CHARACTERISTICS AND REACTIVITY OF HUMIC ACIDS EXTRACTED FROM FOREST AND SAVANNA SOILS OF NIGERIA

BY

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(PhD/AGRIC/004/2009-2010)

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA IN PARTIAL FULFILMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN SOIL SCIENCE

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NIGERIA

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DECLARATION

I solemnly declare that the work in the project dissertation entitled "Characteristics and Reactivity of Humic Acids Extracted from Forest and Savanna Soils of Nigeria" has been performed by me in the Department of Soil Science under the supervision of Professors J.O. Agbenin, E.O. Uyovbisere and E.N.O. Iwuafor.

The information derived from the literature has been fully acknowledged in the text and a list of references provided. No part of this project dissertation was previously presented for another degree or diploma in any University.

Ayorinde Michael Olusegun

_____

Date

CERTIFICATION

This dissertation titled "Characteristics and Reactivity of Humic Acids Extracted from Forest and Savanna Soils of Nigeria" by Michael Olusegun Ayorinde meets the regulations governing the award of the degree of Doctor of Philosophy (Soil Science) for which it is presented. The thesis is acceptable in form and content and is approved after an oral examination of the candidate, for its contribution to knowledge and literary presentation.

_____ _____

Prof. J.O. Agbenin Date

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DEDICATION

To God the Father, God the Son and God the Holy Spirit who began this work and has brought it to completion, to my parents Late Pa Deacon Gabriel Ayorinde (JP) and Evang. (Mrs.) Esther M. Ayorinde, who in spite of their very meagre resources made it a point of duty to lay for me a sound educational foundation without which the present achievement would have been impossible, and to my beautiful wife Rev (Mrs) Tinuade Bukola Ayorinde and my children Tolu, Ibukun, Precious, Goodness and Mercy, this work is affectionately dedicated.

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ABSTRACT

The study was carried out to determine the characteristics and reactivity of humic acids extracted from soils sampled from three ecological zones of Nigeria: Forest, Southern Guinea savanna and Northern Guinea savanna. Surface soil samples were obtained from undisturbed (virgin) plots and from fallowed plots for not less than twenty five years. Soil samples were analyzed for selected physico-chemical properties. Humic acid was extracted from each soil sample under atmospheric N_2 atmosphere. Chemical properties and reactivity of the extracted humic acid were investigated by chemical analysis and infra-red spectroscopic methods. Attempts were also made to examine the effect of environmental gradients on the characteristics and reactivity of humic substances extracted from the soil samples. Soil acidity increased from forest to Northern Guinea savanna. Metal bound and water-soluble carbon, nitrogen content and available P all decreased from Forest to Northern Guinea savanna. Humification ratio (HR) and humification degree (HD) of the extracted humic substances increased from Forest to Northern Guinea savanna, while colour quotient (E_4/E_6 ratio) decreased in the same direction. Analysis of some functional groups in the humic acid indicated that phenolic group was significantly higher than carboxylic group. Total acidity of the humic substances was significantly lower for soils from the Northern Guinea savanna than the other ecological zones. The same trend was observed for the concentration of phenolic and carboxylic groups in the humic substances (HS). Reactivity of the HS was investigated by adsorption of Cu, Zn, Pb and Cd added to HS at the pH range of 4-6. In almost all cases, metal adsorption increased with increasing pH and increasing concentration of the metal adsorptive. Except for Cu adsorption at pH 6 which exhibited the H-isotherm curve, the adsorption isotherm curves of the other metals were either the S-type or L-type. Using the Langmuir equation to model the adsorption isotherm of the metal was generally unsuccessful probably because of the heterogeneity of the adsorption sites in contrast to the homogeneity of adsorption sites assumed in the derivation of the Langmuir equation. However, the Freundlich

isotherm of the form $q = KC^n$ where 'q' was the metal sorbed, 'k' and 'n' adjustable coefficient and C, the concentration of the added metal in solution successfully described the adsorption pattern of the metal with coefficient of determination (R^2) ranging from 0.59 to 0.99 or 56% to 99%. Variations in the adsorption of Cu, Zn, Pb and Cd across the three ecological zones were accounted for by the ratio of humic acid (HA) to fulvic acid (FA), humification ratio and the concentration of phenolic groups and total acidity of the humic substances. Indeed, the ratio of HA:FA accounted for between 90 and 94% of the variation of the Freundlich 'k' and 'n' value for Zn adsorption. Based on the analysis of 'n' value of the Freundlich equation, interpreted to represent the heterogeneity of the adsorption sites in the HS, the result indicated that the sites for the adsorption of Cu and Zn were more variable than those of Pb and Cd. In other words, Cu and Zn could be adsorbed unto a wide range of sites (heterogeneous) in contrast to a narrow range of sites (localized) for Cd and Pb. Spectroscopic studies, using infra-red spectroscopy to examine functional groups most active in the retention of metals or ions in HS in soils showed that the carboxylic (-COOH) and the carbonyl (=C=O) groups were most active in metal complexation, This was deduced from the changes in the stretching and bending vibrations of the functional groups in the HS as inferred from infra-red absorption bands. In general, HS from the soils showed strong absorption bands at the regions of 2990 to 2860cm^{-1}at pH range of 4 to 6, indicating the C-H vibration of aliphatic compounds, at between 1460 to 1330 cm^{-1} for OH and C-O stretching vibrations of phenolic group, at 1630 to 1650 cm^{-1}due to C=C vibration of aromatic compounds most likely conjugated to C=O stretching vibrations of groups like amide, ketones, quinines and COO^- symmetric stretching. Differences across zones in the concentration of functional groups of the humic acids were critical enough to accept that there will be differences in their reactivity across zones.

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CHAPTER ONE

1.0 INTRODUCTION

Soil organic matter, albeit a minor component by weight, of the vast majority of soil system is a major contributor to soil physico-chemical properties and ecosystem sustenance (Tate, 1996). Organic matter (OM) is a source of, and temporary sink for, several plant nutrients (Anderson and Coleman, 1985). Results of various research efforts have confirmed that soil OM determines and controls the supply and release of most plant nutrients. The study of characteristics and reactivity of humic substances is important in weathered soils where OM plays a vital role in soil fertility (Sanchez, 1976; Agboola, 1982) and optimizing ecosystem stability (Tate, 1996).

There is some information on the quantitative changes in OM in soils under cultivation in Nigeria. (Jaiyeoba, 1988; Jaiyeoba, 1995; Agbenin and Goladi, 1997, 1998). Adejuwon and Ekanade (1988) have shown that replacement of tropical rainforest with cocoa (*Theobroma cacao*), fallow or Kola (*Cola nitida*) resulted in losses of OM, nutrients and water holding capacity. Similarly, Aweto (1981), Jaiyeoba (1988) and Ekanade (1989) showed that forest fallows lead to increased OM in soils.

In the dry savanna, Jaiyeoba (1995); Agbenin and Goladi (1997) and Goladi and Agbenin (1997) showed that cultivation not only reduces OM and total-N content but also increases soil acidity. Furthermore, research on savanna soils revealed that soil OM is significantly correlated with CEC (Jones and Wild, 1975) and with N and P content (Kowal and Kassam, 1978; Agbenin and Goladi, 1998).

Some workers (Nigam *et. al.,,* 2001) have shown that emphasis is now laid on the quality rather than the quantity of OM in soil. It has been established that some OM fractions in the soil have radiocarbon age of over 2000 years (Anderson and Paul, 1984); while Bol *et. al,* (1996) found organic matter component aged between 13000-14000 years. These observations suggest that organic matter might interact with certain soil components to form resistant moieties that tend to maintain humic substances in soils for thousands of years. Formation of such recalcitrant OM is most probably controlled by its reactivity.

Interactions of humic substances with metals and surface cations contributed to aggregate stability in West African soils (Piccolo and Mbagwu, 1990; Dutartre *et. al,* 1993). Reaction of humic substances (HS) with cations has long been recognized (Bresnahan *et. al*, 1978; Gamble *et. al*, 1980; Stevenson and Chen, 1991). Humic-metal reactions are known to control the behavior of pollutant metals and micro- nutrients in the soil environment (Tyler and Mc Bride, 1982; Sauvé *et. al*, 1995; 1997). The reactions are highly complex (Stevenson and Chen, 1991; Fish *et. al*, 1986) and depend on the metal (Tipping, 1993; Logan, 1995), humic materials (Town and Powel, 1993; Stevenson *et. al*, 1993) and pH (Evans *et. al*, 2003; Mc Bride *et. al*, 1997).

Soil organic matter is composed of different compartments, which differ from each other in biochemical composition, biological stability (Piccolo *et al*, 2005) and their reactivity (Logan et. al.,, 1997). The humified OM fractions, namely humic acid (HA), fulvic acid (FA) and humin (HU) represent the most microbially recalcitrant portion of organic matter. They represent the stable reservoir of organic cation as well as natural

sink for plant nutrients, and are important components for the control of soil erosion by water and wind (Piccolo, 1996).

In the soil environments, naturally occurring humic substances exert strong binding strength with heavy metals (Chang Chien *et. al*, 2003b). Organic ligands derived from humic substances react with heavy metals to form complexes that influence the concentrations of free and labile heavy metal ions in soil (Vinkler *et. al*, 1978, Piccolo and Stevenson, 1981).

Humic-metal reactions have environmental implications for the mobility and migration of the metal: first, the reaction may produce soluble organic complexes with the potential to contaminate ground water and to retain such metal in soil solution and consequently ecotoxicity of such metals (Eby, 2004). Further, the reaction may also produce insoluble complexes resulting in reduction in bioavailability (Stevenson, 1994). An important factor in this regard is the strength of binding of the metal to the humic material, which is part of the focus of this study.

In the tropics, soil organic matter determines the fertility and productivity of soils, especially in highly weathered soil with small or no reservoir of weatherable minerals (Feller and Beare, 1997; Zech *et. al*, 1992). Moreover on a global scale the type of climate will affect not only the quantity but also the quality of organic matter formed (Hatcher *et. al*, 1981; Novak and Smeck, 1991).

Most of the studies on organic matter dynamics in the tropics have focused on its absolute amount (quantity) without recourse to the chemical fractions (Ekanade, 1989; Jaiyeoba, 1988; Agbenin and Goladi, 1997). In Nigeria there is an acute need to replenish soil organic matter (Spaccini *et. al*, 2001), Results of research works carried out in the

country (Agboola 1982, Agbenin and Goladi, 1997) have indicated that soil organic matter determines the extent of soil fertility. Kaolinite, which is the predominantly clay mineral in Nigerian soils is low in cation exchange capacity. This essentially, is responsible for the high dependence of the soils on their OM content as the major source of cation exchange capacity and therefore the main nutrient depot (Piccolo *et. al*, 2004).

In the highly weathered, humid tropical and savanna soils of Nigeria, there is no information on the qualitative dynamics of the stable humic pool of these soils. Yet more than 70% of the cation exchange capacity (CEC) of these soils is attributed to soil organic matter (SOM) and to humic acid (HA) in particular (Asadu *et. al*, 1997). This study was intended therefore to:

1. evaluate the quality and quantity of humic substances in forest and savanna (grassland) soils
2. determine the effect of climate and vegetation (ecological) zones on the quality and quantity of organic matter in these soils
3. examine the reactivity of humic acid extracted from these soil with trace ions (Cu^{2+} Zn^{2+}, Pb^{2+} and Cd^{2+}).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CONCEPTS OF SOIL ORGANIC MATTER

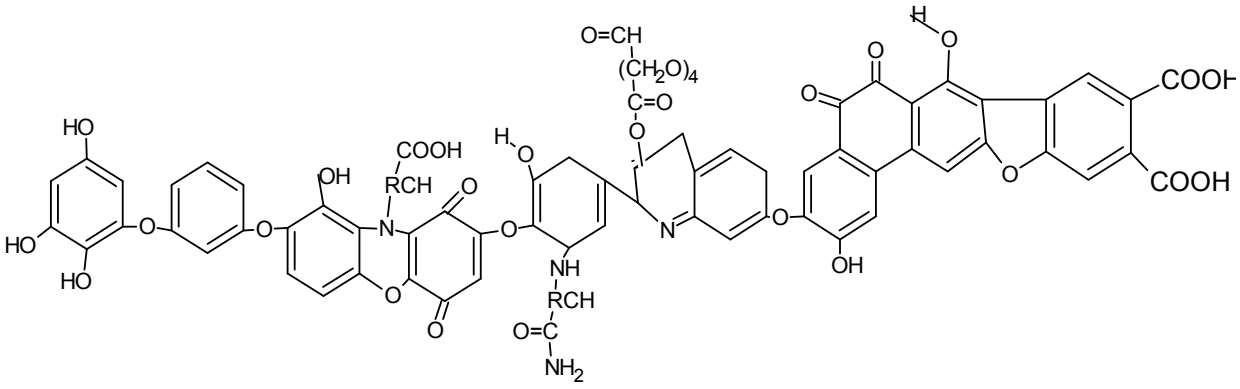
Understanding the soil as a chemical entity would be grossly inadequate without recourse to study its composition such as OM. This need is particularly urgent in the humid tropics where organic matter plays a very vital role in soil fertility (Agboola, 1982; Nigam *et. al*, 2001).

Soil OM has been broadly recognized to include newly fallen materials to the thoroughly decomposed and polymerized residual matter (Kononova, 1966). The modern concept, however, sees soil OM as consisting of two major groups of compounds: the non-humified substances and the humified remains of plant and animals (Stevenson, 1994). The non-humic substances are represented by recognizable plant debris, carbohydrates, proteins, fats, oils, waxes, alcohols, lignin, and vitamins. The humified materials (humic substances) consist of series of highly acidic, yellow to black amorphous hydrophilic and chemically complex organic substances (Schnitzer, 1978; Stevenson, 1994). Fresh and undecomposed organic materials have no vital role in soil functions (Yormah, 1980). Partially decomposed plant and animal materials form an important constituent of the soil. Their further decomposition (humification) leads to release of nutrients (Kononova and Alexandrova, 1973; Schnitzer and Khan, 1972). Humic substances are products of microbial degradation of plant and animal residues (Stevenson, 1994) and represent a range of chemical compounds whose detailed structures are still largely unknown (Wu *et. al*, 2002). However, humic substances are

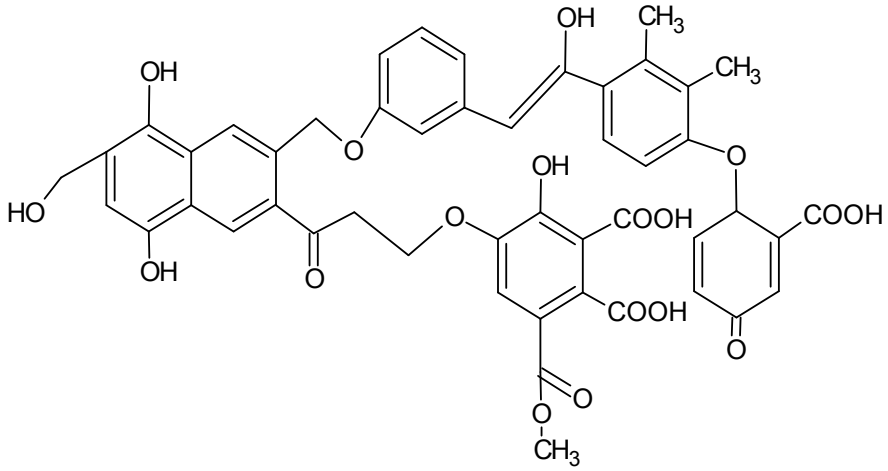
regarded as chemically heterogeneous mixture of products or compounds that cannot be assigned unique chemically defined molecular structures. Nonetheless, Stevenson (1994) depicted a generalized typical molecule of soil humic acid and fulvic acid (Fig. 1).

On the basis of solubility and pH, humic substances are classified into three fractions: humic acid (HA), fulvic acid (FA) and humin (Hu) (Stevenson, 1994). Humic acids and fulvic acids have molecular weight range of 50,000 to 100,000 and 500 to 2000 Daltons respectively (De Nobili and Chen, 1999). Fulvic acids have a higher proportion of hydrophllic to hydrophobic functional groups than humic acid (Stevenson, 1994). This makes fulvates soluble over a pH range of 2 to 12 (Wu *et. al*, 2002). Humins are insoluble in alkali or acid medium at standard temperature and pressure (Wu *et. al*, 2002) but can be dissolved using strong acids at elevated temperatures (Yong and Mourato, 1988; Wu *et. al*, 2000). Humins are high molecular weight polymers (Stevenson, 1994) with a lower proportion of functional groups than humic acid and fulvic acid (Wu *et. al*, 2002).

The macromolecular structure of HA and FA is made up of condensed aromatic, phenolic, quinonic and heterocyclic building blocks linked together by aliphatic, oxygen, nitrogen and sulphur bridges as illustrated in Figure 1 (Stevenson, 1994). The macromolecular structure consists of several functional groups mainly carboxylic, phenolic, alcoholic, hydroxyls, carbonyls, amino, sulfhydrryl and others that tend to confer acidic character on humic substances polymers. The presence and abundance of these functional groups that can be protonated and deprotonated depending on the pH of the medium, confers high reactivity, cation and anion exchange capacity on the humic



(a)



(b)

Figure 1. Proposed model structure of soil humic acid (a) and fulvic acid (b)

(Adapted from Stevenson, 1994).

substances. As can be seen in Figure 1 the macromolecular structure of HA is more complex than that of FA.

Advances in instrumentation have now generally improved the understanding of OM. However, much remains to be known about OM in soil both in the context of its quantity and quality. For example, absolute value can not be placed on the amount of OM present in any soil sample (Charles and Simmons, 1986). After determination of the total carbon content of a particular soil sample, it is still difficult to know what conversion factor to use to convert carbon value into OM content because of different C: H: O: N ratios in different organic components. In order to obtain the OM status of soils from organic carbon, a factor of 1.724 is usually employed, based on assumption that organic matter contains 58% C (Howard and Howard, 1990). This is the conventional 'van Bemellen factor' which according to Allison and Moodie (1965) has become established through constant use by workers rather than by authenticity.

