Diego A. Moreno, Gemma Víllora, Joaquín Hernández, Nicolás Castilla, and Luis Romero. Accumulation of Zn, Cd, Cu, and Pb in Chinese Cabbage As Influenced by Climatic Conditions under Protected Cultivation. *Journal of Agricultural and Food Chemistry* 2002 *50* (7), 1964-1969.

DOI: 10.1021/jf011142v

Accumulation of Zn, Cd, Cu, and Pb in Chinese Cabbage As Influenced by Climatic Conditions under Protected Cultivation

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Accumulation of heavy metals from agricultural soils contaminated by low levels heavy metals has important implications in the understanding of heavy metal contamination in the food chain. Through field experiments (1994-1996), the influence of thermal regime under different treatments on the accumulation of zinc, cadmium, copper, and lead in Chinese cabbage [Brassicapekinensis (Lour) Rupr. cv. Nagaoka 50] grown in a Calcareous Fluvisol (Xerofluvent) in Granada (southern Spain) was examined. Two floating row covers were used: T_1 (perforated polyethylene, 50 μm thick) and T_2 (17 g m^{-2} polypropylene nonwoven fleece). An uncovered cultivation (T_0) served as control. Zn, Cd, Cu, and Pb levels in the whole tops of experimental plants were analyzed. Treatments T_1 and T_2 gave rise to differences in environmental conditions with respect to T_0. The influence of environmental factors manipulated by floating row covers (particularly under T_1) increased total heavy metal accumulation in the aboveground plant biomass with respect to the open-air crop. The total contents of Zn, Cd, Cu, and Pb were 30, 50, 90, and 40% higher in T_1, respectively, than in T_0. This technique could be used in contaminated zones for different plant species because the thermal effect favors the process of phytoextraction and thus reduces the contamination.

KEYWORDS: Brassica pekinensis; heavy metals; temperature; phytoremediation

INTRODUCTION

Heavy metal contamination of soil causes a variety of environmental problems, and metal-acumulating plants have been used to remove toxic metals from soil (*1*, *2*). Phytoremediation is the use of plants to extract, sequester, and/or detoxify pollutants (*3*, *4*). In Spain, indiscriminate application of inorganic fertilizer and pesticides has led to a buildup of heavy metal residues in many agricultural soils, reducing agricultural productivity. Soils contaminated with low levels of heavy metals are now frequently used for vegetable growing, and heavy metals from these polluted soils may accumulate in the agricultural plants being grown in them and thereby enter the human food chain (*5*, *6*). Adverse consequences may ensue, such as phytotoxicity or quality deterioration of edible portions from metal enrichment (*7*, *8*). Consequently, growing interest has focused on how heavy metals affect plant metabolism, stemming from the idea that plants can be used for phytoremediation (*9*). In this situation, heavy metal buildup is a major need. However, because greenhouse or pot studies may not be representative of field conditions, metal acumulation in soil and plant uptake under natural open-field conditions (with rural or industrial influence) is of great interest (*10*).

Chinese cabbage [*Brassica pekinensis* (Lour) Rupr, Brassicaceae], a major leafy vegetable in eastern Asia because all of the aboveground biomass is edible and highly nutritious (heading types such as wong baak, napa, or won bok), is gaining worldwide market demand. However, in less suitable environments in southern Spain, early spring field seeding may result in a high percentage of flower stalks, and thus to produce a good spring crop, seedlings raised in the greenhouse and transplanted to the field may be protected with plastic row covers used as a season-extending technology (*11*, *12*). The use of row covers is expanding as a simple, cheap, and effective semiprotecting technique, which can promote early growth by creating a mini-greenhouse effect and thus improve thermal conditions for both the root and the shoot zone (*12-14*).

The objectives of this study are to elucidate the effects of growth conditions, manipulated by the floating row covers, on the uptake of Zn, Cu, Cd, and Pb in Chinese cabbage. It is hypothesized that growth conditions under floating row covers contribute to higher uptake of Zn, Cd, Cu, and Pb in a highbiomass vegetable crop belonging to Brassicaceae, the Chinese cabbage.

MATERIALS AND METHODS

Field Site and Experimental Design.

