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Uptake and distribution of lead in tobacco (*Nicotiana tabacum L.*)

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Summary

Six *Nicotiana tabacum* varieties were *in vitro* cultured and experimentally exposed to lead in order to estimate lead uptake and distribution in tobacco plantlets and to observe possible differences depending on variety. Fifty-day-old plantlets were exposed to four Pb rates ranging from 0 to 200 mg dm^{-3}. No statistically significant effect of lead on dry matter weight was observed for any variety or plant part. Tissue lead concentration was determined on upper leaves, lower leaves, stems and roots. Lead accumulation in the plant positively correlated with lead exposure level. Lead concentration in the different plant parts decreased as follows: root, lower leaves, stem and upper leaves. All tobacco varieties showed similar behaviour with respect to lead treatment level and revealed the same distribution pattern of lead concentration in the different plant part. The highest values of tissue lead concentration were found in tobacco variety Pr61, while the lowest in G94-2.

Introduction

In nature lead is a non-essential element found in all environmental media and in all components of the biosphere. In the earth's crust the average natural concentration of Pb is 16 mg kg^{-1} (NRIAGU, 1978). In the last sixty years large amounts of lead have been extracted, concentrated, used, and re-emitted into the environment, resulting in a much higher lead concentration than it used to be. Indeed lead is one of the most frequently encountered heavy metals in polluted environments. In soil, in rural, urban and industrial (smelters) areas Pb concentrations (in the upper layer) range from 5 to 60, from 50 to 300 and from 300 to 20,000 mg kg^{-1} respectively (EWERS and SCHLIPKÖTER, 1991). Moreover lead is one of the most persistent metals being estimated to have a soil retention time of 150-5,000 years (SHAW, 1990).

Lead is a hazardous element with a long biological half-life and accumulates in bones and teeth (STEENHOUT, 1982). In humans it induces diverse physiological and biochemical disorders, as well as mutagenic and carcinogenic effects (GERBER, 1980, Ewers and SCHLIPKÖTER, 1991).

In addition to food, beverage and inhaled air, cigarette smoking may be a substantial source of lead and other heavy metal intake (CHIBA and MASIRONI, 1992; EWERS and SCHLIPKÖTER, 1991). These elements pass from tobacco to the smoke as they are only partially retained by cigarette filters, therefore smokers have higher lead levels than non-smokers (GRASMICK, 1985; HUNTER, 1986; QUINN and DELVES, 1987; WATANABE et al., 1985). Moreover, cigarette smoke is a source of environmental pollution not only from the smoke exhaled by the smokers, but also from sidestream smoke emitted by burning cigarettes. Sidestream smoke, which is inhaled by non-smokers via passive smoking, usually contains relatively high concentrations of many noxious substances including heavy metals (CHIBA and MASIRONI, 1992). Passive smoking plays an important role in the exposure of children to lead. Parental smoking has a significantly greater influence on blood lead levels than other environmental or dietary factors (ANDREN et al., 1988).

Nicotiana tabacum L. is recognized to be an accumulator of metals from the soil (CLARKE and BRENNAN, 1983; MENCH et al., 1989; WAGNER and YERGAN, 1986; WAGNER et al., 1988; DOROSZEWSKA and BEBEC, 2004). The concentration of metals in tobacco leaves may be influenced by soil type, pH, agricultural practices (ADAMU et al., 1989a; ADAMU et al., 1989b; BELL et al., 1988; BELL et al., 1992; TSADILAS et al., 2005) and genotype (WAGNER and YERGAN, 1986; WAGNER et al., 1988). It is reported that tobacco leaves may have a lead content varying from 0 to 200 mg kg^{-1} dry weight (TSO, 1991). Little information is available concerning lead tissue distribution and varietal variation in tobacco.

The present study aims at evaluating lead uptake and distribution within plantlets of *N. tabacum* L. and revealing possible differences depending on variety. The use of a method based on culture solution was preferred as a model system before extending the trial to soil at the greenhouse and field levels.