2.2 FORMATION OF SOIL ORGANIC MATTER (HUMIC SUBSTANCES)

Several theoretical pathways have been proposed for the formation of soil humic substances (Stevenson, 1994). The major ones are:

1) Lignin-Protein theory,

2) Sugar-Amine theory,

3) Polyphenol theory.

In practice, all the three pathways may operate for the synthesis of HS in all soil, but not to the same extent since one pathway may be dominant in a given soil. For

example, in poorly drained soil, lignin may represent the major precursor (Stevenson, 1994; Senesi and Lofredo, 1999). Polyphenols synthesized by micro organisms may be predominant in forest soil, while sugars and amines may be important precursors for humic substance synthesis in soils where frequent and sharp fluctuation in continental climate factors occur (Stevenson, 1982). Furthermore, in any given soil, not all humic substance components may form by the same mechanism. For example, humic acid may originate from plant-lignin and microbially synthesized polyphenols while fulvic acid might be formed from sugar-amine condensation mechanism (Stevenson, 1994). However no satisfactory mechanism has been offered in soil chemistry and biochemistry for the formation of humic substance in soils. Briefly, each of the mechanism enumerated above involve the followings:

1. **The Lignin-Protein theory** (LPT). This is the classical theory, also known as pathway 1. Lignins are made of phenyl propane units (C_6-C_{30}) including coniferyl alcohol, P-hydroxycinamyl alcohol and sinapyl alcohol (Orlov, 1985). The latter is contained in relatively high proportion where herbaceous plants are well represented.

According to this theory, lignin is incompletely utilized by soil microorganism and undergoes a series of modifications: loss of methoxyl (OCH_3) groups, formation of O-hydroxyphenols from cyclic and COOH- groups from side chain (Flaig et. al.,, 1975). The quinones formed undergo condensation reactions with amino compounds yielding first humin, then humic acid and finally fulvic acid (Figure 2).

Waksman (1932) first proposed this theory and has been modified (Hatcher and Spiker, 1988) to include macromolecules other than lignin: cutin, suberin, melanin and

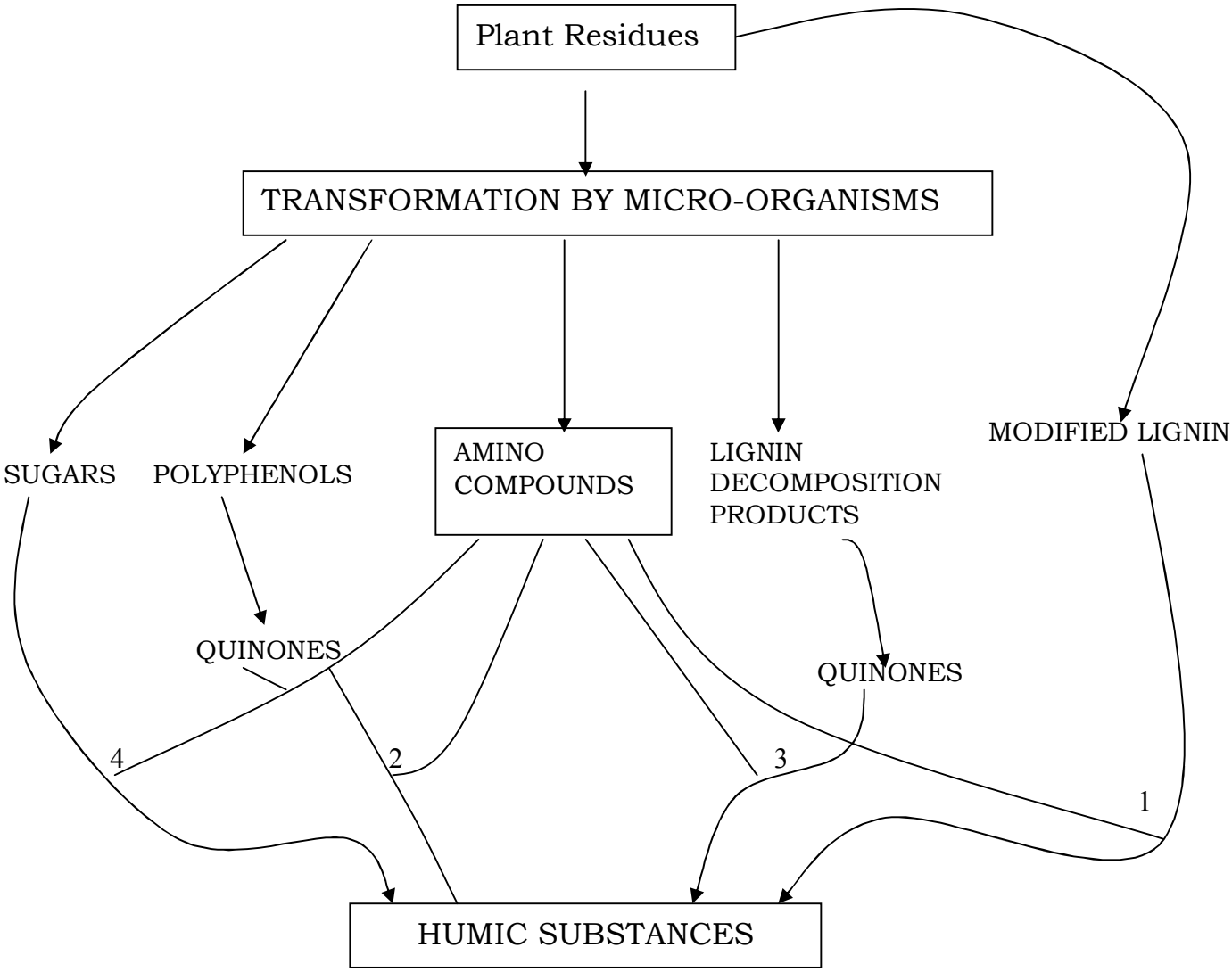


Figure 2. Major pathways proposed for the formation of soil humic substances. Adapted from Stevenson (1994).

parafinic macromolecules. However, oxygen is required for the complete microbial breakdown of lignin (Stevenson, 1982), it is feasible that in poorly drained soils modified lignin will contribute substantially to the formation of humic substances (Stevenson, 1994).

2. **The Polyphenol theory (PPT).** This is made of pathways 2 and 3 (Fig 3).

This is the most accepted view that the major building blocks of humic substances derive from polyphenols of lignin origin or synthesized by microorganisms. Here, humic substance is believed to form in soil mainly through a pathway in which the initial step consists of degradation of all plant biopolymers, including lignin into their monomeric structural units (Stevenson, 1994).

All the mechanisms (pathways) enumerated above are feasible for the synthesis of humic substances; however the overriding mechanism depends on the environment. In poorly drained soils lignin-protein theory predominates, whereas, synthesis from polyphenols is of great importance in certain forest soils (Weber, 2005).

3. **The Sugar-Amine theory (SAT).** This consists of pathway 4 (Fig 3).

According to this theory, humic substances may be formed by purely chemical reactions in which microorganisms do not play a direct role other than producing sugars and amino acids from carbohydrates and proteins respectively. These microbial by-products undergo polymerization reactions and have been postulated to play an important role in the formation of humic substances (Hayes and Swift, 1978; Ikan *et. al*, 1986). According to Weber (2005) this mechanism seemed to be favoured by drastic and frequent changes (wetting and drying, freezing and thawing) occurring in the soil

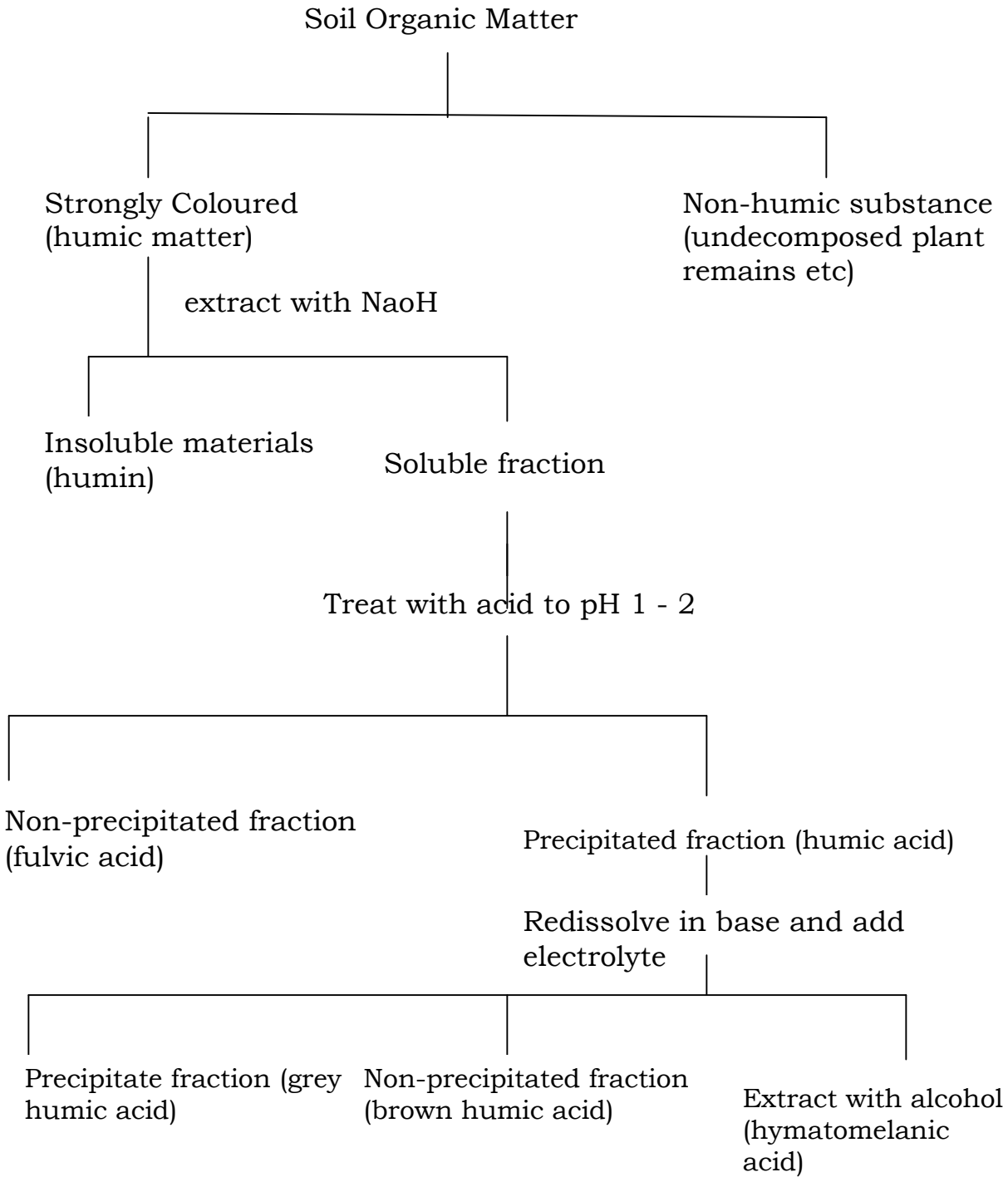


Figure 3. A Flowchart showing the extraction and fractionation of humic substances (Adapted from Bohn et al., 1985)

environment. In addition, intermixing of mineral materials having catalytic properties may also facilitate this mechanism (Weber, 2005).

2.3 EFFECTS OF ENVIRONMENTAL GRADIENT ON SOIL ORGANIC MATTER

The OM content of surface soils in the savanna is generally low compared with that in forest soils (Ahn, 1974; Jaiyeoba, 1988). Differences in OM content between a soil under closed forest and that under open savanna are largely due to much greater weight of vegetation in the forest, and the annual burning of the above-ground parts of the savanna grasses. Much of the above-ground parts of savanna grasses may also be eaten by grazing animals (Kowal, 1973), the organic matter in the savanna soils is therefore derived from the roots (Kowal and Kassam, 1978).

The factor having the most influence on organic matter is climate and, in particular, the mean annual rainfall and its seasonal distribution (Jones and Wild, 1975). Kadeba (1978) observed that increasing organic matter content correlated with increased rainfall; therefore, as we move from savanna to the coast, the organic matter content also increases. Jones (1973) obtained a relationship indicating a mean increase of 0.17% of soil organic matter per 100mm increase in rainfall in savanna region.

The seasonal character of the savanna climate affects accumulation of organic matter in several ways. Firstly, duration of wet period determines total dry matter produced, and hence, the quantity of plant residues returned to the soil (Jaiyeoba, 1995; Wild, 1995). Secondly, the destruction of vegetation by burning affects the return of residues to the soil (Hopkins, 1968; Ekanade, 1993). Further, one effect of burning is to encourage growth of grasses at the expense of trees and bushes which, according to Hopkins (1968)

and West (1965) affects the nature of organic residues returned to the soil. This has an overall effect on the quality of humus formed (Novak and Smeck, 1991).

A second factor affecting soil organic matter status is the nature of soil parent material, which may determine the nature of the soil and the size of its clay fraction (Kowal and Kassam, 1978). Differences between soils of savanna and forest zones are as a result of difference in the density of the natural vegetative cover (Ahn, 1970). The latter determines the degree of soil erosion and leaching. The quantity of nutrients cycled through vegetation is greater in the forest zone than in the savanna (Nye and Greenland, 1964) and becomes progressively less as one moves up in the savanna area. The presence of mineralizable organic matter reduces rate of biodegradation of aromatic hydrocarbon (Manilal and Alexander, 1991) and is highly correlated with denitrification capacity of the soil (Bufford and Bremner, 2002).

In soil environment, humic substances (HS) form part of a complex system in which they interact with ions, mineral, colloids and non humified organic materials. Humic substances can be studied in detail only when they are isolated from the soil in a free state (Kononova, 1963), from the inorganic materials and from non-humic compounds. This can only be achieved by extraction. Because of the complexity and heterogeneity of HS (Bohn *et. al*, 1985), its extraction and fractionation procedure would be limited. Lack of clear-cut distinction in the physico-chemical properties of the molecules that make up humic substances (Stevenson, 1994; 1982) makes the extraction difficult. This is probably part of the reason why the study of HS is more complex. It is therefore not surprising that in the literature diverse solvents are used for extracting humic substances.

2.4 EXTRACTION, FRACTIONATION AND PURIFICATION OF HUMIC SUBSTANCE

2.4.1 Extraction

Humic substances can only be successfully separated from the soil inorganic matrix by taking cognizance of the properties of the extractant as well as type of association existing between HS and other soil constituents. White-head and Tinsley (1964) proposed that the molecular size of the extractant should be small enough to penetrate into humic structure. The authors also stressed that the extractant should be able to disperse the charged molecules, disrupt the existing hydrogen-bond and provide alternate humic-solvent hydrogen bonds.

The objective of extraction method is to include isolation of unaltered HS free from inorganic contamination representing fraction from entire molecular weight range (Stevenson, 1982). It must also be applicable to all soils. However, there is no evidence in the literature that these objectives have been realized. The extraction method employed in isolation of HS from the soil varies.

Alkaline solvents were the earliest reagents used for extraction of HS (Tyurin and Kononova, 1962; Whitehead and Tinsley, 1964). This method remains the most widely used till date as revealed in the method employed by different workers: Schnitzer (1982), Stevenson (1994), Yuan and Xing (2001), Li *et. al*, (2003). By this method a soil: extraction solution ratio ranging from 1:2 to 1:10 (W/V) is commonly employed. Some authors (Goh, 1974; Li *et. al*, 2003; Stevenson and Kashem *et. al*, 2007) advocated repeated extraction for maximum recovery. Some others (Schnitzer, 1972; Stevenson, 1994; Swift, 1996) suggested the use of aqueous salt solution of Na and K. According to

Schnitzer (1972) aqueous salts are milder and less selective than the alkaline solvents. However, the HS isolated with this solvent usually contain high amount of Fe and Al which are difficult to remove as impurities. According to Schnitzer (1982), it is practically impossible to remove phosphate from the extracted HS, which causes problem with its subsequent characterization. The most widely used aqueous salt is 0.1M $Na_2P_2O_7$ at pH 7. Others are sodium fluoride (NaF), sodium metaphosphate (Na_3PO_4), sodium borate ($Na_2BO_4O_7$) sodium chloride (NaCl) and sodium bromide (NaBr). Sun et. al., (2001) used organic chelate ethylene-diamine-tetra-acetic acid (EDTA) to extract OM from soil. Organic solvents such as pyridine and dimethyl-sulfur-oxide (DMSO) are also employed as extractants (Song and Hayes, 2004). Whitehead and Tinsley (1964) extracted up to 55% of HS from soils using formic acid as the extracting agent. Formic acid in the presence of LiF, LiBr, and HBF_4 is also employed (Sinclair and Tinsley, 1981). Extraction by formic acid dissolves large amount of impurities like Fe and Al along with HS (Stevenson, 1984; Senesi and Lofredo, 1999).

2.4.2 Fractionation

Humic substances contain a wide range of macromolecules exhibiting a wide variation of values for any given molecular property (Senesi *et. al*, 1991; Li *et. al*, 2007). It is [therefore, necessary to subdivide HS into distinct fractions that exhibit a much narrower variation in properties. A flow chart for fractionation of humic substances on basis of solubility as well as basic properties of the fractions is shown in Figure 3.

Several methods are employed in fractionation of HS and include solubility, molecular size, reactions of the fractions with different metal ions, charge density and adsorption properties.

The classical procedure for fractionation involves acid precipitation of some fractions obtained from the strong base extract (Bohn *et. al*, 1985). The acid used is usually strong acids such as HCl or H_2SO_4 to a pH of 1-2. This is the most common method employed by several authors. (Hatcher *et. al*, 1981; Swift, 1996; Logan *et. al*, 1997; Chang-chien, 2003a). Three major fractions are obtained by this method: Humic acid-the fraction soluble in alkali but insoluble in acid, fulvic acid – soluble in both alkali and acid media and humin which is insoluble in both alkali and acid media. Humin is the portion of organic matter not extracted with alkali and strongly associated with oxyhydroxides and clays. Some earlier workers separated the HA and FA fractions into sub fractions by means of organic solvents, salts, and metal ions. Oden (1919) used ethanol to extract ethanol-soluble fraction (hymatomelanic acid) from HA. Berzelius (1839) used copper ions to fractionate HA into apocrenic and crenic acids. Sprengel (1826) used KCl to salt-out gray and brown fractions from HA. Theng et. al., (1968) employed ammonium sulphate at pH 7 to fractionate HA.

