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Root-Zone Temperature Influences the Distribution of Cu and Zn in Potato-Plant Organs

MOURAD BAGHOUR,*[,†] DIEGO A. MORENO,[‡] GEMMA VÍLLORA,[‡]
INMACULADA LÓPEZ-CANTARERO,[‡] JOAQUÍN HERNÁNDEZ,[§]
NICOLAS CASTILLA,[||] AND LUIS ROMERO*[,‡]

Department of Forest Genetics and Plant Physiology, SE-901 83 Umeå, Sweden,
Department of Plant Biology, Faculty of Sciences, University of Granada, E-18071, Granada, Spain,
Department of Plant Production, EUP Ingeniería Técnica Agrícola, 04120 Almería, Spain, and
Department of Horticulture, Centro de Investigación y Formación Agraria (CIFA),
Camino de Purchil S/N, 18004 Granada, Spain

Root-zone temperatures (RZT) in relation to Cu and Zn uptake and tissue accumulation, and to total biomass, in potato plants (*Solanum tuberosum* L. var. Spunta) were studied. Using five different plastic mulches (no cover, transparent polyethylene, white polyethylene, coextruded white−black polyethylene, and black polyethylene) resulted in significantly different RZT (16 , 20, 23, 27, and 30 °C, respectively). These RZT significantly influenced Cu and Zn content (concentrated) and the biomass in various potato organs. Root-zone temperature at 20 °C resulted in significantly high Cu content in leaflets, and soluble Cu content in leaflets and stems, whereas 23 and 27 °C resulted in significantly high Cu content in roots. However, RZT had no effect on Cu content in tubers or stems or on soluble Cu in roots or tubers. The RZT at 20 °C resulted in significantly high Zn and soluble Zn in stems, roots, and tubers; whereas, at 27 °C Zn and soluble Zn content were significantly highest in leaflets. The most biomass occurred in roots and tubers at 27 °C; whereas in leaves and stems, the RZT influence was highly variable. Total accumulation of both Cu forms was affected by RZT at 20 °C, with roots and tubers having significantly the least Cu and stems and leaflets having the most. Total accumulation of both Zn forms by RZT in potato organs was highly variable, but tubers consistently accumulated the most.

KEYWORDS: Mulching; heavy metals; phytoextraction; *Solanum tuberosum*

INTRODUCTION

As essential micronutrients, Cu and Zn form part of the enzymatic structures of numerous important metabolic processes (*1*, *2*), and their function is based on their participation in enzymatic reactions (*3*). Nevertheless, high concentrations of these elements can be toxic for plants and animals (*4*).

Agricultural soil and water pollution, a current problem for human health, could be partially solved by phytoremediation (*5*), using plants to phytoaccumulate certain contaminants (*6*). One technique to boost the uptake of certain nutrients is the control of root-zone temperatures (*7*, *8*), because root temperature is a key factor in alteration of the ion accumulation (*9*). One method of manipulating the root-zone temperature is the application of polyethylene covers (mulch) of different colors to generate a microenvironment for the plants, and thereby trigger thermal changes in the root zone (*10*).

Glass (*11*) estimated the U.S. market for phytoremediation at $16.5−29.5 million in 1998. The largest markets are for treatment of organic contaminants in groundwater, which is estimated at $5−10 million, and control of landfill leachate, estimated at approximately $3−5 million. Smaller current markets are for remediation of organics in soil and organics in wastewater, each estimated at $2−3 million, and radionuclides in soil and groundwater, estimated at $0.5−1 million. In general, the markets involving metals or radionuclides are projected for dramatic growth as the technological efficacy improves and becomes more widespread, with predictions of reaching $214−370 million by 2005 (*11*).

The aim of the present work with potato plants is to determine the effects of the different root-zone temperatures (RZT), controlled by the application of plastic mulches, on the content and total accumulation of Cu and Zn forms, to provide a reliable diagnosis of the nutritional state of this crop in the field and possibilities for phytoextraction practices.

