Phytoextraction of Cd and Pb and Physiological Effects in Potato Plants (*Solanum Tuberosum* Var. Spunta): Importance of Root Temperature

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Three consecutive years of field experiments were carried out to investigate the effect of different root-zone temperatures, induced by the application of mulches, on the concentration and accumulation of Cd and Pb and on bioindicators (chlorophylls, catalase, peroxidase and cell wall fractions) in different organs of potato plants (roots, tubers, stems, and leaflets). Four different plastic covers were employed (T1, transparent polyethylene; T2, white polyethylene; T3, white and black coextruded polyethylene, and T4, black polyethylene), using uncovered plants as the control (T0). The different treatments had a significant effect on the mean root-zone temperatures (T0 = 16 °C, T1 = 20 °C, T2 = 23 °C, T3 = 27 °C, and T4 = 30 °C) and induced significantly different responses in the Cd and Pb concentrations and phytoaccumulation, with T2 (23 °C) and T3 (27 °C) giving high concentrations of Cd in the roots and low concentrations in other organs. In relation to Pb, T2 and T3 reached higher levels in the tubers and lower levels in the roots, stems, and leaves. In terms of phytoaccumulation, the roots and tubers were the most effective organs for Cd and Pb. On the other hand, the highest values of peroxidase and catalase activities were obtained for T3. In addition, most of the carbohydrate fractions in both the roots and the tubers were highest for T3. Meanwhile, the lowest pigment values were registered for T1 (20 °C). For phytoremediation, it is necessary to ascertain the relevance and control of the thermal regime of the soil to optimize the phytoextraction of pollutant elements (Cd and Pb).

INTRODUCTION

Cadmium (Cd) and lead (Pb) are nonfunctional elements that are highly toxic for plants (*1*). Although both are nonessential, they can be taken up easily by the roots and transported through the xylem to the vegetative and reproductive organs, negatively affecting the crops (*2*) by upsetting processes such as photosynthesis (*3*), DNA synthesis (*4*), mitosis and cell division (*5*), and germination (*6*). In short, rising concentrations of these metals inhibit most basic physiological processes (*7*, *8*). Larsson et al. (*9*) observed a reduction in the chlorophyll concentrations of plants exposed to Cd, while Kastori et al. (*10*) found that the treatment of sunflower plants with lead diminished the concentrations of chlorophylls and carotenes. Studying enzymatic activities, Xiong (*11*) found that the peroxidase activity increased with rising Pb concentrations, while Mohan and Hosetti (*12*) suggested that both Cd and Pb drastically depressed catalase activity but stimulated peroxidase activity. Also, toxicity can induce these two metals to bond to the cell wall, weakening their toxic effects (*13*–*15*).

Current problems of agricultural soil and water pollution, which cause difficulties for human health, can be partially solved by the application of phytoremediation technologies (*16*), designed to eliminate pollutants from the environment by the use of green plants (*17*).

Root-zone temperatures strongly influence plant growth and ionic uptake (*18*–*20*). In economic terms, crops as important as the potato require optimal root-zone temperatures for maximum growth and yield (*21*). One of the techniques used to increase and control root-zone temperature is the application of polyethylene covers (mulches) of different colors and characteristics, which generate a microenvironment for optimal root-zone temperatures that favors crop development (*22*).

In the present work, we examine how the different root-zone temperatures generated by the application of plastic mulches affect the concentrations and phytoaccumulation of Cd and Pb in different organs of field-grown potato plant (*Solanum tuberosum* L. var. Spunta), as well as assessing the response of certain physiological and biochemical indicators under such conditions.

MATERIAL AND METHODS

Crop Design and Plant Sampling. The experiment was conducted for three consecutive years (1993–1995) in the field (Granada, Spain), using *Solanum tuberosum* L. var. Spunta, planted at the beginning of March with a crop cycle of about

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4 months. The climate was semiarid, and the area was intensively used for agriculture. The soil used had the following characteristics: silt, 45.3%; clay, 43.2%; pH 8.6 (H_2O 1:2.5); electrical conductivity, 1.10 dS m^{-1}; $CaCO_3$, 11.2%; total N, 0.1%; P_2O_5, 58 μg g^{-1}; K_2O, 115 μg g^{-1}; DTPA + TEA + $CaCl_2$ (pH 7.3) extractable Cd, 3 μg kg^{-1}; and Pb, 11 mg kg^{-1}. The characteristics of the irrigation water were: pH 7.6; electrical conductivity, 1.05 dS m^{-1}; Cl$^-$, 58 mg L^{-1}; Na$^+$, 25 mg L^{-1}; K$^+$, 4 mg L^{-1}; H_2CO_3, 369 mg L^{-1}; Cd, 1 μg L^{-1}; and Pb, 1 μg L^{-1}.