Other workers achieved fractionation using other means such as gel chromatography (Swift, 1996: Wu *et. al*, 2002), ion exchange (Bolto *et. al*, 2002) and ultra-filtration (Aster *et. al*, 1996: Wershaw and Aiken, 1985). The main fractions obtained are humic acid (HA) soluble in alkali, insoluble in acid, fulvic acid (FA) soluble in both alkali and acid; and humin insoluble in both alkali and acid. Flaig *et. al* (1975) used changes in pH to obtain narrower fractions.

2.4.3 Purification of Humic Substances

Acid fractionation is usually accompanied by purification as the extracted humic substances contain both organic and inorganic impurities. Clay impurities are removed from crude humic acid by repeated dissolution in alkaline solvent followed by high speed centrifugation and acid re-precipitation. Mixture of dilute mineral acids (HCl + HF) solution is used for repeated precipitation of the humic acid to reduce its ash content (Schnitzer, I968). The humic acid is shaken in the acid mixture for 24 hours followed by filtration and washing of the residue with distilled water until free of chlorine. Further, the humic acid may be passed through an ion exchange resin such as Amberlite IR-120 (Malcom, 1990) or Dowex 50 in H form (Senesi and Loffredo, 1996; Logan *et. al*, 1997). Other workers (Khan, 1971; Swifts, 1996; Xing, 2001) used dialysis to purify humic acid extracted from soils. Schnitzer and Khan (1978) observed that dialysis could not separate complexed or strongly adsorbed metal hydroxide from humic material. Hartworth (1971) boiled humic substance with water to remove polysaccharide and polypeptide impurities. Lipids (fats, waxes, resins) are easily removed with ether or alcohol benzene. Inorganic impurities can be eliminated in fulvic acid by selective absorption, by passage through a cation exchange resin or by repeated dialysis (Senesi and Lofredo, 1996).

2.5 ANALYSIS OF HUMIC SUBSTANCES

2.5.1 Chemical Methods of Analysis

As the name implies the method employ chemical reagents to analyze and probe into the nature of humic substance extracted in the soil and sediments. By this method

chemical bonds in the body of the material under study are broken down with or without formation of new bonds.

The most commonly used tool in characterization of humic substances is elemental analysis. This provides information on the distribution of major elements: C, H, N, O, and S in the humic substance. Stevenson (1994) affirmed the use of elemental analysis in estimating the efficiency and purity of HS preparations. Elemental composition is also often useful in distinguishing different classes of humic substance (Stevenson, 1994). The result of elemental analysis is usually computed to give atomic ratios of H/C, O/C and N/C which according to Schnitzer (1978) can be used to monitor structural changes in HS.

Information about acidity and reactivity of HS is often provided by functional group analysis. Humic substances contain many functional groups of different types. Oxygen-containing functional groups such as phenols, carboxyl and carbonyls are the most abundant and ultimately the most important for reactivity. Logan *et. al*, (1997) noted that the involvement of other groups in reactivity of HS cannot be discounted. Sikora and Stevenson (1988) obtained a positive correlation between maximum binding capacities of humic acid and their nitrogen content suggesting that other groups such as amine and amino groups are quite reactive in HS.

2.5.2 Instrumental Analysis

The most commonly used tool in instrumental analysis is the use of different spectroscopic measurements in different regions of the electromagnetic spectrum. This method provides valuable information about the nature of soil humic substances. The

types of spectroscopy used include ultraviolet and visible spectroscopy, infra-red nuclear magnetic resonance, diffuse reflectance spectroscopy and others. Several of these instruments have been used independently or combined by several authors. Hatcher et. al., (1982) elucidated the differences in humic acid from different ecological regions using a combination of solid state ^{13}C nuclear magnetic resonance (NMR) and infra-red spectroscopy. Evidence from these studies indicated that humic acids from different ecological zones are different from each other. Li *et. al*, (2003) used the same combination of ^{13}C-NMR and infra red spectroscopy to suggest that humic acid is composed of aliphatic and aromatic subunits.

Recently, rapid and non destructive methods such as diffuse reflectance infra-red spectroscopy have been employed in analysis of whole soil and soil organic matter. Fritz *et. al*, (1994) obtained positive correlation between humus content and microbial humus of burnt soil using near infra- red (NIR) spectroscopy.

Chodak *et. al*, (2002) employed NIR spectroscopy for characterization of organic matter in peat, and using NIR. Kemper and Sommer (2002) obtained accurate prediction of trace element (Pb, Hg) in soils. Mid infra-red (MIDIR) spectroscopy has also been used for quantitative analysis of soil chemical properties (Ehsani *et. al*, 2001). Mid infra-red calibrations performed significantly better than near infra red calibrations for the determination of C content (McCarthy *et. al*, 2002).

The infra-red (IR) spectra of humic substances and their derivatives contain a variety of bonds that are typical of specific molecular structures (Stevenson and Goh, 1974). IR spectroscopy provides key information about the nature and reactivity of oxygen-containing functional groups (Lofredo and Senessi, 1999). Schnitzer *et. al*,

(1991) concluded that X-ray diffraction can successfully be used to characterize HA-metal complexes. Sanchez et. al., (2002) proved by infra red spectroscopy the absence of any significant differences between purified humic acid and the untreated materials.

2.6 INTERACTIONS OF METALS WITH HUMIC SUBSTANCE

Humic-metal binding may result in the formation of soluble organic complexes with the potential of retaining the metal in soil solution or may form insoluble complexes resulting in a reduction in bioavailability of such metal (Logan *et. al*, 1997). Petruzzelli *et. al*, (1985) reported that bioavailability of heavy metals in soils is more important than their content to better understand the possibility of transferring such metal into food chain. The amphiphatic nature of humic substances enable them to interact with a wide variety of inorganic and charged organic pollutants via chemical bonding and less polar organics through non specific interactions (Li *et. al*, 2004).

Based on their bioavailability, Bohn and Fish (1993) grouped humic-metal complexes into two groups. The first group known as outer sphere complexes is purely electrostatic in nature with both the ligand and the metal retaining their hydration sphere. The alkali and the alkaline earth metals are classified into this group. The second group is known as the inner sphere complexes which is as a result of formation of a covalent or coordination bond between the ligand and the metal ions, with partial or total breakdown of the hydration sphere of the metal. The transition metal elements such as copper (Cu), lead (Pb), zinc (Zn), molybdenum (Mo) and cadmium (Cd) are classified into this group.

In his own review, Pearson (1963) advanced the concept of hard and soft acids, and hard and soft bases to classify humic-metal reactions. Hard acid such as Al^{3+} and

hard bases (Na^{+}, Mg^{2+}) according to this concept are small, compact metal cations, not very polarizable in nature, while soft acids and soft bases are metal cations such as Pb^{2+}, and Cu^{2+} respectively. Further, according to Pearson (1963), hard acids prefer to react with hard bases and soft acids prefer soft bases.

Humic and fulvic acids are extremely important complexing agents for heavy metals (Baird, 1999; White, 2002). The abundance of oxygen–containing functional groups in humic and fulvic acids are responsible for their ability to attract polyvalent metal ions (Eby, 2004). The solubility of the resulting complex, however, is often different with humic acids yielding water-insoluble acid complexes and fulvic acids yielding water soluble ones (Baird, 1999; Eby, 2004).

Binding of metals to humic substances is an important factor that can potentially control the mobility of heavy metals in soils. Variations in quality of humic substances influence the speciation of heavy metals (Carnazutto *et. al*, 1991). Working on compost maturation, Carnazutto *et. al*, (1991) concluded that heavy metals are complexed by humic substances and reach the soil in less mobile chemical form. Consequently, such complexed metals are less available for plant absorption. Ciavatta *et. al*, (1993) demonstrated that organic compost as a source of humified organic matter can decrease availability of heavy metals in amended soils. Huang *et. al*, (2005) showed that the bioavailability of Cu and Zn in organic compost was dependent upon the HA/FA ratio. This is attributed to different preferential binding for HA and FA fractions by the metals. Karathanasis (1999) observed that Cu solubility increased in soil as the pH increased from pH 5 to 6.5. Organic residues in soil significantly contribute to complexation and mobilization of soil metals (Evangelou and Marsi, 2001).

The conclusion of Karathanasis (1999) that presence of OM and other colloids significantly facilitates metal transport in soils is of great interest. This translates .to binding interaction of metals on the colloids and OM. This is in consonance with several other works. Logan *et. al*, (1997) observed that binding constants (K_1) for Cu^{2+} increased with increasing pH whether in peat or humic acid. Sorption of metals on mineral-humic acid system is a function of sorption of humic acid by the mineral (Spark *et. al*, 1997). Therefore, the more humic acid is sorbed on the mineral, the more the uptake of metals by the humic acid-mineral system. These authors also observed that pH is a master variable in the sorption system. The presence of chelates such as ethylene diamine tetraacetic acid (EDTA) and ionic strength reduces copper desorption from humic acids (Chen and Lin, 2005).

Guggenberger et al (1994) studied hydrophobic and hydrophilic fraction of soil dissolved organic C. The authors observed that the hydrophilic fraction had a higher Cu complexation capacity than the hydrophobic fraction. Due to its relative soil mobility, hydrophilic fraction played a significant role in Cu leaching (Guggenberger *et. al*, 1994). Kuiter and Mulder (1993) observed that low molecular weight fraction of forest litter layer and humus horizon was responsible for 50-99% and 30-60% of Cu-binding capacity respectively.

Humic acids and fulvic acid are the main reactive fractions of humic substances, thus their high affinity for metals is no longer in doubt. This coupled with the findings of other authors on the role of low molecular weight organic C in metal complexation of humic substances, makes suggestions of Nigam *et. al*, (2001), and Huang and Schnitzer (1986) that low molecular weight organic acids are probably the building blocks of humic

substances more plausible. The authors also suggested that low molecular weight organic acids play a key role not only in mineral uptake but also in alleviating metal-induced plant-rot with humic acid acting as a detoxifier as it produces insoluble complexes with metals as against soluble ones of fulvic acid-metal complexes.

Huang *et. al*, (2003) obtained results pointing to the existence of two subunits of HA: an aliphatic subunit having larger apparent molecular size and an aromatic subunit having smaller apparent molecular size. The authors concluded that HA fractions are a mixture of these two subunits. Hydrogen binding and hydrophobic interactions are some of the mechanisms of interactions between humic substances and inorganic materials (Schulten, 1996). Some other workers like Gu *et. al*, (2001) observed that interactions between humic substances and metals are results of ligand exchange and or cation bridging.

Using different techniques for predicting metal uptake by plants, Nolan *et. al*, (2005) proved that uptake of metals such as Zn and Cd from soil by wheat (*Triticum aestivium* L.) plants were greatly influenced by the labile solid phase pool of metal. These workers (Nolan et. al.,, 2005) obtained poor relationships of $CaCl_2$–extractable concentrations with Zn, Pb, and Cu availability. This indicates Ca competition is too weak for these metals and therefore plant-available pools of Zn, Pb, and Cu in the soils are not just the easily exchangeable fraction. Since HA forms insoluble complexes with metals, its role in releasing such metals may be crucial.

An important feature of chemical characterization of humic acids is their content of COOH, C=O and phenolic-OH in determining their humification level (Coles and Yong, 2006) Higher contents of COOH and C=O group indicates well humified humic

acids (Yong and Mourato, 1988), whereas a high alcoholic content indicate poorly decomposed humic acid (Lavti and Paliwal, 1981).

2.7 MODELLING METAL-BINDING IN HUMIC SUBSTANCES

Most of the works on humic acid interactions with metals (Garcia *et. al*, 1991, 1995; Lena *et. al*, 1997; Chen *et. al*, 2003) have been experimental, but there have also been a significant number of modeling studies (Bryan *et. al*, 2005; Van Riemsdijk and Weng, 2005). Many of the models have addressed the rapid uptake of metal ions by solution phase humic substances (Tipping and Hurley, 1992; Kinniburrgt *et. al*, 1996; Evans *et. al*, 2003). Choppin and Clark (1991) opined that though the initial uptake is rapid, not all the metal loadings may be removed instantaneously. The uptake of humic substances by surfaces has been studied mostly via batch experiments (Tipping, 1993; Schlautman and Morgan, 1994). A number of studies (Tessier *et. al*, 1996; Logan *et. al*, 1997) have found that the adsorption step may be simulated by Langmuir isotherms. Other workers (Attanosova and Okazaki, 1997; Sparks *et. al*, 1997) obtained data perfectly fitting into Freundlich isotherm. There are two distinct steps to the reaction of humic substances with metals: sorption and desorption (Spark, 1996; Yuan and Xing, 2001). The sorption step has been characterized as fast (Logan *et. al*, 1997) whilst desorption is described as slow (Olks *et. al*, 1998).

Lenhart and Honeyman (1999) elucidated a number of mechanisms for the binding of humic substances to inorganic surfaces. These include ligand exchange; cation bridging, hydrogen bonding and van der Waals (hydrophobic) interactions. The use of infra-red spectroscopy has shed light on the mechanism of reactions (Gu *et. al*, 1994)

pointing to the fact that ligand exchange mechanism does take place. By this mechanism, according to Gu *et. al*, (1994), the oxygen donors from humic acid replace surface–OH groups and a covalent bond forms between humic substance and the surface. However, other mechanisms cannot be precluded. Bryan *et. al*, (2005) suggested that different fractions of humic substances may have predominantly physical or chemical interactions with the surface. Avena and Koopal (1999) showed that sorption and desorption isotherms are not the same. Sorption isotherm is not necessarily linear and may be competitive (Pignatello, 1997, 1998; Webber and Huang, 1999; Xing and Xing and Chen, 1999). Xing and Pignatello (1998) proved that competition does occur among the sorbates. Yuan and Xing (2001) obtained evidence showing variation in energy distribution of sorption sites.

Gueu *et. al*, (2007) obtained results indicative of the fact that desorption as a result of pH change was rapid but slow as a result of changes in humic concentrations. .This points to the fact that electrostatic interactions are crucial to the binding mechanism (Wu *et. al*, 2003). Gueu *et. al*, (2007) suggested that species with ortho functional groups might form strong chelate interactions with the surface. Slow desorption kinetics may be due to formation of multiple strong bond between humic substances and the surface (Wu *et. al*, 2003) which must all be broken before desorption can take place.

Several studies (Perdue and Lyttle, 1983; Logan *et. al*, 1997) outlined major models commonly used. These studies are summarized into four models below:

1. Discrete ligands: these are multi ligand models that require only a few ligands to fit data and ignore the effect of variation in humic charge and molecular configuration.

The ligands used in this model do not represent distinct classes of binding site with similar binding affinities (Dzombark *et. al*, 1986).

2. The electrostatic model: this attempts to take into consideration the electrostatic effects that influence metal binding, particularly as titration proceeds (Wilson and Kinney, 1977). The model assumes that all binding sites are chemically the same, with variation in apparent log K (Freundlich coefficient). By this model, binding occurs as a result of electrostatic interactions resulting from the change in humic charge on metal binding. Backes and Tipping (1987) used this basis to develop a model used for the study of Al and proton binding.

3. Discrete and electrostatic models: these are regarded as the most advanced model for metal binding. Electrostatic models have been used by several workers (Ephraim *et. al*, 1986) and further developed by others (Backes and Tipping, 1987; Tipping *et. al*, 1988, 1991; Tipping and Hurley, 1992; Tipping, 1993). The integral concept of this model is that metals and protons bind at severally chemically discrete sites, with electrostatic secondary effects being accounted for by the Scatchard multisite approach.

4. Continuous distribution model: Due to complexity of humic materials and the variety in type and affinity of the binding sites, continuous distribution models were developed. These models assume that a stability constant *K* represent a continuum of binding sites on the humic substance, rather than the discrete classes of binding sites (Dzombark *et. al*, 1986). The continuum is produced from secondary effects which alter the affinities of the binding sites as binding proceeds. The ligand frequency distributions are then integrated over the distribution of *log K*. Examples of such models include the

affinity spectrum approach model by Thakur *et. al*, (1980), the constant stability function model (Gamble *et. al*, 1980), and the normal distribution model (Perdue and Lytle, 1983).

However, Stevenson and Chen (1991) adapted the Scatchard multi site approach from a discrete model into a continuum distribution model with the incremental stability constant model.

CHAPTER THREE

MATERIALS AND METHODS

3.0 SITE DESCRIPTION

The soils for this study were obtained from plots located in three different ecological zones of Nigeria, namely: Forest, Southern and Northern Guinea Savanna.

3.1 FOREST ZONE

The soil from the forest zone was obtained from Ago-Atsaviya in the high forest zone of Ondo State. Ago-Atsaviya is a village located South-West of Owo, about 12km on Owo-Ore highway, approximately on Latitude 7^0 12`N and Longitude $5^0$ 30`E. The site is about 4 kilometers from the highway. The plot was a `sacred` plot, about 2 kilometers from the village which has been undisturbed for about 200 years. According to the village tradition, this site is specifically reserved for the burial of the demised village heads only, as such it has only been used 4 or 5 times in its existence. Samples were taken from the undisturbed part of the `sacred` plot. The vegetation on this site was observed to be in four distinct layers:

The first layer is made of trees of about 35-40 meters tall that included trees like `obeche` (*Triplochiton seleroylon)* , *K*haya spp*,(Tectonia grandis), Cordia melanii, Nuclea didiereshii* and other timber trees. It was observed that this layer of trees did not form canopy as found in lower layers. The second layer is also made of trees about 28m tall, with their crowns forming a canopy with the lower branches of the first layer. This layer included trees like *Terminalia superb, Lovoa trichloides* and *Entadrophrayma spp*. Below this second layer are other shorter trees about 10m tall, while the fourth layer is

made of shrubs and herbs of various heights, such as *Avengea gabonensis*, *Momordica charantia, Laphira lanceolata, Moringa oliefera* and *Tetracarpidium lonapharium*. Average weight of fallen vegetation or litter was about 1600g/m^2. Ago-Atsaviya falls within the very few high forest found only in Ondo and Cross-River States (Areola, 1984). There is no meteorological station in either Ago-Atsaviya or Owo. The climatic data of Akure, which is the closest metrological station was used. Rainfall in this area is about 2200mm per annum with double peaks: one in July and the other in September. The month of August is distinctly drier than either July or September. The rain is spread from February to November, December and January experience minimal rainfall. The sample taken from this plot was labeled HFV.

