

# The effect of lead on the phytochemistry of *Tithonia diversifolia* exposed to roadside automotive pollution or grown in pots of Pb-supplemented soil

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*Tithonia diversifolia*, a ruderal weedy species utilized in agroforestry, is widely distributed in the tropics, often being found in wasteland, margins of polluted city rivers and roadsides. The objective of this study was to compare plants sampled at a light and heavy traffic roadside for metal concentration and foliar compounds which may be related to senescence and/or detoxification of metals. The field survey was followed by an experiment in pots of Pb-acetate-supplemented soil to verify if the same response is obtained under controlled conditions with regard to Pb. The concentration of Pb in leaves and roots of plants sampled at a heavy traffic roadside was higher than in samples from a light traffic site. The content of Cr, Al, Fe, Cu and Ni was also higher in leaves but not of roots in plants from the polluted site. No significant differences were found in the concentrations of chlorophyll, carotenoids, UV-absorbing compounds and proteins, nor in the chlorophyll *a/b* ratio and lipid peroxidation of leaves collected at both sites. On the other hand, Pb-contaminated leaves and roots showed higher acid phosphatase activity while the foliar nitrate reductase activity and specific leaf mass were lower. An increase of leaf phenol concentration, thought to be involved in the detoxification of metals, was observed in the polluted field plants and in roots in the pot experiment, where Pb-acetate was supplied to the soil, but the changes observed in acid phosphatase and specific leaf mass in the field were not found in the pot experiment.

**Key words:** Asteraceae, metals, Mexican sunflower, phenols, pollution, weeds.

**Efeito do chumbo na fitoquímica de *Tithonia diversifolia* exposta à poluição de rodovia ou crescidas em solo suplementado com chumbo:** *Tithonia diversifolia*, uma espécie ruderal utilizada em sistemas agroflorestais, amplamente distribuída nos trópicos, é freqüentemente encontrada em locais de descarte, margens de rios poluídos próximos a cidades e ao longo de rodovias. O objetivo deste estudo foi comparar plantas coletadas de rodovias com pouco e com intenso tráfego em relação à concentração de metais e compostos foliares que podem estar relacionados à senescência e/ou à destoxificação de metais. Também fez-se um experimento com vasos contendo solo suplementado com chumbo para verificar se a mesma resposta é obtida sob condições controladas usando esse metal. A concentração de Pb nas folhas e raízes de plantas amostradas em rodovia de tráfego intenso foi maior que nas amostras de rodovia de pouco tráfego. As concentrações de Cr, Al, Fe, Cu e Ni foram maiores nas folhas, mas não nas raízes de plantas da rodovia de tráfego intenso. Não houve diferença significativa nos conteúdos de clorofila, carotenóides, compostos absorvendo no UV e proteínas, como também na razão clorofilas *a/b* e peroxidação de lipídeos das folhas coletadas em ambas as rodovias. Por outro lado, folhas de plantas contaminadas com Pb mostraram maior atividade de fosfatase ácida, enquanto a atividade foliar da redutase do nitrato e a massa foliar específica foram menores. Aumento na concentração de fenóis, o que se pensa estar envolvido na destoxificação de metais, foi observado nas plantas coletadas na rodovia de tráfego pesado e nas raízes do experimento com vasos, no qual Pb foi adicionado ao solo. Todavia, as alterações de fosfatase ácida e massa foliar específica não foram observadas nesse experimento.

**Palavras-chave:** Asteraceae, fenóis, girassol mexicano, metais, plantas daninhas, poluição.

## INTRODUCTION

Mexican sunflower, *Tithonia diversifolia* is widely distributed in the tropics, from Mexico to Brazil, and is also found in Asia and Africa, where it has been introduced in view of its multiple uses, for example, as animal forage, green fertilizer, a natural insecticide, a honey-plant and an ornamental shrub (Rios, 1999). The species enhances the availability of P to crops, which led to its recommendation for biomass transfer technologies in Kenya (George et al., 2002 a, b). However, it behaves as an aggressive weed with high invasive capacity in Africa (Ayeni et al., 1997). This species, adapted to a variety of ecological zones, is present in Venezuela, from montane environments in the Coastal and Andean ranges to the savanna areas, and is very common in wasteland, margins of polluted city rivers and roadsides. Studies of biochemical responses to environmental pollution of *T. diversifolia* are important, since this species is recommended in agroforestry for animal production in the tropics and is also a weed capable of inhabiting polluted sites.