The experiments were conducted in 1994, 1995, and 1996 at Granada, Spain (37° 10′ 11″ N; 3° 38′ 10″ W; altitude 600 m). Seeds of Chinese cabbage [*B. pekinensis* (Lour) Rupr. cv. Nagaoka 50, heading type, generally produceing an elongated, compact head composed of wrinkled leaves with broad veins] were sown on 8 February 8 in polyethylene trays, containing a mixture of compost and vermiculite (4:1) and kept under controlled greenhouse conditions (24 (4 °C; 60-80% relative humidity). The seedlings, at the four-leaf stage and with a fresh weight exceeding 2 g, were transplanted into experimental plots.

The experimental design was a randomized complete block with four replicates per treatment. Each plot, oriented east-west and measuring 4 m × 1.5 m, had 4 rows of 12 plants each spaced ∼33 cm apart in both directions (11.11 plants/m^2). For the determinations, only plants from the two central rows were used. The treatments were two different floating row covers: perforated polyethylene (T_1, 50 μm thick and 500 holes/m^2, each 10 mm in diameter, Repsol Quı´mica S.A.-Alcudia S.A.) and polypropylene (T_2, a floating nonwoven sheet, Agryl-P17, Sodoca Manufacturing). The control (T_0) plants were uncovered. Floating row covers are used by cutting a piece of netting larger than the area to be covered and laying the piece out over the bed, leaving some slack for growth. The edges of the netting are secured by scooping a little soil onto the cloth, completely covering the edge. The cover is anchored with rocks or other weights. It is important that there not be any openings under the cloth. Plots were covered on the day of transplanting (35 days of age), and covers were kept on as long as possible to generate environmental differences between treatments. They were removed permanently when they began to interfere with further plant growth (80 days). Fifteen days after the covers were removed, the last sample was taken (95 days).

A portable agrometeorological station was installed in the test plots to record, both in the open air and under the row covers, the following parameters: soil temperature at 5 and 15 cm depths, air temperature, relative humidity, and solar radiation. The station consisted of a Campbell Scientific CR-21X datalogger programmed to take measurements every 15 min and average these each hour for all of the sensors except solar radiation sensors, from which data were recorded every minute. The temperature sensors used both in the open and under the two floating row covers were Campbell Scientific 107 probes, with a maximum error of (0.4 °C within the range of -23 to 48 °C. The sensors used to determine the solar radiation in the open air and under the covers were LI-COR LI-200 pyranometers. The sensors used for measuring relative humidity were of two types: ventilated aspirator psychrometers (wet bulb-dry bulb) and Rotronic MP 100 sensors to measure moisture in the solid state. Table 1 lists the mean values recorded for environmental conditions during the growing seasons.

The fertilizer program used was the same as that used by local farmers. A complete NPK fertilizer (15-15-15) was mixed into the soil before planting at 750 kg ha^{-1}. Subsequently, a total of 170 kg ha^{-1} of ammonium nitrosulfate (26% N) and 360 kg ha^{-1} of KNO_3 (13% N, 45% K) was supplied in two applications throughout plant growth.

At seeding, the fungicide ethylene bis(ditiocarbamate) of zinc (Zineb) was used at 2 g m^{-2}, and at transplanting γ-1,2,3,4,5,6-hexachlorocyclohexane (Lindane) was used at 4 g m^{-2}. After the plastic covers were removed, a solution of $CaCl_2$ (0.3 g L^{-1}) was applied to the foliage to prevent tipburn.

The air-dried soil samples were taken before planting (0-30 cm) and showed the following characteristics: Xerofluvent or calcareous Fluvisol; 453 g kg^{-1} sand, 432 g kg^{-1} silt, and 112 g kg^{-1} clay; pH (1:2.5 soil/water) 8.6; electrical conductivity (EC) in saturated paste, 1.10 dS m^{-1}; $CaCO_3$ (112 g kg^{-1}); organic matter 14 g kg^{-1}; extractable Olsen-P (58 mg kg^{-1}); exchangeable K (1 M ammonium acetate), 115 mg kg^{-1}; total (HNO_3 digested) Zn (65 mg kg^{-1}), Cd (50 μg kg^{-1}), Cu (16 mg kg^{-1}), and Pb (24 mg kg^{-1}); DTPA-extractable (*15*) Zn (5 mg kg^{-1}), Cd (3 μg kg^{-1}), Cu (5 mg kg^{-1}), and Pb (12 mg kg^{-1}).

The characteristics of the irrigation water were as follows: pH 7.6; EC, 1.05 dS m^{-1}; Cl$^-$, 58.5 mg L^{-1}; Na$^+$, 25 mg L^{-1}; Zn, 12 μg L^{-1}; Cu, 24 μg L^{-1}; Cd, 1 μg L^{-1}; and Pb, 2 μg L^{-1}. The plants were flood irrigated at transplanting to aid establishment and weekly during growth.