A second plot located about 2 km NW of the forest plot was also sampled. The plot was an abandoned plantation under kola (*Cola Nitida)* since 1976. The plantation is overgrown by various herbs and weeds such as *Pallisota* sp, *Baphia nitida, Baphia pubscens* and with trees like *Triplochiton scleroxylon.* The weight of leaf litter in this plot was about 1100g/m^2. The soil sampled from this plot was labeled HFF.

3.2. SOUTHERN GUINEA SAVANNA

The plot sampled in the Southern Guinea Savanna was located in Osara about 15 kilometer North-West of Okene along Okene-Lokoja highway at approximately Latitude7^o 41'N and Longitude 6^o 26"E. The plot is located South-West on the Okene-Lokoja highway about 6 kilometer from the highway. The terrain is gently undulating lowland with various herbs and climbers growing. Some of the plant species identified at the sampling sites include *Uapaca guinensis, Chromolaena odorata, Terminalian glaucescens, Khaya senegalensis, and Danielia olivieria.* Rainfall in this area is about

1250mm/annum with two peaks in July and September. August is distinctly dry, having lesser rain than both July and September. Vegetation cover is a mixed leguminous wooded savanna lacking in gigantic trees. There are few herbs, dense with climbers and grasses weight of litter of 960g/m^2. The sample taken from this plot was labeled SGV.

A second sample was obtained in Osara about 3 kilometer South-East of Osava at approximately Latitude7^o 43'N and Longitude 6^o 28'E. The plot has been under palm tree plantation since 1976. The undergrowth of the plantation has been taken over by various grasses and weed such as *Uapacca togoensis, Chromolaena odorata, Nauclea latifolia.* Weight of fallen leaf litter was about 800g/m^2. The sample taken from this plot was labeled SGF.

3.3 NORTHERN GUINEA SAVANNA

Sample from this ecological zone was obtained at Kagoro. The field sampled was a virgin plot under grasses. The plot was located at approximately Latitude 9^o 36' N and Longitude 7^o 16'E. The vegetation was open grassland with a few scattered shrubs within the grasses. Some of the vegetation identified included *Chromolaena odorata, Penisetum pupureum, Pilliostigma thoniningii*.

The landscape was mostly flat with slope of about 1^o. There was evidence of bush burning but the soil was practically uncultivated. Rainfall in this area is about 1250mm per annum with a double peak in July and September (Areola, 1984). August is distinctly dry, having lesser rain than both July and September. Vegetation cover was mixed leguminous wooded savanna lacking in gigantic trees. There are few herbs, dense with

climbers and grasses. Mass of litter was about 850g/m^2. The sample taken from this plot was labeled NGV.

Another field sample was taken along Kachia-Kagoro road/highway at approximately Latitude 9^0 48'N and Longitude 7^0 50' E. The field plot was situated about 1.5Km from the road and had been left under fallow since 1968. The vegetation was woodland savanna with abundant grasses. The woods were mainly shrubs that included species like *Parkia clapertoniana, Euphorbia hirta, Ficus spp.* Grasses identified include *Pennisetum pupureum, Andropogon gayanus,* and *Dannielia olivieria.* The vegetation was made of more than 70% grasses. Rainfall is about 1250mm per annum with a single peak in July. The dry season spans from October to March. Mass of litter measured about 860g/m^2. The soil from this plot was labeled NGF.

3.4 SAMPLE COLLECTION

Soil surface samples (0-10 cm) were collected from each of the study areas. Soil samples were collected from both virgin (undisturbed) and fallow fields of not less than 25 years in each of the ecological zones. Sampling consisted of 20 auger cores spread over an estimated area of not more than 100m x 100m in each field. This procedure was repeated three times. The collected samples were bulked together to form a single composite sample. The collected fresh vegetation was weighed and the weight recorded. The soil samples were air-dried, ground and sieved through a 2mm sieve and stored in a plastic container for further analyses and experimentation. In each of the sampled field, fresh vegetation was collected in a quadrant (grid) of 1m^2.

3.5 SOIL ANALYSIS

The following analyses were made on the sieved soil samples.

3.5.1 Soil Texture

Textural analysis of the soil was determined by the hydrometer method in accordance with suggestion of (Gee and Bauder, 1986). Fifty grams of 2 mm sieved soil samples were weighed into 250ml polythene bottles in three replicates. To this were added 100ml of freshly prepared calgon solution and 400 ml of distilled water. The mixture was left on a shaker for about 20 min and quickly transferred into a 1-L cylinder and made up to mark with distilled water. The soil suspension was adequately stirred with the aid of a plunger before taking the hydrometer readings. Hydrometer readings were taken at 40 seconds and 2 hours for silt and clay fraction readings respectively. A blank (without soil) was also prepared for comparison. The percentage composition of the various soil particles was calculated an textural classes assigned in accordance with the USDA. All experiments were done at room temperature.

3.5.2 Soil pH

The soil pH was measured in water and 0.01M $CaCl_2$ solution. Ten grams of 2mm sieved soil sample were weighed into plastic beakers. To this was added 20 ml of distilled water gradually and the soil suspension was thoroughly mixed using a glass stirring rod. For the $CaCl_2$, 20ml of 0.01M $CaCl_2$ solutions were added to the soil. In both cases, the soil suspension was allowed to stand for about 30 min with occasional stirring before the pH was determined using digital pH meter (Crison micropH-2000, United Kingdom). The determinations were replicated three times and the average reading taken.

3.5.3 Exchangeable Acidity

Exchangeable acidity was determined by the KCl extraction method (Thomas, 1982). Ten grams of < 2mm sieved sample was leached with 1 N KCl solution and filtered through a Whatman filter paper No. 42. The leachate was titrated with 0.1N NaOH solution using phenolphthalein indicator. Appearance of the first pink colour was considered to be the end point. A blank determination (without soil sample) was also run.

3.5.4 Effective Cation Exchange Capacity (ECEC)

The effective cation exchange capacity (ECEC) was determined by the summation of exchangeable bases and exchangeable acidity. Exchangeable cations were determined by the ammonium acetate method (Chapman, 1965). Ten grams of <2mm sieved sample were leached with 50 ml of 1N NH_4OAC solution adjusted to pH 7. K and Na in the leachate were determined using flame photometer while Ca and Mg were determined by atomic absorption spectrophotometer (Phoenix-986AA).

3.5.5 Total Nitrogen

Total nitrogen was determined by the micro-Kjedahl method (Bremner and Mulvaney, 1982). One gram of 0.5mm sieve sample was digested in 50-ml Kjedahl digestion flask. The cooled digest was made to 20ml with distilled water and the suspension transferred into a Kjedahl flask and 20 ml of 10N NaOH solution was added. Distilled water was added to make up the volume to 80 ml. The suspension was distilled into a 50-ml Erlenmeyer flask containing 5-ml boric acid indicator solution. Nitrogen in the distillate was determined by titrating with 0.1N HCl solution.

3.6.0 CHARACTERIZATION OF CARBON IN THE SOIL

3.6.1 Organic Carbon

Organic carbon (OC) in the soils was determined by wet dichromate oxidation method of Walkley-Black (Nelson and Sommers, 1996). One gram of < 2 mm sieved samples was weighed in triplicates into 250ml conical flask. Ten milliliters of 1 N $K_2Cr_2O_7$ solution were added to the soil in the flasks and gently swirled to allow the soil disperse in the solution.

To this were quickly added 20 ml concentrated H_2SO_4 and the flask shaken to allow a thorough mixing of the reagents. The flasks were allowed to cool for about 30 min by placing the flask on an asbestos pad. On cooling, 100 ml of distilled water was added with 5 drops of ferroin indicator. The mixture was titrated with freshly prepared 0.4M $Fe(NH_4)_2SO_4$ solution. A blank sample (without soil) was also determined concurrently. Organic carbon was calculated as:

$$OC(g/Kg\ soil) = \frac{M_{eq}K_2Cr_20_7 - M_{eq}Fe(NH_4)_2S0_4}{Weight\ of\ sample} x4$$

3.6.2 Water Soluble Carbon

Water soluble carbon was extracted by shaking 10g of finely ground sample (< 0.5mm) in 50 ml distilled water for 24 hrs (Burford and Bremner, 1975). The mixture was then centrifuged at 7650g for 30 mins. Carbon content in the filtrate was determined by wet oxidation method as described in section 3.6.1.

3.6.3 Metal Bonded Organic Carbon (MBOC)

Metal bonded organic carbon was extracted with 0.1N $Na_4P_2O_7$ solution. One gram of finely ground sample soil (< 0.5 mm) was shaken with 100 ml of 0.1N $Na_4P_2O_7$ solution in 200ml extraction bottle for 16hrs (Defelipo and Ribeiro, 1981). The soil mixture was then carefully transferred into 250 ml centrifuge tube and centrifuged at 5000 rpm (revolution per minute) for about 30mins. The amount of organic C in the filtrate was determined by the dichromate oxidation method as described in section 3.6.1.

3.6.4 Labile Organic Carbon (LOC)

Labile C was determined by extracting the soil with $CaHPO_4$ solution. One gram of < 0.5 mm sieved soil was extracted with 100 ml of 0.02 M $CaHPO_4$ solution for 16hrs (Defelipo and Ribeiro, 1981). Carbon in the extract was determined by the dichromate oxidation method as described in section 3.6.1.

3.7 EXTRACTION OF ORGANIC MATTER

Organic matter extraction was done by the method of Schnitzer (1982) as modified by Xing (2001). To 100g of air-dried soil sample passed through 2-mm sieve in a 2-L extraction bottle was added one litre of freshly prepared 0.5 M NaOH solution. Nitrogen gas was bubbled into this mixture for 3 minutes; the bottle was quickly stoppered, and shaken on a mechanical rotator. Extraction was allowed to proceed for 18hrs, after which the mixture was centrifuged at 10000rpm for 30 minutes. The supernatant was carefully decanted, stored in plastic bottle under N_2 atmosphere. To the remaining soil filtrate was added 50 ml of distilled water and shaken for 20 mins and centrifuged at 10000rpm for 30 minutes. The supernatant was added to the previous extract in the plastic bottle.

3.7.1 Fractionation of Extracted Organic Matter

The solution containing the humic substances was acidified to pH 2 with concentrated HCl. The HCl was added in small amounts with about 10 drops initially. After each set of drops of HCl, the mixture was stirred to allow complete reaction before taking the pH readings. As the pH approached 2 only a droplet was added each time and stirred before measuring the pH. At pH 2, the mixture was well stirred and left overnight for complete precipitation of humic acid, under N_2 atmosphere. The mixture was centrifuged at 7000rpm for 15 minutes and the solution containing the fulvic acid (FA) was carefully decanted and stored under N_2 atmosphere. The precipitate containing the humic acid (HA) was carefully transferred into 100 ml extraction bottle with the aid of stainless steel spatula. The residue was washed once with 25ml of distilled water and added to the HA in the extraction bottle.

3.7.2 Purification of the Extracted Humic Substances

To the HA precipitate was added 50ml of a mixed solution of HCl-HF (5ml conc. HCl + 5ml HF made up to 1L mark with distilled water) and left on a reciprocating shaker for 16hrs. The mixture was then centrifuged at 10000rpm and the supernatant carefully decanted. This procedure was repeated two more times. The HA was dried at standard temperature and stored.

3.8 ANALYSES OF THE EXTRACTED HUMIC SUBSTANCES

The following analyses were carried out on the extracted humic substances (humic acid, fulvic acid).

3.8.1 Determination of Carbon in Humic Acid (C_{HA})

0.1g of finely ground humic acid (obtained in 3.4.1) was weighed into 100ml Erlenmeyer flask. Carbon in the sample was determined by the dichromate oxidation method as described in section 3.6.1

3.8.2 Determination of Carbon in Fulvic Acid (C_{FA})

Dry sample of FA was obtained by carefully drying about 250ml of the FA solution obtained during fractionation (section 3.7.1) over water bath. 0.5g of the dried fulvic acid was weighed into 100ml Erlenmeyer flask and carbon in the sample determined by the dichromate oxidation method as described in section 3.6.1.

3.8.3 Determination of Humification Parameters

Humification degree (HD) and humification ratio (HR) were computed for the samples using the following relationships.

$$HD = \frac{C_{HA} + C_{FA}}{TEC}$$ (Petrussi et. al.,, 1988),

and

$$HR = \frac{C_{HA} + C_{FA}}{TOC}$$ (Petrussi et. al.,, 1988)

Where: HD = Humification degree

C_{HA} = Carbon content in humic acid

C_{FA} = Carbon content in fulvic acid

TEC = Total extracted carbon

HR = Humification ratio

TOC = Total organic carbon

3.9. FUNCTIONAL GROUP ANALYSES

The humic substances extracted were analyzed to determine the functional groups. The following functional groups were determined.

3.9.1 Determination of Total Acidity

Total acidity was determined by barium adsorption method or the indirect method (Schnitzer and Khan, 1972) as modified by Schnitzer (1990). 0.1g of the HA was weighed into 125 ml Erlenmeyer flask and allowed to equilibrate in 20 ml of 0.1M $Ba(OH)_2$ solution. The suspension was shaken for 24 h under N_2 atmosphere, before being filtered through Whatman No.42 filter paper. The residue was washed with distilled water and the solution titrated with 0.5M HCl solution potentiometrically. Total acidity was determined by:

$$\text{Total acidity (mmol}_c\text{/g)} = \frac{(V_{blank} - V_{sample})xM_{acid}}{weight\ of\ sample}$$

Where V_{blank} = titre volume for blank

V_{sample} = titre volume for sample

M_{acid} = molarity of acid used

3.9.2 Determination of Carboxyl Group.

Carboxyl group in HA was determined by the acetate method (Wright and Schnitzer, 1959). To 100 mg of the sample was added 10 ml of 0.5M calcium acetate ($Ca(OAC)_2$) solution and 20 ml distilled water. The suspension was shaken for 24 h under N_2 atmosphere. The suspension was filtered through Whatman No. 42 filter paper and the precipitate washed with distilled water. The filtrate and the washing were combined and titrated potentiometrically with 0.1M NaOH solution. The same procedure

was done for a blank solution containing no humic acid. Carboxyl group was calculated as

$$\text{COOH acidity (mmol}_c\text{/g)} = \frac{(V_{sample} - V_{blank})xM_{base}}{weight\ of\ sample}$$

Where V_{sample} = titre volume for sample

V_{blank} = titre volume for blank

M_{base} == Molarity of base used

3.9.3 Determination of Phenolic Group

Phenolic-OH groups in the extracts were computed as the differences between total acidity and carboxylic group (Swift, 1996).

3.9.4 Determination of Colour Quotient (E_4/E_6) Ratio

One milligram of HA was dissolved in 10 ml of 0.05N $NaHCO_3$ solution and adjusted to pH 8 with few drops of 0.1M NaOH solution (Chen et. al.,, 1977). The absorbance of the solutions was determined at 465nm and 665nm on a 6405 UV/Vis spectrophotometer (Jenway, United Kingdom). The values obtained were used to calculate the E_4/E_6 ratio.

3.10 ADSORPTION REACTION OF HUMIC ACID WITH METALS

About 0.3g of the purified HA obtained in 3.7.2 was weighed into 100 ml flask and titrated to pH 8 with 0.1M NaOH solution so as to bring the HA into solution. The solution of sodium humate was then made up to 200 ml with distilled water and protonated in an ion exchange resin column using Dowex 50W–X8 resin in (H) form. The resins were packed in a glass column of 15 cm length and 2 cm diameter fitted with a

valve tap and plugged with glass wool. A slow and regular flow rate of 1 ml min-^{1} was maintained to ensure complete and uniform protonation (Logan et. al.,, 1997). The protonated humic acid was adjusted to pH 4 using few drops of 0.01M KOH solution. Ten millilitres of the HS suspension was pipetted and 20ml of a mixed solution containing copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) metals at 500µg of each metal/g of HS was added and made to 50 ml with de-ionised distilled water. Adsorption was allowed to proceed for a period of 30 minutes and the solution quickly centrifuged at 10000g for 20 minutes (Logan et. al.,, 1997). The supernatant was carefully decanted into 60 ml extraction bottle and stoppered under N_2 atmosphere. The precipitate (metal-humate) was dried in desiccators under N_2 atmosphere The above procedure was repeated for metal concentrations of 1000µg, 2000µg, 3500µg, 7500µg and 15000µg/g HS. Concentrations of Cu, Zn, Pb, and Cd in the supernatant were determined by atomic absorption spectroscopy (AAS). Metal adsorbed by HS was calculated by the difference in the supernatant solution and the metal added to the HS. The same procedure was repeated for HS at pH 5 and 6.

Blanks were run using the same method but without humic acid. Sorption data obtained were fitted into Langmuir equation given as follows:

$$q = KbC/1+KC$$

where q = mass of adsorbate (metal ion) per unit weight of the adsorbent (HS) (g/Kg)

C = equilibrium concentration of the metal ion (mmol/L)

K = parameter related to the affinity of the adsorbent for the adsorbate

b = maximum adsorption.

