

Effects of Natural Hydrosoluble Chelates of Three Plant Species on the Mobilization of Heavy Metals

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Heavy metals are concentrated in the superficial horizon of the soil profile due to cycling through vegetation, atmospheric deposits and adsorption on organic matter in the soil. Their presence at these levels is due to the fact that pedogenetic processes require hundreds of years to redistribute them in the profile (Alloway, 1990). However, human intervention has altered this pattern in time. At present, the plant-soil system has undergone short-term accumulation of these metals due to indiscriminate use of fertilizers and pesticides, as well as to air pollution. This occurs despite of on going phenomena such as leaching, erosion, volatilization, and exportation of minerals by cultivation, which bring about a loss of metals from the soil. The organic horizon of forest soils near urban centers acts as a sink for heavy metals, specially for those that have been carried in the air as suspended particles, originated by combustion of fossil fuels (Pouyat and McDonnell, 1991). The possible ecotoxicity of heavy metals is primarily due to their concentration and speciation. Metal ions are distributed in the aqueous and solid phases of the soil according to the laws of chemical equilibrium. However, several factors influence this equilibrium, among them the presence of organic complexes that can chelate metal ions (Kuiters and Mulder, 1990). Forest soils produce a large amount of hydrosoluble organic substances, particularly in the more superficial horizons. These substances may be leached products of litter decomposition, radical exudates, and compounds resulting from microbial neosynthesis. Their complex forming capacity is related to the amount and types of chemical functional groups they possess, such as carboxyl, phenyl, hydroxyl, and amino radicals (Kuiters and Mulder, 1990). Since some chelating agents are hydrosoluble, it is quite possible that, on the one hand, when they percolate they can transport ions to the water table or to deeper soil strata (Driscoll, et al., 1994), and on the other hand they may increase the metals bio-availability. Several investigations (Pouyat and McDonnell, 1991; Pohlman and McColl, 1988) have shown that leached products from leaf litter and other organic substances such as weeds, grasses, and blood meal can differentially dissolve and chelate Cd, Cu, Pb, Zn, Mn, Fe and Al. The differences are caused by each substance's content of aliphatic carboxylic acids and amino acids, which facilitate metals movement in the soil profile. Likewise, several studies (Kögel, et al., 1998; Kuiters and Mulder,

1990) present clear evidence that polyphenols and labile carbohydrates are the principal compounds responsible for metal mobilization. However, it is safe to say that, in general, there are few studies relating to the desorption of ions by the action of litter lixiviates in forest soils (Kuiters and Sarink, 1986; Malcom and McCracken, 1968; Pohlman and McColl, 1986, 1988). On the other hand, during the past decade there has been a growing interest in lixiviates obtained from animal and vegetable matter, since it has been suggested that they can be used in the recovery of soils polluted with heavy metals. Forest soils near urban areas are subject to continually operating negative impacts, such as deforestation, induced fires, pollution, grazing and urbanization. Priority should be given to understanding the role that vegetation types play in the dynamics of heavy metals in these soils, since the increase or decrease of the metal bio availability and lixiviation depend on the plant species established in the area.

High rates of metal leaching in the Ajusco Mountains, near Mexico City, may have a great environmental impact. The Ajusco is the physiographical area that most contribute to recharging the aquifers of the Mexican basin, and these aquifers are used to provide Mexico City with potable water. Increased availability of heavy metals may have an inhibitory effect on microbial communities that mineralize organic matter (Quiroz and Ilangovan, 1999), specially on microorganisms involved in the nitrogen cycle (Gupta, 1991). The study area selected in the Ajusco Mountains has been subject to fires and illegal logging. These events have, in some places, caused the oak grove dominated by *Quercus rugosa* to be displaced by clump forming grasses such as *Muhlenbergia macroura*. *Buddleia cordata* dominates small basins, as well as the transition zone between grassland and oak woodland. These three species have a clear influence on the chemical speciation of heavy metals (Quiroz and Ilangovan, 1999); therefore, what is needed is a more precise understanding of the role played by the mentioned plant communities in heavy metal dynamics. The main objective of this study is to identify differences in the desorption of the metals Pb, Mn, and Zn by leaf litter lixiviates coming from the *Quercus rugosa*, *Buddleia cordata*, and *Muhlenbergia macroura* communities. Also measured the relative levels of total hydrosoluble phenols and total labile polysaccharides, present in the organic-mineral horizons (Ah) to analyse its contribution to heavy metal mobilization.

