# Soil Nitrogen Fertilization Effects on Phytoextraction of Cadmium and Lead by Tobacco (*Nicotiana tabacum* L.)

## QUERY SHEET

**Q1:** Au: Bennet et al., Brooks 1998. Location of publisher?
**Q2:** Au: Forstner 1995. Location of publisher?
**Q3:** Au: Olsen 1983. Location of publisher?
**Q4:** Au: Reeves & Baker. Chapter page range?

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# Soil Nitrogen Fertilization Effects on Phytoextraction of Cadmium and Lead by Tobacco (*Nicotiana tabacum* L.)

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**ABSTRACT** A greenhouse experiment using 24 plastic pots filled with 6 kg of Pb- and Cd-contaminated soil was carried out. In all 24 pots, soils were heavy metal–contaminated with 10 mg Cd $kg^{-1}$ soil and 500 mg of Pb $kg^{-1}$ soil by using CdCl and $PbNO_3$. Two-month-old tobacco (*Nicotiana tabacum* L.) plants were used to extract these heavy metals. Results showed that tobacco is able to remove Cd and Pb from contaminated soils and concentrate them in its harvestable part, that is, it could be very useful in phytoextraction of these heavy metals. Increasing additions of ammonium nitrate to soil (50, 100, and 150 mg N $kg^{-1}$ soil) significantly ($p \leq .05$) increased aboveground Cd and Pb accumulation during a 50-day experimental period, whereas increasing additions of urea to soil (50 and 100 mg N $kg^{-1}$ soil) did not show these effects at the same significance levels. Increasing additions of ammonium nitrate to soil shows as dry matter increases, both accumulated Cd and accumulated Pb also increase when tobacco plants are growing under Pb- and Cd-contaminated soil conditions. Higher Pb concentrations depress Cd/Pb ratios for concentrations and accumulations, suggesting that Pb negatively affects Cd concentration and/or accumulation.

**KEYWORDS** Cd-Pb nteraction nutrient accumulation, nutrient concentration, phytoextraction

## INTRODUCTION

Heavy metals have been widely used in many different activities, such as agriculture, mining, smelting, electroplating, and ore refining. In agriculture, for instance, lead arsenate was used as an insecticide to control the apple codling moth (*Cydia pomonella*) in orchards starting in the 1900s (Merwin et al., 1994; Shepard, 1951). By this way, many orchard soils are contaminated with lead (Pb) and arsenic (As) (Peryea and Creger, 1995). Additionally, Pb is often used in paints, gasoline, explosives, and antispark linings as well as municipal sewage sludge (Levine et al., 1989). Sharma and Shanker-Dubey (2005) reported that significant increases in the Pb content of cultivated soils have been observed near industrial areas. Thus, many sites around the world are Pb contaminated.

Like nitrogen, potassium, calcium, magnesium, and other nutrients, phosphorus (P) is an essential nutrient, and plants respond favorably to the

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application of phosphate fertilizers, which often contain cadmium (Cd). Therefore, agricultural soils all over the world are slightly to moderately contaminated by Cd (Vassilev, 2002). Moreover, soils are also Cd contaminated by sewage sludge application and smelter dust spreading (Vassilev, 2002).

Problems associated with heavy metals contamination of soils have been well documented. The accumulation of heavy metals in plants inhibits or activates certain enzyme processes, affecting their productivity from both qualitative and quantitative aspects (Miteva et al., 2001). Chang et al. (2001) reported increments in toxic levels due to heavy metals, eutrophication in fields, and reductions of 30% to 70% in rice yield when paddy fields have been sewage irrigated in China. Lead and As (Codling and Ritchie, 2005), Cd and zinc (Zn) (Brown et al., 1994), and other heavy metals can be taken up by plants. Such situation may be one possible avenue of these heavy metals entry into the human food chain through the consumption of plants directly or indirectly by human beings (Mortvedt, 1996; Chien et al., 2003). There is evidence that Pb and Cd have been the cause of human health effects, animal fatalities, and the disruption of natural ecosystems and agro-ecosystems. In general, heavy metals may be potentially toxic to human health.

Phytoremediation is being developed as a potential remediation solution for heavy metal–contaminated sites around the world. Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless. Phytoremediation of heavy metals is a cost-effective 'green' technology based on the use of metal-accumulating plants to remove them from soils (Raskin et al., 1997). Many investigations on phytoremediation have studied one heavy metal. However, 70% of all heavy metal-contaminated soils involve two or more metals (Forstner, 1995). Therefore, the possibility of effects of interactions may be of considerable importance at some heavy metal–contaminated soils, and the phytoremediation behavior of a plant (i.e., uptake of metals) may be different for mixtures of metals than for one metal alone (Ebbs et al., 1997).

Phytoremediation is essentially an agronomic approach and its success depends ultimately on agronomic practices applied at the contaminated site. It has been suggested to use high biomass species such as oat (*Avena sativa* L.), maize (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.) (Kayser et al., 2000), or sugar cane (*Saccharum* spp.) (Segura-Muñoz et al., 2006). The use of fertilizers is also a common practice when applying phytoremediation technique in heavy metal-contaminated soils. Inorganic fertilizers are considered as soil additives to provide nutrients needed for high-yielding plants, and to acidify the soil for greater metal bioavailability (Lasat, 2000).