García-Miragaya et al. (1981) reported strong Pb pollution in roadside soils at Caracas, Venezuela, due to heavy traffic of motor vehicles and the utilization of leaded gasoline. At present, only motor vehicles bought after the year 2000 use unleaded gasoline in Venezuela and consequently the same or higher levels of Pb contamination prevail.

Lagerwerff and Specht (1970) reported that the concentrations of Cd, Ni, Pb and Zn in roadside soil and grass samples from several locations decreased with the distance from vehicular traffic. Contamination was related to the composition of gasoline, motor oil and automobile tires, and to roadside deposition of residues of these materials. Contamination from motor vehicle emissions within 100 m of the roadway has been reported in vegetation (Motto et al., 1970; Ward et al., 1975), soils (Yassoglou et al., 1987), and spider webs (Hose et al., 2002). Lead added to fuel as tetra ethyl, to act as a lubricant during combustion, is discharged into the environment in exhaust fumes largely as minute particles of inorganic Pb compounds and about 50 % of this falls within a region of 100 m from the road (Mengel and Kirkby, 2001).

In the year 2000, the cumulative industrial age anthropogenic global production of Pb was 235 million tonnes (Han et al., 2002). According to Weiss et al. (1999) since the industrial revolution and until 1950, the main sources of Pb in the biosphere have been industrial activity mostly coal combustion and ferrous and non-ferrous smelting, as well as open waste incineration, whereas from 1950 onwards it has been the use of leaded gasoline. The largest Pb emissions into the

atmosphere throughout Europe took place between 1950 and 1980 due to automotive exhausts. The marked drop in atmospheric Pb fluxes, recorded since the 1980s, has been attributed to the phasing out of leaded gasoline.

Diverse biochemical changes in green plants in response to Pb have been reported by several authors: decreases in chlorophyll content (Ewais, 1997; Xiong, 1997; Kastori et al., 1998; Fargašová, 2001), carotenoids (Fargašová, 2001), proteins (Kevresan et al., 2001), nitrate reductase activity (Singh et al., 1997; Kevresan et al., 2001), lamina and mesophyll thickness, epidermal cell size and diameter of vessels (Kovačević et al., 1999); increases in chlorophyll *a/b* ratio (Fargašová, 2001) and concentration of phenols (Lummerzheim et al., 1995; Lavid et al., 2001 a, b). In the presence of other metals, such as Al, Cu and Cd, lipid peroxidation has been found to increase (Cakmark and Horst, 1991; Gallego et al., 1999; Pereira et al., 1999; Baryla et al., 2000; Hartley-Whitaker et al., 2001; Sandalio et al., 2001; Shah et al., 2001).

Thus, in metal contaminated plants we expect to find senescence symptoms, such as lower concentrations of chlorophyll, carotenoids and proteins, diminished nitrate reductase activity and increased acid phosphatase activity, lipid peroxidation and chlorophyll *a/b* ratio compared to plants not stressed by metals. We also expect detoxification through phenols.

The objective of the present study was to evaluate metals in foliar and root material from field samples of *T. diversifolia*, collected at two sampling sites with contrasting pollution levels due to automotive traffic, as well as evaluate foliar compounds sensitive to phytotoxic concentrations of metals. The field study was followed by an experiment using pots of Pb-acetate-supplemented soil to learn whether the parameters affected in the field also change when the plants are exposed to the same air and soil, but to a different concentration of Pb in the soil. This experiment was carried out in order to verify whether the parameters affected in the field were responding to Pb.

## MATERIALS AND METHODS

*Plant material and sites:* *Tithonia diversifolia* (Hemsl.) Gray (Asteraceae) was sampled in two agricultural fields near satellite towns of Caracas, Venezuela, where the plant grows wild on roadsides. The control site (Site 1) is located at 10° 21' N, 66° 56' W, in a rural area (El Amarillo) close to the town of San Antonio, while the polluted site (Site 2) is located at 10° 22' N, 67° 05' W, where automotive traffic is

heavy because it is close to the entrance of the town of San Pedro. Soils from both sites presented a sandy clay loam texture, a pH (KCl) between 5 at Site 1 to 6 at Site 2, together with large concentrations of Fe ( $36 \text{ mg.g}^{-1}$ ) and Al ( $17 \text{ mg.g}^{-1}$ ) in similar concentrations at both sites (Olivares et al., 2002a).