MATERIALS AND METHODS

The study area is part of a 730 hectare lot known as Lomas del Seminario, in the middle point of the Ajusco Mountains, south of Mexico City. Altitude above sea level is between 2,400 and 2,800 meters. The climate of the area is Cb(w), continental sub humid with summer showers. Average rainfall is around 1000 mm annually (Soberón et al., 1991), as interpolated from pluviometric data obtained from a peripheral weather stations (Ciudad Universitaria, Ajusco-Tlalpan, Desviación Alta and Vivero Peña Pobre). The vegetation types of the study area are mainly, pine-oak forests (*Quercus rugosa*, *Pinus montezumae*, *P. teocote*) at the

highest elevation, followed by oak forests (*Q. rugosa*, *Q. laurina*, *Q. crassipes*) and scrub (where *Sedum oxypetalum*, *Senecio praecox*, and several species of Asteraceae belonging to the genus *Dahlia*, *Tagetes* and *Eupatorium* are the dominants). As far as soils are concerned, litosols and andosols are the most abundant, with feozems in some places (DDF, 1989). Soil and leaf litter samples were collected according to the following protocol. For each species, six 100 meter transect lines were placed, and samples were taken at 25 meters intervals. Only three samples were taken per transect in order to avoid edge effect. Leaf litter samples of *Quercus rugosa*, *Buddleia cordata*, and *Muhlenbergia macroura* were taken during the dry season. Lixiviates were obtained in laboratory conditions using the procedure suggested by Kuiters and Sarink (1986), which simulates the effect of rainfall on dry leaf litter. Triplicates of five grams fresh litter were mixed with 250 mL de-ionized water, and the mixture was left to rest for 20 hours in the dark. Afterwards they were filtered through a Whatman number 40 filter paper, followed by filtration through a 0.45 µm membrane. This treatment was repeated eight days later using left over saturated litter from the first treatment, stored at 10°C. pH values and concentrations of Pb, Mn, and Zn were obtained for all leached solutions. In order to determine the desorption capacity, samples of the organic Ah horizon (0-20cm) were taken at the same locations where litter was collected. The samples were subdivided into two sets: in one set, total hydro soluble phenols were quantified using the Folin-Cicolteau method (Box, 1983); total labile polysaccharides were also quantified (Dubois et al., 1956). The samples of the second set (10 g) were mixed with 25 mL of lixivate and the suspensions were stored 20 hours in the dark. When the rest period was over, pH values of the suspension were determined, later the suspension was put in a centrifuge at 2,500 r.p.m., for 20 minutes, and the aqueous part was filtered with a 0.45 µm membrane. Finally, heavy metals in the filtrate were quantified by means of an inductive coupling emission plasma spectrometry, and by atomic absorption. De-ionized water was used as a control sample. Analysis of variance (ANOVA) was used to detect significant differences between the chelated metal content of lixiviates from different plant species. Correlation analysis was applied to pH and concentration of heavy metals in solution, for each species.

RESULTS AND DISCUSSION

The lixiviates of the three studied vegetal species and after lixivate mobilization on soil samples heavy metal concentration (Pb, Mn, and Zn) were showed in tables 1 and 2. Considering the detected heavy metals levels in the fractions exchangeable and adsorbed to manganese reported by Quiroz and Ilangovan (1999), the lixiviates of *M. macroura* mobilized a greater proportion of lead, an amount of zinc similar to *Q. rugosa*, and a smaller amount of manganese than *B. cordata*. Lixiviates of *Q. rugosa* was the one that mobilized the smaller proportion of lead of the three species considered in this study. Nevertheless this mobilization stayed constant throughout the time of experiment. After eight days of initiate the experiment, it mobilized a similar amount of zinc that *M. macroura*. The lixiviates

Table 1. Heavy metal concentration in leaf litter extract samples of *Buddleia cordata*, *Muhlenbergia macroura* and *Quercus rugosa*.

| Plant species | Pb | Mn | Zn | Pb | Mn | Zn |
|--------------------|-------------------------|----------------|----------------|-----------------------|---------------|---------------|
| | $\mu\text{g/L}$ (20 hr) | | | $\mu\text{g/L}$ (8 d) | | |
| <i>B. cordata</i> | 7 \pm 5.7 | 114 \pm 90.1 | 141 \pm 50.0 | n.d | 36 \pm 21.2 | 53 \pm 21.9 |
| de-ionized water | n.d | 2 | 14 | n.d | n.d. | 25 |
| <i>M. macroura</i> | 7 \pm 4.9 | 55 \pm 7.7 | 104 \pm 12.7 | n.d | 51 \pm 25.1 | 78 \pm 14.8 |
| De-ionized water | n.d | 2 | 14 | n.d | n.d | 25 |
| <i>Q. rugosa</i> | n.d | 75 \pm 33.2 | 63 \pm 3.5 | n.d | 34 \pm 28.3 | 44 \pm 10.0 |
| de-ionized water | n.d | 2 | 14 | n.d | n.d | 25 |

