Pergamon

PII: S0031-9422(97)00159-3

Phytochemistry, Vol. 45, No. 7, pp. 1343–1350, 1997

0031-9422/97 $17.00+0.00

CADMIUM- AND COPPER-INDUCED CHANGES IN TOMATO MEMBRANE LIPIDS

OMAR OUARITI,* NAIMA BOUSSAMA, MOKTAR ZARROUK,† ABDELKADER CHERIF‡ and MOHAMED HABIB GHORBAL

Laboratoire de Physiologie Végétale 'Nutrition et Métabolisme Azotés', Département des Sciences Biologiques, Faculté des Sciences de Tunis Campus Universitaire-1060, Tunis, Tunisia; †Unité de Biotechnologie Végétale et de Ressources Génétiques, INRST BP 95, 2050, Hammam-Lif, Tunisia; ‡Ecole Supérieure des Industries Alimentaires, Tunis El Khadra 1002 Tunis, Tunisia

(*Received in revised form* 9 *December* 1996)

Key Word Index—*Lycopersicon esculentum*; Solanaceae; cadmium; copper; fatty acids; lipids; heavy metal-stress.

Abstract—Cadmium and copper uptake and distribution, as well as their effects on growth and lipid composition were investigated in 17-day-old tomato seedlings (*Lycopersicon esculentum* Mill. cv. 63/5 F1) grown in culture solution supplied with two concentrations of Cd or Cu (0, 5 and 50 μM). The accumulation of Cd and Cu increased with external metal concentrations, and was considerably higher in roots than in primary leaves. Biomass production of the growing roots and primary leaves was strongly depressed at high metal levels. Also, significant decreases in the content of lipid classes and changes of fatty acid composition were recorded in heavy metal-stressed plants in comparison with controls. Glycolipid contents were decreased more in leaves than in roots by Cd-treatment, but copper decreased both to similar extents in both organs. Likewise, both metals reduced the phospholipid and neutral lipid contents more in roots than in leaves. In almost all lipid classes the proportion of palmitic acid (16:0) increased, and that of linoleic (18:2) or linolenic (18:3) acid decreased, suggesting that heavy metal treatment induced an alteration in the fatty acid desaturation processes. Furthermore, the accumulation of palmitate (16:0) rather than stearate (18:0) indicated an alteration in the ratio of products from the fatty acid synthase. Copper was found to be the most unfavourable for plant growth and lipid metabolism. The possible mechanisms by which heavy metals, especially Cu, induce a strong lipid shift are discussed.

INTRODUCTION

High heavy metal contamination of soils is one of the major environmental stresses. Increasing industrial pollution, urban activities and agricultural practices can lead to a build-up of toxic levels of cadmium and copper in the growth medium of plants [1, 2]. When accumulated in excess in plant tissues, these metals cause alterations in various vital growth processes, such as mineral nutrition [3, 4], transpiration [5, 6], photosynthesis [7–10], enzyme activities-related to metabolism [11, 12] and biosynthesis of chlorophyll [7, 13] and nucleic acids [14, 15]. In spite of the considerable literature on the subject, the fundamental mechanism of heavy metal phytotoxicity has not yet been characterized and is still an open question.

Apparently, cell decompartmentalization and modification of membrane functions represent the first targets for metal toxicity [16, 17]. To evaluate this, we really need to know to what extent metal can induce changes in acyl lipid and fatty acid composition of the major lipid components of the cell membranes. Only a few recent studies have examined the effects of heavy metals on membrane lipid metabolism. Most of these reports have been focused mainly on lipid peroxidation [13, 18] and fatty acid composition in isolated chloroplasts of stressed plants [19].

The present paper reports on changes of membrane lipid composition in root and leaf cells, accompanying the accumulation of cadmium as well as copper in tomato seedlings. Cadmium and copper were chosen as probe metal-ions because they are particularly toxic for most crop species [20]. To date, no comparative study on heavy metal-mediated changes in lipid metabolism has been reported at the whole plant level.

* Author to whom correspondence should be addressed.

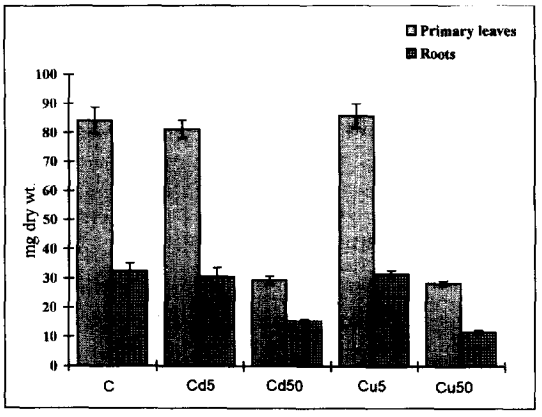

Fig. 1. Dry weights of roots and primary leaves of tomato seedlings after 7 days exposure to two concentrations of cadmium or copper. Data shown are means ± s.e. ($n = 6$). C, control; Cd5, Cd50, Cu5 and Cu50, metal species and its concentration (μM) in the medium.

