SOIL METAL INTERACTIONS AND INVESTIGATION OF EXTRACTIVE METAL REMOVAL PATHWAYS

by

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The only stability is change…
Robert Sardello

Dedicated to my Family and Beloved…

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ABSTRACT

The aim of this work was to interprete the interaction of zinc with soils varying in composition and investigate methods for the removal of zinc from soil.

The adsorption behaviour of zinc was evaluated with isotherms, kinetic studies and sequential fractionation. The findings derived from isotherms were in consistence with the results of sequential extraction experiments, suggesting the presence of more than two sites participating in the adsorption of zinc. Thus, it was presented that high affinity sites with high binding energies like iron and manganese oxides and carbonates were occupied at lower zinc concentrations, and low affinity sites with low binding energies like exchangeable sites at higher zinc concentrations.

The removal of zinc with naturally occurring humic and fulvic acids was found to be low. The reason of this could not be explained by the number of functional groups involved in the extractions. The adsorption of humic and fulvic acid was proposed as the mechanism hindering the removal of zinc.

The removal of zinc with synthetic chelating agents such as EDTA and DTPA increased with increasing EDTA and DTPA concentrations, revealing efficiencies over 100 %. These observations were well explained with the stoichiometric molar ratios and the pH dependent speciation of EDTA and DTPA.

Sequential extraction experiments helped to understand from which soil fraction zinc was taken by humic acid, EDTA and DTPA. Thus, humic acid preferred to remove zinc adsorbed by the exchangeable sites of soils, whereas EDTA and DTPA took the zinc associated with carbonates and iron and manganese oxides.

ÖZET

Bu çalışmanın amacı çinkonun toprak ile olan etkileşimini yorumlamak ve çinkonun topraktan giderimini incelemektir.

Çinkonun toprağa bağlanma davranışı adsorpsiyon isotermleri, kinetik ve ardışık ekstraksiyon çalışmalarıyla değerlendirilmiştir. İsotermlerden elde edilen bulgular ardışık ekstraksiyon çalışmasının sonuçlarıyla desteklenmiştir. Buna göre, çinkoyu daha yüksek bağlanma enerjileriyle tutacak demir ve mangan oksit ve karbonatlar gibi elemanlar çinkonun daha düşük konsentrasyonlarında kullanılırken, daha düşük bağlanma enerjileriyle çinkoyu değiştirilebilir durumda tutacak ögeler daha yüksek çinko konsantrasyonlarda devreye girmekte olduğu anlaşılmıştır.

Çinkonun, doğal olarak bulunan hümik ve fulvik asitlerce topraktan giderimi oldukça düşük düzeyde bulunmuştur. Bu durum, hümik ve fulvik asitlerde bulunan ve pH ile değişkenlik gösteren fonksiyonel grupların ekstraksiyon sırasındaki bulunuş durumlarıyla açıklanamamıştır, ancak hümik asidin toprağa tutunmasının konsantrasyonla artmasına dayanarak, hümik ve fulvic asitlerin toprağa bağlanması nedeniyle olduğu öne sürülmüştür.

Çinkonun topraktan EDTA ve DTPA ile gideriminin ise bu maddelerin konsantrasyonunun artmasıyla artmakta olduğu görülmüştür. Bu yüksek giderimler, molar kesir ve pH'ya bağlı EDTA ve DTPA'nın değişik formlarda dağılmış olmasıyla açıklanmıştır.

Yapılan ardışık ekstraksiyonlar yardımıyla çinkonun hümik asit, EDTA ve DTPA ile toprağın hangi fraksiyonlarından alınabildiği anlaşılmıştır. Buna göre, hümik asitin toprak elemanlarının yüzeyinde değiştirilebilir durumda bulunan çinkoyu aldığı, EDTA ve DTPA'nın ise demir ve mangan oksitlere ve karbonatlara bağlı çinkoyu aldığı öğrenilmiştir.

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1. INTRODUCTION

Soil heavy metal interaction has become an increasing interest, not only because of the danger of groundwater pollution but also the bioaccumulation of metals in crops (Borůvka *et al.*, 1997; García Sánchez *et al.*, 1999). Besides mining activities, increased agricultural practices like usage of sewage sludges and application of artificial fertilizers and pesticides resulted in deposition of heavy metals in soils from agricultural areas. Sewage sludges disposed off on land or used for soil amendment introduce appreciable amounts of copper, zinc, cadmium, and nickel into soil (Polo *et al.*, 1999). Fertilizers and pesticides, on the other hand, are sometimes overused or continuously applied in order to increase crop production as much as possible. The introduction of zinc to soils of agricultural interest, for example, can be through continuous supplement of zinc fertilizers like zinc sulfate, zinc oxide, zinc chelate and spraying of fungicides like mancozeb, etc. In the soil, zinc, a chemically stable metal showing only a single valence, is present in combined form resulting from its interactions with soil minerals and organic matter (Krauskopf, 1972).

Metals in soils can be associated with various components: adsorbed on surfaces of clay minerals, iron and manganese oxides; complexed with organic matter; present in lattice of secondary minerals like carbonates, phosphates, sulfates or oxides, etc. Possible reactions of metals with different adsorption sites were well described by Evans (1989). Generally, adsorption isotherms are used to evaluate the interaction of metals with soils. The influence of soil properties like clay, organic matter and carbonate contents, pH and CEC on the metal soil interaction was also explained with adsorption isotherms. Fitting the data into Freundlich, Langmuir and BET equations provide a better understanding of the interaction. On the other hand, the adsorption coefficients help to differentiate between the adsorption behaviour of metals in different types of soils. In general, isotherms offer useful information about the soil retention capacity, however this information is limited since it does not include the partitioning of metals with the soil constituents. Generally, to provide a deeper insight into the adsorption phenomenon and into its environmental and agricultural meanings, sequential extraction procedures are used. Sequential extractions with selective extractants fractionate the metals bound to different soil constituents and thus provide information about their origin, bioavailability and potential mobilizability and transport in the environment. Among the many sequential extraction procedures described (Shuman, 1985; Ure, 1996; Maiz *et al.*, 1997) the procedure developed by Tessier *et al.* (1979) is the most widely used speciation procedure reported in literature (García Sánchez *et al.*, 1999; Gómez Ariza *et al.*, 2000). The procedure distributes the metals into five fractions: exchangeable, carbonate, iron and manganese oxide, organic and residual.

Soils contaminated by heavy metals have been remedied either by landfilling after excavation or by *in-situ* or *ex-situ* treatment methods. Treatment methods are based on the same principles: 1) physicochemical stabilization/immobilization and 2) extraction. Extraction or mobilization of metals is accomplished by using acidic or chelating agent solutions, which are able to recover the metals bound to soil particles by either changing the pH or by forming stable complexes. The inconvenience of acid treatment is that it is not selective for heavy metals and destroys important soil components, especially in carbonate rich soils (Theodoratos *et al.*, 2000). Chelating agents such as nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) are less damaging the soil matrix and therefore are often suggested as alternatives to straight mineral acid solutions (Jianzhen and Klarup, 1994).

In this study the properties of soils from greenhouses located in three different regions of Turkey, namely Fethiye (Mediterrenian region), Izmir (Aegean region) and Samsun (Black Sea region) were examined and the influence of soil constituents on zinc adsorption was indirectly evaluated. The adsorption behaviour of zinc was evaluated with isotherms, kinetic studies and sequential fractionation. The influence of soil composition and soil pH on the adsorption behaviour and on the removal efficiency was presented. The removal of zinc from greenhouse soils, by using naturally occurring humic and fulvic acids and synthetic chelating agents EDTA and DTPA, was examined. Humic and fulvic acids are polydentate, high molecular size chelating agents, whereas EDTA and DTPA are oligodentate, low molecular size chelating agents. With the help of sequential extraction experiments, it was possible to assess from which fraction zinc could be removed by chelating agents. These successive steps may lead to the interpretative assessment of the interaction between the soil components and model metal, zinc.

2. THEORETICAL BACKGROUND

2.1. Soil

2.1.1. Definition of Soil

Soil, the upper part of soil crust of earth, is formed over time by the weathering of parent materials and decomposition of organic matter from plant and animal litter. It is differentiated into horizons, of variable depth, which differs from the material below in morphology, physical make-up, chemical properties and composition, and biological characteristics. The definite horizons of soil make up the soil profile. Mostly, the boundary between the horizons in a soil profile is recognizable due to changes in color, texture, structure, consistence, porosity, presence and absence of certain metals like iron, moisture, etc. A hypothetical soil profile, showing the various horizons, which might be possible in a highly developed soil, is presented in Figure 2.1.

The horizons in a soil profile are shown by letters. The topsoil, 01 and 02, is composed of 20-30 % or more of fresh and partially degraded organic matter. The A horizon, the upper part of profile, demonstrates the highest biological activity. Clays and oxides of iron and aluminium are leached from this horizon and are translocated to lower horizons by internal washing in the soil. This process of rinsing through upper horizons and removing finer particles and minerals is termed eluviation. The B horizon, in contrast to the A horizon, is the layer where clays and oxides of iron and aluminium accumulate. This depositional process is termed illuviation. A and B horizons make up the solum. The third layer, the C horizon, is weathered bedrock or weathered parent material, excluding the bedrock itself. The C horizon is not affected by the soil operations in the solum and lacks clay concentrations. Soil roots and soil microorganisms are rare in this layer.

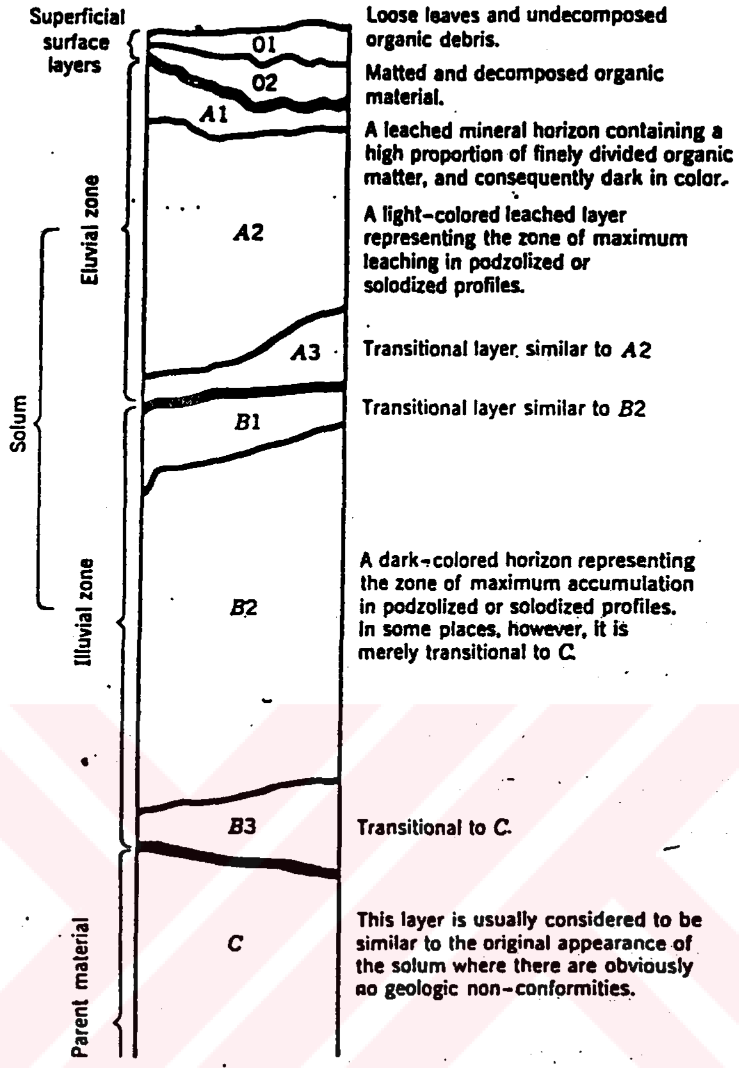


Figure 2.1. Hypothetical soil profile showing the principal horizons (Millar *et. al*, 1958).

2.1.2. Components of Soil

Soil, as a heterogeneous porous medium, is made up of solid, liquid and gaseous phase. Depending on its texture, the solid phase roughly makes up approximately 50 % of soil as mainly mineral and organic constituents. The mineral portion is derived from parent material, whereas the organic largely from vegetation. The porous phase, on the other hand, might be occupied nearly equally by water and air, depending on the texture and structure of soil, and on climatic conditions. In Figure 2.2 an approximate volume composition of a representative silt loam surface soil can be seen.

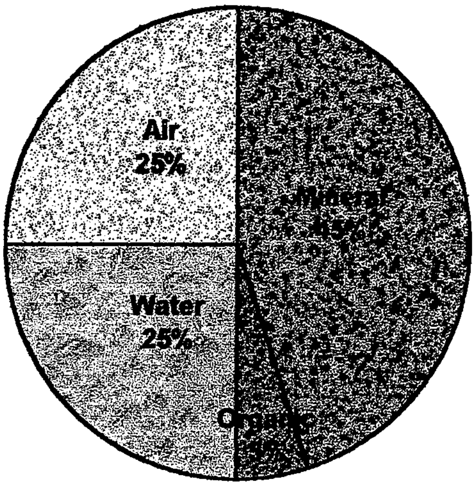


Figure 2.2. Composition by volume of a typical topsoil (Ellis and Mellor, 1995).

2.1.2.1. Soil Minerals: Soil minerals that are more or less unchanged in composition from the original rock are called *primary* minerals. Quartz, feldspar, mica, etc. are primary minerals. Other minerals that are formed by weathering of less resistant minerals or during soil formation process are called secondary minerals. Silicate clays and iron oxides are examples of secondary minerals. Generally, primary minerals dominate in the coarse fraction of soil, like sand and silt, while secondary minerals in the fine materials.

Gravel and sand, which are made up of primary minerals, are chemically inactive. However, they support drainage and air movement. Silt particles, on the other hand, are actually microsand particles. Their relatively low particle size and related higher surface area make it possible to carry a clay film enabling them to take part in chemical reactions to a lesser extent. Sand and silt, which are made up weatherable minerals like feldspar and mica, are able to slowly release ions that supply plant needs or recombine to form secondary minerals like clay. Among mineral constituents of soil the most chemically active fraction are clay minerals. This feature is attributed to their low particle size (< 0.002 mm) and consequently high surface area. The chemical structure of clay minerals is explained in more detail below.

Clay Minerals: Clay minerals are formed by either simple alterations of the original mineral, which keeps its structure maintained, or by complex transformations. In the latter process, the clay minerals are formed by precipitation of dissolved minerals. Minerals like hydrous oxides of iron and aluminium are also formed by this recrystallisation process. A diagram showing the general conditions for the formation of various silicate clay minerals and the oxides of iron and aluminium can be seen in Figure 2.3.

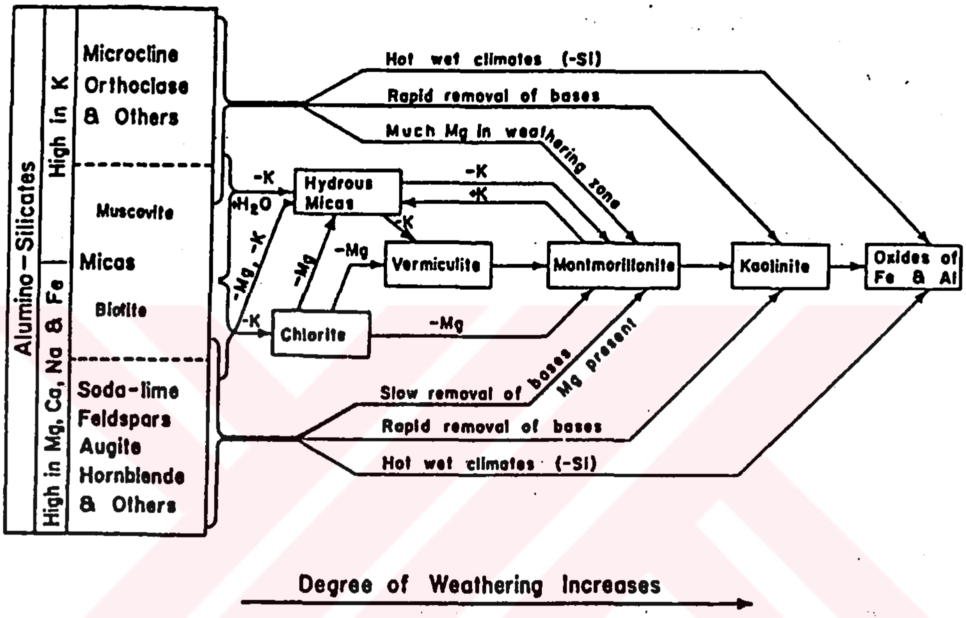


Figure 2.3. General conditions for the formation of various silicate clays and the oxides of iron and aluminium (Buckman and Brady, 1961).

Clay minerals, in general, represent layers of silica and aluminium sheets. Silica sheets are made up of various Si-O tetrahedral structures, in which one silicon atom is centered between four oxygen atoms. Aluminium sheets, on the other hand, are made up of Al-O octahedral structrures, in which one aluminium atom is centered between six oxygen atoms. Different arrangements of these sheets result in the formation of clay mineral like kaolinite, montmorillonite, illite and vermiculite. Kaolinite is composed of one silica sheet and one aluminium sheet, indicated by the ratio of 1:1. Monmorillonite, illite and vermiculite are examples of 2:1 silica-aluminium sheet. Clays that have two silicate sheets and two aluminium sheets are known as 2:2 clay minerals like chlorite. The silicon and aluminium atoms in the sheets, called the coordinating atoms, are replaced by other atoms with lower valence like iron, manganese and magnesium. The permanent negative charge or cation exchange of clay colloids is due to this cation substitution. The location of cation substitution, whether it takes place in the silica sheet or aluminium sheet, also controls the expansion and specific surface of 2:1 clay minerals. 1:1 and 2:2 clay minerals (e.g. kaolinite and chlorite) do not exhibit significant expansion.

Minerals other than clay: In addition to clay minerals soils contain at minor amounts a variety of minerals like oxide minerals, calcite ($CaCO_3$), calcium sulfates ($CaSO_4$), and anatase (TiO_2) and amorphous silica. Oxide minerals present in soil are the free oxides and hydroxides of iron, aluminium and manganese. Iron oxides occurring in soil are goethite (α-FeOOH), hematite (α-Fe_2O_3), lepidocrocite (β-FeOOH) and maghemite (γ-Fe_2O_3). They vary greatly in size, shape and surface morphology. The surface of iron oxides is often hydroxylated, either structurally or through hydration of the Fe ions. Aluminium oxides present in soil are gibbsite [γ-$Al(OH)_3$] and boehmite (γ-AlOOH). Aluminium oxides in comparison to iron oxides are larger. Manganese oxides like birnessite and pyrolusite, on the other hand, occur in soil as very small (~10 nm) structurally disordered crystals. Metal oxides are present in soil as free oxides, clay edges and clay mineral coatings. They carry a charge due to the protonation and deprotonation of the oxygen coordinated to the metal. Depending on the pH of soil solution and point of zero charge (PZC) of oxide, this charge can be positive or negative. However, the number of this pH dependent charge is smaller than that of clays and therefore, cause to adsorption at a much lower degree. Depending on the parent materials, some soils may contain significant quantities of relatively high surface, soluble calcite or calcium sulfate. It is generally agreed that $CaCO_3$ surfaces provide sites for metal-surface interaction via specific adsorption or speciation reaction (Martinez and Motto, 2000).

2.1.2.2. Soil Organic Matter: Soil organic matter is defined as the nonliving portion of the soil organic fraction, and it is formed by decomposition of plant and animal tissue and by microbial synthesis. Although it makes only a small part of soil, about 3-5 % by weight of a representative mineral topsoil (Buckman and Brady, 1961), it has a great influence on the physical, chemical and surface properties of soil material. Soil organic matter is a major soil source of three important mineral elements, nitrogen, phosphorus and sulfur and therefore supports plant growth. It also tends to increase the water holding capacity of soil and is the main energy source for soil microorganisms.

The soil organic matter may be considered to consists of two general groups: (1) original tissue and its partially decomposed equivalents; and (2) the humus. Humus, also known as humic substances, is produced at the end of the humification process, which involves the complex reaction of various decomposition products to produce large, complex molecular chains, or polymers. The number of molecules involved in this process, as well as the number of ways in which they combine is almost unlimited, which explains the heterogeneity of humic material in soil. A schematic representation of humification process can be seen in Figure 2.4.

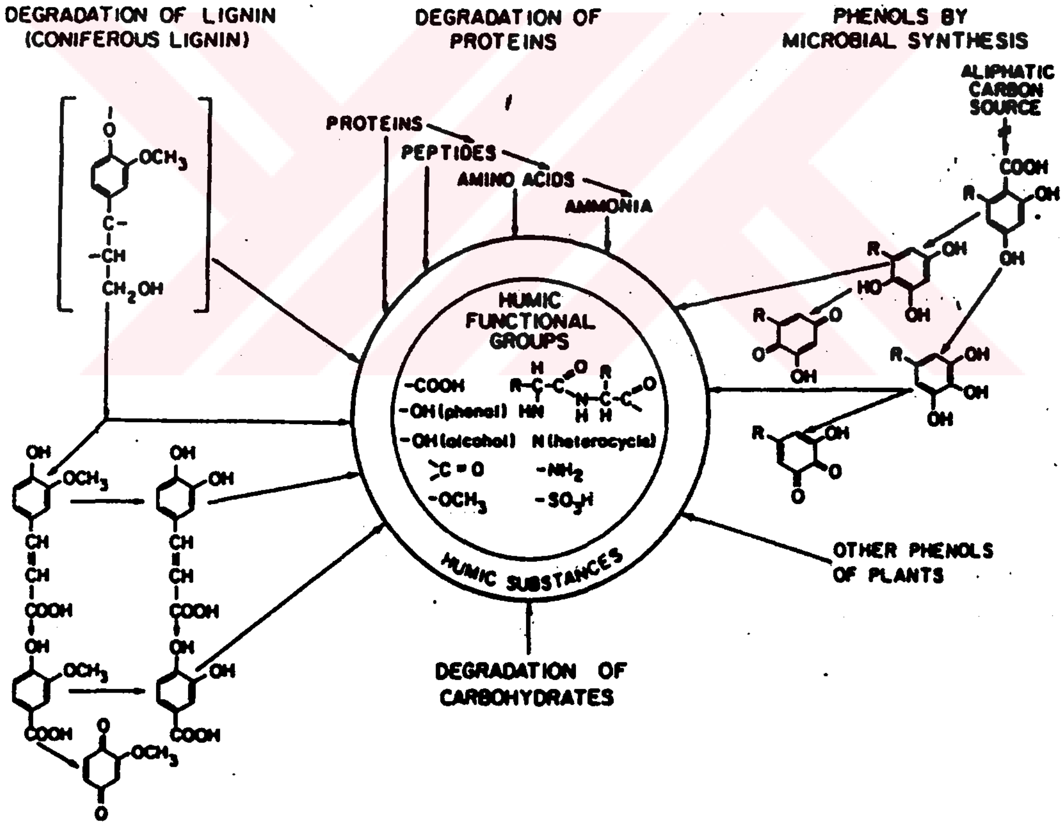
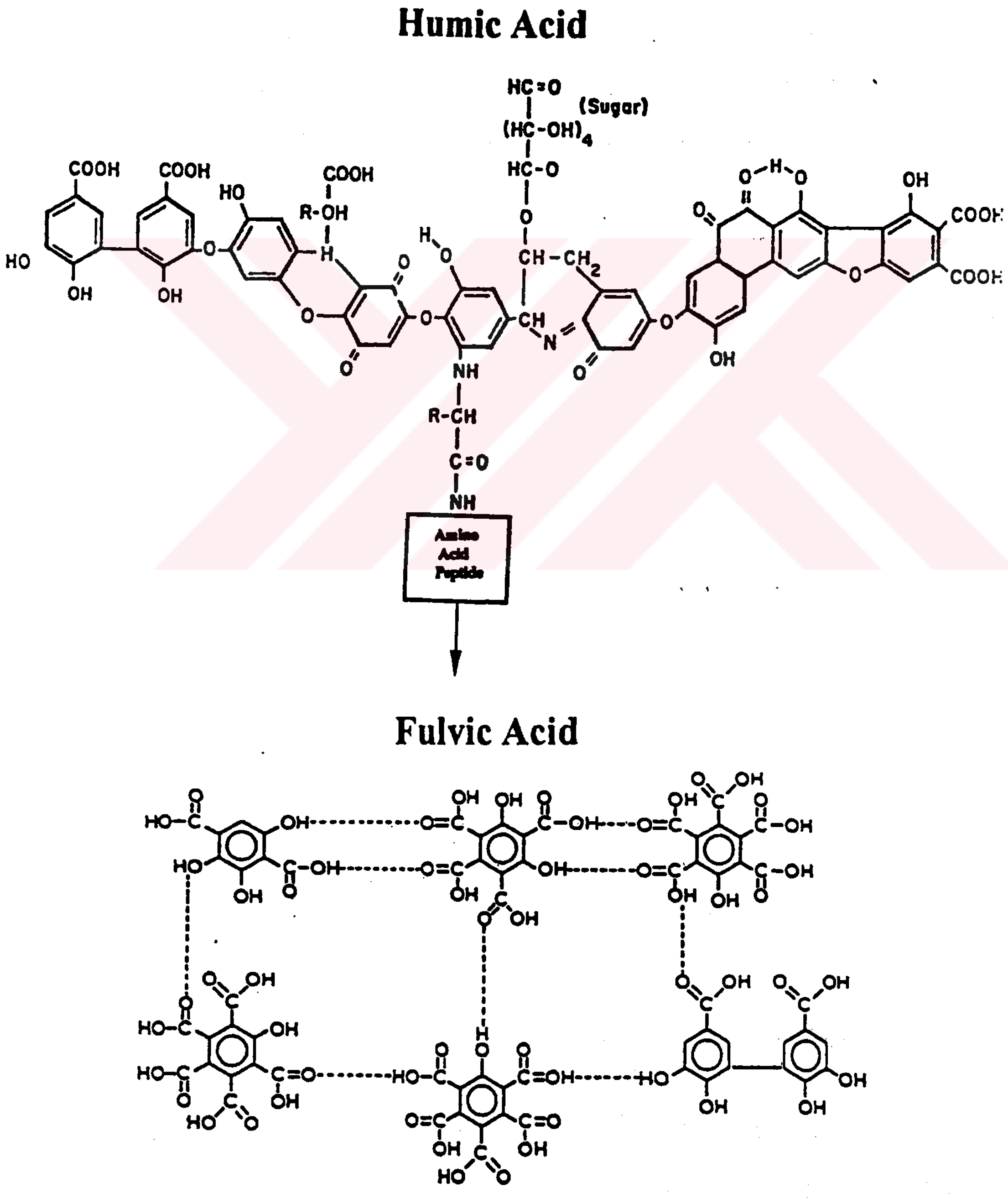


Figure 2.4. Schematic representation of humic substances in soil (Yaron *et al.*, 1996).

Humic substances are polar, amorphous, brown or black, hydrophilic, acidic, polydisperse substances with a wide range in molecular weight from a few hundreds to several thousands. Their approximately elemental composition on an ash free basis is as follows: 44-53 % carbon, 40-47 % oxygen, 3.5-5.5 % hydrogen and 1.5-3.5 % nitrogen (Ellis and Mellor, 1995). They contain various functional groups like phenolic OH, carboxylic and aliphatic OH groups. Based on their solubility behaviour humic substances can be differentiated into three main fractions: humic acid, which is soluble in dilute alkali, but precipitates in acid solution; fulvic acid, which is soluble in both alkali and acid solution; and humin, which is insoluble both in alkali and acid solutions. Proposed structural formulae for humic and fulvic acid can be seen in Figure 2.5.


Humic Acid

Fulvic Acid

Figure 2.5. Model of soil humic and fulvic acids (Evangelou, 1998).

Generally, humic and fulvic acids have a similar structure but differ in molecular weight and elemental and functional group contents. Humic acids are higher in molecular weight (10000-50000) and contain less oxygen-containing functional groups and elemental oxygen when compared to fulvic acids, which have a molecular weight in the range of 500-7000. A high portion of oxygen in humic acids occurs as a structural component of the nucleus, whereas the oxygen in fulvic acids is taking part in functional groups. Additionally, humic acids contain higher nitrogen and carbon per unit weight.

In general, humic substances possess structural lability due to intermolecular association and molecular conformation changes in response to changes in pH, etc. (Evangelou, 1998). Under neutral or slightly alkaline conditions, the molecules are in an expanded state as a result of the repulsion of the charged acidic groups, whereas at low pH and high salt concentration, contraction and molecular aggregation occur due to charge reduction (Yaron *et al.*, 1996). Humic substances have a negative charge originating from the dissociation of the hydrogen ions from the –OH of carboxyl, aliphatic and phenolic groups.

2.1.2.3. Soil Solution: The soil water together with its dissolved salts makes up the soil solution. Sources of soil water are precipitation and ground water. Precipitation might take place in various forms of which rain, snow and hail are the main forms. It introduces dissolved atmospheric ions like sodium, chloride, and molecules like carbon dioxide and sulfate into the soil medium. Other inorganic and organic ions and unionised molecules present in soil solution arise from mineral weathering and organic matter decomposition. Examples are bicarbonate, iron, aluminium, silica, nitrate, phosphate, etc. Besides the mineralization process during organic matter decomposition, nitrate and phosphate are also added to the soil medium by fertilizer application like calcium, potassium and ammonium. The composition of soil solution is continuously changing depending on the intimate association between the water, clay and humus and plant roots. It involves the exchange of ions between these components.

2.1.2.4. Soil Air: Pores, which are not occupied by water, are filled with air. The soil air is continuous with the atmosphere only when compaction or crusting is not occurring; otherwise the soil air is entrapped in the soil pores making its composition variable from place to place in the soil. The gases move along gradients of partial pressure, so that oxygen migrates from the atmosphere into the soil, and carbon dioxide and water vapour from the soil into the atmosphere. Movement of gases occur mainly by diffusion but also by mass flow or in dissolved form. Besides from the atmosphere, the gaseous constituents of soil air are also derived from the respiration and metabolism of soil organisms and from the evaporation of moisture. Thus, the soil air differs from that of the atmosphere in several aspects. The carbon dioxide content is higher than that of atmosphere, and oxygen lower. Nitrogen is present in amounts similar to that present in the atmosphere.

2.1.3. Properties of Soil

2.1.3.1. Physical Properties of Soil: Interactions and transformations in soil are mainly governed by the physical and chemical properties of soil in general. Physical properties of soil are texture, structure, consistence, porosity, density, color and temperature.

Texture: Texture, which refers to the fineness and coarseness of soil, is the relative proportions of the different size groups namely sand silt and clay. Since the particle size distribution determines the amount of surface on which physical and chemical reactions occur, the texture of soil is very important. Soil group or separates are formed on the basis of their size, without taking into account their chemical composition, color, weight or other properties. As can be seen from Table 2.1, they consist of mineral particles less than 2 mm in diameter.

Since a soil separate rarely makes up the soil, they are grouped on the basis of proportion to create the soil textural classes. Simply when the percentage of clay, silt and sand is known the soil class can be determined by the use of textural triangle (Foth, 1990).

Table 2.1. Some characteristics of soil separates (Foth, 1990)

Separate	Diameter, mm[a]	Diameter, mm[b]	Number of Particles per gram	Surface Area in 1 gram, cm^2
Very coarse sand	2.00-1.00	-	90	11
Coarse sand	1.00-0.50	2.00-0.20	720	23
Medium sand	0.50-0.25	-	5,700	45
Very fine sand	0.25-0.10	0.20-0.02	46,000	91
Fine sand	0.10-0.05	-	722,000	227
Silt	0.05-0.002	0.02-0.002	5,776,000	454
Clay	< 0.002	< 0.002	90,260,853,000	8,000,000[c]

[a] United States Department of Agriculture System.

[b] International Soil Science Society (ISSS) System.

[c] The surface area of platy-shaped montmorillonite clay particles determined by the glucol retention method by Sor and Kemper.

<u>Soil Structure:</u> As the term texture is referred to the size of soil particles, structure is used in the reference of arrangements of the particles. Structure refers to the aggregation of primary soil particles (sand, silt, clay) into compound particles or clusters of primary particles, which are separated from the adjoining aggregates by surfaces of weakness (Millar *et al.*, 1958). Due to different arrangements of sand, silt and clay into secondary particles, the aggregates, the soil structure can be divided into four principal types namely platy, prismlike, blocklike and spheroidal. Soil structure influences mainly the infiltration rate of water. Spheroidal soils, for example, have rapid infiltration rates, whereas blocky and prismatic soils have moderate rates. Platy and massive soil structure, results in slow infiltration rates.

<u>Soil Consistence:</u> Consistence is the resistance of the soil to deformation and rupture. It deals with the strength and nature of forces between sand, silt and clay particle. Consistence is more important for tillage and traffic considerations (Foth, 1990).

Porosity: The solid fraction of soil, which is made up of mineral and organic particles, is about 50 % of soil. The remaining fraction of soil is the pore space within the particles. The proportion of soil occupied by pore space depends on both the texture and structure of the soil, because space exists between soil grains as well as aggregates. Pore spaces are important, because they contain air and moisture and allow movement.

Density: Particle density and bulk density are used to describe the density of soil. Particle density is usually defined as the mass of a unit volume of soil solids. Since only solid particles are considered, it is a constant and does not vary with the amount of space between the particles. For many soils the particle density will average about 2.65 g/cm^3 (Millar *et al.*, 1958). Bulk density is defined as the mass of unit volume of dry soil and is the density of the bulk soil in its natural state, including both the particles and pore space. The bulk density of a given soil is variable due to its varying volume of pore space. The bulk density of a mineral soil ranges between 1.0-2.0 g/cm^3 (Buckman and Brady, 1961) and for organic soils between 0.1-0.6 g/cm^3 (Foth, 1990).

Color: The color of soil is an indirect measure of other important characteristics such as water drainage, aeration and organic matter content. Major coloring agents of soil are organic matter and iron compounds in various states of oxidation and hydration. Organic matter gives a black color whereas iron oxides a reddish color. Generally, color in each horizon mainly depend on the nature, amount and distribution of coloring agents.

Temperature: Temperature affects many processes in the soil; not only life processes of plants and animals, but also plays a role in rock weathering, structure formation, etc. The temperature of soil, besides many factors, is also influenced by soil color and texture.

2.1.3.2. Chemical Properties of Soil: Two main chemical properties are ion exchange and soil pH.

Ion Exchange: Ion exchange involves cations and anions that are adsorbed from solution onto negatively and positively charged surfaces, respectively. The dominating exchange is cation exchange rather than anion exchange. In the cation exchange process, the cation in solution replaces another cation on the surface of a negatively charged colloid. The colloid can be a clay or an organic colloid.

The exchange of a cation adsorbed on these negatively charged colloids by a cation in solution is influenced by factors such as (a) relative concentration or numbers of the ions, (b) the number of charges on the ions and (c) the speed of movement or activity of the different ions. The speed or activity of an ion is primarily a function of its size, but the degree of hydration must also be considered. A listing of the more common ions in descending order of replacing power is: H Sr Ba Ca Mg Rb K NH_4 Na Li (Millar *et al*, 1958).

The cation exchange capacity (CEC) of soils is defined as the sum of positive (+) charges of the adsorbed cations that the mineral fraction (clays, etc.) and organic fraction (humic substances) of soil can adsorb at a specific pH (Foth, 1990). The CEC of clay minerals is directly dependent on surface area, which is different for each clay type. As can be seen from Table 2.2, 1:1 type clays like kaolinite, have low surface area and thus lower CEC. The expandable 2:1 type clays like montmorillonite and vermiculite, on the other hand, possess higher surface area and consequently higher CEC. 2:1 type clays representing a non-expanding nature like illite have again a reduced surface area and therefore limited CEC.

Table 2.2. Characteristics of three clays (Haque, 1975)

Clay	Type*	Surface area, m^2/g	Cation exchange capacity, meq/100 g
Kaolinite (non-expanding)	1:1	25-50	2-10
Montmorillonite (expanding)	2:1	700-750	80-120
Illite	2:1	75-125	15-40

* number of silica sheets:number of alumina sheets

Soil organic matter usually have a large surface area (500-800 m^2/g) and thus exhibit high cation exchange capacity (200-400 meq/100 g), many times higher than various clay minerals. The high CEC of organic matter is not only due to its relatively high surface area but also related to the presence of various functional groups.

The pH of soil highly influences both the CEC of soil organic matter and clay minerals. Generally, the CEC of soil increases with increasing soil pH. In the case of soil organic matter, for example, the CEC is about 36 $cmol_c/kg$ at a pH of 2.5; whereas at pH 8 about 213 $cmol_c/kg$ (Evangelou, 1998). The influence of pH on the CEC of clay is more dependent on the type of clay; kaolinite and oxidic clays are more sensitive than the 2:1 clays.

The anion exchange of soil is more due to the replacement of hydroxyls in the clay minerals by anions like nitrate, phosphate and sulfate. Such a capacity of soil is inversely related to the soil pH.

Thus, it appears that the anion exchange capacity (AEC) of mineral decreases as the pH rises above 3, but cation exchange capacity (CEC) remains unaffected up to pH 5 and increases significantly above 5 (Evangelou, 1998).

Soil pH: The soil pH is an important property since it influences the ion exchange capacity and the availability of nutrients. High soil pH, for example, decreases the solubility of iron resulting in iron deficiency for plant growth. The pH of soil mainly depends on the parent material, the soil organic content and biological activity of microorganisms, etc. Parent material, which contain calcium carbonate or sodium carbonate result mostly in alkaline soils due to the release of hydroxyl ions via hydrolysis of these compounds. Mineralization of organic compounds leads to the formation of organic acids and some other nitric and sulfuric acids. Biological activity, on the other hand, generates carbon dioxide contributing to the acidity of soil.

Buffering Capacity: The soil exhibit great resistance towards changes in pH. This is due to the buffering capacity of soil, which is defined as the ability of ions associated with the solid phase to buffer changes in ion concentration in the solution phase. The higher the exchange capacity of a soil the greater will be its buffering capacity. This is because more H^+ ions and metallic cations must be interchanged to result in a rise or lowering in pH.

Decomposition of plant and animal litter to various organic acids like humic and fulvic acids, or addition of fertilizers or agricultural lime to soils introduces H^+, OH^- and various other cations and anions to soil solution. The ions will be exchanged or bound by other ions in solution, thus avoiding their influence on soil pH and biological activities. For example, H^+ introduced into the soil solution results in the replacement of exchangeable bases adsorbed on mineral surface. Adsorption of H^+ on mineral surface eliminates a sharp decrease in pH, thus maintaining solution pH at approximately condition. On the other hand, addition of lime results in release (desorption) of hydrogen ions from mineral surface into the soil solution. As a consequence, the pH rise will be negligible.

2.1.3.3. Surface Charges of Soil Components: Soil components like clay and humic compounds are due to some mechanisms electrically charged. These charges can be divided into two groups, namely permanent structural charges and variable charges.

Permanent charges at the clay mineral surface are generated by lattice substitution within the layers of tetrahedral or octahedral sheets. Substitution of Si^{4+} by Al^{3+}, or Al^{3+} by Mg^{2+} will create negative charges while the substitution of Al^{3+} by Ti^{4+} positive charges. The overall charge on the unit structures of clay minerals is, however, always negative (Evans, 1989).

Variable charges arise from the OH at the edges of clays, or from the surfaces of Al, Fe and Mn oxides and hydroxides ($\equiv S$) able to adsorb H^+ and OH^-. When protons are adsorbed positive charges are created (2.a), and when they are dissociated negative charge (2.b). Thus, variable charges are pH dependent, whereas permanent charges are not. Variable charges also develop in humic constituents of soil ($\equiv R$) due to the ionization of functional groups like phenolic OH, carboxylic and aliphatic OH groups, the dissociation of carboxylic acid group can be seen in (2.c).

$$\equiv S - OH^0 + H^+ \leftrightarrow \equiv S - OH_2^+ \qquad (2.a)$$

$$\equiv S - OH^0 \leftrightarrow \equiv S - O^- + H^+ \qquad (2.b)$$

$$\equiv R\text{—}COOH \leftrightarrow \equiv R\text{—}COO^- + H^+ \qquad (2.c)$$

The ionization of functional groups depends on their pK_a, the negative logarithm of dissociation constant K_a. Each functional group on different surfaces has a different pK_a value. The clay mineral, for example, has three different OH functional groups; the –Al-OH (at the octahedral sheet), the –Si-OH (at the tetrahedral sheet) and the intermediate –Si-Al-OH_2 (OH shared between both sheets) with pK_a of around 5, 9 and 6-7; respectively. A surface functional group with pK_a of 5 signifies that at pH 5 half of the surface is dissociated and, therefore, its negative charge is one half of the potential maximum. At approximately two pH units above the pK_a, all of the surface groups are dissociated and thus the negative charge approaches maximum. At approximately two pH units below the pK_a, all of the surface groups are protonated and thus the negative charge approaches zero. The pK_a value of aliphatic and phenolic OH functional group of soil organic matter is near 9, whereas that of the carboxylic group is around 4.

The negative and positive charges of mineral surfaces might be equal at a specific pH. This pH at which the surface charge is zero is referred to as the point of zero charge (PZC). Below this pH the surface is positively charged and above this pH it is negatively charged. Most variable charge soils with low organic matter have a zero point of charge in the pH range of 5-5.5 (Harter and Naidu, 1995). In Table 2.3, the PZC of various minerals commonly found in natural soil are presented.

Table 2.3. Examples of oxides and pH of point of zero charge (Evangelou, 1998)

Oxide	pH_{pzc}
Aluminium oxide	9.1
Aluminium trihydroxide	5.0
Iron oxide	6-8
Manganese oxide	2-4.5
Silicon oxide	2
Kaolinite	4.5
Montmorillonite	2.5

2.1.4. Interactions in Soil Medium

Soil medium represents a multi-component system, each component having different surface characteristics and binding affinities to inorganic and organic compounds. Considering surface interactions of individual components separately, without taking into account symbiotic and competitive relationships, might bring an insight to the complex sorption phenomenon of soil.

