AN EXPERIMENTAL STUDY OF FRACTIONATION OF THE RARE EARTH ELEMENTS IN POPLAR PLANTS (POPULUS EUGENEI) GROWN IN A CALCIUM-BEARING SMECTITE SOIL

by

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ABSTRACT

Rare earth element (REE) concentrations were measured in a source (reference) clay Ca-smectite standard and in the roots, stems, and leaves of a species of poplar plant (Populus eugenei). The poplar plant was grown in the clay standard under controlled laboratory conditions during a period of about three months. REEs were shown to fractionate in the clay mineral and plant materials with greater fractionation observed in plant materials.

The REE data provide insight into the process of weathering of clay minerals such as a Ca-bearing smectite and provide insight into the degradation of and the composition of clay minerals in the plant environment. The degradation process is not followed by significant interlayer ion exchange effect on remaining clay minerals in the root environment. REEs were found to be transported into complex forms, potentially as REE-carboxylic anion pair complexes. The plant materials in this study were in general heavy REE (HREE) enriched relative to the source clay minerals due to the complexation effect. The REE anomalies observed in this study, in addition to the Ce and Eu anomalies, may be explained by the selective uptake by the plant by an enzyme effect rather than due to the influence of oxidation-reduction. The enzyme influence was more evident in the REE distribution when compared among the plant organs. These REE characteristics described for the plants may eventually be incorporated with data from numerous other studies and also used as a guide in the assessment of the contribution of plant materials to dissolved REE content in surface water and groundwater.

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Chapter 1 - Introduction

1.1 Scope of the Study

The primary purpose of this research was to conduct a laboratory-controlled plant-growth study to gather information concerning the degree or nature of fractionation among the rare-earth elements (REEs) in the process of their translocation from clay minerals into a given plant. The plant selected for this study was a species hybrid poplar plant (*Populus eugenei*). This plant was selected for research due to the fact that it has a relatively fast growth rate and that it has been frequently a subject of study for environmental bioremediation of various chemical contaminants. The clay mineral chosen for this investigation was a sample of smectite (Cheto Saz-1). This mineral is commonly present in natural soil materials. It has a high ion-exchange capacity and it accommodates significant amounts of rare-earth elements in its interlayer sites.

Although a number of studies have been conducted to examine the REE fractionation between soils and plants in natural environments (Wyttenbach et al., 1997), very few experimental data exist for the fractionation of REEs between soil minerals and plants. Plant-mineral interactions can lead to fractionation of REEs in the soil environment. This fractionation may be reflected in the accumulation of REEs in plants. Natural plant studies can provide information regarding the fractionation. However, the difficulties with investigating the natural environment include the variations in soil components which can change significantly over short distances. This can lead to difficulties in depicting the causes of the REE fractionation.

The plant samples used in previous research were grown in heterogeneous soils and background conditions could not be evaluated. Different results have come from studies of REEs in different plant-soil natural environments. Given that soils in the natural environment are spatially heterogeneous with respect to mineral composition, the results of studies on natural systems could not be properly evaluated

in terms of the impact of different minerals in a soil environment defined by plant-mineral interaction. Multiple laboratory studies are needed before the degree by which different soil chemical and physical parameters (pH, oxidation-reduction, mineral chemistry, crystallinity, temperature, water availability, microorganisms, etc.) impact the fractionation of REEs between plants and soils can be understood. Multiple laboratory studies are needed to fully characterize the geochemical characteristics of individual minerals and their interactions in soil.

The results of this study are intended to create a small basic foundation with potential applications to variety of different areas of investigation of surface and subsurface processes. Although the REEs are trace elements, their geochemical paths may provide additional insight into the process of weathering of minerals. Plants can be a source of a number of solutes to both surface water and groundwater. The REE signatures in plants as compared to the dissolved chemical components of surface water and groundwater could then serve as an additional indicator of the role of plants in the solute budget of surface and groundwater.

1.2 Previous Studies

All plants contain naturally occurring lanthanides, also commonly known as REEs, which include 14 elements from lanthanum (La) with atomic number 57 to lutetium (Lu) with atomic number 71 and exclude promethium (Pm) with atomic number 61 because of its absence in all naturally occurring terrestrial materials. Cossa (1870) was the first to report the occurrence of lanthanides in plants. Subsequent works by many different investigators have established the universal occurrence of lanthanides in all living materials. Limited by analytical difficulties in detecting low amounts of lanthanides in plants, nearly all early work on lanthanides in plants reported the concentrations of only some selected lanthanide elements. Improved analytical conditions have occurred in relatively recent years; particularly with the introduction of neutron activation analytical techniques and later of inductively coupled plasma spectroscopy. The scope of investigations of lanthanides in plants has expanded to include not only the concentrations of individual elements within the lanthanide group, but also the characteristics of relative distributions of lanthanides in individual plants and their various parts.

Lanthanide concentrations in plants are widely varied. The variations exist not only among different plants, but also among species of the same kind but in different growth environments. Moreover, the concentrations have been found to be considerably varied among different parts of an individual plant. The concentrations commonly range from few tens of nanograms (ng) to few micrograms (μg) per gram of dry plant material (Bangfa et al., 1995; Borneman-Starinkevitch et al., 1941; Robinson, 1943; Erametsa and Haukka, 1970; Henke G., 1977; Koyoma et al., 1987; Miekeley et al., 1994; Markert and Li, 1991; Fu et al, 1998; Fu et al., 2001; Wang et al., 1997; Wei et al., 2001; Wyttenbach et al., 1996; Wyttenbach et al., 1998a, 1998b; Zhang et al., 2002). Some uncommonly high concentrations have been reported. Robinson et al. (1938) found lanthanide concentrations of 300 to 2,300 μg/g in the leaves of hickory trees in an area in the eastern U.S. Milton et al. (1944) reported lanthanide concentrations of nearly 600 μg/g in the leaves of chestnut trees; whereas they found nearly non-detectable amounts of lanthanide in the leaves of adjacent oak trees in a locality in Virginia. Even though the concentration variations can be attributed to a number of different physical, chemical, biological, and mineralogical factors, the extent of influences of these factors individually or collectively as yet remains largely unknown.

The distribution characteristics of lanthanides have now become a critical parameter in the search for a key to an understanding of geochemical pathways by which lanthanides are taken up by plants from the source or sources of these elements and are then translocated for their accumulation in different parts of the plants. An interest in the study of the distribution stems from the fact that the lanthanides are a chemically homogeneous group of elements, all having 3+ valence states with only Eu and Ce also of 2+ and 4+ valence states, respectively. Despite a broad chemical similarity, the lanthanides may become subject to fractionation because there is often a progressive change in the binding constants in the associations of the lanthanides with a common naturally occurring inorganic or organic ligands. Studies on natural plants have produced mixed results as to fractionation of the lanthanides in plants relative to their associated soils. Some have found that in relation to soils, plants produced either no fraction or hardly any fractionation of the lanthanides (Henke, 1977; Laul and Weimer, 1982; Miekeley et al., 1994). Others have found clear

indications of lanthanide fractionation between plants and associated natural soils (Chaudhuri et al, 2007; Schneider, 2006; Wyttenbach et al., 1996, 198a, 1998b; Wang, 1997) or between different parts of plants (Chaudhuri et al, 2007; Schneider, 2006; Zhang et al., 2002). As yet, no clear understanding has emerged regarding a process or processes which govern the magnitude or the nature of lanthanide fractionations between plants and associated soils or the nature of intraspecies lanthanide fractionation.

Chapter 2 - Geochemical Properties of the Rare Earth Elements

In most common terrestrial environments, lanthanides typically occur as trivalent ions (Ln^{3+}), with the exceptions of Eu, which can occur both as a divalent ion (Eu^{2+} or Eu II) and a trivalent ion (Eu^{3+} or Eu III) and Ce, which can occur both as a tetravalent ion (Ce^{4+} or Ce IV) and a trivalent ion (Ce^{3+}). The loss of the single 5d electron plus the two 6s electrons from La, Gd, and Lu atoms results in the formation of their trivalent ionic forms, whereas the loss of one 4f electron plus the two 6s electrons from Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu atoms results in the formation of their trivalent ionic forms. Hence the valence electrons for the trivalent lanthanides are: $6s^2$ and $5d^1$ electrons for La, Gd, and Lu, whereas $6S^2$ and $4f^1$ electrons for the remaining lanthanides.

There are several well known coherent chemical properties of lanthanide atoms or ions in their interactions with compounds. These make them as good tracers for defining many different natural inorganic and organic geological processes. A major property of the lanthanides is the well known "lanthanide contraction", that is the progressive decrease in the size of the atom, or decrease in the ionic radius, with increasing atomic number (Smith, 1963; Evans, 1990). For example, the ionic radii of lanthanide ions range from 1.14 angstroms, or 0.114 nm, for La^{3+} at 6-fold coordination (or 1.18 angstroms, or 0.118 nm, for La^{3+} at 8-fold coordination) to 0. 85 angstrom, or o.085 nm, for Lu^{3+} at 6-fold coordination (or 0.87 angstrom for Lu^{3+} at 8-fold coordination). The ionic radii change not only with the coordination number, but also with the ionic charge. As stated earlier, Eu exists in common natural environments both in Eu^{3+} and Eu^{2+} valence states and Ce both in Ce^{4+} and Ce^{3+} states. The ionic radius of Eu^{2+} at 8-fold coordination is about 1.12 angstroms (or 0.112 nm) and at 10-fold coordination is about 1.15 angstroms (or 0.115 nm) , whereas that of Eu^{3+} at 6-fold coordination is about 0.98 angstrom (or 0.098 nm) and at 8-fold coordination is about 1.02 angstroms (or 0.102 nm). In the case of Ce ions, the ionic radius of Ce^{3+} at 6-fold coordination is 1.07 angstroms (or 0.0107

nm) and at 8-fold coordination is 1.11 angstroms (or 0.111 nm), whereas the ionic radius of Ce^{4+} at 6-fold coordination is 0.94 angstrom (or 0.094 nm) and that at 8-fold co-ordination is 0.97 angstrom (or 0.097 nm).

The lanthanide contraction or the progressive decrease in the size arises from insufficient shielding of the increasing nuclear attractive force with each additional electron that fills the 4f orbitals. As both the nuclear charge and the number of 4f electrons increase at each step with the continued process of orbital fillings by electrons, the shielding of one 4f electron by another from the nuclear positive charge with the process of electron filling remains imperfect, due to the shapes of the 4f orbitals. Hence at each increased filling of the 4f orbitals, the effective nuclear attraction on the electrons increases, thus causing a reduction in size of the entire 4f subshells and a general steady contraction in the ionic radii (Cotton and Wilkinson, 1962). Because lanthanides vary primarily in the number of 4f electrons, these elements are very similar in chemical properties as they are always found occurring together in natural materials. Their occurrences together in natural materials do not imply that they respond equally to chemical changes of natural systems. In fact, the lanthanide ions become involved in some degree of separation among themselves when they are either confronted with optimum atomic radii for accommodations in many mineral structures or are in solutions and become involved in complex formations, especially in formation of chelations, with different kinds of ligands. In some mineral structures, such as in amphibole and garnet, lanthanides with smaller ionic radii (or heavier lanthanides) are accommodated, whereas in some other mineral structures, such as in feldspar minerals, lanthanides with larger ionic radii are more favored. In solutions, some degree of separation among lanthanides occurs because the stability constants of many different lanthanide-ligand complexes are very often varied in a gradual or steady fashion, but not necessarily in a smooth pattern, across the lanthanide series. The variations that have been observed in natural materials have led studies on lanthanides to subdivide the elements into three groups: the light lanthanide group (from La to Eu), the middle lanthanide group (from Sm to Ho), and the heavy lanthanide group (from Gd to Lu). The middle lanthanide group covers the two end-members (Sm and Eu) of the light lanthanide group and the first four members (Gd, Tb, Dy, and Ho) of the heavy lanthanide group (Topp, 1965).