Material and methods

Plant culture

Seeds of six *N. tabacum* L. varieties, belonging to the Tobacco Institute of Scafati collection, Burley IST Bu23, Burley IST G94-2, Bright IST G19, Bright IST G165, Perustitza IST Pr61, and Perustitza IST P2B, were aseptically germinated in Petri dishes on solidified (agar 8 g dm^{-3}) Murashige and Skoog medium (MS) (MURASHIGE and SKOOG, 1962). After two weeks, the plantlets were transferred into solidified MS (agar 4 g dm^{-3}) in glass vessels and cultured for three weeks. The plantlets were then transferred into liquid MS, in other glass vessels, containing a perforated plastic raft to hold up the plantlets, and cultured for two more weeks. At this stage, the plants were utilized for lead exposure experiments. The following growing conditions were used: 26 °C, 4000 lux (Philips TL 33 40 W), and 16/8 hours light/dark cycle.

Lead exposure experiments

The MS culture medium was removed from the vessels and the roots of the plantlets were rinsed three times with sterile distilled water. Then plantlets, selected for uniformity, were exposed for 10 days to Pb, at the concentration of 0, 2, 20 and 200 mg dm^{-3}, supplied with aqueous solutions of lead nitrate or potassium nitrate, calculated to contain the same amount of nitrate as in each lead rate.

At the end of the experiment, plants were thoroughly washed in distilled water, placed on filter paper and divided into four parts: upper leaves, lower leaves, stem and roots. Samples for each plant part were prepared by collecting ten plants into one sample and dried in a forced ventilated oven at 60 °C for 24 hours and then at 100 °C for three hours. Dry matter of each plant part was determined.

Chemical analysis of lead

Dried samples were powdered and wet digested with nitric acid, using a microwave oven (CEM, mod. MDS 2000). Lead content was

measured by Atomic Absorption Spectrophotometry (VARIAN mod. SPECTRA AA20), equipped with graphite furnace.

Statistical Analysis

Analysis of variance (ANOVA) was performed using Statistica software (Version 5.0 for Windows, 1996, StatSoft, Inc. Tulsa, USA). The lead concentrations in plant tissue were log transformed to normalize the frequency distributions. Post hoc comparisons to determine the significant differences between means were performed by Newman-Keuls test.

Results

Plantlets exposed to lead nitrate as well as potassium nitrate showed no evident sign of suffering and had the same appearance as before being exposed. For each plant part and each tobacco variety no statistically significant effect of lead nitrate as well as potassium nitrate rates on dry matter weight was revealed.

For all varieties, lead concentration was determined for the upper leaves, lower leaves, stem, and root of plants exposed to the four lead rates. For all the examined varieties, tissue Pb concentration increased as the lead exposure rate increased, for all plant parts. For all treatments, the highest lead concentrations were revealed in the root, the lowest in the upper leaves (Fig. 1).

The different rates of lead application had significant effects on the lead concentration determined in various plant parts and varieties ($P<0.001$). Lead accumulation in the plant positively correlated with the lead treatment level; moreover, significant differences between the mean values at each lead rate were revealed (Tab. 1A). The comparison of mean values for the lead concentration in the various plant parts showed that the differences were statistically significant. The lead accumulation in the different plant parts decreased as follows: root, lower leaves, stem and upper leaves (Tab. 1B). In