The experimental design was a factorial arrangement in a randomized complete block with five treatments replicated four times (20 plots). Each plot occupied an area of 78.4 m^2, with a planting density of 4.2 plants m^{-2}. The plants were spaced 30 cm apart, with 80 cm between rows. The soil temperature was measured at a depth of 15 cm using probes (107 type) from Campbell Scientific TM. The root-zone temperature was measured (six measurements at 4-h intervals) every 3 days of the crop cycle.

The different treatments consisted of covering the soil surface of each plot with plastic mulches (polyethylene sheets), making a tight seal with the soil: transparent polyethylene (25 μm in thickness, T1), white polyethylene (25 μm in thickness, T2), coextruded black and white polyethylene (50 μm in thickness, T3), and black polyethylene (25 μm in thickness, T4). Finally, no plastic was applied in the control treatment (T0).

The fertilization used was the same as is typically applied by farmers in the zone. In the month of February in all three years, N (NH_4NO_3) and P and K (K_2HPO_4) (27 g m^{-2}) were applied. At the end of the month of April, 25 g m^{-2} of NH_4NO_3 was applied. Fertigation was complemented with the following micronutrients: Fe, 0.5 mg L^{-1}; B, 0.1 mg L^{-1}; Mn, 0.1 mg L^{-1}; Zn, 0.075 mg L^{-1}; Cu, 0.075 mg L^{-1}; and Mo, 0.05 mg L^{-1}. Iron was applied as FeEDDHA, B as H_3BO_3, and the remaining micronutrients as sulfates.

The plant material (stems, leaflets, roots, and tubers) were sampled once every 2 weeks for 12 weeks throughout the plant development for the three years of experiments. For each sampling, 10 plants were collected from each replicate per treatment. Foliar samples (leaflets) were taken only from plants with fully expanded leaves of the same size, and leaflets were picked at about one-third of the plant height from the plant apex. Roots, leaflets, stems, and tubers were rinsed three times in distilled water after decontamination with nonionic detergent at 1% (*23*) and then blotted on filter paper. Then, a sample was dried in a forced-air oven at 70 °C for 24 h, ground in a wiley mill, and placed in plastic bags for the further analyses.

Plant Analysis. *Cd and Pb Determination.* For the assay of Cd and Pb concentrations, oven-dried and pulverized plant material was digested with concentrated nitric acid and hydrogen peroxide, and measurements were made using an atomic absorption spectrophotometer equipped with a graphite furnace (*24*). Reagent blanks for analysis were also prepared by performing the entire extraction procedure but in the absence of the samples.

Catalase (*CAT*) *and Peroxidase* (*POD*) *Assays.* POD activity was determined from fresh leaf samples following the change of absorbency at 485 nm due to guaiacol oxidation at 30 °C (*25, 26*). The CAT activity was determined from fresh tissue by following the consumption of H_2O_2 at 240 nm (*27*). All of these procedures were carried out at 4 °C. To determine whether the reaction was enzymatic, the sample extract was boiled and assayed.

Chlorophylls and Carotene Determination. The leaf sections used for the measurement of A_{max} were extracted in 6 mL of 80% acetone for spectrophotometric determination of the concentration of chlorophylls (total chl, chl *a* and chl *b*) and total carotenoids according to Lichtenthaler (*28*).

Cell Wall Fractionation. The cell wall fractions were extracted by a standard procedure reported elsewhere and previously described in Wakabayashi et al. (*29*) and Muramatsu et al. (*30*). The sugar concentration in each fraction was measured by the phenol−sulfuric acid method (*31*) using a

Table 1. Effect of Mulch Treatments on Root-Zone Temperature (RZT) and Biomass in Different Organs of Potato Plants

treatment	RZT(°C)	biomass (g^{-1} of plant)			
		roots	tubers	stems	leaflets
T0	16 e^a	1.75 bc	19.94 c	1.82 b	2.48 b
T1	20 d	1.04 c	10.89 d	2.19 a	1.93 c
T2	23 c	1.97 b	22.42 b	1.85 b	2.59 b
T3	27 b	2.34 a	26.93 a	1.70 c	2.84 a
T4	30 a	1.63 bc	20.70 c	2.09 ab	2.51 b