Plant Sampling and Analysis.

Plants were sampled at 15-day intervals throughout the biological cycle, and samples of four plants were taken from each plot. The roots were cut off and, in the laboratory, the samples (whole tops) were washed thoroughly in distilled water after washing with 1% nonionic detergent and then blotted on filter paper. For the assay of heavy metals, oven-dried and pulverized plant material was digested with concentrated nitric acid and 30% hydrogen peroxide until the evolution of the nitrous gas stopped and the digest became clear (*14*, *16*). After dilution, the digest was analyzed for Zn, Cd, Cu, and Pb using atomic absorption spectrophotometry (PerkinElmer model 5100) with a graphite furnace (Perkin-Elmer model 5100 ZL Zeeman) as well as pyrolytic graphite tubes depending on the element being determined. The standard addition calibration subroutine, with a three-point calibrataion line, was used (*16*). For the soluble fraction of Zn, Cd, Cu, and Pb, dry matter (0.15 g) was extracted with 10 mL of 1 M HCl for 30 min and then filtered, and extracts were determined using the method indicated above. Appropriate blanks and standards for both analyses were also prepared by performing the entire extraction procedure but in the absence of the samples.

Statistical Analysis.

All data were subjected to analysis of variance (ANOVA) procedures accomplished using the Statgraphics 7.0 DOS version program and Duncan's multiple-range test was used to compare the significance of the differences (LSD $P < 0.05$) (*17*, *18*).

The results of the parameters measured varied slightly between years, and homogeneity of variance was tested by accepted methods (*17*, *18*) and found to be not significant. Therefore, the statistical analyses of the data from each year were pooled to avoid duplication of the calculations and to simplify the presentation of the results.

RESULTS

The air temperatures registered over the three experimental years reached the highest mean in the perforated polyethylene T_1 treatment, whereas in the nonwoven sheet with no perforations, T_2, the values were intermediate between those of T_1 and T_0 (Table 1). The root temperatures at 5 cm depth were higher in T_1 and T_2 than in T_0, with an average increase of some 5 °C in both cases (Table 1). Root temperatures at 15 cm soil depths were 4 °C lower in T_0 than in T_1 and T_2 (Table 1). The relative humidity values under the covers (T_1 and T_2) were 8 and 10% higher, respectively (Table 1), than in the open-air treatment (T_0). During the cultivation, rainfall averaged 0.86 L m^{-2}. As expected, T_1 and T_2 partly screened the sunlight reaching the plants, reducing instantaneous solar radiation by 13% (Table 1). Cumulative solar radiation during the entire cycle was reduced 17 and 16%, respectively, by T_1 and T_2 with respect to T_0.

The influence of the thermal regime on the production of fresh weight was most notable in T_1, the treatment that reached the highest values, surpassing T_0 by 123%, whereas T_2 surpassed T_0 by 105%. Similarly, the dry weight of the shoot was highest for T_1, exceeding T_0 values by 34%, whereas T_2 exceeded T_0 values by 18%. The lowest yield of Chinese cabbages at harvest was given by T_0, whereas T_1 gave the highest total yield, 111% higher than T_0, and T_2, 108% higher (Table 2). The accelerated growth was probably the result of greater foliar expansion, which provides better distribution of mineral nutrients as well as photoassimilates in the shoot (*11*, *22*, *27*). We found that fresh weight as well as dry weight increased ($P < 0.001$, data not presented) with plant age. Between the third and fourth samplings (65-80-day-old plants), both fresh and dry weights represented half of the total biomass recorded at the end of the crop cycle (mean values at 95 days of age: 934.4 g of fresh weight/plant and 34.3 g of dry weight/plant).

The imposed microenvironmental conditions induced significant differences in the Cd concentration (**Table 3**), which in T_2 proved higher, surpassing T_0 by 28%, but in T_1 did not differ significantly from control. The Cu concentration showed no differences between T_1 and T_2, both exceeding control (70 and 50%, respectively). For Pb, T_1 and T_0 were similar in concentration, and the lowest value was registered in T_2, some 23% lower than control. Finally, Zn declined in the following manner: $T_2 > T_1 > T_0$ (Table 3). The soluble fraction of the elements is closely related to their physiological availability (*12*, *14*, *16*) and is a good indicator of plant status together with the total content (*16*, *19*). The Cd and Cu soluble concentrations greatly surpassed the control in T_1 and T_2, whereas soluble Zn showed decreasing concentrations as follows: $T_2 > T_1 > T_0$. Meanwhile, the soluble Pb did not differ between treatments.