2.1.4.1. Interactions Between Soil Constituents: Soil as a heterogeneous medium involves multi-component association among humic substances, clays, metal oxides, $CaCO_3$, and other minerals. Generally, the surface of clay minerals is covered by metals oxides, organic matter, etc. For instance, in soil horizons rich in organic matter, amorphous iron oxides are hydrated and are present as positively charged iron hydroxides. Due to this positive charge they are either attached on the negatively charged clay surfaces or are chelated by organic acids. Coating by mineral oxides mostly blocks the access to active sites leading to a decrease in CEC of clays. As iron oxides are negatively charged, they attract positively charged metals in solution. The nature of clay surfaces is also altered when organic matter adsorb on the clay surface. Depending on the its chemical structure, organic compound in most cases increasing the net surface negative charge density, but sometimes also reducing the availability of sites to metal ions.

The most extended interactions between soil constituents are those between clay minerals and humic substances like humic acid and fulvic acid. These acids are capable of combining with their various functional groups to the clay surface depending of pH of soil solution. Theng (1979) emphasized that the quantity of dissolved organic matter adsorbed tends to decrease as pH increases above 4. When both the organic acid and the clay surface are negatively charged, they expel form each other, thus adsorption of humic and fulvic acid occur only when polyvalent cations like Ca, Al, Fe are present. Possible mechanisms of clay-humate complex formation are (Evangelou, 1998):

1. By anion and ligand exchange to clay edges
2. By cation or water bridges to basal clay surfaces
3. By H-bonding to the siloxane or gibbsite sheet
4. By van der Waals forces
5. By trapping in the crystal pores
6. By adsorption in interlayer spaces

A schematic diagram of a clay-humate complex is presented in Figure 2.6.

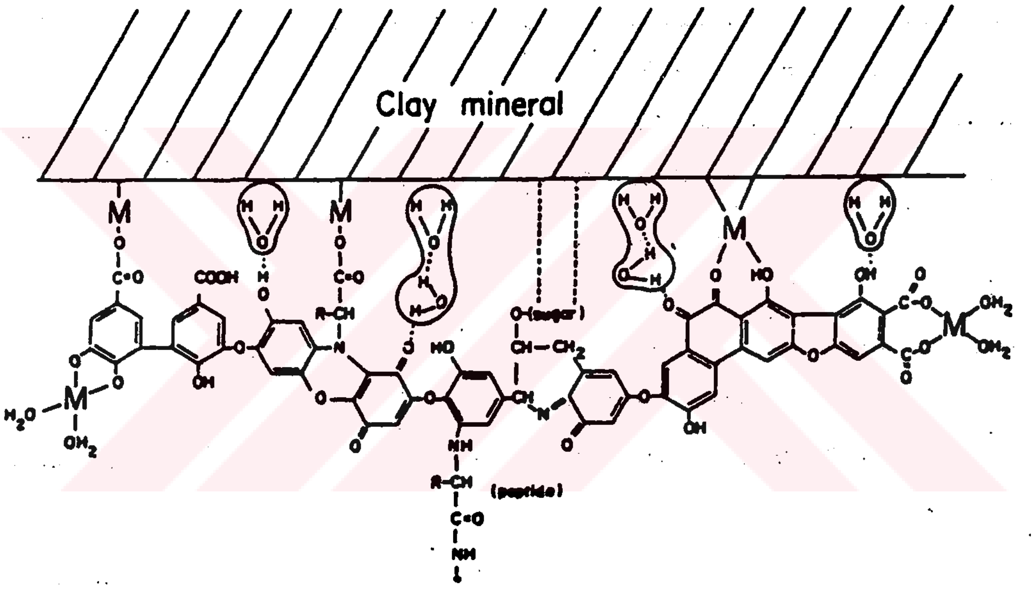


Figure 2.6. Schematic diagram of a clay-humate complex in Soil (Stevenson and Ardakani, 1972).

Sorption of organic compounds by iron, aluminium and manganese oxides also occur, but their effect on the oxide surface is mainly depending on the size of organic molecule, pH_{pzc} of oxide and on the pH of soil solution. Low molecular organic compounds occupy sites on surface thus limiting the sites for metal retention. The sorption of high-molecular-weight organics like humic and fulvic acids, on the other hand, increases sorption sites, as in the case of clays. Sorption of these acids to aluminium oxide surface seems to occur by hydrogen bonding, thus increasing metal sorption sites. New sorption sites are also created at iron and manganese oxide surfaces due to the reduction of iron or manganese by sorption of organic reductants. Silica oxide surfaces, however, are less sorptive toward organic molecules, but if ever occur via metal bridging mechanism.

2.1.4.2. Interactions Between Metals and Soil Constituents: Metals are released from the parent soil material or are introduced into the soil by various activities. The retention of metal ions in soil medium is depending on the concentration and nature of cations, like their valencies and hydrated radii, and on the activity of surface sites of each soil constituent. The activity of soil surface site is determined by the nature of site, whether it is a permanent charge surface site or variable charge surface site, and by the pH of solution. Accordingly, each soil constituent represents a different binding affinity to the metal. In a multi-component system, competition exists among various metal ions for different sorption sites. The presence of organic molecules, like humic and fulvic acids, has an additional influence on competitive metal sorption. Depending on the dissociation of functional groups they react either with the mineral surface or with the metal. Metal ions complexed with organic molecules may still react with the surface or may stay in solution as chelates.

Depending on the conditions, metals may also precipitate with other compounds of soil. Among the most important of these precipitates are the oxides, oxyhydroxides, hydroxides, carbonates; sulfides, phosphates and silicates probably are of lesser importance. The hydrolysis reaction can precipitate hydroxides and oxyhydroxides if the soil solution becomes supersaturated by the metal ion and the OH^- ion. Metals that might be expected to occur as hydroxides are Fe^{3+}, Al^{+3}, Cu^{2+}, Fe^{2+}, Zn^{2+}, and Cd^{2+}. Similarly are metallic carbonates and sulfides formed, however supersaturation of carbonates and sulfides is dependent on the partial pressure of gaseous CO_2 and H_2S since these control the concentration of CO_3^{2-} and S^{2-} in soil. Metals that might be expected to occur as carbonates in soil are Ca^{2+}, Sr^{2+}, Ba^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}. Metals that might be expected to occur as sulfides under reducing conditions are Ag^{+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Fe^{3+}.

2.2. Adsorption

2.2.1. Adsorption Process in General

Adsorption is a process by which ions or molecules present in one phase tend to concentrate on the surface of another phase. The material being concentrated or adsorbed is the adsorbate and the surface on which it accumulates is the adsorbent.

Adsorption of a molecule on surfaces results from its relative affinity for each surface, which is related to the nature of forces between the molecule and the surface. Type of interactions occurring during physical adsorption may be hydrogen bonding, direct and induced ion-dipole interactions, dipole-dipole interactions, etc. According to the nature of interaction dominating in the process, adsorption can be physical and/or chemical. Physical adsorption, also called physisorption, comprises all weak interactions like electrostatic forces, e.g. van der Waals interactions (von Oepen *et al.*, 1991). Electrostatic forces, for example, result in the attraction of an ion to sites with opposite charge. Physical adsorption is categorized as non-specific adsorption and characterized by exhibiting lower heat of adsorption (< 10 kcal/mol). The molecule is not affixed to a particular site of the surface. The chemical adsorption or chemisorption, on the other hand, involves the formation of much stronger bonds, generally covalent bonds, between the molecule and the surface site than in physisorption. Since adsorption occurs at a specific site chemical adsorption is categorized as specific adsorption, seldom reversible. Additionally it is differentiated from physical adsorption by the heat of adsorption, which exceeds 20 kcal/mol.

2.2.2. Types of Metal Adsorption on Exchange Sites of Soil

Metals are adsorbed specifically or non-specifically on the exchange sites of clays, iron, aluminium and manganese oxides and natural organic compounds like humic and fulvic acids. Specific adsorption results in inner-sphere complexes, which reside in a plane near the surface. Non-specific adsorption, on the other hand, generates outer-sphere complexes, which reside in an adjacent plane that is further from the surface. Those metals, which are not complexed in solution reside in a diffuse layer (Evans, 1989).

Non-specific adsorption: When the adsorption reactions involve only weak associations like electrostatic forces between the positively charged cation and negatively charged soil surface then weak outer sphere complexes are formed. This type of bonding is called non-specific adsorption or cation exchange. The metals adsorbed are easily exchanged by other metals, since non-specific adsorption does not involve covalent bonding. Metals retained on soil particles mostly by non-specific adsorption are alkali and earth alkali metals like Na^+, Ca^{2+}, and Mg^{2+}.

Non-specific adsorption on soil particles can develop both on its variable charge surface and its permanent charge surface. Since the charge on permanent surfaces is stronger, cation exchange on clay minerals is accepted to occur more. For soil organic constituent, non-specific adsorption happens by the exchange of proton of functional groups by the metals in solution.

Specific adsorption: When the adsorption reactions occurring between metallic ions and the charged surface of soil particle involve ligand exchange reactions, strongly bound inner-phere complexes are formed. This types of complexes occur most readily on oxide and hydroxide surface, such that of goethite and gibbsite (Evans, 1989). Specific adsorption, includes formation of covalent bonds, thus the adsorbed species are not readily displaced. On variable charge surfaces, specific adsorption is particularly important for metals that readily hydrolyse in water and for metals that form oxyanions. At constant charge surfaces associated with phyllosilicate clay minerals, specific adsorption is important for the larger monovalent cations.

Metals are specifically adsorbed by organic constituents when associations between them and the coordinating functional group of humic substance are formed. These functional groups behave like complexant organic ligands since they contain more than one donor atom. When the metal is bound to at least two of the donor atoms chelated complexes can be produced. Donor atoms of organic constituents are O, N, and S which are contained within basic groups like $-NH_2$ (amino), =O (carbonyl), –OH (alcohol), –S– (thioether) or within acidic groups like –COOH (carboxyl), –OH (phenolic) and –SH (thiol) of humic substances. Generally, Metal-organic complexes experience three types of interactions, which can be summarized as follows;

1. Protons (H^+) compete with cations for organic binding sites
2. Hydroxyl ion (OH^-) competes with humic substances for the cationic metal-ion
3. Soft metals compete with hard metals for organic functional groups

A simple schematic presentation of metal-humic substances complexation can be seen in Figure 2.7. Accordingly, 1 explains electrostatic interaction taking place between the metal ion and the oxygen atom of the carboxylate group. 2a and 2b explain inner-phere complexation formed between the metal ion and the carboxylate groups of the humic substance. 3 explains a weak interaction via bridging between the oxygen of water and metal through hydrogen bonding

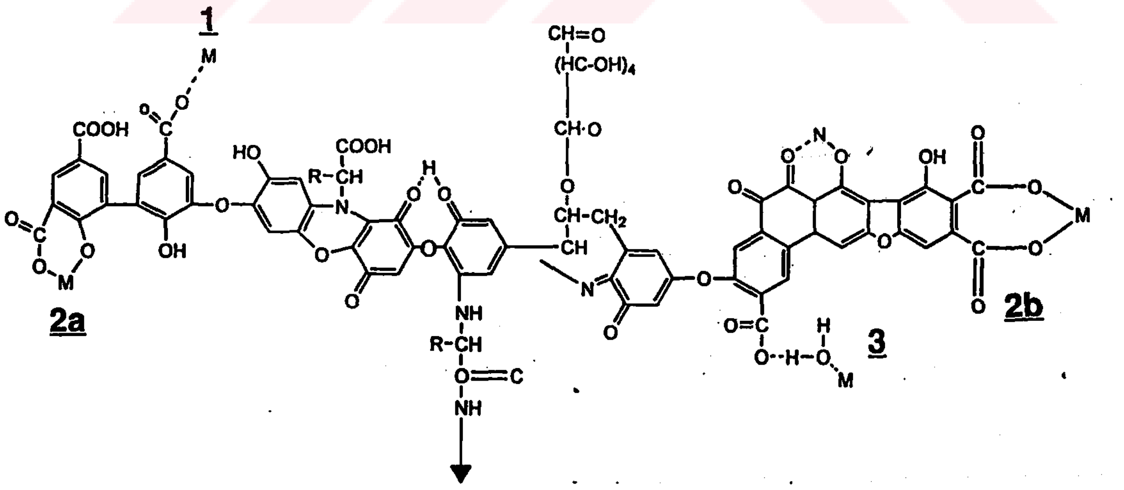


Figure 2.7. Schematic presentation of metal-humic substances complexation (Evangelou, 1998).

2.2.3. Adsorption Isotherms

An adsorption isotherm or curve is a graphic representation showing the amount of solute adsorbed by an adsorbing surface as a function of the equilibrium concentration of the solute (Roy *et al.*, 1991). Thus, when the amount of adsorbed compound per unit weight of adsorbent is plotted against the concentration of adsorbate at equilibrium, the arising shape of curve gives an idea about the adsorption mechanisms involved. It should be stated that equilibrium is the point at which the rate of the adsorption reaction equals the rate of desorption reaction, meaning that the concentration of adsorbate in solution is equal to that on the adsorbing surface. Different solutes show different adsorption behaviour on an adsorbent, reflecting this variation in the shapes of their adsorption curves. The shape of curve also varies when the same adsorbate is adsorbed on different adsorbents, e.g. soils with different clay and/or organic matter content. Depending on their shapes, adsorption isotherms can be classified into four types, Figure 2.8:

- *L-type* isotherm is characterized by an initial slope that does not increase with adsorbate concentration in solution. This behaviour points out the high relative affinity of adsorbent for the solute at low concentration and a decrease of free adsorbing surface. It usually indicates chemisorption, e.g. phosphate-soil interaction.
- *S-type* isotherm is characterized by an initial slope that increases with adsorbate concentration in solution. This suggests that the relative affinity of the adsorbent for the adsorbate at low concentrations is less than the affinity of the adsorbant for solution ligands, meaning that adsorption proceeds after the saturation of ligand, e.g. aluminium-fulvic acid-clay interactions.
- *C-type* isotherm is characterized by an initial slope that remains independent of the adsorbate concentration in the solution under possible experimental condition. This describes partitioning mostly observed for interactions between a generally hydrophobic adsorbate with a hydrophobic adsorbent, e.g. pesticide organic matter interaction.
- *H-type* isotherm is actually an extreme case of *L-type* isotherm, describing strong chemisorption interactions. It is characterized by a large initial slope, which

indicates the high affinity of adsorbent for the adsorbate, e.g. phosphate-iron oxide interactions.

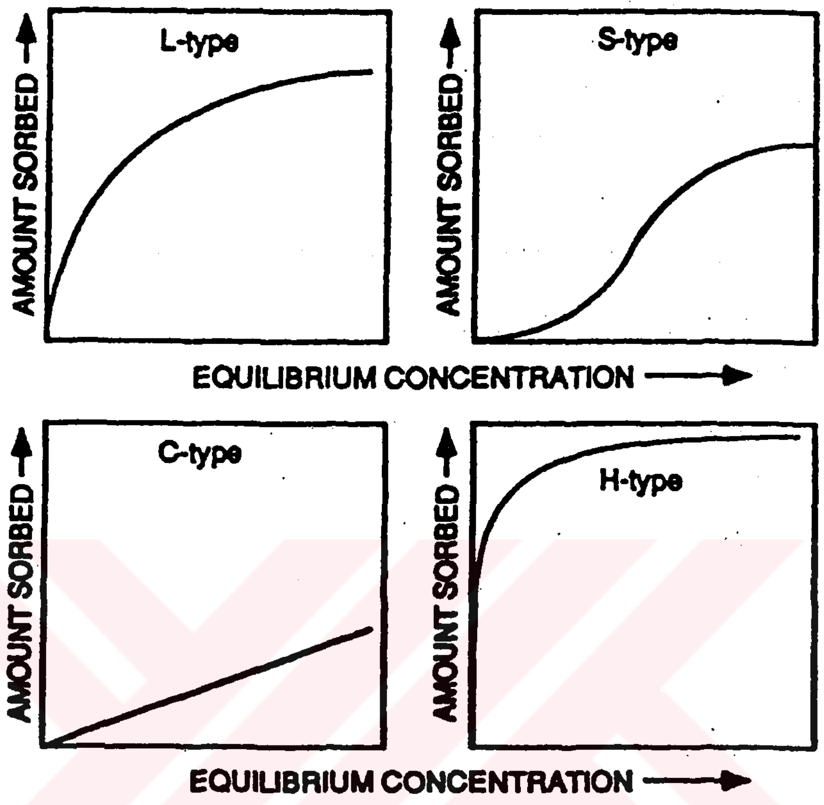


Figure 2.8. Classification of adsorption isotherms (Evangelou, 1998).

These isotherms are mathematically described by various equations, among them Freundlich, Langmuir and Brunauer, Emmet and Teller (BET) equations are the most used equations.

Freundlich Equation: The Freundlich equation was empirically derived to allow for the logarithmic decrease in adsorption energy with increasing coverage of the adsorbing surface (Yaron *et al.*, 1996). It represents the relation between the adsorption density of metal on a heterogeneous solid surface and aqueous concentration of metal:

$$q_A = K_F C_e^{1/n} \qquad (2.1)$$

where q_A is the amount of solute adsorbed per unit weight of solid adsorbent, K_F is the sorption capacity, n is the adsorption intensity and C_e is the equilibrium concentration of metal in solution. K_F can be determined from the intercept and 1/n from the slope of its linear form which is obtained when Log q_A is plotted against log C_e ;

$$\log q_A = \log K_F + 1/n \log C_e \tag{2.2}$$

The Freundlich equation is the most widely used non-linear equation, agreeing well with the Langmuir equation except for very low or very high solute concentrations. The main limitation of the Freundlich equation is the fact that it does not predict a maximum adsorption capacity.

Langmuir Equation: The Langmuir equation was developed to describe adsorption on a homogeneous surface. Several assumptions are incorporated when it is employed to the adsorption of chemical species in soil solution suspensions. These are stated by Evangelou (1998) as follows;

1. The number of surface adsorption sites are fixed
2. Adsorption results in the formation of a monolayer.
3. Adsorption behaviour is independent of surface coverage
4. All adsorption sites are represented by similar types of functional groups
5. The isotherm displays L-type behaviour

Another important assumption of Langmuir equation is that the heat of adsorption is constant over the entire monolayer.

The most commonly used Langmuir equation may be generalized as;

$$q_A = K_L M C_e/(1 + K_L C_e) \tag{2.3}$$

where q_A is again the amount of solute adsorbed per unit weight of solid adsorbent, K_L is a term related to the binding energy of sorption and M is the adsorption maximum. K_L can be determined from the intercept and M from the slope of its traditional linear Langmuir form when C_e/q_A is plotted against C_e;

$$C_e / q_A = 1/K_L M + C_e / M \qquad (2.4)$$

BET Equation: The BET equation assumes that more than one layer is formed by the adsorption of solute molecule. It also assumes that a given layer does not need to completely formed prior to the initiation of subsequent layers. The BET equation includes the application of Langmuir equation to each layer. The BET equation can be generalized as:

$$q_A = BC_eQ^0 / (C_s - C_e) [1 + (B-1) (C_e/C_s)] \qquad (2.5)$$

where q_A is again the amount of solute adsorbed per unit weight of solid adsorbent, C_e is the equilibrium concentration of solute molecule, C_s is the saturation concentration of solute molecule that can exist before precipitation can occur, Q^0 is the monolayer capacity of solute molecule calculated as the amount of solute molecule adsorbed per unit weight of solid adsorbent, and B is related to the free energy of transfer of solute molecule from the bulk solution to the surface of adsorbent. Its linearized form is as follows:

$$C_e / [(C_e - C_s) q_A] = 1/BQ^0 + [(B-1)/BQ^0 \times C_e/C_s] \qquad (2.6)$$

All three adsorption isotherm equations have been used to evaluate the adsorption process. Among these, the Freundlich equation can be accepted as a rather general expression with no particular molecular mechanistic interpretation. It is probably the most commonly used equation. The Langmuir equation, on the other hand, is mainly used to describe L-type adsorption isotherms. In addition to a surface adsorption affinity constant, it also yields the adsorption maximum. In comparison to the Freundlich and Langmuir equations, BET equation does not apply as well as the two equations. In general, all equations cannot be interpreted to indicate any particular adsorption mechanism. Thus, they should be regarded as curve-fitting models without particular molecular significance, but with predictive capability under limited conditions (Sposito, 1989).

2.3. Sources of Heavy Metal Pollution and Remediation of Sites Contaminated by Heavy Metals

2.3.1. Sources of Heavy Metal Pollution

Contamination of soils by heavy metals has received increased attention with regard to increased accumulation in soils, uptake by plants and groundwater pollution. Mining and smelting operations (crushing, grinding, washing, etc.) have been accepted as main sources resulting in elevated heavy metal concentrations in both soils and plants. Almost ten to hundred times higher concentrations than the limit values of E.C. directive 86/278/EEC have been determined especially in tailings (García Sánchez *et al.*, 1999; Papassiopi *et al.*, 1999). The limit values for the concentration of heavy metals in soils were reported by the E. C. directive to be 3 mg/kg for cadmium, 200 mg/kg for copper, 300 mg/kg for lead and zinc. Contamination of agricultural soils surrounding the mining areas has also been reported to occur due to atmospheric deposition of dust and aerosol particles from metal smelters (Kedziorek and Bourg, 2000; Zheljazkov and Nielsen, 1996) and from metal producing factories (Barona and Romero, 1997; Gommy *et al.*, 1998). Higher levels of heavy metal concentrations were found in soils from sites used for deposition of industrial wastes, mine tailing wastes, etc. Sites used for automobile battery reclamation are also known to be intensively contaminated by especially copper, lead and cadmium (Elliot and Brown, 1989). The inventory about trace metal emissions from different industrial/municipal sources into the soil by Nriagu and Pacyna (1988) mentioned the disposal of ash residues from coal combustion as another main source of trace metals in soils. The inventory also showed that on local scale, municipal sewage sludge represents one of the most important sources of metal contamination in soils. Sewage sludges (Ghestem and Bermond, 1998; Ramachandran and D'Souza, 1997) and sometimes dredged sediments (Singh *et al.*, 1996) are applied to soils to improve fertility of soils that are marginally suitable for agricultural production. Heavy metal contents of sludges might be higher when industrial wastewater is treated together with municipal wastewater resulting in increased accumulation in soils. Soils located along motorways have been found to contain higher amounts of heavy metals like lead, zinc and cadmium in comparison of background values (Lee and Touray, 1998; Katahira *et al.*, 2000). Lead, copper, cadmium, zinc contents of various soils contaminated by different sources are presented in Table 2.4. It should be mentioned that the given values are values of soils which are most contaminated within a group of soils examined.

Table 2.4. Heavy metal contents (mg/kg) of soils contaminated from different sources

Source	Cadmium	Lead	Zinc	Copper	Reference
Mining area	90	69 300	66 000	-	García Sánchez *et al.*, 1999
Metal production	80	12 500	10 200	-	Papassiopi *et al.*, 1999
Dust from smelter	24.7	1063.7	1039.2	167.2	Zheljazkov and Nielsen, 1996
Metal producing factory	17.4	1060	965	-	Gommy *et al.*, 1998
Battery reclamation	332	211 271	655	1383	Elliot and Brown, 1989
Sewage sludge	94.9	44.8	151	45.3	Ghestem and Bermond, 1998
Dredg. sediment der. soil	17	274	1447	210	Singh *et al.*, 1996
Motorway	2.8	1461.2	2830.4	-	Lee and Touray, 1998

Recently, heavy metal loading of agricultural soils has also increased. Besides application of sewage sludges (Polo *et al.*, 1999), over usage of pesticides and commercial fertilizers (Graham-Bryce, 1973) are known to be the main sources. In Bavaria (Germany), for example, copper contents of agricultural soils reached a value as high as 421 mg/kg due to intensive application of copper containing fungicides (Schramel *et al.*, 2000). In addition to inorganic fertilizers and pesticides, heavy metals are also released from organic pesticides through their chemical and biological decomposition. Other sources are waste disposal sites (Kim and Kim, 1999) and atmospheric fallout from non-ferrous metallurgic activities (Charlatchka and Cambier, 2000).

2.3.2. Remediation of Sites Contaminated by Heavy Metals

Soils contaminated by heavy metals have been remedied either by landfilling after excavation or by treating with *in-situ* or *ex-situ* treatment methods. In *in-situ* treatment the contaminated soil is treated in place and is not moved from the ground as it is done in *ex-situ* treatment. It consists of the injection of stabilizing or flushing (washing) solution into the soil. In *ex-situ* treatment the soil is generally passed through sieves in order to obtain the soil fraction containing the highest heavy metal concentration, which is actually the finer portion of soil, and thus to lower the cost of treatment in a prepared bed or in-tank system. Treatment in a prepared bed system can be carried out in a different area previously prepared or at the original site, which is prepared after the soil is excavated and kept on a storage site and finally is replaced. Preparation of the bed generally includes the construction of an impermeable liner (clay or plastic) to prevent transport of contaminant. Enhancement of the treatment, on the other hand, is mostly accomplished with physical/chemical methods. While prepared bed systems are systems prepared and/or constructed on an area, the in-tank systems consist of a vessel or other system designed to optimise treatment efficiency.

Both *in-situ* and *ex-situ* treatment methods are based on the following principles; 1) physicochemical solidification/stabilization and 2) extraction. Solidification or stabilization are immobilization techniques that are applicable in *in-situ* or prepared bed systems. Solidification is achieved by injecting or mixing cement, lime, thermoplastic and soluble silicate reagents to fix the contamination in low permeable matrices (Cairney and Hobson, 1998). Similarly, stabilization aims the reduction of the hazardous potential of waste materials by converting contaminant into their least soluble, mobile, or toxic form. For both systems immobilization of contaminants is designed to be permanently. Temporary immobilization can also be achieved by increasing adsorption, ion exchange or precipitation of pollutants in in-situ and prepared bed treatments systems. The ion exchange capacity of soil, for example, can be increased by addition of clays, synthetic resins and zeolites. These techniques reduce the rate of contaminant release from the soil environment for a desired time period, until total removal of contaminant can be accomplished.

Techniques involving extraction or mobilisation of metals are soil flushing (*in-situ*) and soil washing (in-tank). Extracting solutions used for the remediation of polluted sites are generally water, acidic and basic solutions, surfactants and solvents. For the remediation of heavy metal polluted sites, acidic or chelating agent solutions are preferred. These are able to recover the metals bound to soil particles by either changing the pH or by forming stable complexes. The inconvenience of acid treatment is that it is not selective for heavy metals and destroys important soil components, especially in carbonate rich soils (Theodoratos *et al.*, 2000). Soils high in humic matter, silt and clay contents may also face some problems. In the soil flushing system the leachate and partially polluted ground water requires to be contained to prevent further transport of contaminant and contamination of soil and groundwater offsite. The extracting solution also needs to be recovered in the soil washing system. Treatment of solution and/or recovery of both extractant and pollutant if possible is necessary.

2.4. Alternative Removal of Heavy Metals by Chelating Agents

Chelating agents are used for many purposes for years. Phosphonates, for example, are used as scale and erosion inhibitors in cooling towers. Ethylenediaminetetraacetic acid solution, on the other hand, is used to inhibit fauling by hardness salts, such as calcium or magnesium carbonates, sulfates, etc. in boilers of chemical process industry (CPI) plants, nuclear power plants, breweries and dairies. The aim is always to keep the metal ion in solution by producing soluble metal chelates. The ability of chelating agents to form strong complexes with metals resulted in their involvement in many other applications and more importantly in new innovative technology developments.

Since the end of 1990's, chelating agents are examined for their use in remediation of contaminated soils. The efficiency of various chelating agents on the removal of heavy metals from contaminated soils has been investigated by various scientists. Generally, it was found out that the soil matrix, degree of contamination, dose of chelating agents, ambient conditions like pH of soil solution are the main parameters governing the efficiency of metal removal. Remediation techniques involving extraction with chelating agents are also under research. David and Singh (1995) tried to wash zinc (II) from a contaminated soil column with chelating agents like ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid, and water with a pH of 2. For full-scale applications it was proposed that the treatment aggressiveness can be elevated until the desired balance of remediation and cost is achieved.

Real field applications have not been reported yet; this may be due to the challenge for the recovery of both extracted metals and chelating agents for reuse. Especially the recovery of chelating agents enabling their reuse is very important. Reuse of the chelator at least three or four times is necessary for the process to be economical. Removal of metals from ethylenediaminetetraacetic acid and their possible reuse was investigated by photocatalytic decomposition of ethylenediaminetetraacetic acid and subsequent separation of metal by acidification with HNO_3 and HCl by Borrell-Damián and Ollis (1999). However, this technique does not involve recovery and reuse of ethylenediaminetetraacetic acid. Lately, some innovative techniques involving precipitation and electrodeposition of metals have been proposed. Hong *et al.* (1999) studied recovery of ethylenediaminetetraacetic acid metal chelates with the use of cationic (calcium and sodium sulfide) and anionic precipitants. IT Corporation (2001), on the other hand, separated metals from chelating agent by using an electromembrane reactor.

For full-scale applications, other factors influencing the process efficiency and feasibility have to be considered. One factor considered was the biodegradation of organic chelate compound by the indigenous soil bacteria, which could make the process less feasible. It was found out that some chelating agents were more biodegradable than others (Hong *et al.*, 1999).

Improved recovery techniques and reuse of chelating agents in alternative fields and applications however still need to be investigated.

2.4.1. Chelating Agents and Possible Mechanisms Involved in Metal Removal

2.4.1.1. Chelating Agents: Chelating agents are mostly organic molecules, which have the ability to bind to metal cations at several coordinating sites and form inner-phere complexes. Inorganic chelating agents also exist, but their number is relatively small in comparison to the organic ones. Examples are the polyphosphonates, such as pyrophosphate $(P_2O_7)^{4-}$, trimetaphosphate $(P_3O_9)^{3-}$, tripolyphosphate $(P_3O_{10})^{4-}$, hekzametaphosphate $(PO_3)_6^{3-}$. Polyphosphates tend to be less expensive than organic chelating agents, but they are often hydrolytically unstable at high temperatures and pH levels. Three commonly used groups of organic chelating agents are phosphonic acids, polycarboxylic acids and aminopolycarboxylic acids. Phosphonic acids are organic acids modified with phosphate groups, examples are ethylenediaminetetramethylene phosphonic acid (EDTMP), diethylenetriaminepentamethylene phosphonic acid (DTPMP) and nitrilotrimethylene phosphonic acid (ATMP). These are generally more expensive, but have high stability constants. Polycarboxylic acids, which include gluconates, citrates, polycrylates and polyaspartates, are hydrolytically stable, but tend to be weak chelating agents and have low stability constants. In comparison to the other chelating agents aminopolycarboxylic acids are stable over broad pH and temperature ranges and possess strong affinity for metals. Their relatively high stability constants with most metal ions and their moderate cost make them more favourable. Examples of aminopolycarboxylic acids are nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), N-hydroxyethylethylenediaminetriacetic acid (HEDTA) and diethylenetriaminepentaacetic acid (DTPA).

Complexation with metals occurs through ionic, covalent and coordinate covalent bonds between the metal cation and the coordinating site in the chelating agent. The number of sites, which a molecule can provide, mainly depends on the number of multiple ligand atoms like O, N, S or functional groups present in the chelating agent. When a chelating agent has only one site in each molecule capable of forming a bond with metal, this agent is a monodentate, when it has two sites it is a bidentate agent and when it has more than two sites it is a polydentate chelating agent. The more sites are involved in the complexation/chelation reactions the more stable are the formed complexes/chelates (when more than one site is involved the process is called chelation and the product is called chelate), thus the chelating agent is known to be a stronger compound.

The affinity of a chelating agent towards a metal depends on the activity of sites during the interaction. For functional groups like carboxylic groups the degree of deprotonation is very important. The sites provided by functional groups of a chelating agent molecule are only totally available when they are completely dissociated. Deprotonation of functional groups and generation of ionic species are influenced by the pH of medium. The dissociation of hydrogen ions from a tetrabasic chelating agent like EDTA, notified as H_4Y, takes place in following steps:

$$H_4Y \leftrightarrow H^+ + H_3Y^- \tag{2.d}$$

$$H_3Y^- \leftrightarrow H^+ + H_2Y^{2-} \tag{2.e}$$

$$H_2Y^{2-} \leftrightarrow H^+ + HY^{3-} \tag{2.f}$$

$$HY^{3-} \leftrightarrow H^+ + Y^{4-} \tag{2.g}$$

Below, species of EDTA and DTPA resulting from the dissociation of functional groups can be seen in relation to medium pH. As can be seen from Figure 2.9, total dissociation occurs mostly at elevated pH, where competition with hydrogen ions is eliminated and so all sites are available to the metals. Under neutral conditions, however, partial deprotonation results in the formation of protonated species.

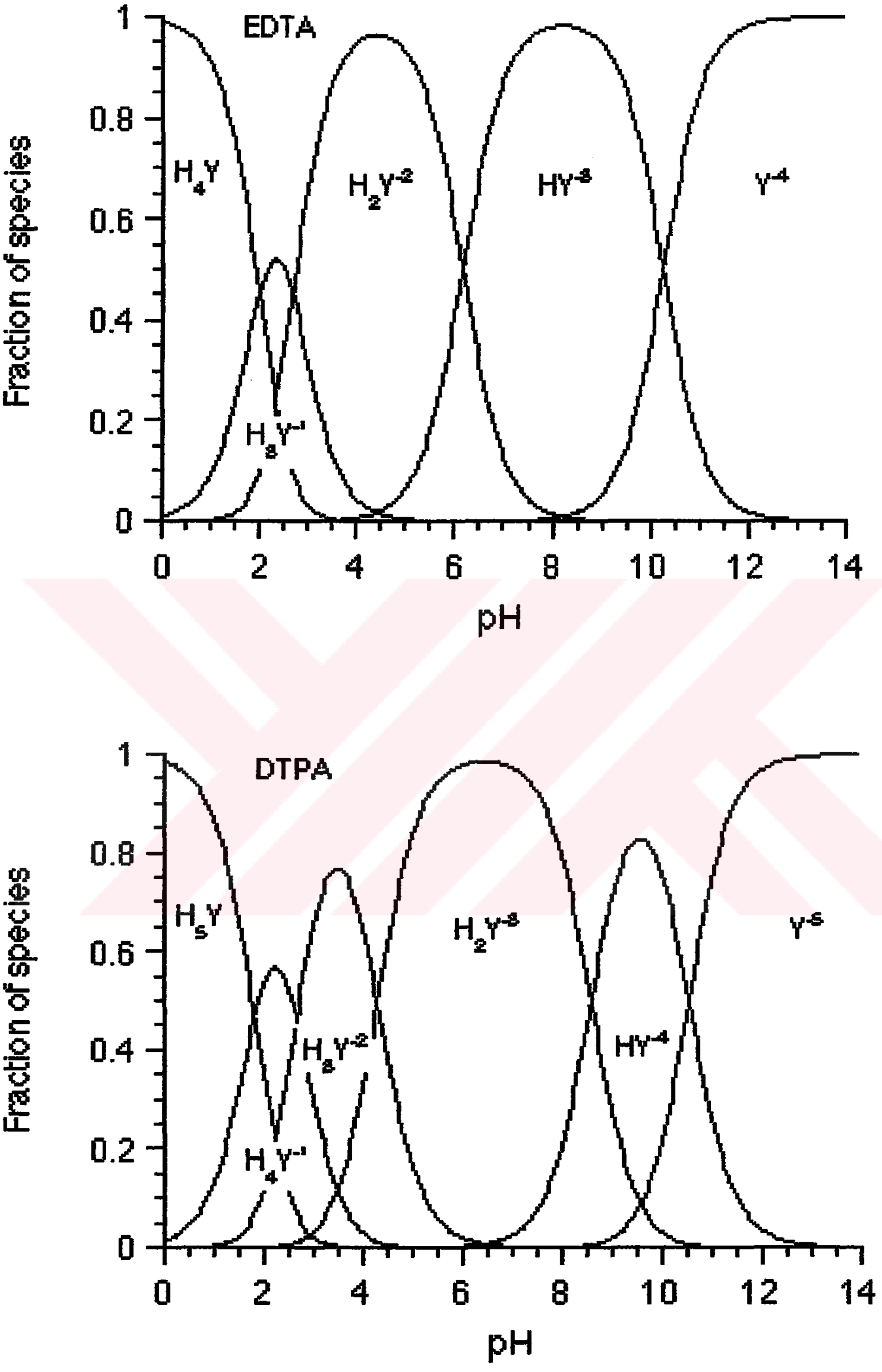


Figure 2.9. Fractionation of EDTA and DTPA species in relation to medium pH.

The affinity of all species of a chelating agent is not equal towards a metal, but the complexation of metal (M) and chelating agent (Y^{4-}) species goes to completion as represented below,

$$M^{n+} + Y^{4-} \leftrightarrow MY^{(4-n)-} \qquad (2.h)$$

$$M^{n+} + HY^{3-} \leftrightarrow MY^{(4-n)-} + H^{+} \qquad (2.i)$$

Depending on the conditions of medium, presence of different chelating agents, etc. other forms of complexes like protonated complexes ($MH_aY^{(4-n-a)-}$), hydroxocomplexes ($MY(OH)_x^{(4+x-n)-}$), and mixed complexes ($MYA^{(4-n)-}$) can also be formed.

As can be seen from Figure 2.10, the presence of various organic and inorganic compounds in natural water systems makes the complexation process more complex and unpredictable.

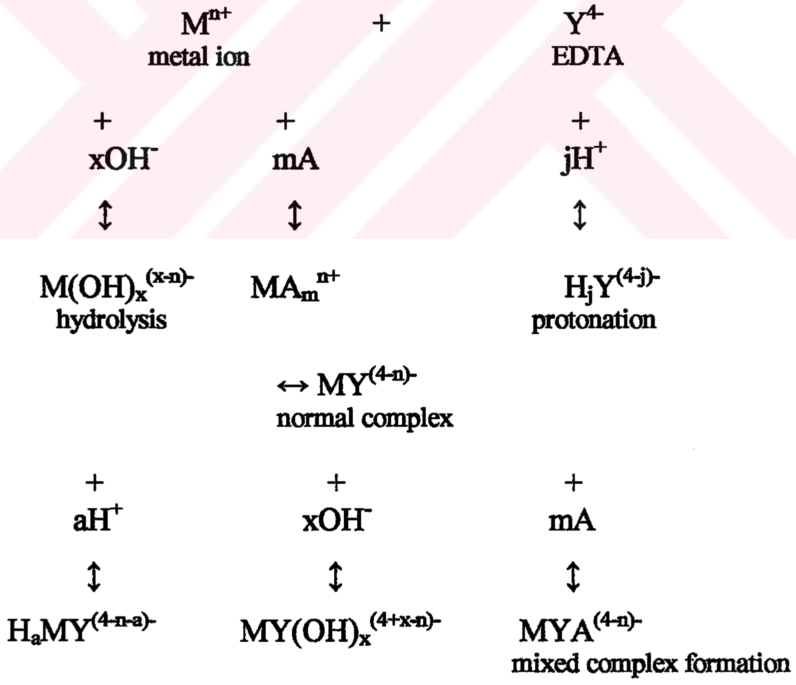


Figure 2.10. The possible equilibria between a metal ion and chelating agent (Pataki and Zapp, 1980).

2.4.1.2. Possible Mechanisms Involved in Metal Removal: The interaction of metal with the surface of soil constituents occurs in many ways depending on the properties of both the metal and the surface of soil constituents. As explained before, various mechanisms are involved, but to simplify the reactions the interaction of metal (M) with soil (S) can be presented as below;

$$S + M \leftrightarrow S\text{-}M \qquad (2.j)$$

A definite mechanism about the extraction of heavy metal from soil has not been reported, due to the difficulty in discrimination among various reactions involved in the soil medium. However, when the general reaction of chelating agent with metal, e.g. EDTA with metal (2.h), is considered together with the above reaction (2.j) a possible simplified reaction can be suggested for the removal of metal from soil surface:

$$S\text{-}M + Y^{4-} \leftrightarrow MY^{2-} + S \qquad (2.k)$$

Considering only the possible reactions illustrated in Figure 2.10, it can be understood how different the number and type of reactions can be, resulting in variable mechanisms.

2.4.2. Humic and Fulvic Acids as Chelating Agents and Their Relation with Dissolution of Heavy Metals from Soil Constituents

Humic and fulvic acids are organic acids containing various coordinating atoms and functional groups, which enables the molecule to behave like a natural chelating agent. Senesi (1992) stated that carboxylic acids, alcohols, phenols, carbonyls, phosphates, sulfates, amides, and sulfides present in humic acid are able to interact with metal species in solution. Prasad and Sinha (1980) similarly reported that the metal-binding capacity of natural occurring humic and fulvic polyelectrolytes emanates from the presence of a number of functional groups like –COOH, phenolic-OH, -NH_2 and =C=O, which may impart several kinds of structural configuration. Generally, attention was paid more to the carboxylic and hydroxylic functional groups in humic and fulvic acids. Tipping (1993) concluded that these ionizable groups confer metal binding properties on to the humic and fulvic acids. Spark *et al.* (1997a) reported that the nature and relative solubility of humic acid components influence the interactions between the humic acid and heavy metals in soil. Like many other complexation reactions, it is obvious that differences in the number and type of components and/or functional groups of humic substances affect the complexation mechanism, but the interaction of humic substance with metal is definitely known.

The ability of dissolved humic substances, like humic and fulvic acids, to chelate positively charged multivalent ions was especially taken into consideration in the mobilization of heavy metals in soil medium. It seems that soluble organics are able to raise the trace metal carrying capacity of soil solution (Almås *et al.*, 2000). Cesco et al. (2000) found that the presence of water extractable humic substances increased the solubility of iron from soil particles. In the study, iron was solubilized of about 2 to 25 % depending on the soil type. It was concluded, that low molecular weight humic substances (e.g. fulvic acids) can form soluble complexes with iron and move to the roots, whereas higher molecular weight humic substances can act as reservoir of easily accessible iron in the soil. Especially, when the soil organic matter, like humic acids, gets soluble at higher soil pH, dissolution of metals from adsorption sites on clay minerals is more promoted due to complexation with dissolved organic matter. Chairidchai and Ritchie (1990) found a 49 % reduction in zinc adsorption in the presence of organic ligands. It was concluded that most of the zinc was in a form complexed with humate.