A major property of lanthanides is that lanthanide ions are highly electropositive. The bonds formed with compounds or complex forming compounds have a high degree of ionicity. The outer orbitals of Ln are of high energy and hence they are not very favorable to the formation of covalent bonding. La, Gd, and Lu each has a single electron at a 5d orbital. The extra d electron tends to impart only a very small degree of covalency to the bonding made by these three atoms, but the bonding in each case is still essentially ionic. Notwithstanding the small tendency of the lanthanides to form covalent bonding, the degree of covalency for the lanthanides increases only very slightly with increase in the atomic number.

A prominent chemical aspect of the lanthanides is that they form strong complexes with a number of different ligands. In the Pearson (1963) terminology, the Ln^{3+} ions are classified as "hard" ions. Water is a very strong ligand for Ln^{3+} ions. In aqueous environments, other ligands would form strong bonds with Ln^{3+} ions only if the different ligands become able to dislodge or displace water molecules from the coordination sphere. These ligands should be those which are considered to be "hard" bases, using the Pearson terminology, that are not easily polarized and form highly predominantly ionic bonds with little or no covalent bonding and have high affinity for bond formation with "hard" acids such as the lanthanide ions. High ionicity of "hard" acid lanthanide ions causes them to bond preferentially with "hard" base ligands such as H_2O, OH^-, CO_3^{2-}, NO_3^-, SO_4^{2-}, PO_4^{3-}, O^{2-}, F^-, CH_3COO^-, R-OH (alcohols), NH_3, R-NH_2 (amines), among many others, containing highly electronegative donor atoms such as O and F. Lanthanides, like many other "hard" acid ions, have strong preference for O donor atoms. Bonding to Cl^- ion has been known, but it is relatively weak compared to bonding to O and F anions. Complexes solely with NH_3, R-NH_2 (amines), HS^- and CN^- are extremely weak and are unknown in aqueous solutions (Evans, 1990; Wood 1990). Lanthanide coordination with N alone is weak and such a complex is easily hydrolyzed by water. But in a polydentate complex formation or chelation, N can serve as an effective donor when Ln^{3+} is also coordinated to at least an O donor atom and this can happen under physiological conditions. In general, the lanthanide ion preference for donor atoms is O > N > S (Thompson, 1979).

Another major chemical property of the lanthanides is that in aqueous solutions the Ln^{3+} ions attract around them water molecules in the form of a hydration shell by way of forming Ln^{3+}-H_2O complexes. The basic properties of lanthanides in natural aqueous solutions, for example in soil solutions, depend heavily on the degree of removal of the hydration shell by other coordinating compounds that may be present in the aqueous system.

Coordination spheres with 4 and 6 coordination numbers are common for many metals, but higher coordination numbers of 7, 8, 9, 10, and 12 have been reported for metals of second and third transition series, the lanthanides and actinides. Studies on different lanthanide complexes have established that water molecules associated with lanthanide complexes are not waters of hydration but are coordinated (Thompson, 1979).

There had been very little agreement among studies prior to the mid-1960s as to the lanthanide coordination numbers in aqueous solutions. Suggestions during this period for the hydration numbers of Ln^{3+} ions in aqueous solutions ranged from 6 to 12. But a strong consensus concerning the hydration numbers of Ln^{3+} ions developed in later years. Many relatively recent studies have supported the view that the hydration number, in reference to the first coordination sphere which is commonly known as the inner sphere, varies across the lanthanide series ranging from a hydration number of 9 for the La^{3+} to Nd^{3+} series, to 8 for Tb^{3+} to Lu^{3+} and mixed numbers of 9 and 8 for Sm^{3+}, Eu^{3+}, and Gd^{3+} (Spedding et al., 1974, 1977; Wood, 1990; Evans, 1990).

Chapter 3 - Materials and Methods

3.1 Materials

Three *populus eugenei* (poplar) plants were grown in a calcium-rich montmorillonite (smectite) from Apache County, Arizona (Saz-1 "Cheto") in separate containers. The poplar plants were obtained from Hybrid Poplar Tree Farm located in Washington State. These plants were chosen due to their rapid growth, ability to grow in harsh conditions, availability for sample replication, and use in environmental bioremediation activities for removal of contaminants. The smectite was obtained from the The Source Clays Repository of the Clay Mineral Society located in Columbia, Missouri. The Source Clays Repository of the Clay Mineral Society relocated to Purdue University in West Lafayette, Indiana.

The plants were grown indoors in a clean environment, kept at a near constant temperature of 25º C. The plants were watered using deionized water and the growth of the plants occurred under constant lighting for approximately 3 months from March through June. No additional nutrients, beyond what the clay could provide, were used during the growing process. The requisite nitrogen for plant growth most likely came from ammonia present in the indoor air. After the plants had grown large enough so that samples could be obtained, the leaves, stems, roots, and root (root-attached) clay were separated and segregated for sample preparation. In addition, the non-planted reference stem material (cuttings) that was not planted in the clay and the non-planted source clay reference material were also prepared for analysis. Table 1 presents a description of the nomenclature used to label the samples prepared for analysis.

3.2 Methods

Non-planted source (reference) clay and root-attached clay were prepared for Cu K alpha x-radiation (X-ray diffraction). Three different analytical sample sets were prepared with the preferred orientation for the dry particles. The first sample set was untreated. The second set was treated by glycolation (glycol solvation) which is known to increase the basal (d001) spacings of smectites. The third set was treated by heating the sample to 470^{o}C for one hour. The resultant effect of this treatment is known to cause

decreases in the basal spacings of smectites relative to the spacing for the corresponding untreated clay sample.

A combination of wet and dry destruction of the plant material was used to prepare the samples for laboratory chemical analysis. Wet destruction was completed by using vacuum double distilled nitric acid to digest the plant material. After the material was digested, the solution was evaporated to dryness. The evaporated sample was subsequently dried and ashed in an oven at 500^{o}C. The material was then redissolved in vacuum double distilled concentrated nitric acid and evaporated on a hot plate to dryness. The final step included redissolving the evaporated mass into a known volume of vacuum double distilled 0.2 *N* nitric acid. The final solution of each plant sample was then submitted for elemental analysis by Inductively Couple Plasma – Atomic Emission Spectroscopy (ICP-AES) and Inductively Couple Plasma - Mass Spectroscopy (ICP-MS).

A combination of physical and chemical methods was used to prepare clays for laboratory chemical analysis. Root (root-attached) clays for this experiment are defined as the clay material in direct contact and within one to five millimeters of the root. Clay materials were sorted by grain size prior to analysis using physical separation in deionized water and extracting an aliquot comprising the <2 micron fraction. A bulk set was also collected. Following physical grain size sorting, a silicate dissolution process was used prepared the clay samples for laboratory analysis. The acid reagents used for the solution were double distilled hydrofluoric acid and nitric acid Following the hydrofluoric and nitric acid dissolution, the solution was evaporated to dryness and redissolved into a solution with a known volume of distilled water and 0.5 mol nitric acid. The sample solution was then submitted for analysis by ICP-AES and ICP-MS.

The precision of the analyzed data were based on replicate analyses of standards during the period of this analytical study. The precision of the major chemical data were within ±five percent and of the trace element data were within ±ten percent at 2Φ (95 percent) level. Replicate analysis of three aliquots of a sample of plant stem reaffirmed the analytical precision established by the repeated analyses of a standard sample. Plant and clay samples were analyzed at the Kansas State University, Department of Chemistry Laboratory and Centre de Geochimie de la Surface at Louis Pasteur University in Strasbourg, France.

Chapter 4 - Results

4.1 X-Radiation Analysis

The <2-micron-fraction untreated non-planted source clay d001 spacing was 15.1 Angstroms (Å). The glycol solvation increased the d-spacing to 16.9 Å. The heating to 470 ºC decreased the d-spacing to 9.7 Å.

The <2-micron-fraction untreated poplar root clay (i.e. the clays following the growth of the plants) d-spacing was 15.1 Å. The glycol solvation increased the d-spacing to 17.1 Å. The heating to 470 ºC decreased the d-spacing to 9.6 Å.

The two batches of clay fractions before and after the growth of plants had a nearly identification d-spacing. The lack of difference in the d-spacing will be discussed subsequently.

4.2 Total REE Content

The total REE contents for different materials are: the bulk non-planted source clay at 364 µg/g, <2-micron-fraction non-planted source clay at 274 µg/g , bulk root clay at 355 µg/g, <2-micron-fraction root clay at 203 µg/g, roots at 39.3 µg/g, original non-planted (reference) stems 0.288 µg/g, planted old stems 0.221 µg/g, new stems 0.195 µg/g, and leaves 13.6 µg/g. Therefore, the REE content of the plant materials increasing order were: new stems > planted old stems > leaves > roots. The relative total REE concentrations are shown in Table 2 and Figure 1.

4.3 Distribution Characteristics of REEs in Clays and Plants

The distribution characteristics of REEs in clay and plant materials for this study were evaluated by the trend of relative distribution, which is given as the ratios of the concentration of the lanthanides of the sample to the respective concentrations of a selected standard. This will then be called a normalized distribution characteristic for the sample with respect to the chosen standard or relative to another sample.

A deviation for any particular lanthanide element from the general distribution trend is called an anomaly for that element. The anomalies were calculated by dividing

the actual analytical result by the theoretical result. The theoretical result was calculated by multiplying the analytical results and taking the square root of this product.

For this study, a negative anomaly is less than one and a positive anomaly is greater than one. Due to an analytical error margin of 10 percent in this study, an anomaly represents negative if it is less than 0.8 and positive if it is greater than 1.2.

The REE in clays were normalized to the REE in Post Archean Australian Shale (PAAS) (Taylor and McLennan, 1988). The REE distribution characteristics of a plant organ were examined relative to source clays or root clays or another plant organ.

Anomalies for Cerium (Ce) and Europium (Eu), if any, were calculated using the following formulas: $Ce^* = Ce_N/(La_N \times Pr_N)^{1/2}$, and $Eu^* = Eu_N/(Sm_N \times Gd_N)^{1/2}$, where Ce* and Eu* are the calculated values or theoretical values and the symbols with subscript N refer to the measured values.

4.3.1 Bulk Clay

The distribution of the PAAS-normalized REEs in the bulk source and root clays can be described as one with light rare earth element (LREE) enrichment (Table 3 and Figure 2). There may appear from this figure to be a small cerium anomaly but it should be discounted in light of the experimental error. Nevertheless, there is a strong indication of an Eu-negative anomaly of about 0.64.