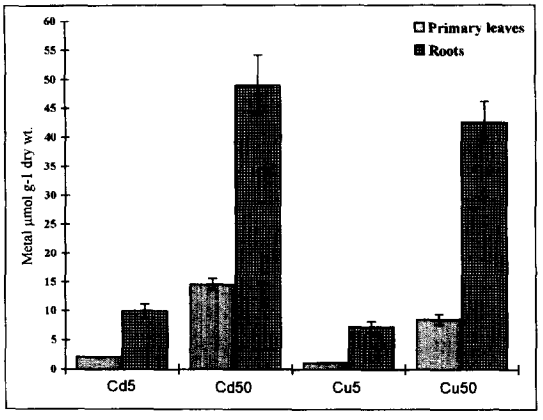

Fig. 2. Cadmium and copper contents in roots and primary leaves of tomato seedlings after 7 days exposure to two concentrations of cadmium or copper. Data shown are means ± s.e. ($n = 6$). C, control; Cd5, Cd50, Cu5 and Cu50, metal species and its concentration (μM) in the medium.

RESULTS

Seedling growth

At the end of the treatments, the growth of tomato plants was unaffected by 5 μM Cd or Cu (Fig. 1), whereas the presence of 50 μM Cd or Cu in the medium, reduced the root dry weight by 52 and 64%, respectively, compared to the control, whereas that of primary leaves was reduced by approximately 65% with both metals.

Heavy metal accumulation and distribution

Tomato plants exposed to Cd or Cu in the nutrient solution accumulated substantial amounts of either Cd or Cu, in the roots and the primary leaves (Fig. 2). At both concentrations tested, the distribution of Cd between roots and primary leaves resembled that of Cu. At 5 μM about 90% of total plant content of either Cd or Cu was found in the roots. At 50 μM, this proportion decreased to 71% for Cd and to 84% for Cu.

Lipid content and fatty acid composition

In control plants, significant differences in the lipid composition of roots and primary leaves were observed (Tables 1 and 2). In the leaves, glycolipids were the major constituent lipid classes, accounting for 53% of the total lipids (Table 2). Moreover, in this lipid class the proportion of monogalactosyl diacylglycerol (MGDG) was higher than that of digalactosyl diacylglycerol (DGDG) (*ca* 29 and 21% of total lipids, respectively) (Fig. 3). However, the root lipids were characterized by a high level of phospholipids, about 61% of the total lipids, followed by neutral lipids and glycolipids (Table 2). The most abundant phospholipids in both leaf and root tissues were phosphatidylcholine (PC) and phosphatidylglycerol (PG) (Fig. 4). It is also interesting to note that the percentage of MGDG in total lipids of roots was lower than that of DGDG (*ca* 5 and 8% of total lipids, respectively). Some fatty acid species, palmitic (16:0), linoleic (18:2) and linolenic (18:3) acids were commonly higher in root and leaf lipids, and only differed

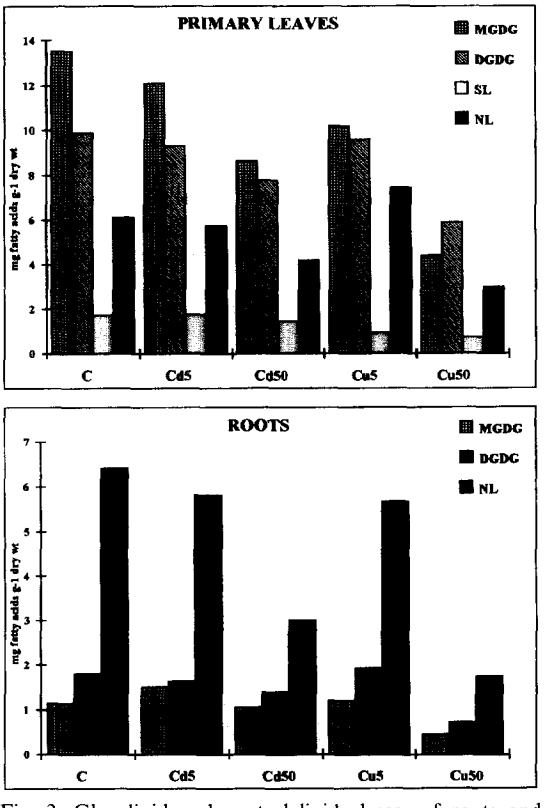

Fig. 3. Glycolipid and neutral lipid classes of roots and primary leaves of tomato seedlings exposed for 7 days to two concentrations of cadmium or copper. C, control; Cd5, Cd50, Cu5 and Cu50, metal species and its concentration (μM) in the medium. MGDG, monogalactosyl diacylglycerol; DGDG, digalactosyl diacylglycerol; SL, sulpholipid; NL, neutral lipid.

Table 1. Total fatty acid composition of primary leaves and roots of tomato seedlings after 7 days exposure to two concentrations of cadmium or copper*