The prevention of precipication of heavy metals and formation of soluble humic-metal complexes, on one hand, facilitates the uptake of microelements by plants, on the other hand, however increases the possibility of leaching into ground water. Contamination of ground water, especially happens for sites with higher ground water levels, otherwise, adsorption of metal-humic complexes under way is likely to occur.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Soil Samples

Several soils samples were taken of the upper 15 cm from different locations of the greenhouses and thoroughly mixed to observe a final composite sample. Fethiye Soil, Samsun Soil and Izmir Soil are representing the properties of greenhouse soils of Fethiye, the Mediterranean region (Southwest Turkey); Samsun, the Black Sea Region (North Turkey); Izmir, the Aegean region (West Turkey); respectively. According to the grain size analyses, described in Section 4.1.1, the greenhouse soils were found to be sandy loams; Fethiye and Samsun soils with higher clay content. All greenhouses were used for vegetable cultivation like tomatoes, cucumber, aubergine, green pepper, etc.

3.1.2. Clay Samples

Two different clays, kaolin and montmorillonite, were purchased from the Source Clay Repository established by the Clay Mineral Society of University of Missouri. The Kaolin KGa-1b is a well-crystallized kaolinite taken from Washington County, Georgia. The Ca-Montmorillonite SAz-1, on the other hand is a calcium rich montmorillonite taken from the Apache County, Arizona. Physical/chemical data of both clays are presented in Table 3.1.

Table 3.1. Physical/chemical data of clays

	Kaolin KGa-1b	Ca-Montmorillonite SAz-1
Chemical Composition, %		
SiO_2	44.2	60.4
Al_2O_3	39.7	17.6
TiO_2	1.39	0.24
Fe_2O_3	0.13	1.42
FeO	0.08	0.08
MnO	0.002	0.099
MgO	0.03	6.46
CaO	-	2.82
Na_2O	0.013	0.063
K_2O	0.05	0.19
F	0.013	0.287
P_2O_5	0.034	0.02
CEC, meq/100 g	2.0	120.0
Surface Area, m^2/g	10.5 +/- 0.02	97.42 +/- 0.58

3.1.3. Zinc

Zinc solutions were prepared daily from hydrous zinc nitrate ($Zn(NO_3)_2$ x 6 H_2O), which was provided from Riedel-de Haën AG. For the adsorption experiments generally stock zinc solutions (200 and 500 mg/L) were prepared from which standard solutions were obtained by dilution. For the extraction experiments carried out with humic acid, fulvic acid, EDTA and DTPA, adsorption experiments were conducted with individually prepared zinc solutions. All solutions were only acidified when the samples were stored for atomic adsorption spectrometric analyses.

3.1.4. Humic Acid

The humic acid was purchased from Carl Roth GmbH & Co. KG and prepared according the procedure described by Urano *et al.* (1983). All other humic acid solutions

(10-300 mg/L) were obtained by dilution of stock solution (1000 mg/L) with distilled and deionized water.

3.1.5. Fulvic Acid

The soil fulvic acid standard (Code: 2S102F) was purchased from the International Humic Substances Society (IHSS). 100 mg/L and 200 mg/L fulvic acid solutions were obtained by dilution from 300 mg/L stock solution, which was simply prepared by dissolving appropriate amount in distilled and deionized water.

3.1.6. EDTA

EDTA solutions were prepared from ethylenediaminetetraacetic acid disodium salt (Titriplex III), which was provided from Merck KGa-A. Generally, EDTA solutions (0.0001-0.005 M) were obtained by dilution from a stock 0.01 M solution; 0.015 M was prepared as a separate solution to avoid disposal of unused chemical.

3.1.7. DTPA

DTPA was provided from Sigma-Aldrich Chemie GmbH. Stock solution (0.01 M) was prepared by dissolving appropriate amount in distilled deionized water; 0.0001-0.005 M were obtained by dilution. 0.015 M was prepared as a separate solution to avoid disposal of unused chemical.

3.1.8. Magnesium Chloride

Magnesium chloride solutions (1 M), which were used in the sequential extraction experiments, were carefully prepared from anhydrous magnesium chloride purchased from Fluka Chemie AG. For the extraction of zinc bound to the exchangeable surface sites the pH of solution was adjusted to ~ 7 with dilute nitric acid solution.

3.1.9. Sodium Acetate

Sodium acetate, which was obtained from Merck KGa-A, was used in the sequential extraction experiments to determine the amount of zinc present as carbonate complex. Solution pH was adjusted to ~ 5 with acetic acid.

3.1.10. Hyrdoxylammonium Chloride

Hyrdoxylammonium chloride solution (0.04 M) was used in the sequential extraction experiments in order to determine the amount of zinc bound to iron and manganese oxides. Hyrdoxylammonium chloride, which was provided from Merck KGa-A, was prepared in 25 % acetic acid.

3.1.11. Ammonium Acetate

Ammonium acetate was used in the sequential extraction experiments in order to prevent adsorption of zinc extracted from the organic fraction of soil. Ammonium acetate solution (3.2 M) was prepared in 25 % nitric acid solution.

3.1.12. Potassium Dichromate

Potassium dichromate was used both in the determination of organic matter content of soil and in the determination of the amount of humic acid adsorbed on soils. Potassium dichromate was purchased from Merck KGa-A.

3.1.13. Ammonium Iron (II) Sulfate (FAS)

Ammonium iron (II) sulfate was used in the determination of cation exchange capacity of soil, organic matter content of soils and in the determination of the amount of humic acid adsorbed on soils. Ammonium iron (II) sulfate was prepared from ammonium iron (II) sulfate hexahydrate, which was obtained from Merck KGa-A.

3.1.14. Other Reagents

Other reagents like sulphuric acid, hydrochloric acid, nitric acid, boric acid, etc. were of analytical grade. All reagents were prepared with distilled and deionized water, which was produced by Labcomco Waterpro PS.

3.1.15. Laboratory Equipment

Nuve Temperature Controlled Water Bath Shaker Model ST400: Adsorption experiments on greenhouse soils and source clays were performed overnight at room temperature with this Nuve Shaker.

Memmert Temperature Controlled Shaking Water Bath WB14: This shaker was used for all extraction experiments conducted at room temperature with humic acid, fulvic acid, EDTA and DTPA and humic acid adsorption experiments.

Julabo ShakeTemp SW22: This special temperature controlled shaking water bath was used during the sequential extraction experiments. Extraction experiments were performed at elevated temperatures of 96 ± 3 °C and 85 ± 2 °C.

Bibby SB1 Rotator: The rotator was mainly used in the first two steps of sequential extraction experiments and for the adsorption of 10 mg/L carried out as the preliminary step of sequential extraction experiment.

Sartorious Balance: Balance was used for weighing certain amounts of soils, clays and all other chemicals.

Hettich EBA 8S Centrifuge: Centrifuging was carried out in all experiments in order to separate soil from soil suspensions.

WTW 526 pH meter. pH of solutions and soil suspensions were measured during the adsorption and extraction experiments. pH adjustments were also performed with the help of this pH meter.

Perkin Elmer AAnalyst 300: Atomic absorption spectrophotometer was mainly used to determine the concentration of zinc in solutions.

Nuve Vortex: The vortex was used during all extraction experiments to suspend adsorbed soils in centrifuge tubes.

Ultrasonic LC 30: This equipment was used for the homogenisation of reagents by sonication.

Framo-Geraetetechnik M 21/1 Magnetic Stirrer: Stirrer was used during the adjustment of pH and back titrations of excess dichromate in humic acid adsorption experiments and determination of soil organic matter.

HACH COD Reactor: The reactor was used in humic acid adsorption experiments and determination of soil organic matter.

Gerhardt Vapodest 12: In the cation exchange capacity analysis, excess ammonium was measured with the help of this equipment.

Memmert Oven: It was used for drying glassware.

Hot plates: Digestion of soil samples was accomplished with hotplates.

3.1.16. Glassware

Many types and sizes of glassware, e.g. erlenmeyer flasks, volumetric flasks, beakers, pipettes, etc., were utilized during the experiments. These were cleaned each time with 1:1 laboratory grade nitric acid, rinsed several times with tab water and three times with distilled and deionized water. Before being used they were dried in the oven at 103 °C.

3.2. Experimental Procedures

3.2.1. Characterization of Soil Samples

Characterization of soil samples was both conducted at the Soil Department of Faculty of Agriculture of Ege University and at the Institute of Environmental Sciences of Boğaziçi University. Soil properties determined at Boğaziçi University were grain size distribution, soil pH, soil organic matter content and cation exchange capacity (CEC). Exchangeable bases, carbonate content, salt content and some important microelements, on the other hand, were determined at Ege University according to the soil laboratory handbook by Saatçi *et al.* (1983). The mineralogical composition of soils was determined with a PHILIPS PW/1040 X-ray diffractometer and the geochemical composition of soils with a RIGAKU X-ray fluorescence analyzer at Istanbul Technical University, Faculty of Mines, Department of Geological Engineering. All analyses were performed on air-dried samples (< 2 mm), which were prepared according to a standard practice outlined in ASTM-D 421 (1993).

Grain size analysis: Particle size analysis was conducted to determine the relative proportions of the different grain sizes that make up soil. Therefore, two analyses, mechanical and hydrometer analysis, were carried out as outlined in ASTM D 422-63 (1990). The mechanical method simple consists of a sieve analysis which measures the mass of soil retained on any sieve each having different mesh sizes. The hydrometer analysis, on the other hand, determines the distribution of particle sizes smaller than 75 µm by a sedimentation process, using a hydrometer.

Soil pH: The pH of greenhouse soils was measured in soil solution suspensions obtained with distilled and deionised water and 0.01 M $CaCl_2$ solution at a ratio of 1:2.

Soil Organic Matter Content: The organic matter content of soil was determined by oxidation with dichromate in H_2SO_4 as described in the standard method of Turkish Standards Institute (TSE) TS 8336 (1990) which is actually based on the Walkley-Black

Method. Some modifications were made in order to eliminate variable results arising due to temperature variations and reaction periods. Therefore, the experiments were performed in closed flasks with increased reaction time resembling the closed reflux COD method in the APHA-AWWA-WPCF (1989). The procedure includes the reaction of approximately 0.3 g soil with 3 mL 1N dichromate solution and 3 mL concentrated H_2SO_4 in COD tubes for 2 h. Excess dichromate was back titrated with standard Fe^{2+} solution to determine the amount that has reacted. Standardization of 0.5 N $(NH_4)_2SO_4FeSO_4x6H_2O$ (FAS) was previously performed by using ferroin solution as indicator. Assuming that 77 % of organic matter was easily oxidisable, the carbon content was calculated as follows;

$$\%\ \text{Organic Carbon} = [(B-S) \times N_k \times 0.389] / T \tag{3.1}$$

B = mL of FAS used for blank
S = mL of FAS used for sample
N_k = normality of standard FAS
T = weight of sample, g

Assuming that 58 % of soil organic matter consists of carbon, the organic matter content can be calculated as follows;

$$\%\ \text{Organic Matter} = \%\ \text{Organic Carbon} \times 1.724$$

Cation Exchange Capacity (CEC) of Soil: The CEC analysis was carried out as described by Rump and Krist (1992). For the analysis, 10 g of air dried soil was mixed with 2.4 g charcoal and 0.25 g $CaCO_3$ and 125 mL ammonium acetate solution in an Erlenmyer flask. The mixture was shaken for 2 h. After centrifuging, the ammonium in aliquot was determined as described in APHA-AWWA-WPCF (1989). To see the effect of charcoal and $CaCO_3$, samples consisting only of charcoal and $CaCO_3$ were treated in the same way. The ammonium present in this aliquot was accepted as the ammonium available to the soil constituents.

Exchangeable Bases: Exchangeable bases like free iron and aluminium, carbonates and heavy metals are held in the ion exchange complex of soil and are usually extracted using ammonium acetate, which is later analysed by flame emission or atomic adsorption spectrometric methods (Ellis and Mellar, 1995).

For the determination of exchangeable bases 10 g of air-dried soil was washed with 45 % ethyl alcohol to remove all chloride from soil. The soil was then mixed with ammonium acetate and shaken for 1 h. Sodium and potassium ions were then measured in ammonium acetate solution Eppendorf flame photometer.

Carbonate Content of Soil: The carbonate content of greenhouse soils was determined volumetrically by using Scheibler Calsimeter. This calsimeter measures volumetrically the carbon dioxide produced during the reaction of soil with hydrochloric acid.

$$MCO_3 + 2\,HCl \rightarrow MCl_2 + H_2O + CO_2$$

For the generation of carbon dioxide 1g of soil was mixed with 10 mL of 1:3 hydrochloric acid and shaken until evolution of carbon dioxide was not volumetrically monitored. The carbonate content was calculated with the below given formula.

$$\%\ \text{Carbonate} = \{[V_t \cdot (b-e) \cdot 273]/[760\,(273 + T)]\} \cdot 0.4463 / w \qquad (3.2)$$

V_t: volume of carbon dioxide recorded from the calsimeter, cm^3
b: atmospheric pressure at that moment, (taken as 759 mm Hg)
e: maximum pressure of water vapour; temperature dependent. (taken as 16.32 mm for 19 °C)
T: temperature at the moment of measurement, (taken as 19 °C)
w: weight of sample, g
0.4463: g of calcium carbonate corresponding to 1 cm^3 carbon dioxide at 0 °C and 759 mm Hg

Salt Content of Soil: In order to determine the salt content of soil, a paste-like mixture was prepared by saturating the soil with distilled water. The electrical resistance of this paste was measured as ohm (Ω). The total salt content was derived from the relationship between the resistance and salt content from a simple diagram in Toprak ve Su Analiz Yöntemleri (Saatçi *et al.*, 1983).

Available Microelements: Microelements like copper, iron, zinc, manganese and magnesium, which are available to plants, were determined by adding 40 mL DTPA (pH ~ 7.3) to 20 g soil and shaking for 2 h. After filtration the microelements were detected by Perkin Elmer 2380 atomic absorption spectrometer.

3.2.2. Adsorption Experiments

In order to determine the interaction of zinc with greenhouse soils (< 2 mm) and clays, batch adsorption experiments were conducted at soil buffered pH (no pH adjustment) with various zinc concentrations (10-500 mg/L). The solutions were prepared from 500 mg/L $Zn(NO_3)_2 \cdot 6H_2O$ stock solutions and were added to 2 g air dried soil (< 2 mm) in screwed Erlenmeyer flasks at a soil:solution ratio of 1:25 (Hanafi and Sjiaola, 1998). pH values of the suspensions were recorded immediately after the solutions were added to soil (before shaking started), carefully avoiding any loss from soil mass. Shaking of suspensions at room temperature started right after pH measurement and continued for 24 h (Barrow, 1986; Taylor *et al.*, 1995; Wilkins *et al.*, 1998; Polo *et al.*, 1999). To see the effect of soil buffering on medium pH, the pH of suspensions were again recorded and finally centrifuged for 30 min at 10000 cycles/min. The supernatants were only acidified with HNO_3 (pH<2) when the samples were stored for further metal analysis by Perkin Elmer AAnalyst 300 atomic absorption spectrometer (AAS). Results of duplicate analyses were reported as average values.

3.2.3. Preparation of Soil Samples for Extraction Experiments

To assemble polluted agricultural soils, greenhouse soils were treated with zinc solutions revealing zinc loads ranging between 250 mg/kg and approximately 7000 mg/kg. For this purpose, preliminary adsorption experiments were conducted with 10 and 500 mg/L zinc solutions as described above. After centrifuging, the supernatants were analysed by AAS and the soil in Erlenmeyer flasks and centrifuge tubes were kept for one day at room temperature and further used in the extraction experiments.

3.2.4. Batch Extraction Experiments Conducted with Chelating Agents

The extractability of zinc from previously treated greenhouse soils was examined with natural, humic and fulvic acid, and synthetic, EDTA and DTPA, chelating agents. The extractions were carried out in duplicate by carefully washing the soil constituents in centrifuge tubes back into the Erlenmeyer flask with a total volume of 50 mL chelating agent. Adjustment of pH was neither performed for the solutions nor for the soil suspensions and the experiments were conducted at soil buffered pH. pH of soil suspensions were recorded immediately after the solution was added and after stopping of shaking. After shaking for an appropriate time period (24 h for humic and fulvic acid and 60 min for EDTA and DTPA) when the pH was measured, the suspensions were centrifuged for 30 min at 10000 cycles/min. The supernatants were analysed for zinc content on the same day by AAS.

The concentrations of humic acid used in the extraction experiments were ranging between 20-1000 mg/L, and that of fulvic acid between 100-300 mg/L. EDTA and DTPA concentrations were varying between 0.0001 and 0.015 M.

3.2.5. Equilibration of Humic Acid Solution with Zinc

In order to see if any precipitation of humic acid-zinc complexes occurs, 50 mL of humic acid-zinc solutions, with varying humic acid concentration (20-500 mg/L) and a constant zinc concentration, were equilibrated for 24 h. Since extraction experiments were carried out for 24 h, a time period of 24 h was also chosen for these experiments. Humic acid-zinc solutions were simply obtained by first mixing 50 mL of 20 mg/L zinc solution with appropriate volume of stock humic acid solution (2-50 mL) and finally diluting with distilled deionised water to obtain a final volume of 100 mL. pH adjustment of solutions was not performed. Shaking of humic acid-zinc solutions at room temperature started right after pH measurement. pH of equilibrated solutions were again measured right before the mixtures were centrifuged for 30 min. at 10000 cycles/min. The concentration of zinc in initial and equilibrated solutions was determined on day by AAS.

3.2.6. Humic Acid Adsorption Experiments

The interaction of humic acid with greenhouse soils was evaluated by performing batch adsorption experiments at soil buffered pH with exactly the same time of batch extraction experiments conducted with humic acid. The humic acid solutions (10-200 mg/L) were prepared by diluting appropriate amounts of 200 mg/L humic acid solution in 100 mL volumetric flasks. 50 mL of each humic acid solution was added to 2 g air dried soil (<2 mm) in screwed Erlenmeyer flasks maintaining the soil:solution ratio of 1:25. The remaining solutions were kept for the determination initial organic matter concentration. Immediately after solution addition, the pH of suspensions was carefully recorded. The suspensions were shaken at room temperature for 24 h. As the aim of conducting adsorption experiment is to evaluate the probable interaction of humic acid with soil during extraction experiments the same equilibration time of 24 h was selected. An equilibration time of 24 h was also found sufficient by Moore *et al.* (1992) who reported that > 90 % of sorption occurred in 24 h. To see the effect of soil buffering on medium pH, the pH of suspensions were again recorded and finally centrifuged for 30 min at 10000 cycles/min.

The organic matter content of humic acid solutions and supernatants were determined by first digesting the solutions according to the closed reflux COD method and then titrating the excess dichromate with standard ferrous ammonium sulfate solution as described in the APHA-AWWA-WPCF (1989). To be able to record even slight changes in the samples, humic acid solutions with concentrations below 40 mg/L were digested with 1/10 diluted digestion solution. Additionally, the back-titration of excess dichromate was done with diluted ferrous ammonium sulfate solutions, which were each time standardized as outlined in the APHA-AWWA-WPCF (1989). Results of duplicate samples were reported as average values.

In order to find out the amount of humic matter released from greenhouse soils within 24 h of shaking, additional experiments were performed. For this purpose, 50 mL of distilled/deionised water were added to 2 g of greenhouse soils and were shaken for 24 h. After centrifuging for 30 min at 10000 cycles/min, the supernatants were analysed for their dissolved organic matter content as explained above. Since the released organic matter was supposed to be low, digestion of supernatants were preformed with 1/10 diluted digestion solutions.

3.2.7. Sequential Extraction of Greenhouse Soils

A sequential extraction procedure, which was developed by Tessier *et al.* (1979), was carried out for the speciation of zinc in greenhouse soils. This procedure separates heavy metals from soil samples into five groups: exchangeable, carbonate bound, Fe-Mn oxide bound, organic bound and residual fractions. The procedure was slightly modified in relation to the laboratory conditions. Accordingly, the extractions in i) and ii) were performed in centrifuge tubes placed into Bibby SB1 Rotator (30 ± 5 cycles/min), and in iii) and iv) in Erlenmeyer flasks placed into a Julabo ShakeTemp SW 22 shaking water bath. Additionally, the determination of residual fraction was not performed by digestion with a mixture of hydrofluoric acid and perchloric acid as described by Tessier *et al.* (1979). Since most heavy metals are not silicate-bound, a 'pseudototal' analysis of strong acid digests was found to be sufficient. As was done by Ure (1996), Schramel *et al.* (2000), and Theodoratos *et al.* (2000) aqua regia digestion procedure was carried out instead as described below. Sequential extractions were performed on duplicate samples; experiments with variable results (> ± 5 %) were repeated. The extraction steps are as follows:

i) Exchangeable: 8 mL of 1 M $MgCl_2$, pH previously adjusted to 7, was added to 0.4 g soil which was then rotated for 1 h at room temperature

ii) Carbonate bound: 8 mL of 1 M NaOAc, pH previously adjusted to 5 with acetic acid, was added to the residues of i) which was then rotated for 5 h at room temperature

iii) Fe/Mn Oxide bound: the residue of ii) was washed with 20 mL 0.04 M $NH_2OH{\cdot}HCl$ in 25 % (v/v) HOAc into an Erlenmeyer flask and was occasionally agitated for 6 h at 96 ± 3 °C

iv) Organic bound: the residue of iii) was occasionally agitated with 3 mL 0.02 M HNO_3 and 5 mL of 30 % H_2O_2, pH previously adjusted to 2, at 85 ± 2 °C for 2 h. After a second addition of 3 mL of 30 % H_2O_2 agitation continued intermittently for 3 h. When the samples were cooled 5 mL of 3.2 M NH_4OAc in 20 % HNO_3 (v/v) was added and the sample was diluted to 20 mL and agitated continuously for 30 min.

v) Residual: aqua regia digestion (with a HNO_3 and HCl mixture of 1:3 (v/v) and a soil to solution ratio of 1/10) was applied to the residue of iv).

To maintain the suspension pH at especially 7 and 5, pH control was provided at specific time periods by pH adjustment with dilute HNO_3 and HOAc solutions.

Between each extraction, suspensions were centrifuged in their tubes at 10000 rpm for 30 min. Supernatants, which were removed by pasteur pipettes, were analysed by AAS for zinc right after the extraction. The residues were washed after each step with 8 mL of distilled/deionised water; after centrifuging for 30 min., this second supernatant was discarded.

In order to understand on which soil fraction zinc is adsorbed in the adsorption experiments, the sequential extraction procedure was performed on 10 mg/L and 500 mg/L zinc treated soil samples. The treatment was carried out by addition of 10 mL of zinc solution into tubes containing 0.4 g soil, maintaining the soil solution ratio of 1:25. The tubes were rotated for 24 h at room temperature and were then centrifuged for 30 min at 10000 cycles/min. Supernatants removed with pasteur pipettes, were analysed by AAS for zinc. The residues were washed as described above and further used in the sequential extraction experiments.

To evaluate if humic acid, EDTA and DTPA extractions take the zinc from a specific fraction of soil, sequential extractions were accomplished on humic acid, EDTA and DTPA extracted soil samples. For this purpose, 0.4 g of soil samples were initially treated with 10 mg/L zinc solutions as described above. The next day 10 ml of either 300 mg/L humic acid, 0.0001 M EDTA or 0.0001 M DTPA solutions were added to the samples in tubes, which were then finally rotated for the same time period, which was performed in the actual extraction experiments (24 h for humic acid extractions and 1 h for EDTA and DTPA extractions) at room temperature. After centrifuging for 30 min at 10000 cycles/min the residues in tubes were washed as described above and further used in the sequential extraction experiments. The supernatants were analysed by AAS for zinc.

4. RESULTS AND DISCUSSION

In the following section the properties of three different greenhouse soils, which were determined as outlined in Section 3.2.1, have been presented. The adsorption of zinc to greenhouse soils, on the other hand, was evaluated with isotherms and described by Freundlich and Langmuir adsorption models. The influence of soil properties and soil suspension pH on the adsorption behaviour was examined. Removal of zinc was accomplished with naturally occurring (humic and fulvic acids) and synthetic (EDTA and DTPA) chelating agents. Efficiencies obtained by humic and fulvic acids were interpreted in sense of soil suspension pH and humic/fulvic acid adsorption. Removal of zinc by EDTA and DTPA, on the other site, was evaluated with stoichiometric molar ratios and the effect of suspension pH. Sequential extraction experiments were carried out to determine the distribution of zinc in natural and pretreated greenhouse soils. In order to see from which soil fraction zinc was removed by chelating agents, additional sequential extraction experiments were also performed.

4.1. Soil Properties

The properties of greenhouse soils (< 2 mm), which were determined according to the methods outlined in Section 3.2, are presented in Table 4.1.

4.1.1. Grain Size Distribution of Soils

In order to determine the grain size distribution of greenhouse soils two analysis, mechanical and hydrometer analysis, were carried out. The results of sieve analysis can be seen in Table 4.2.

Table 4.1. Characterization of greenhouse soils

Soil components, %	Fethiye Soil	Samsun Soil	Izmir Soil
Sand (2-0.02 mm)	69.45	72.24	75.88
Silt (0.02-0.002 mm)	14.87	9.10	15.02
Clay (< 0.002 mm)	15.68	18.66	9.10
$CaCO_3$	1.28	6.83	0.83
Organic Carbon	1.64	3.07	3.15
Organic Matter	2.83	5.29	5.43
Salt	0.26	0.23	0.09
pH, soil/deionised water	7.03	7.85	7.28
soil/$CaCl_2$	6.96	7.65	7.09
CEC, meq NH_4^+/100 g soil	16.95	22.57	18.64
Exchangeable bases, Na^+	0.30	14.25	11.48
meq/100 g soil K^+	0.40	1.66	0.71
Available microelements, mg/kg			
Copper	16.73	2.07	2.12
Iron	4.89	26.48	11.93
Zinc	1.42	3	10.2
Manganese	4.64	2.46	4.61
Magnesium	540	*	*

* not measured

The results of hydrometer analysis were calculated as described in ASTM D 422-63 (1990). The grain size distribution of greenhouse soils, which can be seen in Figure 4.1, were obtained by combining the results of both analysis as explained by Bowles (1992). Fethiye and Izmir soil are well distributed soils, however the particle size distribution of Samsun soil is dominated in the finer fractions. Especially, the sand fraction is mostly made up of fine sand particles.

Sand, silt, and clay fractions of soils (< 2 mm) represented in Table 4.1, were calculated from the grain size analysis by taking into account the ISSS standards. According to the textural triangle all greenhouse soils can be classified as sandy loams.

Table 4.2. Sieve analysis of greenhouse soils

Sieve No	Opening, mm	Fethiye Soil % Retained	Fethiye Soil % Passing	Samsun Soil % Retained	Samsun Soil % Passing	Izmir Soil % Retained	Izmir Soil % Passing
1 ½	38.10	7.82	92.18	-	100.00	-	100.00
1	25.40	10.04	82.14	-	100.00	-	100.00
¾	19.05	7.27	74.87	-	100.00	15.19	84.81
3/8	9.50	5.81	69.06	-	100.00	10.27	74.54
4	4.75	6.27	62.79	-	100.00	7.61	66.93
10	2.00	8.12	54.67	0.16	99.84	7.01	59.92
16	1.18	4.17	50.50	0.34	99.50	5.10	54.82
30	0.60	6.17	44.33	0.97	98.53	7.11	47.71
40	0.43	3.39	40.94	1.73	96.80	2.98	44.73
50	0.30	3.41	37.53	11.43	85.37	3.62	41.11
70	0.21	4.75	32.78	26.29	59.09	2.39	38.72
100	0.15	4.42	28.37	12.78	46.30	1.86	36.86
230	0.06	3.08	25.29	5.25	41.05	2.05	34.81
pan		25.01	-	40.65	-	34.45	-

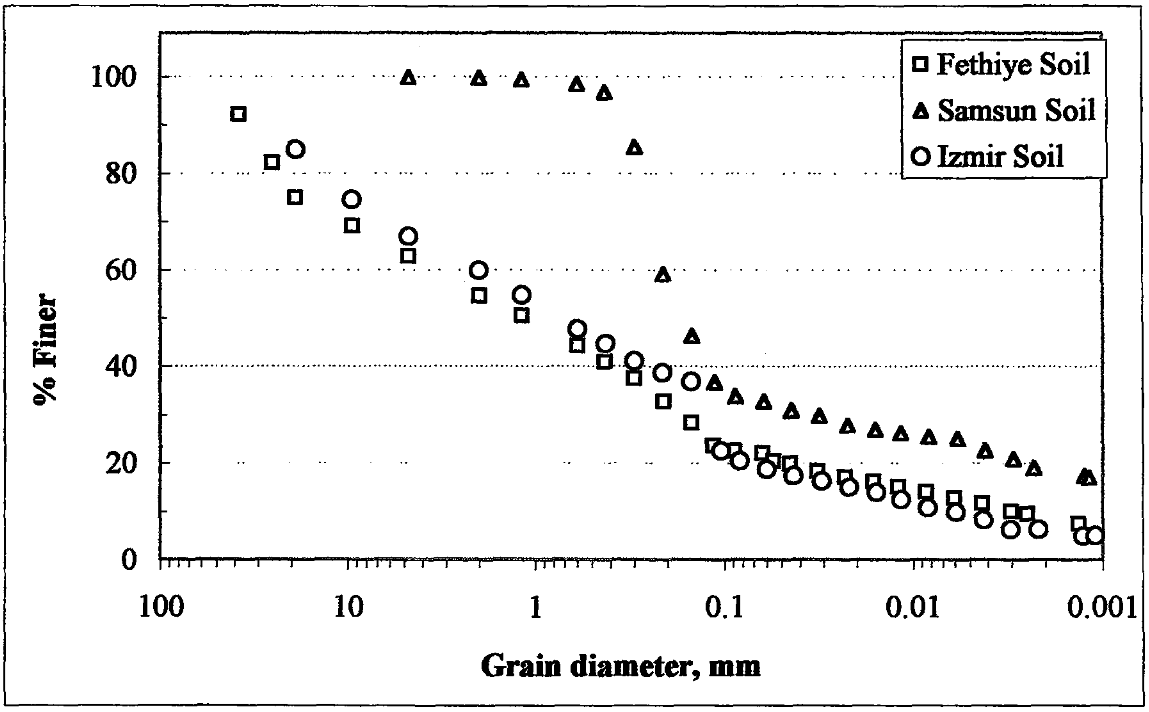


Figure 4.1. Grain size distribution of greenhouse soils.

4.1.2. Soil pH

Soil pH is normally measured in soil-water slurry. However sometimes the soluble salts of soils may affect pH. In order to mask the effect of differential soluble salt concentrations in individual samples excess salt is provided in the slurry by using 0.01 M $CaCl_2$ solution instead of water. The effect of differential soluble salt concentrations can be recognized from Table 4.1. For all soils the pH was higher for soil-water slurries than for soil-$CaCl_2$ slurries. The Fethiye and Izmir soils can be regarded as a neutral soils, whereas as Samsun Soil as a slightly alkaline soil. The higher pH of Samsun Soil is mainly due to its high carbonate content.

4.1.3. Soil Organic Matter Content

The organic matter content of greenhouse soils was determined in four samples according to procedure outlined in Section 3.2.1. The variations between results were calculated to be below 5 %; average values of results can be seen in Table 4.1. Considering that the organic matter content of a representative mineral topsoil ranges between 3 % and 5 %, Fethiye Soil can be accepted as a soil with moderate organic matter content and Samsun and Izmir soils as soils with relatively high organic matter. Generally, organic matter is present in higher amounts in fine-textured soils (clays, clay loams, and so forth) and in lower amounts (~1 %) in coarse textured soils (sands and sandy loams), since these do not support a dense vegetative growth. The organic matter contents could not be related to their textural properties, since all are sandy loams. Thus, the variation in organic matter contents might be related to the origin of greenhouse soils (regional changes) and to the agricultural practices, e.g. support with organic amendments.

4.1.4. Cation Exchange Capacity (CEC) of Soils

Triplicate analyses were performed and the results were found to be slightly above the experimental error. Average values of Fethiye, Samsun and Izmir soils were presented as 16.95, 25.78 and 18.64 meq NH_4^+/100 g soil, respectively. CEC of soil can be well related to its texture; heavier soils with higher clay and organic matter contents have higher CEC. This relation can also be recognized for the greenhouse soils. The low CEC of Fethiye Soil can be mainly attributed to its relatively low organic matter content and its clay content, which is mainly composed of kaolinite (Section 4.1.9). The contribution of organic matter can be better recognized from the CEC of Samsun Soil, having almost the same clay content but an organic matter content almost twice as high than that of Fethiye Soil. The role of clay in the CEC, on the other hand, can be seen from the CEC of Izmir Soil. Its lower CEC in comparison to that of Samsun Soil, is due to its lower clay content. The CEC of greenhouse soils with respect to the type of clay present in each soil is discussed in more detail in Section 4.1.9.

4.1.5. Exchangeable Bases of Soils

Exchangeable bases were extracted with ammonium acetate as outlined in the soil laboratory handbook by Saatçi *et al.* (1983). As can be seen from Table 4.1, the predominantly measured ions were sodium and potassium. Samsun Soil represented higher amounts of sodium than potassium, as was also observed for Fethiye and Izmir soils. Both ions were highest in Samsun Soil and lowest in Fethiye Soil.

4.1.6. Total Carbonate Content of Soils

The carbonate content of greenhouse soils was determined in duplicate by measuring the generated carbon dioxide volumetrically with the Scheibler Calsimeter.

Average values of carbonate contents of Fethiye, Samsun and Izmir soils were found to be 1.28 %, 6.83 % and 0.83 %, respectively. The high carbonate content of Samsun Soil is due to the presence of calcareous minerals in soil at relatively higher amounts. The low carbonate of Izmir Soil, on the other hand, indicates that calcareous minerals are present in trace amounts. Carbonates in soil mainly affect the soil pH and the buffering capacity of soils as is reflected by the high soil pH of Samsun Soil.

4.1.7. Total Salt Content of Soils

The total salt content of Fethiye, Samsun and Izmir soils were found to be quite low, determined as 0.26 %, 0.23 % and 0.09 %, respectively. Since these soils were taken from greenhouses, the low salt contents are reasonable. Generally, the presence of water-soluble salts like sulfates chlorides, bicarbonates of sodium, calcium, magnesium etc. in soil from cultivated lands in elevated degrees is not preferred since this restricts the uptake of water and thus the plant growth.

4.1.8. Available Microelements

Microelements like copper, iron, zinc, manganese and magnesium available to plants were determined by extraction with DTPA (pH ~ 7.3). As can be seen from Table 4.1, copper is present in Fethiye Soil almost eight times higher than in Samsun and Izmir soils. Iron, on the other hand, is more available to plants in Samsun Soil and less available in Izmir and especially Fethiye soils. Zinc is more present in Izmir Soil than in Samsun and Fethiye soils. In Fethiye and Izmir soils manganese is available to the plants at the same degree, but much lesser in the Samsun Soil.

Since the microelements are mainly introduced to the soils by fertilizers and to some extent by pesticides, differentiation by considering the origin of soil does not seem meaningful.

4.1.9. The Mineralogical Composition of Soils

The mineralogical composition of soils was determined with a PHILIPS PW/1040 X-ray diffractometer at Istanbul Technical University, Faculty of Mines, Department of Geological Engineering. The mineralogical composition of the soils can be seen in Table 4.3.

Table 4.3. Mineralogical composition of greenhouse soils

	Kaolinite	Illite	Smectite	Dolomite	Calcite	Quartz	Feldspar
Fethiye Soil	xxx	-	x	xx	(x)	xxx	x
Samsun Soil	-	-	x	x	xx	xxxx	xx
Izmir Soil	x	xx	-	-	-	xxxxx	x

(x) = trace; x = rare; xx = present; xxx = rather abundant; xxxx = abundant; xxxxx = predominant

Considering the type of clay minerals present in soils, it can be concluded that the clay fraction of Fethiye Soil is mainly consisting of kaolinite and rarely smectite group clays like bentonite and montmorillonite. Kaolinite may be present at higher amounts however its contribution to the cation exchange capacity of soil will not be high since it is a 1:1 type clay possessing very low CEC (Section 2.1.3.2.). The XRD analyses reveal that Samsun Soil contains only smectite type clays at a similar amount to Fethiye Soil. Thus, the clay fraction of Samsun Soil will only contribute to a relatively reduced degree to the CEC of soil. The clay minerals present in Izmir Soil are mainly illite and rarely kaolinite. Since the CEC of illite is even less than that of smectite group clays its contribution to the CEC of soil will be again at a reduced degree.

Considering the calcareous minerals present in soil, it can be recognized that Samsun Soil contains the highest amount, mainly consisting of calcite and rarely of dolomite. This is also reflected by its soil pH, which was also the highest among soils. For Fethiye Soil the reverse situation was observed meaning that dolomite was dominating instead of calcite, which is only present at trace amounts. Izmir Soil contains non of the calcareous minerals, calcite and dolomite, however others might be possibly present in trace amounts, since the carbonate content of soil was still at a measurable degree.

Primary minerals determined were quartz and feldspar; among these, quartz is dominating in all soils. The quartz content of soils is highest for the Izmir Soil and lowest in the Fethiye Soil. Feldspar, on the other hand, is more present in Samsun Soil than in Fethiye and Izmir soils.

4.1.10. The Geochemical Composition of Soils

The geochemical composition of soils was determined with a RIGAKU X-ray fluoresence analyzer at Istanbul Technical University, Faculty of Mines, Department of Geological Engineering. can be seen in Table 4.4.

Table 4.4. Geochemical composition of greenhouse soils

Soil components, %	Fethiye Soil	Samsun Soil	Izmir Soil
SiO_2	47.73	55.60	65.74
Al_2O_3	6.01	12.60	12.99
Fe_2O_3	10.22	5.97	6.80
TiO_2	0.32	0.72	0.41
CaO	3.05	7.14	1.56
MgO	20.68	2.57	0.98
Na_2O	0.63	1.52	0.73
K_2O	0.40	1.70	1.78
LOI	9.58	11.89	9.47

Both SiO_2 and Al_2O_3 are present at higher amounts in the Izmir Soil, and to a lesser degree in the Samsun and Fethiye soils. On the other site, the Fe_2O_3 and MgO contents of Fethiye Soil are higher than that of Izmir Soil and Samsun Soil, which has the lowest Fe_2O_3. In general, TiO_2 was present at very low amounts especially when compared to the other oxides. As expected, the CaO content of Samsun Soil was the highest and that of Izmir Soil the lowest, as was also determined in the total carbonate analyses conducted at Ege University. MgO was found to be extremely high in the Fethiye Soil and comparatively very low in the Izmir Soil. The Na_2O contents of both Fethiye and Izmir soils were close, almost half of that of Samsun Soil. On the other hand, K_2O contents of Samsun and Izmir soils were found to be equal.

4.2. Zinc Adsorption Behaviour

The adsorption of zinc on three greenhouse soils was examined by studying the adsorption of zinc within a concentration range of 10-500 mg/L. For comparison purposes zinc adsorption was additionally performed on two different clays, KGa-1b and SAz-1. When the results of adsorption experiments were evaluated, the dependence of equilibrium concentration of zinc associated with the solid phase (q_A, mg/g) on the aqueous solution concentration of zinc (C_e, mg/L) was described by adsorption isotherms. As can be seen from Figure 4.2, the adsorption of zinc exhibited a L-type isotherm with a high initial slope decreasing with increasing zinc concentration (Sposito, 1989). This initial increasing trend ($q_A \cong 1$ mg/g corresponding to a zinc concentration < 50 mg/L) was highest for Samsun Soil and lowest for Fethiye Soil. Considering the changes in q_A values with respect to C_e, it can be recognized that the q_A values were continuing to increase with a decreasing trend. At an equilibrium concentration of about 20 mg/L, the highest q_A value, 4.3 mg/kg, was observed for Samsun Soil and the lowest, 2.4 mg/kg, for Fethiye Soil. These values were in accordance with their CEC values in Table 4.1. Increasing the equilibrium concentration to about 130 mg/L revealed q_A values of only 7 mg/kg, 5.1 mg/kg and 3.8 mg/kg for Samsun, Izmir and Fethiye soils, respectively, reflecting the declining increase in adsorption. Sposito (1989) stated that this may be attributed to high initial affinity of soil particles for the adsorbate, followed by a decreasing amount of the adsorbing surface remaining as the excess in solution increases. A plateau formation was not observed at increased initial zinc concentrations for all soils, consequently not revealing a distinct Langmuirian type adsorption pattern.

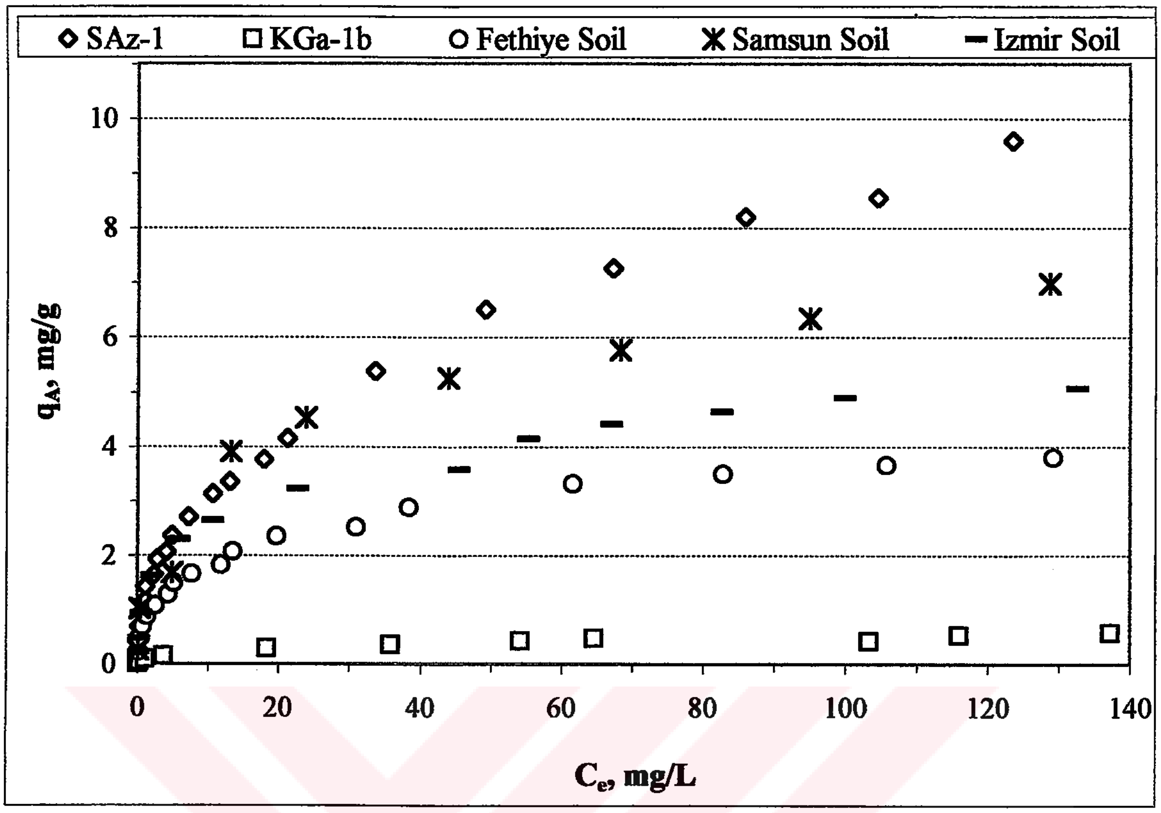


Figure 4.2. Isotherms for zinc adsorption by greenhouse soils and clays.

