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MOLECULAR INDICATORS OF SOIL HUMIFICATION AND INTERACTION WITH HEAVY METALS

Teresa W.-M. Fan[1], Richard M. Higashi[2], Teresa Cassel[3], Peter Green[4], and Andrew N. Lane[5]
[1]Dept. of Chemistry, Univ. of Louisville, Louisville, KY 40208; e-mail: teresa.fan@louisville.edu
[2]Center for Health & Environment, Univ. of California, Davis, CA 95616
[3]Crocker Nuclear Lab., Univ. of California, Davis, CA 95616
[4]Civil & Environ. Engineering, Univ. of California, Davis, CA 95616
[5]The J.G. Brown Cancer, Univ. of Louisville, Louisville, KY 40202

For stabilization of heavy metals at contaminated sites, interaction of soil organic matter (SOM) with heavy metal ions is critically important for long-term sustainability, a factor that is poorly understood at the molecular level. Using ^{13}C- and ^{15}N-labeled soil humates (HS), we investigated the turnover of five organic amendments (celluose, wheat straw, pine shavings, chitin, and bone meal) in relation to heavy metal ion leaching in soil column experiments. The labeled molecular substructures in HS were examined by multinuclear 2-D NMR and pyrolysis GC-MS while the element profile in the leachates was analyzed by ICP-MS. Preliminary analysis revealed that peptidic and polysaccharidic structures were highly enriched, which suggests their microbial origin. Cd(II) leaching was significantly attenuated with humification of lignocellulosic materials. Correlation of ^{13}C and ^{15}N turnovers of HS substructures to metal leaching is underway.

Introduction

The myriad of human activities including strategic and energy development at various DOE installations have resulted in the contamination of soils and waterways that can seriously threaten human and ecosystem health. Development of efficacious and economical remediation technologies is needed to ameliorate these immensely costly problems. Bioremediation (both plant and microbe-based) has promising potential to meet this demand but still requires advances in fundamental knowledge. For bioremediation of heavy metals, the three-way interaction of plant root, microbial community, and soil organic matter (SOM)[1] in the rhizosphere is critically important for long-term sustainability but often underconsidered. Particularly urgent is the need to understand processes that lead to metal ion stabilization in soils, which is crucial to all of the goals of bioremediation: removal, stabilization, and transformation.

This work builds on the knowledge and tools that we have generated relating root exudation and metabolism (Fan, Lane et al. 1997; Fan, Baraud et al. 2000; Fan, Lane et al. 2001; Shenker, Fan et al. 2001) as well as SOM structures (Higashi, Fan et al. 1998; Fan, Higashi et al. 2000) to metal mobilization and accumulation. Both root exudates and SOM (humic substances or HS in particular) are the major organic players in metal ion interactions in soils and sediments. We found that root-exudated phytosiderophores (i.e. mugineic acids, known to be involved in transition metal uptake into plants) (Clark, Römheld et al. 1988; Marschner 1995) were not associated with the accumulation of heavy metals such as Cd.

On the other hand, isolated soil HS appeared to facilitate metal sequestration while alleviating Cd-induced growth reduction in wheat plants (Baraud, Fan et al. submitted). The ability of HS to protect plants from toxic effects of excess metals has also been reported previously (Strickland, Chaney et al. 1979; Kinnersley 1993). The above two effects are seemingly contradictory and are difficult to explain based on the strong affinity of HS towards metal ions and their presumed inability to penetrate plant cell membranes. Due to the complex nature of HS, it is conceivable that different parts of the HS structure may mediate different functions regarding metal ion mobilization or immobilization. To make matters even more complex is the fact that different HS substructures have varying turnover rates by microbes, mainly due to physical or chemical protection by soil matrix and/or innate biochemical stability. Strongly bound metal ions can be released from HS if the interacting HS substructures are degraded by microbes, which makes HS turnover a crucial factor in the stability of HS-metal complexes. Therefore, to gain a mechanistic understanding of HS functions in metal ion bioavailability, it is most fruitful that the structure basis for metal ion interactions and the associated effect on HS turnover be unraveled.

To investigate HS structure in relation to metal ion binding, we prepared ^{13}C and ^{15}N-labeled soils from the DOE Savannah River Site (SRS), which were in turn employed in soil column experiments where different organic amendments are being humified for up to one year. The labeled HS in these soils was then used as turnover indicators for the humification process of various organic amendments. In this report, we describe some preliminary results on ^{13}C and ^{15}N labeled HS structure characterization and effect of humification on metal ion leaching properties.