Nitrogen fertilizers containing N in the form of ammonium can acidify the soil and decrease rhizosphere pH by causing $H^+$ extrusion (Tisdale et al., 1993). The ammonium ion can lead to desorption of heavy metals from exchange sites or soil colloids via ion exchange (Lorenz et al., 1994). Urea has been shown to increase exchangeable and water-soluble Cd in the soil, illustrating the effect of acidification on the solubility of Cd (Brown et al., 1994). It deserves be pointed out that there may be some negative side effects associated with soil acidification. For instance, due to increased solubility, some heavy metals may leach into the groundwater, creating an additional environmental risk. The aims of this research work were (i) to identify the effects of ammonium nitrate and urea as fertilizers on concentration and accumulation of Cd and Pb in *Nicotiana tabacum* L. plants, and (ii) to define important relationships between Cd and Pb concentrations and accumulations in aboveground biomass.

## MATERIALS AND METHODS

This study is based on data acquired in 2001 from a greenhouse experiment that was carried out at the Facultad de Agronomía of the Universidad Autónoma de Nuevo León' at Marín, Nuevo León state, in Mexico.

### Soil Preparation and Experimental Setup

A soil sample was collected from a 0- to 30-cm surface layer at a site near Monterrey, Nuevo León, México, which is perhaps the most important industrial city in the country. The soil was a sandy loam (hydrometer method) containing 4.5 g organic matter $kg^{-1}$ soil (Walkley-Black procedure). The pH was about 7.5 at a solution of 1:2 soil:water ratio. Heavy metals were extracted from 5 g soil using 25 ml of a mixture of concentrated nitric acid and hydrogen peroxide (1:1; *v:v*), and Whatman no. 41 filter paper following the procedure of Ebbs et al. (1997). Total Pb was of 24 mg $kg^{-1}$ soil, and Cd concentration was negligible.

Six kilograms of soil, previously mixed, were introduced into each of the 24 plastic pots. Once all 24 pots were prepared, soil was heavy metal–contaminated with 10 mg Cd $kg^{-1}$ soil and 500 mg of Pb $kg^{-1}$ soil by using CdCl and $PbNO_3$ as sources of Cd and Pb, respectively. These levels of heavy metals are 10 times more than levels in uncontaminated soil (Temmerman et al., 1984).

After this, a 2-month-old tobacco plant was planted in each of the 24 pots. Three days after planting, pots were fertilized with ammonium nitrate ($NH_4NO_3$) or urea (CO $(NH_2)_2$). A control set of pots were not fertilized. There were six treatments: five with chemical fertilizer plus the control (no fertilization) with four replications. Three levels of N (50, 100, and 150 mg N $kg^{-1}$ soil) were considered as treatments when applied to the pots in the case of ammonium nitrate, and two (50 and 100 mg N $kg^{-1}$ soil) in the case of urea. All 24 pots were randomly distributed inside the greenhouse.

Pots were daily irrigated with distilled water to maintain the soil moisture at 80% of field capacity, and to avoid water excess during all the experimental period.

## Heavy Metals Determination

Fifty days after planting, all aboveground fresh matter was harvested from each pot, then washed with distilled water, and dried at 75°C in an oven to constant weight. Weight for each pot was registered as aboveground dry matter (DM).

Metal concentrations in plants were determined in DM samples as consigned by Brooks (1998). DM was ashed in the furnace at 350°C. Ash was digested with concentrated HCl and taken to dryness, and residue was dissolved with 1 N HCl. Analyses of Cd and Pb concentrations were performed using an atomic absorption spectrophotometer (model UNICAM-SOLAAR 969). By taking into account DM and heavy metal concentrations, accumulation for both Cd and Pb in aboveground biomass was computed. According to Depledge et al. (1994), accumulation refers to the amount of Cd and Pb that remain in the tobacco plants following exposure over a certain time period, 50 days in the present case. This variable takes into account metal uptake patterns as well as the negative effect that excessive metal concentrations can have on yield. Reeves and Baker (2000) used this variable to describe total shoot Ni in a study on the accumulation patterns of Ni by *Thlaspi goesingense* Hálácsy, and Brown et al. (1994) used it in a study with the hyper-accumulator specie *Thlaspi caerulescens* J. Presl & C. Presl

## Statistical Analyses

Statistical analyses were performed using the STATISTICA software, Kernel release 5.5 (StatSoft Inc., 2000). Data were processed for analysis of variance (ANOVA–Tukey test), estimation of Pearson correlations, and principal components analysis.

## RESULTS AND DISCUSSION

## Analysis of Variance

### Plant Aboveground Dry Matter

In general, nitrogen applications using ammonium nitrate or urea as fertilizer enhance the production of aboveground dry matter (DM) in tobacco plants (Table 1). Treatments with added N through urea and ammonium nitrate produced significantly ($p \leq .05$) greater DM (almost 1.5 and 2 times more DM, respectively) than control (Table 1). These results are explained by the general knowledge of the effects of nitrogen on plant growth. It is known that ammonium nitrate contains $NH_4^+$and $NO_3^-$ ions, and both are available for plants (Marschner, 1986), but $NO_3^-$ is more easily used by plants because it does not compete with other ions. However, urea, once incorporated in the soil, reacts (through hydrolysis process) to form $NH_4^+$ ions which compete with other cations for exchange sites. It appears that increases in the rate of $NH_4^+$ ions merely increase this competition, and then more cations (including Cd and Pb) became phytoavailable as pointed out by Mitchell et al. (2000).