	Treatments* C	Cd5	Cd50	Cu5	Cu50
Fatty acid of leaves (% of total)					
16:0	17.1±0.1	19.6±0.0	23.0±0.1	18.8±0.4	26.9±0.4
16:1	2.7±0.1	2.8±0.1	1.6±0.1	2.8±0.1	1.0±0.1
16:3	11.7±0.3	11.2±0.5	8.6±0.2	12.2±0.3	8.0±0.4
18:0	1.4±0.0	1.6±0.1	1.7±0.0	1.5±0.0	2.6±0.2
18:1	2.3±0.0	2.2±0.2	2.1±0.0	2.0±0.1	2.6±0.1
18:2	15.0±0.1	15.5±0.1	18.7±0.4	14.5±0.6	18.2±0.6
18:3	49.8±0.3	47.1±0.1	44.3±0.2	48.2±0.7	40.6±0.4
C_{16}/C_{18}	0.46	0.51	0.50	0.51	0.56
Fatty acid of roots (% of total)					
16:0	26.4±0.7	26.5±0.4	30.3±0.1	26.1±0.8	36.0±0.5
16:1	3.4±0.1	4.4±0.1	3.7±0.4	3.5±0.2	2.8±0.1
18:0	2.6±0.1	2.3±0.1	3.4±0.5	2.9±0.4	16.0±0.4
18:1	6.1±0.4	4.9±0.2	10.0±0.6	6.9±0.4	28.3±0.0
18:2	48.1±0.4	48.4±0.1	40.8±0.4	47.6±0.5	12.7±0.7
18:3	13.4±0.2	13.5±0.2	11.8±0.9	13.0±0.2	4.2±0.6
C_{16}/C_{18}	0.42	0.45	0.51	0.42	0.63

* Data shown are given as % of total fatty acids. Values are means±s.e. ($n = 6$).
† C, control; Cd5, Cd50, Cu5 and Cu50, metal species and its concentration (μM) in the medium.

Table 2. Lipid content (mg g^{-1} dry wt) of primary leaves and roots of tomato seedlings after 7 days exposure to two concentrations of cadmium or copper*

Treatments†	Glycolipids	Phospholipids	Neutral lipids	Total lipids
		Primary leaves		
C	25.13±0.46 (100)	15.86±0.27 (100)	6.12±0.09 (100)	47.12±0.83 (100)
Cd5	23.19±0.37 (92)	15.11±0.46 (95)	5.70±0.18 (93)	43.96±0.37 (93)
Cd50	17.91±0.09 (71)	12.79±0.54 (80)	4.20±0.63 (68)	35.19±0.36 (74)
Cu5	20.77±0.55 (82)	13.54±0.18 (85)	7.42±0.27 (120)	41.92±0.18 (89)
Cu50	11.05±0.53 (44)	10.34±0.71 (65)	3.03±0.53 (49)	24.61±0.62 (52)
		Roots		
C	2.89±0.24 (100)	14.46±0.12 (100)	6.38±0.48 (100)	23.74±0.72 (100)
Cd5	3.13±0.36 (108)	13.49±0.72 (93)	5.78±0.36 (90)	22.53±0.24 (95)
Cd50	2.36±0.11 (82)	7.67±1.06 (53)	2.95±0.82 (46)	13.09±0.82 (55)
Cu5	3.01±0.36 (104)	12.77±0.48 (88)	5.66±0.24 (89)	21.57±0.36 (91)
Cu50	1.19±0.11 (41)	3.82±0.71 (26)	1.79±0.95 (28)	6.78±0.35 (28)

* Data shown are means±s.e. ($n = 6$).
† C, control; Cd5, Cd50, Cu5 and Cu50, metal species and its concentration (μM) in the medium.
Values relative to control are given in parentheses.

in their relative proportions (Table 1). The contribution of 18:3 in the leaves was significantly higher (*ca* 50% of total fatty acids) and that of 16:0 and 18:2 were lower (*ca* 17 and 15% of total fatty acids, respectively), and vice versa in the root lipids. Also present were palmitoleic (16:1), hexadecatrienoic (16:3), stearic (18:0) and oleic (18:1) acids. Furthermore, the fatty acid composition of leaf lipids was also dominated by the presence of an appreciable content of 16:3, approximately 12% of the total fatty acids. This profile is generally characteristic of the so-called 16:3 plants and is in agreement with previous work on *Lycopersicon esculentum* [21, 22].

The amount of total lipids, which corresponds to the total fatty acids, was lower in treated plants than in control ones, and was remarkably dependent on metal doses as well as metal species. The results (Tables 1 and 2) indicate a decrease in leaf lipids from 7 to 26% and from 11 to 48% of the control with Cd and Cu treatments, respectively (Table 2). In roots, there was however a marked drop in total lipids and this ranged from 5 to 45% and from 9 to 72% of the control with both treatments (Table 2). The fatty acids most subject to variation were palmitic (16:0), linoleic (18:2) and linolenic (18:3) acids (Table 1). In leaves of plants treated with the high Cd and Cu doses, the percentage of 18:3, the abundant fatty acid, decreased and that of 16:0 and 18:2 increased. In addition, a