As a general expression the adsorption data were fitted into the Freundlich adsorption model simply describing the relationship between the zinc adsorbed on soil (q_A, mg/g) and that remaining in solution (C_e, mg/L) with the help of K_F and 1/n coefficients, Table 4.5. K_F, the sorption capacity, can be determined from the intercept and 1/n, indicating to the degree of non-linearity between the capacity of adsorbent and equilibrium solute concentration, from the slope of its linear form. The K_F value of Samsun Soil, calculated as 1.22 mg^{1-n} L^n/g, was found to be the highest among soils, followed by the Izmir Soil with a K_F value of 0.96 mg^{1-n} L^n/g. The lowest K_F value, on the other hand, was observed for the Fethiye Soil, which was about 0.76 mg^{1-n} L^n/g. Considering the 1/n values, it can be seen that all soils revealed a similar value, which was ranging between 0.36 and 0.38. Sorption of zinc could be better explained by Feundlich equation when the data was resolved into two linear parts identified as Part 1 and Part 2, especially for Samsun Soil and Izmir Soil. Separation to two parts was not really necessary for Fethiye Soil, but for Samsun Soil definitely helpful (Figure 4.3). The zinc concentration at which the lines, representing both parts, meet was found to be 30 mg/L, 40 mg/L and 100 mg/L for Fethiye, Izmir and Samsun soils, respectively. Hence, Part 1 represents the sorption pattern up to these concentrations and Part 2 the pattern remaining above these concentrations. This zinc concentration might be related to the adsorption capacity of soils, which represent the same order of CEC of soils. Elrashidi and O'Connor (1982) similarly explained that soils with higher CEC had a wider range of Freundlich isotherm linearity (Part 1) than those with lower CEC. Resolution of the linear form of Freundlich equation into two parts was also reported by Kuo and Mikkelsen (1979), Taylor *et al.* (1995) and Falatah and Sheta (1999) for the explanation of zinc adsorption. Resolution of zinc adsorption data into two linear portions may indicate that zinc adsorption is controlled by two different sites varying in their binding energies.

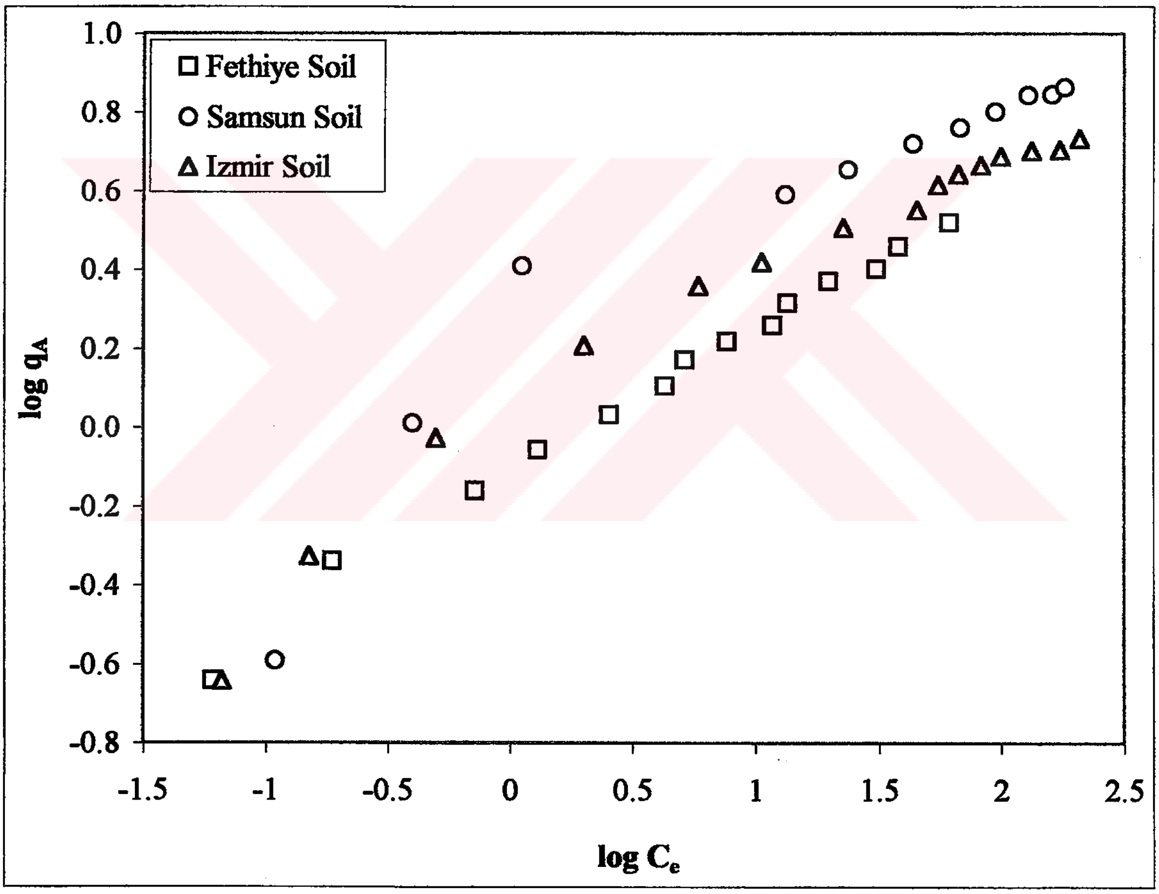


Figure 4.3. Zinc adsorption expressed by linear Freundlich equation.

For Fethiye and Izmir soils, the K_F values for the first linear part were higher than that for the second part, whereas Samsun Soil represented a different situation by having a slightly lower K_F value for the first and a higher K_F for the second linear part. This might indicate that the adsorption mechanisms involved are proceeding at different rates for soils with different composition. Higher K_F values for Part 2 were also reported by Falatah and

Sheta (1999) who studied the adsorption of zinc on two sandy loams having 4 and 8.2 % $CaCO_3$. The K_F value of Part 1 of Gizan Soil (2.64 mg^{1-n} L^n g^{-1}) was in the same range of the K_F value of Samsun Soil (2.38 mg^{1-n} L^n g^{-1}). The K_F values for Part 2, on the other hand, were much higher than those of Samsun Soil. This might be related to the studied zinc concentrations, which were much lower than the concentrations of this study. Thus in the case of Samsun Soil, complexation and/or coprecipitation with carbonates is obviously more pronounced than in the Fethiye and Izmir soils, thus resulting in higher K_F values for both parts.

The exponential term of Freundlich equation is an indicator of how the capacity of the adsorbent varies with the equilibrium solute concentration. Part 1 of all greenhouse soil exhibited higher 1/n values than Part 2, meaning that the high affinity sorption sites are occupied at low concentration, whereas at higher concentrations also sites of lower affinity are occupied (Wilkins *et al.*, 1998). Generally, 1/n values were found to be less than unity (< 1), ranging between 0.21 and 0.99, and thus reflecting the decreasing affinity of surfaces for zinc. For Part 1, Samsun Soil almost reached unity (0.99), thus the highest adsorption intensity, and Fethiye Soil the lowest, whereas for Part 2 the order was reverse.

When the K_F values of soils, calculated for overall data or separately for both parts, were evaluated in relation to their soil properties it was found out that soils with higher CEC reflected higher K_F values than those with lower CEC. Similarly, K_F values increased for soils with higher pH. The greater sorption of metals with increase in CEC and pH is known (Szymura, 1988; Taylor *et al.*, 1995; Wilkins *et al.*, 1998). A direct relation of organic matter and clay content with K_F values could not be established. Despite its lower clay content, Izmir Soil presented higher K_F values than Fethiye Soil, reflecting the contribution of organic matter and the importance of clay type. The low K_F value of Fethiye Soil might be attributed to its low organic matter content and the presence of 1:1 type clay kaolinite possessing low CEC. The highest K_F value was obtained for Samsun Soil due to its higher clay (mainly smetite), organic matter and carbonate contents. Martinez and Motto (2000) stated that $CaCO_3$ surfaces provide sites for metal-surface interaction via specific adsorption or speciation reactions. Madrid and Diaz-Barrientos (1992) found that removing the carbonate from soil reduced the adsorption capacity of soil.

Table 4.5. Freundlich coefficients for zinc adsorption by greenhouse soils

		K_F, mg^{1-n} L^n/g	1/n	r^2
Fethiye Soil	Part 1	0.85	0.44	0.980
	Part 2	0.8	0.35	0.997
	Overall	0.76	0.38	0.996
Samsun Soil	Part 1	2.38	0.99	0.998
	Part 2	2.40	0.21	0.995
	Overall	1.22	0.38	0.944
Izmir Soil	Part 1	1.59	0.69	0.993
	Part 2	1.29	0.28	0.993
	Overall	0.96	0.36	0.980

The general trend observed in adsorption profiles in Figure 4.2 reveal that the adsorption data might be better interpreted with the Langmuir equation. To get deeper insight into the adsorption behaviour of zinc the data were fitted to the linearized Langmuir equation (Figure 4.4). A better fit ($r^2 > 0.98$) was obtained when the curve was resolved into two portions. Similarly, two portions of the curve were considered separately due to their differences in slope. Resolution of the linearized Langmuir equation into two parts was also reported by Shuman (1975), Shukla and Mittal (1979) and Taylor *et al.* (1995) for the explanation of zinc adsorption. The initial zinc concentration, at which the two lines (representing both parts) intersect, was found to be different for each soil. For the Fethiye soil the initial zinc concentration was found to be about 90 mg/L, corresponding to the C_e = 11.75 mg/L and C_e/q_A= 6.45 g/L values of the data adapted to the linear Langmuir equation. Accordingly, Part 1 represents the adsorption pattern up to 90 mg/L and Part 2 the adsorption pattern remaining above this value. The initial concentration found for the Samsun Soil, on the other hand, was about 160 mg/L, corresponding to the C_e = 10.6 mg/L and C_e/q_A= 3.4 g/L values. Thus, Part 1 was reflecting the adsorption pattern remaining below 160 mg/L and Part 2 the pattern present above 160 mg/L. For the Izmir Soil, this value was about 140 mg/L corresponding to the C_e = 17.33 mg/L and C_e/q_A= 6.36 g/L values of the data adapted to the linear Langmuir equation. Hence, the linear low concentration portion (Part 1) was the portion up to 140 mg/L and the linear high concentration portion (Part 2) the portion remaining above 140 mg/L. These initial zinc concentrations were almost congruent with the initial concentrations corresponding to the Point B values of adsorption isotherms of all the soils, which were about 90 mg/L and 1.8 mg/g for the Fethiye Soil, 170 mg/L and 4.2 mg/g for the Samsun Soil and 140 mg/L and 2.87 mg/g for the Izmir Soil, respectively. Point B was deduced from the adsorption isotherm from the intersection of tangents to the origin and plateau of isotherms. The Point B was defined by Echeverría *et al.* (1998) as the initial metal concentration corresponding to the monolayer capacity. Some of the Point B values were close to those reported by Echeverría *et al.* (1998) for the monometal zinc adsorption by similar sandy clay loams.

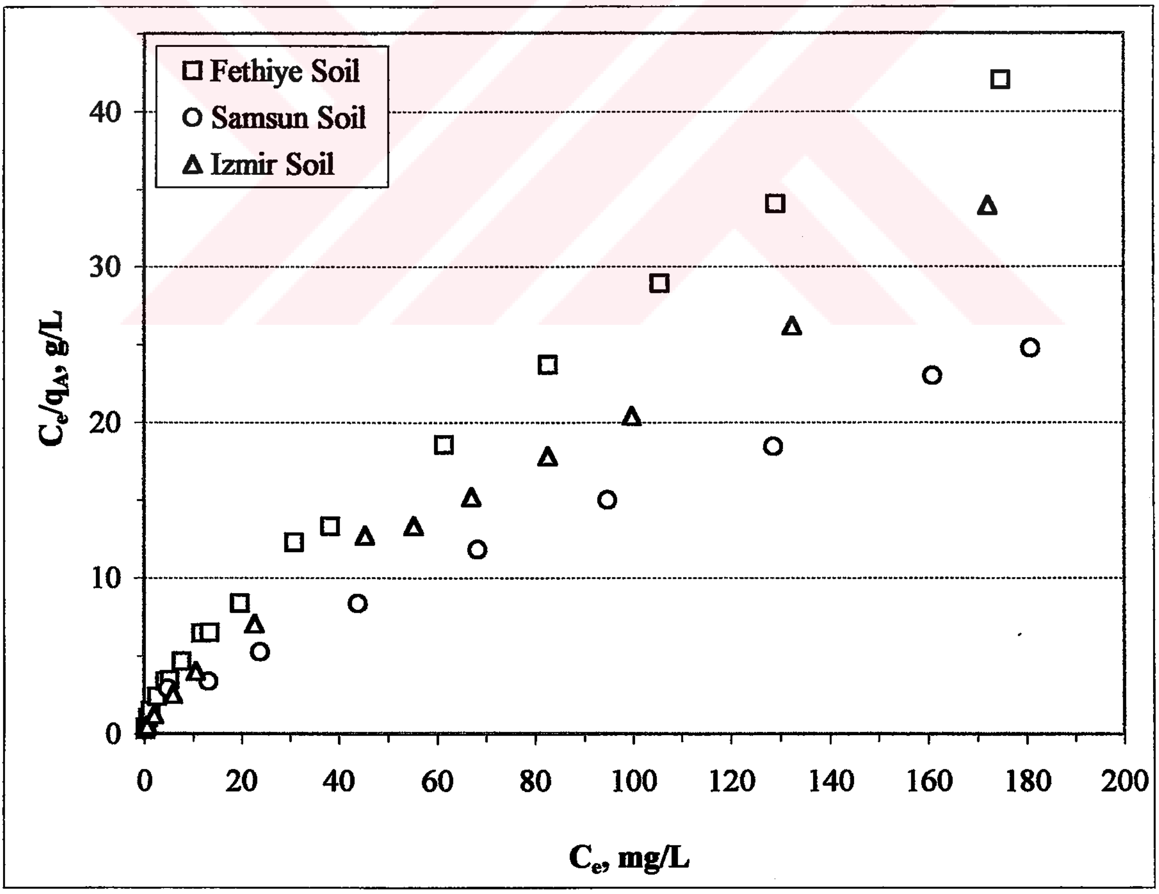


Figure 4.4. Zinc adsorption expressed by linearized Langmuir equation.

The K_L values, representing the relative bonding energy, and M values, the adsorption maximum, were evaluated separately for both parts and for the overall data. Subtraction of the adsorption maximum of Part 1 from q_A values of Part 2, as proposed by Shuman (1975), resulted in erratic values; thus correction for Part 2 was not performed. The K_L and M values derived for the parts of the adsorption isotherms of soils can be seen in Table 4.6. The K_L values were generally higher for the first part and lower for the second part, again suggesting the presence of two different adsorption sites. These results are similar to the findings of Shuman (1975) and Taylor *et al.* (1995). Accordingly, high affinity adsorption sites are occupied at lower concentrations, whereas at higher concentrations (Part 2) site of lower affinity are also occupied. This was also reflected by the higher K_L values for Part 1. Shuman (1975) similarly explained that the sites responsible for zinc sorption at low concentration are characterized by much higher energies than for those at higher concentrations. The M values were all higher for the second part of all soils, slightly exceeding the M values of overall data. In general, the M values of Part 2, but especially that of the overall data were in consistence with their highest q_A value of adsorption isotherms of all soils in Figure 4.2. Additionally, the M value of Samsun Soil was again the highest among soils, which can be directly related to its physicochemical properties. Izmir Soil represents a lower M value than Samsun Soil, due to its lower clay and carbonate contents and consequently lower CEC. As expected, the M of Fethiye Soil was the lowest.

Shuman (1975) proposed that Part 1 sites might be more important for their high bonding energies and Part 2 sites more important for their high adsorptive capacities. M and K_L values of these greenhouse soils indicate to the same findings. However, Shuman (1975) concluded that organic matter is involved in the high bonding energy of Part 1 sites, whereas organic matter content was higher for Samsun Soil and Izmir Soil having higher adsorption capacities (Part 2) than Fethiye Soil. Similarly, bonding energies were higher for Fethiye Soil and Samsun Soil having also relatively higher clay contents. Therefore, it should be evaluated how reliable it is to make a correlation between bonding energy and organic matter, as it is known that many other solution conditions may influence the reactions undergoing in the soil solution.

Table 4.6. Langmuir coefficients for zinc adsorption by greenhouse soils

		M, mg/g	K_L, L/mg	r^2
Fethiye Soil	Part 1	1.93	0.78	0.985
	Part 2	4.49	0.05	0.994
	Overall	3.33	0.18	0.984
Samsun Soil	Part 1	4.29	0.34	0.998
	Part 2	7.94	0.05	0.998
	Overall	7.55	0.08	0.995
Izmir Soil	Part 1	2.82	0.99	0.997
	Part 2	5.94	0.04	0.998
	Overall	5.40	0.10	0.995

Changes in pH of soil suspensions were recorded by measuring the pH of suspensions at the beginning and the end of the shaking period, defined as the initial and the equilibrium pHs, respectively. Generally, the equilibrium pH decreased with increasing initial zinc concentration. Thus, the highest equilibrium pH values were recorded for the lowest zinc concentration (10 mg/L), as 7.04, 7.95 and 7.25 for Fethiye, Samsun and Izmir soils, respectively. For higher zinc concentrations the lowest equilibrium pH was about 6.03 for the Fethiye Soil; all other equilibrium pH were above 6. The equilibrium pH of Samsun Soil was always the highest among soils, which was directly related to its high soil pH and carbonate content. Similarly, the low suspension pH of Fethiye Soil through out the adsorption experiments were attributed to its lower soil pH.

Considering the changes between initial and equilibrium suspension pH, Table 4.7, the highest difference was observed for Samsun Soil and lowest for Izmir Soil, indicating to the influence of carbonates. The reason of the general increase in suspension pH was assumed to be the dissolution of carbonates, resulting in the formation of $ZnHCO_3^-$ and $ZnCO_3$ species. Dang *et al.* (1996) who have evaluated the zinc species present in soil solution, stated that $ZnCO_3$ species begin to form at pH > 7, a pH where $ZnHCO_3^-$ is already present. Schnitzer and Kerndorf (1981), on the other hand, showed that zinc starts to precipitate at a pH about 6.2. Taking into account the higher suspension pH at lower zinc concentrations, it can be suggested that at low zinc concentrations precipitation of zinc is more involved than that at higher concentrations. Mesquita and Vieira e Silva (1996) stated that zinc in soils having high levels of $CaCO_3$ and soil-solution pH can be retained by surface precipitation or sorption of $Zn(OH)^+$ ion and adsorbed by carbonate surfaces, reflecting the situation of Samsun Soils. Sorption of the metals as metal hydroxide ions was also supported by Barrow (1986). Petrović *et al.* (1999) also reported that at higher pH levels heavy metals are removed from solution by coprecipitation with calcite.

Table 4.7. pH values of the suspensions before and after equilibrium

for 100 ppm*	Initial suspension pH	Equilibrium suspension pH
Fethiye Soil	5.98	6.36
Samsun Soil	6.37	7.15
Izmir Soil	6.42	6.58

* pH of 100 mg/L zinc solution was 5.23.

Comparison of Zinc Adsorption on Soils with Sorption on Special Clays: In order to evaluate the adsorption capacities of greenhouse soils with respect to adsorption isotherms representing higher adsorption capacities, zinc adsorption was additionally performed with clays having different cation exchange capacities. The clay possessing high CEC (120 meq/100 g) was selected as a Ca-montmorillonite clay, SAz-1, and that with very low CEC (2 meq/100 g) was selected as a kaolinite, KGa-1b. As expected zinc sorption was highest for the montmorillonite and lowest for the kaolinite, represented by their adsorption isotherms in Figure 4.2. The high zinc adsorption on SAz-1 was due to its expanding nature resulting in an elevated CEC. On the contrary, the non-expanding nature of KGa-1b resulted in a quite lower adsorption of zinc.

Considering the adsorption of zinc by greenhouse soils, it can be recognized that their isotherms are placed between the isotherms of clays. The adsorption isotherm of Samsun Soil is found to be the closest to the isotherm of SAz-1, especially considering the adsorption pattern up to a zinc concentration of 200 mg/L ($q_A \sim 4.25$ mg/g, $C_e \sim 20$ mg/L). The adsorption pattern above this concentration reveals a variation indicating to the difference in their adsorption capacities. This is also reflected by the percentage of zinc adsorbed by Samsun Soil and SAz-1, which is equal (88 %) at 200 mg/L zinc. Increasing the concentration to 400 mg/L results in a decrease in zinc adsorption to 68.5 % for Samsun Soil and to 79.3 % for SAz-1. At a concentration of 500 mg/L these values further drop to 61.9 % and 75.7 % for Samsun Soil and SAz-1, respectively, representing especially the declining adsorption capacity of Samsun Soil in comparison to the clay. The corresponding percentages of zinc adsorbed by KGA-1b clay, Fethiye Soil and Izmir Soil are 13 %, 68 % and 76 %, respectively.

Zinc Adsorption Kinetics: Changes in the zinc concentrations (10 mg/L) remaining in solution were evaluated with respect to time. It was found that the adsorption reactions proceed very fast for all soils. Over 90 % of zinc was retained on surfaces within 5 min, however adsorption continued with decreasing trend reaching equilibrium in 4 h for Samsun and Izmir soils. Fethiye Soil reached equilibrium in 8 h. The lower adsorption capacity of Fethiye Soil could be distinguished by its lower initial slope. These results are in accordance with the previously reported findings on the cation adsorption reactions in soil, which were also found to be rapid, operating on time scales of minutes or hours, but also able to exhibit long-time “tails” that extend over days or even weeks (Sposito, 1989).

The kinetic data were presented in terms of zinc concentration in solution divided by the initial zinc concentration (normalized concentration values, expressed as C_s/C_i) in Figure 4.5. The application of the first order kinetic rate model ($-dC/dt = k \cdot C$; C: concentration of zinc, mg/L; k: first order rate constant, min^{-1}) revealed no significant correlations ($r^2 < 0.70$) for all of the soils. Therefore, adsorption of zinc with respect to time was examined by the initial rate method. The initial rates, which were calculated as 2.12 ± 0.05 mg/L/min for the first 5 minutes, presented that the initial trend of adsorption of 10 mg/L zinc was the same for all soils. However, zinc adsorption behaviour of the soils were found to be comparatively different between 5 min and 4 h. For a contact time of 8 h, on the other hand, the data of all three soils overlapped revealing the same degree of adsorption.

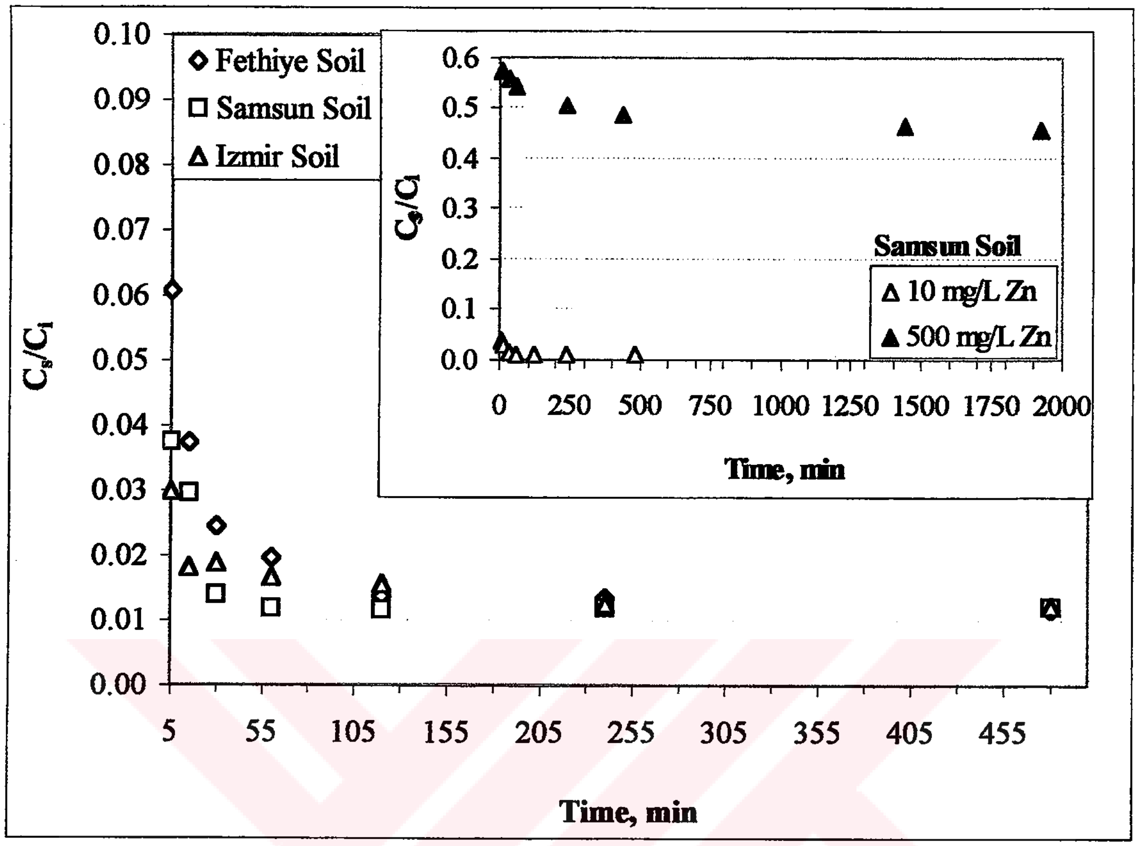


Figure 4.5. Comparison of normalized values of concentration of zinc remaining in solution versus time; C_i: 10 mg/L Zn. Inserted Figure: Samsun Soil, zinc concentration C_i: 10 and 500 mg/L Zn.

The kinetics of 500 mg/L zinc adsorption on Samsun Soil was assessed in order to evaluate the differences between the adsorption behaviour of low and high zinc concentrations (inserted figure in Figure 4.5). For 500 mg/L zinc, the initial rate method revealed an adsorption rate of 42.9 mg/L/min for the first 5 minutes, indicating again to a fast interaction. In comparison to the findings of 10 mg/L zinc adsorption, only 40 % of zinc was adsorbed in 5 minutes. An additional adsorption of 14 % was observed when the reaction time was increased to 24 h, reflecting the declining adsorption capacity previously defined as "long-time tail". Huang and Liao (1970), on the other hand, explained the rapid initial sorption followed by a slow increase in sorption by a fast sorption to the outer sites of particles and further slower sorption to the inner parts of the particles. A further change in zinc adsorption was not recorded for a contact time of 32 h, denoting that the equilibration time could be accepted as 24 h for the adsorption of 500 mg/L zinc on soils.

A reaction time of 24 h was also proved to be adequate for the equilibration of elevated metal concentrations (5000 mg/L) by Polo *et al.* (1999).

4.3. Removal of Zinc with Chelating Agents

The removal of zinc from greenhouse soils was accomplished with two different groups of chelating agents, naturally occurring and artificially produced chelating agents. Among the naturally occurring chelating agents humic and fulvic acid were chosen since these are the best known chelators naturally occurring in soil and water ecosystems. Both humic and fulvic acids are classified as polydentate chelating agents with high molecular size varying in the number of functional groups. In comparison to humic acids, fulvic acids contain more functional groups like carboxylic and phenolic groups. Among artificially produced chelating agents, EDTA and DTPA have been selected; EDTA is a well known chelating agent and DTPA is a chelating agent used in agricultural experiments for the determination of plant available microelements. Additionally, EDTA is introduced into soil and water ecosystems either by application as a fertilizer (providing nutrient in the form of chelates) or by waste discharge from food preparation facilities etc. Both are oligodentate chelating agents, of which EDTA is heksadentate and DTPA is octadentate, possessing low molecular weights.

4.3.1. Removal of Zinc with Humic Acid and Fulvic Acid

Results of studies involving the removal of zinc from soil by humic and fulvic acid treatments were not available in literature. However, some publications about metal, humic/fulvic acid and soil interaction have been made indicating to the formation of soluble humic/fulvic acid-zinc complexes. delCastilho *et al.* (1993), for example, stated that the fulvic acid present in cattle manure caused to the solubilization of metals. Petrović *et al.* (1999), on the other hand, examined the interactive sorption of metal ions and humic acid onto mineral particles and concluded that zinc adsorption decreased at pH > 7 by the formation of soluble humic/fulvic acid-zinc complexes. Similar results were also reported by Prasad *et al.* (1976), Chairidchai and Ritchie (1990), Spark *et al.* (1997c) and Zuyi (2000). In the scope of these findings, the removal of zinc from soil by possible formation of humic/fulvic acid-zinc complexes was investigated.

4.3.1.1. Evaluation of Optimum Time for the Removal of Zinc by Humic Acid and Fulvic Acid: The optimum time for the extraction of zinc from Samsun Soil previously treated with 10 mg/L zinc was determined by conducting extraction experiments at times of 1, 2, 4, 8, 24, 32 h with a humic acid solution concentration of 100 mg/L. Results showed that an initial removal of 1.8 % was reached in an hour; an increase to 2.4 % was observed by increasing the extraction time to 24 h and no further increase was detected within 32 h (Figure 4.6). Since humic acid extraction is a slowly proceeding process the extraction time was determined to be 24 h in the extraction experiments carried out with humic acid. Fulvic acid extractions experiments were also performed for 24 h for comparative purposes.

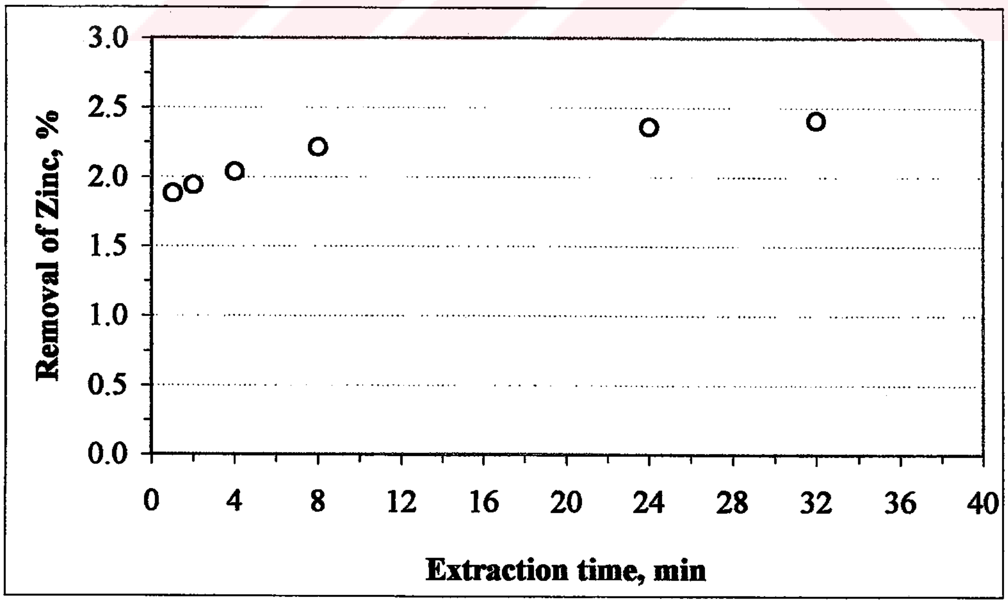


Figure 4.6. Changes in zinc removal efficiencies with respect to time.

4.3.1.2. Removal of Zinc from Greenhouse Soils with Humic Acid: The removal of zinc from greenhouse soils, previously treated with 10 mg/L zinc solution, was accomplished with varying humic acid concentrations ranging between 100-1000 mg/L. As can be seen from Figure 4.7, the removal of zinc from all soil samples with 100 mg/L humic acid was only 2.17 %, 2.47 % and 2.73 % for Fethiye, Samsun and Izmir soils, respectively. Increasing the concentration to 200 mg/L resulted in an increase to 3 % for Fethiye Soil. An additional increase of about 2 % was achieved when zinc was extracted with a humic acid concentration of 300 mg/L. The highest removal efficiency observed for Fethiye Soil was about 7 % at the highest humic acid concentration of 1000 mg/L. For Samsun Soil, a removal efficiency of about 4 % was achieved by a humic acid concentration of 200 mg/L. Increasing the concentration to 300 mg/L resulted in an additional zinc removal of 1.7 %. With a humic acid concentration of 1000 mg/L, the highest removal of zinc accomplished was 7.3 %. The removal of zinc from Izmir Soil with 200 mg/L humic acid concentration was about 4.8 %, which was further increased to 6.2 % when the concentration was raised to 300 mg/L. The highest zinc removal observed for Izmir Soil was 7.7 % achieved with a humic acid concentration of 1000 mg/L. In general, the removal of zinc from soils increases with a declining trend almost approaching equilibrium conditions. The total removal efficiency achieved by 1000 mg/L was approximately 8 % for all of the soils.

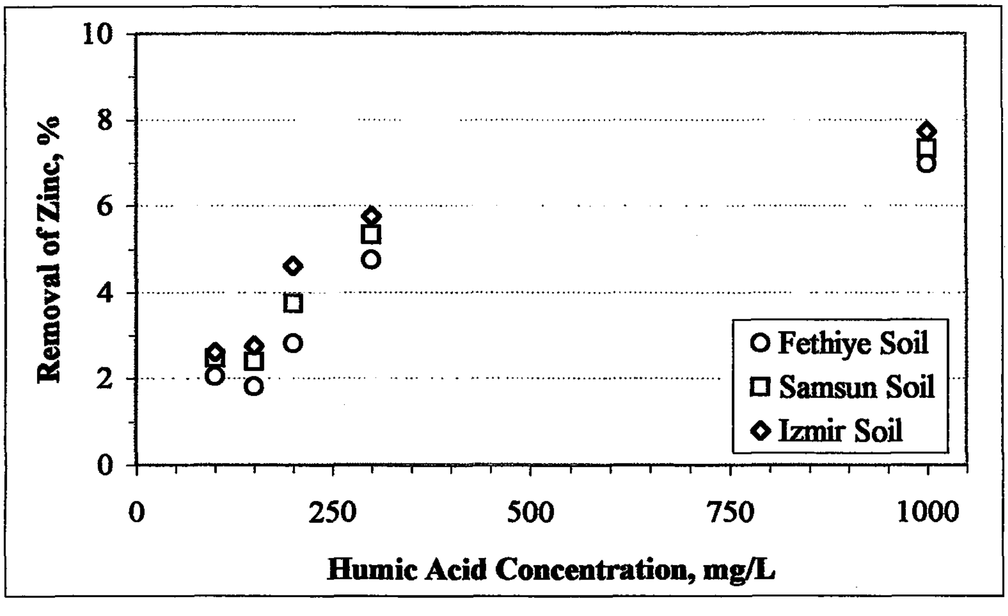


Figure 4.7. Removal of zinc with various humic acid concentrations.

For all soils a different situation was recorded for 150 mg/L humic acid treatments, which did not reflect an additional increase in the removal of zinc despite the increase in humic acid concentration from 100 mg/L to 150 mg/L. A similar situation was observed by Schnitzer and Kerndorff (1981), who studied the complexation of zinc with fulvic acid at different pH ranges. It was reported that fulvic acid-zinc complexes precipitated mostly at fulvic acid concentrations between 50 mg/L and 150 mg/L at pH between 6 and 8. In order to see if the reduced removal efficiencies of 150 mg/L humic acid concentration can be related to a similar precipitation process as was observed for fulvic acid, additional experiments were carried out. For this purpose, humic acid–zinc solutions (20-500 mg/L humic acid and 10 mg/l zinc) were equilibrated under the same conditions of extraction experiments. Similarly, pH adjustments were not performed, thus the pH of humic acid-zinc solutions increased from about 6.5 to 8 as the concentration of humic acid increased from 20 mg/L to 500 mg/L. This pH range resembles the conditions of Schnitzer and Kerndorff (1981). As can be seen from Figure 4.8, a precipitation maxima was observed for a humic acid concentration of 150 mg/L. Further increasing the humic acid concentration resulted in a decrease in precipitation, indicating that zinc was not precipitating as $Zn(OH)_2$ despite the increasing pH. These changes in the precipitate formation were also visually recognized when attention was paid to the remainings in the centrifuge tubes. Thus, it can be stated that a similar situation observed by Schnitzer and Kerndorff (1981) for the fulvic acid-zinc complexes, was prevailing for humic acid-zinc complexes.

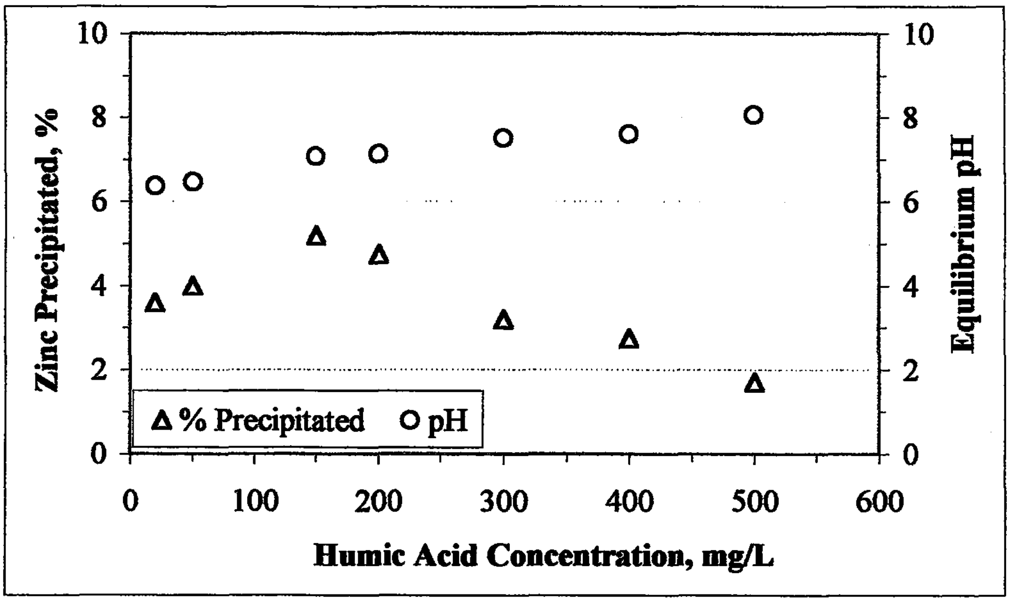


Figure 4.8. Complexation of humic acid with 10 mg/L zinc.

Since a similar increase in the pH of humic acid soil suspensions in extraction experiments, from about 7 (100 mg/L) to 8 (300 mg/L), was recorded it can be accepted that, from pH point of view, the humic acid zinc equilibration conditions reflect the conditions of extraction experiments. Thus, the low zinc removal efficiencies accomplished by 150 mg/L humic acid concentration can be attributed to the precipitation of humic acid-zinc complexes. This was also verified by the visual observations of the extraction experiments. For all soils, a slight film formation on the soil samples in centrifuge tubes was noticed. The highest film thickness was observed in extraction experiments conducted with 150 mg/L humic acid solutions. The film thickness, which was differentiated by the intensity of color, was highest for Fethiye Soil and lowest for Izmir Soil, actually reflecting the order of removal efficiency. A similar situation was observed by delCastilho *et al.* (1993), who reported that 80 to 95 % of cadmium and zinc most probably complexed by organic matter are removed from solution after supercentrifugation.

The effect of zinc load on the extraction efficiency of humic acid was examined by performing an extraction experiment on a sample of Fethiye Soil previously treated with 50 mg/L zinc to reveal a zinc load of 1250 mg/kg zinc. A removal efficiency of 4.05 % was observed by 200 mg/L humic acid in comparison to 3 %, indicating that zinc load had little effect for a concentration of 200 mg/L. When the same experiment was conducted with a humic acid concentration of 20 mg/L, zinc was only removed about 1 % from the Fethiye Soil. At this concentration, a film formation was not observed, presenting that precipitation of humic acid-zinc complexes was not occurring. This also approved the findings of Schnitzer and Kerndorff (1981), who did not observe precipitation for fulvic acid-zinc complexes for concentrations below 50 mg/L. It was explained that the fulvic acid zinc complexes became more water soluble, most likely because of the availability of more fulvic acid-metal binding sites or reactive or functional groups as the fulvic acid concentration increased.