^{a} Values followed by the same letter within a column were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 2. Effect of Root-Zone Temperature on Cd Concentration^a in Different Organs of Potato Plants

treatment	roots	tubers	stems	leaflets
T0	1260 b^b	260 a	550 b	280 c
T1	850 c	210 ab	740 a	500 a
T2	1450 a	170 c	260 c	285 c
T3	1530 a	160 c	190 c	310 bc
T4	930 c	220 ab	500 bc	340 b

^{a} Concentrations measured in nanograms of Cd per gram of dry weight. ^{b} Values followed by the same letter within a column were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

glucose standard and uronic acid was measured by the carbazole method (*32*) using a galacturonic acid standard.

Statistical Analyses. Analysis of variance was used to assess the significance of treatment means. Significant differences according to the Duncan's multiple range test (DMRT) are indicated with different letters in the tables. Levels of significance of the correlation analysis are represented by * at $P < 0.05$, ** at $P < 0.01$, *** at $P < 0.001$, and ns for not significant.

RESULTS

Table 1 shows the mean values for root-zone temperature (RZT) generated by the different cover treatments applied, as well as those for the open-air plots. We found that the treatments significantly affected the mean RZT, giving the highest value for T4 (30 °C), and the lowest for T0 (16 °C).

The biomass results (on a dry-weight basis) for the different organs of the potato plants were significantly affected by the RZT resulting from the treatments (Table 1). Thus, for the roots, leaflets, and tubers, the highest values were found for T3 (27 °C) and the lowest for T1 (20 °C), the latter values being lower than those for T0 (16 °C). On the contrary, in the stems, T1 reached the highest dry weight, while T3 showed the lowest.

Table 2 presents the significant effect of RZT on the Cd concentrations, with the highest Cd concentrations in roots for T2 and T3, surpassing T0 by 15 and 21%, repectively. The lowest Cd concentration in the roots was recorded for T1 (33% lower than that for T0). In tubers, the highest Cd value was reached for T0 and the lowest for T3 (38% lower than T0), whereas in stems, the highest Cd value was recorded for T1 (35% higher than T0). With respect to Cd in the leaflets, T1 also gave the highest concentration.

The highest Pb concentration in the roots was registered for T1 (Table 3) surpassing that for T0 by 26%, whereas the rest of the treatments obtained concentrations similar to that of the control. In the tubers, T2 and T3 gave the greatest values of Pb, surpassing T0 by 50 and 60%, respectively, and the lowest level that

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Table 3. Effect of Root-Zone Temperature on Pb Concentration in Different Organs of Potato Plants

treatment	roots	tubers	stems	leaflets
T0	28.9 b[b]	1.0 b	12.0 a	3.2 a
T1	36.5 a	0.8 b	10.4 a	4.5 a
T2	26.1 b	1.5 a	6.7 b	1.4 b
T3	27.1 b	1.6 a	4.4 b	1.3 b
T4	28.8 b	1.1 ab	8.6 ab	3.5 a

[a] Concentrations measured in micrograms of Pb per gram of dry weight. [b] Values followed by the same letter within a column were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 4. Effect of Root-Zone Temperatures on Foliar Bioindicators

	chlorophylls					
treatment	chl a[a]	chl b[a]	total chl[a]	carot[b]	CAT[c]	POD[d]
T0	65.1 a[e]	18.7 a	83.2 a	34.6 a	23.8 b	474 bc
T1	51.4 b	15.4 b	66.8 b	30.6 b	22.8 b	306 c
T2	65.4 a	19.1 a	83.6 a	35.3 a	27.4 ab	507 b
T3	65.5 a	21.1 a	85.3 a	34.1 a	28.9 a	581 ab
T4	66.6 a	19.1 a	85.6 a	35.4 a	24.8 b	608 a

[a] chl a, chl b, and total chl measured in micrograms per square centimeter. [b] carot = carotenes measured in micrograms per square centimeter. [c] CAT = catalase activity measured in micromoles of H_2O_2 oxidized per gram of full weight per hour. [d] POD = peroxidase activity measured in micromoles of guaiacol oxidized per gram of full weight per hour. [e] Values followed by the same letter within a column were not significantly different at $P < 0.05$ acording to Duncan's multiple range test.

was given in the T1. In the stems, T0 and T1 showed the highest concentrations of Pb, and the lowest level was given by T3 (63% less than the T0). For the leaflets, T1 reached the highest value of Pb (40% higher than T0), whereas the lowest were obtained for T2 and T3 (55 and 59%, respectively, smaller than T0).