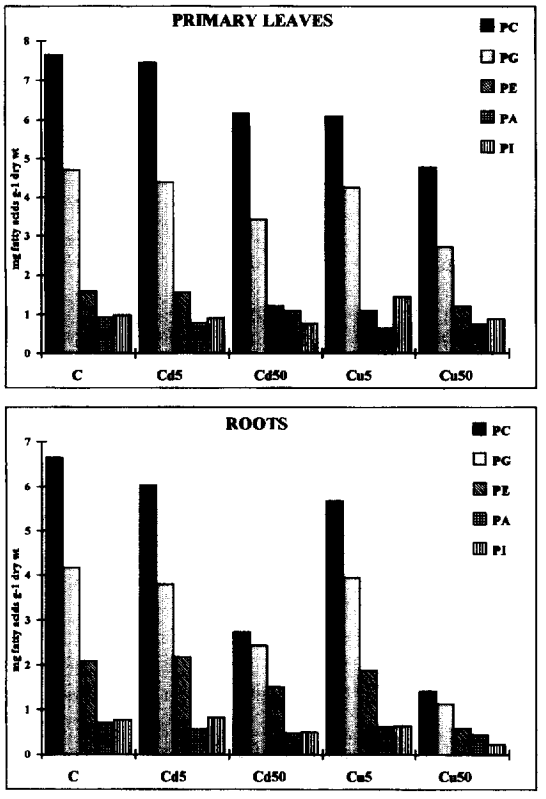

Fig. 4. Phospholipid classes of roots and primary leaves of tomato seedlings exposed for 7 days to two concentrations of cadmium or copper. C, control; Cd5, Cd50, Cu5 and Cu50, metal species and its concentration (μM) in the medium. PC, phosphatidylcholine; PG, phosphatidylglycerol, PE, phosphatidylethanolamine; PA, phosphatidic acid; PI, phosphatidylinositol.

lowering from 12 to 8% in 16:3 was recorded. However, in roots the remarkable impoverishment in both 18:2 and 18:3 was accompanied by enrichment in 16:0. It is noteworthy that the C_{16}/C_{18} ratio was increased in leaves and roots of treated tomato plants (Table 1). Also, it should be noted that in both organs, excess Cu has a strong effect on total lipid content and fatty acid composition.

The data in Table 2 reveal that the decline of total lipid content in treated seedlings was associated with a shift towards a low content of all lipid classes. Again, there were substantial differences in the effect of heavy metals on lipid classes isolated from leaves and roots of stressed plants. Indeed, the glycolipid content was more reduced in leaves (*ca* 29 % of control) than in roots (*ca* 18% of control) by toxic Cd-treatment (Table 2), and the MGDG/DGDG ratio changed from 1.35 in control leaves to 1.11 in treated ones, and from 0.63 to 0.75 in roots (Fig. 3). However, in stressed plants with the high Cu dose, the glycolipid content decreased to 56% of the control in leaves and to 59% in roots (Table 2). The MGDG/DGDG ratio was only reduced in leaves (*ca* 1.36 to 0.74), and, remarkably, remained unchanged in roots (Fig. 3).

Conversely, the decrease in phospholipid content by Cd and Cu treatments was more pronounced in roots than in leaves; and this resulted in a decline in the content of all phospholipid molecules including phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidic acid (PA), and phosphatidylinositol (PI) (Fig. 4). The variation in neutral lipid content was also similar to that of the phospholipids.

The changes in fatty acid composition due to heavy metal-stress differed between individual lipids as well as organs (Tables 3 and 4). In glycolipids of treated plants a decrease of 18:3 percentage was shown, except for DGDG and SL in leaf tissues, where the increase of 18:3 was accompanied by a decrease in the proportion of 16:0. Further, within MGDG of treated leaves the drop of 18:3 content results in an increase of 18:2 and 16:0 percentages and a decline in 16:3. However, in the MGDG of treated roots the 18:2 proportion was reduced. In neutral lipids, the increase in 16:0 percentage was accompanied with a diminution in 18:3 and 18:1 in leaves and in 18:3 and 18:2 in roots. The fatty acid composition of phospholipids was also changed by heavy metal-stress. Therefore, within both PC and PG of leaves the high Cd and Cu concentrations resulted in a decrease of 18:3 and 18:2 proportions with an increase in 16:0. But in PC and PG of stressed roots, the sharp decline of 18:3 and 18:2 percentages was coupled to an increase in almost all other fatty acids.

Although in lipid classes of leaves the changes of fatty acid composition were somewhat different to those of roots, our data generally indicate that toxic level of the heavy metals used leads to a decrease in content of unsaturated fatty acids, essentially in 18:3 and 18:2, and to an increase in that of saturated ones, particularly in 16:0. This shift in fatty acid composition resulted in a lowered degree of fatty acid unsaturation in lipid classes. In contrast to treatments with toxic metal levels, where the changes were clear, in plants treated with the low Cd or Cu dose the effects on lipid contents and fatty acid composition were apparently smaller. Again, it was evident from our results that Cu, in contrast to Cd, was a strong inducer of changes in both content and fatty acid composition of individual lipids.

DISCUSSION

Growth inhibition and reduction of biomass production are general responses of higher plants to heavy metal toxicity [20]. Similarly, in our study significant depression of leaf and root dry weight production in treated tomato plants was observed (Fig. 1), and this effect varied as a function of the nature and the concentration of the metal added to the nutrient solution. Inhibition of both cell elongation and division by heavy metals could explain, in part, the decline in biomass production [23]. The growth reduction observed at the high dose of Cd or Cu closely coincided with a considerable accumulation of these

Table 3. Fatty acid composition of glycolipids, neutral lipids and phospholipids in primary leaves of tomato seedlings after 7 days exposure to two concentrations of cadmium or copper*