The Effect of pH on the Removal of Zinc: Among the various binding sites of humic substances carboxylic and phenolic functional groups are the most important ones. Waller and Pickering (1992) stated that initial uptake of metals occurs at the most active sites and that lower affinity sites are used after consumption of higher affinity sites. The reactivity of these functional groups depends mainly on their dissociation, which is determined with their pK_a values. At solution pH above pK_a, the functional groups of humic acid are deprotonated resulting in a negative charge. Metals in solution are attracted by this negative charge, leading to complexation reactions. McBride *et al.* (1997) stated that soil solution pH is a controlling variable for metal complexation by organic matter. Various pK_a values of humic acid solution from different origins have been reported. Tombácz *et al.* (2000) stated the pK_{a1} value, which presents the pH at which carboxylic groups are dissociated, to be 3.7 ± 0.1 and the pK_{a2} value, which presents the pH at which phenolic groups are dissociated, to be 6.6 ± 0.1 of a coal derived humic acid. Tipping (1993) derived the pK_a values from published results for various soil humic acids with the core Model V program and presented the average pK_{a1} and pK_{a2} values of humic acids as 4.0 and 8.6, respectively. Spark *et al.* (1997a) stated that carboxylic species dissociate around pH 4-5 and phenolic species dissociate at pH > 10. Roughly at pH ~ 4-5 carboxylic type and at pH ~ 9 phenolic type functional groups are dissociated (Schulten and Schnitzer, 1997). pH of soil solution suspensions during the extraction experiments were slightly above 7 at a humic acid concentration of 100 mg/L (pH_{HA} = 10.3) for all soil samples. As the concentration of humic acid solution increased to 300 mg/L (pH_{HA} = 10.7) the pH of soil solution suspensions also increased, approaching a pH of about 8. The reason of lower pH of suspensions in comparison of high humic acid solution pH, was the buffering capacity of soils, which is dependent on the pH of soils and the amount of ions associated with the solid phase. Since pH of suspensions were ranging between 7 and 8, not approaching 9, it can be assumed that only carboxylic functional groups were deprotonated. Low removal efficiencies might be related to the elimination of the participation of phenolic functional groups during extraction.

In order to evaluate the effect of pH on the extractability of humic acid, extraction with 300 mg/L humic acid solution was carried out at suspension pH of 10.5 ± 0.2 which was maintained by hourly pH adjustments. Increasing the suspension pH from about 8 to approximately 10.5 resulted in an additional increase of zinc removal from Fethiye Soil of about 1.7 %, from 5.2 % to 6.9 %. Since at a pH above 9, phenolic functional groups are also deprotonated this increase might be attributed to the involvement of these functional groups in the extraction process. Spark *et al.* (1997a) mentioned that at higher pH humic acid molecules become more open and linear in shape due to decrease in intra and inter molecular hydrogen bonds and increase in repulsive forces between the dissociated functional groups. Under these conditions humic acid functional groups would be more accessible to the metals in soil solution. Naidu and Harter (1998), on the other hand, related the release of cadmium to soil solution to the higher solubility of organics at higher pH.

A much higher removal efficiency was observed when the suspension pH was decreased to about 5. The removal of zinc from Fethiye Soil with 100 mg/L humic acid was increased from 2.2 % to 14.9 %. To examine the contribution of acidity, a control experiment was carried out at exactly the same condition but using distilled/deionised water with approximately the same pH. The results indicated that acidity was the main contributor of zinc removal. By just using distilled/deionised water with a pH of 5, 9.7 % of zinc was removed from soil. High zinc concentration in the labile dissolved organic carbon was also obtained by Almås *et al.* (2000), who concluded that this due to the lower soil pH and the lower content of organic matter in the loam soil. Actually an extraction efficiency of 5.2 % (obtained by subtraction of 9.7 from 14.9) is still high for a humic acid concentration of 100 mg/L, especially when it is taken into account that only carboxylic groups are dissociated. The reason of high removal efficiencies might be the dissolution of zinc bound to different soil constituents like calcite. Martinez and Motto (2000) stated that carbonate compounds dissolve easily by lowering the pH of extraction so that metal solubility from calcareous soils is more susceptible to decreases in pH. Another reason might be the release of bound zinc from iron and aluminium oxides, etc. which are definitely positively charged at pH 5; $pH_{pzc} > 5$ (stated in Section 2.1.3). Zinc released from these surfaces is unable to bind to carboxylic functional groups due to the absence of humic acid in the experiment, thus keeping its soluble state in solution. In the presence of humic acid, zinc is bound to the humic acid molecule, which might be later on adsorbed as humic acid-zinc complexes on mineral surfaces.

Adsorption of Humic Acid on Greenhouse Soils: To determine the degree of humic acid adsorption on greenhouse soils batch adsorption experiments were performed with humic acid concentrations ranging between 10 mg/L and 200 mg/L. The concentration of standard humic acid solutions and supernatants were determined as COD (mg O_2/L). COD results of some supernatants were higher than that of standards initially added to soils, indicating that release of dissolved humic substances from soils occurred. For Fethiye Soil a higher COD was only recorded for the supernatant of 10 mg/L humic acid adsorption, whereas for Samsun Soil supernatants of 10-60 mg/L humic acid adsorptions and for Izmir Soil supernatants of 10-40 mg/L humic acid adsorptions had higher COD values than the COD values of standard humic acid solutions. In order to understand the amount of dissolved humic substance released by soils after 24 h of shaking, supplementary experiments were conducted. The experiments were carried out under the same conditions except that distilled/deionised water was added to soils instead of humic acid solutions. COD of supernatants were highest for Samsun Soil and lowest for Fethiye Soil, representing a decreasing order of release as Samsun Soil > Izmir Soil > Fethiye Soil. Soil organic matter content of Fethiye, Samsun and Izmir soils were 2.7 %, 4.6 % and 4.7 %, respectively. Low dissolution of organic matter from Fethiye Soil might be related to its low organic matter content and to its low soluble organic matter fraction. Since the organic matter content of Samsun and Izmir soils are close, it can be proposed that the soluble organic fraction of soil organic matter is higher for Samsun Soil than for Izmir Soil. The COD values, which were also verified by the TOC values, of released organic substances were 13, 32.7 and 17.6 mg O_2/L corresponding to volume corrected COD values (COD_{corr}) per g soil of 0.33, 0.82 and 0.44 COD/g soil for Fethiye, Samsun and Izmir soils, respectively.

Adsorption and desorption of humic subtances on soil and soil constituents like iron and aluminium oxide is generally assessed as sorption per mass of soil (mg/g) versus initial concentration of humic acid added to soils (Moore *et al.*, 1992; Moore and Matos, 1999; Kaiser and Guggenberger, 2000). Initial humic acid concentrations are used instead of final humic acid concentration in solution, to consider humic acid adsorption hysteresis (Kaiser and Zech, 2000). The results on humic acid adsorption experiments were evaluated by plotting the changes in humic acid concentration, as sorption per mass of soil (COD_{corr}/g), against the initial humic acid concentration added to soils. As can be seen in Figure 4.9, the adsorption of humic acid on all greenhouse soils represent a linear behaviour resembling C-type isotherm; adsorption increased with an increase in the initial humic acid concentration added to soil without any saturation of soil adsorption sites. The adsorption of humic acid on greenhouse soils is highest for Fethiye Soil and lowest for Izmir Soil. It is known that adsorption of dissolved organic matter increases with decreasing organic matter and increasing iron and aluminium oxides/ hydroxides and clay contents (Kaiser and Guggenberger, 2000; Kaiser and Zech, 2000; Moore *et al.*, 1992; Moore and Matos, 1999; Kalbitz and Wennrich, 1998). Fethiye Soil has a higher iron oxide content than Samsun and Izmir soils (Table 4.4). On the contrary, the aluminium oxide contents of Samsun and Izmir soils are two times higher than that of Fethiye Soil. Under these conditions, it seem that the organic matter and clay contents are the controlling factors in humic acid adsorption. Thus the high adsorption of humic acid on Fethiye Soil is mainly due to its quite low organic matter and high clay content. Samsun and Izmir soils have almost the same organic matter contents, however the clay content of Samsun Soil is two times higher than that of Izmir Soil. Thus the adsorption of humic acid on clay surfaces is more pronounced for Samsun Soil. The low humic acid adsorption of Izmir Soil can be related to its high organic matter and low clay contents. The negative values in the isotherms reflect the dissolution of organic matter from greenhouse soils. Organic matter dissolution was also similarly recognisable from the dissolved organic matter adsorption isotherms of Kaiser and Zech (2000) and Moore *et al.* (1992).

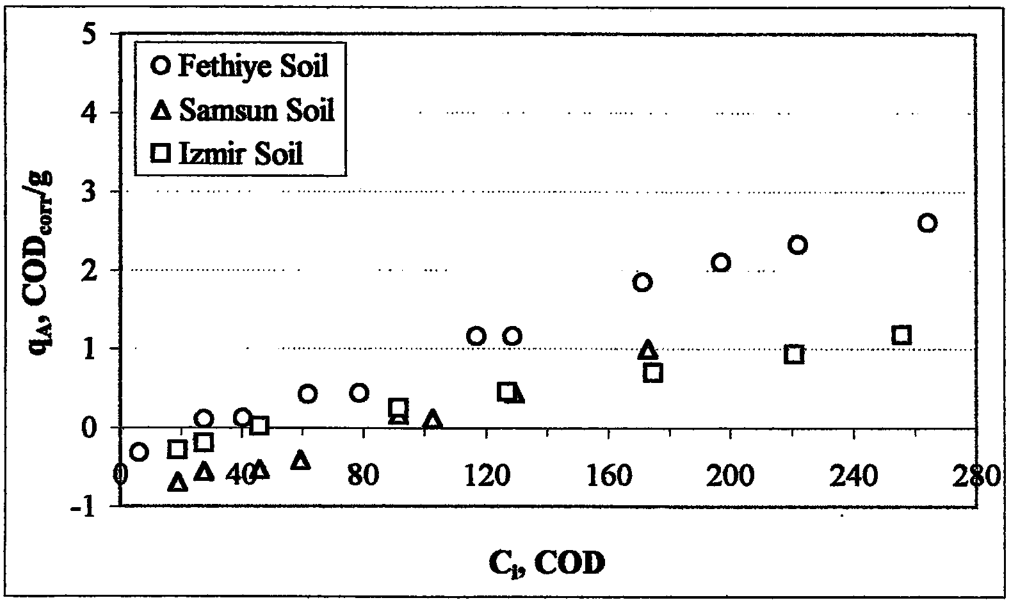


Figure 4.9. Adsorption of humic acid on greenhouse soils.

Effect of pH on Humic acid Adsorption: Adsorption of dissolved organic matter on mineral surfaces increases with decreasing pH (Vermeer *et al.*, 1998). Spark *et al.* (1997b) stated that sorption of humic acid was found to be related to the nature of charge on the mineral surfaces, with positively charged surfaces sorbing the humic acid to a greater extent than negatively charged surfaces. This might be the reason why soil organic matter hinders while oxides/hydroxides in the clay fraction promotes the adsorption of dissolved organic matter on soil surface. Kaiser and Zech (2000) concluded that soil organic matter causes to the occupation of sorption sites and thus to a decrease in positive charges, consequently resulting in a charge reversal on mineral surface (Vermeer et al, 1998). Aluminium and iron oxides/hydroxides, on the other site, are generally positively charged at pH below 8 depending on their pH_{zpc}; pH_{zpc} of goethite (α-FeOOH), for example, is 9.3, that of alumina (α-Al_2O_3) is 8.6 (Spark *et al.*, 1997b) and that of hematite (α-Fe_2O_3) is 9 (Vermeer *et al.*, 1998). The direct contribution of silica-alumina clay minerals on dissolved organic matter adsorption is not well understood. Kaiser and Zech (2000) tried to determine the net effect of clay on dissolved organic matter adsorption by removing the organic matter and oxides/hydroxides on the clay particles. Results showed that the non-expandable layer silicates, e.g. kaolinite, exhibited a weak sorption of dissolved organic matter, especially when compared with that of goethite. AlOH surfaces of the expandable 2:1 layer silicates contribute to the adsorption of humic matter. However, observations of Inoue *et al.* (1990) revealed that the sorption of humic substances onto pure montmorillonite is still small compared to that on AlOH- and SiAlOH- montmorillonite complexes. Thus, conflicts about the direct contribution of clay minerals to dissolved organic matter adsorption make it difficult to specify the effects of varying clay species in Fethiye, Samsun and Izmir soils on the humic acid adsorption.

In general the adsorption is due to both specific (non-Coulombic) and electrostatic (Coulombic) interactions (Vermeer *et al.*, 1998). Differences in the extent of sorption on different sites might be attributed to the extent of each type of bond involved, which is depending on the surface characteristics of minerals. Thus, Kaiser and Zech (2000) reported the possible reason for small sorption onto layer silicates as the involvement of little to no chemisorptive bonds with organic acids. The sorption of dissolved organic matter on oxides/hydroxides, on the other hand, involves at a higher degree ligand exchange binding (formation of stronger inner-phere complexes), especially with hydrophobic fraction of organic matter (Kaiser and Guggenberger, 2000).

The effect of suspension pH on humic acid adsorption was investigated by measuring the pH of humic acid soil suspensions before and after equilibration. For soils with lower carbonate content and soil pH, the difference between both pH were higher; Fethiye Soil > Izmir Soil > Samsun Soil. However, when the pH changes were recorded against time it was observed that equilibrium pH was reached within a low period of time. Equilibrium pH of Fethiye, Samsun and Izmir soils was reached within 55 min, 5 min and 30 min, respectively, representing the same order of pH. From these results it can be concluded that the pH and buffering capacity of soil are the governing parameters of humic acid soil suspension pH.

Considering the equilibrium pH, humic acid adsorptions were proceeding in a pH range from 7 to 8. As can be seen from Figure 4.10, slight differences were only observed for lower concentrations of humic acid solutions, which did not seem to have any influence on sorption. At this pH range, which is close to the pH_{pzc} of aluminium and iron oxides/hydroxides, aluminium and iron oxides/hydroxides still carry a positive charge; humic acid molecules, on the other site, are negatively charged due to the deprotonation of only carboxylic functional groups ($pK_a \sim 4$). Under these conditions, relatively high suspension pH, it can be proposed that the adsorption of humic acid molecules on mineral surface might have occurred directly and with a lesser extent of cation bridging between the humic acid molecule and mineral surface. Thus, the reason of different adsorption behaviours of humic acid on greenhouse soils can only be explained with their organic matter and aluminium and iron oxides/hydroxides contents and not with the effect of pH on humic acid adsorption.

Results of dissolved organic carbon (DOC) adsorption on soils mostly cannot be analysed with Freundlich and Langmuir adsorption equations due to the release of organic matter (Kaiser and Guggenberger, 2000; Vermeer *et al.*, 1998). Freundlich and Langmuir equations might be only used to describe adsorption of humic material on montmorillonite, ferrihydrite etc, since these minerals are pure and release from soil organic matter has not to be considered (Liljestrand *et al.*, 1992). Therefore, "initial mass" approach (Nodvin *et* *al.*, 1986) was used to calculate a linear regression of sorption against humic acid concentrations. Kaiser and Zech (2000) stated that this approach was a useful tool for describing the sorption of DOC in soils because it allowed to consider the release of indigenous organic carbon. Moore *et al.* (1992) also used the initial mass approach for the sorption of DOC by soils and calculated the distribution coefficient K_d by using the regression coefficient (m) from the equations represented below;

$$RE = mX_i - b \qquad (4.1)$$

$$K_d = [m/ (1\text{-}m)] \times (\text{volume of solution})/(\text{mass of soil}) \qquad (4.2)$$

where RE is the release or removal of DOC (mg/g) and X_i is the initial amount of DOC added to soil (mg/g). m, the regression coefficient, is similar to the partition coefficient. When RE is plotted against X_i, m can be obtained from its slope and b (mg/g) from its intercept. K_d is the distribution coefficient (m^3/kg).

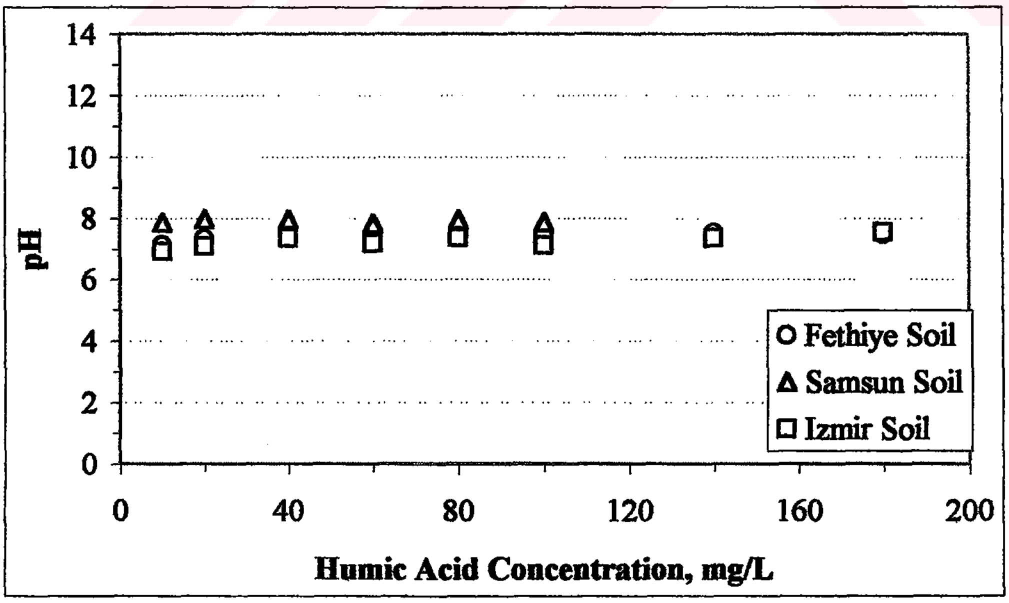


Figure 4.10. Equilibrium pH of humic acid soil suspensions.

In Figure 4.11 adsorption behaviour of humic acid is represented according to the "initial mass" approach. Correlation coefficients (r^2> 0.99) show that humic acid adsorption can be well described by the linear initial mass isotherm. The regression coefficients and distribution coefficients were calculated and compared with the coefficients of Moore *et al.* (1992) and Nodvin *et al.* (1986). The coefficients, that can be seen in Table 4.8, are similar to those reported by Nodvin *et al.* (1992) (m = 0.60 and K_d = 1.50 x 10^{-2} m^3/kg) and Moore *et al.* (1986) (m = 0.45 and K_d = 1.00 x 10^{-2} m^3/kg). As expected m and K_d was highest for Fethiye Soil and lowest for Izmir Soil.

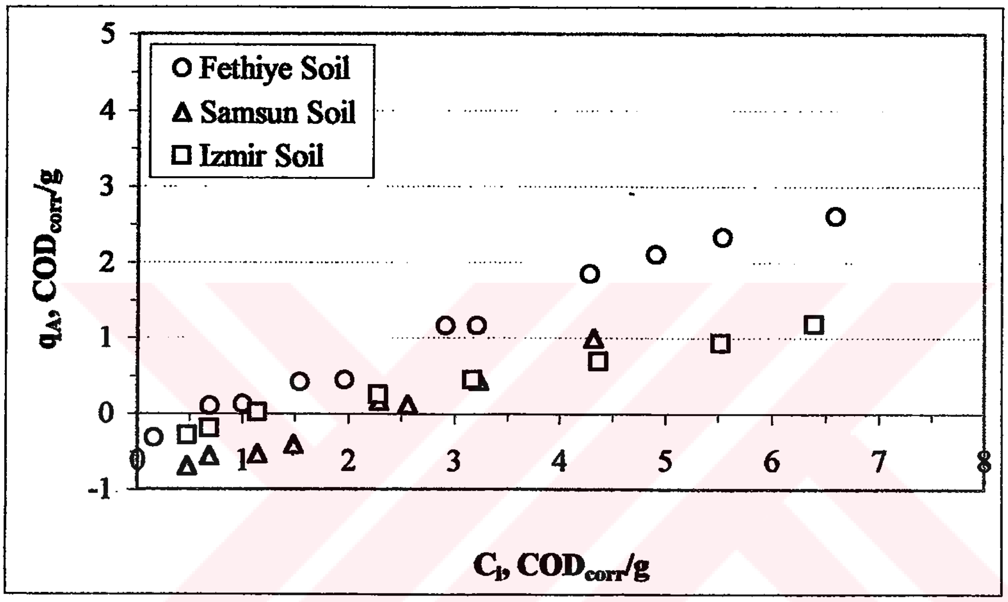


Figure 4.11. Linear initial mass isotherm of humic acid adsorption on greenhouse soils.

Table 4.8. Coefficients of linear initial mass isotherm of humic acid adsorption

Soil	m	Kd, x 10^{-2} m^3/kg	r^2
Fethiye Soil	0.47	2.24	0.994
Samsun Soil	0.44	1.98	0.990
Izmir Soil	0.24	0.77	0.996

The low extractability of zinc from soils seems to be attributable to the adsorption of humic acid on mineral surface, especially when the order of humic acid adsorption is taken into account. It can be proposed that some of humic acid molecules either directly adsorb to the mineral surface or adsorb as humic acid-zinc complexes after extracting some of the zinc bound to minerals with weaker forces. As proposed by Spark *et al.* (1997c), the below given reactions may be involved in the reaction mechanisms of a metal-humic acid-mineral system;

$$M + hu \rightarrow M\text{-}hu_{(sol)} \qquad (4.a)$$

$$M\text{-}hu_{(sol)} + S \rightarrow M\text{-}hu\text{-}S \qquad (4.b)$$

$$hu\text{-}M_{(sol)} + S \rightarrow hu\text{-}M\text{-}S \qquad (4.c)$$

where M stands for metal, hu for humic acid and S for soil minerals.

In order to see to which extent zinc can be adsorbed as humic acid zinc complexes on minerals, an additional control experiment was carried out in duplicate. In this experiment, zinc was equilibrated with Fethiye Soil as humic acid-zinc complexes (100 mg/L humic acid and 10 mg/L zinc) under the same experimental conditions. It was found out that humic acid zinc complexes were definitely not remaining in solution as soluble complexes, but were adsorbed to soil at exactly the same degree. Falatah and Sheta (1999) reported that amounts of zinc sorbed in the presence of dissolved organic carbon were higher than those without dissolved organic carbon. Shuman (1999), on the other hand, observed that adsorption of zinc to sandy soils was increased with humic acid amendment at pH of about 7. Another experiment was performed to see the direct influence of humic acid adsorption on zinc adsorption on soil. For this purpose, zinc adsorption (50 mg/L) was accomplished on a soil sample, which was previously treated with humic acid. Treatment of soil with 200 mg/L humic acid solution was done as in the humic acid adsorption experiments. Under these conditions, the zinc concentration in the supernatant dropped to half of the concentration in supernatant of 50 mg/L zinc adsorption experiments carried out in the absence of humic acid. Desorption of adsorbed humic acid and its complexation with zinc was not observed.

Experiments reveal that the removal of zinc from greenhouse soils with humic acid solutions is relatively low, approaching only 8 % for a humic acid concentration of

1000 mg/L. The influence of soils suspension pH, which determines the types of functional groups involved in the extraction experiments, was found to be insignificant. The interaction of humic acid with soils, on the other hand, revealed an increasing humic acid adsorption with increasing concentration. Additionally, adsorption of humic acid in the presence of zinc indicated rather to the increased adsorption of zinc instead of the formation of soluble humic acid-zinc complexes. Thus, the reason of low removal efficiencies can be attributed to the adsorption of humic acid.

4.3.1.3. Removal of Zinc from Greenhouse Soils with Fulvic Acid: The removal of zinc from greenhouse soils was also accomplished with fulvic acid in the same way as was done with humic acid. Since the soil fulvic acid standard could only be purchased from IHSS at a definite amount (100 mg), extractions were only conducted with concentrations of 100 mg/L, 200 mg/L and 300 mg/L.

As can be seen from Figure 4.12, the removal of zinc from each soil presented almost a linear increase with increasing fulvic acid concentration. The lowest removal efficiencies were observed for a fulvic acid concentration of 100 mg/L, which are about 1 % for all of the soils. Increasing the concentration of fulvic acid to 200 mg/L resulted in an insignificant increase in zinc removal for Fethiye Soil. A further increase to 300 mg/L fulvic acid concentration revealed to the highest removal efficiency, observed for the studied concentration range, of only 1.2 %. For Samsun Soil, on the other hand, a raise in fulvic acid concentration of 200 mg/L revealed an additional increase of only 0.3 %. A zinc removal efficiency of about 2.2 % was achieved when the fulvic acid concentration was increased from 100 mg/L to 300 mg/L. The removal of zinc from Izmir Soil was about 2.5 % for a fulvic acid concentration of 200 mg/L. A further increase in concentration presented the highest removal efficiency, which was about 3.7 %.

Among soils, zinc was most efficiently removed from Izmir Soil, followed by Samsun Soil. The removal of zinc from Fethiye Soil was occurring at least degree, almost independent from the fulvic acid concentration.

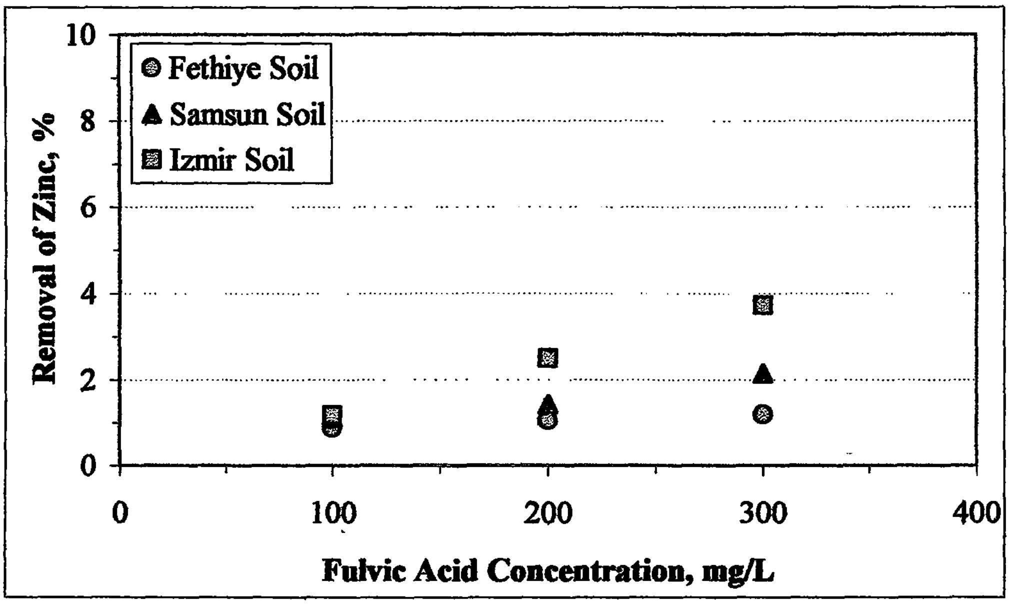
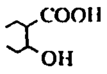
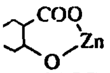
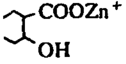
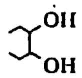
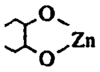
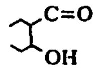
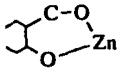


Figure 4.12. Removal of zinc with various fulvic acid concentrations.

Fulvic acids are known to have more functional groups in comparison to humic acids. Vermeer and Koopal (1998) stated that the high O:C ratio of Laurentian fulvic acid indicated to a higher total acidity. Ephraim (1992) reported that fulvic acid can be characterized to have five predominant acidic sites with various dissociation constants and abundances. These acidic sites, their pK_a values and the type of complexation with zinc can be seen in Table 4.9 below.

Table 4.9. Predominant acid sites in Bersbo FA, their envisaged complexes with Zn(II) and the literature complex formation constants (Ephraim, 1992).

Acid site	pK_a	Abundance	Functionality	Complexes with zinc	Constants employed (l mol^{-1})
I	1.7	0.20	Unidentate $-COOH$	$-COOZn^+$	$\beta_1 = 10^{1.1}$
II	3.3	0.25			$K_{exc1} = 10^{-5.2}$
					$\beta_2 = 10^{1.1}$
III	5.0	0.30	Unidentate $-COOH$	$-COOZn^+$	$\beta_3 = 10^{1.1}$
IV	6.5	0.20			$K_{exc2} = 10^{-1.0}$
V	7.0	0.05			$\beta_4 = 10^{7.8}$

Effect of pH on the Removal of Zinc: The pH of standard fulvic acid solutions were quite low (between 3 and 4) and decreased with increasing fulvic acid concentration. This was also reflected in the pH of fulvic acid soil suspensions. However, overall pH of soil suspensions in the fulvic acid extraction experiments were between 6.5 and 7.5, which is also not much different from those (pH = 7–8) in the humic acid extractions. The reason of this is the same buffering capacity of soils as explained before. Under these conditions, it can be assumed that most of the acidic sites of the fulvic acid molecules, with the exception of site V for suspension pH below 7, are deprotonated and thus in an active state. Dissociation of site V might be eliminated for the fulvic acid extraction of Fethiye and Izmir soils, since suspension pH of both were between 6.5-7, due to their relatively low buffering capacity and soil pH. If the participation of site V were that effective, decrease in the removal of zinc should have been observed for both soils, however low removal efficiencies were only obtained for Fethiye Soil, whereas fulvic acid extractions were highest for Izmir Soil. Thus, it seems that deprotonation of functional groups of fulvic acid are not the governing parameter in fulvic acid reaction, since it does not explain the differences in extraction efficiencies.

The differences in zinc removal by humic acid extractions were attributed to the different humic acid adsorption behaviour of soils rather than the deprotonation of its functional groups. Unfortunately adsorption of fulvic acid to soils could not be carried out due to the reason explained before. Adsorption of humic substances on mineral surfaces was related to its aromatic carbon content, O/C ratio and hydrophilicity. Petrović *et al.* (1999) stated that adsorption of humic substances is increased with increased aromatic carbon content and decreased O/C ratio and hydrophilicity. Kaiser and Guggenberger (2000), on the other hand, stated that hydrophobic dissolved organic matter (related to the aromatic carbon content) adsorbs strongly whereas hydrophilic fractions sorbs weakly. Since fulvic acid possesses a higher O/C ratio and a more hydrophilic character, it is expected that fulvic acid molecules are less adsorbed on mineral surfaces than humic acid molecules. This was also presented by Vermeer *et al.* (1998).

As mentioned before, adsorption of humic substances increases with decreasing pH. With increasing concentration the pH of fulvic acid solutions decreased from 3.7 to 3.2 whereas those of humic acid solutions increased from 10.3 to 10.7. This was related to the differences in the solubility characteristics of both solutions; humic acid is soluble in alkaline solution whereas fulvic acid is soluble at any pH –therefore, humic acid solution was prepared in alkaline solution whereas fulvic acid was simply dissolved in distilled/deionised water. Variation in solution pH was also reflected in the suspension pH. However, overall pH of soil suspensions (pH = 6.5–7.5) in the fulvic acid extraction experiments were not much different from those (pH = 7–8) in the humic acid extractions as stated above. Under these conditions, adsorption of fulvic acid cannot be higher than that of humic acid and the low removal efficiencies in comparison to the efficiencies of humic acid can definitely not be related to adsorption of fulvic acid on soils. However, the order of extraction efficiency (Izmir Soil > Samsun Soil > Fethiye Soil) though can still be related to the differences in fulvic acid adsorption on soils, since adsorption is expected even at a lower degree. The adsorption behaviour of fulvic acid is supposed to be the same of humic acid, because the nature of mineral surfaces remain the same.

The low extraction efficiencies could neither be related to the dissociation of functional groups nor to the suspension pH and adsorption behaviour of fulvic acid. In spite of the fact that fulvic acid contains higher amounts of functional groups, removal of zinc from greenhouse soils by fulvic acids is almost half of that of humic acid. Lastly it should be focused on the differences between the stability constants. Schnitzer and Skinner (1966) tried to determine the stability constant of fulvic acid zinc complexes at pH 3.5 and 5.0. The stability constant of fulvic acid zinc complexes at these pH were found to be 1.73 and 2.34, respectively. The stability constants of humic acid zinc complexes, at pH 3.5 and 5.6, on the other hand, were found to be 4.42 and 6.18, respectively (Randhawa and Broadbent, 1965). As can be seen, the stability constants of fulvic acid zinc complexes are quite lower than the stability constants of humic acid zinc complexes. Schnitzer and Skinner (1966) concluded that not the number of bounds but strength of bonds is the parameter determining the magnitude of stability constants. This might be the reason of lower extraction efficiencies, in spite of the higher amount of functional groups present.

4.3.2. Removal of Zinc with EDTA and DTPA

4.3.2.1. Evaluation of Optimum Time for the Removal of Zinc with EDTA and DTPA:

The equilibration time of EDTA extraction was initially determined by conducting the extraction experiments on treated samples of Fethiye Soil for times of 5, 10, 20, 30, 60 and 120 min with an EDTA concentration of 0.01 M. The same procedure was performed for 0.001 M DTPA for extraction times of 5, 15, 30, 60 and 120 min. Results show that an extraction time of 60 min was found to be sufficient to obtain a removal efficiency of 92 % for EDTA and 74 % for DTPA (Figure 4.13). Increasing the extraction time further to 120 min did not affect the efficiency of EDTA and resulted only in additional increase of approximately 5 % in the efficiency of DTPA. Thus, the extraction time was determined to be 60 min throughout the extraction experiments.

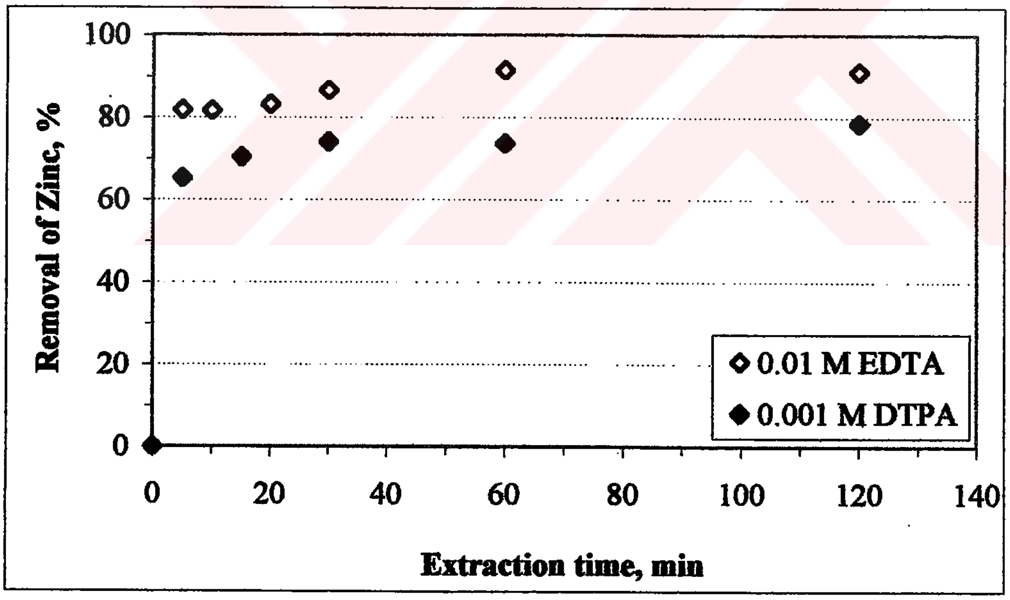


Figure 4.13. Removal of zinc from Fethiye Soil by 0.01 M EDTA and 0.001 M DTPA with respect to time.

The equilibrium of extraction was also very rapidly reached in the experiments conducted by Lo and Yang (1999). Generally, about 90 % of zinc and copper were extracted with EDTA concentrations of 0.05 M or 0.1 M in 15 min. In the extraction experiments performed by Hong *et al.* (1999) an extraction time of 4 h was used in the extractions of lead, cadmium, copper and zinc with EDTA concentrations ranging between

0.003 M and 0.05 M. Much longer extraction times were reported for metal extractions from higher polluted soils (Yu and Klarup, 1994). Ghestem and Bermond (1998), on the other hand, reported that the chosen reaction time of 24 h was not enough to reach equilibrium of extracted zinc. Results of these studies point towards the importance of contaminant load and the EDTA concentration.

4.3.2.2. Removal of Zinc from Greenhouse Soils with EDTA: The removal of zinc from soil samples was accomplished with various concentrations of EDTA ranging between 0.0001 M and 0.015 M; removal efficiencies are presented in Figure 4.14. For all soils the lowest removal efficiencies were obtained for the lowest EDTA concentration, which were 45 %, 39.7 % and 49.9 % for Fethiye, Samsun and Izmir soils, respectively. Among these the highest efficiency was observed for Izmir Soil, followed by Fethiye Soil. Increasing the concentration 10 times, from 0.0001 M to 0.001 M, resulted in an increase to 74 % for Fethiye Soil. An additional increase of about 10 % was observed when the EDTA concentration was raised to 0.005 M. With an EDTA concentration of 0.01 M, on the other hand, 92.5 % of zinc was extracted from the soil. When the EDTA concentration was increased to 0.015 M in order to see if a further elevation will be recorded, an additional increase of only 3.5 % was observed. For the Samsun Soil, a removal efficiency of 60.6 % was obtained when the EDTA concentration was increased to 0.001 M. Raising the concentration to 0.005 M resulted in an additional increase of about 29 %. On the other hand, zinc was removed from Samsun Soil about 98 % with an EDTA concentration of 0.01 M. The removal of zinc from Izmir Soil with an EDTA concentration of 0.001 M was about 89.4 %. Increasing the concentration 5 times, to 0.005 M, resulted in an additional increase of 18 %, reaching an efficiency of 107.5 %. Zinc was removed from soil about 111 %, when the concentration was further increased to 0.01 M. Extraction efficiencies above 100 % for Izmir Soil may also be attributed to its relatively higher zinc content. Supplementary experiments revealed that 0.01 M EDTA was able to extract about 30 % of zinc present in natural (untreated) Izmir Soil.

As can be seen from Figure 4.14, for all EDTA concentrations the highest removal efficiencies were recorded for Izmir Soil, which may be related to its low carbonate and elevated zinc content. Zinc was lowest removed from the Fethiye Soil for EDTA concentrations above 0.005 M. For lower EDTA concentrations, however, zinc removal from Samsun Soil revealed the lowest efficiencies.

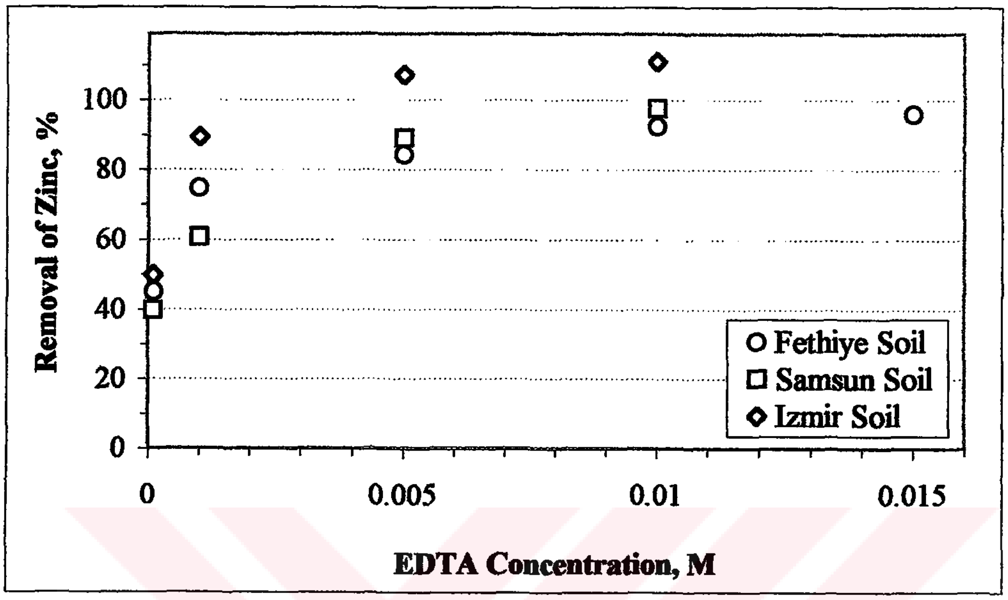


Figure 4.14. Removal of zinc from soil with various concentrations of EDTA.

Similar results were obtained by Mobley (1988) who recovered 80 to more than 95 % of cadmium, copper, nickel and zinc from a Lebanon silty soil containing contaminant levels of 100 to 300 mg/kg. For soils with elevated contents of trace metals the efficiency of extractions is mostly reduced. Ghestem and Bermond (1998) reported a zinc removal efficiency of about 40 % for a soil with a zinc content of 1412 mg/kg, iron content of 20900 mg/kg and a lead content of 1120 mg/kg. They explained that the removal depends on pH, on the EDTA concentration and on the concentration of major cations. They concluded that the extraction of trace metals is governed by competitions with major cations when EDTA is not concentrated enough.

The effect of EDTA concentration on zinc removal can be best interpreted in terms of the stoichiometric molar ratios between the EDTA concentration used and the zinc concentration in soil (EDTA/Zn, molarity of EDTA divided by molarity of Zn) and between the zinc concentration extracted and the EDTA concentration used (EDTA-Zn/EDTA, molarity of extracted Zn divided by molarity of EDTA). Molar ratios can be seen in Table 4.10. Taking into account that EDTA and zinc form complexes at a ratio of 1:1, EDTA/Zn ratios indicate that 0.0001 M EDTA reflects a lack of EDTA and concentrations above 0.0001 M an excess of EDTA for all soils. Excess of EDTA above concentrations of 0.001 M was also defined by Ghestem and Bermond (1998). For all soils, the EDTA/Zn ratio of 0.0001 M EDTA shows that zinc in soil is about twice as high as the EDTA present, meaning that EDTA can only extract half of the Zn bound to soil. Accordingly, the zinc removals found were approximately 45 %, 40 % and 50 % for Fethiye, Samsun and Izmir soils, respectively. The order of these removals can be quite well explained by the EDTA-Zn/EDTA ratios. From Table 4.10 it can be seen that 86 %, 76 % and 95 % of EDTA was used during the extraction of zinc from Fethiye, Samsun and Izmir soils, respectively, meaning that EDTA was most efficiently used for Izmir Soil and less efficiently for Fethiye Soil and especially Samsun Soil, thus reflecting the order of efficiencies. The reason of this is the presence of other cations in Fethiye and Samsun soils, like the relatively high copper content of Fethiye Soil and relatively high calcium content of Samsun Soil, which were leading to additional consumptions of EDTA. The same situation was observed for 0.001 M EDTA with the exception that EDTA was present in excess amounts. Removal efficiencies of about 75 %, 60 % and 89 % were achieved with consumptions of 13 %, 11 % and 16 % of EDTA for Fethiye, Samsun and Izmir soils, respectively. This order again represents the importance of copper content of Fethiye Soil and the calcium content of Samsun Soil under lower EDTA concentrations. On the other hand, about 3 %, 3.2 % and 3.9 % of 0.005 M EDTA was used for 85 %, 89 % and 108 % removal of zinc from Fethiye, Samsun and Izmir soils, respectively. At this concentration the influence of these cations seems to diminish due to presence of excess EDTA. The diminishing influence of copper, iron and calcium can be better recognized from Table 4.11, which represents the EDTA-Me/EDTA molar ratios for copper, iron and calcium. The total of these molar ratios points toward the presence of excess EDTA, despite its consumption by copper, iron and calcium. For all greenhouse soils, these molar ratios also show that calcium consumed most of the EDTA, between 20 and 36 %, followed by iron, which used about 2-3 % of EDTA. As expected, the highest fraction of EDTA consumed by calcium was observed for the Samsun Soil and the EDTA used by iron for the Fethiye and Izmir soils. The highest portion of EDTA consumed by copper was obtained for the Fethiye Soil. With further increase in the concentration of EDTA, the percentage of EDTA complexed with zinc decreased. Approximately 1.6 %, 1.8 % and 2 % of 0.01 M EDTA was consumed for a zinc removal of 93 % 98 % and 111 % from Fethiye, Samsun and Izmir soils, respectively. As can be recognized, the high removal efficiencies observed for Izmir Soil was mainly related to the higher percentages of EDTA involved in the extractions.