At each site, 15 whole plants of approximately 2 m height were collected. The plants were transported to the laboratory with their roots immersed in tap water. In the present study, *T. diversifolia* leaves and roots were washed before analysis, since a previous study conducted on plants exposed to highway dust showed that unwashed plant tissue had a higher Pb content than those analyzed after a 15 min wash with distilled water, indicating that 27 % of the Pb occurred on the surface of the leaves (Isermann, 1977).

*Analysis of mineral elements:* The leaves and roots were dried in a ventilated oven, for approximately 78 h at  $60^\circ\text{C}$  to constant weight and then ground. Total nitrogen concentration was determined by the Kjeldhal method (Tecator Kjeltec Systems from Foss Tecator, Höganäs, Sweden) after digestion with sulphuric acid of the ground dry plant material.

Phosphorus was measured colorimetrically (Murphy and Riley, 1962) in the digested material by means of an UV/visible spectrophotometer (Ultrospec 2000, Amersham Pharmacia, Cambridge, England).

For the measurement of metals (K, Ca, Mg, Mn, Cd, Co, Pb, Cr, Al, Fe, Cu and Ni) samples were digested in a nitric-perchloric acid mixture (Miller, 1998) and analysed with an atomic absorption spectrometer (SpectrAA 55B, Varian Techtron, Victoria, Australia).

*Enzymatic activities:* Nitrate reductase activity (NRA) was determined in fresh tissue of leaves and roots from each site by measuring the amount of nitrite produced from nitrate; nitrite was estimated by formation of the diazonium salt after reaction of nitrite with sulphanilamide in acid medium (Lindblad and Guerrero, 1993). Acid phosphatase activity (APA) in fresh tissue of leaves and roots was determined according to Tabatabai and Bremner (1969). Activities were expressed on a dry mass basis, using the fresh mass/dry mass ratio of leaf discs taken from the same leaves used for enzyme activity determinations.

*Organic compounds affected by metals and leaf specific mass:* For determinations of chlorophyll and carotenoids, fresh tissue of leaves was ground in a mortar with cold 80 % aqueous

acetone. The concentration of chlorophyll *a+b* and the total concentration of carotenoids were calculated according to Lichtenthaler and Wellburn (1983). The amount of UV-B-absorbing compounds was estimated in fresh leaf tissue by measuring the absorbance at 300 nm in acidified methanol solutions (Day and Demchik, 1996). Total phenols were measured colorimetrically in ethanol extracts of ground dried leaves, using the Folin-Ciocalteu method (Amorim et al., 1977); values were standardized against chlorogenic acid. Lipid peroxidation in fresh tissue of leaves was measured by the formation of a 2-thiobarbituric acid (TBA)-malondialdehyde (MDA) complex (van Hasselt et al., 1996). Water-soluble proteins were determined according to Bradford (1976).

Specific leaf mass (SLM) was calculated from the dry mass expressed per unit area using four leaf discs ( $1.41 \text{ cm}^2$  diameter) dried in a microwave oven for approximately 15 min to constant weight. SLM corresponds to the inverse of specific leaf area (SLA) or ratio of leaf area to leaf dry mass, dimension quotients were introduced by Stocker in 1931 and Müller-Stoll in 1947 (Medina et al., 1990).