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* To whom correspondence should be addressed. Telephone: +46 (0) 90-786-5881. Fax: +46 (0) 90-786 5901. E-Mail: moba@nona.slu.se.

[†] Umeå.

[‡] University of Granada.

[§] EUP Ingeniería Técnica Agrícola.

[||] CIFA.

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Table 1. Effect of Root-Zone Temperature on Biomass (fresh and dry weight) of Potato Organs

RZT (°C)	roots	tubers	stems	leaflets
	fresh weight (g plant^{-1})			
16	14.09 c[a]	112.10 b	22.08 b	20.89 b
20	10.21 d	64.44 c	27.53 a	16.23 c
23	16.97 b	122.25 ab	23.06 b	20.30 b
27	19.11 a	143.73 a	20.72 c	23.03 a
30	14.10 c	112.66 b	23.73 b	20.39 b
LSD	1.89	29.79	1.30	1.95
ANOVA-*P* value	0.004	0.009	0.006	0.007
	dry weight (g plant^{-1})			
16	1.75 c[a]	19.94 b	1.82 c	2.48 a
20	1.04 d	10.89 c	2.19 a	1.93 b
23	1.97 b	22.42 b	1.85 bc	2.59 a
27	2.34 a	26.93 a	1.70 c	2.84 a
30	1.63 c	20.70 b	2.09 ab	2.51 a
LSD	0.13	3.00	0.25	0.39
ANOVA-*P* value	0.001	0.007	0.007	0.025

[a] All data correspond to mean values of 3 readings of all data from the 4 replicates of each treatment and the 6 samplings in 3 years of experiments. Mean values followed by the same letter within a column were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

MATERIALS AND METHODS

Crop Design. The experiment was conducted for three consecutive years (1993, 1994, and 1995) in an experimental field in Granada, Spain, using *Solanum tuberosum* L. var. Spunta, planted at the beginning of March. The crop cycle was about 4 months under the Mediterranean climate in a location used intensively for agriculture. The soil used showed the following characteristics: sand 45.3%, silt 43.2%, and clay 11.2%; pH(H_2O 1:2.5) 8.6; electrical conductivity (ec) 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 Cu (3 mg kg^{-1}) and Zn (3 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}; NO_3^- 34 mg L^{-1}, NH_4^+ 14 mg L^{-1}, Na^+ 25 mg L^{-1}; K^+ 4 mg L^{-1}; H_2CO_3 369 mg L^{-1}, Cu 25 μg L^{-1}, Zn 12 μg L^{-1}.

The experimental design was a factorial arrangement in a randomized complete block with 5 treatments replicated 4 times (20 plots). Each plot had an area of 78.4 m^2, with 4 plants per m^{-2} spaced 30 cm, with 80 cm between rows.

The root-zone temperatures (RZT) were measured at 15 cm depth, using 107 temperature probes (Campbell Scientific Co., Logan, UT), at 4-hour intervals every 3 days throughout the crop development (from 45 to 120 days of age) and mean values are represented in Table 1.

The different treatments consisted of covering the soil surface of each plot with plastic mulches (polyethylene sheets), making a tight seal with the soil: no plastic (control treatment); transparent polyethylene (25 μm thick), white polyethylene (25 μm), coextruded black and white polyethylene (50 μm), and black polyethylene (25 μm).

The fertilization used was the same as is habitually 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) were applied (27 g m^{-2}). Afterward, and at the end of the month of April, 25 g m^{-2} was applied in the form of NH_4NO_3. Fertilization 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 was applied as H_3BO_3, and the remaining micronutrients were applied as sulfates.