The sorption data obtained were equally fitted into the linear Freundlich equation given as follows:

$$\log q = \log K + 1/n \log C$$

Where q = mass of metal ion adsorbed per unit weight of HS,

$\log K$ = Freundlich coefficient,

$1/n$ = adjustable coefficient,

$\log C$ = equilibrium concentration of the metal ion

Linear Langmuir isotherms were obtained by plotting c/q versus C, while maximum sorption *(b)* and affinity *(K)* were obtained from the slope and intercept respectively. By the same analogy Freundlich isotherms were also plotted by log q versus log C. Log K and $1/n$ were obtained from the intercept and slope of the plot respectively (Wang and Xing, 2005).

Adsorption on FA was not possible as it is impossible to obtain data by AAS. This is because metal-fulvate could not be precipitated from the reaction medium.

3.11 INFRA RED SPECTROSCOPIC ANALYSIS OF METAL– HUMATE COMPLEXES

The Infra-red (IR) spectroscopy of the humic substances was run on Perkin Elmer Infra-Red spectrophotometer. The samples used for analyses were:

(i) Humic acids after protonation as obtained in section 3.10. This is labeled HA.

(ii) Metal-humate precipitates obtained after reaction of HA with each of the metals at pH 4, and pH 6 from section 3.10 above: labeled (HA-Cu, HA-Zn, HA-Pb and HA-Cd).

(iii) Metal-humate precipitates obtained from section 3.10 after reaction of HA with a solution containing all the metals: labeled HA-MX.

About 5mg of dried sample humic acid (HA) obtained in section 3.10 at initial pH 4 was ground into fine powder using mortar and pestle. To this was added one drop of 'nujol' and the mixture ground to a fine dispersion called "Nujol mull" (Senesi and Loffredo, 1999). The nujol mull was placed between two pellets of NaCl and the IR spectra obtained on Perkin Elmer IR-spectrophotometer (Perkin Elmer model 399B) in the regions of 4000-400cm^{-1} (Senesi and Loffredo, 1999). The above procedures were repeated for HA sample at initial pH 6. Methylene chloride and hexane were used to clean the NaCl pellets before mounting the next sample. Good quality spectra were obtained by 50 scans using 2cm^{-1} resolution. Interferences in the C-H stretching region of nujol were compensated for by previously calibrating the instrument against 'nujol' (Schnitzer, 1991).

The above procedures were then repeated for HA-Cu, HA-Zn, HA-Pb, HA-Cd and HA-MX obtained in section 3.10 both at initial pH 4 and pH 6. The spectra obtained were analyzed to determine functional groups in HA involved in metal binding.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 PHYSICO-CHEMICAL PROPERTIES OF THE SOIL

The selected physical properties of the soils under study are presented in Table 1. The pH of the soils was slightly to weakly acidic for the soils except for the Forest virgin sample (HFV) which was about neutral in H_2O. Soil acidity decreased from the latitudes of the Northern Guinea savanna to the southern Forest soils. The pH [H_2O] was consistently higher than pH [$CaCl_2$] in all the studied samples, suggesting that the soils had net negative charge (Balasubramanian et. al.,, 1984). Virgin soils had higher pH than the corresponding fallow sample. Texturally, the sand content of the soils was in narrow range, except for the forest virgin soil (HFV). The sand content ranged from 40.6% to 49.6%, while silt and clay content ranged from 32-46% and 13.8 to 26.6% respectively. The textural class was mostly sandy clay loam.

4.2 ORGANIC CARBON DISTRIBUTION

The organic carbon characteristics including total organic carbon, water soluble organic carbon and labile organic carbon contents of the soils under study are reported in Table 2. Total organic carbon [TOC] increased from the Northern Guinea savanna to the Forest zone and ranged from 15.7 g kg^{-1} in NGV to 40.5 g kg^{-1} in HFF soil. Total organic carbon (TOC) obtained in this study was significantly higher in fallow samples both in forest (HFF) and Northern Guinea savanna (NGF) than their corresponding virgin samples This is in agreement with Jaiyeoba (1988) and Ekanade (1989) who both showed that soils

Table 1. Some selected physico-chemical characteristics of the soils from different ecological zones of Nigeria

Ecological Zones	pH H_2O	pH $CaCl_2$	Sand	Silt	Clay	Texture	Exch. Acid	ECEC
			---------- ($g\ kg^{-1}$) ----------				--------- $mmol\ kg^{-1}$ ---------	
Virgin								
HFV	7.3	6.3	443	321	266	Loamy sand	53	257
SGV	6.6	5.3	418	418	164	Loamy sand	73	200
NGV	5.3	4.0	472	414	159	Sandy loamy	22	84
Fallow								
HFF	5.9	4.5	406	456	138	Loamy sand	102	238
SGF	5.3	4.2	430	461	109	Loamy sand	9	90
NGF	5.1	4.2	496	432	162	Sandy loam	2	110

Key

HFF = High Forest Fallow
HFV = High Forest Virgin
NGF = Northern Guinea Savannah Virgin
NGF = Northern Guinea Savannah Fallow
SGV = Southern Guinea Savannah Virgin
SGF = Southern Guinea Savannah Fallow

Table 2. The influence of environmental gradient on the total organic carbon (TOC) and its characteristics in soils from the different ecological zones of Nigeria

Ecological Zones	TOC[1]	MBOC[2]	WSOC[3]	LOC[4]	Total N	C: N
	---------- g kg^{-1} ----------			mg kg^{-1}	g kg^{-1}	ratio
Virgin						
HFV	28.8	0.80	1.50	96.8	2.7	11.1
SGV	28.0	1.44	0.10	58.1	1.3	21.1
NGV	15.7	1.28	0.50	77.5	0.6	28.1
Fallow						
HFF	40.5	1.22	1.58	19.4	2.1	19.1
SGF	19.2	2.92	0.10	58.1	13.0	15.1
NGF	33.2	1.44	0.10	19.4	1.4	23.1

KEY

[1]Total organic carbon (TOC)
[2]Metal-binding organic carbon (MBOC),
[3]Water soluble organic carbon (WSOC)
[4]Labile organic carbon (LOC)

KEY

HFF = High Forest Fallow
SGF = Southern Guinea Fallow
NGF = Northern Guinea Fallow
NGV = Northern Guinea Virgin
SGV = Southern Guinea Virgin
HFV = High Forest Virgin

under forest fallow had higher organic matter than soils under virgin forest in Western Nigeria. Groendijke *et. al*., (2002) also found lower organic C in sites replaced by trees than open fallow sites. Similar trend was obtained in this study probably because of higher rate of litter decomposition in fallow than in virgin lands under forest or natural vegetation (Qualls *et. al*, 2003). Litter decomposition is slower in virgin than in fallow conditions (Qualls *et. al*., 2003). Differential rate of litter decomposition and organic matter turnover could be responsible for this.

Further, the litters in virgin plots as observed in this study were made of wooded plants and lignified vegetation which would be much slower to decompose by microbes, a position shared by other workers (Qualls et. al.,, 2003; Kumar and Goh, 2002). On the other hand, the fallow plots consisted of few kola trees (*Cola Nitida*), which were overgrown with various weeds and herbs which could be easier to decompose. Even though litter weight as reported in section 3.3 was significantly lower in the fallow plot (1100g/m^2) compared with the virgin (1600/m^2), the rapid rate of decomposition of weeds and herbs confers higher turnover of organic matter in fallow land than in virgin plots (Jaiyeoba, 1988).

Metal-bonded organic carbon (MBOC) varied in the samples under study and ranged from 0.80 mg kg^{-1} in forest virgin (HFV) to 2.92 mg kg^{-1} soil in Southern Guinea Savanna fallow (SGF). Values of Metal-bonded organic carbon were higher in fallow samples than corresponding virgin sample except in Northern guinea savanna (NGV and NGF) samples.

Water soluble organic carbon (WSOC) ranged from 0.1g kg^{-1} in savanna zone to1.58 mg kg^{-1}in forest zone. Water soluble organic carbon [WSOC] was not significantly different between virgin and fallow samples in all ecological zones. This is an indication that soil colloids in both virgin and fallow samples have about the same capacity to sorb

soluble organic carbon on their surfaces. There was however, a significant difference in WSOC between Northern Guinea savanna samples where virgin sample had higher WSOC than fallow samples.

Labile organic carbon (LOC) ranged between 1.94 mg kg^{-1} in fallow savannah (NGF) and 9.68 mg kg^{-1} in forest virgin (HFV) respectively. In both Forest and Northern Guinea savannah, labile organic carbon was significantly lower in virgin than fallow samples (Table 2). This is expected as LOC represent the fraction of soil organic carbon with most rapid turnover (Zou et. al.,, 2005). Labile organic carbon was therefore expected to be higher fallow plots where turnover was more rapid.

Total nitrogen ranged from 0.06 g kg^{-1} in NGV to 0.27 g kg^{-1} in HFV. The N content from SGF sample was higher than N content in the samples from all other ecological zones. The C: N ratio ranged from 11in HFV to 28 in NGV and was significantly higher in virgin indicating lower rate of decomposition than in fallow samples across the ecological zones.

4.3 CHEMICAL PROPERTIES OF THE EXTRACTED HUMIC SUBSTANCES

Humification parameters varied widely in the samples under study and are presented in Table 3. The range of carbon concentration in humic acids (C_{HA}) was from 181g Kg^{-1} in forest fallow sample to 683 g kg^{-1} in southern guinea savanna sample. The carbon in fulvic acids (C_{FA}) ranged from 147 g kg^{-1} in SGV to 568 g kg^{-1} in NGV samples both from savanna zones. Humification degree (HD) increased from forest to

Table 3. The influence of environmental gradient on the total organic carbon (TOC) and its characteristics in soils from the different ecological zones of Nigeria

Ecological Zones	C_{HA}[1]	C_{FA}[2]	C_{HA}:C_{FA} ratio	HR[3]	HD[4]	E_4	E_6	E_4 / E_6
	----------g kg^{-1}----------							
Virgin								
HFV	380	247	1.54	0.22	2.61	0.41	0.07	5.55
SGV	351	147	2.39	0.18	3.11	0.21	0.05	5.85
NGV	327	568	0.58	0.57	4.97	0.10	0.03	3.57
Fallow								
HFF	181	554	0.33	0.18	2.61	0.37	0.06	6.11
SGF	683	482	1.42	0.61	3.79	0.22	0.04	4.89
NGF	250	529	0.47	0.23	4.84	0.05	0.02	2.50

[1]Carbon concentration in humic acid fraction
[2]Carbon concentration in fulvic acid fraction
[3]Humification ratio (HR)
[4]Humification degree (HD)
E_4 = Optical density at 465nm
E_6 = Optical density at 665nm

KEY
HFF = High Forest Fallow
SGF = Southern Guinea Fallow
NGF = Northern Guinea Fallow
NGV = Northern Guinea Virgin
SGV = Southern Guinea Virgin
HFV = High Forest Virgin

NGS and ranged from 2.61 to 4.97. There was no significant difference in humification degree within each ecological zone. Humification ratio (HR) was however significantly different between Northern Guinea (NGS) and Southern Guinea savanna (SGS) ecological zones, ranging from 0.18 in HFF to 0.61 in SGF.

The ratio C_{HA} : C_{FA} is an indicator of the type of humification process going on in the soil. The results showed that C_{HA}/C_{FA} ratio was greater than unity in the forest and NGS samples indicating humification process in these samples seemed to favor the humate type. In the SGS and HFF samples fulvate type of humification seemed to be favored as the value of C_{HA}/C_{FA} was less than unity (Kononova, 1965). Values of C_{HA}/C_{FA} ratio were 1.54 and 2.39 for HFV and SGV respectively, indicating humification process in favour of humate (Table 3). The higher C_{HA}/C_{FA} ratios also indicated slow mineralization of organic matter (Fagbenro, 1988; Chen et. al.,, 2001). This supports the low OM content obtained in virgin plots (Table 2). In soils where mineralization is high more fulvic acid than humic acid is produced, hence lower HA: FA ratios.

The E_4/E_6 ratio which is an indication of optical density of HS was 6.11 in HFF and decreased from the forest plot to 2.50 in NGS (Table 3). Except in forest ecological zone E_4/E_6 ratio was significantly higher in virgin than in fallow plots indicating significantly higher contents of carboxylic groups in the virgin plots than their corresponding fallow plots (Table 4). The optical density parameter (E_4/E_6 ratio) therefore, expresses molecular condensation of HS (Stevenson. 1994; Chen et. al.,, 1977).

A value of E_4/E_6 ratio ≥ 5.0 indicates a reduced hydrophobicity while that ≤ 5.0 suggests an increased hydrophobic structure (Chen et. al.,, 1977). Results of E_4/E_6 ratio in the present study ranged from 6.11 in HFF to 2.50 in NGF indicating that the HA in HFF was less

hydrophobic. Fagbenro (1988) obtained E_4/E_6 ratio ranging from 5.5 to 6.1 for HA extracted from forest and savanna soils. Increase in hydrophobic character of the HA observed in this study from forest to the grassland of NGS was probably as a result of reduced woody trees from forest zone to NGS as observed on sample sites. Hydrophobic skeletons such as phenols are capable of influencing metal sorption in soils.

Table 4 shows the influence of environmental gradient on the concentrations of some functional groups in the extracted humic acids at pH 4. Across the ecological zones concentrations of phenolic groups were consistently and significantly higher than concentrations of carboxylic groups in both virgin and fallow plots. Concentrations of phenolic groups ranged from 4.59 cmol Kg^{-1} in NGF to 11.5 cmol Kg^{-1} in SGV. Phenols are part of major ligand sites for metal ion in humic acids and one of the major oxygen-containing functional groups (Adekunle et. al.,, 2007). These nucleophillic sites expressed in percent to total acidity constitute from almost 100% in NGF to a minimum of 67.2% in HFV (Table 4). This observation coupled with the values of total acidity obtained in this study are in consonance with values obtained for humic acid from Nigerian soils (Enenje and Mbagwu, 2006; Onianwa et. al.,, 2001). Concentrations of carboxylic groups in HFV, SGV and NGV were 3.33, 2.17 and 1.42 cmol Kg^{-1} respectively and were higher than their corresponding values in fallow plots. Carboxylic groups were almost indiscernible in NGF, a situation attested to by a very low E_4/E_6 ratio as seen in Table 3.

Table 4. The influence of environmental gradient on the concentrations of some functional groups in the humic acid at pH 4.0

Ecological Zones	Carboxylic group	Phenolic group	Total acidity	Percent phenol
	←---------- cmol kg^{-1} ----------→			
Virgin				
HFV	3.33	6.86	10.2	67.2
SGV	2.17	11.5	13.7	84.1
NGV	1.42	4.59	6.01	76.4
Fallow				
HFF	2.33	9.52	11.9	80.3
SGF	1.10	10.8	11.9	90.2
NGF	NA	5.01	5.01	100

KEY

HFG = High forest Virgin
SGV = Southern Guinea Savanna Virgin
NGV = Northern Guinea Savanna Virgin
HFF = High Forest Fallow
SGF = Southern Guinea Savanna Fallow
NGF = Northern Guinea Savanna Fallow

4.4 ADSORPTION OF METALS BY HUMIC SUBSTANCES

Metal adsorption to humic acids varied widely and depended on initial pH and the concentration of metal. Results of adsorption isotherms of Cu, Zn, Pb, and Cd on humic acid extracted from Forest virgin soil (HFV) are shown in Fig 4. Copper adsorption onto humic acid at pH 4, 5 and 6 exhibited distinct pH effect with increase in Cu adsorption as pH increased (Fig 4a). At pH 4 and 5 of the reaction medium, Cu adsorption followed the S-curve, while at pH 6 of the reaction medium the adsorption isotherm was the H- curve (Sposito, 1984). Adsorption of Pb (Fig 4c) followed the same trend as that of Cu but at pH 6, Pb adsorption did not follow the H-type isotherm observed for Cu but remains the L-type isotherm. Adsorption isotherm curves for Zn and Cd overlapped with no consistent trend with pH. The tendency for higher adsorption of Cd at either pH 4 and 5 than at pH 6 is quite abnormal for metal adsorption.

The HA extracted from HFF Cu and Pb adsorption distinctly reflected the increasing adsorption with increasing pH (Fig 5) although Zn adsorption exhibited similar pattern as Cd adsorption at low concentration of the added metal.

For HA extracted from NGV, Cu and Pb adsorption patterns were distinctly greater at pH 6 than at pH 5 or 4 (Fig.6). However, adsorption curves at pH 6 for Cu and Pb followed the H-type isotherm while at pH 4 and 5 adsorption was either the C-type or L-type isotherm. However, the adsorption of Cd and Zn did not follow a consistent trend with pH of the medium. As obtained for HA extracted from HFF or HFV, there were overlaps in the adsorption isotherm at different pH especially at equilibrium concentrations of Zn or Cd greater than 1.5 mmol L^{-1} (Fig.5).