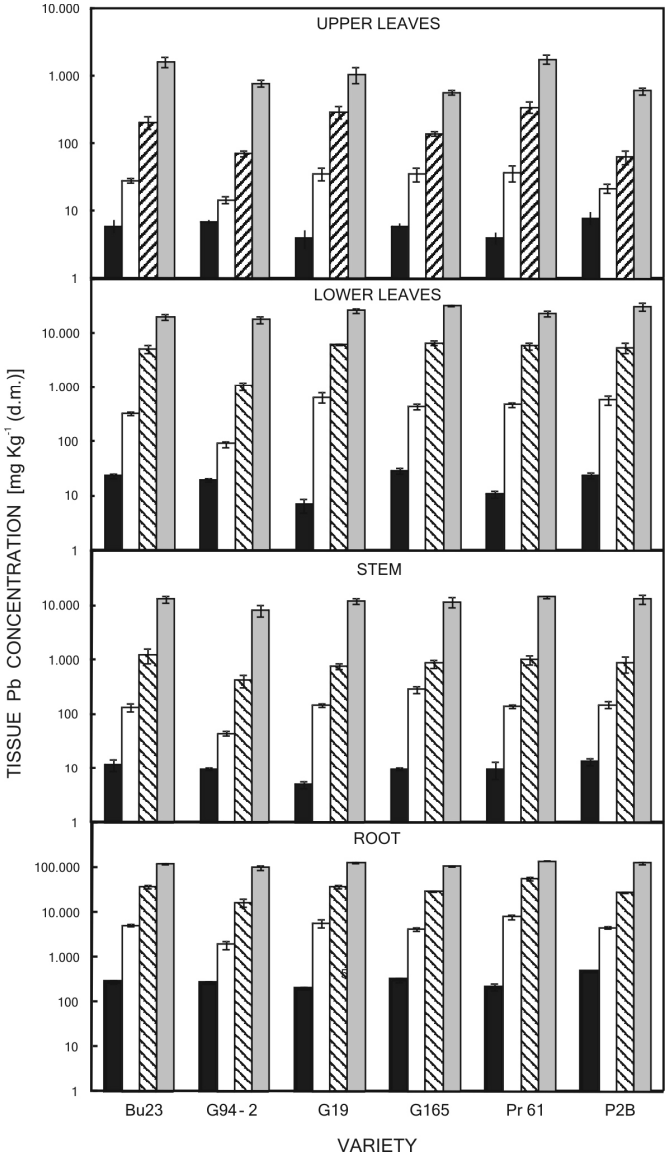

Fig. 1: Tissue Pb concentration [mg kg^{-1} (d.m.)] in different plant parts of six tobacco varieties exposed to four Pb rates for 10 days. Values represent mean (± S.E.) of six replicates, each collected from 10 plants. Black column: 0 mg dm^{-3} Pb; white column: 2 mg dm^{-3} Pb, striped column: 20 mg dm^{-3} Pb, grey column: 200 mg dm^{-3} Pb.

Tab. 1: Effect of lead rate (A), plant part (B) and variety (C) on tissue Pb concentration [mg kg^{-1} (d.m.)] in tobacco plantlets exposed to four Pb rates for ten days. Values are the mean of six replicates, each collected from ten plants. Values shown are the back-transformed concentrations calculated from log-transformed data. Values within columns followed by the same letter were not significantly different at P= 0.01 according to the Newman-Keuls test.

(A) Lead rate (mg dm^{-3})		(B) Plant part		(C) Variety	
0	21d	Upper leaves	63d	Bu23	697a
2	265c	Lower leaves	877b	G94-2	369b
20	1919b	Stem	304c	G19	614a
200	12972a	Root	8072a	G165	705a
				Pr61	728a
				P2B	622a

Tab. 2: Effect of plant part (A) and variety (B), for each lead rate, on tissue Pb concentration [mg kg^{-1} (d.m.)] in tobacco plantlets exposed to four Pb rates for ten days. Values are the mean of six replicates, each collected from ten plants. Values shown are the back-transformed concentrations calculated from log-transformed data. Values within columns followed by the same letter were not significantly different at P= 0.01 according to the Newman-Keuls test.