*Pot experiment with Pb-supplemented soil:* Twenty young plants were selected in the field according to their uniformity in size (0.5 m height) and were transplanted with their native soil to pots (2 kg soil capacity) and kept in the grounds of the “Instituto Venezolano de Investigaciones Científicas” (I.V.I.C.), a site with low traffic and low Pb ( $0.41 \mu\text{g.m}^{-3}$ ) in the air (Escalona and Sanhueza, 1981). The soil also presented a low Pb concentration (42 to  $64 \text{ mg.kg}^{-1}$ ). The soil was described in Olivares et al. (2002 b). The pots were supplemented with solutions of analytical grade Pb-acetate at 21 and 29 days after transplantation ( $1 \text{ g.kg}^{-1}$  soil at each time). During the 3 weeks without Pb, the plants become adapted to the pot condition. Lead was added in two progressive sessions in order to increase the probability of Pb incorporation through roots. Pb-acetate was used by Ewais (1997), Xiong (1997) and Singh et al. (1997/98). A gradient was used by Xiong (1997): 0, 0.8, 1.6 and  $3.2 \text{ g.kg}^{-1}$  Pb. Based on this gradient two treatments were selected, 0 and  $2 \text{ g.kg}^{-1}$  soil. The plants were watered daily and care was taken that no water was lost from the dishes under the pots. The plants were maintained under natural conditions of light and air temperature and humidity. The maximal radiation in the greenhouse as reported by Suarez (2000) was  $1550 \pm 370 \text{ mmol.m}^{-2}.\text{s}^{-1}$ , with daily temperatures of  $30.5 \pm 3.4^\circ\text{C}$  during the day and  $16.3 \pm 2.0^\circ\text{C}$  during the night and with a relative humidity from 50 to 78 %.

After 34 days of the first metal application measurements were made of the concentration of phenols, APA of leaves and roots and SLM (variables that suffer changes in response to Pb in the field plants) using 5 plants selected randomly from the 10 plants in each treatment.

*Statistical analysis:* Student's t-test was used for mean comparisons using the software Sigma plot 2001 (V7.0). Regression analysis was used for the APA and Pb data. Two-way ANOVA was carried out to compare the total concentration of mineral elements and enzymatic activities in *T. diversifolia* grown in the field at two sites (with light and heavy automotive traffic) and between two organs (leaves and roots) using the software Statistica 6.0 (Statsoft Inc.). The same analysis was done to compare levels of Pb, phenols, APA and SLM in plants grown in pots between two treatments (with or without Pb-acetate) and two organs. One-way ANOVA was carried out to compare biochemical parameters and SLM in leaves with different foliar Pb content from plants grown in the field.

## RESULTS

*Analysis of mineral elements in leaves and roots:* In leaves, the content of heavy metals (Pb, Cr, Al, Fe, Cu and Ni) was higher in samples from the polluted Site 2 than in those from the control Site 1. The Pb concentration was found to be 15 times higher for Site 2 than Site 1 (table 1). In roots, higher values were also found for Pb at Site 2, while the other metals occurred in similar or lower amounts in samples from Site 2 compared with Site 1. The metals Co and Cd were not detected, the detection limits being  $0.05 \mu\text{g}\cdot\text{g}^{-1}$  for Co and  $0.02 \mu\text{g}\cdot\text{g}^{-1}$  for Cd.

Roots and leaves from Site 2 showed higher concentrations of P, K and Ca than those from the less polluted Site 1 (table 1). The same relationship between sites held for Mn in leaves, and for Zn, N and Mg in roots. The ANOVA showed differences between sites in the mineral elements except for Fe, Mn and N, and between organs except for K.

*Enzymatic activities:* The NRA was lower in leaves from Site 2 as compared with those from Site 1 but was similar in roots from both sites (table 1). APA was higher in both leaves and roots of the more polluted Site 2, where higher values of P were also found. The following regressions were found between APA and P concentrations:  $y = 0.11x + 1.84$ ,  $r^2 = 0.60$  (roots and leaves);  $y = 0.07x + 0.28$ ,  $r^2 = 0.73$  (leaves); and  $y$

$= 0.07x - 0.03$ ,  $r^2 = 0.57$  (roots). The ANOVA showed significant differences between sites and organs.

*Organic compounds affected by metals and leaf specific mass:* Chlorophyll, chlorophyll *a/b* ratio, carotenoids, compounds absorbing at 300 nm such as flavonoids and proteins, lipid peroxidation and water-soluble proteins were similar at both sites, while the concentration of phenols was higher and SLM was lower for Site 2 (table 2). The t-test and one way ANOVA showed similar results.