### Heavy Metal Concentrations

Using Cd and Pb concentrations in the control treatment as reference, it is shown there was significantly ($p \leq .05$) reduced Cd and Pb in tobacco plants as nitrogen fertilizer increased for both nitrogen sources, except for treatment of 150 mg N $kg^{-1}$ soil when using ammonium nitrate fertilizer and for treatment of 100 mg N $kg^{-1}$ soil when using urea fertilizer (Table 1). These effects on Cd and Pb concentrations may be attributed to heavy metal dilution due to increased plant biomass production (Olsen, 1983). This is confirmed by the data in Table 1, because there are no significant ($p \leq .05$) differences in Cd and Pb concentrations in tobacco plants when comparing with the control and

**TABLE 1** **Means ± Standard Deviations of Dry Matter (DM), Cd and Pb Concentrations, and Cd and Pb Accumulations in *Nicotiana tabacum* L. Associated with Six Soil Nitrogen Fertilization Treatments and Four Replications***

| | Fertilization treatment (mg N $kg^{-1}$ soil) | | | | | |
|---|---|---|---|---|---|---|
| | Control | Ammonium nitrate | | | Urea | |
| Variable | 0 | 50 | 100 | 150 | 50 | 100 |
| Dry matter (g per plant)§ | 5.7 ± 0.26 c | 12.32 ± 0.41 a | 11.8 ± 0.76 a | 13 ± 0.73 a | 7.6 ± 0.39 b | 7.8 ± 0.51 b |
| Cd concentration (mg Cd $kg^{-1}$ DM) | 35 ± 3.81 a | 27 ± 4.74 b | 26 ± 1.58 b | 32.1 ± 5.39 ab | 28.95 ± 1.78 b | 31 ± 4.81 ab |
| Cd accumulation ($\mu$g Cd per plant) | 200.25 ± 24.72 c | 333.6 ± 63.84 b | 307 ± 10.39 b | 419.25 ± 66.69 a | 220.12 ± 9.85 c | 241.87 ± 37.08 c |
| Pb concentration (mg Pb $kg^{-1}$ DM) | 180 ± 22.25 a | 159 ± 26.81 b | 142 ± 12.96 b | 180 ± 18.65 a | 111 ± 10.80 c | 166 ± 26.42 ab |
| Pb accumulation ($\mu$g Pb per plant) | 1030.5 ± 132.49 c | 1953.11 ± 271.69 b | 1672 ± 142.20 b | 2345.75 ± 122.39 a | 870.25 ± 119.53 c | 1290.5 ± 190.96 c |

*$n = 4$ observations for each treatment.

§Values having the same letter within a row do not differ statistically at a $p \leq .05$ significance level (Tukey test).

the treatment of 150 mg N $\text{kg}^{-1}$ soil by using ammonium nitrate fertilizer, but certainly there are significant ($p \leq .05$) differences between these treatments for DM. This result is similar to that of Bennet et al. (1998), who reported a decrease of Ni and Zn concentrations in hyperaccumulator plants *Alyssum bertolonii* Nyar. and *Thlaspi caerulescens* when applying 100 mg N $\text{kg}^{-1}$ soil. However, other authors have reported the opposite effect (e.g., Lorenz et al., 1994; Mitchell et al., 2000; Kulli et al., 1999).

Meneses et al. (1999) reported Cd concentrations for various vegetation samples collected near a municipal waste incinerator were lower than 0.11 mg Cd $\text{kg}^{-1}$ DM. In Table 1, higher levels than this are shown for all treatments under study. On the other hand, lead was found at higher levels in tobacco plants (Table 1) than the typical levels which vary between 1 and 12 mg Pb $\text{kg}^{-1}$ DM, in plants reported by Fleming and Parle (1977) and Turkan et al. (1995). Thus, tobacco is able to remove Cd and Pb from contaminated soils and concentrate them in its harvestable parts, indicating that it could be very useful in phytoextraction of these heavy metals.

## Heavy Metal Accumulations

Additions of ammonium nitrate to soil increased aboveground Cd and Pb accumulation more than urea additions (Table 1). Treatment of 150 mg N $\text{kg}^{-1}$ soil using ammonium nitrate as N source induced the highest Cd and Pb accumulation, with $419 \pm 66.70$ $\mu$g Cd per plant and $2346 \pm 122.40$ $\mu$g Pb per plant (Table 1). It is also interesting to point out that no significant ($p \leq .05$) statistical differences were found between treatments with urea and control (Table 1) and that Pb accumulation due to the treatment of 50 mg N $\text{kg}^{-1}$ soil using urea as N source was lower than Pb accumulation from the control (Table 1).