Plant Sampling. The plant organs (roots, leaflets, stems, and tubers) were sampled 6 times every 2 weeks, throughout the plant development, for the three years of experiments. For each sampling, 10 plants were collected from each replicate per treatment from all samplings. Leaf samples were taken only from plants with fully expanded leaflets of the same size. Leaflets were picked at about one-third of the plant height from the plant apex. In the roots, we chose all the material possible (primary as well as secondary roots with diameters from less than 1 mm to 1 cm). Roots, leaflets, stems, and tubers were rinsed three times in distilled water after they were disinfected with nonionic detergent at 1% (*12*), then blotted on filter paper. At each sampling, a subsample was oven dried (70 °C for at least 24 h), ground in a Wiley mill, and then placed in plastic bags for further analyses.

Plant Analysis. *Cu and Zn Contents*. Oven-dried and pulverized plant material was digested with concentrated nitric acid, and hydrogen peroxide measurements were made by atomic absorption spectrophotometry (*12*).

Soluble Cu and Zn. For these determinations dry matter (0.15 g) was extracted with 10 mL of 1 M HCl for 30 min and then filtered, and Cu and Zn were determined using an atomic absorption spectrophotometer (*13*).

Reagent blanks for both analyses were also prepared by performing the entire extraction procedure, but in the absence of the samples.

Total Accumulation. Elemental uptake (concentration × dry matter) was also calculated using the dry-weight data.

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

The results of the parameters measured, regardless of the year, varied slightly between years, and thus the variance was homogeneous enough to pool the results for the 3 years. Therefore, the statistical analyses of the data from all samplings during the plant cycle were pooled to avoid duplication of the calculations and to simplify the presentation of the results.

RESULTS

Biomass. The mulch treatments significantly affected the mean RZT, with the black mulch having the highest value (30 °C), and the open-air plots having the lowest value (16 °C).

The biomass on a fresh and dry weight basis was also affected by the RZT (Table 1). Thus, for the roots, leaflets, and tubers, the highest fresh and dry weights were found at 27 °C RZT, and the lowest at 20 °C, with the latter values being lower than those found at 16 °C. On the contrary, in the stems the highest fresh and dry weights were found at 20 °C and the lowest occurred at 27 °C.

Cu and Zn Contents. The roots registered the highest Cu contents at 23 °C/27 °C, being 26%/22% over the control, respectively; whereas at 20 °C we found the lowest level (45% lower than in the open). In tubers and stems, no significant effect was found with regard to the RZT. With respect to the leaflets, 23 °C/27 °C gave the lowest Cu contents. In relation to the soluble Cu, in the roots and tubers, the values did not statistically differ between RZT (Table 2). In stems and leaflets, the highest contents were found at 20 °C, surpassing those of the control (16 °C) by 24% and 31%, respectively.

In relation to the effect of RZT on Zn (Table 3), in roots higher values were recorded at 16 °C/20 °C. In tubers and stems Zn reached the highest levels at 20 °C, exceeding the 16 °C results by 44% and 45%, respectively, whereas at 23 °C/27 °C the lowest Zn content appeared for both organs. In leaflets the results were the opposite: at 23 °C/27 °C the highest contents were recorded and at 20 °C we found the lowest contents (31% lower than that of the control). Soluble Zn (Table 3) in the roots reached the highest content at 20 °C; they were 16% higher than those found at 16 °C. In tubers and stems, at 16 °C/20 °C we found the highest soluble Zn. In leaflets, the lowest content was detected at 20 °C (31% lower than at 16 °C), whereas the rest of the RZT showed no significant effect.