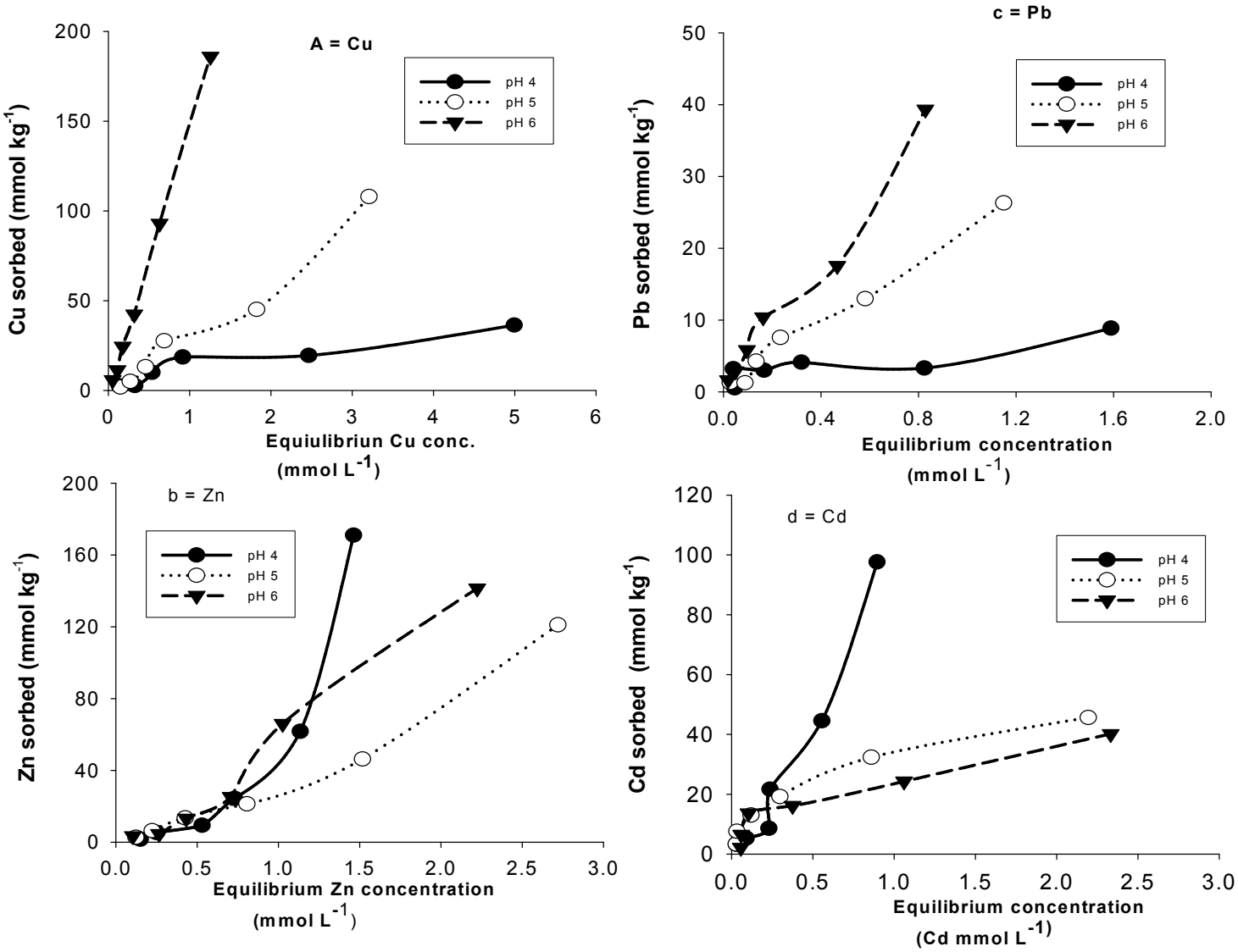


Figure 4. The adsorption isotherms of Cu, Zn, Pb and Cd by humic acid isolated from HVF samples as influenced by pH.

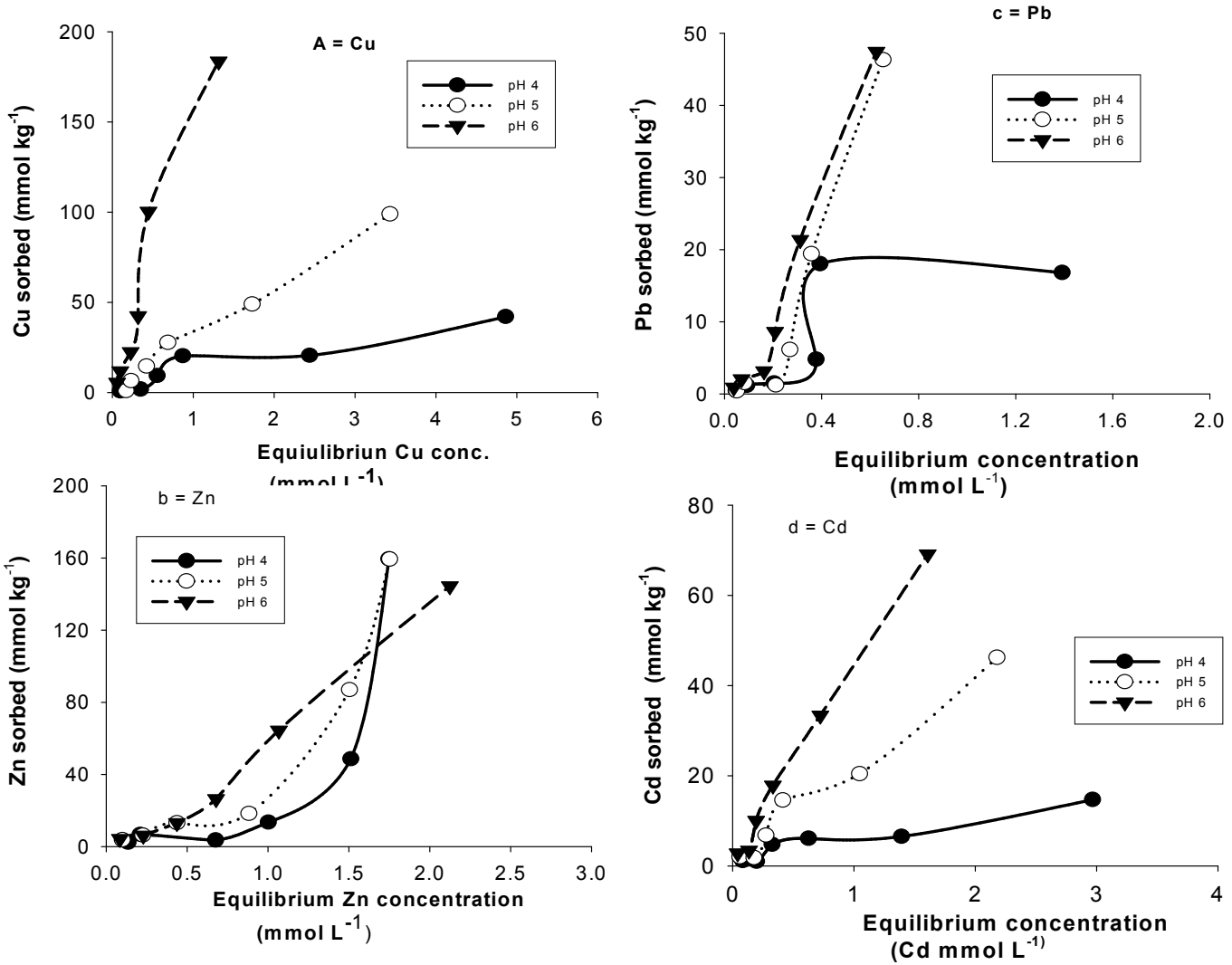


Figure 5. The adsorption isotherms of Cu, Zn, Pb and Cd by humic acid isolated from HFF samples as influenced by pH.

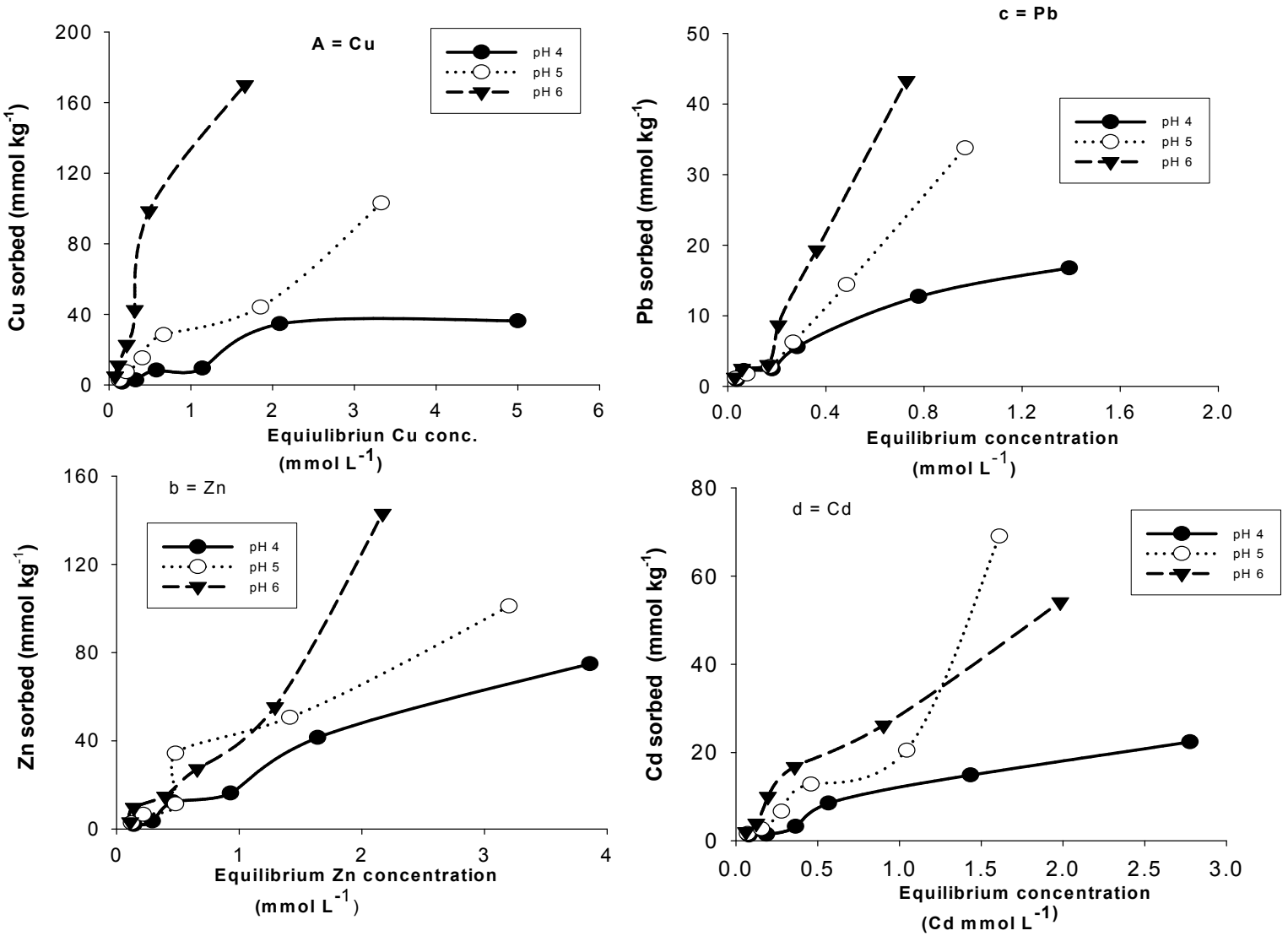


Figure 6. The adsorption isotherms of Cu, Zn, Pb and Cd by humic acid isolated from NGV samples as influenced by pH.

Expectedly, the adsorption of Cu, Pb, Cd and Zn to HA extracted from NGF actively showed the distinct effect of pH on metal adsorption (Fig 7). The adsorption of the metals was consistently greater at pH 6 than at pH 5 and at pH 5 than at pH 4, although there was no apparent difference between adsorption of Zn at pH 5 and 6. Except for Cu adsorption at pH 6 that exhibited the H type isotherm, adsorption of other metals generally followed the L or S curve isotherm (Fig 7).

The adsorption patterns of Cu, Pb, Zn and Cd in HA extracted from SGV and SGF are shown in Fig. 8 and Fig. 9 respectively. For HA derived from SGV (Fig. 8) adsorption increased with increasing pH of the adsorption medium with adsorption at pH 6 > 5 > 4 but there was no difference for adsorption of Pb at pH 5 and 6. Similarly, Zn adsorption at pH 5 and 6 was not different except at equilibrium concentration ≥ 2 mmol L^{-1} (Fig. 8).

Whereas the adsorption of Cu at pH 6 and Pb at pH 5 and 6 followed the H type isotherm, adsorption of Zn and Cd at all pH, and for Cu at pH 5 and 4 were generally the L- or S curves isotherms with the L-isotherm dominating the adsorption patterns. It is quite surprising to find that there was no effect of pH on Cu sorbed to HA extracted from the SGF samples (Fig. 9). This appeared to be indicative of competitive adsorption between Cu and other metals with one or more of such metals blocking Cu from prospective adsorptive sites. Adsorption of Pb, Cd and Zn increased with increasing pH.

The adsorption pattern for all the metals to HA extracted from SGF were generally the S type of isotherm (Fig. 9). Apart from Cu adsorption, the adsorption of Zn, Pb and Cd were either the S- or L- type whereas the C- type was less frequent.

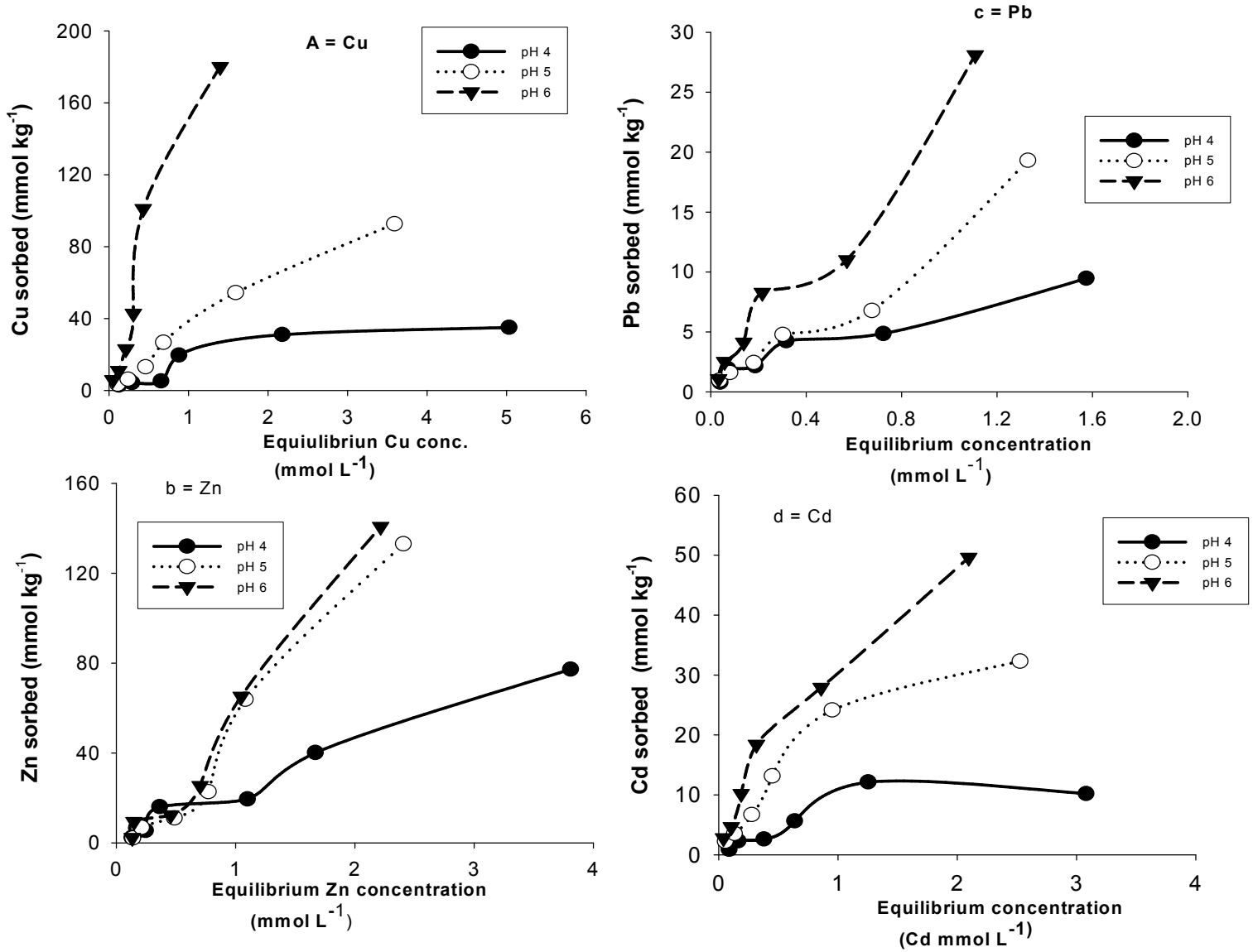


Figure 7. The adsorption isotherms of Cu, Zn, Pb and Cd by humic acid isolated from NGF samples as influenced by pH.