With respect to the thermal effect on foliar biochemical parameters (Table 4), the chlorophyll a and chlorophyll b showed the lowest concentrations for (21 and 18% lower than T0, respectively). The total chlorophyll (chl a + chl b) also registered lower levels for T1, as did the carotene concentrations. The remaining treatments did not statistically differ from each other.

For the foliar catalase (CAT) activity, the highest values were registered for T3 and T2, surpassing T0 by 21 and 15%, respectively (Table 4). The peroxidase activity (POD) reached the highest level for T4 (28% higher than T0; Table 4), whereas T1 induced the lowest activity, falling to 36% lower than T0 value.

The different treatments significantly affected the cell wall composition of the differents potato organs. In roots (Table 5), T2 and T3 induced the highest concentration of neutral sugars in the hydrosoluble and pectic (EDTA) fractions. However, the hemicellulose and cellulose fractions were very similar for the different treatments. The lowest level of hemicellulose was allways recorded with T1 for the different carbohydrates fraction. For the concentration of uronic acids in roots (Table 5), the highest and lowest levels were reached with T3 and T1, respectively, for all the extracted fractions. The uronic acids were higher in the pectic fraction (EDTA), and lower in the hemicellulose and hydrosoluble carbohydrate fractions.

The RZT significantly affected the cell wall composition of the tubers (Table 6), with T3 achieving the highest levels of neutral sugars and uronic acids. The lowest concentrations of neutral sugars and uronic acids in all cell wall fractions were always obtained for T1.

Table 5. Effect of the Root-Zone Temperature on Cell Wall Composition of Potato Roots

treatment	hydrosoluble	EDTA	hemicellulose	cellulose
	neutral sugars (mg g^{-1} of dry weight)			
T0	47.39 ab[a]	43.19 ab	47.02 a	95.73 a
T1	44.59 b	35.12 b	43.45 a	94.25 a
T2	50.23 a	50.10 a	46.76 a	96.19 a
T3	51.49 a	51.23 a	47.73 a	97.83 a
T4	47.13 ab	38.01 b	46.24 a	96.81 a
	uronic acids (mg g^{-1} of dry weight)			
T0	0.98 b[a]	3.02 ab	1.05 b	2.12 ab
T1	0.96 b	2.76 b	1.02 b	2.05 b
T2	1.12 ab	3.03 ab	1.05 b	2.13 ab
T3	1.24 a	3.27 a	1.26 a	2.24 a
T4	0.98 b	3.03 ab	1.14 a	2.15 b

[a] Values followed by the same letter within a column were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 6. Effect of the Root Temperature on Cell Wall Composition of Potato Tubers

treatment	hydrosoluble	EDTA	hemicellulose	cellulose
	neutral sugars (mg g^{-1} of dry weight)			
T0	36.13 ab[a]	107.25 c	47.81 b	48.69 b
T1	23.91 b	104.15 c	44.91 b	40.11 c
T2	37.08 ab	121.25 b	59.65 a	64.69 a
T3	46.25 a	146.85 a	60.51 a	69.85 a
T4	37.01 ab	118.76 b	47.63 b	56.15 ab
	uronic acids (mg g^{-1} of dry weight)			
T0	0.83 ab[a]	2.81 b	1.09 ab	1.84 ab
T1	0.75 b	2.43 c	0.97 b	1.71 b
T2	0.83 ab	2.83 ab	1.05 b	1.89 ab
T3	1.10 a	3.14 a	1.23 a	2.15 a
T4	0.85 ab	2.86 ab	1.08 ab	1.84 ab

[a] Values followed by the same letter within a column were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 7. Effect of the Root Temperature on Cell Wall Composition of Potato Stems

treatment	hydrosoluble	EDTA	hemicellulose	cellulose
	neutral sugars (mg g^{-1} of dry weight)			
T0	28.21 a[a]	48.33 b	45.62 a	95.90 a
T1	29.60 a	50.07 a	46.72 a	96.59 a
T2	20.35 b	37.52 b	41.53 b	86.08 ab
T3	18.99 b	37.14 b	39.08 c	87.17 b
T4	18.31 b	39.34 b	41.03 b	86.20 b
	uronic acids (mg g^{-1} of dry weight)			
T0	1.17 ab[a]	3.43 ab	1.13 ab	2.35 a
T1	1.18 a	3.78 a	1.15 a	2.36 a
T2	1.03 ab	3.25 b	0.81 b	2.07 b
T3	0.96 b	3.21 b	0.85 b	1.98 b
T4	1.03 ab	3.40 ab	1.05 ab	2.01 b

[a] Values followed by the same letter within a column were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

In relation to the carbohydrate composition of the stem cell walls (Table 7), the RZT showed a significant influence, with T0 and T1 giving the highest levels of neutral sugars and uronic acids in the different cell wall fractions and the rest of treatments showing significantly lower values.