Acyl lipids	Treatments†	Fatty acid composition (% of total) 16:0	16:1	16:3	18:0	18:1	18:2	18:3
MGDG	C	6.8±0.2	1.6±0.1	26.4±0.9	0.9±0.1	1.9±0.1	3.6±0.3	58.8±0.9
	Cd5	6.4±0.1	1.3±0.2	24.6±1.1	1.2±0.2	1.9±0.0	4.4±0.2	6.2±1.3
	Cd50	21.3±1.2	0.9±0.0	19.1±0.5	1.4±0.2	1.9±0.2	7.2±0.5	47.6±0.8
	Cu5	7.1±0.0	1.1±0.2	27.3±0.7	0.6±0.3	2.4±0.3	3.5±0.0	58.0±1.6
	Cu50	25.3±0.9	0.9±0.1	16.2±1.3	1.5±0.1	2.0±0.5	9.1±0.4	45.0±0.3
DGDG	C	31.5±1.1	0.6±0.0	2.6±0.6	4.8±0.2	3.4±0.7	8.4±1.2	48.7±0.5
	Cd5	30.3±1.7	0.6±0.2	2.8±0.8	4.8±0.4	3.5±0.6	8.9±0.3	49.1±1.2
	Cd50	20.1±0.6	0.9±0.1	2.5±0.2	4.9±0.3	4.2±0.2	7.5±0.8	59.9±0.8
	Cu5	32.1±0.5	0.7±0.2	2.0±0.0	4.2±0.1	3.7±0.1	8.7±0.5	48.6±1.5
	Cu50	15.7±1.16	2.0±0.5	3.1±0.5	5.9±0.8	5.2±0.3	12.4±1.1	55.7±0.7
SL	C	55.6±2.3	1.1±0.3	1.0±0.2	2.3±0.2	10.8±0.4	5.3±0.9	23.9±0.9
	Cd5	56.8±3.1	0.3±0.0	1.4±0.2	2.1±0.5	10.3±0.2	5.7±0.2	23.4±1.2
	Cd50	52.4±0.3	0.3±0.0	1.6±0.6	2.7±0.2	6.2±0.1	7.4±0.5	29.4±2.6
	Cu5	50.2±0.9	0.3±0.1	1.2±0.1	2.0±0.1	9.7±0.5	6.4±0.5	30.2±3.2
	Cu50	28.9±1.5	0.1±0.0	0.6±0.1	2.4±0.3	4.4±1.0	8.6±0.1	55.0±2.8
NL	C	27.5±0.5	10.3±2.5	4.8±0.5	7.6±0.8	12.0±1.1	11.1±0.2	26.7±1.5
	Cd5	27.7±0.3	10.0±0.6	4.2±0.6	7.5±0.5	11.8±1.0	11.3±0.6	27.5±2.1
	Cd50	35.9±1.6	12.6±0.2	4.1±0.1	5.3±0.1	8.4±0.2	12.4±0.9	21.3±0.3
	Cu5	28.4±1.3	10.1±2.3	4.5±0.4	7.2±0.4	12.6±0.0	11.5±1.5	25.7±2.5
	Cu50	42.5±1.5	13.5±0.7	4.9±0.7	4.9±0.3	5.4±0.3	11.8±1.8	17.0±3.1
PC	C	32.8±1.1	0.6±0.3	1.4±0.6	4.6±0.2	6.2±0.7	25.7±0.6	28.7±1.0
	Cd5	30.3±0.2	0.5±0.1	1.1±0.1	4.3±1.0	5.4±0.9	28.3±1.8	30.1±2.5
	Cd50	41.1±0.5	0.7±0.3	2.7±0.7	4.6±1.2	4.4±0.0	20.3±0.4	26.2±0.1
	Cu5	35.0±1.2	0.5±0.0	1.5±0.3	4.6±0.3	5.9±0.4	24.0±0.8	28.5±3.4
	Cu50	44.9±1.7	0.5±0.1	0.7±0.4	7.3±0.5	13.2±1.9	13.3±1.3	20.1±0.5
PG	C	29.6±1.1	26.8±3.6	0.7±0.2	4.8±0.6	7.8±0.3	11.7±0.2	18.6±0.8
	Cd5	29.7±0.8	26.2±0.5	1.0±0.1	4.6±0.4	8.0±0.5	11.3±0.6	19.2±1.5
	Cd50	47.6±0.2	20.9±0.2	—	4.2±0.8	9.8±0.1	9.0±0.7	8.5±1.4
	Cu5	28.9±1.8	27.1±0.0	1.3±0.6	4.6±0.7	7.4±1.1	11.6±1.0	19.1±2.0
	Cu50	57.1±2.2	13.3±1.2	—	8.4±1.1	10.7±0.6	8.1±0.5	2.4±0.3

* Data shown are means±s.e. ($n = 3$).

† C, control; Cd5, Cd50, Cu5 and Cu50, metal species and its concentration (μM) in the medium.

metals, especially in the roots (Fig. 2). The distribution profile of heavy metals reported here is in good agreement with the original reports [24, 25], demonstrating that metals are obviously immobilized to a far greater extent at the site of metal uptake. This is an important factor, since the differential accumulation of either Cd or Cu between leaves and roots (Fig. 2) might explain the lower damage on lipid metabolism in the aboveground parts of stressed tomato plants (Tables 1 and 2). On the other hand, the excess of absorbed metals in roots can be pumped largely into intracellular compartments [26, 27, Boussama, N., Ouariti, O. and Ghorbal, M. H., data not shown], resulting in a high metal concentration in the biochemically active compartments; so, this again confirms the marked damage recorded in the root systems.

