0.06	1.15 ± 0.11	4.29 ± 0.16
GNA	0.09 ± 0.01	0.08 ± 0.01	0.15 ± 0.02	0.11 ± 0.02	0.12 ± 0.03
4-OHGBS	0.14 ± 0.01	0.19 ± 0.01	0.50 ± 0.04	0.24 ± 0.03	0.52 ± 0.03
GBS	0.12 ± 0.03	0.12 ± 0.02	0.49 ± 0.03	0.20 ± 0.02	0.22 ± 0.03
4-OMeGBS	1.38 ± 0.17	0.70 ± 0.08	2.89 ± 0.08	1.14 ± 0.15	1.41 ± 0.09
neo-GBs	0.12 ± 0.02	0.10 ± 0.01	0.37 ± 0.06	0.09 ± 0.01	0.22 ± 0.01
Medium-late cultivar KG					
GIB	0.51 ± 0.04	0.64 ± 0.03	0.40 ± 0.11	0.25 ± 0.11	0.55 ± 0.11
PRO	0.53 ± 0.02	0.50 ± 0.02	1.13 ± 0.27	0.39 ± 0.10	1.10 ± 0.26
GRA	0.24 ± 0.07	0.27 ± 0.02	0.11 ± 0.01	0.07 ± 0.02	0.13 ± 0.09
SIN	2.31 ± 0.29	2.39 ± 0.04	2.88 ± 0.42	1.57 ± 0.26	3.58 ± 0.63
GNA	0.08 ± 0.01	0.15 ± 0.01	1.11 ± 0.32	0.68 ± 0.10	1.31 ± 0.28
4-OHGBS	0.05 ± 0.01	0.08 ± 0.01	0.38 ± 0.09	0.16 ± 0.06	0.23 ± 0.06
GBS	0.19 ± 0.07	0.54 ± 0.01	0.69 ± 0.09	0.85 ± 0.12	0.55 ± 0.04
4-OMeGBS	1.18 ± 0.40	1.01 ± 0.02	0.44 ± 0.30	0.74 ± 0.14	0.97 ± 0.24
neo-GBs	0.22 ± 0.05	1.39 ± 0.11	2.88 ± 0.42	0.82 ± 0.18	0.47 ± 0.14

[a] Values are means of four determinations ± SD (μmol/g dry wt).
C = control sample (untreated soil); Zn1 = soil spiked with Zn (50 mg/kg dry wt); Zn2 = soil spiked with Zn (200 mg/kg dry wt); Cd1= soil spiked with Cd (10 mg/kg dry wt); Cd2 = soil spiked with Cd (40 mg/kg dry wt); GIB = glucoiberin; PRO = progoitrin; GRA = glucoraphanin; SIN = sinigrin; GNA = gluconapin; 4-OHGBS = 4-hydroxyglucobrassicin; GBS = glucobrassicin; 4-OMeGBS = 4-metoxyglucobrassicin; neo-GBS = neoglucobrassicin.

CONCLUSIONS

The purpose of the present study was to verify the theoretical grounds for an approach in which white cabbage (*Brassica oleracea* var. *capitata* f. *alba*) is employed to couple phytoremediation and biofumigation processes into one sustainable biotechnology. In this approach, the first phase consists of growing cabbage in soil that requires recultivation to extract contaminating heavy metals in the process of phytoremediation. The phytotron experiments indicated that both cultivars studied were able to absorb Zn and Cd with only limited initial toxic effects, which were overcome altogether at later stages of growth. The estimates of phytoremediation efficiency of cabbage to remove heavy metals from the environment, predicted based on greenhouse conditions, markedly exceeded those reported for other plant species tested in field studies. The second, biofumigation stage of the proposed biotechnology takes advantage of the fact that chemical stress can stimulate the biosynthesis of glucosinolates, which are precursors of isothiocyanates, exhibiting strong biocidal activity. The present results indicate that the exposure to Zn and Cd strongly, and in a dose-dependent manner, stimulated biosynthesis of GLS, especially in the case of the medium-late cultivar. This increase was particularly visible for sinigrin, a precursor of AITC, the most effective biofumigant. All these results point to the high phytoremediation and biofumigation potential of white cabbage. Thus, this initial stage of investigation confirms the soundness of the proposed biotechnology in which cabbage cultivated for phytoremediation purposes can be used as a raw material in the production of natural pesticides. The technology of isolation of bioactive compounds obviously has to ensure the eradication of heavy metals from the final biofumigation product.

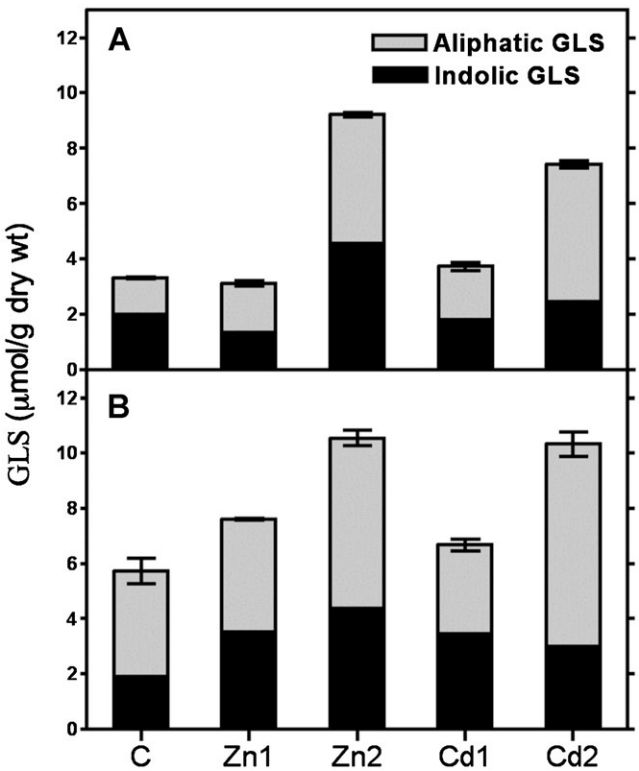

Fig. 5. Total content of indolic and aliphatic glucosinolates (GLS; μmol/g dry wt) in leaves of early DN (**A**) and medium-late KG (**B**) cabbage cultivars grown in untreated soil (C) or soil spiked with Zn (Zn1 50 mg/kg dry wt, Zn2 200 mg/kg dry wt) or Cd (Cd1 10 mg/kg dry wt, Cd2 40 mg/kg dry wt). The values are means of four determinations ± SD.

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