In the leaflets (Table 8), the hydrosoluble cell wall fraction had the highest neutral sugar concentrations for T1 (57% higher than T0) and the lowest for T0, whilewhereas the pectic fractions for T2 and T3 reached the highest neutral sugar concentrations surpassing that of T0 by 13 and 15%, respectively. T1 presented the highest neutral sugar levels in both the hemicellulose and cellulose fractions, and the rest of the treat-

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Table 8. Effect of the Root Temperature on Cell Wall Composition of Potato Leaflets

treatment	hydrosoluble	EDTA	hemicellulose	cellulose
	neutral sugars (mg g^{-1} of dry weight)			
T0	9.93 b[a]	39.85 b	26.45 b	64.25 ab
T1	15.65 a	37.63 b	30.33 a	68.43 a
T2	10.72 b	45.10 a	25.13 b	52.79 b
T3	10.13 b	45.93 a	25.98 b	55.68 b
T4	10.49 b	34.79 c	26.14 b	58.89 b
	uronic acids (mg g^{-1} of dry weight)			
T0	0.94 ab[a]	3.20 a	1.03 ab	2.06 a
T1	0.82 b	2.98 b	0.93 b	2.05 a
T2	0.97 ab	3.27 a	1.17 a	1.81 b
T3	1.07 a	3.25 a	1.19 a	1.81 b
T4	0.96 ab	3.22 a	1.03 ab	2.06 a

[a] Values followed by the same letter within a column were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

ments did not statistically differ from each other. The RZT significantly influenced the uronic acid content in the leaflets. Thus, the hydrosoluble fraction showed higher concentrations for T3 (exceeding T0 by 14%), whereas the T1 values fell 13% below the T0 values. For the EDTA fraction, the uronic acid was the lowest for T1 (7% lower than T0), whereas for the hemicellulose fraction, the T2 and T3 values exceeded the T0 values by 14 and 15%, respectively. Finally, for the cellulose fraction, T2 and T3 showed the lowest values of uronic acids (12% lower than T0).

The phytoaccumulation of Cd in roots (Figure 1) was the greatest for T3 (62% higher than T0) and the lowest for T1 (60% lower than T0). In tubers, the main accumulation occurred for T4, without statistical differences with T3 and T0. In the stems and leaflets, the highest content was reached for T1. In terms of the relative distribution of Cd (all the organs according to the treatments), it was striking that the highest accumulation occurred in the root zone (roots and tubers), oscillating between 55 and 89% within the plants.

In relation to the phytoaccumulation of Pb (Figure 2) in roots, T3 induced the highest Pb content (39% higher than T0), and the lowest was found for T1 (17% lower than T0). In tubers, the highest value was recorded for T3 and the lowest for T1 (48% lower than T0). The stems presented the greatest levels for T0 and T1 and the lowest for T3. Finally, in leaflets, T2 and T3 registered the lowest values falling to 53 and 52% lower than the T0 values, whereas T1 reached the highest Pb content, surpassing that for T0 by 9%. The relative distribution of Pb in the different organs of potato plants followed the sequence: roots (48–55%) > tubers (12–36%) > stems (6–29%) > leaflets (3–11%).

DISCUSSION

The results of the effect of different mulches on root-zone temperatures (RZT, Table 1) were similar to those obtained by Ham et al. (*33*), who reported that black polyethylene (similar to our T4) absorbs roughly 96% of the short-wave radiation while reflecting very little and conducts the absorbed radiation to the underlying soil, thus warming the soil (*34*). The white polyethylene (T2) induces a cooler soil temperature than does black (T4) because the former reflects most wavelengths of solar radiation (*35*). Schmidt and Worthington (*36*) demonstrated that transparent mulches such as T1 do not cause soil warming, presenting mean temperatures

of 18–20 °C during the crop cycle, whereas the white–black coextruded covers generate higher RZTs (27 °C in our T3).