*Pot experiment with Pb-supplemented soil:* Young plants transplanted to pots with Pb-acetate in the soil showed a highly significant increase in phenol concentration in roots compared to the controls without Pb-acetate (table 3). The concentration of lead in roots was very high and higher than in leaves, whereas in the field, where the main source of Pb would be from the emissions of motor vehicles, a higher concentration was observed in leaves than in roots. For the plants from the greenhouse experiment there were no significant differences in SLM and the APA did not increase in Pb-contaminated plants in contrast to the field plants, where these responses may depend on multiple factors rather than Pb alone. However, a very highly significant increase of phenols was observed in Pb-contaminated roots.

The ANOVA showed differences between treatments and organs for Pb. The difference in phenol concentration in roots was highly significant for both treatments but not in leaves. The two-way ANOVA therefore did not show differences between treatments because it includes both organs.

## DISCUSSION

*Analysis of mineral elements in leaves and roots:* The concentration of Pb in leaves from heavy traffic roadside sites found in this study is higher than those reported by Ross (1994) as typical values for Pb-contaminated plants (30-300  $\mu\text{g}\cdot\text{g}^{-1}$ ). Mengel and Kikby (2001) reported values of  $50 \mu\text{g}\cdot\text{g}^{-1}$  Pb in vegetation at the side of the road in motorway areas, with roots being very little affected, but only 2 to 3  $\mu\text{g}\cdot\text{g}^{-1}$  Pb at a distance of 150 m from the motorway.

The concentrations of Cr, Cu and Ni in leaves from polluted Site 2 were at the lower limit of the range typical of contaminated plants, whereas at Site 1 leaves showed concentrations of Cr, Cu and Ni below this range.

*T. diversifolia* from Site 2 had a higher concentration of Pb in leaves than in roots. This may be explained by the fact that there are two major pathways for the incorporation of

**Table 1.** Mineral concentrations, nitrate reductase activity (NRA) and acid phosphatase activity (APA) in leaves and roots of *T. diversifolia* from two sites: with light (site 1) or heavy (site 2) automotive traffic.

Mineral element	Leaves <sup>a</sup>			Roots			ANOVA	Factor 1: Site	Factor 2: Organ	Interaction Factor 1 x 2
	Site 1	Site 2	t-test	Site 1	Site 2	t-test				
Pb ( $\mu\text{g.g}^{-1}$ )	7.79 (0.81)	120.79 (9.30)	***	3.40 (0.52)	34.94 (5.58)	***	F p significance	9.0093 0.0049 **	13.2330 0.0009 ***	36.3822 0.0000 ***
Cr ( $\mu\text{g.g}^{-1}$ )	0.20 (0.20)	9.39 (1.33)	***	9.79 (1.56)	7.19 (0.80)	ns	F p significance	8.8428 0.0052 **	11.1659 0.0020 **	28.3899 0.0000 ***
Al ( $\mu\text{g.g}^{-1}$ )	561.20 (43.48)	2484.50 (141.26)	***	2619.95 (352.62)	1974.78 (187.45)	ns	F p significance	9.0093 0.0049 **	13.2330 0.0009 ***	36.3822 0.0000 ***
Fe ( $\mu\text{g.g}^{-1}$ )	561.63 (56.85)	1949.60 (133.01)	***	3360.07 (384.10)	1226.91 (95.74)	***	F p significance	3.1264 0.0855 ns	24.2579 0.0000 ***	69.8020 0.0000 ***
Cu ( $\mu\text{g.g}^{-1}$ )	6.59 (0.73)	19.37 (0.67)	***	19.58 (1.76)	13.98 (1.23)	*	F p significance	9.1993 0.0045 **	10.3526 0.0027 **	60.5216 0.0000 ***
Ni ( $\mu\text{g.g}^{-1}$ )	1.20 (0.44)	10.20 (0.76)	***	11.20 (0.74)	7.20 (0.61)	***	F p significance	14.7638 0.0005 ***	28.9370 0.0000 ***	99.8031 0.0000 ***
Mn ( $\mu\text{g.g}^{-1}$ )	49.20 (4.16)	62.10 (4.01)	*	36.98 (5.39)	20.17 (1.94)	**	F p significance	0.2277 0.6363 ns	43.6736 0.0000 ***	13.1470 0.0009 ***
Zn ( $\mu\text{g.g}^{-1}$ )	107.05 (5.29)	116.39 (4.45)	ns	51.55 (2.28)	83.44 (3.48)	***	F p significance	26.1164 0.0000 ***	120.2599 0.0000 ***	7.8123 0.0083 **
N ( $\text{mg.g}^{-1}$ )	38.31 (1.09)	38.55 (2.09)	ns	8.45 (0.39)	13.89 (0.88)	***	F p significance	2.8241 0.0986 ns	260.5296 0.0000 ***	2.3641 0.1300 ns
P ( $\text{mg.g}^{-1}$ )	2.33 (0.15)	6.38 (0.39)	***	1.41 (0.10)	4.06 (0.23)	***	F p significance	5.4341 0.0255 *	81.2202 0.0000 ***	34.6945 0.0000 ***
K ( $\text{mg.g}^{-1}$ )	5.13 (0.34)	9.02 (0.86)	***	3.86 (0.17)	8.01 (0.61)	***	F p significance	51.2518 0.0000 ***	4.0841 0.0508 ns	0.0507 0.8231 ns
Ca ( $\text{mg.g}^{-1}$ )	22.20 (0.67)	28.11 (1.57)	*	3.67 (0.23)	6.33 (0.35)	***	F p significance	14.4877 0.0005 ***	715.2939 0.0000 ***	0.9961 0.3249 ns
Mg ( $\text{mg.g}^{-1}$ )	6.83 (0.40)	4.46 (0.30)	***	2.53 (0.24)	3.56 (0.15)	**	F p significance	5.4341 0.0255 *	81.2202 0.0000 ***	34.6945 0.0000 ***
Enzymatic activities										
NRA ( $\text{mmol.g}^{-1}.\text{h}^{-1}$ )	2.50 (0.54)	1.03 (0.42)	*	0.30 (0.11)	0.53 (0.16)	ns	F p significance	8.8428 0.0052 **	11.1659 0.0020 **	28.3899 0.0000 ***
APA ( $\mu\text{mol.g}^{-1}.\text{min}^{-1}$ )	0.44 (0.04)	0.77 (0.06)	**	0.07 (0.02)	0.24 (0.06)	*	F p significance	27.4349 0.0001 ***	89.5080 0.0000 ***	2.5823 0.1276 ns