The <2-micron fraction was separated from the bulk source clay and has a relative REE distribution pattern to PAAS that is closely similar to that of the bulk source clay with LREE enrichment, except that the finer fraction has a prominent Ce-negative anomaly of 0.43 (Table 3 and Figure 3). A closer examination of the distribution of the REEs between the fine fraction and the bulk source clay reveals that the fine fraction is not only more depleted in Ce, but is also relatively LREE enriched (Table 3 and Figure 4).

4.3.2 Root Clay REE Distribution

The root clay here refers to the clay that was collected in the vicinity of the roots (root-attached plus adhering material in close proximity to the roots) after the growth of plants. The chemical data have been collected from both the bulk material and the < 2-micron fine clay fraction of the root clay portion.

The REE distribution trend of the bulk root clay after the growth of plants relative to the bulk source reference clay before plant growth is nearly horizontal (Figure 5). The REE distribution trend of the <2 micron fraction root clay relative to the bulk root clay is one that is LREE enriched with a Ce-negative anomaly of 0.28 (Figure 6). As shown in Figure 4 and Figure 6, this comparison of fine fraction relative to the bulk shows is not a good basis for determining the impact of REE fractionation occurring within the soil due to the plant growth effect. However, the REE distribution trend of the < 2-micron fraction root clay relative to the <2-micron fraction source clay reveals LREE enrichment superimposed with some HREE enrichment and a Ce-negative anomaly of 0.63 (Figure 7).

4.3.3 Plant Root REE Distribution

The REE distribution trend of the roots relative to the <2-micron source clay is one that is HREE enriched with a Ce-positive anomaly of 2.21 and an Eu-positive anomaly of 1.52 (Figure 8). The REE distribution trend of the roots relative to the <2-micron fraction root clay is one that is HREE enriched with a Ce-positive anomaly of 3.52 and an Eu-positive anomaly of 1.47 (Figure 9). Figures 8 may also show a potential Gd-positive anomaly of 1.3. The REE distribution trend of the roots relative to the planted old stems is one that is HREE enriched superimposed with MREE enrichment and accompanied by an Eu-negative anomaly of 0.23 (Figure 10). If MREE enrichment were accepted, then this will require besides the Eu-anomaly, there should be an Sm-negative depletion of at least about 0.59.

4.3.4 Plant Stem REE Distribution

Original (non-planted) reference stems here refer to the Populus eugenei plant cuttings prior to planting in the clay soil. The planted old stems here refer to the original non-planted reference stems that were planted in the clay soil. The new stem here refers to the new stem growth from the planted old stem.

The REE distribution trend of the planted old stems relative to the original reference stems was one that is enriched in HREE with a Ce- and Pr- positive anomalies

of 1.70 each, an ND-positive anomaly of 1.2, an Eu-positive anomaly of 1.23, and a Gd-positive anomaly of 1.2 (Figure 11). The REE distribution trend of the new stems relative to the <2-micron source clay was nearly horizontal with a Ce-positive anomaly of 2.39 and an Eu-positive anomaly of 8.92 (Figure 12). The REE distribution of new stems relative to original reference stems is one that is MREE enriched with a Ce-positive anomaly of 1.73, a Pr-positive anomaly of 1.42, an Eu positive anomaly of 1.69, a Sm-negative anomaly of 2, and a Gd-positive anomaly of 1.45 (Figure 13). The REE distribution trend of the new stems relative to the planted old stems is one that is LREE enriched with an Eu-positive anomaly of 1.37 (Figure 14). The REE distribution trend of the new stems relative to the roots was one that is enriched in LREE with an Eu-positive anomaly of 6 (Figure 18).

4.3.5 Plant Leaf REE Distribution

The REE distribution trend of leaves relative to new stems is one that is HREE enriched accompanied by positive anomalies of Pr and Nd and negative anomalies of Sm (0.7), Eu (0.12), Gd (0.7), and Th (0.7). (Figure 15). The REE distribution pattern of leaves relative to planted old stems is very similar to the distribution pattern of the leaves to the new stems except that the Gd anomaly is not apparent (Figure 16). The REE distribution trend of leaves relative to the <2-micron source clay is one that is HREE enriched with a Ce-positive anomaly of 2.28 and a Gd-positive anomaly of 1.4 (Figure 17). The REE distribution trend of leaves relative to roots is one that is HREE depleted with a Ce-negative anomaly 0.7 (Figure 19).

4.3.6 Calcium and Total REE

The total calcium for the plant organs presented in increasing order is as follows: original reference stem at 3,336.97 µg/g, planted old stem at 4,035.91 µg/g, new stem at 6,896.01 µg/g, roots at 8997.99 µg/g, and leaves at 18,556.53 µg/g (Appendix B).

The ratio of the calcium to total REE for each plant organ in increasing order is as follows: roots (229.11), leaves(1,359.81), original reference stems(11,598.72), planted old stems(18,290.58), and new stems(35,413.39).

Chapter 5 - Discussion

5.1 X-Ray Diffraction and Clay

The X-ray diffraction results indicated no change in the basal d-spacing of the clay. The plants received at least some of the elements from the smectitic clays. There had to be some alteration of the clays to provide the elements for plant growth. The alteration of the clays which are well known for their interlayer ion exchange and no change in the d-spacings need to be rationally linked.

Weathering of the clay minerals in the rhizosphere could proceed either through a congruent dissolution path or an incongruent dissolution path. An incongruent dissolution path would be dominated by a significant change in the stoichiometry of the clay mineral composition as there could be secondary products much different from the original reactant mineral. The major element chemistry, for example Ca/Al and K/Ca ratios for the fine clay fraction before and after the plant growth remain essentially unchanged (Appendix B). Therefore, the scale of alteration of the clays remained confined to the very limited zone of rhizospere. Such a small change would not have been detected by the X-ray analysis procedure that was followed in this study.

There is no denying that alteration did occur and it produced key REE signatures in support of plant-mineral alterations. There were changes both in the total REE content and the distribution of the REEs. The <2 micron root clay fraction had a total REE concentration of 203 ug/g, whereas the <2 micron source clay fraction before the planting had a total REE concentration of 275 µg/g. The <2-micron fraction root clay was enriched in LREE with a Ce-negative anomaly (0.63) relative to the <2-micron fraction source clay. Therefore the absence of any change in the d-spacings of the clays is most likely attributable to the alteration of the smectitic clays by a very small amount so much so that it could not be detected by the X-ray analysis. The alteration effect would need to be five percent or greater before X-ray diffraction would detect these changes.

5.2 Roots

The roots have higher REE concentrations than the other plant organs such as stems and leaves. Some previous studies have shown similar trends of REE concentrations in plant organs (Cao et al., 2000, Li et al., 1998, Wen et al. 2001, Xu et al., 2002).

The plant roots relative to the <2-micron source clay or the <2-micron root clay were HREE enriched with both Ce- and Eu-positive anomalies. The HREE could be related to carboxylic acids which are commonly reported as a product of plant exudation in the root environment. The HREE-carboxylate anions are known to have higher stability constants than LREE-carboxylate anions. While the root clay became LREE enriched and Ce-depleted, the plant roots became HREE enriched and Ce and Eu enriched. The translocation of REE from clays to roots was accompanied by a fractionation effect. The fractionation that accompanied the plant uptake was marked by sharp enrichment in HREE. The enrichment in HREE is often attributed to higher stability constants for Ln-ligands or Ln-chelates with increasing atomic numbers. The enrichment trend in this case of the plant roots relative to the <2-micron root clays or <2-micron source clays was unlike the normal effect one expects from simple Ln-carboxylic anion complexation or Ln-carbonate complexation. One possible explanation for this sharp increase in HREE in the plant roots could be that translocation was influenced by an enzyme effect. Epstein et al (1973) reported association constants of lanthanide-trypsin (a proteinase) that had a very high values for the HREE compared to the LREE (Appendix C). The plant root REE trend was also characterized by an Eu-positive anomaly. Eu anomalies, positive or negative, have very often been explained in the rhizosphere in terms of oxidation reduction. However, the enzyme effect may suggest that the anomaly is at least in part due to selective transport of the REE from the rhizosphere clay source to the plant roots. As stated earlier, there is a possibility that the plant roots had a Gd anomaly in addition to the Eu anomaly. An enzyme effect could also account for this prospect of Gd anomaly in the root REE. REE has been reported often that lanthanides bind at calcium sites (Evans, 1990). Maximum binding of lanthanide ions would occur at an optimum ionic radius. Considering the Ca-specific bindings sites, lanthanide ions whose radii are closest to the Ca ion would be preferred.

The optimum condition occurs between calcium with 7-fold coordination bound to an enzyme with Eu and Gd with 8-fold coordination. Therefore enzymes providing this kind of coordination would cause Eu- and Gd-positive anomalies.

5.3 Stems

The new stems relative to the roots are slightly LREE enriched with a potential accompaniment of MREE enrichment and had a noticeable Eu-positive anomaly. The news stems relative to planted old stems are LREE enriched. Relative translocation of REEs is influenced by the complexation effect showing greater mobilities for the HREE. To translocate any amount of REE from planted old stem to new stem, a carrier enzyme may play a significant role. The Eu enrichment, and potentially also Gd enrichment, can be explained in terms of preferential inclusion of Eu in the cation sites within the enzyme that share a similar ionic radius to Ca. The Eu enrichment can also be related to respiration related oxidation-reduction influence. If the Eu anomaly is all due to Eu oxidation-reduction, Eu2+ to Eu3+, the potential Gd anomaly would not be possible. Hence, at least part of the Eu anomaly will be attributable to enzyme effect.

5.4 Leaves

The leaves REE concentrations (13.65 µg/g) is higher than new stems (0.19 µg/g) but lower than roots (39.3 µg/g). As previously stated, the roots had a preferential uptake of HREE from the rhizosphere solution. This imprint of preferential HREE uptake has been carried through to the leaves. But this does not imply that the roots and the leaves had the same relative distribution pattern of REE relative to the clays in the rhizosphere environment. A shown in Figure 19, the leaves were relatively HREE depleted with a relative Eu depletion. This relative HREE depletion may be due to the part of the downward translocation of the REE from the leaves to the roots.

Chapter 6 - Summary and Conclusions

REE fractionation was observed between Ca-bearing smectite, serving as soil, and poplar plant materials. The experimental condition didn't allow for the determination of the interlayer ion change that could have been associated with alteration of the clay particles.

The relative distribution of the plant relative to the clay suggests that there has been alteration of the clay particles. This alteration remained confined essentially within the rhizosphere. The REE signatures provide an insight into the weathering process. Apparently no significant interlayer ion exchange was shown subsequent to the weathering or degradation process.

The total REE concentrations in plants increased in the following order: new stems (0.19 µg/g), planted old stems (0.22 µg/g), leaves (13.65 µg/g), and roots (39.27 µg/g). This sequence is similar to observations made by other investigators.

The roots relative to the fine fraction source and roots clays showed little variation indicating limited REE depletion from plant growth in the clays. This may be due to the scale of the effects of roots on clays or to the duration of the experiment. An experiment with a longer duration may show greater fractionation in clays.