During development, the concentrations of Cd, Cu, Pb, and Zn significantly fell ($P < 0.001$; Figure 1). Consequently, in all cases the concentrations were higher in the first phases of the cycle (35-50 days of age) and lower at the end of the cycle.

In terms of the environmental effects of potentially toxic elements, estimations of accumulated amounts are more meaningful than are concentrations in plant parts (*20*). Thus, the effect of the microclimate under the floating row covers with respect to accumulation of heavy metals (**Table 4**) shows clear differences according to the treatments, as total Cd in T_1 and T_2 exceeded 50% at the accumulated level in control, whereas total Cu in T_1 surpassed the accumulation of T_0 by 90%, although T_2 also accumulated some 55% more Cu than did control. However, total Pb in T_0 and T_2 did not significantly differ, and T_1 registered the highest level of accumulated Pb (some 43% more than T_0). The total Zn followed the sequence $T_1 > T_2 > T_0$ (Table 4). The total contents of Zn, Cd, Cu, and Pb were 30, 50, 90, and 40% higher in T_1, respectively, than in T_0. In the soluble forms, the responses were similar, T_1 being notable for reaching higher accumulation in soluble fractions of Zn, Cd, Cu, and Pb.

In terms of the changes in accumulation of Zn, Cd, Cu, and Pb during development (Figure 2), all metals showed increasing accumulation with plant age ($P < 0.001$).

Table 5 presents the significant effect of the treatments on the Cd and Zn concentrations on a fresh weight basis, with the highest concentrations for T_2, surpassing T_0 by 33 and 18%, respectively. The soluble metal concentration showed analogous response with higher Cd and Zn in T_2 than in the control. The Cu and Pb concentrations in the treatments proved to be not significantly different on a fresh weight basis (Table 5).

DISCUSSION

The perforated polyethylene (T_1) and polypropylene (T_2) floating row covers raised temperatures both in the air and in the root zones (Table 1) in comparison with the plants grown in the open air. These increases under the covers resulted from different factors (e.g., chemical composition, thickness, permeability) of the covers (*11*, *14*, *21*). However, the soil temperature did not usually rise as much as the air temperature, due to the thermal inertia of the soil (*22*). The higher temperatures in

T_1 and T_2 induced a greater relative humidity under the covers than in T_0, due to reduced evapotranspiration in the protected zone, which produced a mini-greenhouse effect (*11*, *13*). Our results agree with those of Choukr-Allah et al. (*23*), who reported average humidity increases of 5 and 15% under conditions comparable to ours. The chemical composition and the permeability of T_2, without perforations, favored slightly higher humidity than found under the T_1 cover, although the higher values in the former may be due simply to condensation (*13*, *20*, *24*).

Because the covers partially reflect solar radiation (*25*), the instantaneous solar radiation and consequently the accumulated solar radiation in T_1 and T_2 (Table 1) were reduced with respect to the open air experiment (T_0), but the transmissivity of >80% is not considered limiting for the crop in this climate zone (*26*). The thermal regime under T_2 covers provided intermediate conditions between T_1 and T_0, promoting significantly less biomass production than in T_1 but notably more than in T_0 (Table 2) as the result of greater foliar expansion, which provides better distribution of nutrients as well as photoassimilates in the shoot (*27*). Thus, the increase in fresh weight resulted both from the dry weight accumulation and from increased water content.

The smallest or poorest yield at harvest, in T_0 (Table 2), coincided with the lowest temperatures, whereas T_1 and T_2 encouraged growth by promoting biomass production during cooler conditions and thereby ultimately boosted yield (*11*, *25*) at harvest (Table 2).

Metals such as Pb and Cd are often cited as primary contaminants of concern, but Zn and Cu are also problematic at some sites. These latter two metals can be toxic to plants if the concentration of available metal in the growth medium is high enough. Because most metal-contaminated sites involve two or more metals, the possibility of synergistic effects may be of considerable importance at some sites contaminated with heavy metals (*1*). These Chinese cabbage plants probably take up Cd, and the other elements, because they have large leaf surfaces and hence high rates of transpiration. If the stomata are open, Cd probably moves in the transpiration stream (*28*).