Klock et al. (*37*), studying tomato plants, reported an increase in total biomass in plants for the root-zone temperature range of 24–27 °C, whereas outside this range, the dry weight fell. However, the decrease in dry weight for T4 could be due to the high RZT exceeding 29 °C, with resulting retarded development and lower dry-weight accumulation (*38*).

The results show that the Cd uptake is significantly affected by the RZT (Table 2), as T2 (23 °C) and T3 (27 °C) induced strong increases in the root concentration of this heavy metal. In tubers, the Cd concentrations were lower for T2 and T3. In the aerial parts of the plants, stems and leaflets, the treatments with low RZT (T0 = 16 °C and T1 = 20 °C) favored higher concentrations of Cd. T2 (23 °C) and T3 (27 °C), which are more appropriate temperatures for plant development, affected the Cd levels and thus Cd mainly concentrated in the root part of the plant and was retained in the roots (*39*), so that the portion that is transported toward other organs, in this case, tubers, stems, and leaflets, is smaller (*40*).

Concerning the possible phytotoxicity of Cd, Pais and Jones (*41*) found that 3 mg of Cd kg^{-1} of dry weight can reduce plant growth. De Pieri et al. (*42*) reported that the Cd concentrations in the tubers of the potato varied between 0.05 and 1.74 μg g^{-1} of dry weight. The "normal" nontoxic content for Cd was established as 0.1–1 mg kg^{-1} of dry weight. In our plants, the highest foliar concentrations (0.5 mg of Cd kg^{-1} of dry weight for T1) fell below this range. Although plants do not require Cd for growth, the bioaccumulation exceeds that of other elements, and plants can accumulate high quantities of Cd without suffering adverse effects on growth (*43*), although this represents a high risk for food (*44*).

In the roots, the capacity to concentrate Pb was affected significantly by the RZT (Table 3), and the lower temperatures induced by T0 (16 °C) and T1 (20 °C), favored higher levels of this heavy metal. In tubers, the concentrations are much lower, as Pb is considered to have low mobility within the plant (*41*). Neverthless, treatments T2 and T3 increased the Pb concentration with respect to that of the control. In leaflets and stems, T2 (23 °C) and T3 (27 °C) significantly reduced the Pb concentration, which probably exhibited lower absorption and transport under these temperature conditions, whereas T0, T1, and T4 favored higher levels of Pb in the stems and leaflets.

The highest foliar content of Pb in our plants was near 5 mg kg^{-1} of dry weight, very high compared with levels permitted for human consumption (2 mg kg^{-1} of dry weight; *45*). Kabata-Pendias and Pendias (*43*) suggested that Pb concentrations higher than 30 mg kg^{-1} of dry weight in forages could be highly toxic for livestock; meanwhile, potato tubers cultivated at different sites varied between 0.03 and 0.16 μg g^{-1} of dry weight (*42*). In our experiment, the Pb concentration in the tubers ranged from 0.8 to 1.6 μg g^{-1} of dry weight.