<sup>a</sup>Mean (SE) (n = 5) expressed by dry mass. Statistically significant differences (t-test and two way ANOVA) between both sites and organs are denoted as: ns = not significant, \*p<0.5, \*\*p<0.1, \*\*\* p<0.01.

**Table 2.** Chlorophyll content, chlorophyll *a/b* ratio, carotenoids, UV-B absorbing compounds (flavonoids), total phenols, lipid peroxidation measured by the formation of a malondialdehyde (MDA)-2-thiobarbituric acid (TBA) complex, water-soluble proteins and specific leaf mass (SLM) in leaves of *T. diversifolia* from two sites: with light (1) or heavy (2) automotive traffic, with different foliar Pb concentrations ( $7.79 \pm 0.81$  and  $121.79 \pm 9.30$  respectively, see Table 1).

Parameter	Site 1 <sup>a</sup>	Site 2	Statistics t-test	ANOVA F	p	significance
Chlorophyll (mg.g <sup>-1</sup> )	9.32 (1.18)	9.11 (0.66)	ns	0.0230	0.8832	ns
Chl <i>a/b</i>	3.58 (0.09)	3.40 (0.08)	ns	2.0422	0.1908	ns
Carotenoids (mg.g <sup>-1</sup> )	1.82 (0.14)	1.68 (0.24)	ns	0.2659	0.6200	ns
UV-B Absorb. compounds ( $\Delta A_{300} \cdot g^{-1}$ )	233.48 (27.84)	280.61 (48.40)	ns	0.7126	0.4231	ns
Phenols (mg.g <sup>-1</sup> )	6.72 (0.63)	8.82 (0.59)	*	5.8766	0.0416	*
MDA-TBA complex (nmol.g <sup>-1</sup> )	203.37 (23.30)	187.57 (9.68)	ns	0.3918	0.5488	ns
Water sol. proteins ( $\mu g \cdot g^{-1}$ )	431.30 (43.60)	491.27 (29.08)	ns	1.3099	0.2855	ns
SLM (mg.cm <sup>-2</sup> )	5.23 (0.23)	3.05 (0.10)	***	77.6470	0.0000	***

<sup>a</sup>Mean (SE) (n = 5) expressed by dry mass. Statistically significant differences (t-test and one way ANOVA) are denoted as: ns = not significant, \*p<0.5, \*\*\* p<0.01.