In zones of temperate climate, the physiological activity of the plants and the hydric and mineral availability of the soil show noticeable seasonal changes as well as changes in short periods of time in the climatic parameters (*29*, *30*). The potential influence of the environmental factors in the concentrations of the pollutants within the plants has been studied, with results, in most cases, conflicting (*15*, *30*, *31*). In this respect, it is important to emphasize that the thermal treatments affected biomass and yield results (Table 2) by means of improving thermal conditions (creating a mini-greenhouse effect in the protected chamber), and these improved thermal conditions increased the concentration of heavy metals, mainly Zn and Cd.

It was surprising that the Pb concentrations in T_0 and T_1 were very similar, and greater than in T_2, and soluble Pb did not differ between treatments (Table 1), a result that can be explained as the constant response of the plant with respect to Pb; that is, it appears that the absorption was similar in the three treatments, and it was the difference in growth (biomass production; Table 2) that gave rise to the different concentrations.

B. pekinensis Rupr cv. JF-1 plants in a pot experiment under controlled conditions accumulated an unusually high level of Pb in their tissues during a 2 week growth period (*32*). In this experiment with *B. pekinensis* Lour (Rupr) cv. Nagaoka 50, grown under field conditionsswith normal or nontoxic levels of Pb in the soil and irrigation water, the source for plant uptakesthe accumulated Pb level reached nearly 0.6 mg plant^{-1} in T_1 (Table 4). Thus, phytoextraction may require technologies such as floating row covers given that open-field conditions in controls gave significantly lower values.

However, the Pb concentration found in all of the treatments far exceeded the level permitted for human foods (2 mg kg^{-1} of dry weight), and even Cd and Pb exceeded the toxicity limit established in fodder plants of 0.5 and 30 mg kg^{-1} of dry weight, respectively (*33*). In general, the average levels of the heavy metals (Tables 4 and 5) were much lower than the corresponding values for maximum human intake (*33*, *34*).

Tissue concentration alone, however, should not be used to evaluate the ability of a species to extract heavy metals from the growth media, because it does not take plant biomass into consideration (*18*, *35*). The concentration and content of Zn were highest in these Chinese cabbage plants, but concentrations of all elements vary in plants; Cd might be in lower concentration, yet it might be accumulated better than Zn. The important fact is the relationship between what is in the soil and what is in the plant (*36*), and it is this ratio (≈1 for Zn, Cu, and Pb; ≈12 for Cd) of concentrations that is important.

There was also a striking positive effect of T_1, with the highest accumulation in all cases (Table 4). Thus, the T_0 plants removed the lowest content of heavy metals, and differences in concentrations and total metal content (micrograms per plant) can be partially explained by the effect of the treatments' increasing growth, leading to increased metal content. However, once the metal reaches the shoot, it has to be processed or physiologically inactivated and probably general tolerance mechanisms could take place (vacuolar compartmentalization, cell wall binding, and precipitation, etc.; *37*-*39*), which could explain at least in part the ability to accumulate metals in this plant; thus, verification of these responses requires the subsequent study of locating this accumulation at the physiological level.

The extent of heavy metal removal in the field was appreciably more favored in T_1 and T_2. This technique of floating row covers could be used in contaminated zones for all plants, not just plants efficient at taking up metals and other elements (*40*, *41*), because the thermal effect favors the process of phytoextraction and thus reduces the contamination by using established crop production and management practices. Nevertheless, toxic substances accumulated in plants (Pb and Cd; *5*, *34*), due to agricultural soil and irrigation water pollution (phytoextraction), make the plant material less suitable for use as fodder and for human consumption.

ACKNOWLEDGMENT

We thank David Nesbitt for review, constructive comments, and translation into English.

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Table 1. Mean Values of Environmental Parameters Recorded during the 1994−1996 Spring Growing Seasons of Chinese Cabbage[a]

	air temp (°C)	root zone temp (°C) at depth of		relative humidity (%)	irradiance (W m^{-2})	radiant exposure (kJ m^{-2})
		5 cm	15 cm			
T_0	14.1 c	18.8 b	18.9 b	57.5 b	237.1 a	31334 a
T_1	20.4 a	24.0 a	23.2 a	61.9 a	207.2 b	26007 b
T_2	18.9 b	23.8 a	23.1 a	63.4 a	205.2 b	26257 b

[a] All data represent the means for three growing seasons. Means within ac olumn followed by the same letter are not significantly different at the $P< 0.05$ level by Duncan's multiple-range test.