Treatment with 150 mg N $\text{kg}^{-1}$ soil using ammonium nitrate as N source allows tobacco plants to accumulate 93% and 129% more Cd and Pb, respectively, than the control (Table 1). Moreover, treatment of 150 mg N $\text{kg}^{-1}$ soil using ammonium nitrate as N source allows tobacco plants to accumulate 76% and 179% more Cd and Pb, respectively, than by the effect of treatment of 50 mg N $\text{kg}^{-1}$ soil using urea as N source (Table 1).

## Pearson Correlations Between Variables

To better understanding of the Cd and Pb interaction and possible relationships between these heavy metals and DM production, correlation analyses were carried out using the five variables plus computed Cd/Pb ratios for concentrations and accumulations.

No significant ($p \leq .05$) Pearson correlations were found when correlations were performed for observations associated to control. However, significant ($p \leq .05$) bivariated correlations are found when all data were considered; that is, data corresponding to treatments with ammonium nitrate and urea as N sources, and control treatment (Table 2), or data considering only treatments with each fertilizer, ammonium nitrate (Table 3) or urea (Table 4).

In Table 2, the significant ($p \leq .001$) Pearson correlations between DM and Cd accumulation, and between

**TABLE 2** **Pearson Correlation Coefficients between Dry Matter (DM), Cd and Pb Concentrations, Cd and Pb Accumulations, and Cd/Pb Ratios for Concentrations and Accumulations in *Nicotiana tabacum* L. Associated with Six Soil Nitrogen Fertilization Treatments and Four Replications***

| Variable | DM | Cd concentration | Pb concentration | Cd accumulation | Pb accumulation | Cd/Pb (concentrations) |
|---|---|---|---|---|---|---|
| Cd concentration | −.395 | | | | | |
| | $p = .056$ | | | | | |
| Pb concentration | .025 | .294 | | | | |
| | $p = .908$ | $p = .164$ | | | | |
| Cd accumulation | .844 | .140 | .176 | | | |
| | $p \leq .0001$ | $p = .516$ | $p = .412$ | | | |
| Pb accumulation | .867 | −.169 | .498 | .829 | | |
| | $p \leq .0001$ | $p = .429$ | $p = .013$ | $p \leq .0001$ | | |
| Cd/Pb (concentrations) | −.371 | .383 | −.742 | −.151 | −.635 | |
| | $p = .074$ | $p = .064$ | $p \leq .0001$ | $p = .481$ | $p = .001$ | |
| Cd/Pb (accummulations) | −.366 | .394 | −.734 | −.141 | −.633 | .991 |
| | $p = .079$ | $p = .057$ | $p \leq .0001$ | $p = .512$ | $p = .001$ | $p \leq .0001$ |

*$n = 24$ observations.

**TABLE 3** **Pearson Correlation Coefficients between Dry Matter (DM), Cd and Pb concentrations, Cd and Pb Accumulations, and Cd/Pb Ratios for Concentrations and Accumulations in *Nicotiana tabacum* L. Associated with Three Soil Nitrogen Fertilization Treatments and Four Replications Using Ammonium Nitrate as N Source***

| Variable | DM | Cd concentration | Pb concentration | Cd accumulation | Pb accumulation | Cd/Pb (concentrations) |
|---|---|---|---|---|---|---|
| Cd concentration | .258 | | | | | |
| | $p = .418$ | | | | | |
| Pb concentration | .086 | .417 | | | | |
| | $p = .791$ | $p = .178$ | | | | |
| Cd accumulation | .545 | .950 | .385 | | | |
| | $p = .067$ | $p = .0001$ | $p = .216$ | | | |
| Pb accumulation | .455 | .485 | .925 | .564 | | |
| | $p = .137$ | $p = .110$ | $p \leq .0001$ | $p = .056$ | | |
| Cd/Pb (concentrations) | .142 | .602 | −.468 | .570 | −.350 | |
| | $p = .660$ | $p = .038$ | $p = .125$ | $p = .053$ | $p = .264$ | |
| Cd/Pb (accummulations) | .134 | .598 | −.473 | .564 | −.357 | .999 |
| | $p = .678$ | $p = .040$ | $p = .121$ | $p = .056$ | $p = .254$ | $p \leq .0001$ |

*$n = 12$ observations.

DM and Pb accumulations, suggest that Cd accumulation and Pb accumulation strongly depended on DM production in tobacco plants growing in Pb- and Cd-contaminated soils. Pb accumulation depends on Pb concentration ($p \leq .05$), and both Pb concentration and Pb accumulation showed significant ($p \leq .001$) negative correlations with Cd/Pb ratios for concentrations and accumulations. Additionally, significant ($p \leq .001$) correlations between Cd accumulation and between Pb accumulation, and Cd/Pb concentration and Cd/Pb accumulation ratios, were found (Table 2). These results suggest that N fertilization affects Cd and Pb concentrations and accumulations, their ratios, and aboveground biomass production (DM) in tobacco plants.