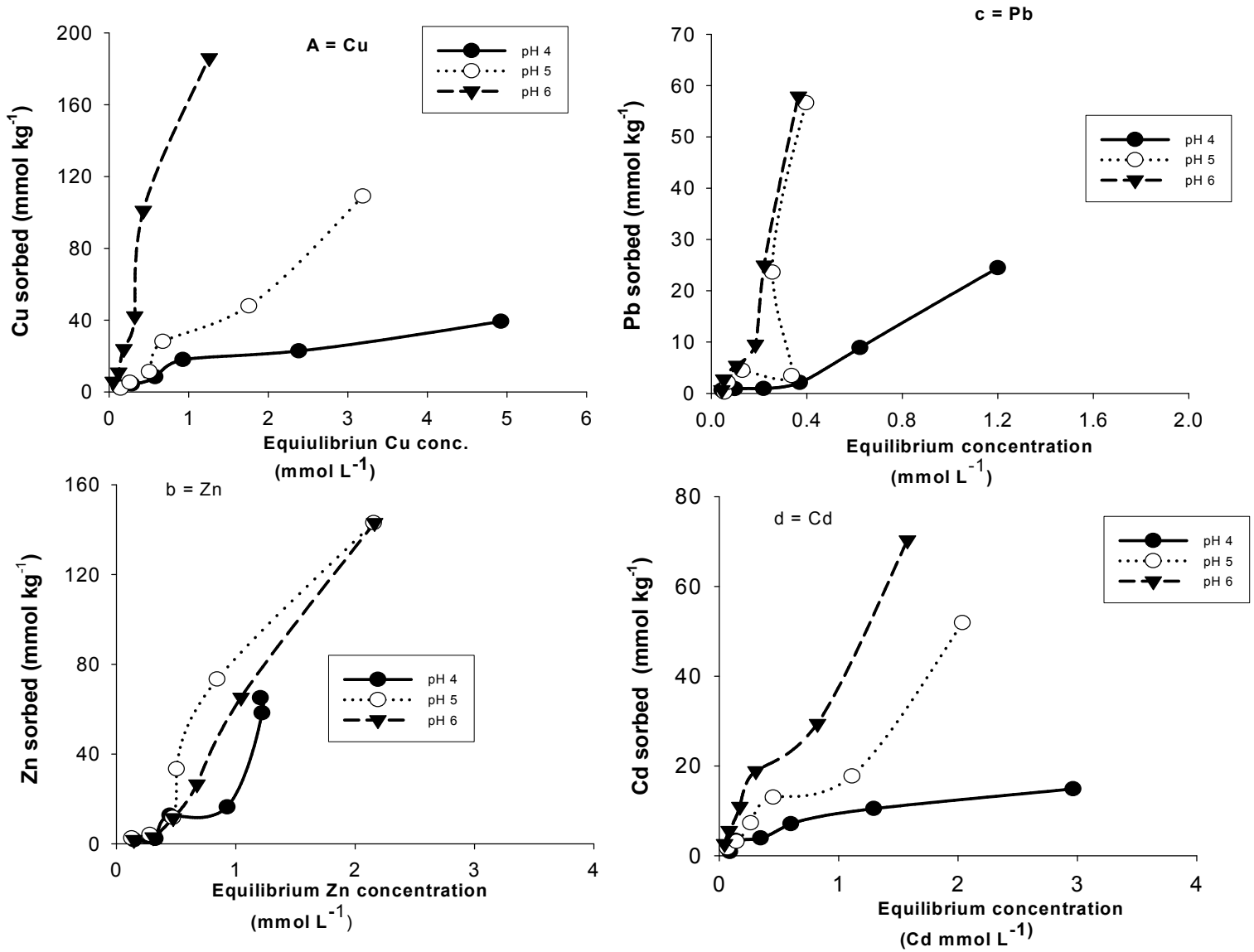


Figure 8. The adsorption isotherms of Cu, Zn, Pb and Cd by humic acid isolated from SGV samples as influenced by pH

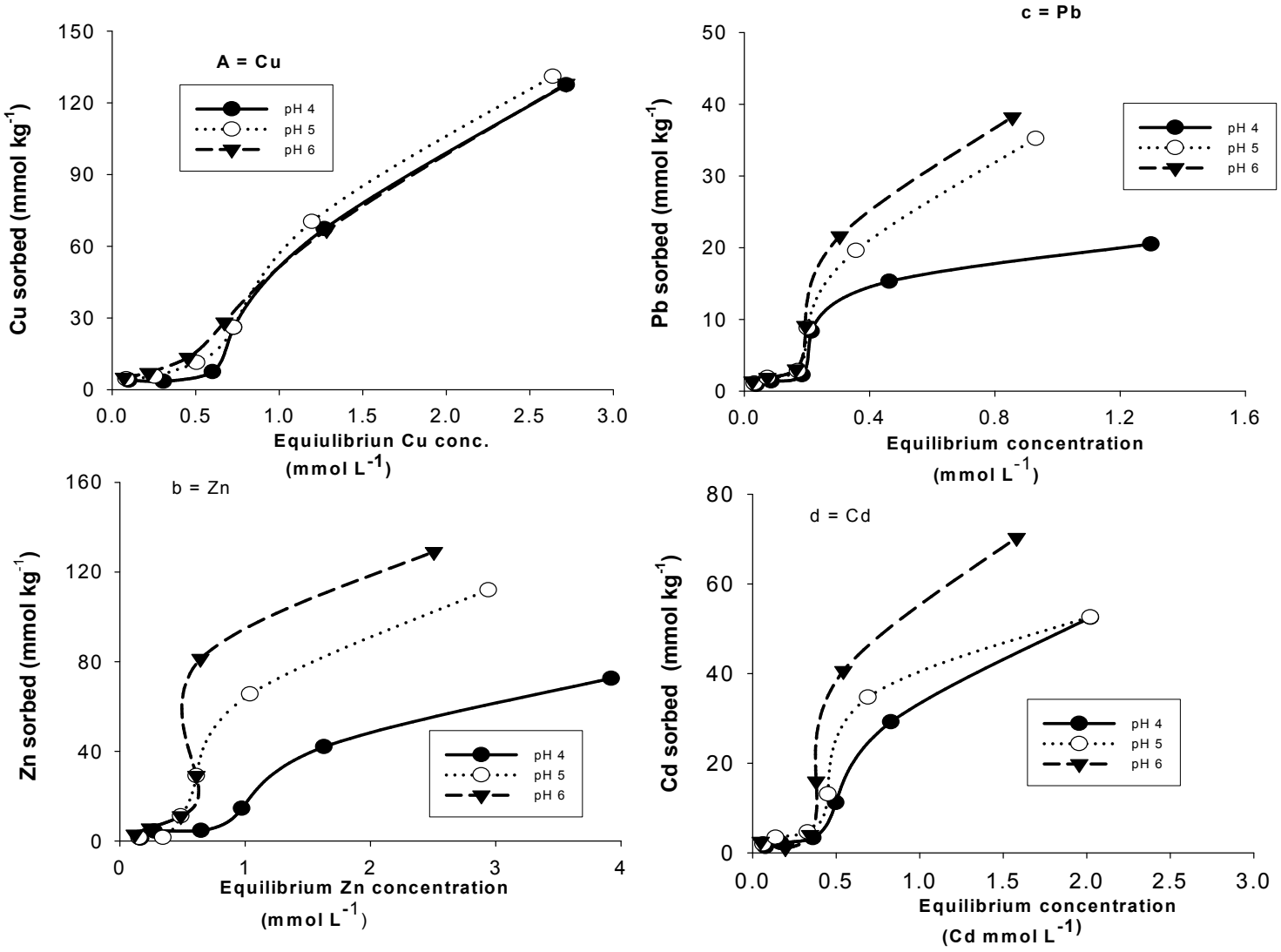


Figure 9. The adsorption isotherms of Cu, Zn, Pb and Cd by humic acid isolated from SGF samples as influenced by pH

The adsorption isotherms corresponding to L-isotherms refer to a great affinity between the metallic cations and adsorbent surface (Sposito, 1984). This type of isotherm curve is frequently reported (Fontes and Gomes, 2003: Agbenin and Olojo, 2004).

The H-isotherms refer to strong affinity between metallic cation and the HA adsorbent surface (Msaky and Calvet, 1990). A typical example in this case is Cu- HA at pH 6 except for SGF. The isotherm is suggestive of a very high affinity of the HA for Cu (Sposito, 1985). The distribution coefficient (K_d) of this curve decreases as the adsorbed metal increases (Msaky and Calvet, 1990).

The S-curve isotherm was the most common shape observed in this study. The S isotherm had an initially small slope that increased with increasing concentration of the adsorptive metal. This isotherm can result from competition between metal cations for adsorptive surfaces (Reddy and Dunn, 1986, Msaky and Calvet, 1990), or as a result of complexation by organic molecules (Sposito, 1984) such as humic acid (Msaky and Calvet, 1990). Buffle et. al., (1977) obtained S-curve isotherm for the complexation of HA and FA with Cu and Pb. Msaky and Calvet (1990) observed that adsorption of Zn on soil surface produced S-curve isotherm at pH > 4. Lu and Xu (2008) obtained results showing that in competitive adsorption system, adsorption isotherms for Cd, Cu, Pb and Zn exhibited significant differences in shape and quantity of adsorbed metal. With increase in metal loading of Cd, Cu, Pb and Zn, competition among the metals for sorptive sites becomes very strong in acidic conditions (Lu and Xu, 2008), but the tendency of the isotherms to form S-shape suggested competition for adsorption sites that were limited. In this competition, Cu seemed to have an upper hand with its characteristic H-type isotherm for its adsorption to HA extracted from the three ecological zones. H-type isotherms is characterized by an initially huge slope that suggest a very high affinity of the metal for the HA

adsorbent. H-type isotherm can also be produced by inner sphere complexation. According to Sposito (1989) large organic molecules mostly produce H-curves isotherms resulting either from van der Waal interaction between the adsorbate and the adsorbent or by inner sphere complexation. The affinity of macro molecule such as HA or dissolved organic carbon for Cu is well documented in the literature (Barancikova and Makovnikova, 2003; Vulkan *et. al*., 2002; Teminghoff *et. al*., 1998).

4.5 MODELLING THE ADSORPTION OF METALS TO HUMIC ACIDS

Modeling the adsorption of Cu, Zn, Cd and Pb by HA extracted from different ecological zones was attempted with two empirical adsorption models namely: the Langmuir equation and the Freundlich equation as described in section 3.6 of this thesis.

The goodness-of-fit of each model to the adsorption data was assessed by the magnitude of coefficient of determination r^2 and residual mean square error (RMSE). The greater the r^2 or the lower the RMSE value, the greater the fit of the model to the metal adsorption data. Although neither the Langmuir nor the Freundlich equation lends itself to any mechanistic interpretation, the two equations provide a means of summarizing adsorption data. Of the two equations, however, the parameters of the Langmuir equation have some physical significance in soils with reference to soil sorption capacity.

Generally, Cu, Zn. Pb and Cd adsorption by HA was poorly described by Langmuir models except for some isolated cases such as the adsorption of Cu on HA at pH 4 in SGV (Table 5), Cd adsorption on HA from HFV and NGF (Table 8). In such cases, the coefficient of determination (R^2) ranged from 0.85 – 0.98.

Fitting the adsorption data to either the Langmuir or Freundlich equation for Cu adsorption (Table 5) or Zn adsorption (Table 6) by HA showed that Langmuir equation was inadequate to characterize the adsorption isotherms evidenced by insignificant r^2 values that ranged from approximately 0.03 – 0.05 for Cu (Table 5) and from 0.13 – 0.62 for Zn (Table 6). In other words, the Langmuir equation was only predicting the adsorption of Cu by HA to between 3 and 45% and to between 13 and 62% for Zn. As observed for Cu and Zn adsorption, the Langmuir equation also gave poor description of Pb adsorption by HA (Table 7) as well as Cd adsorption by HA (Table 8).

The mean r^2 values for Pb adsorption as described by the Langmuir equation ranged from approximately 0.07 – 0.52 (Table 7) while that of Cd adsorption was between 0.11 and 0.87 (Table 8). This means a prediction capability of between 7 and 52% for Pb adsorption and 11 and 87% for Cd adsorption. The Langmuir equation can be regarded as a poor model for Pb and Cd sorption by HA extracted from soils in the ecological zones of Nigeria.

However, the Freundlich equation fairly well predicted adsorption of Cu by HA up to between 89 and 96% (Table 5) and that of Zn up to between 78 and 96% (Table 6) as can be seen in the r^2 values. As already observed for Cu and Zn, the Freundlich model gave relatively good prediction of Pb and Cd adsorption by HA extracted from the soils.

The r^2 values for Pb adsorption as described by Freundlich equation varied from 0.80 – 0.96 (Table 7) and from 0.82 – 0.95 (Table 8) for Cd. The Freundlich model predicted Pb adsorption up to between 80 and 96% and between 82 and 95% for Cd.

Table 5. The range and means of Langmuir and Freundlich adsorption parameters for Cu-humic acid adsorption at pH 4-6 from soils of different ecological zones of Nigeria

Ecological Zones	Langmuir[a]			Freundlich[b]		
	k	*b*	r^2	log *k*	*n*	r^2
Virgin						
HFV	0.009 – 0.067 (0.043)	0.002 – 0.015 (0.009)	0.259 – **0.440** (0.361)	0.79 – 1.27 (1.06)	0.46 – 0.94 (0.70)	0.87 – 0.99 (0.94)
SGV	0.009 – 0.054 (0.031)	0.002 – 0.016 (0.009)	0.178 – **0.819** (0.446)	0.75 – 1.26 (1.06)	0.45 – 0.90 (0.68)	0.96 – 0.97 (0.96)
NGV	0.008 – 0.061 (0.046)	0.001 – 0.015 (0.008)	0.032 – **0.443** (0.036)	1.01 – 1.32 (1.07)	0.46 – 1.00 (0.68)	0.93 – 0.96 (0.95)
Fallow						
HFF	0.009 – 0.157 (0.061)	0.002 – 0.019 (0.011)	0.071 – 0.188 (0.111)	1.16 – 1.32 (1.23)	1.04 – 0.70 (0.73)	0.84 – 0.96 (0.89)
SGF	0.025 – 0.059 (0.040)	0.002 – 0.016 (0.008)	0.050 – 0.218 (0.161)	0.95 – 1.20 (1.08)	0.62 – 0.67 (0.64)	0.82 – 0.97 (0.90)
NGF	0.010 – 0.096 (0.034)	0.003 – 0.026 (0.005)	0.007 – 0.084 (0.170)	0.77 – 1.16 (0.97)	0.46– 0.91 (0.68)	0.87 – 0.98 (0.93)

[a]*k* is affinity term L mol^{-1}); *b* is adsorption maximum (mmol kg^{-1}), r^2 is the coefficient of determination.
[b]log *k* and *n* are adjustable coefficients in the Freundlich equation.

KEY

HFG = High forest Virgin
SGV = Southern Guinea Savanna Virgin
NGV = Northern Guinea Savanna Virgin
HFF = High Forest Fallow
SGF = Southern Guinea Savanna Fallow
NGF = Northern Guinea Savanna Fallow

Table 6. The range and means of Langmuir and Freundlich adsorption parameters for Zn-humic acid adsorption at pH 4-6 from soils of different ecological zones of Nigeria

Ecological Zones[c]	Langmuir[a]			Freundlich[b]		
	k	*b*	r^2	log *k*	*n*	r^2
Virgin						
HFV	0.040 – 0.082 (0.054)	0.006 – 0.055 (0.025)	**0.451 – 0.775 (0.624)**	1.16 – 1.90 (1.47)	0.53 – 0.86 (0.71)	0.94 – 0.99 (0.96)
SGV	0.013 – 0.076 (0.046)	0.020 – 0.082 (0.046)	**0.372 – 0.572 (0.482)**	1.60 – 1.97 (1.79)	0.51 – 0.63 (0.56)	0.83 – 0.96 (0.90)
NGV	0.038 – 0.054 (0.041)	0.001 – 0.005 (0.004)	0.012 – 0.230 (0.126)	1.09 – 1.14 (1.11)	0.88 – 0.92 (0.89)	0.91 – 0.96 (0.94)
Fallow						
HFF	0.031– 0.094 (0.053)	0.002 – 0.030 (0.017)	0.094 – 0.424 (0.205)	1.16 – 1.26 (1.22)	0.79 – 0.86 (0.82)	0.67 – 0.94 (0.78)
SGF	0.036 – 0.109 (0.084)	0.009 – 0.035 (0.021)	0.199 – 0.291 (0.244)	1.31 – 1.70 (1.45)	0.59 – 0.77 (0.70)	0.84 – 0.93 (0.87)
NGF	0.027 – 0.062 (0.047)	0.001 – 0.015 (0.009)	0.001 – 0.477 (0.382)	0.93– 1.41 (1.20)	0.71 – 1.08 (0.91)	0.90 – 0.96 (0.90)

[a] *k* is affinity term L mol^{-1}); *b* is adsorption maximum (mmol kg^{-1}), r^2 is the coefficient of determination.
[b] log *k* and *n* are adjustable coefficients in the Freundlich equation.

KEY

HFG = High forest Virgin
SGV = Southern Guinea Savanna Virgin
NGV = Northern Guinea Savanna Virgin
HFF = High Forest Fallow
SGF = Southern Guinea Savanna Fallow
NGF = Northern Guinea Savanna Fallow

Table 7. The range and means of Langmuir and Freundlich adsorption parameters for Pb-humic acid adsorption at pH 4-6 from soils of different ecological zones of Nigeria

Ecological Zones[c]	Langmuir[a]			Freundlich[b]		
	k	*b*	r^2	log *k*	*n*	r^2
Virgin						
HFV	0.015 – 0.058 (0.043)	0.011 – 0.146 (0.088)	**0.484 – 0.559 (0.520)**	0.45 – 0.88 (0.72)	1.13 – 2.22 (1.52)	0.52 – 0.99 (0.80)
SGV	0.014 – 0.155 (0.113)	0.079 – 0.317 (0.011)	0.210 – 0.439 (0.286)	1.13 – 1.95 (1.64)	0.51 – 0.88 (0.65)	0.69 – 0.93 (0.82)
NGV	0.032 – 0.058 (0.045)	0.015 – 0.080 (0.039)	0.032 – 0.191 (0.165)	0.59 – 1.09 (0.90)	0.92 – 1.71 (1.20)	0.69 – 0.95 (0.85)
Fallow						
HFF	0.043 – 0.124 (0.094)	0.019 – 0.182 (0.083)	0.023 – **0.551 (0.308)**	1.11 – 1.78 (1.44)	0.56 – 0.90 (0.72)	0.75 – 0.95 (0.85)
SGF	0.032 – 0.048 (0.039)	0.006 – 0.019 (0.172)	0.020 – 0.115 (0.071)	1.00 – 1.12 (1.06)	0.89 – 1.01 (0.95)	0.87 – 0.92 (0.89)
NGF	0.029 – 0.057 (0.047)	0.015 – 0.080 (0.038)	0.238 – **0.827 (0.474)**	0.61 – 0.85 (0.75)	1.17 – 1.64 (1.35)	0.94 – 0.97 (0.96)

[a] *k* is affinity term L mol^{-1}); *b* is adsorption maximum (mmol kg^{-1}), r^2 is the coefficient of determination.
[b] log *k* and *n* are adjustable coefficients in the Freundlich equation.