The roots relative to clays, stems, and leaves show HREE enrichment. The enrichment was marked by sharp increase in the HREEs, possibly related to carboxylic acids exuded by the plants in the rhizosphere. The HREE-carboxylate anions are known to have higher stability constants than LREE-carboxylate anions. Additionally, Eu-positive and Gd-positive anomalies superimpose on this trend. Enzymes may have influenced the HREE enrichment. REEs with similar ionic radii to Ca may have bound to the Ca-specific sites on the enzymes increasing their mobility.

The new stems relative to clays, roots, and planted stems show LREE enrichment with an Eu anomaly. In some there cases as related to roots and planted stems, there is an additional anomaly such as Gd anomaly. An enzyme with preferential ionic sites similar to Ca for Eu^{2+} may explain the Eu anomaly. The Eu anomaly may also be related in part to the oxidation-reduction of Eu. The anomaly besides Eu cannot be explained in terms

of oxidation reduction. Therefore, part of this anomaly may be shared by enzyme or oxidation-reduction effects.

The leaves relative to the stems and clays show HREE enrichment. The leaves relative to the roots show HREE depletion. This relative HREE depletion may be due to the downward translocation of the REE from the leaves to the roots.

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FIGURES

Figure 1
Total REEs of Samples

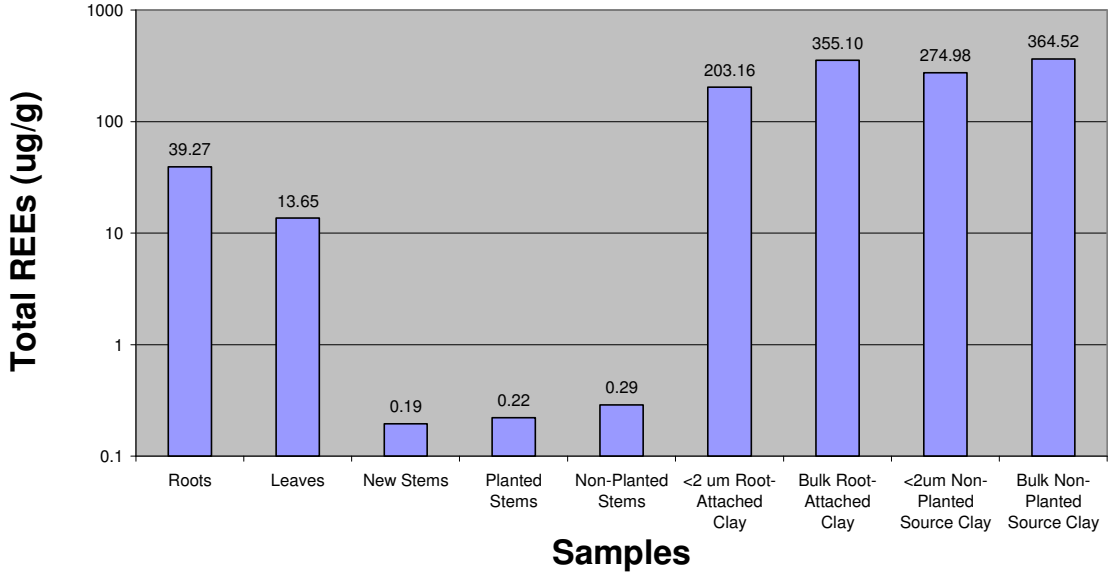
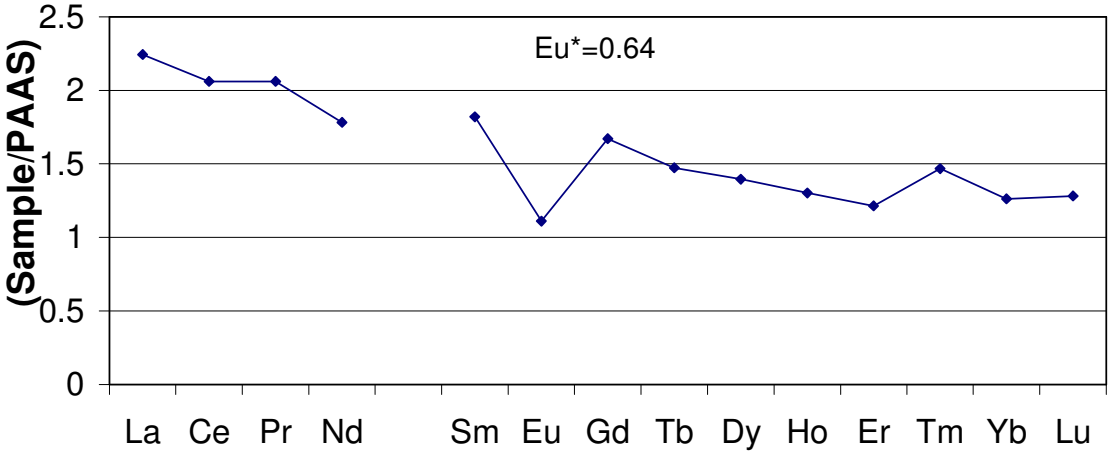
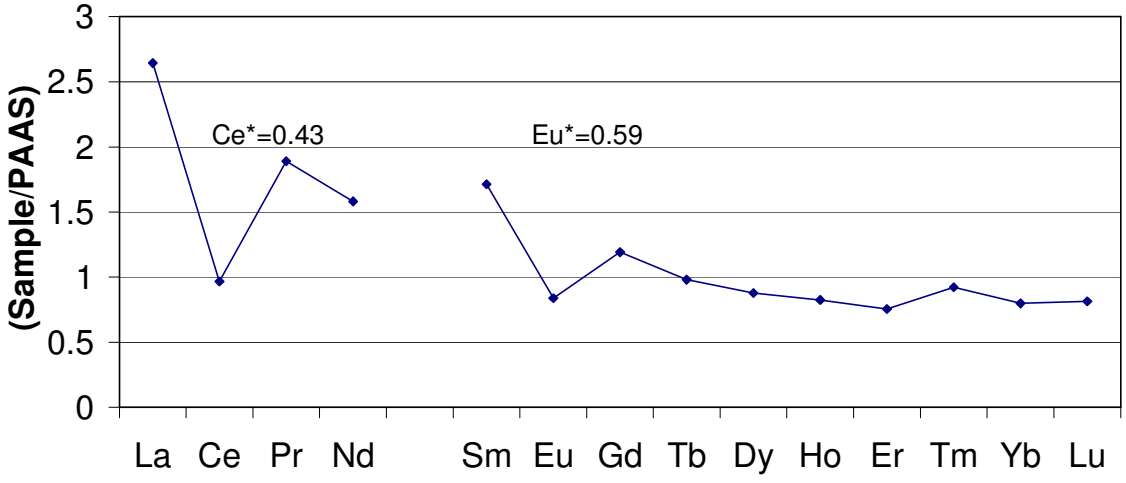


Figure 2
REE Distributions of Bulk Non-Planted Source Clay Relative to PAAS



* = calculated anomaly value

Figure 3
REE Distributions of <2 Micron Fraction Non-Planted Source Clay Relative to PAAS



* = calculated anomaly value

Figure 4
REEs of <2 Micron Fraction Non-Planted Source Clay Relative to Bulk Non-Planted Source Clay

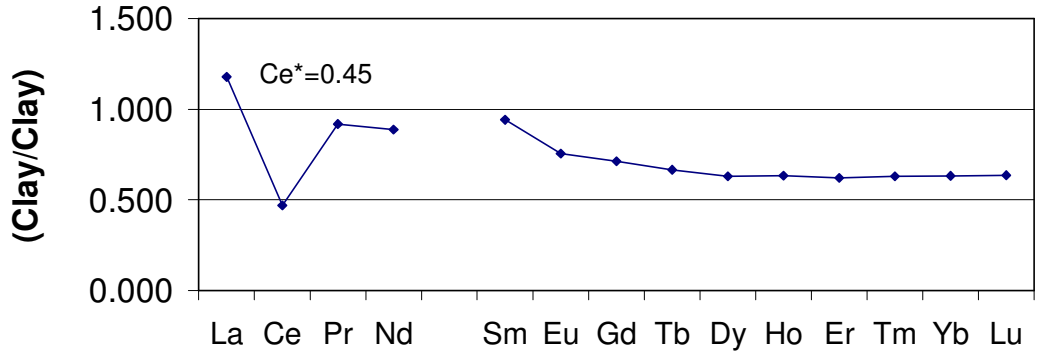


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Figure 5
REEs of Bulk Root-Attached Clay Relative to Bulk Non-Planted Source Clay

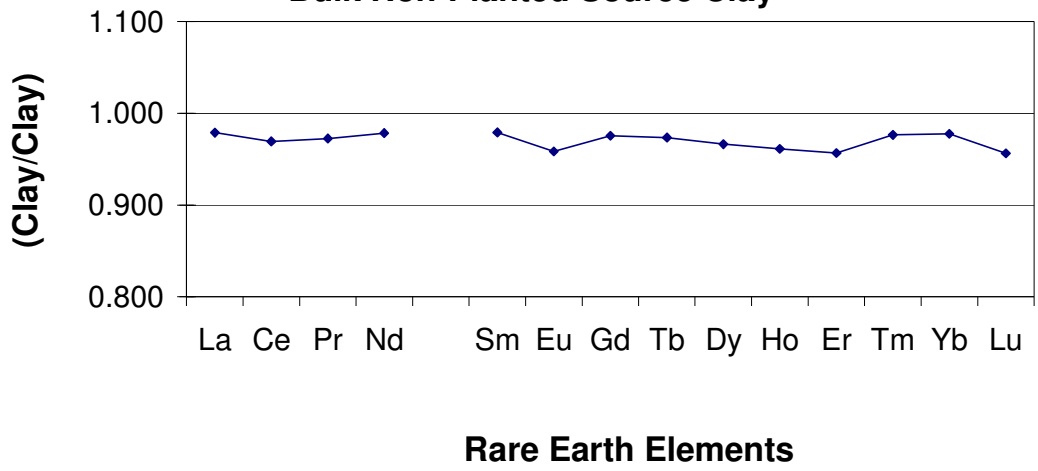

Figure 6
REEs of <2 Micron Fraction Root-Attached Clay Relative to Bulk Root-Attached Clay