As expected, strong significant relationships between Cd concentration and accumulated Cd, and between Pb, concentration and accumulated Pb, were found (Table 3) when ammonium nitrate was used as N source. It is demonstrated that Cd concentration has significant ($p \leq .05$) correlations with Cd/Pb concentration and accumulation ratios, and that correlation between both ratios is also significant ($p \leq .001$) (Table 3). Furthermore, accumulated Cd ($r = .545, p = .067$) depends more on DM production than does accumulated Pb ($r = .455, p = .137$) (Table 3), which may indicate that Cd is more easily phytoavailable than Pb (Lasat, 2000). It is known that Cd occurs primarily in exchangeable form, whereas Pb occurs as soil precipitate (Lasat, 2000).

Significant ($p \leq .05$) correlations between Cd concentration and accumulated Cd, and between Pb concentration and accumulated Pb, were found (Table 4) when urea was used as N source. Both Pb concentration and Pb accumulation showed significant ($p \leq .001$) negative correlations with Cd/Pb concentration and accumulation ratios. It is unclear if Cd or Pb concentration and/or accumulation in urea-fertilized plants depend on DM production as occurred in the case of ammonium nitrate-fertilized tobacco plants.

Additions of ammonium nitrate fertilizer is a more effective agronomic practice to promote aboveground DM production, Cd accumulation, and Pb accumulation in tobacco plants than additions of urea fertilizer. It is perhaps due, in part, to ammonium nitrate fertilizer directly provides $NO_3^-$ ions to soil solution. However, it is unclear what happens with Cd and Pb concentrations in tobacco plants because there are no significant statistical relationships with aboveground DM due to fertilization practice with ammonium nitrate and urea, as shown in Tables 3 and 4, respectively.

In order to have a better understanding about the effects of soil nitrogen fertilization on Pb and Cd and DM production in tobacco plants, data were pooled for subsequent statistical analysis.

## Principal Components Analysis Results

All previous results suggest that tobacco plants are complex systems when used as metal accumulators. Thus, in order to simplify possible relationships

**TABLE 4** **Pearson Correlation Coefficients between Dry Matter (DM), Cd and Pb Concentrations, Cd and Pb Accumulations, and Cd/Pb Ratios for Concentrations and Accumulations in *Nicotiana tabacum* L. Associated to Two Soil Nitrogen Fertilization Treatments and Four Replications Using Urea as N Source***

| Variable | DM | Cd concentration | Pb concentration | Cd accumulation | Pb accumulation | Cd/Pb (concentrations) |
|---|---|---|---|---|---|---|
| Cd concentration | −.207 | | | | | |
| | $p = .623$ | | | | | |
| Pb concentration | .073 | .144 | | | | |
| | $p = .863$ | $p = .734$ | | | | |
| Cd accumulation | .283 | .879 | .173 | | | |
| | $p = .497$ | $p = .004$ | $p = .683$ | | | |
| Pb accumulation | .262 | .119 | .972 | .242 | | |
| | $p = .531$ | $p = .780$ | $p \leq .0001$ | $p = .564$ | | |
| Cd/Pb (concentration) | −.218 | .199 | −.926 | .096 | −.918 | |
| | $p = .604$ | $p = .637$ | $p = .001$ | $p = .821$ | $p = .001$ | |
| Cd/Pb (accumulation) | −.178 | .180 | −.921 | .096 | −.929 | .982 |
| | $p = .673$ | $p = .669$ | $p = .001$ | $p = .822$ | $p = .001$ | $p \leq .0001$ |

*$n = 8$ observations.

between and among all seven variables and 24 cases, principal component analysis (PCA) was conducted on whole database ($n = 24$). PCs were extracted from a correlation matrix constructed with the 24 observations for all seven variables under study, which are shown in Tables 2 to 5. Significant factor loadings were obtained as suggested by Ovalles and Collins (1988) and Gutiérrez-Acosta et al. (2002), after varimax rotation.

Three PCs explained 99% of total variance (Table 5). The first PC explains almost 41% of total variance and was positively correlated with Pb concentration and negatively correlated with Cd/Pb ratios for concentrations and accumulations, suggesting that higher Pb concentrations depress both ratios, that is, higher Pb concentrations could be negatively affecting Cd concentration and/or accumulation. The second PC explains 37% of total variance and is negatively correlated with DM, Cd accumulation, and Pb accumulation, indicating that tobacco plants are Cd and Pb accumulators and are useful for removing these metals from soils, which agree with the previous studies by Kayser et al. (2000). It is very apparent that Cd concentration defines the PC3 structure, explaining 21% of total variation.

**TABLE 5** **Loadings or Correlations between the First Three Principal Components (CPs) and the Variables Dry Matter (DM), Cd and Pb Concentrations, Cd and Pb Accumulations, and Cd/Pb Ratios for Concentrations and Accumulations in *Nicotiana tabacum* L. Associated with Six Soil Nitrogen Fertilization Treatments and Four Replications Extracted from a Varimax-Normalized Matrix***

| Variable | PC1§ | PC2 | PC3 |
|---|---|---|---|
| DM | 0.110 | **−0.931** | −0.341 |
| Cd concentration | −0.123 | 0.053 | **0.987** |
| Pb concentration | **0.899** | −0.078 | 0.421 |
| Cd accumulation | 0.021 | **−0.977** | 0.196 |
| Pb accumulation | 0.499 | **−0.856** | −0.057 |
| Cd/Pb (concentrations) | **−0.941** | 0.185 | 0.267 |
| Cd/Pb (accumulations) | **−0.939** | 0.178 | 0.278 |
| Explained variance | 20.852 | 20.629 | 10.458 |
| Proportion of total | 0.407 | 0.375 | 0.208 |
| Cumulative variance | 0.407 | 0.782 | 0.990 |

*$n = 24$ observations.

