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Heavy Metal Tolerance in *Ocimum basilicum* var. *purpurascens* Benth. I. Subcellular Distribution of Zinc, Copper, Cobalt and Nickel in Leaves and Roots*

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Ocimum basilicum var. *purpurascens* Benth. is tolerant to high concentrations of copper and zinc and is susceptible to cobalt and nickel. Sub-cellular distribution studies of these metals in leaves and roots revealed selective partition of high concentrations of copper and zinc in cell walls, and cobalt and nickel in cytoplasmic supernatant fraction. Isotopes ^{65}Zn and ^{63}Ni distribution studie confirmed the inorganic analysis in subcellular fractions. Accumulations of ^{65}Zn in cell wall was increased from 1 to 14 days of exposure, while high ^{63}Ni activity was recorded in the cytoplasmic fraction. Further analysis of cell wall components to ^{65}Zn and ^{63}Ni revealed that pectins and cell wall proteins are the binders of the metals.

Key Words: Heavy metals, Tolerance, *Ocimum basilicum*, Cell wall components

Introduction

The phenomenon of heavy metal tolerance in plants growing on mines has been of interest for several decades (Bradshaw 1952, Antonovics et al. 1971). Tolerance to the heavy metals among these mine populations has been reported as a genetically controlled factor and specific to the contaminant metal (Gregory & Bradshaw 1965, Antonovics et al. 1971 and Gartside & McNeilly 1974). Although the genetic aspects have been studied rather elaborately, the physiological mechanisms to the metal tolerance was poorly understood. A few investigations have revealed the physiological mechanisms in mine populations (Turner 1969, 1970, Peterson 1969, 1971, Reilly et al. 1970, Reilly 1971, Antonovics et al. 1971, Mathys 1975, 1977, Ernst 1976 and Hart & Bertran 1980).

In our previous article we have reported the tolerance potentials of some local

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plants growing on normal soil (Veeranjaneyulu & Das 1981). In the present study we report the intracellular distribution of heavy metals Cu, Zn, Co, and Ni in the copper tolerant plants of *Ocimum basilicum*.

Materials and Methods

Plants of *Ocimum basilicum* var. *purpurascens* Benth. were raised in 12" × 18" earthenware pots containing 5 kg of air dry soil and farmyard manure in 4:1 proportions. Metal treatments were given to the 4 weeks old plants as described elsewhere (Veeranjaneyulu & Das 1981). Metals were added as their salt solutions (4% W/V) three times at an interval of 4 days to the soil to get required soil metal concentration of Cu 900 ppm, Zn 1450 ppm, Co or Ni 450 ppm. Plants were watered once in a day. Leaching was avoided by watering the plants approximately to the field capacity.

After 12 weeks the plants were harvested and washed thoroughly with tap water and distilled water. Later roots were washed in 0.02M $Cacl_2$ and again in distilled water and were subcellularly fractionated.

Isotopes Administration

Plants were grown in water culture using a full nutrient medium (Hewitt 1966) in a growth chamber at 27°C under light intensity of 150 WM^{-2} with a 12 hr photoperiod. The nutrient solutions were replaced for every 2 days. After 7 days, roots were washed in distilled water, rinsed in 0.02M $CaCl_2$ solutions and again in distilled water. Then plants were transferred to metal solutions containing ^{65}Zn (200μ Ci/litre, 4ppm Zn) or Ni (30μ Ci/litre, lppm Ni) prepared in 0.05% (W/V) Ca $(No_3)_2$. The metal solutions were replaced for every 4 days. At the required time intervals plants were removed and divided into roots, stems and leaves. The roots were washed in cold deionised water and twice with carrier zinc or nickel (0.2 mm) solutions for 5 to 10 min. Finally the roots were rinsed with distilled water.

Subcellular fractionation of roots and leaves was done according to Turner (1970). The samples were wet digested (Humphries 1956), and zinc and cobalt were estimated according to Sandell (1950). Copper was estimated by carbamate method as per Cheng and Bray (1953), Nickel was estimated by diethyldithiocarbamate method, after separating with demethylglyoxime according to Snell and Snell (1949).

Chemical fractionation was done according to Diez-Altares and Bornemisza (1967) as outlined by Peterson (1969). Radio activity was counted in a continuous gas flow proportional counter.

Results

O. basilicum is tolerant to copper and zinc and is susceptible to cobalt and nickel. The subcellular distribution studies of these metals in leaves and roots were made to understand the differential sensitivity of this plant to these 4 metals.

^{65}Zn activity was more in roots and stems than in leaves, while ^{63}Ni activity was high in roots and leaves (table 1).