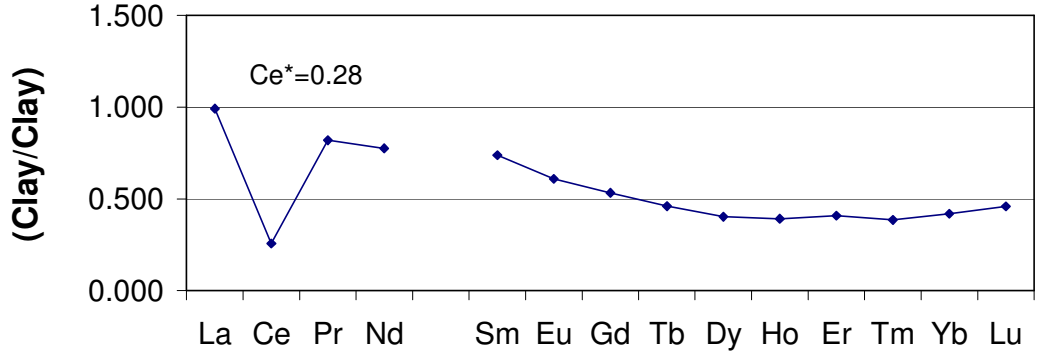

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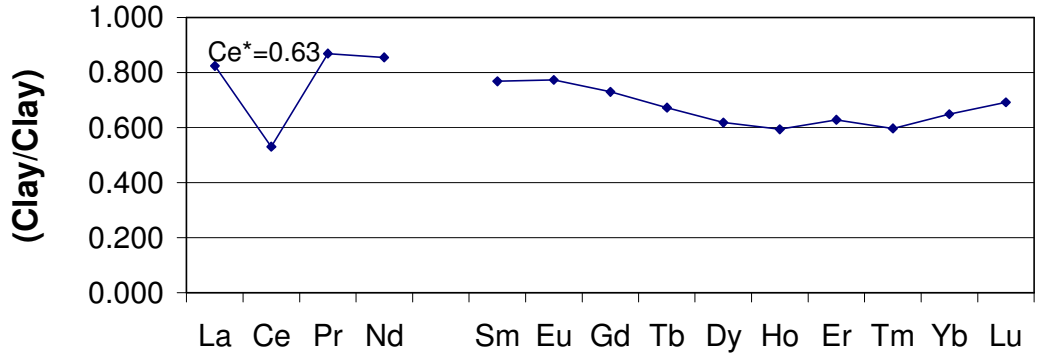
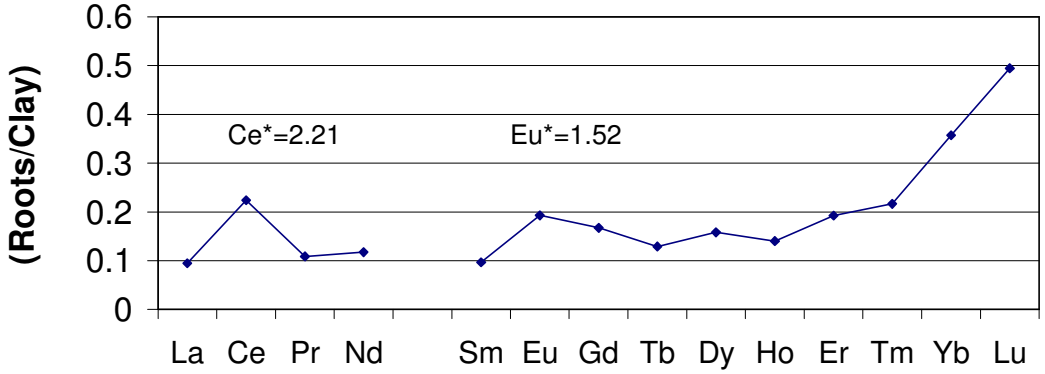
Figure 7
REEs of <2 Micron Fraction Root-Attached Clay
Relative to <2 Micron Fraction Non-Planted Source Clay



Rare Earth Elements
*=calculated anomaly value

Figure 8
REEs of Roots Relative to <2 Micron Fraction
Non-Planted Source Clay



Rare Earth Elements
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Figure 9
REEs of Roots Relative to <2 Micron Fraction
Root-Attached Clay

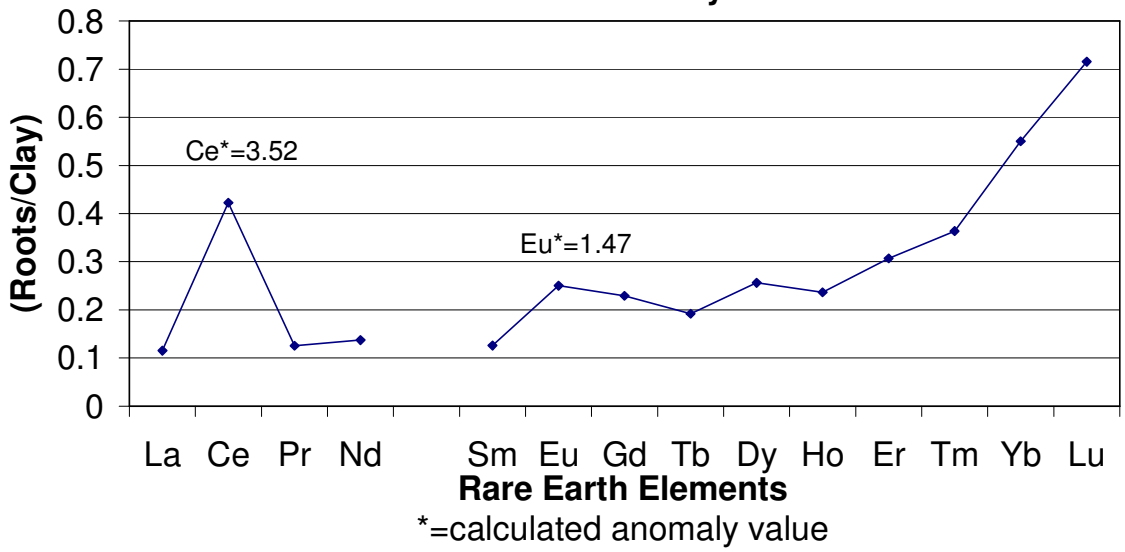

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Figure 10
REEs of Roots Relative to Planted Stems

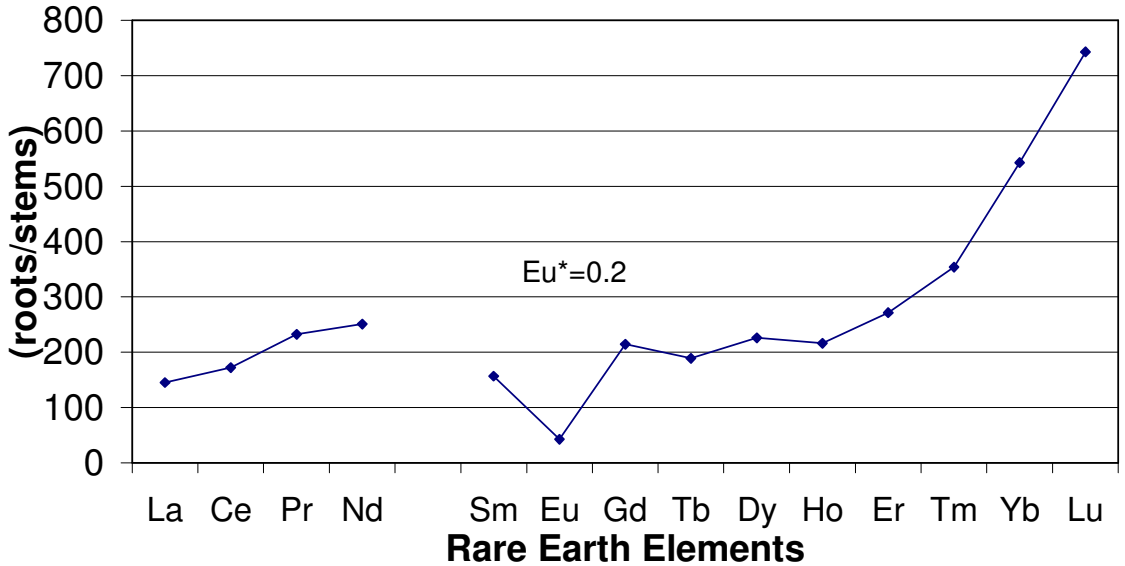

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Figure 11
REEs of Planted Old Stems Relative to Original Reference Stems

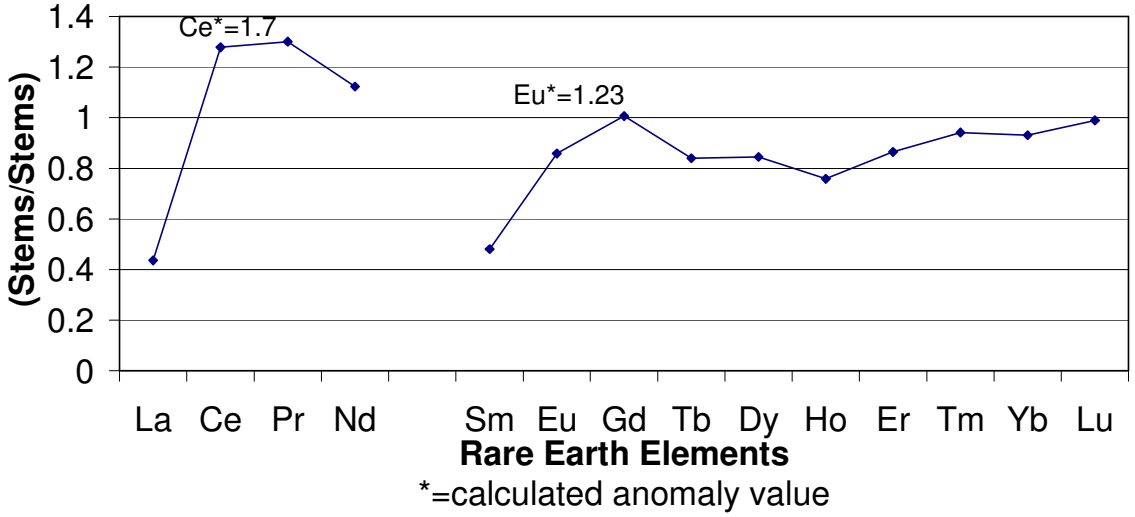

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Figure 12
REEs of New Stems Relative to <2 Micron Fraction Non-Planted Source Clay

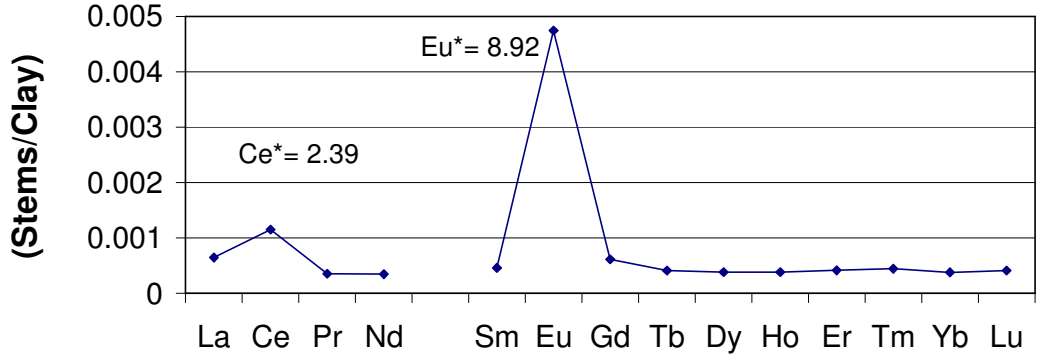

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Figure 13
REEs of New Stems Relative to Original Reference Stems