Table 2. Response of Biomass Components in Shoots of Chinese Cabbage Grown under Different Treatments during the 1994−1996 Growing Seasons of Chinese Cabbage[a,b]

	fresh wt (g $plant^{-1}$)	dry wt (g $plant^{-1}$)	total yield[c] (Tm ha^{-1})
T_0	236.2 c	11.8 c	58.2 c
T_1	525.6 a	15.9 a	122.9 a
T_2	484.9 b	14.0 b	120.9 b

[a] Data represent the means of four replications per treatment and five samplings.

[b] Means within a column followed by the same letter are not significantly different at the $P< 0.05$ level by Duncan's multiple-range test.

[c] Ten plants were severed (the roots were cutoff) at the soil surface, and each plant (whole tops) was weighed fresh from all experimental plots.

Table 3. Influence of Thermal Treatments on Concentration and Soluble Fraction of Heavy Metals in Shoots of Chinese Cabbage[a]

	mg kg^{-1} of dry wt			
	Cd	Cu	Pb	Zn
	Metal Concentration			
T_0	0.58 b	10 b	35 a	63 b
T_1	0.59 b	17 a	37 a	68 ab
T_2	0.74 a	15 a	27 b	72 a
	Soluble Metal Concentration			
T_0	0.02 b	6 b	2 a	34 b
T_1	0.04 a	10 a	2 a	38 ab
T_2	0.05 a	10 a	1 a	44 a

[a] Data represent mean values (1994−1996) of the four replications per treatment and five samplings (n)180). Means within a column followed by the same letter are not significantly different at the $P< 0.05$ level by Duncan's multiple-range test.

Table 4. Influence of Thermal Treatments on Total Content and Soluble Fraction of Heavy Metals in Shoots of Chinese Cabbage[a]

	μg plant^{-1}			
	Cd	Cu	Pb	Zn
		Metal Content		
T_0	6 b	124 c	388 b	715 c
T_1	9 a	236 a	555 a	928 a
T_2	10 a	192 b	381 b	864 b
		Soluble Fraction		
T_0	0.4 b	110 b	16 b	371 b
T_1	0.9 a	133 a	24 a	536 a
T_2	0.9 a	116 b	18 b	501 ab

[a] Data represent mean values (1994−1996) of the four replications per treatment and five samplings (n)180). Means within a column followed by the same letter are not significantly different at the P< 0.05 level by Duncan's multiple-range test.