Table 4.10. Stoichiometric molar ratios between EDTA and zinc

	Fethiye Soil		Samsun Soil		Izmir Soil	
	EDTA/Zn	EDTA-Zn/EDTA	EDTA/Zn	EDTA-Zn/EDTA	EDTA/Zn	EDTA-Zn/EDTA
0.01 M	56.69	0.016	55.22	0.018	55.14	0.020
0.005 M	28.38	0.030	27.61	0.032	27.55	0.039
0.001 M	5.68	0.131	5.53	0.110	5.51	0.162
0.0001 M	0.52	0.862	0.52	0.758	0.52	0.952

Table 4.11. Stoichiometric molar ratios between 0.005 M EDTA and other cations present in soils

	EDTA-Zn/EDTA	EDTA-Cu/EDTA	EDTA-Fe/EDTA	EDTA-Ca/EDTA
Fethiye Soil	0.030	0.0053	0.032	0.202
Samsun Soil	0.032	0.0004	0.019	0.365
Izmir Soil	0.039	0.0011	0.029	0.282

The effect of EDTA concentration on the removal of trace elements like zinc, lead, cadmium and arsenic was also explained with their EDTA/metal molar ratios by Papassiopi *et al.* (1999). They stated that the metal extraction was seen to double when the operating concentration of EDTA was increased 10 times, i.e. from 0.025 to 0.25 M, and the corresponding EDTA/metal molar ratios from 2.3 to 23 mole/mole. The increase in EDTA resulted in an increase of zinc extraction from 20 to 45.4 %. Further analysis showed that the low efficiencies were related to the high consumption (89 %) of EDTA by calcium arising from the dissolution of $CaCO_3$ in calcareous soil. It was concluded that predictions for EDTA consumption must be based on the calcium content of the soil.

4.3.2.3. Removal of Zinc from Greenhouse Soils with DTPA: The removal of zinc from soil samples was accomplished with DTPA solutions at concentrations ranging between 0.0001 M and 0.015 M. As can be seen from Figure 4.15, the lowest removal efficiencies were again obtained for the lowest DTPA concentration, which were 37.6 %, 56.4 % and 50.8 % for Fethiye, Samsun and Izmir soils, respectively. Increasing the DTPA concentration 10 times, from 0.0001 M to 0.001 M, resulted in an increase of almost 2 times in the removal of zinc from Fethiye Soil, which was found to be 73.8 %. An additional increase of about 13 % was achieved for Fethiye Soil when the extraction was performed with 0.005 M DTPA. With a further increase in DTPA concentration to 0.01 M, an additional increase was not observed. Extraction with 0.015 M, on the other hand, revealed a further rise in removal efficiency approaching a value of 95 %. For the Samsun Soil, a removal efficiency of 78 % was observed, when the DTPA concentration was increased to 0.001 M. Raising the concentration to 0.005 M resulted in an additional increase of about 20 %. More than 100 % of zinc was removed with a DTPA concentration of 0.01 M. The removal of zinc from Izmir Soil was above 100 % for DTPA concentrations above 0.001 M. For a concentration of 0.001 M a removal efficiency of 101 % was obtained. An additional increase of 14 % was observed when the concentration was increased to 0.005 M. A further increase in DTPA concentration to 0.01 M did not present an important increase in zinc removal. Efficiencies above 100 % may be again related to its higher zinc content. The extraction performed on natural (untreated) Izmir Soil with 0.005 M revealed that 34 % of zinc could be additionally removed. Thus, percentages above 100 % were achieved by extracting the zinc initially present in natural soils.

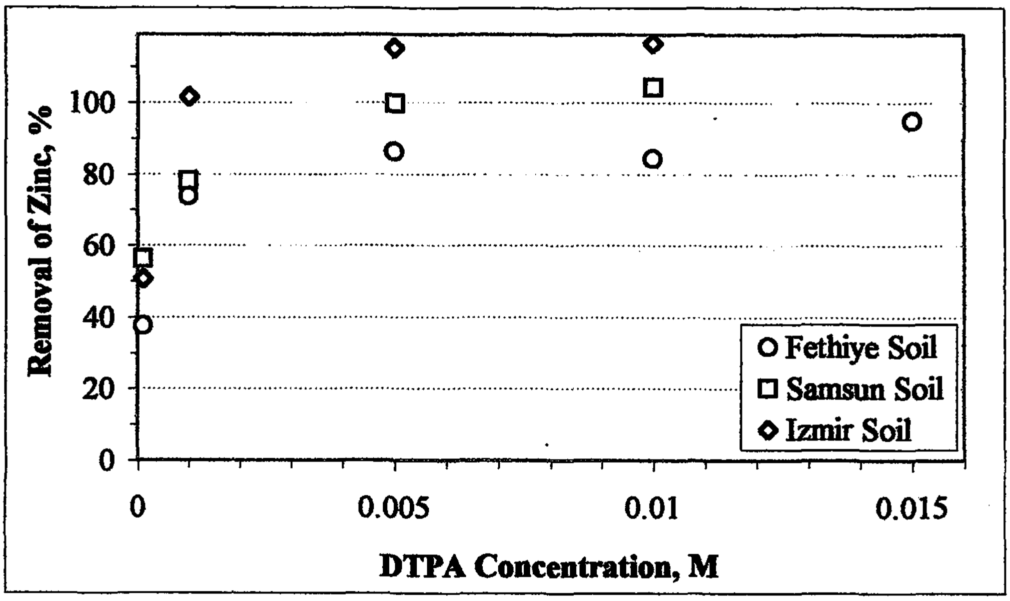


Figure 4.15. Removal of Zn from soil with various concentrations of DTPA.

In general, the highest removal efficiencies were obtained for the Izmir Soil for DTPA concentrations above 0.001 M, followed by the Samsun Soil. Fethiye Soil, on the other hand, represented again the lowest removal efficiencies, as was encountered for the EDTA extractions.

Since DTPA extractions are mostly accomplished as a mixture composed of 0.005 M DTPA, 0.01 M TEA and 0.01 M $CaCl_2$ (pH adjusted to 7.3), in order to determine the micronutrients available for the plant, many publications presenting only the efficiency of DTPA were not available except that of Davis and Singh (1995), who studied the washing of from a contaminated soil column. With a flow rate of 3 mL/min the zinc removal efficiency approached 90 % with 0.003 M DTPA, which is in accordance with the above presented results.

Considering the variations in removal efficiencies achieved for each soil by different DTPA concentrations, a better interpretation could be made by examining the stoichiometric molar ratios DTPA/Zn (molarity of DTPA divided by molarity of Zn) and DPTA-Zn/DPTA (molarity of extracted Zn divided by molarity of DPTA), Table 4.12. Taking into account that DTPA and zinc form complexes at a ratio of 1:1, DTPA/Zn ratios indicated that 0.0001 M represent again a lack of chelator and concentrations over 0.0001 M an excess. Again at this concentration the DTPA present in suspension was approximately half that of zinc bound to soil for Samsun Soil and Izmir Soil and even less for Fethiye Soil. DTPA-Zn/DTPA ratio of Fethiye Soil implies that only 81 % of 0.0001 M DTPA was complexed with zinc yielding to a removal efficiency of only 38 %. On the other hand, almost all of DTPA was used to extract zinc from Izmir Soil. In the case of Samsun Soil some other factors increased the extracting effect of DTPA. This might be the release of soil organic matter due to higher suspension pH and organic matter content. The interaction of DTPA with soil organic matter has been previously reported by Falatah and Sheta (1999) and Száková *et al.* (2000). The ability of DTPA to extract the fraction of metals complexed by organic matter was also stated by Ure (1996), Piccolo (1989) and McGrath (1996). Especially, an effect of calcium could not be noticed at this concentration and 0.001 M DTPA. For a concentration of 0.005 M, removal efficiencies of about 86.3 %, 99.9 % and 115.4 % were achieved with a consumption of 3.7 %, 3.9 % and 4.5 % of DTPA for Fethiye, Samsun and Izmir soils, respectively. An influence of calcium was also not recorded at this concentration, which can also be recognized from Table 4.13. The total of DTPA-Me/DTPA molar ratios reflects the presence of excess DTPA, despite its increased consumptions by calcium, iron and copper. As can be seen, for all soils calcium again used most of the DTPA, approaching 60 %, whereas iron utilized mostly 6 % of DTPA, which was observed for Fethiye and Izmir soils. As expected, DTPA mostly consumed by calcium was again obtained for Samsun Soil. As was also observed for the EDTA extractions, the higher removal efficiencies of Izmir Soil can be mainly related to the higher percentages of DTPA involved in the extractions. The low removal efficiencies recorded for Fethiye Soil, on the other hand, can be attributed to the lower amounts of DTPA used.

Table 4.12. Stoichiometric molar ratios between DTPA and zinc

	Fethiye Soil		Samsun Soil		Izmir Soil	
	DTPA/Zn	DTPA-Zn/DTPA	DTPA/Zn	DTPA-Zn/DTPA	DTPA/Zn	DTPA-Zn/DTPA
0.01 M	46.11	0.018	51.89	0.020	51.63	0.023
0.005 M	23.04	0.037	25.92	0.039	25.84	0.045
0.001 M	4.62	0.160	5.17	0.151	5.16	0.197
0.0001 M	0.46	0.813	0.52	1.099	0.52	0.980

Table 4.13. Stoichiometric molar ratios between 0.005 M DTPA and other cations present in soils

	DTPA-Zn/DTPA	DTPA-Cu/DTPA	DTPA-Fe/DTPA	DTPA-Ca/DTPA
Fethiye Soil	0.037	0.0055	0.059	0.235
Samsun Soil	0.039	0.0007	0.036	0.612
Izmir Soil	0.045	0.0015	0.059	0.368

<u>4.3.2.4. Effect of pH on the Removal of Zinc:</u> For the interpretation of the reactivity of EDTA and DTPA, the parameters to be considered seem to be pH and concentration. As can be seen from Table 4.14 and Table 4.15, the pH of DTPA solutions was lower for each concentration in comparison to EDTA solutions. The pH of both EDTA and DTPA solutions decreased as their concentration increased. Additionally, the pH of DTPA solutions were lower than the corresponding pH of EDTA solutions. Since pH adjustment

was not performed, decline in pH of soil solution suspensions was also recorded for each soil with increase in EDTA and DTPA concentration. Again, pH of soil DTPA solution suspensions was lower than pH of soil EDTA solution suspensions. Recording the pH before and after the extraction showed that the pH of suspensions was altered by the natural acid-neutralizing capacity of soil. Generally, the pH of soil solution suspensions increased for all soils and EDTA and DTPA concentrations, after an hour of shaking. In both EDTA and DTPA extractions, the highest suspension pH were obtained for Samsun Soil, which has also the highest soil pH, and the lowest for Fethiye Soil, which has also the lowest soil pH. The results indicate that soil pH is another important parameter influencing the suspension pH.

The influence of suspension pH on the removal of zinc from greenhouse soils was examined by conducting extraction experiments with distilled and deionised water, whose pH was adjusted to 2.4. A pH of 2.4 was chosen to reflect the conditions of lowest solution pH observed in the extraction experiments conducted with both EDTA and DTPA, which was the pH of 0.01 M DTPA. In order to provide the same pH range observed in the extractions with 0.01 M DTPA for each soil, pH adjustments were performed every 15 min with dilute HNO_3 solution. Thus the pH of distilled deionised water soil suspensions were retained about 2.86, 4.8 and 3.10 for Fethiye, Samsun and Izmir soils, respectively. The corresponding zinc removal efficiencies were found to be about 50 % for Fethiye and Izmir soils, and 21 % for Samsun Soil, showing that the highest contribution of pH to removal of zinc from soils was about 50 % for a pH of 3. Increasing the pH to about 5 reduced the contribution to about 20 %. Taking into account that such low pH (< 3) were recorded only for a few experiments, it can be concluded that the contribution of suspension pH to the removal of zinc was only at reduced degrees. Additionally, the contribution of pH was more possible in the extraction experiments carried out with DTPA, since almost all of the experiments with EDTA had suspension pH > 5. The influence of pH > 6 will be more limited, being acceptable as insignificant.

Table 4.14. pH of EDTA solutions and soil suspensions

Concentration	EDTA	Fethiye Soil		Samsun Soil		Izmir Soil	
		before	after	before	after	before	after
0.01 M	4.55	4.18	4.36	5.04	6.52	4.36	4.58
0.005 M	4.65	4.32	4.54	6.15	6.95	4.60	5.10
0.001 M	4.75	5.12	6.15	7.09	7.53	6.29	6.39
0.0001 M	5.34	6.13	6.68	7.13	7.43	6.18	6.61

Table 4.15. pH of DTPA solutions and soil suspensions

Concentration	DTPA	Fethiye Soil		Samsun Soil		Izmir Soil	
		before	after	before	after	before	after
0.01 M	2.40	2.58	2.81	3.80	4.89	2.81	3.04
0.005 M	2.55	2.88	3.42	4.91	6.08	3.40	4.01
0.001 M	3.01	*	*	6.95	7.31	5.91	5.74
0.0001 M	3.81	6.33	6.81	7.98	8.08	6.51	6.80

*: not measured

These suspension pH influence the distribution of EDTA and DTPA species present during the extractions and thus the extraction efficiencies of zinc from soils. The reactivity of EDTA and DTPA can be compared in this sense. Despite the fact that DTPA is known as a stronger complexing agent than EDTA in accordance to the number of functional groups (Finnen *et al.*, 1991; and Přibil, 1972), its speciation under some pH conditions may cause to equivalent activity to that of EDTA. This situation was observed for Fethiye Soil for which some of the extraction efficiencies are nearly superimposed (Figure 4.16). The reason of close extraction efficiencies was the type of EDTA species (H_4Y, H_3Y^-, H_2Y^{2-}, HY^{3-} and Y^{4-}) and DTPA species (H_5Z, H_4Z^-, H_3Z^{2-}, $H_2Z^{3-}HZ^{4-}$ and Z^{5-}) present at a specific concentration, Table 4.16. For example, the lower removal of zinc by 0.01 M DTPA from Fethiye Soil was mainly due to the presence of a less active species (H_4Z^-) in solution in comparison to the more active species (H_2Y^{2-}) of EDTA. Lower removal efficiencies of DTPA were also resulting from lower amounts of species present in comparison to the species of EDTA with the same activity. Accordingly, a lower extraction efficiency was obtained for Fethiye Soil at a concentration of 0.0001 M, which was due to the lower amount of H_3Z^{2-} present compared to H_2Y^{2-}. On the other hand, close results were due to the presence of species with similar activity, as was the case of Izmir Soil for which the zinc removal efficiencies were found to be close for an EDTA and DTPA concentration of 0.01 M. Additionally, the higher removal efficiencies of DTPA extractions were related to the amounts of DTPA species with higher activity present in suspension in comparison of those of EDTA, as was recorded for the removal of zinc from Samsun Soil with DTPA concentration of 0.005 M.

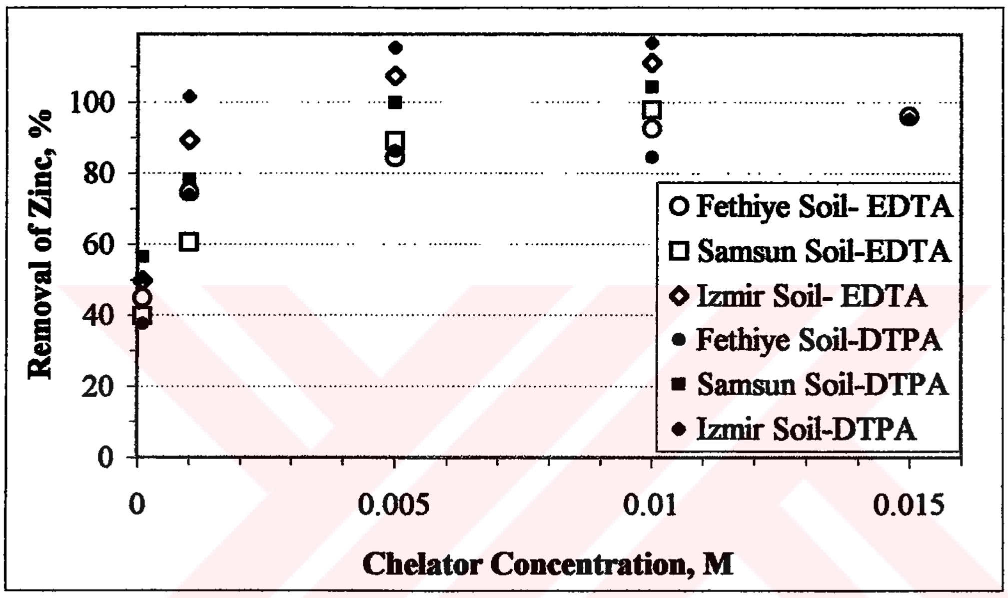


Figure 4.16. Comparison of extraction efficiencies of EDTA and DTPA.

Table 4.16. Type of EDTA and DTPA species present at specific pH

Concentration	Fethiye Soil		Samsun Soil		Izmir Soil	
	EDTA	DTPA	EDTA	DTPA	EDTA	DTPA
0.01 M	$\mathbf{H_2Y^{2-}}$	H_3Z^{2-}, $\mathbf{H_4Z^{-}}$	$\mathbf{H_2Y^{2-}}$, HY^{3-}	H_3Z^{2-}, H_2Z^{3-}	$\mathbf{H_2Y^{2-}}$	$\mathbf{H_3Z^{2-}}$, H_4Z^{-}
0.005 M	$\mathbf{H_2Y^{2-}}$	$\mathbf{H_3Z^{2-}}$, H_4Z^{-}	H_2Y^{2-}, HY^{3-}	H_3Z^{2-}, $\mathbf{H_2Z^{3-}}$	$\mathbf{H_2Y^{2-}}$, HY^{3-}	$\mathbf{H_3Z^{2-}}$, *H_2Z^{3-}
0.001 M	$\mathbf{H_2Y^{2-}}$, HY^{3-}		H_2Y^{2-}, $\mathbf{HY^{3-}}$	$\mathbf{H_2Z^{3-}}$, HZ^{4-}	H_2Y^{2-}, HY^{3-}	H_3Z^{2-}, $\mathbf{H_2Z^{3-}}$
0.0001 M	H_2Y^{2-}, $\mathbf{HY^{3-}}$	$\mathbf{H_2Z^{3-}}$, HZ^{4-}	H_2Y^{2-}, $\mathbf{HY^{3-}}$	H_2Z^{3-}, HZ^{4-}	H_2Y^{2-}, $\mathbf{HY^{3-}}$	$\mathbf{H_2Z^{3-}}$, HZ^{4-}

Note: from the fractionation of EDTA and DTPA species graph presented in Section 2.3.1.1. it was determined that bold typed species are present in higher amounts

* amount of H_2Z^{3-} was higher of that of HY^{3-}

Explaining the variation between the extraction efficiencies encountered for Fethiye, Samsun and Izmir soils in relation to the types of species present seems to be rather a difficult task. According to the types of species present, the extraction efficiency of both EDTA and DTPA should be higher for Samsun Soil than Izmir Soil, however a reverse situation can be seen in Figure 4.16. The reason of this may be the variation in elemental composition of each soil suspension. Especially, the high carbonate content of Samsun Soil appears to influence the extraction efficiency of both EDTA and DTPA.

4.3.2.5 Effect of Zinc Load on the Removal Efficiency of EDTA and DTPA: Removal efficiencies of zinc extractions showed that 0.005 M could be accepted to be the optimum concentration for both EDTA and DTPA. The effect of zinc load on the extraction efficiencies of EDTA and DTPA was determined with experiments conducted on Fethiye Soil with this optimum concentration. As can be seen from Figure 4.17, increasing the zinc load of Fethiye Soil did not change the extraction efficiency of both EDTA and DTPA within this range. For each increase in zinc load it can be seen that the stoichiometric molar ratios between the Zn concentration extracted and the EDTA concentration used (EDTA-Zn/EDTA) and between the Zn concentration extracted and the DTPA concentration used (DTPA-Zn/DTPA) increased, meaning that more and more EDTA and DTPA were needed to achieve the same efficiencies. Thus, it can be suggested that the conservation of same removal efficiency is a result of excess EDTA and DTPA.

The experiments of Fethiye Soil also represent that at higher zinc load the extraction values of EDTA and DTPA are nearly superimposed. Since the species present at a concentration of 0.005 M represent almost the same activity (Table 4.16), this can be explained in relation with the corresponding EDTA-Zn/EDTA and DTPA-Zn/DTPA molar ratios calculated separately for every zinc load. Generally, they imply that for a given zinc load, the molar ratio needed for a similar zinc removal is approximately the same for both EDTA and DTPA, also meaning that under these conditions approximately the same portion of EDTA and DTPA is consumed for the removal of zinc.

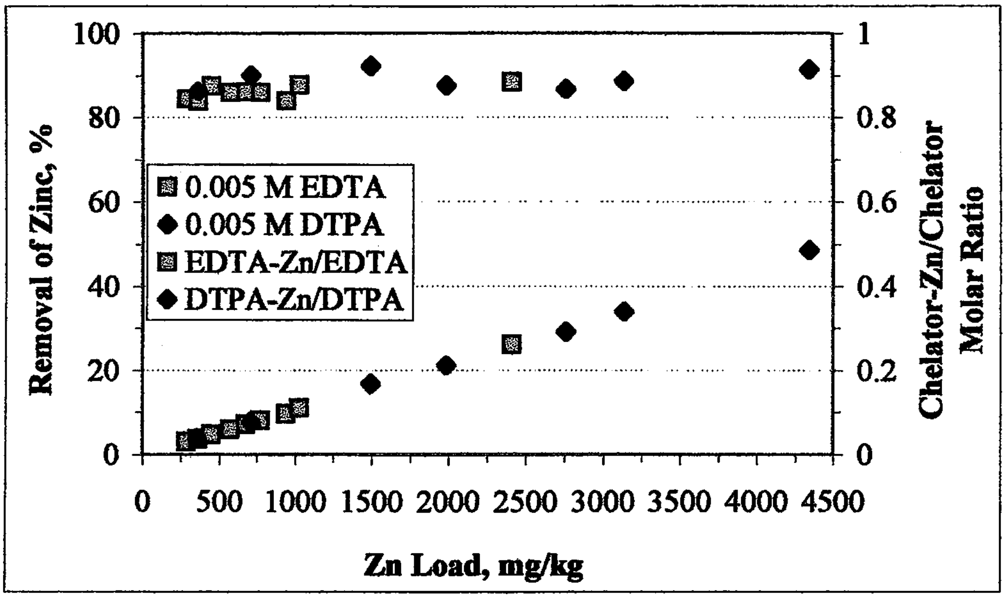


Figure 4.17. Effect of zinc load on the extraction efficiency of EDTA and DTPA.

4.5. Distribution of Zinc to Soil Fractions

Metals in soils can be associated with various components: adsorbed on surfaces of clay minerals, iron and manganese oxyhydroxides; complexed with organic matter; present in lattice of secondary minerals like carbonates, phosphates, sulfates or oxides, etc. Adsorption isotherms provide useful information about the soil retention capacity; however this information is limited since it does not include the partitioning of metals with the soil constituents. Generally, to provide a deeper insight into the adsorption phenomenon and its environmental meaning, sequential extraction procedures are used.

Sequential extractions with selective extractants are applied to fractionate metals bound to different soil constituents and thus obtain information about their origin, bioavailability and potential mobilizability and transport in the environment. Among the many sequential extraction procedures described (Shuman, 1985; Ure, 1996; Maiz *et al.*, 1997) the sequential extraction procedure developed by Tessier *et al.* (1979) is the most widely used speciation scheme reported in literature (García Sánchez *et al.*, 1999; Gómez

Ariza *et al.*, 2000). As mentioned in Section 3.2.7, the procedure distributes the metals to five fractions: exchangeable, carbonate, iron and manganese oxide, organic and residual. The exchangeable (EXC) fraction includes metals which are bound via electrostatic interactions to the surface of clays, hydrated oxides of iron and manganese, humic acids, etc. and thus are easily exchanged under neutral pH. The carbonate (CAR) fraction includes metals associated with calcium and magnesium carbonates and are readily released with a decrease in medium pH. Iron and manganese oxides, which are present as a coating on clay particles, are excellent scavengers of metals. Metals, which are bound to these oxides, are represented by the third fraction (Fe/MnOx fraction). The organic (ORG) fraction includes metals that are bound to various forms of organic matter like living organisms, other nonhumified organic acids, humic and fulvic acids, etc. The last fraction includes metals, which are hold in crystal structures of primary and secondary minerals and refractory organic compounds which are not easily decomposable. These metals are not released into solution over a reasonable time span under natural conditions. Since these were also not released during the previous extraction steps of sequential extraction procedure they are termed as the residual (RES) fraction.

Sequential extraction was carried out to gain information on the distribution of zinc in natural and treated soil samples, and to see from which fraction zinc has been removed by humic acid, EDTA and DTPA during extraction experiments. Since zinc removal by these chelating agents was performed on soils treated with 10 mg/L zinc, sequential extraction experiments were also performed on soils treated with zinc at the same degree. To evaluate the accuracy of the sequential extraction procedure, the sum of each step was compared with the total zinc contents of soil (Lee and Touray, 1998). Generally, the sum of extraction steps was above total zinc contents, despite the corrections made for zinc contents of each extractant. The total of percentage zinc distributed to each fraction were mostly 106 ± 4 % of the total zinc contents. Much higher total distributed values were reported by Barona *et al.* (2001) who stated that the differences between the sum of each extraction step and total zinc content in soils were not greater than 17 %. Schramel *et al.* (2000), on the other hand, found the copper recovery rate of the whole sequential extraction procedure in relation to the total copper amounts determined by aqua regia digestion between 101 and 111 % indicating that for most sequential extraction procedures higher recovery rates were found for most metals. The total zinc contents of natural greenhouse soils were found to be 55.5 mg/kg, 82.5 mg/kg and 178.5 mg/kg for Fethiye, Samsun and Izmir soils, respectively.

4.5.1. Distribution of Zinc in Natural and Treated Greenhouse Soils

The distribution of zinc in natural (untreated) and treated greenhouse soils was assessed. Since the distribution of zinc in soils can change depending on the zinc input, sequential extraction experiments were carried out for soils having both high and low zinc inputs. The effect of aging and the reaction time of zinc with soil on the distribution of zinc were also interpreted. Changes in the distribution of zinc in soils treated with humic acid, EDTA and DTPA were finally determined.

4.5.1.1. Distribution of Zinc in Natural Greenhouse Soils and in Greenhouse Soils Having Low Zinc Input: Sequential extraction experiments were performed on natural (untreated) and treated greenhouse soils. By determining the distribution of the background zinc, it was possible to examine to which fractions zinc was adsorbed after treatment with 10 mg/L zinc solution. The results of both sequential extraction experiments were presented as mg Zn/kg soil in Figure 4.18-4.20.

As can be seen from Figure 4.18, the background zinc of Fethiye Soil was mainly present as the RES fraction and little more in the Fe/MnOx fraction. Zinc was not distributed to the EXC and CAR fractions and only to a negligible degree to the ORG fraction. Distribution of zinc after treatment with 10 mg/L mainly occurred to the Fe/MnOx fraction, increasing from about 35 mg/kg to 197 mg/kg, and to a lesser extent to the CAR and EXC fractions, approaching to 76 mg/kg and 48 mg/kg, respectively. Increase in zinc distributed to the RES fraction was not observed, however the amount of zinc bound to the ORG fraction increased from 3.6 to 6.4 mg/kg, even only composing a very small part of soil.

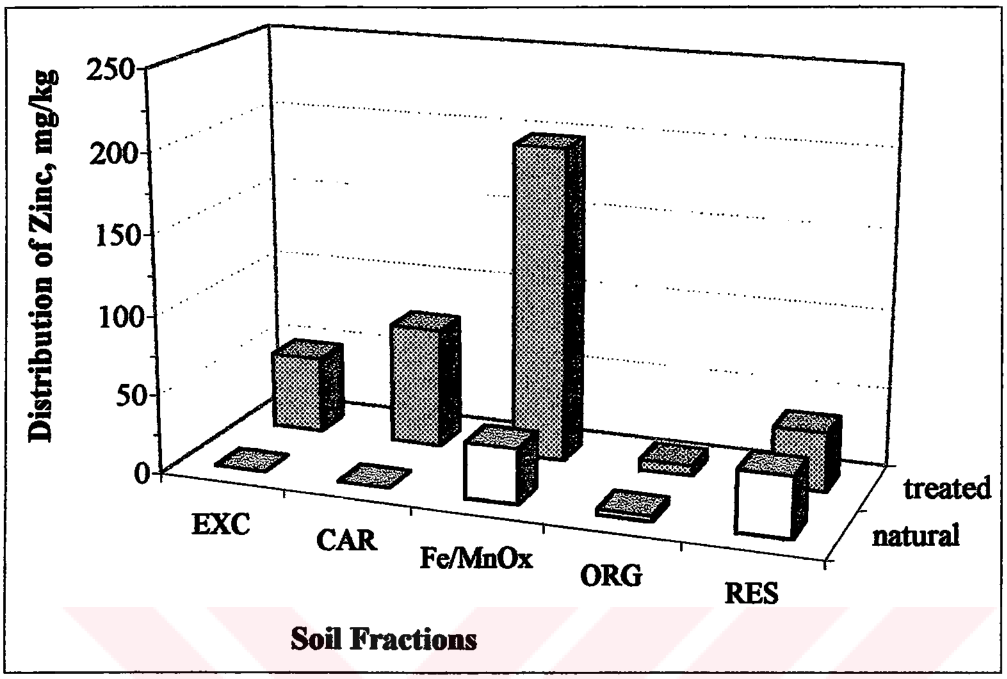


Figure 4.18. Distribution of zinc in natural and treated Fethiye Soil.

The background zinc in the Samsun Soil was mainly associated with the Fe/MnOx fraction and to a lesser extent with the RES fraction (Figure 4.19). Zinc in Samsun Soil was also bound to the CAR and ORG fractions at much lower amounts in comparison to the Fe/MnOx and RES fractions. The distribution of zinc in treated soils was mainly to the CAR fraction, increasing from 5 mg/kg to 184 mg/kg, and to the Fe/MnOx fraction, increasing from about 40 mg/kg to 123 mg/kg. After treatment, the EXC and ORG fractions increased to 21 mg/kg and 8 mg/kg, respectively. Any increase in the RES fraction was not observed.

On the other hand, in the natural Izmir Soil (Figure 4.20), zinc was mainly present in the Fe/MnOx fraction, followed by the RES fraction. Zinc was also bound at much lesser amounts to the CAR and ORG fractions. After treatment with 10 mg/L zinc, the Fe/MnOx fraction continued to dominate by increasing to 222 mg/kg zinc. In comparison, the CAR fraction increased to 154 mg/kg and the EXC fraction to 43 mg/kg. Only a slight increase was observed in the RES fraction, whereas the change in ORG fraction was insignificant. Among soils, the highest amounts of zinc fractionated to the soil components were observed for the Izmir Soil, which was mainly related to its high background zinc content (three times more than that of Fethiye Soil and two times more than that of Samsun

Soil). Thus, despite its low carbonate content, the amount of zinc associated with the carbonates was more than even that of Samsun Soil, which actually has the highest carbonate content.

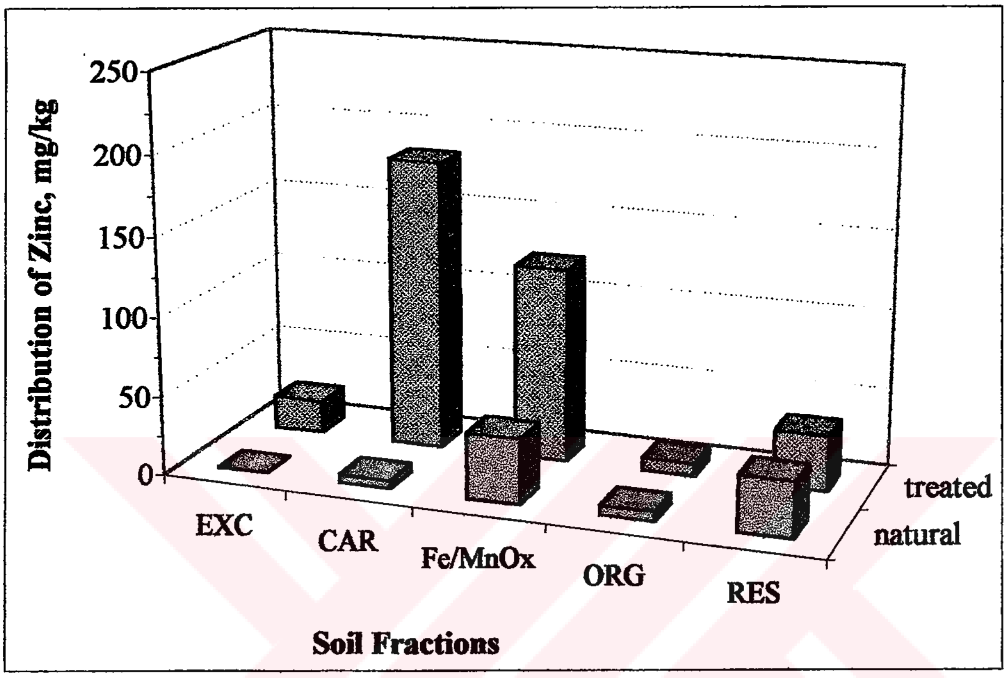


Figure 4.19. Distribution of zinc in natural and treated Samsun Soil.

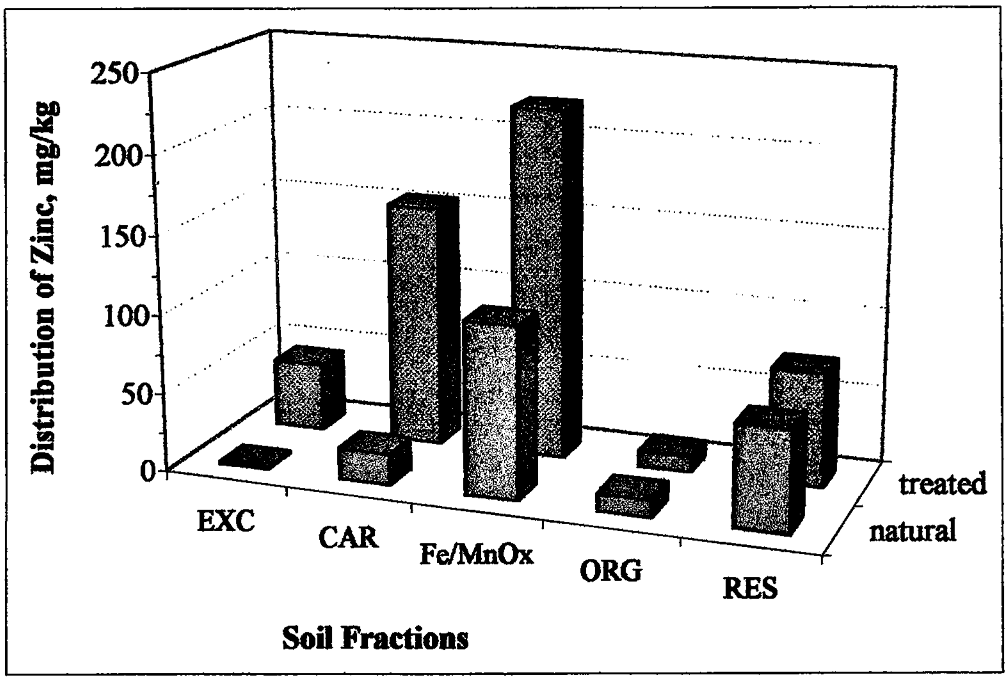


Figure 4.20. Distribution of zinc in natural and treated Izmir Soil.

As a general view, sequential extraction of zinc in natural soils revealed that zinc was mainly bound to the Fe/MnOx and RES fractions of all soils. Li and Shuman (1996b) who used a different sequential procedure developed by Shuman (1985), found similar results for soils with different properties. It was concluded that when compared to natural soils, the metal-amended soils usually had a larger proportion in the exchangeable, organic and manganese oxide fractions, similar or less proportion in the iron oxide fractions and smaller proportion in the residual fraction. Results of Morera *et al.* (2001), on the other hand, presented that zinc was dominantly bound to the RES fraction of natural soils and lesser to Fe/MnOx fractions.

Contribution of each fraction to zinc adsorption and the influence of different carbonate, iron and manganese oxide, organic matter and clay contents on variation in zinc fractionation among different soils were more apparent in treated soil samples. In Figure 4.21, zinc distributed to each fraction is represented as percentage of total zinc (mg Zn/kg soil associated with each fraction divided by the total mg Zn/kg soil), which changes according to the background zinc contents. Comparison of sequential extraction results as percentage values gives a better insight to the distribution of zinc to the soil constituents in relative to each other. So, sometimes the amount of zinc as mg/kg might be higher for specific soils but when the total bound zinc is considered this amount might reveal lower percentage values, as for the Fe/MnOx fraction of Izmir Soil; 221 mg/kg resulted in a value of only 48 %, whereas for Fethiye Soil 197 mg/kg resulted in a value of 69 %. Thus, the difference between percentage values and mg/kg values should be well recognized.

From Figure 4.21 it can be seen that the distribution profile of zinc in Fethiye and Izmir soils is similar; in both soils zinc was preferentially adsorbed by iron and manganese oxides (> 48 %) and to a lesser extent bound to the exchangeable sites (< 15 %) and carbonates (< 34 %) of Fethiye and Izmir soils, indicating that the iron and manganese oxide contents of soils are relatively higher and/or that zinc is preferentially adsorbed by the iron and manganese oxides rather than complexed with the carbonates. Among soils, Fethiye Soil has the highest Fe_2O_3 content (Table 4.4), thus representing higher values in the Fe/MnOx fraction. Similar results were also obtained by Fanfani *et al.* (1997) who reported the distribution of zinc, as percentage of total amount, to be 52 %, 24 % and 20 %

for the Fe/MnOx, ORG and CAR fractions, respectively. Ma and Rao (1997) stated that the partitioning of zinc to iron and manganese oxides might be partially due to the high stability constants of zinc oxides, which was also supported by Ramos *et al.* (1994). For Samsun Soil the reverse situation was obtained; its relatively high carbonate content and relatively low Fe_2O_3 content resulted in higher amounts of zinc association with carbonates (~ 51 %), thus exceeding the Fe/MnOx fraction (~ 34 %). The influence of low Fe_2O_3 content can be better recognized when the results are compared with the results of Theodoratos et al (1997), who carried out the same sequential extraction procedure on calcareous soils (18 % CO_3) and found the zinc distribution as Fe/MnOx fraction (53.9 %) > CAR fraction (29.5 %) > RES fraction (10.7 %) > ORG fraction (5.8 %) > EXC fraction (0.1 %). When only its high carbonate content is taken into account domination of zinc in CAR fraction could be expected, however the dominating fraction was Fe/MnOx fraction. The reason of this was the possible high Fe_2O_3 content coated on the high silt and clay fraction (75.6 %), thus depressing the domination of carbonate content. Results of a sequential extraction study of Lee and Tourney (1998) showed that zinc distribution in a polluted artificial roadside soil was mainly in the carbonate and slightly lesser in the Fe/MnOx fraction, which might be related to the relatively high calcite (between 5.75 and 13.08 %) and lower clay contents. It was concluded that carbonates and Fe-hydroxides in roadside soil act as major scavengers of zinc. In a similar study on roadside soils, Norrström and Jack (1998) reported that the oxide-bound fraction was greater than the other fractions in the roadside soil samples (42-77 %), except some soils from deeper layers where the residual fraction were of similar size. Morera *et al.* (2001) reported different zinc distributions for different soils amended with zinc, thus indirectly representing the influence of different carbonate, Fe/MnOx, organic matter and clay contents on zinc distribution. Considering the organic fraction, it can be concluded that zinc was not preferentially bound to soil organic matter, which is already reported by various scientists (McGrath, 1996; Spark *et al.*, 1997; Almås *et al.*, 2000). A recognizable increase in zinc amounts associated with the RES fraction was not observed in all treated soils, resulting in lower percentage values of total zinc contents. This also shows that zinc added to soils did not enter into the crystal lattice of layer silicates through isomorphous substitution as was proposed by Li and Shuman (1996b) but was distributed to the non-residual fractions.