Cu and Zn Total Accumulation. The highest total Cu in the roots was recorded at 27 °C ($P < 0.001$; Figure 1B),

Table 2. Effect of Root-Zone Temperature on Cu Contents in Potato Organs

	Cu (μg g^{-1} d.w.)				soluble Cu (μg g^{-1} d.w.)			
RZT (°C)	roots	tubers	stems	leaflets	roots	tubers	stems	leaflets
16	76 b[a]	14 a	93 a	210 b	9 a	5 a	29 b	72 b
20	42 c	12 a	98 a	252 a	8 a	5 a	36 a	94 a
23	96 a	15 a	83 a	176 d	10 a	6 a	19 c	57 c
27	93 a	15 a	84 a	189 cd	10 a	6 a	18 c	50 c
30	79 b	14 a	93 a	207 bc	9 a	5 a	28 b	79 b
LSD	11.28	3.25	15.27	20.12	4.70	2.30	4.31	11.37
ANOVA-*P* value	0.007	0.300	0.210	0.001	0.810	0.690	0.001	0.001

[a] All data correspond to mean values of 3 readings of all data from the 4 replicates of each treatment and the 6 samplings in 3 years of experiments. Mean 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 3. Effect of Root-Zone Temperature on Zn Contents in Potato Organs

	Zn (μg g^{-1} d.w.)				soluble Zn (μg g^{-1} d.w.)			
RZT (°C)	roots	tubers	stems	leaflets	roots	tubers	stems	leaflets
16	34 a[a]	36 b	150 b	35 c	19 b	10 a	81 a	13 a
20	35 a	52 a	218 a	24 d	22 a	10 a	86 a	9 b
23	28 b	25 c	115 c	40 b	15 c	5 b	73 b	14 a
27	28 b	24 c	114 c	49 a	14 c	6 b	70 b	14 a
30	30 b	35 b	141 b	36 bc	15 c	7 b	74 b	13 a
LSD	2.93	6.86	24.08	4.38	2.44	2.57	7.16	2.70
ANOVA-*P* value	0.041	0.005	0.001	0.004	0.009	0.038	0.003	0.041

[a] All data correspond to mean values of 3 readings of all data from the 4 replicates of each treatment and the 6 samplings in 3 years of experiments. Mean values followed by the same letter within a column were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

surpassing the control by 64%; and the lowest was recorded at 20 °C (67% lower than that at 16 °C). In tubers, we obtained the highest Cu at 27 °C ($P < 0.001$; 44% higher than in T0), but at 20 °C the level was markedly lower (some 52%) than that of the control. With respect to the stems, the highest total Cu ($P < 0.05$) was given at 20 °C (27% higher than that at 16 °C) and the lowest at 23 °C (15% lower). In leaflets, the RZT effect on total Cu was not significant. Comparing the distribution in different organs, the Cu accumulation followed this order: leaflets (40−55%), tubers (15−29%), stems (12−25%), and roots (5−18%). The accumulated soluble Cu in roots was greatest at 27 °C ($P < 0.05$; 50% higher than that found at 16 °C; Figure 1B), and the lowest value was recorded at 20 °C (49% lower). In the tubers, the highest content ($P < 0.01$), surpassing the control by 59%, was detected at 27 °C, whereas at 20 °C the content proved 44% lower than that at 16 °C. In stems ($P < 0.01$) and leaflets ($P < 0.05$) the 23 °C/27 °C RZT reduced the accumulation of soluble Cu, whereas the greatest value was reached at 20 °C/30 °C, exceeding the control by 49%/11%, respectively. The accumulation of soluble Cu was as follows: leaflets (42−53%), tubers (16−38%), stems (9−24%), and roots (3−6%).

The highest root concentration of total Zn ($P < 0.01$; Figure 2A) was found at 27 °C, and the lowest was found at 20 °C (40% lower than that at 16 °C). In tubers, the RZT effect was not significant. In stems, the total Zn at 20 °C RZT proved 75% higher than that at 16 °C ($P < 0.01$). Finally, in leaflets, the highest value was found at 27 °C, exceeding the 16 °C value by 61%. The Zn in all organs according to the treatments (Figure 2A), was greatest in tubers (50−63%). The rest of the organs accumulated low levels of Zn (stems, 19−43%; leaflets, 4−13%; roots, 4−6%). The greatest content of soluble Zn in roots was reached at 16 °C/27 °C ($P < 0.01$), and the lowest was reached at 20 °C (31% lower). In tubers, at 16 °C the greatest soluble Zn accumulation was found ($P < 0.05$). In stems, the highest soluble Zn was found at 20 °C (28% higher than that at 16 °C), and in the leaflets the highest value appeared at 27 °C (by 21%), but the different RZT treatments showed little difference. The accumulated soluble Zn in organs was distributed as follows: tubers (33−49%), stems (32−55%), leaflets (5−11%), and roots (6−8%).