Table 1 *Distribution of ^{65}Zn ^{63}Ni in different organs of plant fed either ^{65}Zn or ^{63}Ni (CPM mg^{-1} Plant Tissue)*

Days	^{65}Zn			^{63}Ni		
	Roots	Stem	Leaves	Roots	Stem	Leaves
1	1050	348	220	1440	568	653
7	2760	447	349	3724	1080	1460

The results of the sub-cellular fractionation experiments are presented in tables 2 and 3. Both in leaves and roots the cell wall fractions contained comparatively higher concentrations of zinc and copper than the cobalt and nickel. In roots of treated plants 46% of zinc and 42% of copper were found in cell wall fraction. Only 30% and 29% of cobalt and nickel, respectively, were seen in cell wall fraction of roots. However the supernatant cytoplasmic fractions showed high concentrations of cobalt and nickel, up to 47% and 50% respectively. But in zinc or

Table 2 *Sub-cellular distribution of Zn, Cu, Co and Ni in roots of* O. basilicum *var.* purpurascens

Fraction	Zn-treated plant		Cu-treated plant		Co-treated plant		Ni-treated plant	
	μg Zn	%	μg Cu	%	μg Co	%	μg Ni	%
Entire homogenate	465.00 ±18.34	100	510.65 ±10.38	100	235.45 ±9.32	100	285.63 ±10.46	100
Crude debris	104.16 ±5.64	22.40	131.32 ±5.72	25.75	44.33 ±2.86	18.95	51.61 ±1.39	18.11
500 g (Cell wall + Nuclear)	216.69 ±9.23	46.60	217.00 ±6.42	42.55	71.91 ±1.45	30.60	84.90 ±2.54	29.79
10,000 g (Mitochondrial)	7.57 ±0.09	1.63	10.20 ±0.75	2.00	5.28 ±0.09	2.25	5.47 ±0.25	1.92
10,000 g (Ribosomal)	1.95 ±0.08	0.42	30.09 ±1.49	5.90	1.71 ±0.02	0.73	1.96 ±0.03	0.69
Supernatant	134.61 ±2.32	28.95	121.38 ±2.86	23.80	111.67 ±3.85	47.52	141.04 ±4.59	49.49

Table 3 *Sub-cellular distribution of Zn, Cu, Co and Ni in leaves of* O. basilicum *var.* purpurascens

Fraction	Zn-treated plant		Cu-treated plant		Co-treated plant		Ni-treated plant	
	μg Zn	%	μg Cu	%	μg Co	%	μg Ni	%
Entire homogenate	525.26 ±13.86	100	270.62 ±6.36	100	245.56 ±9.05	100	215.79 ±8.05	100
Crude debris	106.62 ±2.84	20.31	50.76 ±2.35	18.80	42.53 ±3.17	17.36	41.51 ±5.42	19.31
500 g (Cell wall + Nuclear)	194.77 ±6.92	37.12	86.67 ±2.42	32.10	50.05 ±3.76	20.43	46.48 ±3.26	21.62
1000 g (Chloroplast)	22.05 ±0.95	4.20	22.14 ±0.95	8.20	16.95 ±1.26	6.92	17.97 ±1.06	8.36
10,000 g (Mitochondrial)	9.45 ±0.16	1.81	5.67 ±0.09	2.10	5.68 ±0.12	2.32	4.19 ±0.23	1.95
1,00,000 g (Ribosomal)	1.57 ±0.03	0.30	3.51 ±0.08	1.30	1.00 ±0.06	0.41	1.35 ±0.02	0.63
Supernatant	190.57 ±8.92	36.26	78.75 ±2.64	37.50	128.77 ±5.26	52.56	103.47 ±3.56	48.13

copper treated plants, the supernatant fractions contained only 29% and 24% of zinc and copper respectively.

The pattern of distribution in leaves was similar to roots. The supernatant fractions contained 36%, 37%, 52% and 48% of zinc, copper, cobalt and nickel respectively. The distribution pattern of both zinc and copper, in general, was found to be similar. Both metals were found in high concentrations in cell walls, as against to cobalt and nickel, which are seen in high concentrations in cytoplasmic fraction.

Isotopes Distribution

After the exposure of plants to ^{65}Zn and ^{63}Ni for 1, 7 and 14 days, the leaves were harvested and were fractionated into sub-cellular fractions including cell walls containing nuclear debris, chloroplasts, mitochondria, ribosomes and supernatant (figure 1). The incorporation of ^{65}Zn increased in the cell wall fraction from 1 to 14 days exposure, while a slight decline in its activity in the supernatant cytoplasmic fraction was observed. Relatively high ^{63}Ni activity was observed in the supernatant fraction. There was little increase in ^{63}Ni activity in cell wall fractions from 1 to 14 days. The chloroplast fraction also showed an increase in the ^{63}Ni activity. There was slight increase in ^{65}Zn activity in the mitochondrial fraction.