	Lead rate (mg dm^{-3})			
	0	2	20	200
(A) Plant part				
Upper leaves	5 d	25 d	144 d	872 d
Lower leaves	16 b	351 b	4279 b	24412 b
Stem	9 c	128 c	702 c	11179 c
Root	258 a	4398 a	31477 a	119591 a
(B) Variety				
Bu23	25 a	277 a	2387 ab	14488 ab
G94-2	24 a	99 b	790 d	9804 c
G19	11 c	353 a	2601 ab	13829 ab
G165	27 a	355 a	2171 b	12156 bc
Pr61	15 b	349 a	3139 a	16885 a
P2B	29 a	291 a	1505 c	11912 bc

particular, the Pb concentration in the roots was about one and two orders of magnitude higher than in the lower and upper leaves respectively; intermediate values between those of lower and upper leaves were observed in the stem. Tobacco variety Perustitza IST Pr61 had the highest values of Pb concentration and Burley IST G94-2 the lowest. The comparison of mean values between the tobacco varieties showed that the differences were statistically significant for Burley IST G94-2 only (Tab. 1C).

In order to have more detailed information on the behaviour of tobacco plant with respect to lead exposure statistical analysis was also performed on each lead rate, each plant part and each variety separately.

Tab. 3: Effect of lead rate (A) and variety (B), for each plant part, on tissue Pb concentration [mg kg^{-1} (d.m.)] in tobacco plantlets exposed to four Pb rates for ten days. Values are the mean of six replicates, each collected from ten plants. Values shown are the back-transformed concentrations calculated from log-transformed data. Values within columns followed by the same letter were not significantly different at P= 0.01 according to the Newman-Keuls test.

	Plant part			
	Upper leaves	**Lower leaves**	**Stem**	**Root**
(A) Lead rate (mg dm^{-3})				
0	5 d	16 d	9 d	258 d
2	25 c	351 c	128 c	4398 c
20	144 b	4279 b	702 b	31477 b
200	872 a	24412 a	11179 a	119591 a
(B) Variety				
Bu23	82 a	938 b	348 a	8888 ab
G94-2	48 bc	421 c	183 b	5105 c
G19	71 a	862 b	274 a	8529 ab
G165	62 ab	1283 a	390 a	8041 b
Pr61	88 a	893 b	341 a	10486 a
P2B	43 c	1174 a	346 a	8564 ab

For each lead exposure level, significant effects of plant part and variety ($P<0.001$) were revealed. At each lead rate the Pb concentration in the different plant parts decreased as follows: root, lower leaves, stem and upper leaves (Tab. 2A) as occurred when the mean values over all lead rates were compared (Tab. 1B). It is worth noting the presence of lead in each part of control plants, especially in the roots where an amount of about 250 mg kg^{-1} dry matter was detected. These findings can be explained by the uptake of lead present as a contaminant in the MS culture medium during the growth stages of the plantlets. Interestingly, the distribution of lead in the different parts of control plants was the same as that of the other treatments (Tab. 2A).

For each lead rate the tobacco varieties were ranked in a different order (Tab. 2B). However with the increase in lead exposure rate for Perustitza IST Pr61 Pb concentration was trending upwards, while for Burley IST G94-2 it was the lowest for each lead rate.

For each plant part significant effects of lead rate and variety on Pb concentration ($P<0.001$) were observed. The comparisons of means showed that the differences between each lead rate were statistically significant (Tab. 3A), as occurred when the mean values over all plant parts were compared (Tab. 1A). For each plant part, tobacco varieties were ranked in a different order (Tab. 3B). However, it is worth noting that Burley IST G94-2 revealed the lowest Pb concentrations in each plant part.

For each variety significant effects of lead rate and plant part on lead concentration ($P<0.001$) were revealed. All tobacco varieties showed similar behaviour with respect to lead treatment level with significant differences between the mean values at each lead rate (Tab. 4A) and revealed the same distribution pattern of Pb concentration in the different plant part (Tab. 4B).