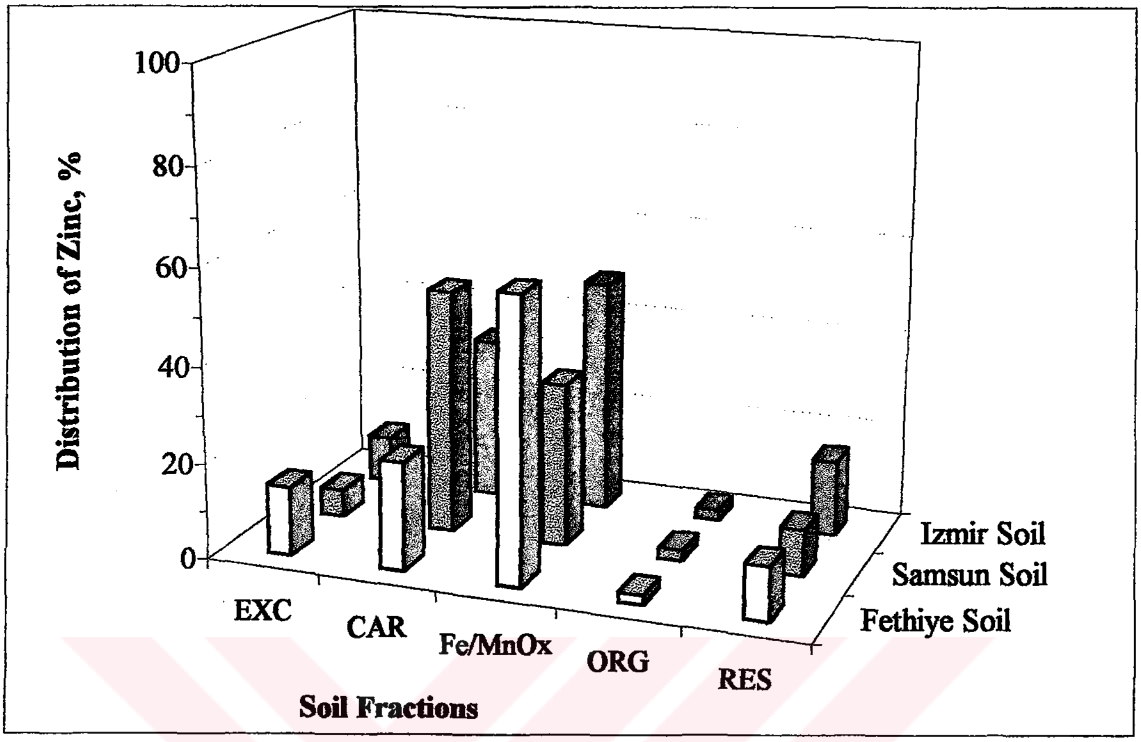


Figure 4.21. Distribution of zinc in greenhouse soils treated with 10 mg/L zinc.

Considering that zinc was not associated with the exchangeable sites in all natural soils, the variation in zinc bound to exchangeable sites of treated soils should be explained by differentiations in type and amount of clays present in the three greenhouse soils (Table 4.3). The clay content of Fethiye Soil and Samsun Soil is almost twice as high as that of Izmir Soil. The effect of amount and type of clay on the distribution of zinc can be distinguished for Fethiye Soil. Its relatively high EXC fraction can be attributed to its high content of clay, which is determined to be present as mainly kaolinite and rarely smectite. Kaolinite represents non-specific interaction with metals, meaning that metals are bound to kaolinite with weaker electrostatic forces. The kaolinite content in Izmir Soil is about one third that of Fethiye Soil. Illite, which is a non-expandable 2:1 type clay, contributes to the EXC fraction. The low EXC fraction of Samsun Soil seems to be related to the presence of low quantities of smectite and the absence of kaolinite. Smectite group clays are expandable 2:1 type clays mainly with high adsorption capacities via specific interactions. It should be kept in mind that exchangeable adsorption does not only occur on clay surfaces, but also on organic components, iron and manganese oxide and other soil constituents. However differentiation in relation to these components could not be assessed in the concept of this study. For Samsun Soil it can be suggested that zinc was more complexed by carbonates than in Fethiye and Izmir soils. This was also approved by the results of soil analysis and X-ray diffractiometry (Table 4.4) representing quite high amounts of calcareous minerals, e.g. calcite and dolomite, in Samsun Soil. An interesting situation was observed for Izmir Soil, because of the high zinc distributed to CAR fraction, despite the low carbonate content determined with soil analysis; calcite and dolomite was even not detected by the X-ray diffractiometer; thus the reason could not be explained under the present conditions. For the distribution of zinc in soils treated with 10 mg/L, the variation in Fe/MnOx fraction can be well explained by the Fe_2O_3 contents of soils. Fethiye Soil, which has the highest Fe_2O_3 content (10.2 %), represented a higher value for the Fe/MnOx fraction. The Fe/MnOx fraction of Samsun Soil and Izmir Soil were less in relation to their relatively lower Fe_2O_3 contents (6 % and 6.8 %, respectively).

Generally, fractionation of zinc to constituents of soil, like carbonates, iron oxides, organic matter and clay was well related to their amounts present in soil. Li and Shuman (1996a) achieved this for soils taken from different depth of a soil profile and related the variation in zinc distribution to the variation of individual constituents within soil profile. Influence of individual fractions on zinc distribution was quite good represented by calculation made by Lo and Yang (1999). Results showed that zinc in soils with higher carbonate content was more found in the CAR fraction, soils with higher Fe/MnOx content in the Fe/MnOx fraction and soils with quite higher organic content in the ORG fraction.

<u>4.5.1.2. Distribution of Zinc in Aged Greenhouse Soils:</u> After the introduction of heavy metals to the soil medium, metals are initially associated with various soil constituents, which are afterwards followed by a long-term process of redistribution. Since the system tends to return to the chemical equilibrium within time, transformation of metals from one fraction to another fraction is likely to occur.

In order to see if any transformation of zinc in treated greenhouse soils proceeds within time, sequential extraction experiments were conducted on samples, which were treated in the same way with 10 mg/L zinc and were aged in the refrigerator for two month. When the distribution of zinc in aged samples, presented in Figure 4.22, are compared with the normal zinc distributions in Figure 4.21, it can be stated that only slight changes were observed in each fraction, from which most of them were accepted to be insignificant. The reason of this might be the reduced transformations of zinc in relation to the short time period, especially when considering that processes within soil medium proceed over a very long time. As a general view, it can be stated that zinc was mostly transferred from the Fe/MnOx fraction of both Fethiye and Izmir soils to mainly the EXC fraction. Zinc was also redistributed to the CAR and ORG fractions, but at much lower degrees. Considering the Fe/MnOx fraction, it was stated that the lowered redox potential in soil, occurring within time, caused to the reduction of iron and manganese from their oxides consequently resulting in the redistribution of heavy metals among the solid-phase components (Han and Banin, 1999). Differently, redistribution of zinc in Samsun Soil was not recognizable, except for the EXC fraction. Generally, for all soils, translocation of the zinc to the most stabile RES fraction did not occur within two months. Transformation from the soluble fraction (CAR and Fe/MnOx fractions) to the EXC fraction was reported for cadmium in a sandy soil by Mann and Ritchie (1994). Many other transformations were reported to occur from mainly the EXC fraction to fractions like ORG, CAR, Fe/MnOx or RES. McGrath and Cegarra (1992), for example found that nickel and zinc in the EXC and NaOH-extractable fractions (mainly organically bound) and cadmium in the EXC fraction decreased and metals in the RES fraction increased. Soon (1994) reported the transformation of zinc from the EXC and ORG fractions to the hydrous-oxide associated fraction. All the different transformations seem to be mostly time dependent, since both examples were presenting the redistribution of heavy metals within a time period of over twenty years. Han and Banin (1999) stated that heavy metals are slowly transferred to the soil components and that the distribution is mainly dependent on the soil properties and the chemical nature of the metal.

4.5.1.3. Distribution of Zinc in Greenhouse Soils Having High Zinc Input:

In order to see if zinc was distributed differently in soils exposed to higher zinc concentrations, sequential extraction experiments were carried out on samples treated with 500 mg/L zinc (Figure 4.23). When compared with the zinc distributions in Figure 4.21, it can be seen that zinc was predominantly bound to the EXC fraction rather than the Fe/MnOx and CAR fractions. In the Fethiye Soil, 71 % of the zinc was associated with the EXC fraction reflecting an increase of 57 %. The increase in zinc concentration from 10 to

500 mg/L did not display a significant change in the CAR fraction, which was found to be about 21 %. On the other hand, a tremendous decrease from 59 % to 14 %, was recorded for the zinc distributed to the Fe/MnOx fraction. The distribution of zinc in Samsun Soil was also found to be different; the amount of zinc adsorbed on the exchangeable sites increased from 6 % to 51 %, whereas zinc complexed with carbonates was reduced from 51 % to 41 %. Zinc associated with the Fe/MnOx fraction was decreased from 34 % to 10 %. Izmir Soil represented a similar distribution pattern to that of Fethiye Soil. Zinc was mainly bound to the EXC fraction, with an additional increase from 9 % to 57 %. The change in the CAR fraction was found to be insignificant, representing a value of 31 %. On the other hand, the zinc in the Fe/MnOx fraction was reduced from 49 % to 15 %. For all soils, the ORG and RES fractions decreased to below 0.6 % and 1.2 %, respectively.

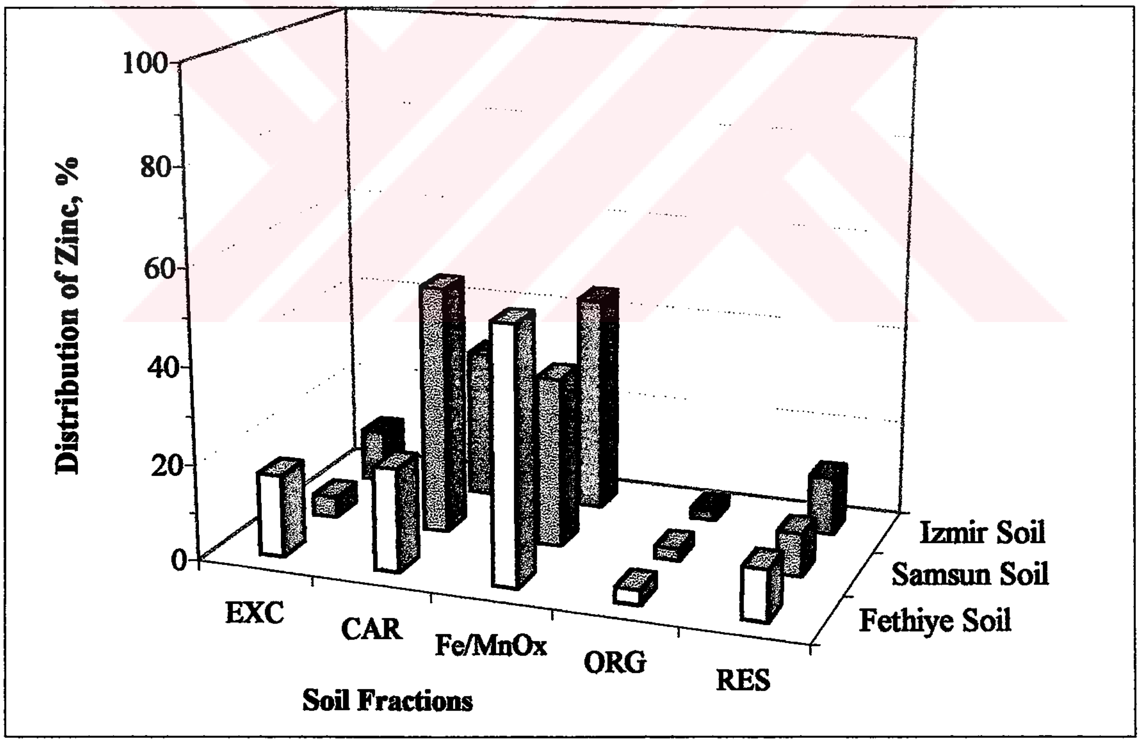


Figure 4.22. Distribution of zinc in aged greenhouse soils treated with 10 mg/L zinc.

Actually the concentration of each fraction increased, except for the RES fraction. Since the amount of zinc retained by the soil increased as well, these increases did not always reflect an elevation in the percentage values, as was the case for some CAR, Fe/MnOx and ORG fractions. For example, the Fethiye and Izmir soils, site-specific co-precipitation or disordered incorporation of zinc to the carbonates occurred almost at the same degree of the increase in total zinc, thus not reflecting any decrease in percentage values. For the Samsun Soil, on the other hand, the increase in zinc complexed by the carbonates occurred at a lower degree, resulting in lower percentage values.

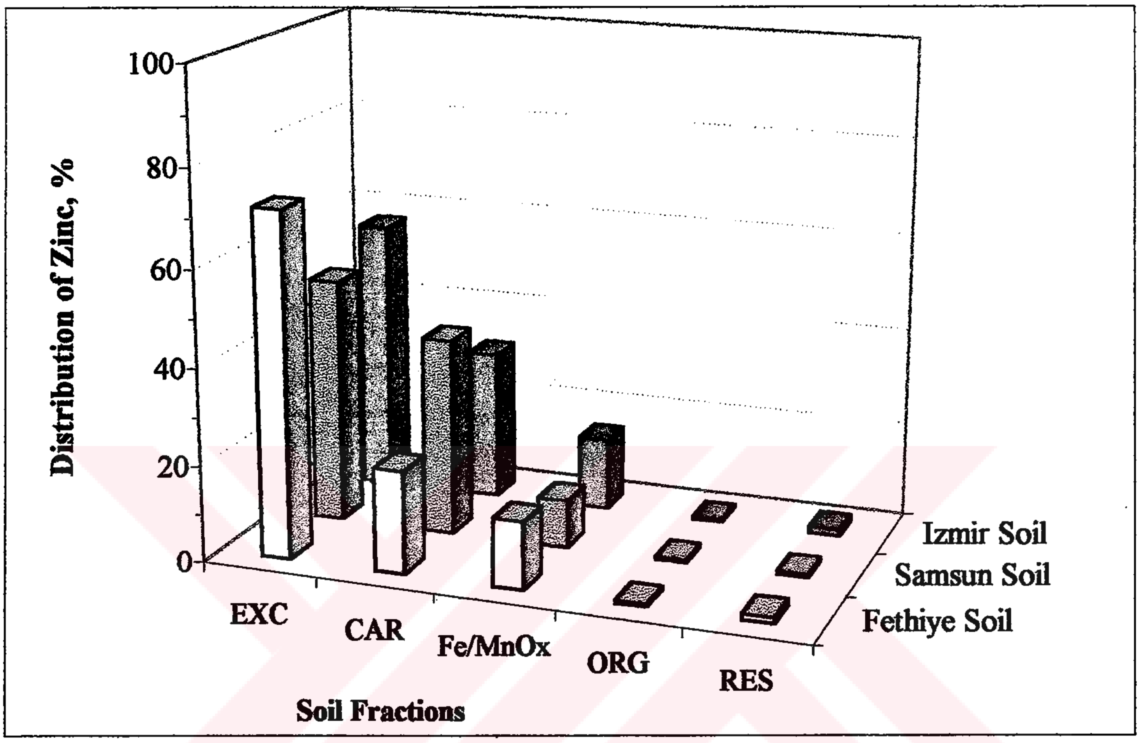


Figure 4.23. Distribution of zinc in greenhouse soils treated with 500 mg/L zinc.

Generally, when both distribution pattern of zinc in greenhouse soils treated with two different concentrations are considered, it can be stated that the adsorption of zinc by soil constituents changes with the concentration of zinc. The results of sequential extraction experiments indicated that the sites of iron and manganese oxides and carbonates were of primary importance in the adsorption of zinc at lower concentrations. However, with increasing zinc concentration as the availability of sites with higher affinity, like sites of iron and manganese oxides, decreases, the importance of sites with lower affinity increases. Thus, with increasing zinc concentration, the distribution of zinc was mainly to the exchangeable sites, which are sites with lower affinity. Adsorption to these sites occurs via electrostatic interactions.

These results are in consistency with the findings derived from the adsorption isotherms of greenhouse soils (Section 4.2.). As previously mentioned, for both Freundlich and Langmuir equations, the data were resolved into two parts, indicating to the presence of more than two adsorption sites participating in the adsorption of zinc in soils. It was concluded that high affinity sites with higher bonding energies are occupied at lower concentrations, whereas at higher concentrations (Part 2) sites of lower affinity are also consumed. Sequential extraction experiments verified these findings by presenting the participation of at least two adsorption sites in the adsorption of zinc; sites available in iron and manganese oxides and calcareous minerals, the higher affinity sites wither higher binding energies, are predominantly occupied at lower metal concentrations, and the exchangeable site, the lower affinity sites with lower binding energies, at elevated concentrations.

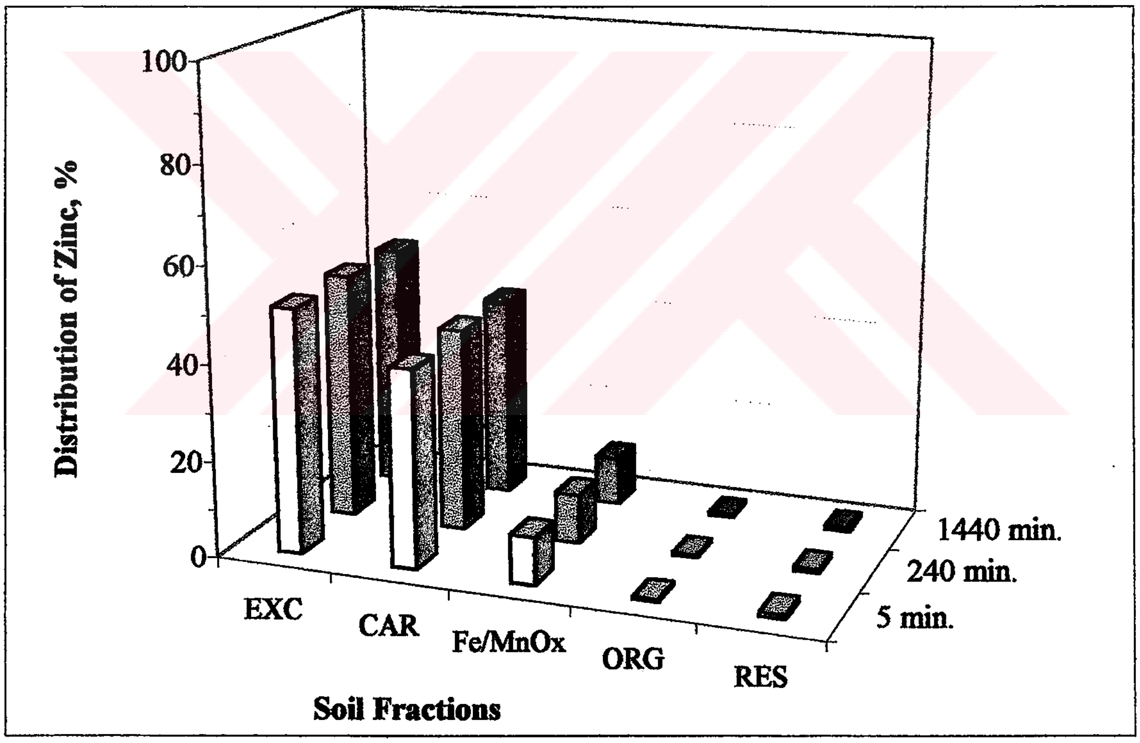


Figure 4.24. Distribution of zinc in Samsun Soil treated with 500 mg/L zinc solution for different times.

In order to see if adsorption of zinc to individual sites (soil fractions) changes with respect to reaction time, additional experiments were performed on samples of Samsun Soil treated with 500 mg/L zinc solution for 5, 240 and 1440 min. Samsun Soil was selected since its retention capacity is the highest among soils and at elevated zinc concentration adsorption still continues with a decreasing rate. As observed in the kinetic study of 500 mg/L treatments, the percentage of zinc adsorbed increased from about 40 % to about 50 % in 240 min and to about 54 % in 1440 min. Results in Figure 4.24 display that the distribution of zinc to a particular fraction was not changing with reaction time. Despite the increasing amounts of extracted zinc with time, differences in partitioning to especially the exchangeable sites was not recognizable. Thus, binding of zinc to exchangeable sites occurred simultaneously with the iron and manganese oxides and carbonates, but with different rates.

4.5.2. Distribution of Zinc in Greenhouse Soils After Treatment with Chelating Agents

Sequential extraction experiments were also performed after zinc removal with humic acid (HA), EDTA and DTPA to see from which fraction zinc was preferentially taken. Results in Figure 4.25-4.27, displaying the per cent distribution of zinc, show the differences between the distribution of zinc in soils treated with 10 mg/L zinc (bars without pattern) and those after treatment with humic acid, EDTA and DTPA (bars with pattern). The abbreviations F, S and I in legends represent the Fethiye, Samsun and Izmir soils, respectively.

4.5.2.1. Distribution of Zinc in Greenhouse Soils After Treatment with Humic Acid:

The removal of zinc from greenhouse soils by treatments with 300 mg/L humic acid was in accordance with the efficiencies previously presented in Section 4.3.1.2. However, when the distribution pattern of zinc in greenhouse soils after treatment with humic acid are compared with those without humic acid treatments (Figure 4.25), it can be recognized that the total of reductions in % distributed zinc values observed for each fraction (Table 4.17) were slightly higher than the removal efficiencies achieved with 300 mg/L humic acid (5.3 %, 5.9 % and 6.5 % for Fethiye, Samsun and Izmir soils, respectively).

In general, most of the zinc was removed from the exchangeable fraction and to a lesser extent from the Fe/MnOx and RES fractions, indicating that weaker electrostatic interactions were easier overwhelmed by the ligand exchange reactions than the stronger specific interactions in the two other fractions. Zinc complexed as carbonates was not easily taken by humic acid, since the suspension pH (~7.5) was not favourable for the dissolution of carbonates. Removal from the ORG fraction did not occur, signifying that zinc was stronger bound to soil organic matter rather than the soluble humic acid. Based on the study on the influence of added humic substances on plant available heavy metals, Piccolo (1989) stated that native soil organic matter exerts a higher metal retention activity than the freshly added and highly reactive humic acid. It was concluded that this is most probably due to a stereochemical arrangement that humic substances assume when in contact with other soil constituents (e.g. clay minerals). This must create complexing sites, which have stronger affinity for metals in comparison to the functional groups of added humic acid alone.

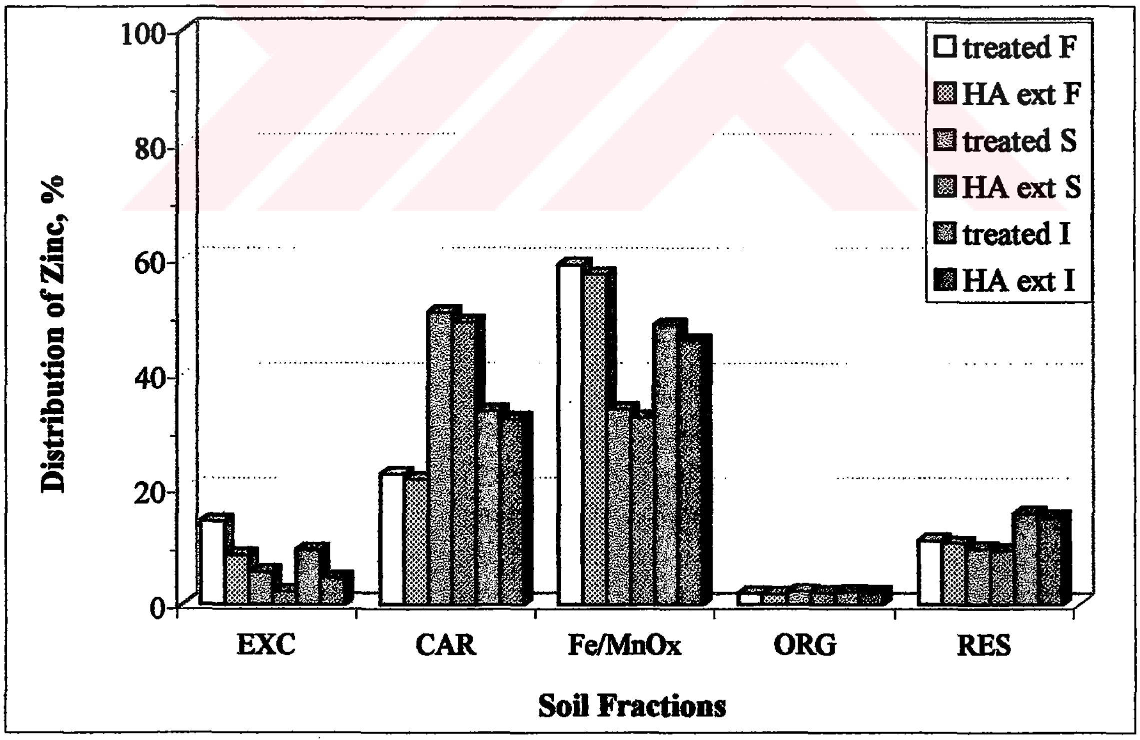


Figure 4.25. Differences in the distribution of zinc in greenhouse soils after treatment with humic acid.

The reductions in each fraction resulting from the removal of zinc by humic acid can be seen in more detail in Table 4.17. The highest reduction in EXC fraction was observed for Fethiye Soil, which can be related to its high EXC fraction in comparison to the other soils. In the CAR fraction the highest reduction was obtained for Samsun Soil, which has a high carbonate content. Remembering that the Fe/MnOx fraction of Izmir Soil was actually higher in mg/kg amount than in percentage values in comparison to the Fe/MnOx fractions of Fethiye and Samsun soils, the highest reduction in Fe/MnOx fraction of Izmir Soil seems to be logical. Reductions in the ORG and RES fractions seem to be insignificant for all soils.

Table 4.17. Reductions in each fraction resulting from the removal of zinc by humic acid

Fractions, %	EXC	CAR	Fe/MnOx	ORG	RES
Fethiye Soil	5.77	0.90	1.70	0.07	0.5
Samsun Soil	3.46	1.64	1.50	0.23	0.4
Izmir Soil	4.79	1.40	2.79	0.06	0.6

In general, the total of percentage reductions were highest for the Izmir Soil and lowest for the Fethiye Soil, which is in accordance with the observed removal efficiencies of soils.

As explained in Section 4.3.1.2, the low removal efficiencies were attributed to adsorption of humic acid molecules either directly or as humic acid zinc complexes (bridged with divalent cations) on the mineral surface. However, since the distribution of zinc in soils after treatment with humic acid did not reveal a transfer from one fraction to other fractions, especially to the Fe/MnOx fraction, the subsequent adsorption of humic acid zinc complexes, after extraction with humic acid, on the Fe/MnOx and RES fractions (possible fractions favoured by adsorption mechanism of humic acid) seems not to be negligible. Thus, it can be concluded that removal of zinc by humic acid was mainly inhibited by its direct adsorption on soil mineral surfaces.

4.5.2.2. Distribution of Zinc in Greenhouse Soils After Treatment with EDTA:

Zinc removal by EDTA was performed with a quite low concentration of 0.0001 M to ensure that zinc concentration of each sequential extraction step was in a detectable range. The removal of zinc by EDTA was lower for the sequential extraction experiments than for the single extraction experiments presented in Section 4.3.2.2. This might be attributed to the changes in shaking device (Rotator was used instead of a horizontal shaker) and experimental procedure (only 1 centrifuge tube was used), since the order of removal efficiency was the same as in the single extractions (Izmir Soil > Fethiye Soil > Samsun Soil for 0.0001 M EDTA). Zinc removal efficiencies decreased to 27 %, 13 % and 29 % for Fethiye, Samsun and Izmir soils, respectively.

In contrast to the results of sequential extraction experiments of humic acid treated soils, results of sequential extraction experiments of EDTA treated soils show that zinc was mostly removed from the CAR fraction of all soils (Figure 4.26). Since the initial pH of soil suspensions were all above 6.2, it can be suggested that the affinity of EDTA towards carbonates is dominating rather than the dissolution of carbonates. Zinc was also removed quite efficiently from the Fe/MnOx fraction, which might be due to the high affinity of EDTA towards Fe^{3+}, resulting in more interactions with the Fe/MnOx fraction. Ability of EDTA to extract zinc bound to Fe/MnOx fraction was also mentioned by Lo and Yang (1999), however no explanation was stated. The quite low removal from the EXC fraction suggests that weaker electrostatic interactions are not that favourable by EDTA, but that special affinities towards Ca^{2+} and Fe^{3+} ions are more effective parameters to be considered. Papassiopi *et al.* (1999) and Ghestem and Bermond (1998) also mentioned the affinity of EDTA for Ca^{2+} and Fe^{3+}. Removal from the RES and ORG fractions only occurred for Izmir Soil, which might be related to the high removal efficiency of EDTA for the Izmir Soil, which was due to the higher percentage of EDTA involved in the extractions (Section 4.3.2.2). According to Barona *et al.* (2001), EDTA is capable of extracting virtually all the metals in the EXC, CAR and ORG fractions. It was concluded that the distribution of zinc before and after EDTA extraction was likewise found to remain similar. In this study, a slight domination in percentage reduction was found in the CAR fraction, but this did not cause a great change in the distribution of zinc within fractions.

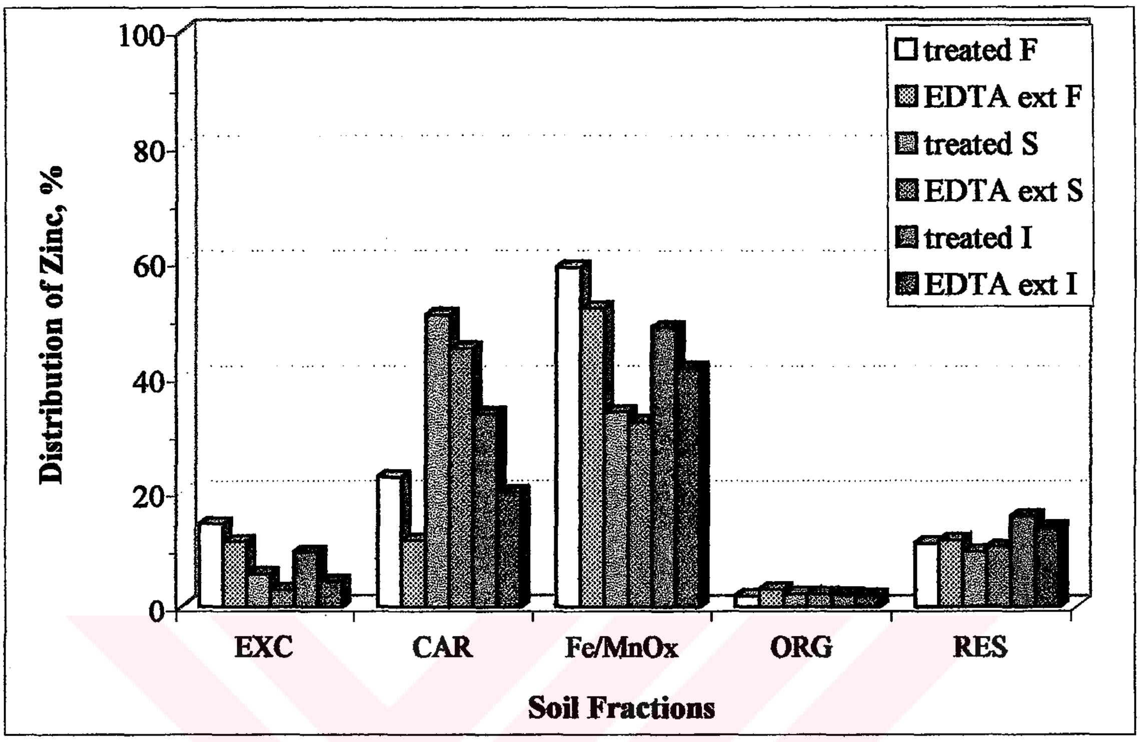


Figure 4.26. Differences in the distribution of zinc in greenhouse soils after treatment with EDTA.

In consistency with the removal efficiencies found for each soil, the highest reductions for each fraction were observed for Izmir Soil, which directly related to its higher zinc removal efficiency (Table 4.18). For the EDTA treatments with a concentration of 0.0001 M, the lowest zinc removal was observed for the Samsun Soil. The reason of this was attributed in Section 4.3.2.2 to the consumption of EDTA by carbonates during extraction.

Table 4.18. Reductions in each fraction resulting from the removal of zinc by EDTA

Fractions, %	EXC	CAR	Fe/MnOx	ORG	RES
Fethiye Soil	3.04	10.95	7.06	-	-
Samsun Soil	2.75	5.81	1.67	-	-
Izmir Soil	5.01	13.62	7.09	0.17	2.01

4.5.2.3. Distribution of Zinc in Greenhouse Soils After Treatment with DTPA:

Due to the same reasons stated for the EDTA experiments, zinc removal by DTPA was accomplished with the same concentration. Again, zinc removal efficiencies encountered by DTPA were lower for the sequential extraction experiments than for the single extraction experiments; they were between 33 % and 40 %.

Similar to the results of sequential extraction of EDTA treated soils, zinc was mostly removed from the CAR fraction, followed by the Fe/MnOx fraction (Figure 4.27). Similarly, this can be explained by its higher affinity towards Ca^{2+} and Fe^{3+} ions, since DTPA is a similar chelating agent. Again, removal from the EXC fraction was quite lower in comparison to the CAR and Fe/MnOx fractions, supporting the explanation made for EDTA treated soils. Removal from the residual fraction occurred only for Izmir Soil, as can be seen in Table 4.19. In contrast to EDTA, DTPA also removed zinc to a minor degree from the ORG fraction of all three soils, showing some additional affinity to soil organic matter, which was also visually observed in the DTPA extraction experiments. The interaction of DTPA with soil organic matter has been previously reported by Falatah and Sheta (1999). Száková *et al.* (2000) concluded that DTPA is able to release a part of the element fraction bound in Fe/MnOx and organic matter of soil sample. The ability of DTPA to extract the fraction of metals complexed by organic matter was also stated by Ure (1996), Piccolo (1989) and McGrath (1996).

Besides their similar extraction behaviour, it can be concluded from Table 4.18 and Table 4.19, that the reduction in each fraction was higher for the DTPA treated soils. Since DTPA is a stronger chelating agent than EDTA, this was something expected. As can be seen from Table 4.19, the highest reduction in the EXC fraction was again observed for Izmir Soil. However, for the Fe/MnOx fraction the highest reduction was obtained for Fethiye Soil rather than for Izmir Soil. Actually, in soil samples extracted by EDTA the persentage reduction in the Fe/MnOx fraction was equally for Fethiye and Izmir soils (Table 4.18); thus it seems quite logical that an additional reduction in the Fe/MnOx fraction caused by DTPA has occurred in the fraction of Fethiye Soil. The reduction observed for the carbonate of Samsun Soil was the highest value, which could not be explained by the effect of pH. For the RES and ORG fractions the highest reductions were accomplished for Izmir Soil.

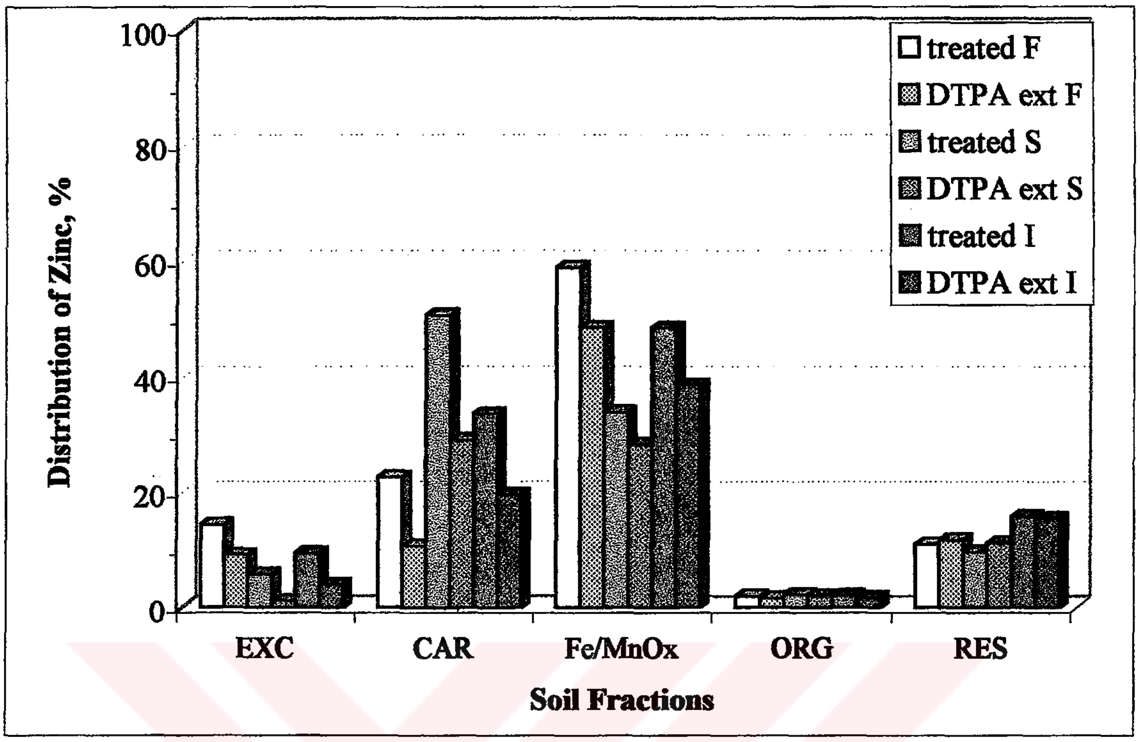


Figure 4.27. Differences in the distribution of zinc in greenhouse soils after treatment with EDTA.

Table 4.19. Reductions in each fraction resulting from the removal of zinc by DTPA

Fractions, %	EXC	CAR	Fe/MnOx	ORG	RES
Fethiye Soil	5.26	11.9	10.43	0.24	-
Samsun Soil	4.57	21.66	4.76	0.19	-
Izmir Soil	5.44	14	9.87	0.37	0.34

In general, the total of percentage reductions were highest for the Samsun Soil and lowest for the Fethiye Soil, which is in consistence with the zinc removal efficiencies.

4.5.3. Comparison of Sequential Extraction Experiments

Below distributions of zinc in the greenhouse soils treated with 10 mg/L (treated), in the greenhouse soils after treatment with humic acid (HA ext), EDTA (EDTA ext), DTPA (DTPA ext) are represented in the same figure to enable a better comparison. For all greenhouse soils it can be seen that the distribution pattern of zinc before and after treatment with humic acid, EDTA, DTPA remained similar.

From Figure 4.28, it can be seen that zinc in Fethiye Soil continued to dominate mainly in the Fe/MnOx fraction. Zinc bound to the CAR fraction decreased to almost half of the % values of soils after treatment with EDTA and DTPA. The highest reduction in EXC fractions was observed for the humic acid treated soils (exceeding even that of DTPA treated soils), thus representing that removal from EXC fraction was favoured by humic acid.

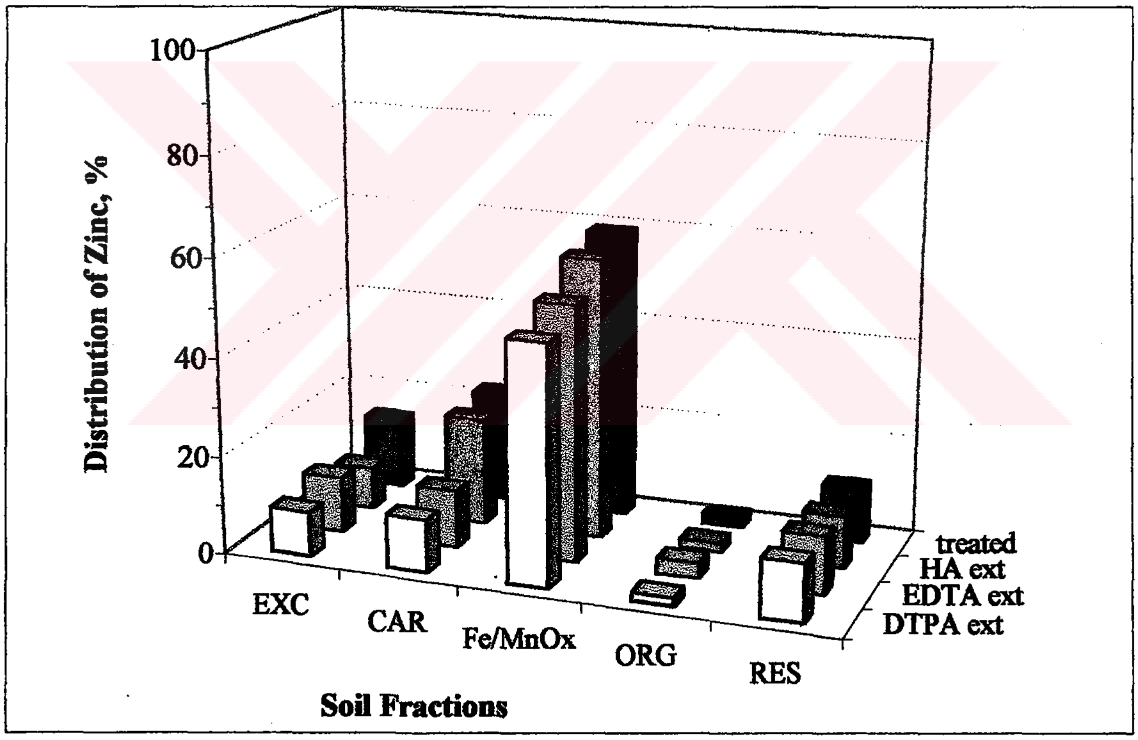


Figure 4.28. Comparison of zinc distribution in Fethiye Soil before and after treatment with chelating agents.

On the other hand, for Samsun Soil zinc continued to dominate in the CAR and Fe/MnOx fractions (Figure 4.29). The highest reductions in both fractions were observed for soils treated with DTPA, which was also recognizable for the EXC fraction of Samsun Soil. The reduction in the EXC fraction resulting from the treatment with humic acid can especially be recognized, when compared with the results of EDTA treatments.

Differences occurring in ORG fraction by treatments with humic acid, EDTA and DTPA were found to be insignificant.