**Table 3.** Specific leaf mass (SLM) and lead content, total phenols and acid phosphatase activity (APA) in leaves and roots of *T. diversifolia* grown in pots without (-Pb) or with 2 g kg<sup>-1</sup> Pb-acetate (+Pb) after 34 days of the Pb application.

Parameter	Leaves <sup>a</sup>			Roots			ANOVA	Factor 1: Treatment	Factor 2: Organ	Interaction Factor 1 x 2
	-Pb	+ Pb	t-test	-Pb	+ Pb	t-test				
Pb ( $\mu g \cdot g^{-1}$ )	1.43 (0.36)	19.48 (6.26)	**	38.37 (4.52)	2376.73 (282.07)	***	F p significance	61.9961 0.0000 ***	63.9998 0.0000 ***	60.1108 0.0000 ***
Phenols (mg.g <sup>-1</sup> )	15.20 (1.28)	15.10 (1.53)	ns	4.64 (0.43)	9.18 (0.54)	***	F p significance	4.4318 0.0514 ns	61.2956 0.0000 ***	4.8539 0.0426 *
APA ( $\mu mol \cdot g^{-1} \cdot min^{-1}$ )	0.87 (0.02)	0.75 (0.06)	*	0.63 (0.04)	0.68 (0.02)	ns	F p significance	0.8650 0.3662 ns	16.5802 0.0009 ***	4.3073 0.0544 ns
SLM (mg.cm <sup>-2</sup> )	3.05 (0.19)	3.13 (0.14)	ns	No aplicable			F p significance	0.0920 0.7694 ns	No aplicable	

<sup>a</sup> Mean (SE) (n = 5) expressed by dry mass. Statistically significant differences (t-test and ANOVA) are indicated: ns = not significant, \*p<0.5, \*\* p<0.1, \*\*\*p<0.01.

heavy metals into plant tissues: soil uptake and the atmospheric deposition from settling dust (Fodor, 2002). On the other hand, similar concentrations of Cr, Al, Fe, Cu and Ni were found in both organs at Site 2, while roots contained larger amounts of these metals than leaves collected at control Site 1. Usually, roots have a higher concentration of Pb than leaves (Wierzbicka, 1999). Higher metal concentrations in roots than in shoots are frequently observed in non-hyperaccumulator plants (McGrath et al., 2001). Generally the majority of Pb is attached to the cell walls in the roots and competes with Ca in the adsorption of cations by the carboxylic groups of the matrix substances, galacturonic and glucuronic acids. However, despite the retention of Pb in the root, there are plants in which a significant amount of the metal is transported to the shoot (Cseh, 2002).

Compartmentalization and sequestration with organic acids have been proposed as mechanisms of tolerance to metals in Asteraceae. In sunflower, trichomes play a role in the tolerance to Mn toxicity (Blamey et al., 1986).

*T. diversifolia* also possesses trichomes in the leaves but the compartmentalization of Pb in the organs was not evaluated in the present study. Nevertheless, the localization of metals in a metabolically inactive form in trichomes is a possible explanation for the unusually higher concentration of Pb found in leaves compared to roots of *T. diversifolia* field plants.

Although the leaves were washed, the hair and cuticle can trap contaminants. Metals, such as Fe and Al, can be used as markers for soil contamination in plant analyses. The metals with higher concentration in the leaves were Al, Fe and Cu (table 1) and these were 3 or 4 times higher for the polluted site compared to Site 1, whereas Pb was 15 times higher, indicating uptake of this metal.

In general, toxic metals may cause deficiency of other elements essential for plants (Fodor, 2002; Krupa et al., 2002). However, this was not observed in the data of table 1. At Site 2 agricultural and urban development is higher than for Site 1, and consequently the input of nutrients through irrigation by streams is larger.

*Enzymatic activities:* The NRA in leaves diminished at the polluted site but no changes were observed in roots (table 1). Kłobus et al. (2002) showed different examples of inhibition of nitrate reductase in plants under heavy metal stress.