Discussion

For this research an *in vitro* culture procedure, based on the use of three different MS culture media (solid, semi-solid and liquid) during the growth, was developed in order to obtain vigorous tobacco plantlets without any agar residues or breaking in the root system. Indeed, 50-day-old plantlets could not be taken from solid medium without damaging the roots and young tobacco seedlings exhibited signs of suffering when cultured directly in liquid medium.

Tab. 4: Effect of lead rate (A) and plant part (B), for each tobacco variety, on tissue Pb concentration [mg kg^{-1} (d.m.)] in tobacco plantlets exposed to four Pb rates for ten days. Values are the mean of six replicates, each collected from ten plants. Values shown are the back-transformed concentrations calculated from log-transformed data. Values within columns followed by the same letter were not significantly different at P= 0.01 according to the Newman-Keuls test.

	Variety					
	Bu23	**G94-2**	**G19**	**G165**	**Pr 61**	**P2B**
(A) Lead rate (mg dm^{-3})						
0	25 d	24 d	11 d	27 d	15 d	29 d
2	277 c	99 c	353 c	355 c	349 c	291 c
20	2387 b	790 b	2601 b	2171 b	3139 b	1505 b
200	14488 a	9804 a	13829 a	12156 a	16885 a	11912 a
(B) Plant part						
Upper leaves	82 d	48 d	71 d	62 d	88 d	43 d
Lower leaves	938 b	421 b	862 b	1283 b	893 b	1174 b
Stem	348 c	183 c	274 c	390 c	341 c	346 c
Root	8888 a	5105 a	8529 a	8041 a	10486 a	8564 a

Lead exposure experiments were performed utilizing water solutions containing lead nitrate only, because lead ions cannot be added to the culture medium as they form insoluble salts with many of the anions present in the medium, such as phosphate and sulphate.

The results of this study revealed no significant effect of lead on dry matter of each plant part for all the tobacco varieties *in vitro* exposed to Pb from 2 to 200 mg dm^{-3}. In research studies previously performed, a general reduction and delay in growth was observed in *Nicotiana tabacum* when 28 day-old plantlets had been treated with lead (BASILE et al., 1992; DEL PIANO et al., 1995), and more than 60 % inhibition of root elongation was shown in seeds exposed to 2 mg dm^{-3} Pb rate (DEL PIANO et al., 1996). It is reasonable to suppose that the growth stage has affected the plant's response to lead, as 50 day-old plants were used instead of younger plants or seedlings. Although a decrease in plant dry weight caused by lead treatment has been generally reported, also for other species, exposed to comparable lead rates, no variation or even an increase in dry matter, has been found (JONES, 1973; WIERZBICKA, 1999).

As regards lead uptake in tobacco plants results showed Pb accumulation mainly in the roots in agreement with the findings widely reported on other species (FODOR, 1998; HUANG and CUNNINGHAM, 1996; KABATA-PENDIAS, 2001; KUMAR et al., 1995; ZIMDAHL, 1976). In this research, 50 day-old plantlets, instead of seedlings, as generally reported in other studies performed in culture solution (FODOR, 1998; HUANG and CUNNINGHAM, 1996; KUMAR et al., 1995; WIERZBICKA, 1995; WIERZBICKA, 1999) were utilized and roots, stem and leaves, divided in upper and lower leaves, were examined. Thus more information about the distribution of lead in the different parts of the plant was obtained, despite using an *in vitro* model system.

For all *N. tabacum* varieties examined, results indicated that lead content in each plant part increased with the increasing lead exposure rate. These findings are consistent with those reported for *Lolium perenne* (JONES, 1973), *Brassica juncea* (KUMAR et al., 1995; JIANG et al., 2000) and *Sesbania drummondii* (SAHI et al., 2002). A concentration-dependent Pb accumulation has also been found both in roots and shoots of *Zea mays*, but not in the shoots of *Ambrosia artemisiifolia*, a weed common on Pb-contaminated sites (HUANG and CUNNINGHAM, 1996).