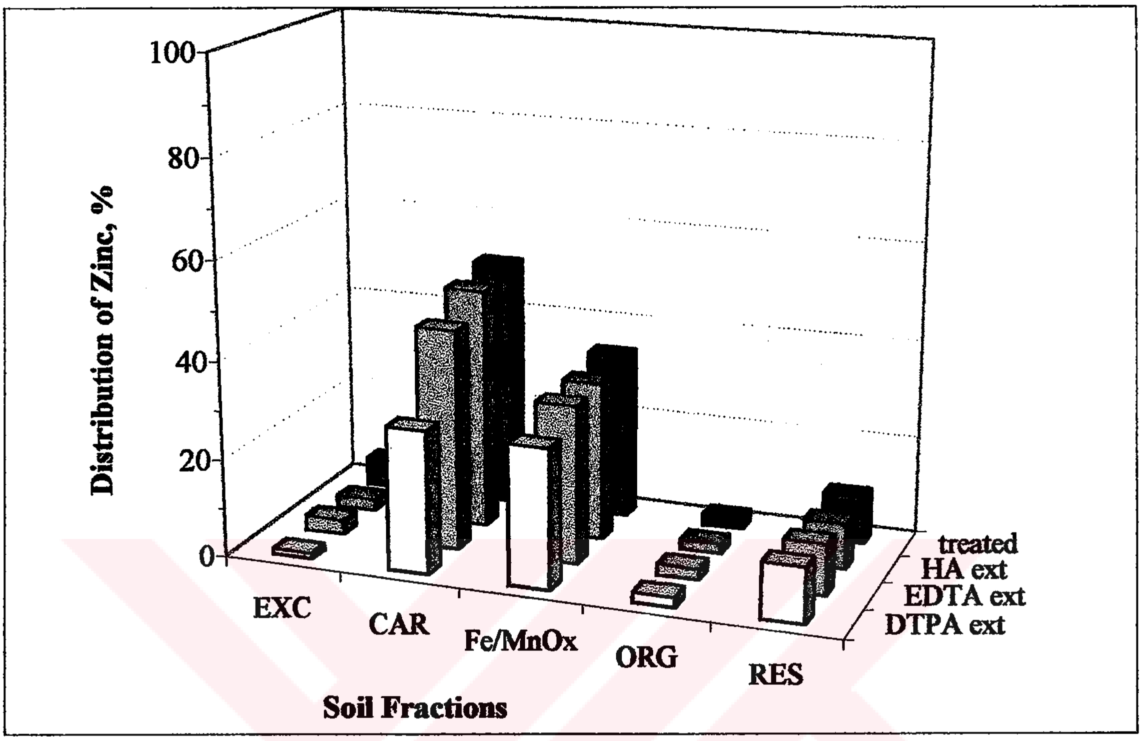


Figure 4.29. Comparison of zinc distribution in Samsun Soil before and after treatment with chelating agents.

Similar to Fethiye Soil, zinc continued to dominate in the Fe/MnOx fraction, followed by the CAR fraction. The highest reduction in Fe/MnOx fraction occurred for DTPA treated soils, in the CAR fraction however for both EDTA and DTPA treated soils. From Figure 4.30, it can be recognized that the highest percentage zinc reduction in the EXC fraction was achieved by DTPA and not humic acid as was observed for Fethiye Soil.

Generally, for all soils it can be seen that the reduction in zinc distribution was lowest for the humic acid and highest for the DTPA treated soil samples. The different removal efficiencies observed for humic acid, EDTA and DTPA could be attributed to their chelating power. As explained before, EDTA and DTPA are both aminopoly-carboxylic acids, having different number of coordinating groups. Since DTPA has three amine nitrogen and five carboxylic groups, one amine nitrogen and one carboxylic group

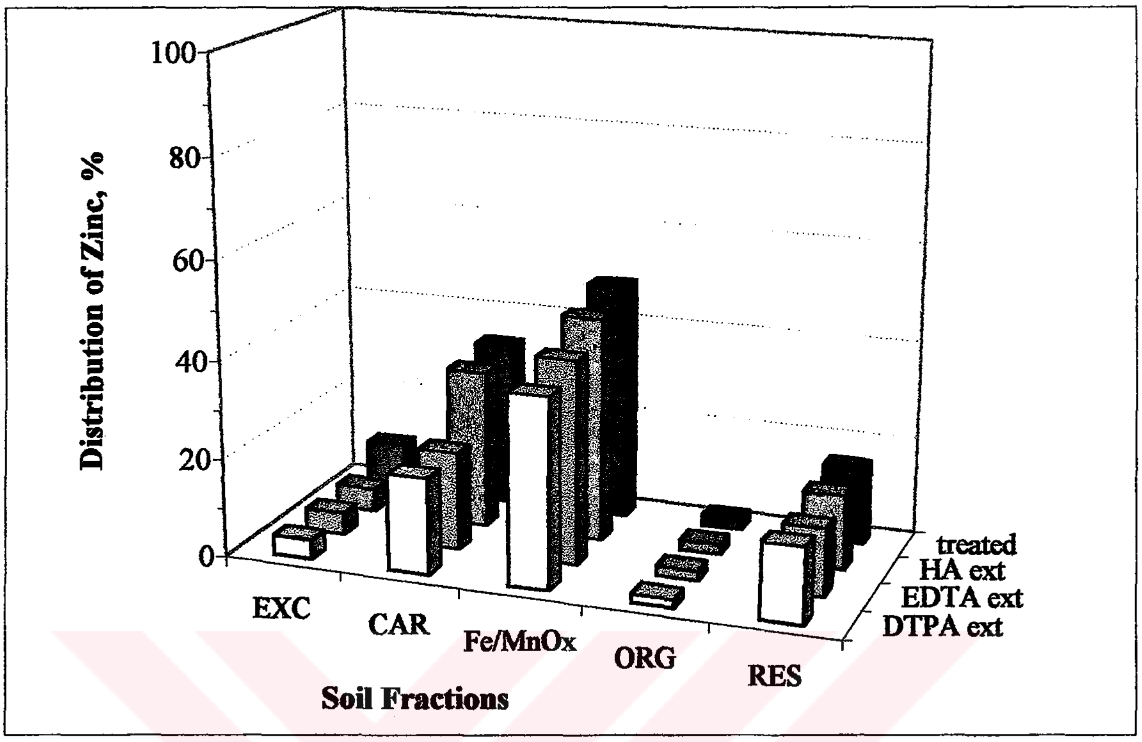


Figure 4.30. Comparison of zinc distribution in Izmir Soil before and after treatment with chelating agents.

more than EDTA, it is a stronger complexing agent than EDTA; thus, representing higher affinity for zinc. Humic acid, on the other hand, has also many functional groups, mainly carboxylic and phenolic groups and therefore possesses the ability to complex with positively charged multivalent ions like zinc. However, despite the high number of functional groups present, the affinity of humic acid towards metals is less than EDTA and DTPA. The measure of affinity of a chelating agent for a specific metal ion is the logarithmic value of equilibrium constant K. Metal-chelate complexes which are more stable have higher stability constants (log K). Stability constants indicate also indirectly to the power of the complexing agent. Stability constants of EDTA, DTPA and humic acid are 16.50, 18.10 (Evangelou, 1998) and 6.80, respectively. Considering their stability constants, it can be concluded that the lower removal efficiencies of zinc from all greenhouse soils by humic acid was due to its low complexing capability for zinc than EDTA and especially DTPA.

5. CONCLUSION

The interaction of heavy metals with soil and consequently their fate in the environment, which mainly depends on both the properties of soil and the contaminant, has received increasing interest. In this study, the interaction of zinc, a chemically stable metal showing only a single valence, with three different greenhouse soils, artificially contaminated to resemble soils contaminated by agricultural practices, was examined and its removal with alternative methods was evaluated.

Three greenhouse soils, Fethiye, Samsun and Izmir soils, were taken from three different regions of Turkey; namely the Mediterranean region, Black Sea region and Aegean region, respectively. The characterization of soils revealed that Fethiye Soil is a soil with relatively high clay (mainly kaolinite) and iron oxide and low organic matter and carbonate contents, representing a low CEC. Samsun Soil was identified as a soil with high clay (smectite), carbonate and organic matter and low iron oxide contents, representing the highest CEC. Fethiye Soil, on the other hand, is a soil with high organic matter and low clay (mainly illite), iron oxide and carbonate contents, representing a moderate CEC. All greenhouse soils were classified as sandy loam with neutral to very slightly alkaline pH.

The adsorption behaviour of zinc was presented by interpreting the adsorption process with isotherms kinetic studies and sequential fractionation. The adsorption of zinc in all greenhouse soils exhibited L-type isotherm with a high initial slope decreasing with increasing zinc concentration. The adsorption data were fitted into the Freundlich and Langmuir equations. For both equations the data were better explained when the data were resolved into two linear parts, differing in slopes. The K_F values, calculated for the overall data or for both parts, increased for soils with higher pH and CEC. A direct relation of the K_F values with the clay and organic matter content could not be established indicating to the importance of especially clay type. In general, the K_F value was highest for Samsun and lowest for Fethiye soils. 1/n values were found to be less than unity (< 1), reflecting the decreasing affinity of surfaces for zinc. The K_L value was found to be higher for the low concentration portion (Part 1) than for the high concentration portion (Part 2), suggesting to the presence of two different adsorption sites, of which the high affinity sites with high binding energies were occupied at lower concentrations and the lower affinity sites with low binding energies were additionally consumed at higher concentrations. The M values, the adsorption maxima, which were higher for the second part, were in consistence with the highest q_A value of isotherms. Kinetic studies, on the other hand, revealed that the initial adsorption of zinc was rapid especially for low concentrations. Adsorption of high zinc concentration exhibited a long-time tail denoting the declining trend in addition to the initial rapid zinc uptake.

The removal of zinc from greenhouse soils was investigated with two different groups of chelating agents, naturally occurring humic and fulvic acids and artificially produced EDTA and DTPA. Experiments conducted for the removal of zinc from greenhouse soils with humic and fulvic solutions did not reveal results which were in accordance with the previously published statements indicating to the solubilisation of heavy metals like zinc, iron etc. from the soil minerals The reason of low removal efficiencies (maximum 8 %) despite of the high humic acid concentration (1000 mg/L) was interpreted by examining the effect of pH on the speciation of functional groups of humic acid. Since for all soils, the pH of soil humic acid suspensions were ranging between 7 and 8 (increasing with increasing humic acid concentration) only carboxylic type functional groups were dissociated and thus involved in the extraction of zinc from soils. Differentiation in the removal efficiencies observed for each soil could not be explained by the deprotonation of functional groups, as they were the same for each soil. Additionally, an experiment, which was conducted at higher pH, enabling the involvement of both carboxylic and phenolic type functional groups, revealed only an additional increase in removal efficiency of about 2 %. Hence, neither the low removal efficiencies nor the variation in removal efficiencies of soils could be related to the soil suspension pH. The adsorption of humic acid on greenhouse soils was evaluated in order to see if the removal of zinc was hindered by the adsorption of humic acid. It was found out that the adsorption of humic acid increased with increasing humic acid concentration, exhibiting C-type isotherm. Humic acid was highest adsorbed by Fethiye Soil and lowest by Izmir Soil, which was mainly related to the organic matter, and iron oxide contents. It seemed as if the low removal efficiency of Fethiye Soil could be explained by its high humic acid adsorption, and on the contrary, the high removal efficiency of Izmir Soil by its low humic acid adsorption.

Zinc removal efficiencies observed by fulvic acid were also low (< 4 %), despite its higher functional group content in comparison to humic acid. The highest removal efficiencies were obtained by Izmir Soil and lowest by Fethiye Soil. Evaluation of the influence of soils suspension pH on the dissociation of functional groups also did not reveal an explanation on the low removal efficiencies. Thus the low removal efficiencies of fulvic acid were attributed to its adsorption on zinc, as was the case of humic acid. Hence, it can be concluded that the reason of low removal efficiencies of natural chelating agents, humic and fulvic acids, can be attributed to the adsorption of humic and fulvic acids on soil minerals, thus limiting the formation of soluble humate zinc complexes

The removal of zinc from greenhouse soils with EDTA was in consistency with the previously published results. Zinc removal efficiencies of DTPA, on the other hand, could not be compared with literature since no data were available. The removal of zinc increased with increasing EDTA and DTPA concentration, yielding efficiencies over 100 %. In general, the removal efficiencies were quite well explained with the stoichiometric molar ratios, which displayed that the highest efficiencies observed for Izmir Soil were resulting from the higher percentage of EDTA and DTPA involved in the extractions. Consumption of EDTA and DTPA by other competing cations was also explained with the stoichiometric molar ratios, which represented that at especially lower concentrations the low removal efficiency of Fethiye and Samsun soils could be related to their higher copper and calcium contents, respectively. The influence of soil suspension pH was also investigated. Results show that the lower and higher efficiencies could be explained with the pH dependent speciations of EDTA and DTPA. Contribution of suspension pH to the removal efficiencies was determined to be higher for the zinc removal by high concentrations of DTPA. Additionally, increasing the zinc load of Fethiye Soil to about 4500 mg/kg did not result in lower removal efficiencies of zinc due to the increasing percentage of 0.005 M EDTA and DTPA involved in the extractions.

The sequential extraction experiments conducted on natural greenhouse soils revealed that background zinc was mainly associated with the iron and manganese oxides and the crystal structures of primary and secondary minerals and refractory organic compounds. Zinc, introduced into the soils at lower degrees (treatment with 10 mg/L zinc), was mainly adsorbed by the iron and manganese oxides and carbonates, and to a lesser extent to the exchangeable sites of soils. Variation in the distribution of zinc to the fractions in accordance with the different soils was well related to their iron and manganese oxide fraction. On the contrary, the carbonate fraction was dominating in the Samsun Soil, which was due to its high carbonate content. When the samples were aged for two months, the distribution of zinc to soil fractions was not found to be different. The distribution pattern changed when zinc was introduced to the soils at much higher levels (treatment with 500 mg/L zinc). For all soils, zinc was mainly associated with the exchangeable sites. The percentage of zinc adsorbed by the iron and manganese oxides, on the other hand, tremendously decreased, whereas complexation with the carbonates was not reduced, except for Samsun Soil. The change in the distribution pattern also indicated to the variation in domination of adsorption sites in relation to the introduced zinc amount. These results present that when soils are treated with lower zinc concentrations adsorption of zinc mainly occurs to the higher affinity sites, sites with higher binding energies like iron and manganese oxides and carbonates, and when treated with higher zinc concentrations on the lower affinity sites, sites with lower binding energies like exchangeable sites, thus verifying the findings derived from the adsorption isotherms. Additionally, the distribution of zinc in Samsun Soil did not change with respect to the reaction time of zinc with soil, suggesting that adsorption to individual soil fractions occurs at the same time but with different rates.

Sequential extraction experiments conducted after the treatment with 300 mg/L humic acid, showed that zinc, even at a lower degree, was mainly removed from the exchangeable sites of soils, indicating that weaker electrostatic interactions were easier overwhelmed by the ligand exchange reactions than the stronger specific interactions in the two other fractions. Sequential extraction experiments conducted after the treatment with 0.0001 M EDTA and DTPA, on the other hand, revealed that the zinc was mainly removed from the carbonates and iron and manganese oxides of soils, which was explained by the high affinity of both EDTA and DTPA to calcium and iron ions. Thereby, the interaction of zinc with the soils constituents could also be more comprehensive interpreted.

REFERENCES

Almås, Å. R., McBride, M. B., Singh, B. R., "Solubility and Lability of Cadmium and Zinc in Two Soils Treated with Organic Matter," *Soil Science*, 165, 250-259, 2000.

APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, Seventeenth Edition, 1989.

ASTM-Designation: D 421-85, "Standard Practice for Dry Perparation of Soil Samples for Particle-Size Analysis and Determination of Soil Constants," Annual Book of ASTM Standards, 8-9, 1993.

ASTM Designation: D 422-63, "Standard Test Method for Particle-Size Analysis Soils," Annual Book of ASTM Standards, 10-16, 1990.

Barona, A., Romero, F., "Relationships among Metals in the Solid Phase of Soils and in Wild Plants," *Water, Air, and Soil Pollution*, 95, 59-74, 1997.

Barona, A., Aranguiz, I., Elías, A., "Metal Association in Soils before and after EDTA Extractive Decontamination: Implications for the Effectiveness of further Clean-up Procedures," *Environmental Pollution*, 113, 79-85, 2001.

Barrow, N. J., "Testing a Mechanistic Model: IV. Describing the Effects of pH on Zinc Retention by Soils," *Journal of Soil Science*, 37, 295-302, 1986.

Borrell-Damián, L., Ollis, D. F., "Conceptual Evaluation of Sequential Extraction and Photocatalytic Decomposition Process for Recovery of Lead from Soil," *Journal of Advanced Oxidation Technologies*, 4, 125-137, 1999.

Bowles, J. E., *Engineering Properties of Soils and Their Measurement*, Fourth Ed., McGraw-Hill, Inc., U.S.A., pp. 57-70. 1992.

Brown, G. A., Elliot, H. A., "Influence of Electrolytes on EDTA Extraction of Pb from Polluted Soil," *Water, Air and Soil Pollution*, 62, 157-165, 1992.

Buckman, H. O., Brady, N. C., *The Nature and Properties of Soils*, The Macmillan Company, U.S.A., pp. 69-104, 1961.

Cairney, T., Hobson, D. M., *Contaminated Land, Problems and Solutions*, Second Ed., E & FN Spon, U.K., 1998.

Cesco, S., Römheld, V., Varanini, Z., Pinton, R., "Solubilization of Iron by Water-Extractable Humic Substances," *Journal of Plant Nutrition and Soil Science*, 163, 285-290, 2000.

Chairidchai, P., Ritchie, G. S. P., "Zinc Adsorption by a Lateritic Soil in the Presence of Organic Ligands," *Soil Science Society America Journal*, 54, 1242-1248, 1990.

Charlatchka, R., Cambier, P., " Influence of Reducing Conditions on Solubility of Trace Metals in Contaminated Soils," *Water, Air and Soil Pollution*, 118, 143-167, 2000.

Dang, Y.P., Tiller, K.G., Dalal, R.C., Edwards, D.G., "Zinc Speciation in Soil Solution of Vertisoils," *Australian Journal of Soil Research*, Vol. 34, pp. 369-383, 1996.

Davis, A. P., Singh, I., "Washing of Zn(II) from Contaminated Soil Column," *Journal of Environmental Engineering*, 121, 174-185, 1995.

delCastilho, P., Chardon, W. J., Salomos, W., "Influence of Cattle-Manure Slurry Application on the Solubility of Cadmium, Copper, and Zinc in a Manured Acidic, Loamy-sand Soil," *Journal of Environmental Quality*, 22, 689-697, 1993.

Echeverría, J. C., Morera, M. T., Mazkiarán, C., Garrido, J. J., "Competitive Sorption of Heavy Metal by Soils. Isotherms and Fractional Experiments," *Environmental Pollution*, 101, 275-284, 1998.

Ellis, S., Mellor, A., *Soil and Environment*, Routledge, U.K., pp. 9-56, 1995.

Elliot, H. A., Brown, G. A., "Comparative Evaluation of NTA and EDTA for Extractive Decontamination of Pb-Polluted Soils," *Water, Air, and Soil Pollution*, 45, 361-369, 1989.

Elrashidi, M. A., O'Connor, G. A., " Influence of Solution Composition on Sorption of Zinc by Soils," *Soil Science Society of Amerca Journal*, 46, 1153-1158, 1982.

Ephraim, J. H., "Heterogenity as a Concept in the Interpretation of Metal Ion Binding by Humic Substances. The Binding of Zinc by an Aquatic Fulvic Acid," *Analytica Chimica Acta*, 267, 39-45, 1992.

Evangelou, V. P., *Environmental Soil and Water Chemistry, Principles and Applications*, John Wiley and Sons Inc., U.S.A., pp. 100-166, 1998.

Evans, L.J., "Chemistry of Metal Retention by Soils," *Environmental Science and Technology*, 23, 1046-1056, 1989.

Falatah, A.M., Sheta, A.S., "Influence of Dissolved Organic Carbon and Initial Moisture on Zinc Sorption by Two Arid Soils," *Arid Soil Research and Rehabilitation*, 13, 133-144, 1999.

Fanfani, L. Zuddas, P., Chessa, A., "Heavy Metal Analysis as a Tool for Studying Mine Tailings Weathering," *Journal of Geochemical Exploration*, 58, 241-248, 1997.

Finnen, D. C., Pinkerton, A. A., Dunham, W. R., Sands, R. H., Funk, M. O., "Structures and Spectroscopic Characteristics of Iron (III) Diethylenetriaminepentaacetic Acid Complexes. A Non-heme Iron (II) Complex with Relevance to the Iron Environment in Lipoxygenases," *Inorganic Chemistry*, 30, 3960-3964, 1991.

Foth, H. D, *Fundamentals of Soil Science*, Eighth Ed., John Wiley and Sons Inc., U.S.A., pp. 22-41, 1990.

García Sánchez, A., Moyano, A., Muñez, C., "Forms of Cadmium, Lead and Zinc in Polluted Mining Soils and Uptake by Plants (Soria Province, Spain)," *Communications in Soil Science and Plant Analysis*, 30, 1385-1402, 1999.

Ghestem, J. P., Bermond, A., "EDTA Extractability of Trace Metals in Polluted Soils: A Chemical-Physical Study," *Environmental Technology*, 19, 409-416, 1998.

Gómez Ariza, J. L., Giráldez, I., Sánchez-Rodas, D., Morales, E., "Selectivity Assessment of a Sequential Extraction Procedure for Metal Mobility Characterization Using Model Phases," *Talanta*, 52, 545-554, 2000.

Gommy, C., Perdrix, E., Galloo, J., Guillermo, R., "Metal Speciation in Soil: Extraction of Exchangeable Cations from a Calcareous Soil with a Magnesium Nitrate Solution," *Journal of Environmental Analytical Chemistry*, 72, 27-45, 1998.

Graham-Bryce, I. J., "Solutions to Problems of Soil Pollution by Agricultural Chemicals," in E. S. Barrekette (Eds.), *Pollution, Engineering and Scientific Solutions, Proceedings of the First International Meeting of the Society of Engineering Science*, Tel Aviv, Israel, June 1972, Plenum Press, NY, U.S.A., pp. 133-147, 1973.

Han, F., X., Banin, A., "Long-Term Transformation and Redistribution of Potential Toxic Heavy Metals in Arid-Zone Soils: II: Incubation at the Field Capacity Moisture Content," *Water, Air and Soil Pollution*, 114, 221-250, 1999.

Hanafi, M.M, Sjiaola, J., "Cadmium and Zinc in Acid Tropical Soils: I. Soil Physicochemical Properties Effect on Their Adsorption," *Communication in Soil Science Plant Analysis*, 29, 1919-1931, 1998.

Haque, R., "Role of Adsorption in Studying the Dynamics of Pesticide in a Soil Environment," in Haque R, Freed U. H. (Eds.), *Environmental Dynamics of Pesticides*, Plenum Press, U.S.A., pp. 65-75, 1975.

Harter, R. D., Naidu, R., "Role of Metal-Organic Complexation in Metal Sorption by Soils," *Advances in Agronomy*, 55, 219-260, 1995.

Hong, P. K. A., Li, C., Banerji, S. K., Regmi, T.,"Extraction, Recovery, and Biostability of EDTA for Remediation of Heavy Metal-Contaminated Soil," *Journal of Soil Contamination*, 8, 81-103, 1999.

Huang, J. C., Liao, C. S., "Adsorption of Pesticides by Clay Minerals," *Journal of Sanitary Engineering Devision ASCE 96*, 1057-1078, 1970.

Inoue, K., Zhao, L. P., Huang, P. M., "Adsorption of Humic Substances by Hydroxyaluminum- and Hydroxyaluminumsilicate-montmorillonite Complexes," *Soil Science Society of America Journal*, 53, 1378-1385, 1990.

IT Corporation, "Chelation/Electrodeposition of Toxic Metals from Soils," 2001, http://www.clu-in.org/PRODUCTS/SITE/ongoing/emertech/itchelat.htm

Kaiser, K., Zech, W., "Dissolved Organic Matter Sorption by Mineral Constituents of Subsoil clay Fractions," *Journal of Plant Nutrition and Soil Science*, 163, 531-535, 2000.

Kaiser, K., Guggenberger, G., "The Role of DOM Sorption to Mineral Surfaces in the Preservation of Organic Matter in Soils," *Organic Geochemistry*, 31, 711-725, 2000.

Kalbitz, K., Wennrich, R., "Mobilization of Heavy Metals and Arsenic in Polluted Wetland Soils and its Dependence on Dissolved Organic Matter," *The Science of the Total Environment*, 209, 27-39, 1998.

Katahira, K., Yoneda, M., Morisawa, S., "Characteristics of Metal Element Distribution in Urban Surface Soil and Plants: Observations in Kyoto City Area and Stochastic Analysis," *Environmental Technology*, 21, 505-513, 2000.

Kedziorek, M. A. M., Bourg, A. C. M., "Solubilization of Lead and Cadmium During the Percolation of EDTA Through a Soil Polluted by Smelting Activities," *Journal of Contaminant Hydrology*, 40, 381-392, 2000.

Kim, Ki-Hyun, Kim, Se-Hyun, "Heavy Metal Pollution of Agricultural Soils in Central Regions of Korea," *Water, Air and Soil Pollution*, 111, 109-122, 1999.

Kuo, S., Mikkelsen, D. S., "Zinc Adsorption by Two Alkaline Soils," *Soil Science*, 128, 274-279, 1979.

Lee, P., Touray, J., "Characteristics of a Polluted Artificial Soil Located Along a Motorway and Effects of Acidification on the Leaching Behaviour of Heavy Metals (Pb, Zn, Cd)," *Water Resources*, 32, 3425, 1998.

Li, Z., Shuman, M., "Heavy Metal Movement in Metal-Contaminated Soil Profiles," *Soil Science*, 161, 656-666, 1996a.

Li, Z., Shuman, M., "Redistribution of Forms of Zinc, Cadmium, and Nickel in Soils Treated with EDTA," *The Science of the Total Environment*, 191, 95-107, 1996b.

Liljestrand, H. M., Lo, I. M-C., Shimizu, Y., "Sorption of Humic Materials onto Inorganic Surfaces for the Mitigation of Facilitated Pollutant Transport Processes," *Water Science and Technology*, 26, 1221-1228, 1992.

Lo, I. M. C., Yang, X. Y., "EDTA Extraction of Heavy Metals from Different Soil Fractions and Synthetic Soils," *Water, Air and Soil Pollution*, 109, 219-236, 1999.

Ma, L. Q., Rao, G. N., "Chemical Fractionation of Cadmium, Copper, Nickel am Zinc in Contaminated Soils," *Journal of Environmental Quality*, 26, 259-264, 1997.

Madrid, L., Diaz-Barrientos, E., "Influence of Carbonate on the Reaction of Heavy Metals in Soils," *Journal of Soil Science*, 43, 709-721, 1992.

Maiz, I., Esnaola, M. W., Millán, E., "Evaluation of Heavy Metal Availability in Contaminated Soils by a Short Sequential Extraction Procedure," *The Science of the Total Environment*," 206, 107-115, 1997.

Mann, S. S., Ritchie, G. S. P., *Australian Journal of Soil Research*, 93, 39, 1994. reference in Han, F. X., Banin, A., "Long-Term Transformation and Redistribution of Potential Toxic Heavy Metals in Arid-Zone Soils: II: Incubation at the Field Capacity Moisture Content," *Water, Air and Soil Pollution*, 114, 221-250, 1999.

Martinez, C.E., Motto, H.L., "Solubility of Lead, Zinc and Copper Added to Mineral Soils," *Environmental Pollution*, 107, 153-158, 2000.

McBride, M., Sauve, S., Hendershot, W., "Solubility Control of Cu, Zn, Cd, and Pb in Contaminated Soils," *European Journal of Soil Science*, 48, 337-346, 1997.

McGrath, D., "Application of Single and Sequential Extraction Procedures to Polluted and Unpolluted Soils," *The Science of the Total Environment*, 178, 37-44, 1996.

McGrath, S. P., Cegarra, J., *Journal of Soil Science*, 43, 313, 1992. reference in Han, F., X., Banin, A., "Long-Term Transformation and Redistribution of Potential Toxic Heavy Metals in Arid-Zone Soils: II: Incubation at the Field Capacity Moisture Content," *Water, Air and Soil Pollution*, 114, 221-250, 1999.

Mesquita, M. E., Vieira e Silva, J. M., "Zinc Adsorption by a Calcareous Soil. Copper Interaction," *Geoderma*, 69, 137-146, 1996.

Millar, C. E., Turk, L. M., Foth, H. D., *Fundamentals of Soil Science*, Fourth Ed., John Wiley and Sons Inc., U.S.A., 1958.

Mobley, K., "Potential Use of Chelating Agents for Decontamination of Soils," M.S. Thesis, Dartmouth, 1988. reference in Allen, H. E. and Chen, P., "Remediation of Metal Contaminated Soil by EDTA Incorporating Electrochemical Recovery of Metal and EDTA," *Environmental Progress*, 12, 4, 284-293, 1993.

Moore, T. R., Matos, L., "The Influence of Source on the Sorption of Dissolved Organic Carbon by Soils," *Canadian Journal of Soil Science*, 79, 321-324, 1999.

Moore, T. R., de Souza, W., Koprivnjak, J-F., "Controls of the Sorption of Dissolved Organic Carbon by Soils," *Soil Science*, 154, 120-129, 1992.

Morera, M. T., Echeverríaa, J. C., Mazkiarán, C., Garrido, J. J., "Isotherms and Sequential Extraction Procedures for Evaluating Sorption and Distribution of Heavy Metals in Soils," *Environmental Pollution*, 113, 135-144, 2001.

Naidu, R., Harter, R. D., "Effect of Different Organic Ligands on Cadmium Sorption by and Extractability from Soils," *Soil Science Society of America Journal*, 62, 644-650, 1998.

Nodvin, S. C., Driscoll, C. T., Likens, G. E., "Simple Partitioning of Anions and Dissolved Organic Carbon in a Forest Soil," *Soil Science*, 142, 27-35, 1986.

Norrström, A. C., Jacks, G., "Concentration and Fractionation of Heavy Metals in Roadside Soils Receiving De-Icing Salts," *The Science of the Total Environment*, 218, 161-174, 1998.

Nriagu, J. O., Pacyna, J. M., "Quantitative Assessment of Worldwide Contamination of Air, Water and Soils by Trace Metals," *Nature*, 333, 134-139, 1988.

Papassiopi, N., Tambouris, S., Kontopoulos, A., "Removal of Heavy Metals from Calcareous Contaminated Soils by EDTA Leaching," *Water, Air and Soil Pollution*, 109, 1-15, 1999.

Pataki, L., Zapp., E., *Basic Analytical Chemistry*, Pergamon Press, Oxford, pp. 196-210, 1980.

Petrović, M., Kastelan-Macan, M.,Horvat, A.J.M., "Interactive Sorption of Metal Ions and Humic Acids onto Mineral Particles," *Water, Air, and Soil Pollution*, 111, 41-56, 1999.

Piccolo, A., "Reactivity of Added Humic Substances towards Plant Available Heavy Metals in Soils," *The Science of the Total Environment*, 81/82, 607-614, 1989.

Polo, M.J., Ordonez, R., Vicente, J., "Copper and Zinc Adsorption by Sewage Sludge-Treated Soil in Southern Spain," *Communicatins in Soil Science Plant Analysis*, 30, 1063-1079, 1999.

Prasad, B., Sinha, M. K., "Physical and Chemical Characterization of Soil and Poultry Litter Humic and Fulvic Metal Complexes," *Plant and Soil*, 54, 223-232, 1980.

Přibil, R., *Analytical Applications of EDTA and Related Compounds*, Pergamon Press, NY, pp. 27-43, 1972.

Ramachandran, V., D'Souza, T. J., "Extractable Zinc and Manganese as Related to Applied Cadmium in Contrasting Indian Soils," *Chemical Speciation and Bioavailability*, 9, 131-144, 1997.

Randhawa, N. S., Broadbent, F. E., "Soil Organic Matter-Metal Complexes: Stability Constants of Zinc-Humic Acid Complexes at Different pH Values," *Soil Science*, 99, 362-366, 1965.

Ramos, L., Hernandez, L. M., Gonzalez, M. J., "Sequential Fractionation of Copper, Lead, Cadmium, and Zinc in Soils from near Doñana National Park," *Journal of Environmental Quality*, 23, 50-57, 1994.

Roy, W. R., Krapac, I. G., Chou, S. F. J., Griffin, R. A., Batch-Type Procedures for Estimating Soil Adsorption of Chemicals, Technical Resource Document, Code:

EPA/530-SW-87-006-F, Office of Solid Waste and Emergencey Response, U.S. Environmental Protection Agency, Washington, D.C. 20460, pp. 59-64, 1991.

Rump, H.H., Krist, H., *Laboratory Manual for the Examination of Water, Waste Water, and Soil*, Second Ed., VCH Verlagsgesellschaft, pp. 154, 1992.

Saatçi, F., Altinbaş, Ü., Tuncay, H., Akıncı, C., *Toprak ve Su Analiz Yöntemleri*, Ege Üniversitesi Zirrat Fakültesi Teksir No: 1877, Ege Üniversitesi, Turkey, 1983.

Schnitzer, M., Kerndorff, H., "Reaction of Fulvic Acid with Metal Ions," *Water, Air and Soil Pollution*, 15, 97-108, 1981.

Schnitzer, M., Skinner, S. I. M., "Organo-Metallic Interactions in Soils: 5. Stability Constants of Cu^{++}, Fe^{++}, and Zn^{++}-Fulvic Acid Complexes," *Soil Science*, 102, 361-365, 1966.

Schramel, O., Michalke, B., Kettrup, A., "Study of the Copper Distribution in Contaminated Soils of Hop Fields by Single and Sequential Extraction Procedures," *The Science of the Total Environment*, 263, 11-22, 2000.

Schulten, H. R., Schnitzer, M., 1997, *Soil Science*, Vol.162, pp. 115-130, 1997. http://www.soils.wisc.edu/virtual_museum/som/

Senesi, N., "Metal-humic Substances Complexes in the Environment: Molecular and Mechanistic Aspects by Multiple Spectroscopic Approach," in Adriano, D. C. (Ed.), *Biochemistry of Trace Metals*, CRC Press: Boca Raton, U.S.A., pp. 429-496, 1992.

Shukla, U. C., Mittal, S. B., "Characterization of Zinc Adsorption in Some Soils of India," *Soil Science Society of America Journal*, 43, 905-908, 1979.

Shuman, L. M., "The effect of Soil Properties on Zinc Sorption by Soils," *Soil Science Society American Proceeding*, 39, 454-458, 1975.

Shuman, L. M, "Fractionation Method for Soil Microelements," *Soil Science*, 140, 11-22, 1985.

Shuman, L. M., "Effect of Organic Waste Amendments on Zinc Adsorption by Two Soils", *Soil Science*, 164, 197-205, 1999.

Singh, S., P., Tack, f. M. G., Verloo, M. G., "Extractability and Bioavailability of Heavy Metals in Surface Soils Derived from Dredged Sediments," *Chemical Speciation and Bioavailability*, 8, 105-110, 1996.

Soon, Y. K., *Canadian Jopurnal of Soil Science*, 74, 179, 1994. reference in Han, F., X., Banin, A., "Long-Term Transformation and Redistribution of Potential Toxic Heavy Metals in Arid-Zone Soils: II: Incubation at the Field Capacity Moisture Content," *Water, Air and Soil Pollution*, 114, 221-250, 1999.

Spark, K. M., Wells, J. D., Johnson, B. B., "The Interaction of Humic Acid with Heavy Metals," *Australian Journal of Soil Research*, 35, 89-101, 1997a.

Spark, K. M., Wells, J. D., Johnson, B. B., "Characteristics of the Sorption of Humic Acid by Soil Minerals," *Australian Journal of Soil Research*, 35, 103-112, 1997b.

Spark, K. M., Wells, J. D., Johnson, B. B., "Sorption of Heavy Metals by Mineral-Humic Acid Substrates," *Australian Journal of Soil Research*, 35, 113-122, 1997c.

Sposito, G., *The Chemistry of Soils*, Oxford University Press, NY, pp. 148-169, 1989.

Stevenson, F. J., Ardakani, M. S., 1972, "Organic Matter Reactions Involving Micronutrients," in J. J. Mordvedt et al. (Eds.), *Micronutrients in Agriculture*, American Society of Agronomy, Madison, WI, pp. 79-114, 1972.

Száková, J., Tlustoš, P., Balík, J., Pavlíková, D., Balíková, M., "Efficiency of Extractants to Release As, Cd, and Zn from Main Soil Compartments," *Analysis*, 28, 808-812, 2000.

Szymura, I., "Use of the Langmuir Isotherm to Description of Zn Sorption by Some Polish Soils," in K. Wolf, W. J. van den Brink, F. J. Colon (Eds), *Contaminated Soil'88*, Vol I, Kluwer Academic Publishers, U.S.A., pp. 47-49, 1988.

Taylor, R.W., Hassan, K., Mehadi, A.A., Shuford, J.W., "Zinc Sorption by Some Alabama Soils," *Communications in Soil Science Plant Analysis*, 26, 993-1008, 1995.

Tessier, A., Campbell, P. G. C., Bisson, M., "Sequential Extraction Procedure for the Speciation of Particulate Trace Metals," *Analytical Che*mistry, 51, 844-851, 1979.

Theng, B. K. G., *Formation and Properties of Clay-Polymer Complexes*, Hilger, London, 1979.

Theodoratos, P., Papassiopi, N., Georgoudis, T., Kontopoulos, A., "Selective Removal of Lead from Calcareous Polluted Soils Using the Ca-EDTA Salt," *Water, Air and Soil Pollution*, 122, 351-368, 2000.

Tipping, E., "Modelling Ion Binding by Humic Acids," *Colloid and Surfaces A: Physicochemical and Engineering Aspects*, 73, 117-131, 1993.

Tombácz, E., Dobos, Á., Szekeres, M., Narres, H. D., Klumpp, E., Dékány, I., "Effect of pH and Ionic Strength on the Interaction of Humic Acid with Aluminium Oxide," *Colloid Polymer Science*, 278, 337-345, 2000.

TS 8336: Topraklar- Organik Madde Tayini, Türkiye Standardları Enstitüsü, 1-4, 1990.

Urano, K., Wada, H., Takemasa, T., "Empirical Rate Equation for THM Formation with Chlorination of Humic Substances in Water," *Water Resources*, 12, 1797-1802, 1983.

Ure, A. M., "Single Extraction Schemes for Soil Analysis and Related Applications," *The Science of the Total Environment*," 178, 3-10, 1996.

Vermeer, A. W. P., Koopal, L K, "Adsorption of Humic Acid to Mineral Particles. 2. Polydiversity Effects with Polyelectrolyte Adsorption," *Langmuir*, 14, 4210- 4216, 1998.

Vermeer, A. W. P., van Riemsdijk, W. H., Koopal, L K, "Adsorption of Humic Acid to Mineral Particles. 1. Specific and Electrostatic Interactions," *Langmuir*, 14, 2810-2819, 1998.

von Oepen, B., Krödel, W., Klein, W., "Sorption of Nonpolar and Polar Compounds to Soils: Processes, Measurements and Experience with the Applicability of the modified OECD-Guideline 106," *Chemosphere*, 22, 285-304, 1991.

Waller, P. A., Pickering, W. F., "Effect of Time and pH on the Lability of Copper and Zinc Sorbed on Humic Acid Particles," *Chemical Speciation and Bioavailability*, 4, 29-41, 1992.

Wilkins, B.J., Brummel, N., Loch, J.P.G., "Influence of pH and Zinc Concentration of Cadmium Sorption in Acid, Sandy Soils," *Water, Air, and Soil Pollution*, 101, 349-362, 1998.

Yaron, B., Calvet, R. and Prost, R., *Soil Pollution, Processes and Dynamics*, Springer Verlag, Berlin, pp. 3-24, 1996.

Yu, J., Klarup, D., "Extraction Kinetics of Copper, Zinc, Iron, and Manganese from Contaminated Sediment Using Disodium Ethylenediaminetetraacetate," *Water, Air and Soil Pollution*, 75, 205-225, 1994.

Zheljazkov, V. D., Nielsen, N. E., "Effect of Heavy Metals on Peppermint and Cornmint," *Plant and Soil*, 178, 59-66, 1996.

Zuyi, T., Taiwei, C., Jinzhou, D., XiongXin, D., Yingjie, G., "Effect of Fulvic Acids on Sorption of U(VI), Zn, Yb, I and Se(IV) onto Oxides of Aluminum, Iron and Silicon," *Applied Geochemistry*, 15, 133-139, 2000.

REFERENCES NOT CITED

Allen, H. E., Chen, P. "Remediation of Metal Contaminated Soil by EDTA Incorporating Electrochemical Recovery of Metal and EDTA," Environmental Progress, 12, 284-293, 1993.

Boulding, J. R., *Practical Handbook of Soil, Vadose Zone, and Ground-Water Contamination, Assessment, Prevention, and Remediation*, Lewis Publishers, Boca Raton, 1995.

Evans, L. J., "Fractionation and Aqueous Speciation of Zinc in Lake Polluted by Mining Activities, Flin Flong, Canada," Water, Air and Soil Pollution, 122, 299-316, 2000.

Kantarcı, D. M., *Toprak İlmi*, İstanbul Üniversitesi Orman Fakültesi Yayınları, İ.Ü. Yayın No: 4261, 2000.

Martell, A. E., Calvin, M., *Chemistry of the Metal Chelate Compounds*, Fourth Ed., Prentice-Hall, Inc., 1959.

Owens, G., Ferguson, V. K., McLaughlin, M. J., Singleton, I., Reid, R. J., Smith, F. A., "Determination of NTA and EDTA and Speciation of Their Metal Complexes in Aqueous Solution by Capillary Electrophoresis," Environmental Science and Technology, 34, 885-891, 2000.

Vermeer, A. W. P., McCulloch, J. K., Van Riemsdijk, W. H., Koopal, L. K., "Metal Ion Adsorption to Complexes of Humic Acid and Metal Oxides: Deviations from the Additivity Rule," Environmental Science and Technology, 33, 3892-3897, 1999.

Weber, W. J., McGinley, P. M., Katz, L. E., "Sorption Phenomena in Subsurface Systems: Concepts, Models, and Effects on Contaminant Fate and Transport," Water Resources, 25, 499-528, 1991.