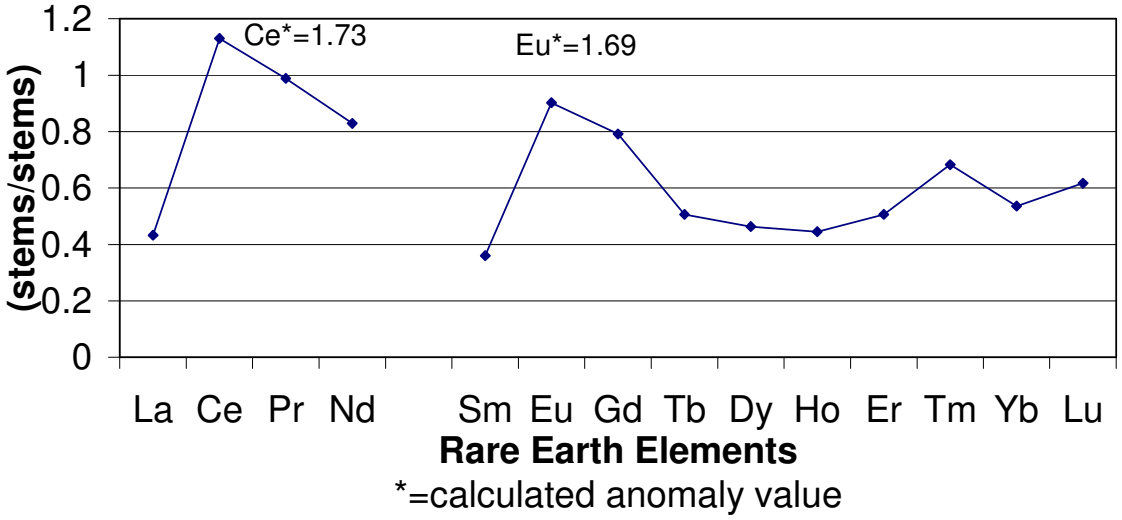

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Figure 14
REEs of New Stems Relative to Planted Stems

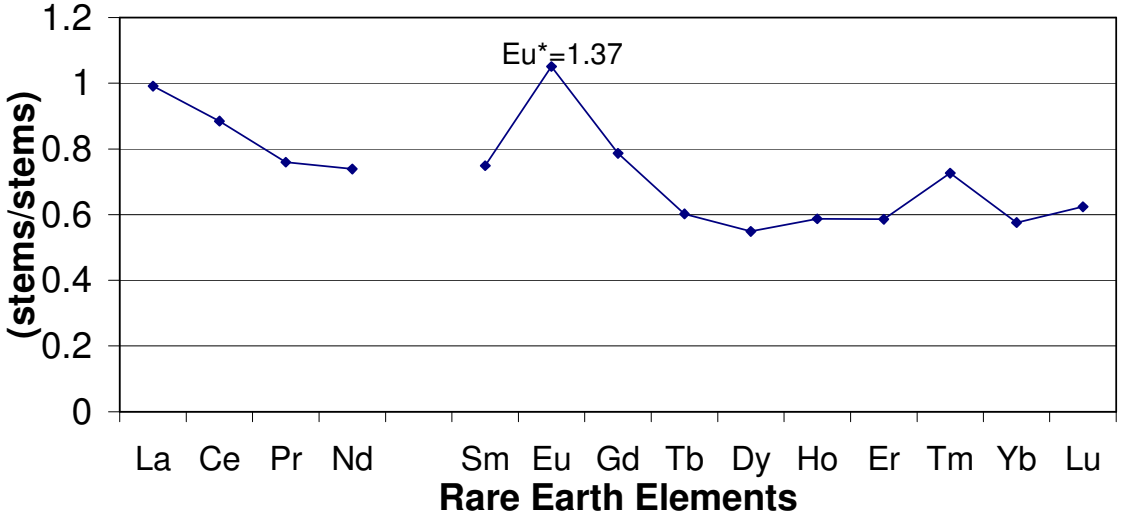

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Figure 15
REEs of Leaves Relative to New Stems

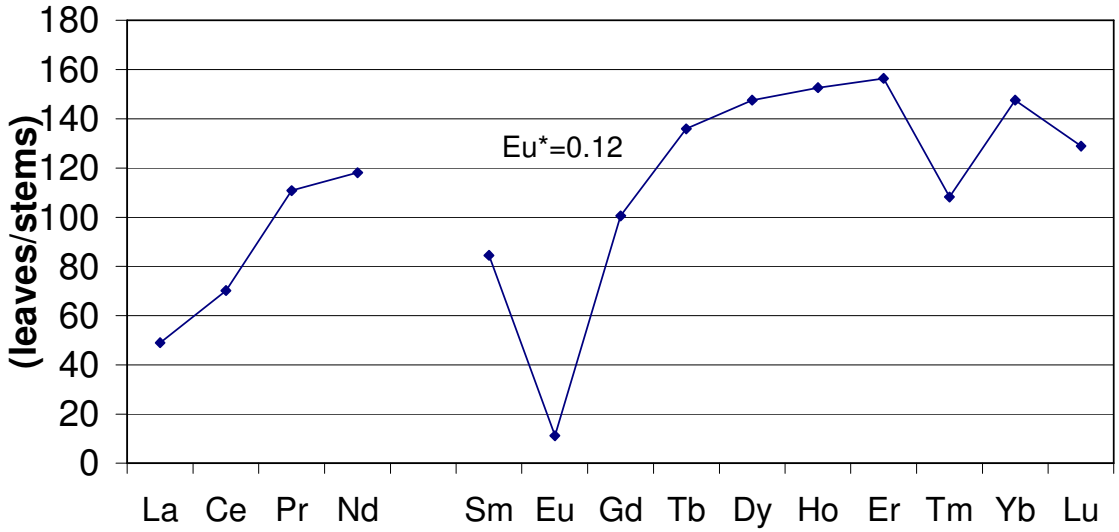

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Figure 16
REEs of Leaves Relative to Planted Stems

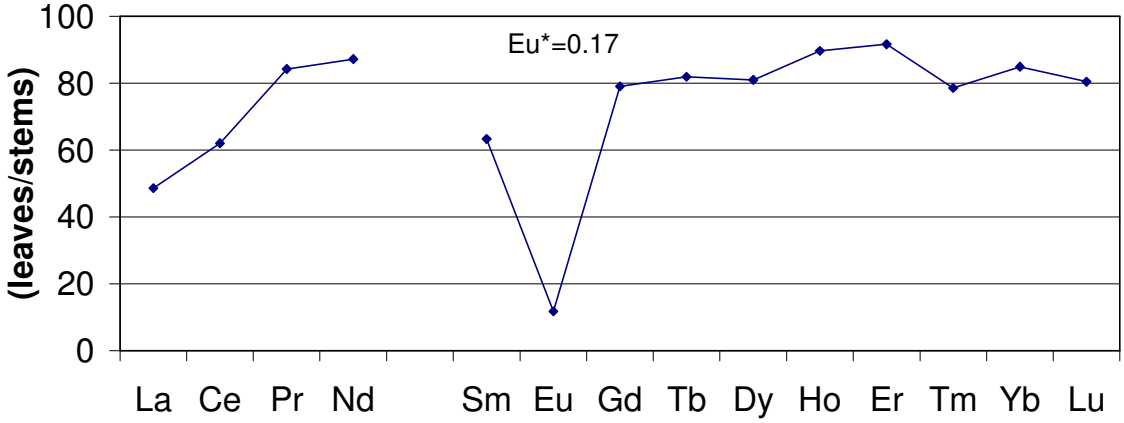

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Figure 17
REEs of Leaves Relative to <2 Micron Fraction
Non-Planted Source Clay

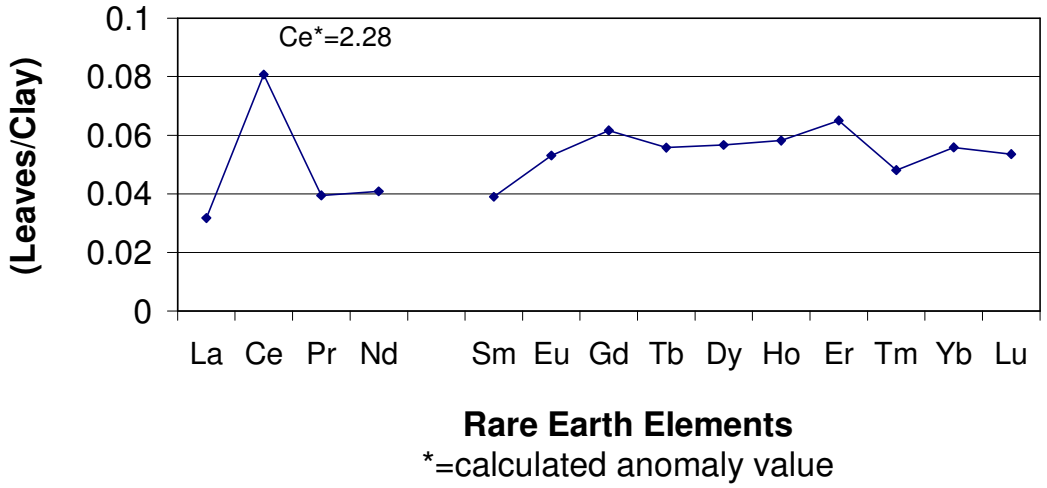

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Figure 18
REEs of New Stems Relative to Roots