Between species as well as between genotypes of the same species, there are great variations in Cd uptake by the roots and transport to the aerial parts of the plants (*40, 46, 47*). Also, Cd can easily be taken up by the roots and transported by the xylem to the vegetative and reproductive organs, negatively affecting the crop
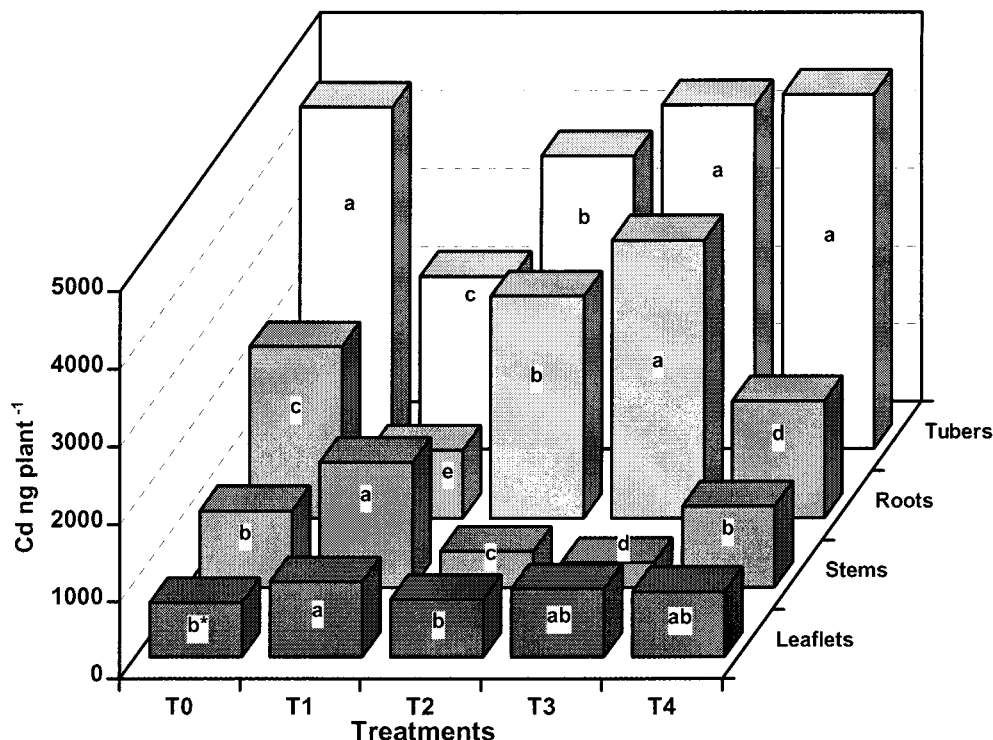
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Figure 1. Effect of root temperature on total quantities of Cd in potato organs. Values followed by the same letter within a series were not significantly different at $P < 0.05$ according to Duncan's multiple range test.
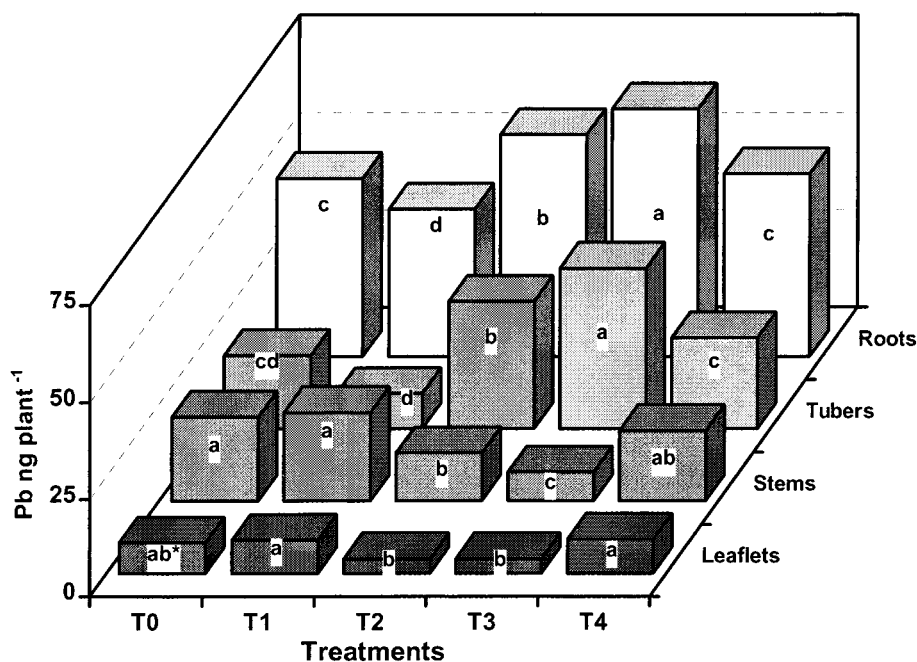

Figure 2. Effect of root temperature on Pb phytoaccumulation in potato organs. Values followed by the same letter within a series were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

health (*1*, *48*), with greater facility than other heavy metals. Maize grown in soils treated with contaminated tailings showed greater increases in Cd concentrations (*49*) than in Pb concentrations (*50*), whereas our potato plants accumulated far higher quantities of Pb than Cd. Most of the Cd taken up in these potato plants was retained in the roots (*39*), and small quantities were translocated toward the aerial parts and other sinks, as previously found (*40*). In addition, the heaviest Pb concentrations were found in the roots. Fodor et al. (*2*), suggested that cucumber roots accumulated high levels of Pb and that only small translocations toward the

aerial parts were observed. Although Pb is transported acropetally, only small amounts of the metal moved from the roots (*51*), perhaps explained by the radial movement of Pb in the root tissues usually being impeded by the endodermis (*52*–*54*), and only a small percentage of Pb was transported toward the aerial part (*8*) by transpiration (*54*). Fodor et al. (*2*) also found that Pb enters the phloem in the leaves to be transported passively downward, although there is no high mobilization of Pb from the leaves of these potato plants, as reflected by the fact that the higher concentrations were found in the roots and stems (Table 3).