§Boldfaced loadings are >0.7.

When distribution of treatments is examined through the orthogonal plane yielded by the first two PC's, three clusters of treatments are found (Figure 1). The control group (treatment 1) is located in the upper-central part of the graph (lower DM values, lower Cd and Pb accumulations, medium Pb concentrations, and medium Cd/Pb ratios for concentrations and accumulations); whereas the urea treatments (treatments 5 and 6) (lower Pb concentration, and medium DM values, and medium Cd and Pb accumulations) are clustered in the middle-left portion; and finally, the treatments with ammonium nitrate (treatments 2, 3, and 4) are grouped in the lower-central portion of the orthogonal plane (higher DM values, higher Cd and Pb accumulations, medium Pb concentrations, and medium ratios for concentrations and accumulations).

The plot yielded by PC2 and PC3 show the same three clusters of treatments (Figure 2). It is remarkable that treatments with ammonium nitrate (treatments 2, 3, and 4) are grouped in the left portion of the orthogonal plane, widely covering the PC3 range, that is,

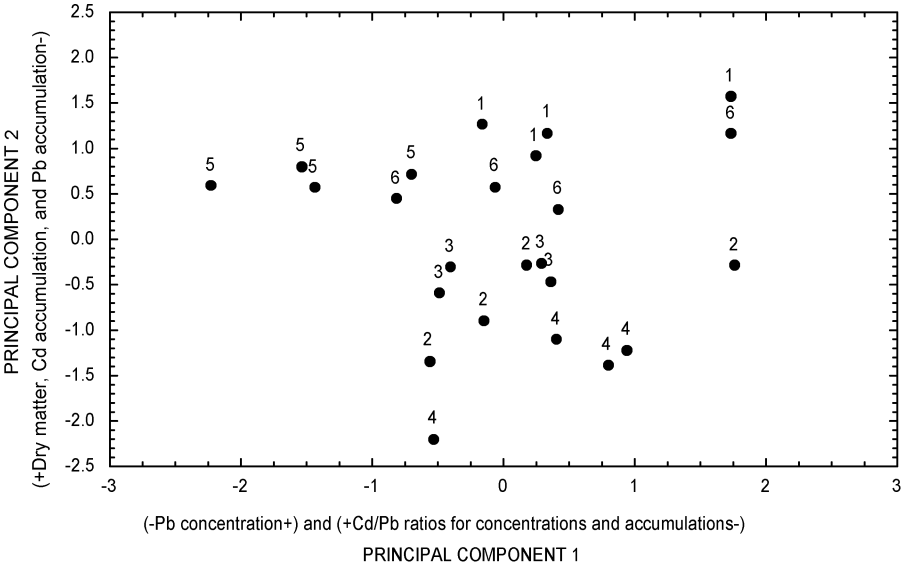

**FIGURE 1** Treatment positions in the orthogonal plane defined by the first two principal components (PCs). PC1 is positively correlated with Pb concentration and negatively correlated with Cd/Pb ratios for concentrations and accumulations. PC2 is negatively correlated with dry matter (DM), Cd accumulation, and Pb accumulation. Numbers are identifying treatments: 1 is control treatment (no fertilization); 2, 3, and 4 correspond to 50, 100, and 150 mg N $kg^{-1}$ soil treatments, respectively, by using ammonium nitrate ($NH_4NO_3$) as N source; and 5 and 6 correspond to 50, and 100 mg N $kg^{-1}$ soil treatments, respectively, by using urea [$CO (NH_2)_2$] as N source.

the range of Cd concentration. This demonstrates that when Cd concentration decreases, DM as well as both accumulated Cd and accumulated Pb also decrease. Additionally, the fact that treatments with both urea (treatments 5 and 6) and control (treatment 1) are clustered on the right portion of the orthogonal plane suggests that tobacco plants growing on soils fertilized with urea or unfertilized were not as able to produce as much aboveground DM and to accumulate as much Cd and Pb as plants growing on soil fertilized with ammonium nitrate.

Results from PCA allow us better understand on the role of Cd and Pb concentrations or accumulations, and their interactions with DM production of tobacco plants used to phytoextract Cd and Pb from soils.