DISCUSSION

Mulch Effects on Root-Zone Temperature (RZT). The effect of the different mulches on RZT according to Ham et al. (*14*) showed that black polyethylene absorbs roughly 96% of the short-wave radiation while reflecting very little, and thus warms the underlying soil (30 °C; *15*). The white polyethylene (23 °C) induced cooler soil temperatures than did black because the former reflected most wavelengths of solar radiation (*16*). Schmidt and Worthington (*17*) demonstrated that transparent mulches (20 °C) do not cause soil warming, presenting mean temperatures of 18−20 °C during the crop cycle, whereas the white and black coextruded covers generate higher RZT (27 °C).

Biomass. The increased fresh and dry weights in our plants within the root-zone temperature range of 24−27 °C agreed with results for tomato plants (*18*), whereas outside this range, the fresh and dry weights fell. However, the decrease in fresh and dry weights at 30 °C could be due to the high RZT exceeding 29 °C with retarded development and lowered the fresh and dry-weight accumulations *(10*, *16*, *18)* (Table 1).

Cu and Zn Content. The RZT acted significantly on Cu content (Cu and soluble Cu; Table 2) in the different organs of the potato plants. However, Hood and Mills (*19*), as well as Engels and Marschner (*8*), studied the effect of RZT on Cu uptake in *Antirrhinum majus* L. cv. Peoria, and in maize, respectively, and demonstrated that the RZT had no effect on the uptake of this micronutrient; by contrast, in our study at 23 °C/27 °C (more appropriate temperatures for the plant development), RZT affected the Cu levels and thus Cu mainly concentrated in roots and stems in comparison with Cu levels found at lower temperatures (*20*, *21*). In addition, the treatments