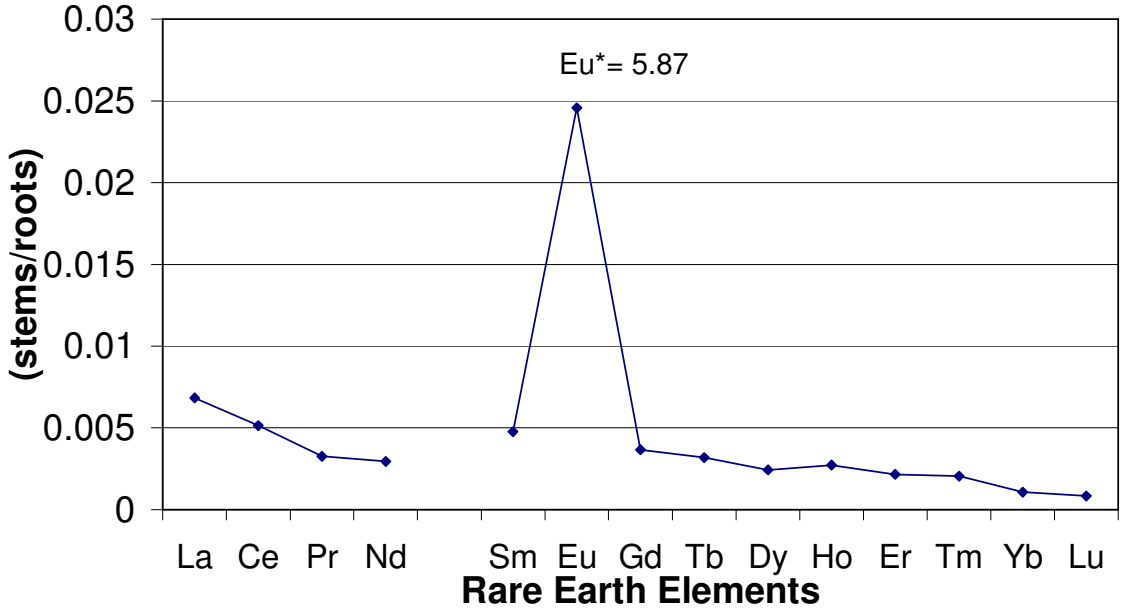

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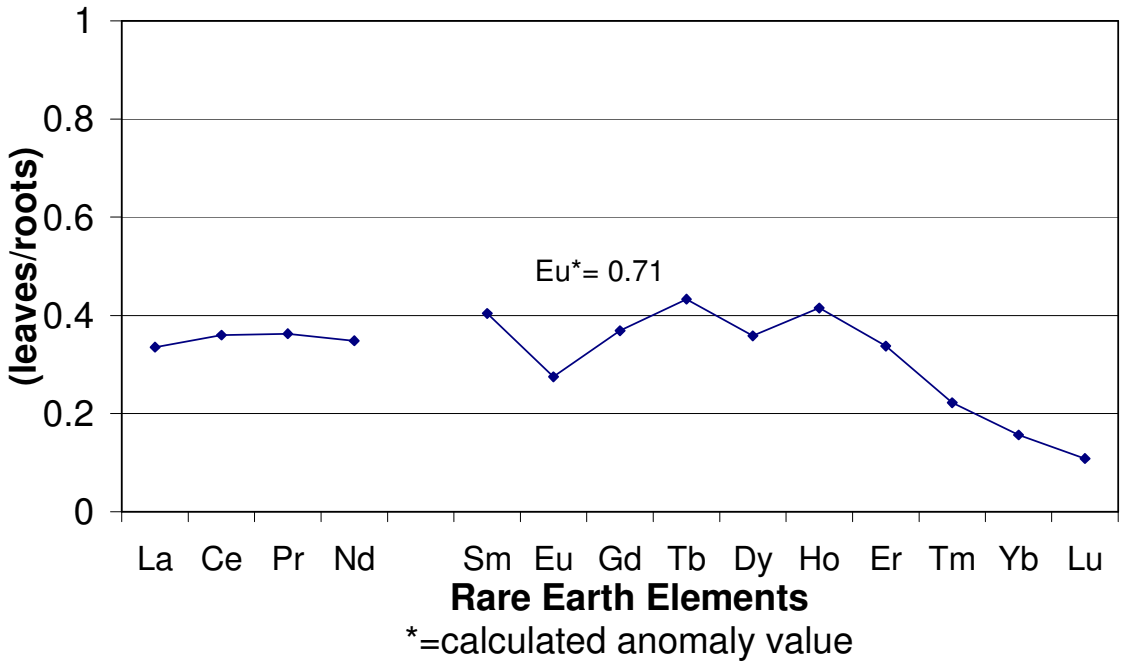
Figure 19
REEs of Leaves Relative to Roots



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TABLES

Table 1

Sample Nomenclature

Sample Nomenclature	Description
Source (Reference) Clay	The original source (reference) clay material. This material was not used during the plant growth cycle and serves as a baseline in which all other materials can be compared.
Less Than (<) 2 micron (µ) Fraction Clay	Clay material that was separated with the particle sizes of < 2 microns remaining.
Bulk Clay	Clay material with no particle size separation.
Root Clay	Clay material that was within one millimeter of the root.
Roots	Root material that grew from the planted old stems planted in the clay.
New Stems	Stems that grew from the planted old stems planted in the clay.
Planted Old Stems	Stems that were planted in the clay. All new plant material grew from these stems.
Original Reference Stems	The original stem cuttings obtained from the hybrid poplar tree farm. These are a portion of the cuttings not used during the plant growth cycle and serve as a baseline in which all other plant materials can be compared.
Leaves	Plant material that developed from the new stem growth.

Table 2

Laboratory Analytical Results

Element	Atomic Number	Atomic Weight	<2µ Source Clay	Bulk Source Clay	<2µ Root Clay	Bulk Root Clay	Roots	Leaves	New Stem	Planted Old Stem	Original (Reference) Stem
Unit			**(µg/g)**	**(µg/g)**	**(µg/g)**	**(µg/g)**	**(µg/g)**	**(µg/g)**	**(µg/g)**	**(µg/g)**	**(µg/g)**
La	57	138.9055	101	85.7	83.2	83.9	9.569293	3.2065	0.065429	0.06602536	0.151266093
Ce	58	140.116	76.9	164	40.8	159	17.24615	6.207177	0.088485	0.100064753	0.078293506
Pr	59	140.90765	16.7	18.2	14.5	17.7	1.817451	0.658632	0.005941	0.007818985	0.006012637
Nd	60	144.24	53.6	60.4	45.8	59.1	6.284312	2.188219	0.018528	0.025083516	0.022347855
Sm	62	150.36	9.51	10.1	7.3	9.89	0.917652	0.370481	0.004383	0.005852011	0.012162098
Eu	63	151.964	0.906	1.2	0.7	1.15	0.174961	0.048097	0.0043	0.004090794	0.004766022
Gd	64	157.25	5.55	7.79	4.05	7.6	0.928364	0.342316	0.003406	0.004329269	0.004302342
Tb	65	158.92534	0.759	1.14	0.51	1.11	0.097835	0.042356	0.000312	0.000517297	0.000615706
Dy	66	162.500	4.11	6.53	2.54	6.31	0.649855	0.232905	0.001579	0.00287695	0.003405387
Ho	67	164.93032	0.817	1.29	0.485	1.24	0.114617	0.047556	0.000312	0.000530435	0.000699321
Er	68	167.259	2.15	3.46	1.35	3.31	0.414193	0.139743	0.000893	0.001524922	0.001763504
Tm	69	168.93421	0.374	0.594	0.223	0.58	0.081053	0.017982	0.000166	0.000228911	0.000243242
Yb	70	173.04	2.25	3.56	1.46	3.48	0.803392	0.12566	0.000852	0.001479274	0.001588674
Lu	71	174.967	0.35	0.551	0.242	0.527	0.173176	0.018741	0.000145	0.000233061	0.000235641

Table 3

REE Distribution Normalized to PAAS - Anomaly Values and Fractionation Description

Sample	Ce*	Eu*	Fractionation Type
Relative to PAAS			
<2µ Source Clay	0.43	0.59	LREE
Bulk Source Clay	---	0.64	LREE
<2µ Root Clay	0.27	0.61	LREE
Root Bulk Clay	---	0.62	LREE

* = calculated anomaly value

Table 4
REE Anomaly Values and Fractionation Descriptions of Sample Materials

Sample Ratios	Figure Number	Ce*	Eu*	Fractionation Type (Enrichment)
Clays to PAAS				
REE Distributions of Bulk Source Clay Relative to PAAS	2	---	0.64	LREE
REE Distributions of <2 Micron Fraction Source Clay Relative to PAAS	3	0.43	0.59	LREE
Clays to Clays				
<2µ Source Clay to Bulk Source Clay	4	0.45	---	LREE
Bulk Root Clay to Bulk Source Clay	5	---	---	None
<2µ Root Clay to Bulk Root Clay	6	0.28	---	LREE
<2µ Root Clay to <2µ Source Clay	7	0.63	---	LREE, HREE
Roots to Clays				
Roots to <2µ Source Clay	8	2.21	1.52	HREE
Roots to <2µ Root Clay	9	3.52	1.47	HREE
Stems to Stems				
Old Stems to Original Reference Stems	11	1.70	1.23	HREE
New Stems to Original Reference Stems	13	1.73	1.69	MREE
New Stems to Planted Old Stems	14	---	1.37	LREE
Roots to Stems				
Roots Relative to Planted Old Stems	10	---	0.23	HREE, MREE
Stems to Clays				
New Stems to <2µ Source Clay	12	2.39	8.92	None
Leaves to Stems				
Leaves to New Stems	15	---	0.12	HREE
Leaves to Planted Old Stems	16	---	0.17	HREE
Leaves to Clay				
Leaves to <2µ Source Clay	17	2.28	---	HREE
Stems to Roots				
REEs of New Stems Relative to Roots	18	---	5.87	LREE
Leaves to Roots				
REEs of Leaves Relative to Roots	19	---	0.71	LREE