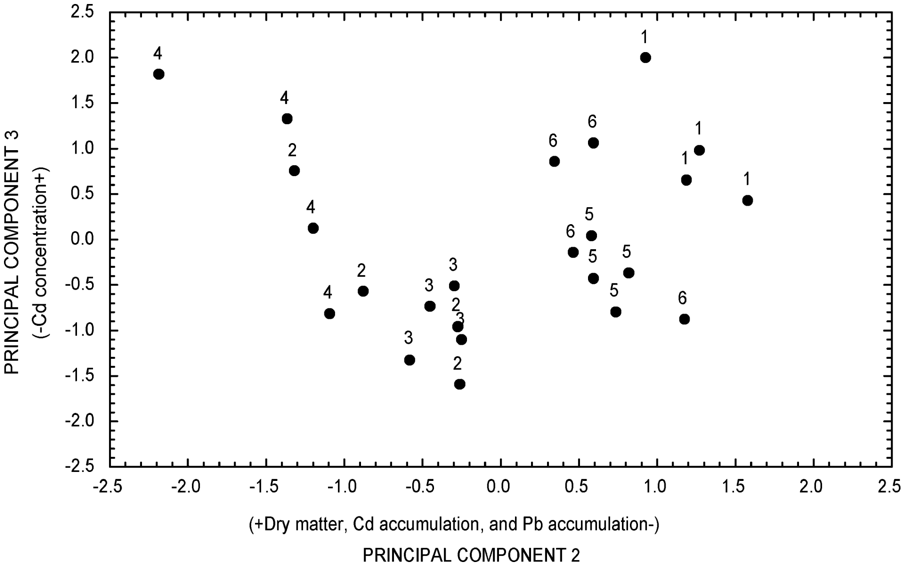

**FIGURE 2** Treatment positions in the orthogonal plane defined by the second and third principal components (PC1 and PC3). PC2 is negatively correlated with dry matter (DM), Cd accumulation, and Pb accumulation. PC3 structure is defined positively by Cd concentration. Numbers are identifying treatments: 1 is control treatment (no fertilization); 2, 3, and 4 correspond to 50, 100, and 150 mg N $kg^{-1}$ soil treatments, respectively, by using ammonium nitrate ($NH_4NO_3$) as N source; and 5 and 6 correspond to 50, and 100 mg N $kg^{-1}$ soil treatments, respectively, by using urea [$CO (NH_2)_2$] as N source.

## CONCLUSIONS

Tobacco is able to remove Cd and Pb from contaminated soils and concentrate them in its harvestable part, that is, it could be very useful in phytoextraction of these heavy metals.

Increasing additions of ammonium nitrate to soil (50, 100, and 150 mg N $kg^{-1}$ soil) significantly ($p \leq .05$) increased aboveground Cd and Pb accumulation during a 50-day experimental period, wherely increasing additions of urea to soil (50 and 100 mg N $kg^{-1}$ soil) did not show these effects at the same significant levels.

Increasing additions of ammonium nitrate to soil shows as dry matter increases, both accumulated Cd and accumulated Pb also increase when tobacco plants are growing under Pb- and Cd-contaminated soil conditions.

Higher Pb concentrations depress Cd/Pb ratios for concentrations and accumulations, suggesting that Pb negatively affects Cd concentration and/or accumulation in above ground tobacco biomass.

As Cd concentration decreases, dry matter as well as both accumulated Cd and accumulated Pb also decrease in aboveground tobacco biomass, when soil is fertilized with ammonium nitrate.

## REFERENCES

Bennet, F. A., E. K. Tyler, R. R. Brooks, P. E. H. Gregg, and R. B. Stewart. 1998. Fertilization of hyperaccumulators to enhance their potential for phytoremediation and Phytomining. *In Plants That Hyperacumulate Heavy Metals*, ed. R. R. Brooks, 249–260. Ed. Cad International.

Brooks, R. R. 1998. Phytochemistry of hyperaccumulators. In *Plants That Hyperacumulate Heavy Metals*, ed. R. R. Brook, 15–53. Ed. Cad International. Q1

Brown, S. L., R. L. Chaney, J. S. Angle, and A. J. M. Baker. 1994. Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc and cadmium contaminated soil. *J. Environ. Qual*. *23*:1151–1157.

Chang, Y., H. M. Seip, and H. Vennemo. 2001. The environmental cost of water pollution in Chongqing, China. *Environ. Dev. Econ*. *6*:312–313.

Chien, S. H., G. Carmona, L. I. Prochnow, and E. R. Austin. 2003. Cadmium availability from granulated and bulk-blended phosphate-potassium fertilizers. *J. Environ. Qual*. *32*:1911–1914.

Codling, E. E., and J. C. Ritchie. 2005. Eastern gamagrass uptake of lead and arsenic from lead arsenate contaminated soil amended with lime and phosphorus. *Soil Sci. 170*:413– 423.

Depledge, M. H., J. M. Weeks, and P. Bjerregard. 1994. Heavy metals. In *Handbook of Ecotoxicology*, Vol. 2, ed. P. Calow, 79–105. Oxford, UK. Blackwell Scientific Publications.

Ebbs, S. D., M. M. Lasat, D. J. Brady, J. Cornish, R. Gordon, and L. V. Kochian. 1997. Phytoextraction of cadmium and zinc from a contaminated soil. *J. Environ. Qual*. *26*:1424–1430.

Fleming, G., and P. Parle. 1977. Heavy metals in soils, herbage and vegetables from an industrialized area west of Dublin City. *Ir. J. Agric. Res. 16*:35–48.

Forstner, U. 1995. Land contamination by metals: Global scope and magnitude of problem. In: *Metal Speciation and Contamination of Soil*, ed. H. E. Allen, C. P. Huang, G. W. Bailey, and A. R. Bowers, 1–33. Lewis Publishers. Q2

Gutiérrez-Acosta, F., R. D. Valdez-Cepeda, and F. Blanco-Macías. 2002. Multivariate analysis of cactus pear (*Opuntia*spp.) fruits from a germplasm collection. *Acta Hort. 581*:111–118.