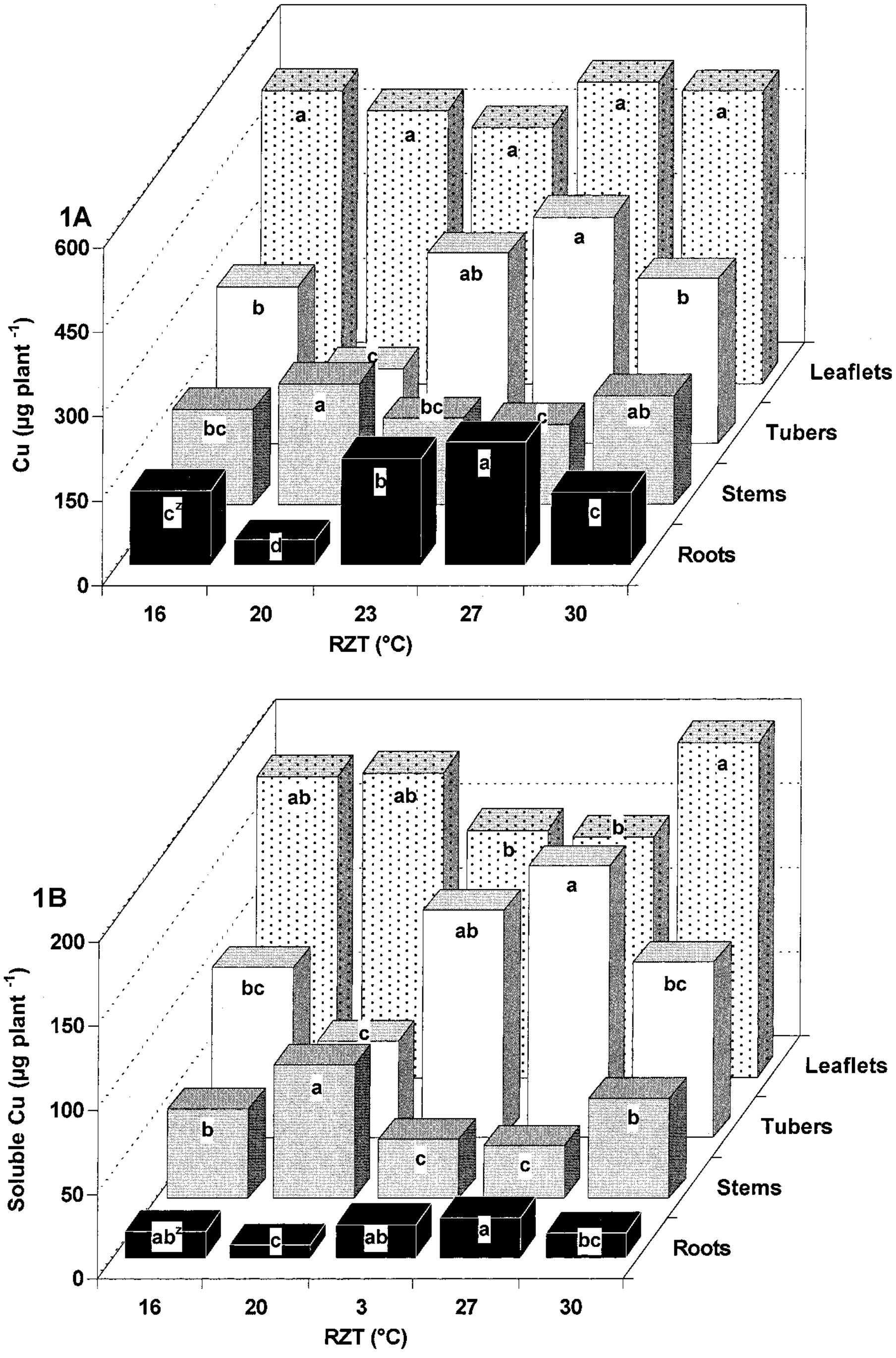

Figure 1. Effect of root-zone temperature on total accumulation of Cu (A) and soluble Cu (B) in potato organs. All data correspond to mean values of 3 readings of all data from the 4 replicates of each treatment and the 6 samplings in 3 years of experiments. Mean values with the same letter within a series were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

also strongly affected Cu distribution in the different organs, with 23 °C and 27 °C negatively affecting the Cu transport toward the leaflets, possibly due to antagonism with Zn or other elements (*8*), such as Fe, like a coadjutant factor of Cu deficiency at high RZT (*9*).

The Cu contents in the roots were within the sufficiency range established by Cook et al. (*22*), and higher than the optimum range previously found for potato plants under analogous conditions (7−11 μg g^{-1} d.w.; *23*), whereas in the leaflets the different treatments were within the optimum range (140−260 μg g^{-1} d.w.; *24*). However, the Cu content in the stems and leaflets showed far higher values than those reported in the work of Cook et al. (*22*), suggesting that the Cu in different plants grown in nonpolluted regions vary between 1 and 10 μg g^{-1} d.w., but when grown in excessive or toxic levels, Cu concentrations can rise to 20 and 100 μg g^{-1} dry weight. Kabata-Pendias and Pendias (*25*) reported that the "normal" Cu content in mature leaves varies from 5 to 30 μg g^{-1} d.w., but our plants showed much higher Cu (*26*, *27*).

In relation to Zn (Table 3), the RZT influenced the Zn content and distribution, possibly acting on uptake mechanisms and membrane permeability (*28*), as well as considerably increasing the growth of the plant and thus favoring the Zn content in leaflets at 27 °C (*28*). However, in the roots, tubers, and stems, we found higher Zn at 20 °C (Table 3), showing probably lower absorption and transport at higher temperatures or induced transport of this nutrient toward aerial parts (*29*), as the Zn content in the leaflets increased with higher RZT (*9*).