Average of nine samples \pm standard deviation.

n.d.: not detectable

Table 2. Total metal concentration in the suspensions of *Buddleia cordata*, *Muhlenebergia macroura* and *Quercus rugosa*.

| Plant species | Pb | Mn | Zn | Pb | Mn | Zn |
|--------------------|-------------------|-----------------|----------------|---------------|---------------|---------------|
| | $(\mu\text{g/L})$ | | | | | |
| | 20 hr | | | 8 d | | |
| <i>B. cordata</i> | 120 \pm 19.4 | 166 \pm 150.3 | 100 \pm 67.9 | 10 \pm 15.4 | 60 \pm 49.6 | 30 \pm 26.8 |
| Control | 80 | 100 | 40 | n.d. | 10 | 20 |
| <i>M. macroura</i> | 80 \pm 15.8 | 60 \pm 25.0 | 90 \pm 35.4 | 10 \pm 8.3 | 20 \pm 7.3 | 60 \pm 14.1 |
| Control | 50 | 40 | 40 | 10 | 10 | 40 |
| <i>Q. rugosa</i> | 80 \pm 25.5 | 60 \pm 48.2 | 30 \pm 24.0 | 30 \pm 17.2 | 50 \pm 40.0 | 20 \pm 15.8 |
| Control | 60 | 40 | 20 | 10 | n.d. | 10 |

Soil samples were mixed with leaf litter lixiviate (25 mL of Leaf lixiviate /10g of soil), after 20 hours and eight days of mobilization process the soil samples were analyzed for heavy metals. Average of nine samples \pm standard deviation. n.d.: not detectable.

of *B. cordata* mobilized a greater amount of manganese than the other two considered species (Table 3). When comparing the amount of heavy metals mobilized by the lixiviates of the species studied in this study against the oak and poplar species studied by Kuiters and Mulder (1990), we found that there was a similar lead mobilization due to *M. Macroura*. The capacity of lead mobilization of the lixiviates varied significantly between species, in the time, and in the interaction (Table 4). In the case of zinc and manganese, the capacity of mobilization of the lixiviates between species and with time did not vary significantly.

The soil with leaf litter of *B. cordata*, showed a greater amount of hydro soluble total phenols in soils than *Q. rugosa* and *M. macroura* (Table 5). The soil with leaf litter of *Q. rugosa* had a greater relative amount of total labile polysaccharides, than of *B. cordata* and *M. macroura*. When carrying out an analysis of correlation between amount of phenols and total labile polysaccharides in the soil, and heavy metals for each species, we found the following result: a moderate positive correlation in phenols ($r = 0.69$) and labile polysaccharides ($r = 0.74$) for the case of *Q. rugosa*.

Table 3. Sequential extraction of heavy metals in the horizon Ah (0-20 cm).

| Fraction | <i>Q. rugosa</i> | | | <i>B. Cordata</i> | | | <i>M. macroura</i> | | |
|--------------------------|------------------|--------------|-----|-------------------|--------------|-----|--------------------|--------------|-----|
| | Pb | Mn (µg/g) | Zn | Pb | Mn (µg/g) | Zn | Pb | Mn (µg/g) | Zn |
| Exchangeable | 0.1 | 14.6 | 1.0 | 0.4 | 16.8 | 2.3 | n.d. | 1.9 | 0.4 |
| Manganese Oxide Bound | 0.2 | 53.7 | 0.9 | 0.2 | 34.5 | 2.4 | 0.2 | 27.2 | 0.8 |
| Total | 0.3 | 68.3 | 1.9 | 0.6 | 51.3 | 4.7 | 0.2 | 29.1 | 1.2 |

Values represent average of nine samples. n.d. = not detectable.

Table 4. Analysis of variance (ANOVA):Relation between Pb in the plant species and time of lixiviate mobilization in the soil.

| Lead | | | | |
|---------------------|------|----------|----------|--------|
| Source of variation | D.F. | S.S. | F | P |
| Specie | 2 | 1111.907 | 23.60877 | 0.0000 |
| Time | 1 | 1791.130 | 38.03047 | 0.0000 |
| Interaction | 2 | 1252.352 | 26.59078 | 0.0000 |
| Error | 48 | | | |