Kayser, A., K. Wenger, A. Keller, W. Attinger, H. R. Felix, S. K. Gupta, and R. Schulin. 2000. Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: The use of NTA and sulfur amendments. *Environ. Sci. Technol. 34*:1778–1783.

Kulli, B., M. Balmer, R. Krebs, B. Lothenbach, G. Geiger, and R. Schulin. 1999. The influence o nitriloacetate on heavy metal uptake of lettuce and ryegrass. *J. Environ. Qual. 28*:1699–1705.

Lasat, M. M. 2000. Phytoextraction of metals from contaminated soil: A review of plant/soil/metal interaction and assessment of pertinent agronomic issues. *J. Hazardous Substance Res. 2*:1–25.

Levine, M. B., A. T. Stall, G. W. Barret, D. H. Taylor. 1989. Heavy metal concentrations during ten years of sludge treatment to an old-field community. *J. Environ. Qual. 18*:411–418.

Lorenz, S. E., R. E. Hamon, S. P. McGrath, P. E. Holm, and T. H. Christensen. 1994. Applications of fertilizer cations affect cadmium and zinc concentrations in soil solutions and uptake by plants. *Eur. J. Soil. Sci. 45*:159–165.

Marschner, H. 1986. *Mineral Nutrition of Higher Plants*. London: Academic Press.

Meneses, M., J. M. Llovet, M. Schumacher, and J. L. Domingo. 1999. Monitoring metals in the vicinity of a municipal waste incinerator: Temporal variations in soils and vegetation. *Sci. Total Environ. 226*:157–164.

Merwin, I., P. T. Pruyne, J. G., Ebel, Jr., K. L. Manzell, and D. J. Lisk. 1994. Persistence, phytotoxicity, and management of arsenic, lead and mercury residue in old orchard soils of New York state. *Chemosphere 29*:1361–1367.

Miteva, E., S. Maneva, D. Hristova, and P. Bojinova. 2001. Heavy metal accumulation in virus-infected tomatoes. *J. Phytopatol. 149*:179–184.

Mitchell, L. G., C. A. Grant, and G. J. Racz. 2000. Effect of Nitrogen application on concentration of cadmium and nutrient ions in soil solution and durum wheat. *Can. J. Soil Sci. 80*:107–115.

Mortvedt, J. J. 1996. Heavy metal contaminants in inorganic and organic fertilizers. *Fert. Res. 43*:55–61.

Olsen, S. R. 1983. Interacciones de los micronutrientes. In *Micronutrientes en la Agricultura*, ed. J. J. Montvedt, P. M. Giordano, and W. L. Lindsay AGT Editor. Q3

Ovalles, F. A., and M. E. Collins. 1988. Variability of northwest Florida soils by principal components analysis. *Soil Sci. Soc. Am. J. 52*:1430–1935.

Peryea, F. J., and T. L. Creger. 1995. Vertical distribution of lead and arsenic in soils contaminated with lead arsenate pesticide residue. *Water Air Soil Pollut*. *79*:1–10.

Raskin, I., R. D. Smith, and D. E. Salt. 1997. Phytoremediation of metals: Using plants to remove pollutants from the environment. *Curr. Opinion Biotechol*. *8*:221–226.

Reeves, R. D., and A. J. M. Baker. 2000. Metal accumulating plants. In *Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment*, ed. I. Raskin and B. D. Ensley. New York: John Wiley. Q4

Segura-Muñoz, S. I., A. da Silva Oliveira, M. Nikaido, T. M. B. Trevilato, A. Bocio, A. M. M. Takanayagui, and J. L. Domingo. 2006. Metal levels in sugar cane (*Saccharum* spp.) samples from an area under the influence of a municipal landfill and a medical waste treatment system in Brazil. *Environ. Int*. *32*:52–57.

Sharma, P, and R. Shanker-Dubey. 2005. Lead toxicity in plants. *Braz. J. Plant Physiol*. *17*:35–52.

Shepard, H. H. 1951. *The Chemistry and Action of Insecticides*, 1st ed. New York: McGraw-Hill.

StatSoft, Inc. 2000. STATISTICA for Windows [computer program manual]. Tulsa, OK: StatSoft.

Temmerman, L. O., M. Hoening, and P.O. Scokart. 1984. Determination of 'normal' levels and upper limit values of trace elements in soils. *Z. Pflanzen. Bodenk*. *147*:687–694.

Tisdale, S. L., W. L. Nelson, J. D. Beaton, and J. L. Havlin (eds.). 1993. *Soil Fertility and Fertilizers*. New York: Macmillan.

Turkan, I., E. Henden, U. Celik, and S. Kivilcim. 1995. Comparison of moss and bark samples as biomonitors of heavy metals in a highly industrialized area in Izmir, Turkey. *Sci. Total Environ*. *166*:61–67.

Vassilev, A. 2002. Physiological and agroecological aspects de cadmium interactions with barley plants: An overview. *J. Central Eur. Agric*. *4*:65–75.