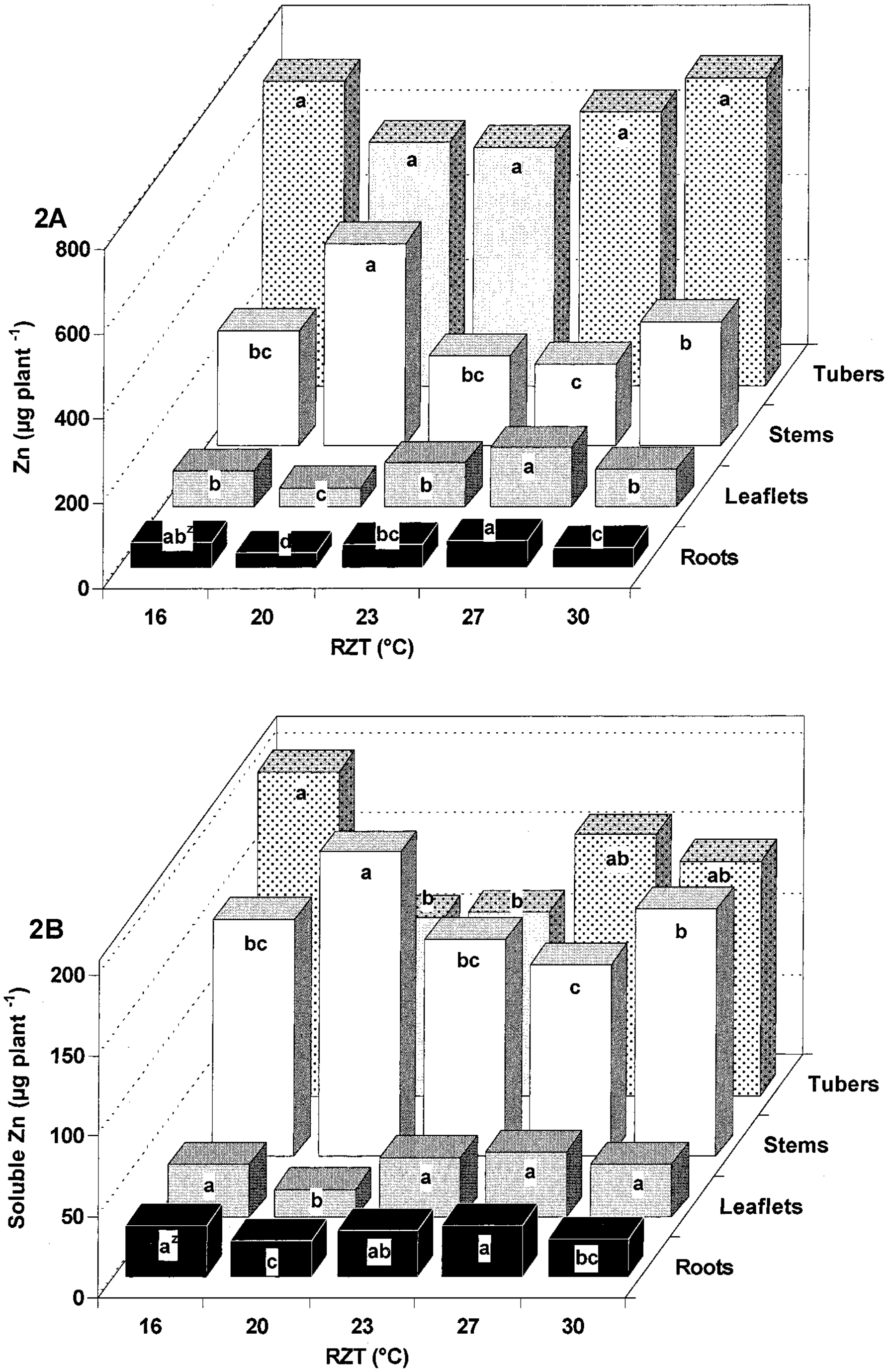

Figure 2. Effect of root-zone temperature on total accumulation of Zn (A) and soluble Zn (B) in potato organs. All data correspond to mean values of 3 readings of all data from the 4 replicates of each treatment and the 6 samplings in 3 years of experiments. Mean values with the same letter within a series were not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Working with tomato, Hurewitz and Janes (*30*), Gosselin and Trudel (*31*), and Tindall et al. (*32*) found that the optimal temperatures for Zn uptake were around 25 °C and 30 °C, whereas lower root temperatures reduced these processes. The Zn distributed mainly in the leaflets at 23 °C/27 °C, probably as a result of a strong influence of Cu, as both could interact in terms of uptake and transport toward the leaves and tubers. However, without neglecting the possible interactions between Zn and Cu in the roots, leaflets, or tubers, we found negative and highly significant correlations between Cu and Zn in the roots (Cu−Zn, $r = -0.85$***; soluble Cu−Zn, $r = -0.81$***;), leaflets (Cu−Zn, $r = -0.87$***; soluble Cu−Zn, $r = -0.91$***), and tubers (Cu−Zn, $r = -0.95$***; soluble Cu−Zn, $r = -0.89$***).

Cu and Zn Accumulation. According to Hocking (*33*), the accumulation of nutrients depends on their mobility and on the size of the sink, and the ideal plants for phytoextraction have rapid growth and efficient transport of elements from the roots to the sinks (*6*, *34*, *35*). For total Cu (Cu and soluble Cu; Figure 1A,B), in roots, leaflets, and tubers, the greatest accumulation was given at 27 °C, where the highest biomass production was also registered (Table 1), whereas in the stems the greatest accumulation was reached at 20 °C, where stem biomass was also highest. Thus, sinks stored the elements during accumulative processes (*6*, *23*, *33*). It is noteworthy that the highest Zn accumulation occurred in the tubers (Figure 2A,B), organs with a high biomass production in comparison with that in other parts of the plant.