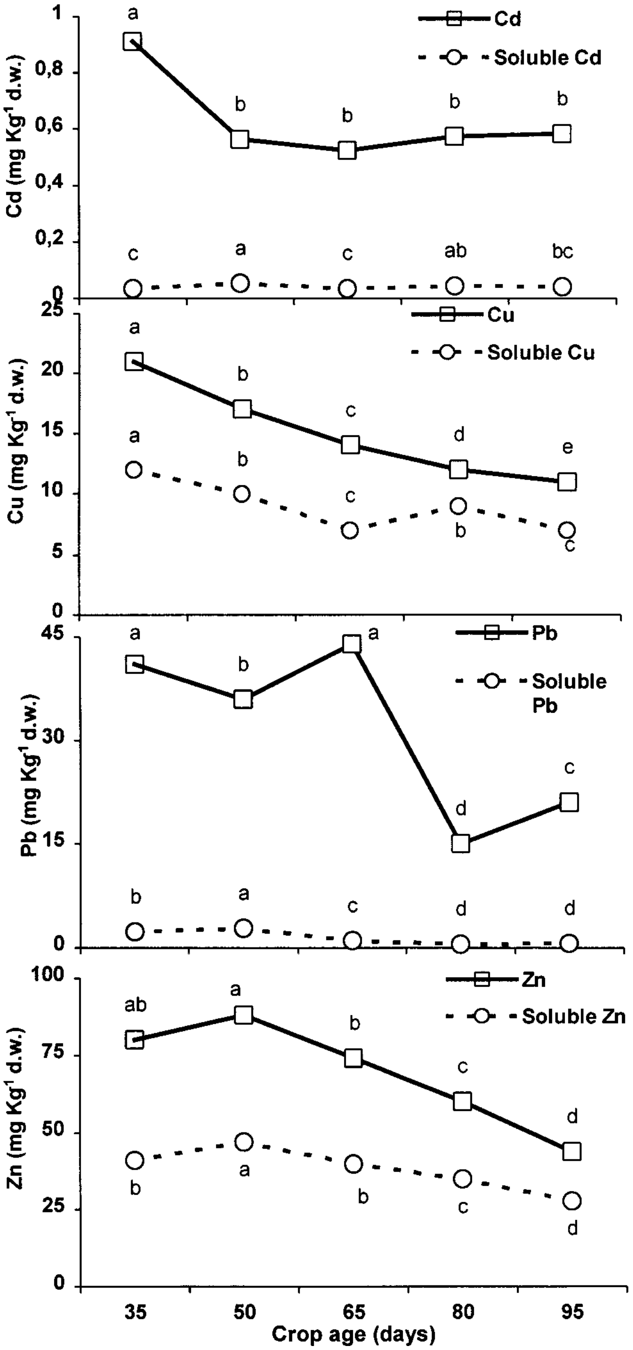

Figure 1. Changes in metal concentrations in aboveground biomass of Chinese cabbage during development. All data represent the means of four replications at every sampling for all treatments (1994−1996). Means within a series followed by the same letter are not significantly different at the P< 0.05 level by Duncan's multiple-range test.

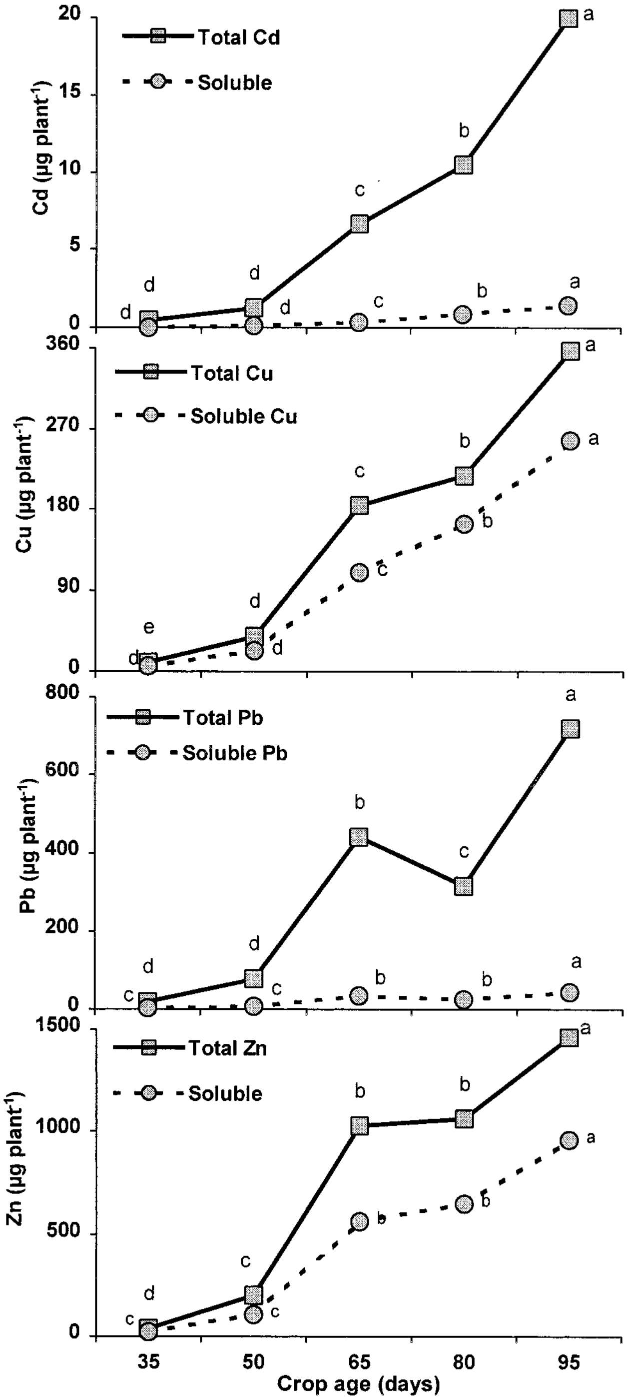

Figure 2. Changes in metal accumulation in aboveground biomass of Chinese cabbage during development. All data represent the means of four replications at every sampling for all treatments (1994–1996). Means within a series followed by the same letter are not significantly different at the $P< 0.05$ level by Duncan's multiple-range test.