Singh et al. (1997) reported that nitrate reduction was inhibited drastically in roots by Pb but in leaves different effects have been observed with various cultivars. Leaf NRA in *Vigna*

*radiata* increased with increasing Pb (Singh et al., 1997/98), while Kevresan et al. (2001) found a significant decrease in the NRA in the aboveground parts of young pea plants.

The APA increased at Site 2, where leaves had a higher concentration of P. The exposure of roots to Ni markedly stimulated phosphatase activity in *Alyssum bertolonii* (Gabbrielli et al., 1989). The deposition of Pb pyrophosphate along cell walls was proposed as the main process responsible for Pb accumulation in root tissues. A favourable P regime reduces the effects of Pb toxicity, since Pb can form insoluble phosphates (Kabata Pendias and Pendias, 1984).

#### *Organic compounds affected by metals and leaf specific mass:*

Plants from Site 2 were exposed to daily traffic during their entire lifespan (long time exposure). The plant samples from the field analysed here did not produce responses such as depletion of chlorophyll, carotenoids and proteins, nor increases in lipid peroxidation, reported in the literature for leaves of plantlets under greenhouse conditions.

Changes of the foliar structure in response to Pb have been reported by Kovačević et al. (1999), who found smaller lamina and mesophyll thickness, epidermal cell size and diameter of vessels. Differences in SLM, related to leaf thickness, were expected for both sites. Detoxification through phenols was also expected (Giertych et al., 1999).

An increase in phenol concentration and a highly significant drop in SLM was observed in Site 2 (table 2). However, phenol concentration in leaves and SLM were not affected, in spite of a significant increase (13.6 times) in foliar Pb observed between the treatments in the pot experiment (see below). On the other hand, Pb concentration in contaminated leaves in the field (table 1) was 6 times higher than in the +Pb treatment in the pot experiment (table 3) presumably because there was an input of Pb from the air due to the traffic, absent in the pot experiment.

*Pot experiment with Pb-supplemented soil:* APA of roots was not affected when comparing both treatments despite the Pb concentration being different by a factor of 62. In leaves a decrease of activity was observed. According to these result the increase of APA observed in the field for contaminated plants is probably not a response to Pb concentration. In this experiment plants were grown in the same soil with or without the Pb supplement.

The highly significant increase of phenols in response to Pb observed for roots has been reported by several authors for other plants in response to metals.

Ruso et al. (2001) found a good correlation between the induced synthesis of phenols, mainly in root tissue, and the tolerance and accumulation of heavy metals in sunflower. It was proposed the selection of lines for their constitutive and induced phenol content to be used for phytoremediation.

Giertych et al. (1999) found higher phenol contents in plants at a polluted site compared with a control site. Total phenols include condensed and hydrolysable tannins, flavonoids, phenolic glucosides and chlorogenic acids. The hydroxyl radicals of tannins have a strong affinity for metals and are used in industry as metal chelators. For example, the high concentrations of tannins in *Psychotria douarrei* leaves might function as a detoxification mechanism for elevated cytoplasmic metal concentrations, in addition to providing defensive benefits (Davis et al., 2001). Lavid et al. (2001 a,b) demonstrated the involvement of phenols in Cd accumulation in aquatic plants. Polyphenols in *Nymphaea* chelate Pb, Cr and Hg *in vitro* (Lavid et al., 2001b). The phenolics are generally thought to prevent oxidative damage by scavenging active oxygen species and by breaking the radical chain reactions during lipid peroxidation. These antioxidative effects require the reduced form of phenolics. In the oxidized form (unstable under physiological conditions but stabilized by Al) act as prooxidants (Sakihama and Yamasaki, 2002).

As a conclusion *T. diversifolia* growing wild on roadsides with heavy urban traffic presented levels of Pb in leaves and roots considered as typical of contaminated plants, but without visible symptoms of damage to the leaves or depletion of chlorophyll, carotenoids, proteins nor increases in lipid peroxidation. These are responses reported in the literature for plants under Pb stress. On the other hand, phenols increased and this was also observed in roots with high Pb concentration after Pb-acetate application. The identification and role of phenols in the tolerance to Pb in this species deserves the attention of future research.

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