For all exposure rates and for all varieties the lead accumulation in the different plant parts decreased as follows: root, lower leaves, stem and upper leaves. It is worth noting that a similar distribution pattern of lead was found also in control plants, owing to the uptake of lead present as a contaminant of the culture media salts (despite using only analytically pure reagents), demonstrating a similar response of tobacco plantlets when exposed to lead in trace or higher rates.

Root lead content in the *Nicotiana tabacum* varieties, at the 2 mg dm^{-3} Pb rate, ranged from 2,000 to 8,000 mg kg^{-1}, most often about 5,000 mg kg^{-1} (Fig. 1). In a study on Pb uptake, performed at the same lead rate in culture medium, a Pb amount of about 3,900 and 2,100 mg kg^{-1} dry matter was reported, in the roots of *Hordeum vulgare* and *Zea mays*, respectively (WIERZIBCKA and ANTOSIEWICZ, 1993). Root lead uptake, for most of the tobacco varieties, was slightly higher than that of *Hordeum vulgare*, except for G94-2 which was similar to that of *Zea mays*.

Moreover, the results indicated that, at the Pb exposure rate of 2 mg dm^{-3}, tobacco plants concentrated Pb in their roots, on average, by 2,200 fold (Tab. 2A). The concentration factor (the ratio between tissue metal concentration and medium metal concentration) decreased as the lead rate increased and it was 600 at 200 mg dm^{-3}, indicating that a possible saturation of the root Pb binding sites occurred.

It is interesting to note that the high potential of root Pb uptake in tobacco plants is confirmed by the large amount of metal (250 mg kg^{-1}) found in control plants (Tab. 2). The presence of high Pb values in the roots of control plants, coming from the contamination of the reagents utilized in culture media has been also reported for *Allium cepa* (WIERZBICKA, 1999).

As regards the aerial parts of plant, at the lowest lead exposure rate (2 mg dm^{-3}) results revealed a Pb content of 350, 25 and 128 mg kg^{-1} for lower leaves, upper leaves and stem respectively, with a corresponding concentration factor of about 170, 10 and 60. At the highest lead exposure rate (200 mg kg^{-1}) Pb concentration factors decreased, always being higher than one unit, also for upper leaves. Also KUMAR et al. (1995), comparing the ability of *brassica* species and other plants to accumulate Pb, reported for tobacco seedlings (17 day-old) exposed to 625 mg of Pb/g DW sand-Perlite mixture, a lead content of about 800 mg kg^{-1} for shoots and a concentration factor higher than one unit.

All tobacco plants examined showed the same response with respect to lead exposure and a similar pattern of lead distribution within the plantlet. As regards tissue Pb concentration, tobacco variety G94-2 showed the lowest values for each exposure rate and for each plant part. It is worth pointing out that G94-2 50-day-old plantlets had higher values of total plant dry matter than other varieties grown under same conditions. Moreover roots had a larger diameter and a higher percentage of dry matter (25 %) on total plant than the other tobacco plants (10-20 %). The dilution effect, caused by the higher root weight, and the larger dimensions of the roots might explain the low Pb concentrations found in variety G94-2. The role of root quality (large and fine) has also been proposed for Cd accumulation traits in *Nicotiana tabacum* and *Nicotiana rustica* (WAGNER and YEARGAN, 1986).

The results of this research highlight the great potential of *Nicotiana tabacum* for lead uptake by the roots, and suggest that, although the transport of lead from root to the aerial parts of the plant is limited, it can be accumulated in the leaves.

It is worth noting that the *in vitro* model system used in this study allows tobacco genotypes to be tested in a relatively short time as regards heavy metal uptake, in the absence of environmental interferences and avoiding complicating soil processes.

However, although the *in vitro* technique may be a useful investigative tool, it might not accurately reflect metal uptake and distribution occurring in soil. Thus investigations should be extended to greenhouse and field cultivation.

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