Indeed, under heavy metal-stress we noted a significant decrease of lipid content and marked alterations of fatty acid composition in all lipid classes (Figs 3 and 4, and Tables 3 and 4). A greater decrease of MGDG content than that of DGDG was observed in metal stressed-tomato leaves, resulting in a decline of the MGDG/DGDG ratio, might be caused by a high galactolipase activity [28], which attacks preferentially MGDG. All those changes would mainly concern the photosynthetic apparatus, in which heavy metals, like Cd and Cu, could disturb the architecture of the thylakoidal membranes [4, 19]. Such disorganization in turn affects some light reaction processes, especially those associated with PS II activity [10]. Conversely, in metal-treated tomato roots, the MGDG/DGDG ratio was increased or unaffected by Cd or Cu, respectively; suggesting that the galactolipid rearrangements depended not only on the plant parts studied but also on the metal species.

The two other types of lipid investigated here, phos-

Table 4. Fatty acid composition of glycolipids, neutral lipids and phospholipids in roots of tomato seedlings after 7 days exposure to two concentrations of cadmium or copper*

Acyl lipids	Treatments†	Fatty acid composition (% of total) 16:0	16:1	18:0	18:1	18:2	18:3
MGDG	C	26.5±1.2	9.8±0.9	3.1±0.6	12.5±0.5	35.3±2.8	12.8±1.6
	Cd5	25.8±0.5	7.1±0.4	2.7±0.7	12.0±0.8	36.8±0.9	15.6±2.3
	Cd50	33.3±1.3	8.7±1.0	2.4±0.3	11.7±1.1	33.2±0.1	10.7±0.6
	Cu5	26.1±2.5	7.2±0.5	2.8±0.0	14.6±1.3	35.0±3.3	14.3±0.5
	Cu50	39.0±0.3	24.7±1.1	2.7±0.2	14.7±0.7	17.4±4.2	1.5±0.2
DGDG	C	22.9±2.2	6.9±1.4	5.4±0.8	11.7±2.1	38.5±3.6	14.6±0.4
	Cd5	23.1±0.6	6.7±0.2	4.9±0.5	12.0±1.3	38.2±2.9	15.1±1.1
	Cd50	30.4±0.5	8.4±0.5	5.9±0.6	15.4±2.2	30.5±0.6	9.4±0.6
	Cu5	22.4±0.1	7.0±1.2	5.1±1.1	12.3±0.5	38.8±0.1	14.3±2.3
	Cu50	32.1±1.1	14.5±0.8	9.0±0.3	16.7±0.3	25.0±1.8	2.7±0.1
NL	C	21.2±0.9	3.8±0.0	7.4±0.1	10.5±0.5	44.1±2.1	13.0±0.9
	Cd5	22.4±0.5	4.0±0.5	6.8±1.0	11.0±0.2	43.2±1.6	12.6±0.7
	Cd50	30.3±3.1	4.1±0.4	7.2±1.1	9.9±0.4	37.7±0.7	10.8±0.2
	Cu5	21.3±0.8	3.5±0.3	7.7±0.5	10.0±1.2	44.4±0.8	13.1±1.2
	Cu50	38.5±1.8	5.4±0.8	6.9±0.3	10.2±1.9	32.7±3.1	6.3±0.9
PC	C	25.3±1.5	6.3±0.6	2.7±0.1	5.5±0.9	53.8±0.7	6.4±0.5
	Cd5	24.0±0.8	5.9±0.3	3.4±0.6	6.1±0.2	53.9±3.1	6.7±0.9
	Cd50	31.4±0.2	16.3±1.5	2.8±0.0	16.1±0.8	29.2±0.9	4.2±0.2
	Cu5	27.7±1.7	6.2±1.0	2.0±0.1	8.3±0.9	49.7±3.7	6.1±0.0
	Cu50	44.5±0.5	20.8±2.3	3.6±0.6	15.7±2.6	15.0±1.6	0.4±0.1
PG	C	22.4±0.1	8.8±0.1	4.3±0.3	7.6±0.1	54.9±4.1	2.0±0.1
	Cd5	23.6±2.0	7.9±1.1	4.5±0.1	7.8±0.7	54.0±0.6	2.2±0.7
	Cd50	27.4±0.5	18.8±0.9	7.8±0.0	9.5±0.8	34.6±0.9	1.9±0.3
	Cu5	22.0±1.0	8.5±0.5	6.1±0.1	11.6±1.4	50.5±2.0	1.3±0.2
	Cu50	32.9±0.7	27.2±2.6	13.5±0.8	14.4±0.1	11.1±0.8	1.0±0.1

* Data shown are means ± s.e. ($n = 3$).

† C, control; Cd5, Cd50, Cu5 and Cu50, metal species and its concentration (μM) in the medium.

pholipids and neutral lipids, behaved similarly to the glycolipids. These components were much more affected in roots than in leaves of tomato plants treated with either Cd or Cu. The same tendency was reported in the roots of sensitive *Silene cucubalus* plants exposed to a toxic concentration of Cu [18]. So the decline in the polar lipid contents (glycolipids and phospholipids) could be associated with losses in intracellular membranes; which would indicate a decrease in the number and size of cellular organelles, such as chloroplasts [4].