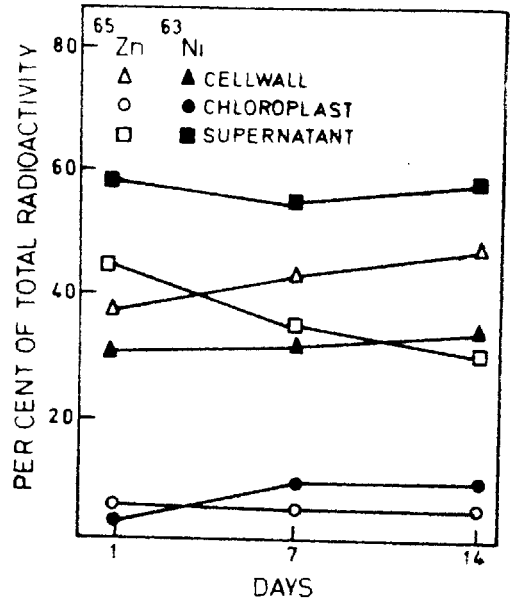

Table 4 *Distribution of ^{65}Zn and ^{63}Ni between 80% ethanol soluble, water soluble and residual fractions of leaves and roots of* O. basilicum *var.* purpurascens

Plant material	^{65}Zn				^{63}Ni			
	Total	Ethanol Soluble	Water	Residue (A)	Total	Ethanol Soluble	Water	Residue (A)
Roots	100	42.2	2.1	55.7	100	61.8	2.2	36.0
Leaves	100	46.0	2.4	51.6	100	66.1	2.9	31.0

Table 5 *Distribution of ^{65}Zn and ^{63}Ni in cell wall components of roots and leaves of plants fed either ^{65}Zn or ^{63}Ni*

Components	Roots		Leaves	
	^{65}Zn	^{63}Ni	^{65}Zn	^{63}Ni
Residue (A)	100	100	100	100
Protein	18.9	20.7	32.4	40.5
Pectin	58.9	60.3	45.6	36.9
Protopectin	3.0	2.9	2.0	2.4
Hemicelluloses	7.0	6.2	7.2	10.8
Lignin	10.2	9.0	12.8	9.5
Oc-cellulose	2.0	0.9	0.0	0.0

There were marked differences in the distribution of ^{65}Zn and ^{63}Ni between the ethanol soluble and residual fractions (table 4), confirming the earlier subcellular studies. In both leaves and roots

high percentage of ^{65}Zn was observed in the residue fraction than the ^{63}Ni. In the ethanol soluble fractions ^{63}Ni activity was higher than the ^{65}Zn. Results of the further extractions of residue showed that nearly 32% of ^{65}Zn and 40% of ^{63}Ni were found in pronase treated extracts (table 5). A major portion of ^{65}Zn (45%) and ^{63}Ni (36%) were seen in pectin fractions. In roots also high percentages of ^{65}Zn and ^{63}Ni were found in proteins and pectins.

Discussion

From the data it is evident that the cell wall fraction has accumulated high concentrations of heavy metals. Specifically zinc and copper were found in high concentrations in cell walls. With the fraction label system in cellular fractions of *Agrostis tenuis*, it was suggested that in the absence of cell wall fractions the particulate could accumulate high concentrations of zinc (Turner & Gregory 1967). Further the sub-cellular organelles including chloroplasts, mitochondria, and ribosomes have been reported as sites for toxic action of metals (Rothstein 1959, Passow et al. 1961, Rao et al. 1966 and Sabnis et al. 1969). Hence the cell walls may exert a protective action over other organelles.

Isotopic distribution studies with ^{65}Zn and ^{63}Ni form a supporting evidence for this preferential localization. The pattern of distribution suggests that the tolerant nature of *O. basilicum* to copper and zinc may be partially due to the deposition of metals in cell walls and higher finite capacity of cell walls to copper and zinc than to cobalt and nickel.

The occurrence of radioactivity in pronase treated extracts of cell wall fractions suggests that metals are bound to proteins. It was reported that zinc possesses high affinity to carboxyl groups (Broda 1965); hence it could bind to the proteins. Further extraction of residue revealed the association of larger percentage of radioactivity with pectin fractions. The pectins and uronic acids in cell walls were known to have cation exchange capacity and can bind the cations in the Donnan free space (Dainty & Hope 1959, Dainty et al. 1960 and Pitman 1965). The differences exist in the total amount of zinc and nickel in the pectate fraction. This phenomenon might inactivate large amounts of zinc than nickel; and therefore plants could tolerate high concentrations of zinc than nickel.

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