The ideal plant for continuous phytoextraction should produce high biomass and accumulate as well as resist high concentrations of metals in shoots (*5*).

Baker and Brooks (*33*) suggested that "hyperaccumulator plants" reach concentrations exceeding 1% of Zn and 0.1% Cu of leaf dry weight, whereas our results showed concentrations below 260 μg Zn g^{-1} d.w.; thus, the potato plant is not a Zn hyperaccumulator. However, with regard to the usefulness of potato plants for phytoremediation of Cu and Zn, as well as for other heavy metals, according to Salt and Krämer (*37*) a plant is a "hyperaccumulator" if it fulfills the following ratio: (metal content in the aerial)/(metal content in the root part) > 1. In our experiment, this ratio was > *1* for both metals, indicating to us that potato plant could have possibilities or potential for phytoextraction practices, with the involvement of RZT controlling and accelerating the plant growth and uptake of elements, and reducing the time of contamination, and, therefore, reducing the cost of the decontamination in agrosystems. Thus, for phytoremediation this mulch technique represents an advantage over other, engineering-based, methods which are costly and may cause more pollutants to be generated (*6*). Furthermore, the mulch technique can be used with other plant species (phytoaccumulators and phytoextractor species), in all cases providing major advantages over industrial or chemical approaches by causing negligible environmental impact, although further detailed studies are needed in this regard.

In terms of human nutrition, the Cu and Zn levels found in our experiment in the tubers (the edible part of the plant) were around 130−400 μg plant^{-1} and between 550 and 727 μg plant^{-1}, respectively, which are both well below the 5-mg plant^{-1} limit for human toxicity.

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