* = calculated anomaly values

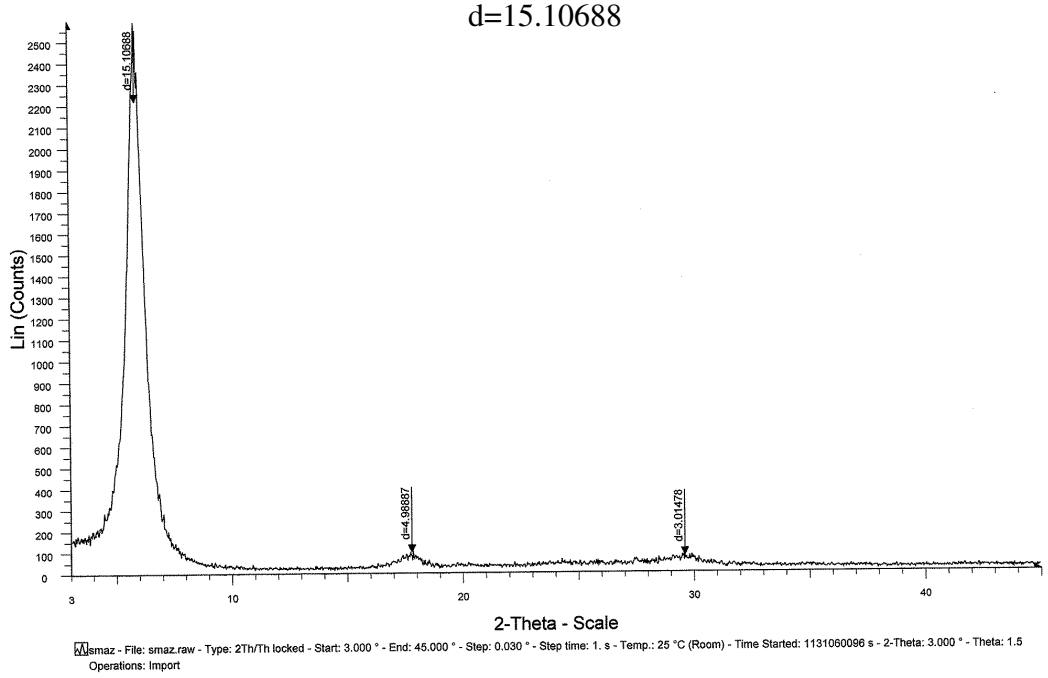
APPENDICES

Appendix A

Cu K Alpha X-Radiation Diagrams

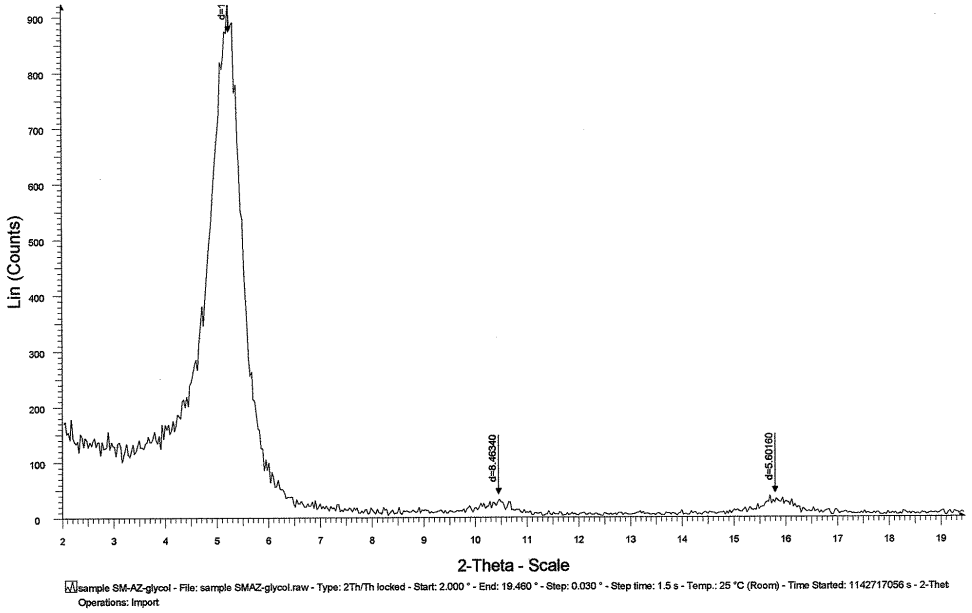
Source Clay – Untreated

d=15.10688



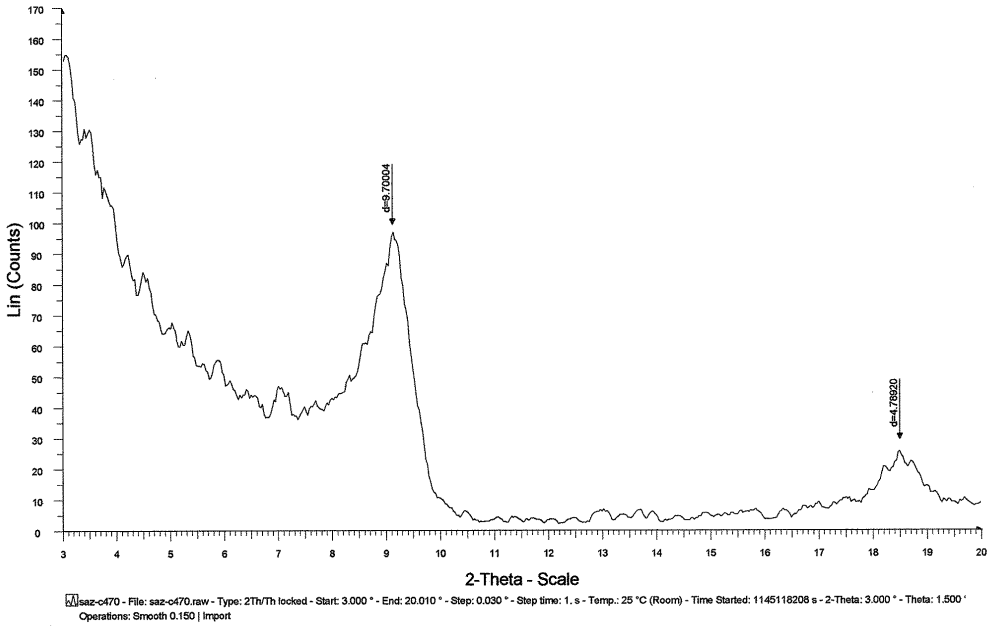
Source Clay – Glycol

d=16.9092



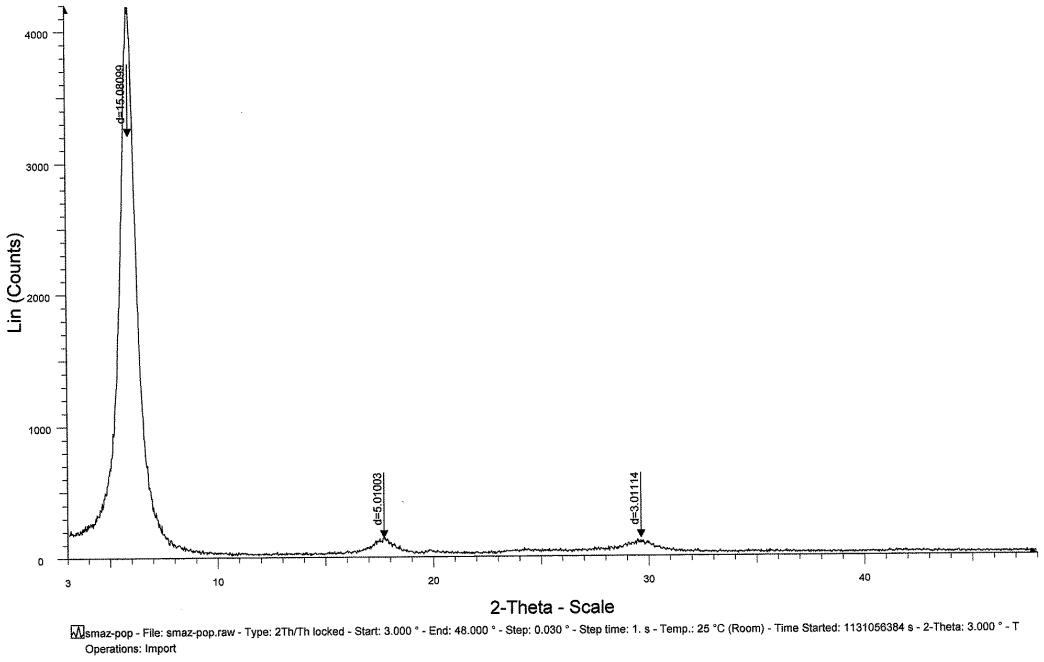
Source Clay – 470 ^{o} C Treated

d=9.7



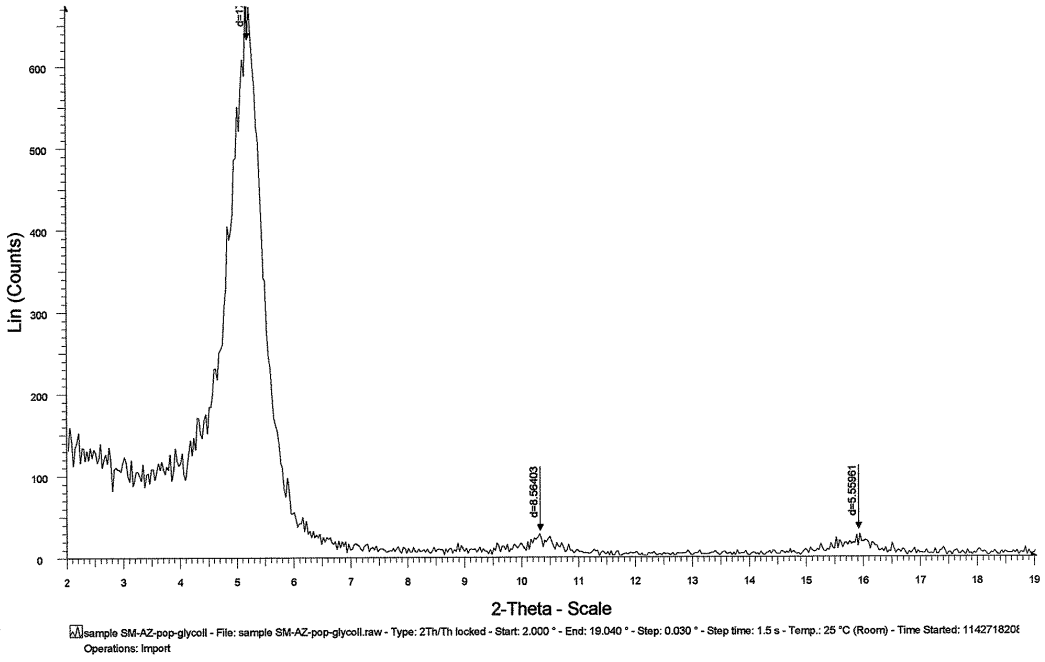
Root Clay – Untreated

d=15.1



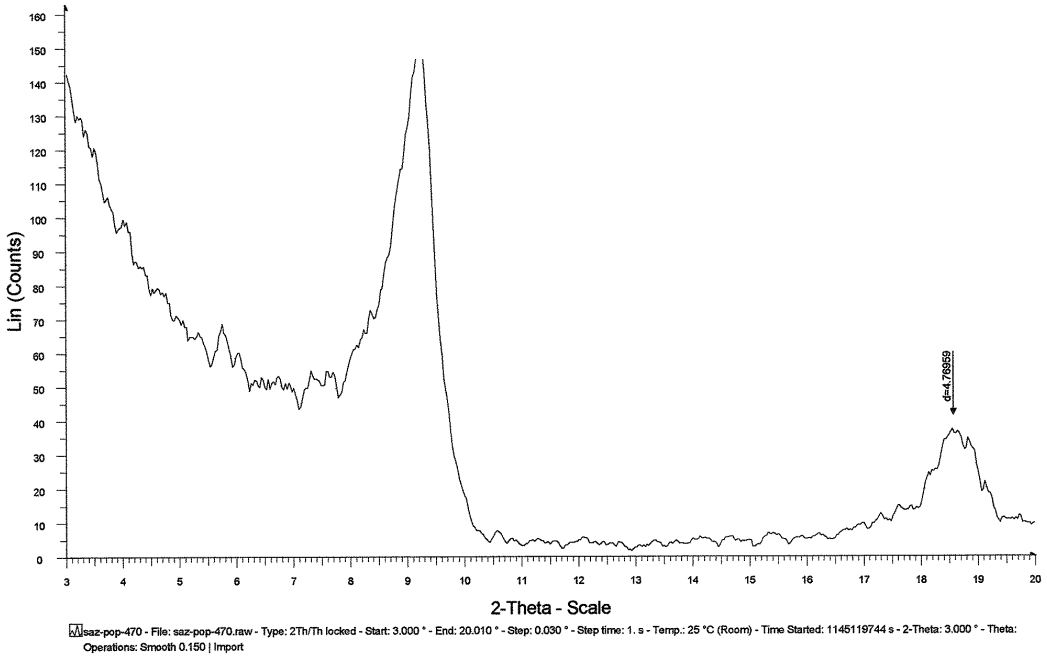
Root Clay – Glycol

d=17.0542



Root Clay – 470 ^{o} C Treated

d=9.56



Appendix B

Major Elements - Laboratory Analytical Results

Element	Atomic Number	Atomic Weight	<2µ Source Clay	Bulk Source Clay	<2µ Root Clay	Bulk Root Clay	Roots	Leaves	New Stem	Planted Old Stem	Original Reference Stem
Unit			(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)
Al	13	26.981538	97,391.2	95,274	95,274	96,332.6	1410.399464	1202.437	17.82163	37.68382	32.98969
Mg	12	24.3050	39,985.53	39,925.22	40,347.79	40,588.63	2188.797143	4744.753	749.8377	1003.677	1444.249
Ca	20	40.078	21,441.0	21,170.68	21,012.18	20,940.71	8997.99152	18556.53	6896.015	4035.918	3336.976
Fe	26	55.845	11,330.28	11,400.22	11,540.1	11,400.22	246.0165142	410.5619	18.07088	20.31765	57.3899
Mn	25	54.938049	929.4	635.09	240.95	503.425	524.8828387	447.3934	98.24744	56.29541	20.82759
Na	11	22.989770	259.665	118.704	356.112	252.246	154.9654095	340.149	149.1367	149.8587	87.41508
K	19	39.0983	1,660.4	2,266.496	1,461.152	2,357.768	5534.478911	20744.75	9554.719	5895.513	7791.344
P	15	30.973761	235.656	257.476	240.02	209.472	1264.003571	1104.942	947.1634	687.3682	1641.883

Appendix C

Lanthanide (3+)- Trypsin Association Constants