Materials and Methods

A soil aging experimental system capable of stable operation for several months, was used for analytical method development and for an initial survey of the effect of organic amendments on soil organic matter transformation (see Figure 1). This system was used to produce ^{13}C and ^{15}N-labeled SRS soils by incubating the soils with either ^{13}C-Glucose or $K^{15}NO_3$ for 34 weeks.

Wheat plants were grown in a Cd-spiked hydroponic media to generate Cd-bioaccumulated wheat root material (Fan, Lane et al. 2001). This material was added to the ^{13}C and ^{15}N-labeled SRS soils along with five different organic amendments: cellulose, wheat straw, pine shavings, chitin, and bone meal). Duplicate columns were prepared for each amendment. The columns were kept at 25°C and irrigated regularly with macronutrients (i.e. Ca, Mg, P) to maintain microbial activities. The leachates were collected and analyzed by ICP-MS for a number of elements including transition metals, Cd, Pb, Hg, Cs, Sr, Ag, and Sn.

HS was extracted from the labeled SRS soils with 0.25 M NaOH following our previous procedure (Fan, Higashi et al. 2000). The isolated HS was then subjected to multinuclear 2-D NMR and pyrolysis GC-MS analysis, as described previously (Olk, Cassman et al. 1995; Fan, Higashi et al. 2000).

Results and Discussion

Figure 1 shows organic matter analysis of an agricultural soil (Ag) low in Cd and Pb concentrations and a Cd and Pb contaminated soil (MC) from the former McClellan Air Force Base, both of which were amended with wheat straw. The high lignin degradation rate observed for the Ag soil indicates that the microbes were active and is consistent with the adaptation of the microbes to lignin breakdown in agricultural soils. In contrast, the subsurface soil from McClellan shows little lignin degradation, which suggests the lack of lignin-degrading microbes

in this soil. The Pb and Cd contamination in the MC soil could contribute to this lack of lignin degradation.