The differences observed for the factor time must to a large extent that the amounts of heavy metal to the eight days of the processing, they diminished in several orders of magnitude in relation to the processing of 20 hours (Table 2), mainly in the case of lead. The significant differences obtained for the factor species, we can attribute to the amount of hydro soluble phenols in the soil, as well as to the values of pH of lixiviates. Since the organic horizon (Ah) of *B. cordata* showed a greater relative amount of phenols, and the soil suspensions with more acidic values was for *M. macroura*, this species were those that comparatively mobilized a greater amount of heavy metals in average. On the other hand, the soil that showed a greater proportion of labile polysaccharides, was that who received the lixiviates of *Q. rugosa*, and it is the one that comparatively mobilized a smaller amount of heavy metals; the previous behavior, we can be attributed to the type of monosaccharides that possibly dominated in the dissolution of the lixiviate, since according to Fischer et al., (1998), "the hexoses that have a greater capacity to mobilize heavy metals, belong to the group of the acid hexoses (uronic acids)", is to say carbohydrates of structural type, which will enter dissolution, after the corresponding polymers like cellulose have been hydrolyzed.

Table 5. Concentration of phenols and labile polysaccharides in the soil with leaf litter samples. of *Buddleia cordata*, *Muhlenbergia macroura* y *Quercus rugosa*.

| Plant species | Phenols (mg/g) | Polysaccharides (%) |
|--------------------|----------------|---------------------|
| <i>B. cordata</i> | 41 | 2.03 |
| <i>M. macroura</i> | 20 | 0.70 |
| <i>Q. rugosa</i> | 34 | 2.53 |

Values represent average of 24 different samples

Table 6. pH changes in the leaf litter lixiviate suspension with soil samples after 20 hours and 8 days.

| Plant species | Leaf litter extract sample pH | Leaf litter extract-soil sample pH | |
|--------------------|-------------------------------|------------------------------------|-------------|
| | | After 20 hr | After 8 d |
| <i>B. cordata</i> | 6.16 | 7.39 (7.23) | 7.00 (7.66) |
| <i>M. macroura</i> | 6.37 | 6.89(7.23) | 5.95(7.66) |
| <i>Q. rugosa</i> | 6.74 | 7.38(7.23) | 6.76(7.66) |

Control pH values are indicated in the parenthesis

Table 7. Correlation coefficient analysis ($p < 0.05$) between heavy metals and pH variation of each plant species.

| Plant species | Pb | Mn | Zn |
|--------------------|---------|---------|-------|
| <i>B. cordata</i> | - 0.708 | - 0.541 | 0.358 |
| <i>Q. rugosa</i> | 0.210 | 0.407 | 0.415 |
| <i>M. macroura</i> | 0.400 | 0.336 | 0.249 |

One of the chemical variables of the soil that contribute to the heavy metal mobilization, is the value of the pH dissolution of the soil (Wytenbach et al., 1991). Most of the authors reported that greater acidity of soil directly related to greater heavy metal mobilization. In our study we found that the values of pH determined in the extracts of leaf litter (Table 6), are ranging from 6.16 - 6.74, whereas the pH values obtained for the mixture of leaf litter extracts and soil oscillated between slightly acid and moderately alkaline (6.89 - 7.39). Analysis of correlation between the pH of the suspension at 20 hours and heavy metals for each species (Table 7) indicate that weak correlation for lead for *M. Macroura*. Similarly, weak correlation for manganese and zinc in the case of *Q. rugosa*; and a moderate negative correlation for lead and manganese in the case of *B. cordata*.

The correlation analysis obtained showed that pH playing important role in the mobilization of heavy metals. Values of pH for the different suspensions (Table 6) were slightly acid and/or moderately alkaline. Our results also showed a

significant negative correlation for of lead and manganese. Initially the extracts of leaf litter show acid values, when contact with the soil the pH changes are due to the buffer capacity of the soil and partly due to hydro soluble organic compounds or microbial activity of the soil (Owen et al., 1994). With respect to the previous results, we can conclude that the metal mobilization differences observed in relation to the variable time might be due to the presence of simple hydro soluble phenolic compounds and labile polysaccharides. The significant participation of *M. macroura* in the heavy metal mobilization was to us very novel, since we hoped that *Q. rugosa* or *B. cordata* was the species that excelled, because they contribute to the soil with important amounts of hydro soluble phenols. The study demonstrate that low molecular weight hydro soluble organic chelates contained in *M. Macroura* and high molecular weight organic compounds that exist in the ecosystem with other variables which play an important role in the heavy metal mobilization in the soil ecosystem. Based on the results of the study it was concluded that disappearance of oak grove vegetation and short term substitution of this community by *M. macroura* or *B. cordata*, would result in significant increase in the mobilization and the bio availability of non essential heavy metals in the soil.

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