A drastic decrease in the phospholipid levels of the root cell membranes will probably have profound effects on such functions as ATPase activity [29] and permeability [16]; and will be still more pronounced if the sterol level changes after heavy metal-stress [30]. Apparently, the quantitative and qualitative loss of lipids in treated tomato plants suggested that Cd and Cu can induce disturbance of the membrane lipid turnover. Both metals enhanced lipoxygenase activity [13, 31], which responsible for catalysing lipid peroxidation by using membrane lipid components as substrates, particularly unsaturated fatty acids. Likewise, the products of the lipoxygenase reaction; mainly peroxy, alkoxy and hydroxyl radicals, are themselves reactive and can result in further membrane lipid deterioration [16], and also affect other macromolecules in the cells [32]. Heavy metals are also involved in many ways in the production of activated oxygen species that actively induce peroxidation of membrane lipids [33]. Therefore, it is conceivable to suppose that a decrease of enzymic free radical scavengers [16, Ouariti, O., Boussama, N. and Ghorbal, M. H., unpublished results] caused by heavy metal-stress may also contribute to the shift in the balance of free radical metabolism towards accumulation, leading further to more breakdown of membrane lipids.

In view of the numerous reports on metal-mediated lipid peroxidation, there are many reasons to suppose that the decrease in unsaturated fatty acid levels, specifically those of 18:2 and 18:3 observed here in the lipid molecular species of treated plants, might be related to direct reaction of oxygen free radicals with unsaturated lipids. However, the decrease of polyunsaturated fatty acid contents does not exclude an inhibition of fatty acid desaturases by Cd or Cu. On the other hand, the observed accumulation of 16:0 rather

than 18:0 suggest an alteration of fatty acid synthase activity. Thus, the C_{16}/C_{18} ratio was modified (Table 1). A similar result has been seen for heavy metal effects on marine algae [34].

An exception was the fatty acid composition of DGDG and SL, where a distinct increase of 18:3 percentage and decrease in that of 16:0, were observed in leaves of metal treated-plants (Table 3). This response has also been found in thylakoid membranes isolated from leaves of excess Cd or Cu treated-plants [28, 19]. Likewise, the question of whether DGDG and SL behave differently to MGDG, regarding fatty acid composition, is worth further exploration.

It is worth mentioning that Cu is always more toxic than Cd. The latter is not a transition metal and hence will not directly generate damaging oxygen species, conversely Cu is the most powerful catalyst of free radical formation and Cu-ions themselves can initiate directly oxidative breakdown of polyunsaturated lipids [18]. According to this, it is evident that excess Cu results in an efficient reduction of lipid content as well as in strong changes in fatty acid composition, compared to excess Cd.

Summing up, we propose that the loss of membrane lipids in tomato plants treated with either Cd or Cu may be related to an enhanced rate of catabolism and/or to the suppression of lipid biosynthesis. Although our data do not exclude the latter hypothesis, the similarity of changes invoked in studies on lipid peroxidation [13, 18] and those obtained in our study favour the first one. Evidence that heavy metal-stress, with respect to lipid metabolism, may cause premature senescence of plant tissues [10], which is essentially a degradative process including the loss of membrane integrity, at least support this idea.

EXPERIMENTAL

Plant material and cultural conditions. Tomato seeds (*Lycopersicon esculentum* Mill cv. 63/5 F1) were washed in distilled H_2O and surface sterilized in 10% H_2O_2 for 20 min. The seeds were next thoroughly washed with distilled H_2O and germinated on moistened filter paper at 25° in the dark. Uniform seedlings were then transferred to plastic beakers (6 l capacity, six plants per beaker) filled with continuously aerated nutrient sols containing 3 mM KNO_3, 0.5 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 2.5 mM KH_2PO_4, 2 mM NH_4Cl, 100 μM Fe-K-EDTA, 30 μM H_3BO_3, 5 μM $MnSO_4$, 1 μM $CuSO_4$, 1 μM $ZnSO_4$, and 1 μM $(NH_4)_6Mo_7O_{24}$. The seedlings grown on this medium constituted the control. After an initial growth period of 10 days, $CdCl_2$ and $CuSO_4$ were separately added to the medium at final concns of 5 and 50 μM. Plants were grown and treated in a growth chamber (26°/70% relative humidity during the day, 20°/90% during the night). A 16 hr (daily) photoperiod was used with a light irradiance of 150 μmol m^{-2} s^{-1} at the canopy level. After 7 days of heavy metal-treatment, the roots and the two primary leaves were harvested and used for chemical analyses.

Cadmium and copper analysis. Cd and Cu contents in primary leaves and roots were analysed by digestion of dried plant material in concd HNO_3–$HClO_4$ (3:1). Metal-ion concns were determined by atomic absorption spectrophotometry.

Lipid analysis. Extraction of total lipids was performed using $CHCl_3$–MeOH–H_2O (1:1:1) [35]. Lipids classes were sepd on silica gel TLC plates 60 (Merck) using the following solvent systems: $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (10:4:2:2:1) [36] and petrol–Et_2O–HOAc (70:30:0.4) [37]. Lipid spots were located by brief exposure to I_2 vapour. Lipid classes were identified by comparison with lipid standards and by specific stains for phospholipids and galactolipids. Fatty acids from total lipids and lipid classes were transmethylated with MeOH–BF_3 [38]. FAMES were analysed by FID-GC on a metal column (1.8 m × 3 mm i.d.) filled with GP 3% SP-2310/2% SP-2300. The column was maintained isothermally at 190°. Carrier gas was N_2 (20 ml min^{-1}), and the operating temp. in the injector and detector were respectively, 210° and 240°. For measuring the amounts of fatty acids, 17:0 was added as int. standard. Calculation of fatty acid quantities was obtained using an integrator.