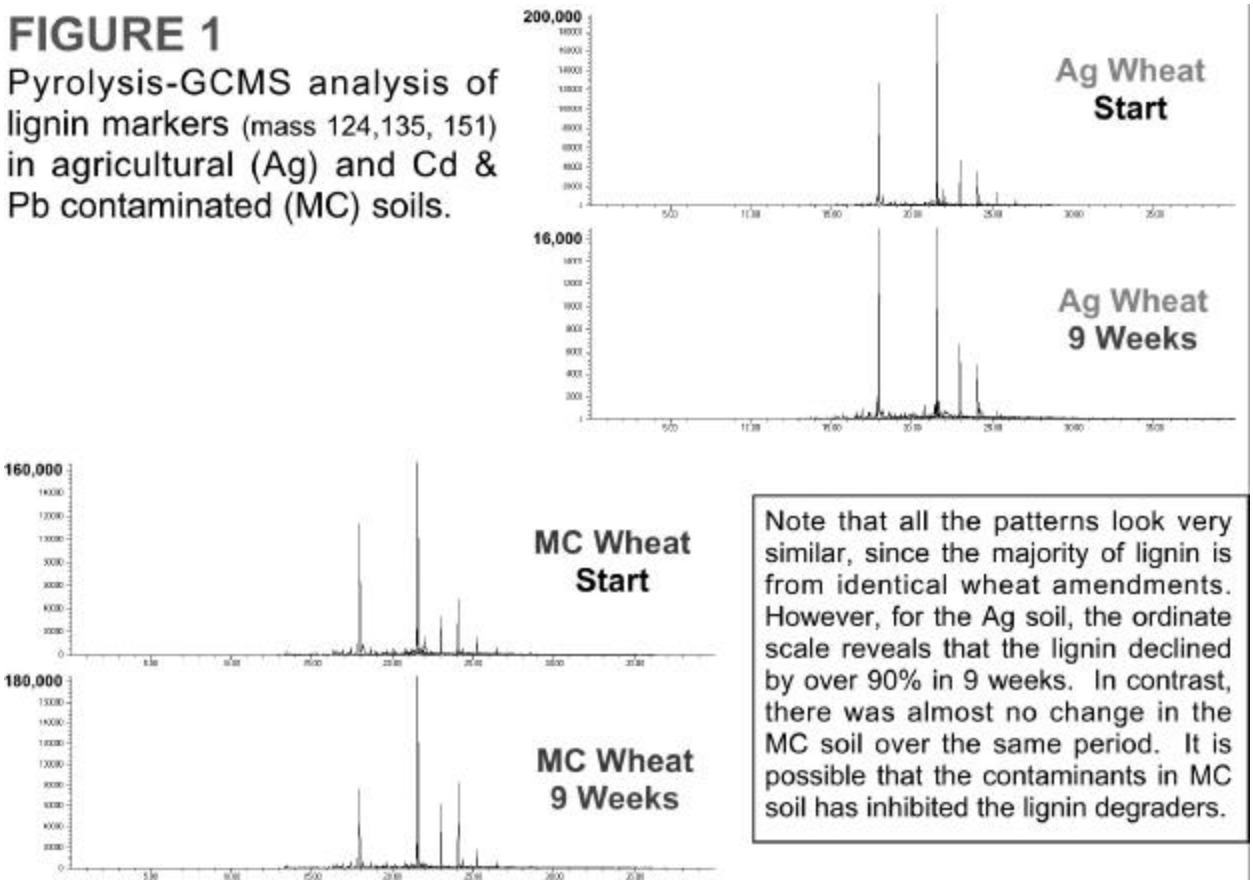


Figure 2 illustrates the 2-D NMR characterization of ^{13}C and ^{15}N-labeled HS isolated from the SRS soil. The ^{1}H-^{13}C HSQC analysis (A) revealed covalent linkages of enriched carbons to protons, from which labeled functional group of certain HS substructures can be deduced. It is interesting to note that the ^{13}C enrichment was high in the alphatic carbons from peptide and ring carbons from carbohydrate substructures of HS. This labeling pattern is consistent with microbial turnover of ^{13}C-glucose and incorporation of microbial biomass into HS. Likewise, the ^{15}N enrichment in peptidic backbone and side chains as illustrated in the ^{1}H-^{15}N HSQC analysis (B) indicates microbial reduction of the precursor ^{15}N-nitrate, synthesis of proteins, and incorporation of protein residues into HS. These labeled patterns were consistent with the pyrolysis GC-MS analysis (data not shown). Namely, pyrolyzed fragments that are of protein and polysaccahride origins were enriched in ^{13}C and ^{15}N. The finding that these biochemically labile residues persisted in HS suggests that some kind of protection (e.g. physical or chemical) mechanism was operative.

When incubating the ^{13}C and ^{15}N-labeled SRS soils with Cd-loaded wheat roots and different organic amendments, different metal leaching property was observed, as shown in Figure 3. Most notably is that Cd leaching was lowest in pine shaving and cellulose-amended soils after 152 days of incubation. It is also interesting to note bone meal amendment led to an initially high Cd leaching (up to 22 days of incubation) but dropped to below control level thereafter. A similar but less pronounced leaching trend was observed for Cu (data not shown).