Root-Zone Temperatures and Phytoextraction of Cd and Pb

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High foliar concentrations of Pb and Cd in plants can reduce the chlorophyll and carotene concentrations (*9*, *10*; Table 4). This idea was also supported in our experiment by the lowest chlorphyll concentrations being found for treatments with higher levels of Cd and Pb and by the negative correlations between foliar pigments and Cd (Cd−chl *a*, *r* = −0.66*; Cd−chl *b*, *r* = −0.83**; Cd−total chl, *r* = −0.68*; Cd−carot, *r* = 0.59*) as well as between foliar pigments and foliar Pb (Pb−chl *a*, *r* = −0.94***; Pb−chl *b*, *r* = −0.84**; Pb−total chl, *r* = −0.92**; Pb−carot, *r* = −0.91**).

We found inversely proportional relationships between CAT and POD (Table 4) and the Pb concentrations: Pb−CAT, *r* = −0.58*; Pb−POD, *r* = −0.82**; Cd−CAT, *r* = −0.95***; Cd−POD, *r* = −0.86**. These results suggest different effects of Pb and Cd on the two activities, as previously reported in Mohan and Hosetti (*12*), who found increased POD and declined CAT activities in the presence of Cd and Pb, respectively. Thus, depending on the species and the crop conditions, Cd and Pb can increase or reduce CAT and POD (*15*), and we found decreased POD with higher Cd and Pb for T1, whereas the opposite was true for CAT, which was higher when Pb and Cd showed lower concentrations for T3.

The RZT significantly affected the growth (biomass, Table 1) and concentrations of neutral sugars and uronic acids of the different fractions of the cell walls in the studied organs. In roots and tubers, the neutral sugar and uronic acid concentrations of the cell wall were reduced for T1, because of the lower temperature induced by these treatment (*55*). In the stems, however, the contrary response appeared, with higher levels of neutral sugars and uronic acids for T1, as well as some cell wall fractions of leaflets with higher neutral sugar levels for T1. This result is probably related to the biomass results, as growth depends on the mechanical changes in the structure of the cell walls (*56*). Such processes can involve alterations in the structure of the polysaccharide matrix and pectin fractions, as well as in the hemicellulose, which serves as an elemental structure for the cell wall (*30*).

Regarding metal toxicity, many works have focused on the bonding of metals to the cell wall (*13*, *14*, *57*), and our experiment revealed a similar response between the concentration of heavy metals and of structural carbohydrates in the cell walls. In the roots, we found similar tendencies between the concentration of Cd and structural carbohydrates, whereas in the leaflets, we found a comparable behavior between the uronic acid concentrations and those of Pb and Cd. With respect to the stems, similar behavior appeared between Cd and the concentrations of uronic acids of the different fractions, and in the leaves and stems, both Pb and Cd proved to be significantly related to the concentrations of uronic acid. The reserve organs store nutrients for plant growth and also accumulate metals (*8*). The potato tubers showed higher total contents of Cd (higher Cd phytoextraction than other tissues, Figure 1), indicating that the accumulation of the metals depended on the biomass of the organ. At the same time, the lower Pb accumulation in these organs (Figure 2) was possibly due to a retention of this metal in other organs of the plant (i.e., roots) or to a low mobility of Pb toward the tubers. This latter idea is supported by Hocking (*58*), who suggested that the accumulation of elements depends on the mobility of each element and the size of the sink.

Salt and Krämer (*59*) suggested that, in heavy metal hyperaccumulator plant species, the ratio between the aerial part and the root part of the metal concentrations should be greater than 1. In our experiment for Cd, this ratio fluctuated between 0.33 (T3) and 1.46 (T1). Thus, the plants should be highlighted as possible accumulators. However, the ratios between the aerial part and the root part for the Pb concentration were 0.2 (T3) and 0.5 (T0), leading to the conclusion that the plants had a lower accumulation potential for Pb. Therefore, it should be emphasized that potato plants cultivated in fields with mulching favored the phytoaccumulation of Cd and Pb for T1, leading to lower concentrations of chlorophylls and carotenes as well as lower CAT and POD activities, whereas the neutral sugar and organic acid concentrations were greater with this treatment. Finally, we conclude that, for phytoremediation, it is necessary to ascertain the relevance and control of the thermal regime of the soil to optimize the phytoextraction of pollutant elements.

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