Acknowledgements—We are grateful to Dr D. Ben Miled (INRST) and Dr S. Rejeb (CRGR) for their help in analytical techniques.

REFERENCES

1. Flemming, C. A. and Trevors, J. T., *Water, Air and Soil Pollution*, 1989, **44**, 143.
2. Wagner, G. J., *Advances in Agronomy*, 1993, **51**, 173.
3. Greger, M. and Lindberg, S., *Physiologia Plantarum*, 1987, **69**, 81.
4. Ouzounidou, G., Eleftheriou, E. P. and Karataglis, S., *Canadian Journal of Botany*, 1992, **70**, 947.
5. Poschenrieder, C., Gunse, G. and Barcelo, J., *Plant Physiology*, 1989, **90**, 1365.
6. Costa, G., Michaut, J. C. and Morel, J. L. *Plant Physiology and Biochemistry*, 1994, **32**, 105.
7. Lidon, F. C. and Henriques, F. S., *Journal of Plant Physiology*, 1991, **138**, 115.
8. Atal, N., Saradhi, P. P. and Mohanty, P. *Plant Cell Physiology*, 1991, **32**, 943.
9. Krupa, Z., Öquist, G. and Humer, N. P. A., *Physiologia Plantarum*, 1993, **88**, 626.
10. Baron, M., Arellano, J. B. and Gorgé, J. L. *Physiologia Plantarum*, 1995, **94**, 174.
11. Nussbaum, S., Shmutz, D. and Brunold, C., *Plant Physiology*, 1988, **88**, 1407.
12. Van Assche, F. and Clijsters, H., *Plant Cell Environment*, 1990, **13**, 195.
13. Somashekaraiah, B. V., Padmaja, K. and Prasad, A. R. K., *Physiologia Plantarum*, 1992, **85**, 85.

14. Shah, K. and Dubey, R. S., *Plant Physiology and Biochemistry*, 1995, **33**, 577.
15. Doncheva, S., Nikolov, B. and Ogneva, V., *Physiologia Plantarum*, 1996, **96**, 118.
16. De Vos, C. H. R. and Schat, H., in *Ecological Responses to Environmental Stresses*, ed. J. Rozema and J. A. C. Verkleij. Kluwer, The Netherlands, 1991, p. 22.
17. Meharg, A. A., *Physiologia Plantarum*, 1993, **88**, 191.
18. De Vos, C. H. R., Ten Boukum, W. M., Vooijs, R. Schat, H. and De Kok, L. J., *Plant Physiology and Biochemistry*, 1993, **31**, 151.
19. Maksymiec, W., Russa, R., Urbanik-Sypniewska, T. and Baszynski, T., *Journal of Plant Physiology*, 1992, **140**, 52.
20. Woolhouse, H. W., in *Encyclopedia of Plant Physiology*, Vol. 12C. Physiological Plant Ecology II, ed. O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler. Springer, Berlin, 1983, p. 245.
21. Douce, R. and Joyard, J., in *The Biochemistry of Plants*, Vol. 4. Lipids: Structure and Function, ed. P. K. Stumpf. Academic Press, New York, 1980, p. 321.
22. Whitaker, B. D., *Planta*, 1986, **169**, 313.
23. Arduini, I., Godbold, D. L. and Onnis, A., *Physiologia Plantarum*, 1994, **92**, 675.
24. Jarvis, S. C., Jones, L. H. P. and Hopper, M. J., *Plant Soil*, 1976, **44**, 179.
25. Hardiman, R. T., Jacoby, B. and Banin, A., *Plant Soil*, 1984, **81**, 17.
26. Lidon, F. C. and Henriques, F. S., *Australian Journal of Plant Physiology*, 1994, **21**, 427.
27. Vazquez, M. D., Poschenrieder, C. and Barcelo, J., *New Phytologist*, 1992, **120**, 215.
28. Skorzynska, E., Urbanika-Sypniewska, T., Russa, R. and Baszynski, T., *Journal of Plant Physiology*, 1991, **138**, 454.
29. Kennedy, C. D. and Gonsalves, F. A. N., *Plant Soil*, 1989, **117**, 167.
30. Ros, R., Cooke, D. T., Martinez-Cortina, C. and Picazo, I., *Journal of Experimental Botany*, 1992, **43**, 1475.
31. Clijsters, H., Van Assche, F. and Gora, L., in *Ecological Responses to Environmental Stresses*, ed. J. Rozema and J. A. C. Verkleij. Kluwer, The Netherlands, 1991, p. 22.
32. Stadman, E. R., *Annual Review of Biochemistry*, 1993, **62**, 797.
33. Halliwel, B. and Gutteridge, J. M., *Biochemistry Journal*, 1984, **219**, 1.
34. Smith, K. L., Bryan, G. W. and Harwood, J. L., *Journal of Experimental Botany*, 1985, **36**, 663.
35. Bligh, E. G. and Dyer, W. J., *Canadian Journal of Biochemistry and Physiology*, 1959, **37**, 911.
36. Trémolières, A. and Lepage, M., *Plant Physiology*, 1971, **47**, 329.
37. Mangold, H. K., *Journal of the American Oil Chemistry Society*, 1964, **41**, 762.
38. Metcalfe, D., Schmitz, A. A. and Pelka, J. R., *Analytical Chemistry*, 1966, **38**, 524.