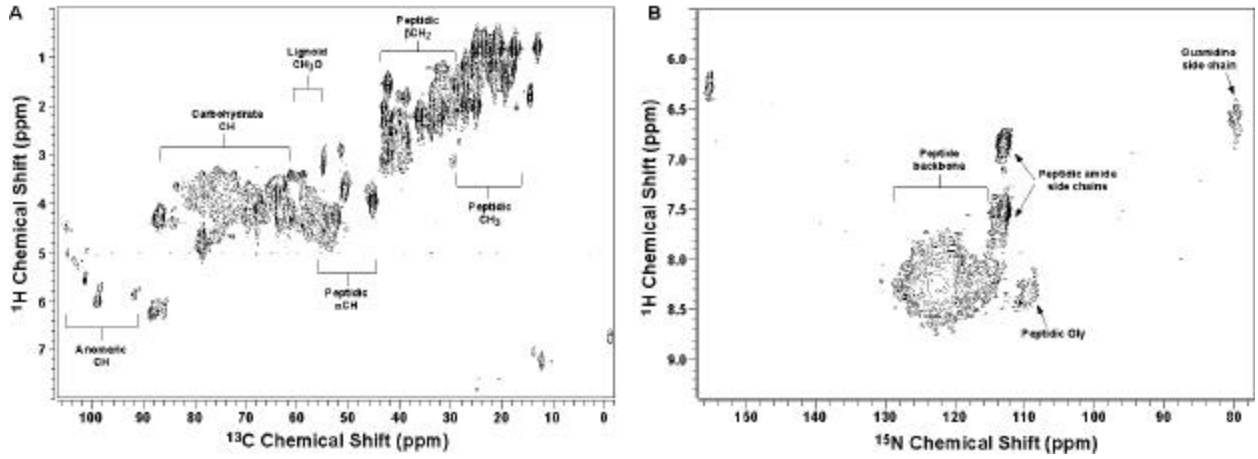
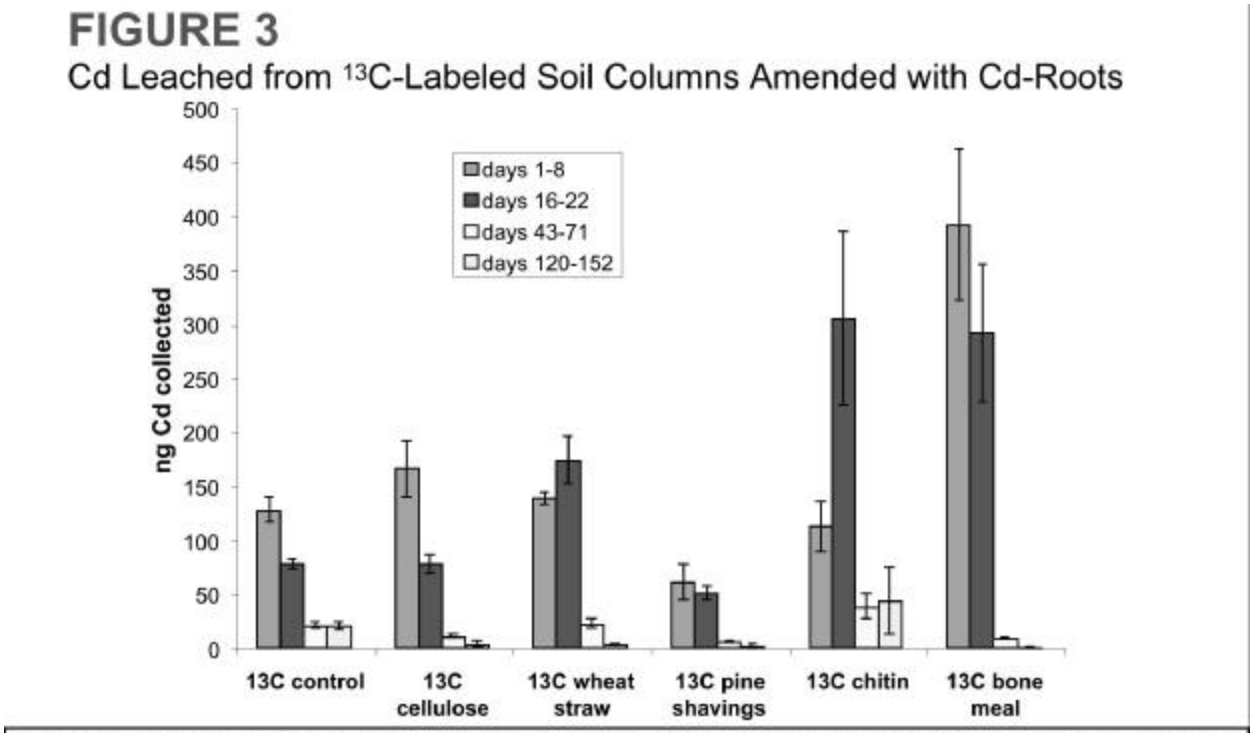


FIGURE 2. 2-D NMR ^{1}H-^{13}C HSQC (A) and ^{1}H-^{15}N HSQC (B) analysis of labeled SRS HS.



Total Cd mobilized by periodic rinsing of ^{13}C labeled SRS soils spiked with Cd-bioaccumulated wheat root and organic amendments as noted. Initial Cd concentrations averaged 2.4 mg/kg (range = 1.6-3.4 mg/kg). The chitin and bone meal caused increased Cd leaching while pine shavings decreased it significantly. As expected, Cd experienced most of its loss in the first four leachings (days 1-22). The corresponding ^{15}N labeled experiments behaved similarly. Cd was analyzed by ICP-MS and each value represent an average of duplicate samples.

In conclusion, it is practical to examine labeled humic substructures using NMR and pyrolysis GC-MS. Humification of different organic materials appeared to influence metal leachability from soils, which is presumably due to a variation in the structure of humified materials. The work on relating humic substructure and turnover to metal mobility is underway.

Acknowledgements

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