KEY

HFG = High forest Virgin
SGV = Southern Guinea Savanna Virgin
NGV = Northern Guinea Savanna Virgin
HFF = High Forest Fallow
SGF = Southern Guinea Savanna Fallow
NGF = Northern Guinea Savanna Fallow

Table 8. The range and means of Langmuir and Freundlich adsorption parameters for Cd-humic acid adsorption at pH 4-6 from soils of different ecological zones of Nigeria

Ecological Zones[c]	Langmuir[a]			Freundlich[b]		
	k	*b*	r^2	log *k*	*n*	r^2
Virgin						
HFV	0.008 – 0.016 (0.013)	0.006 – 0.019 (0.013)	0.086 – **0.972** (**0.630**)	0.53 – 1.00 (0.71)	1.89 – 1.72 (1.53)	0.77 – 0.93 (0.86)
SGV	0.016 – 0.066 (0.041)	0.003 – 0.044 (0.018)	0.036 – **0.857** (**0.451**)	0.71 – 0.96 (0.85)	1.04 – 1.60 (1.20)	0.88 – 0.98 (0.95)
NGV	0.026 – 0.106 (0.061)	0.006 – 0.300 (0.106)	0.132 – 0.394 (0.295)	0.77 – 1.16 (0.95)	1.04 – 1.30 (1.08)	0.82 – 0.96 (0.92)
Fallow						
HFF	0.023 – 0.112 (0.062)	0.000 – 0.036 (0.013)	0.000 – 0.322 (0.110)	0.76 – 0.97 (0.89)	1.03 – 1.31 (1.13)	0.84 – 0.94 (0.89)
SGF	0.041 – 0.079 (0.064)	0.003 – 0.041 (0.021)	0.018 – 0.238 (0.123)	1.04 – 1.23 (1.13)	0.82 – 0.97 (0.89)	0.63 – 0.93 (0.82)
NGF	0.017 – 0.077 (0.041)	0.012 – 0.067 (0.032)	**0.824 – 0.893** (**0.865**)	0.72 – 0.77 (0.75)	1.30 – 1.38 (1.34)	0.89 – 0.97 (0.94)

[a] *k* is affinity term (L mol^{-1}); *b* is adsorption maximum (mmol kg^{-1}), r^2 is the coefficient of determination.
[b] log *k* and *n* are adjustable coefficients in the Freundlich equation.

KEY

HFG = High forest Virgin
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NGV = Northern Guinea Savanna Virgin
HFF = High Forest Fallow
SGF = Southern Guinea Savanna Fallow
NGF = Northern Guinea Savanna Fallow

The failure of the Langmuir equation to adequately describe the sorption of the metal is hardly surprising given the assumptions used to derive this equation. The assumption of energetically homogenous adsorption sites can rarely be applicable to heterogeneous products like HA with a myriad of functional groups with varying reactivity. For instance, the ionization constant for various functional groups in HA such as phenolic, alcoholic, acetic and sulphydryl, quinol and amino groups varies widely, so also is their reactivity including their responses to pH changes (changes in protonation and de-protonation). Consequently, it could hardly be assumed that metals sorption sites in HA are energetically similar.

The Freundlich model was somewhat more successful in describing the adsorption of Cu, Zn, Pd and Cd by HA extracted from soils across the ecological zones than the Langmuir model. The linear transformation of the Freundlich equation requires a log – log transformation such that the log of the concentration of the adsorption metal was plotted against the log of the concentration of the metal in the equilibrium soil solution. The log – log plot of the adsorption data for the metal adsorption by HA extracted from soils across the ecological zones are shown in Figures 10 – 15. In all plots, the log–log transformation produced strongly linear relationship.

Most investigators (Holford, 1982; Barrow, 1985; Polyzoupoulos *et. al.*,, 1985; Ratkowsky, 1986) have, however, pointed out that successful prediction of adsorption of metals on ligands by soils and soil components by Freundlich equation can be attributed to the log – log transformation of adsorption data with a considerable loss of sensitivity of data.

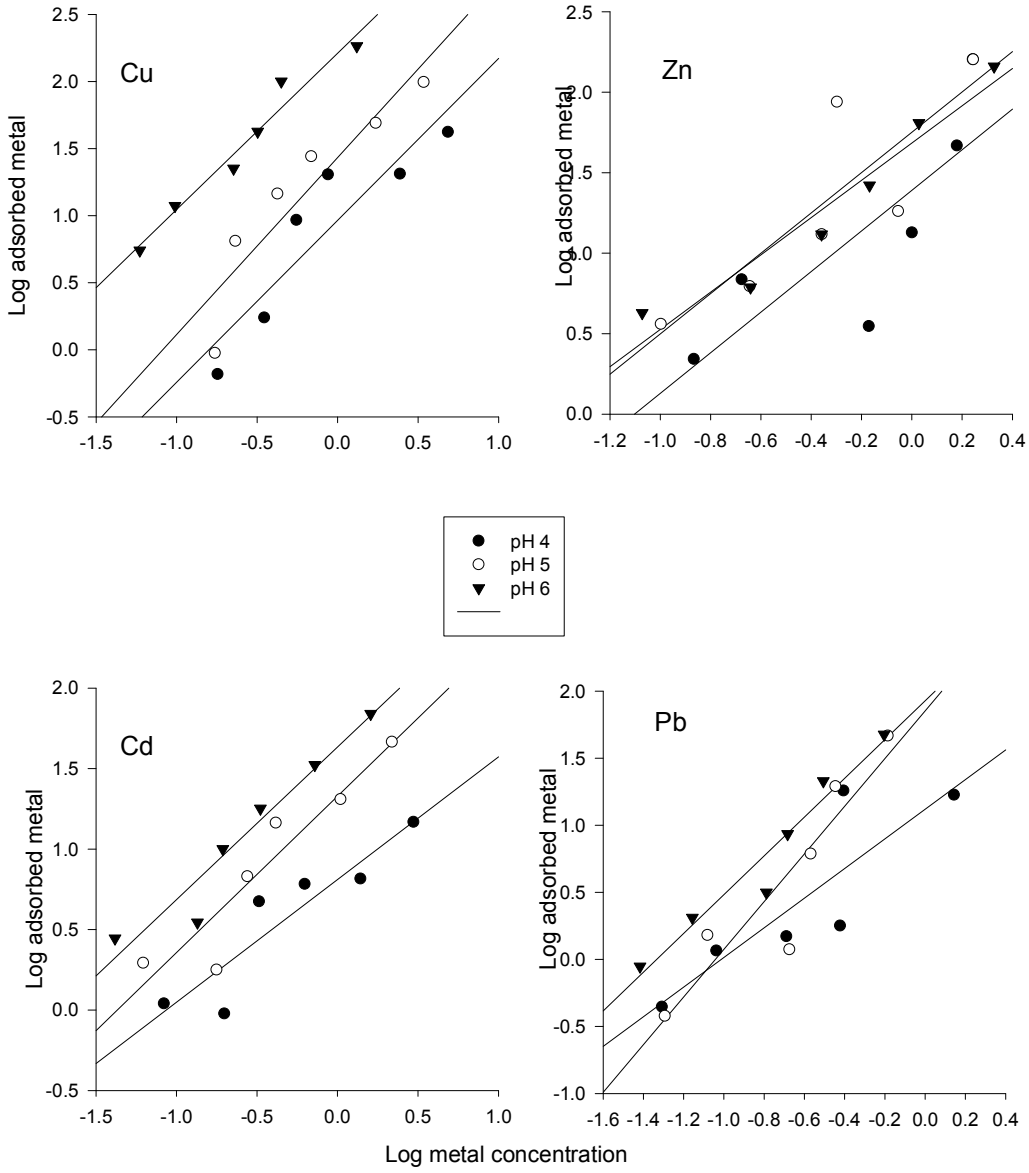


Figure 10. The adsorption of Cu, Zn, Pb and Cd by HA extracted from HFF at pH 4-6 as described by the Freundlich adsorption model

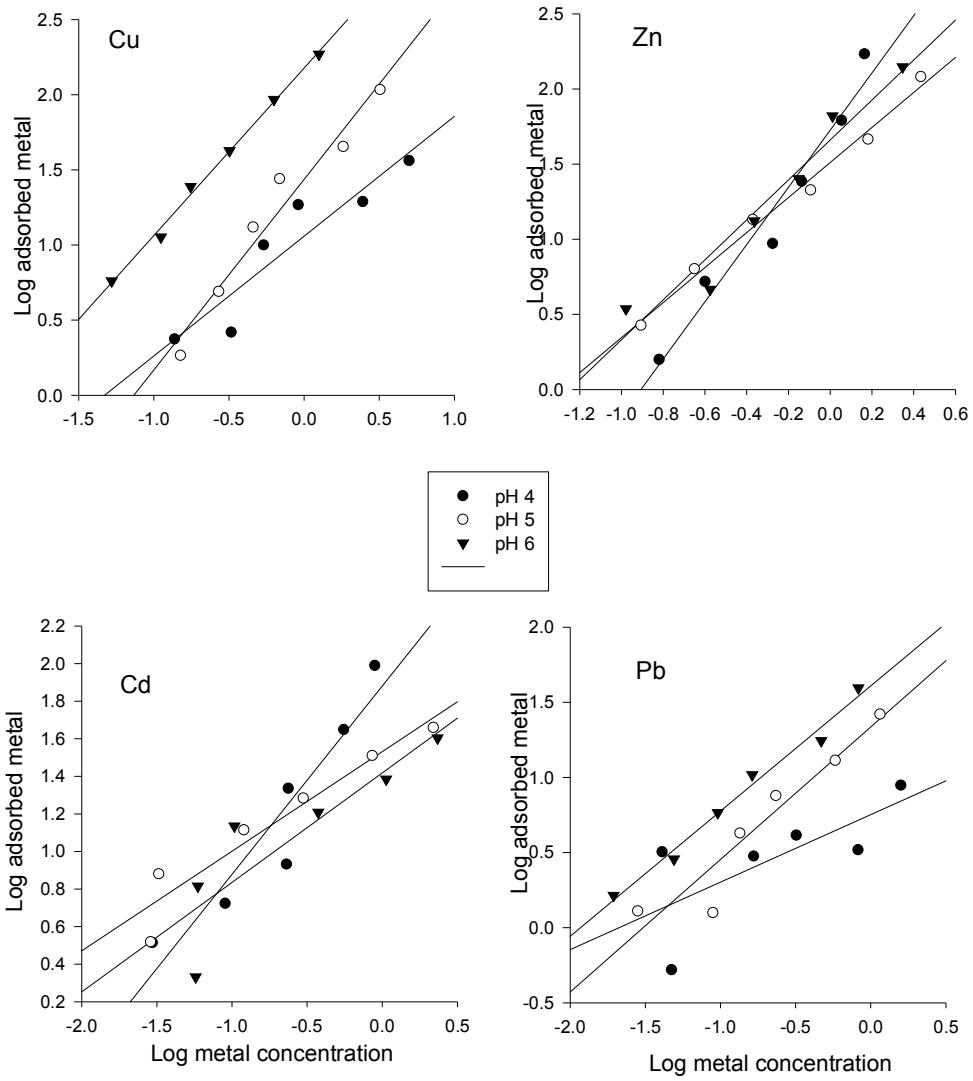


Figure 11. The adsorption of Copper (Cu), Zinc (Zn), Lead (Pb) and Cadmium (Cd) by Humic Acid (HA) extracted from High Forest Fallow (HFF) at pH 4-6 as described by the Freundlich adsorption model.

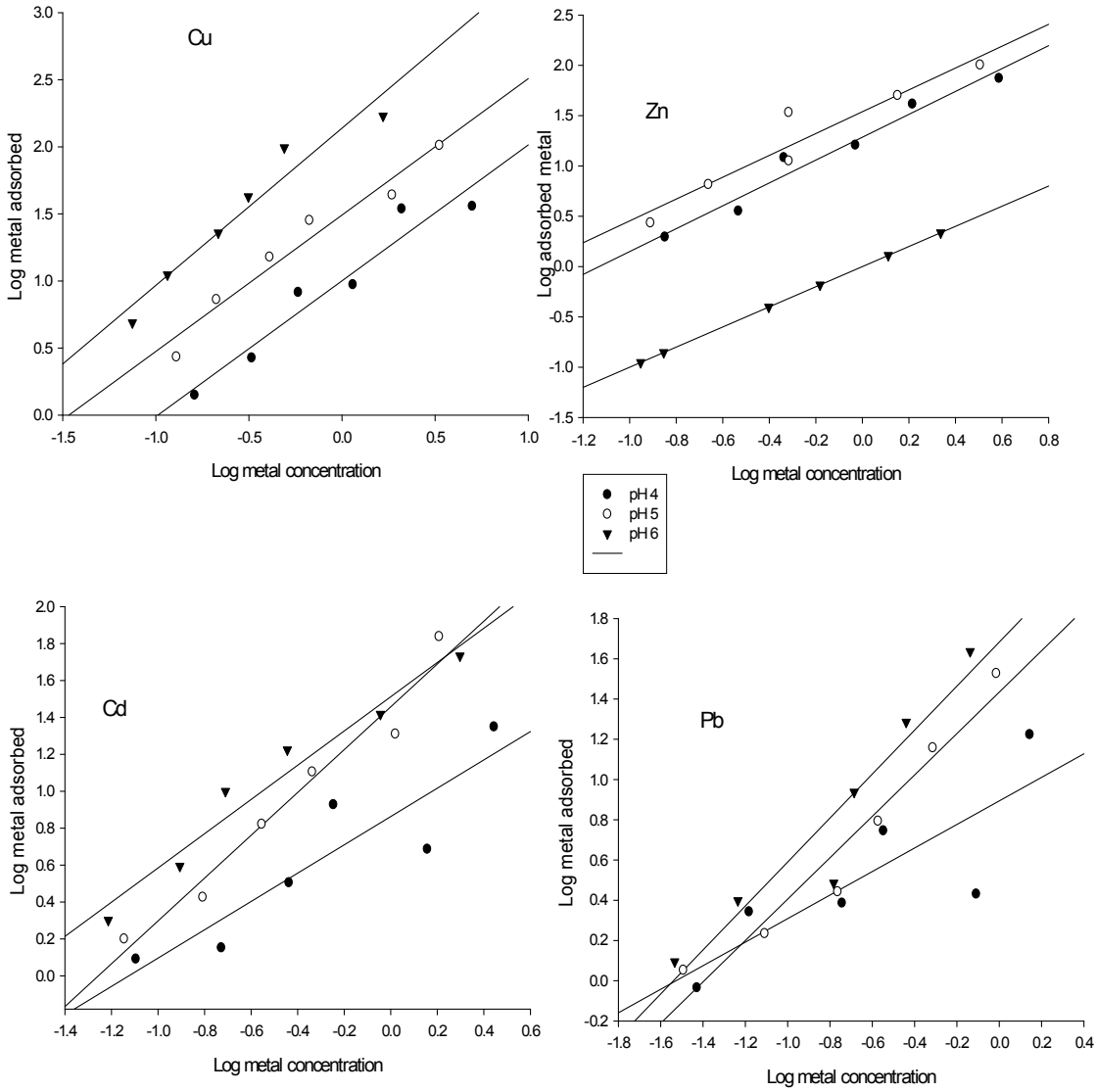


Figure 12. The adsorption of Copper (Cu), Zinc (Zn), Lead (Pb) and Cadmium (Cd) by Humic Acid (HA) extracted from NGV at pH 4-6 as described by the Freundlich adsorption model

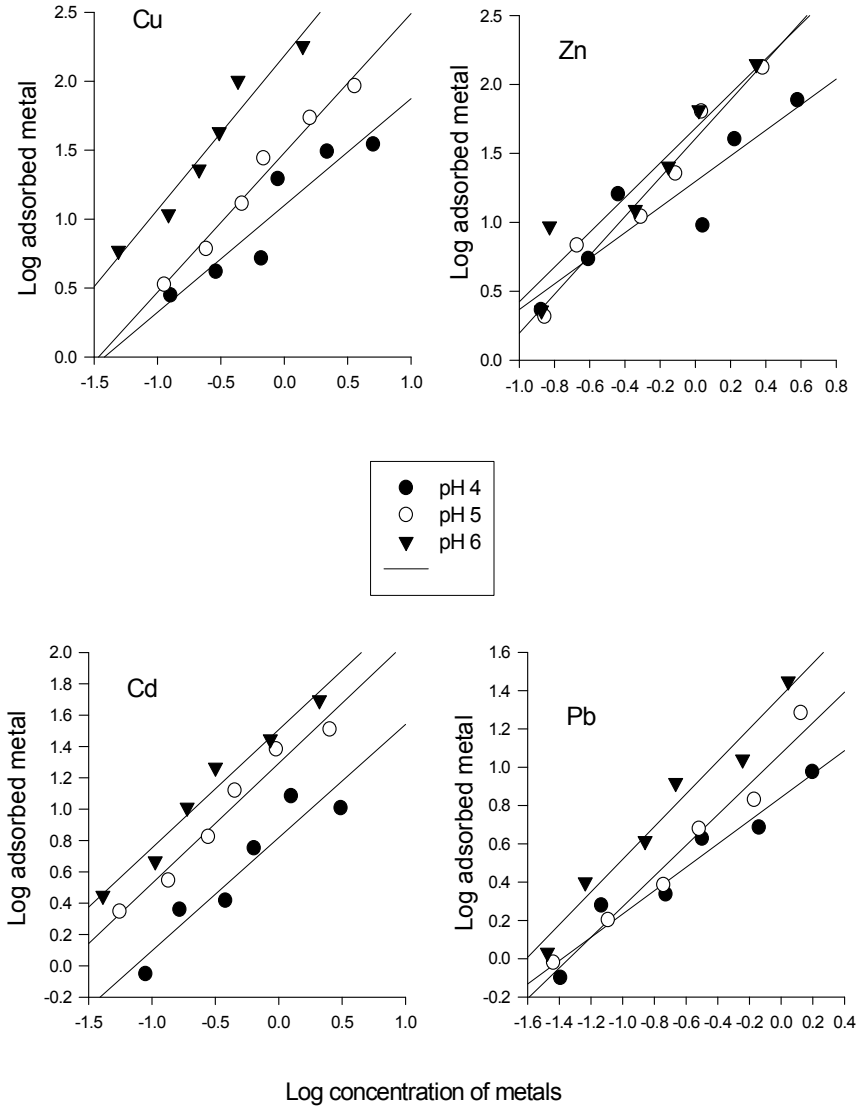


Figure 13. The adsorption of Copper (Cu), Zinc (Zn), Lead (Pb) and Cadmium (Cd) by Humic Acid (HA) extacted from NGF at pH 4-6 as described by the Freundlich adsorption model

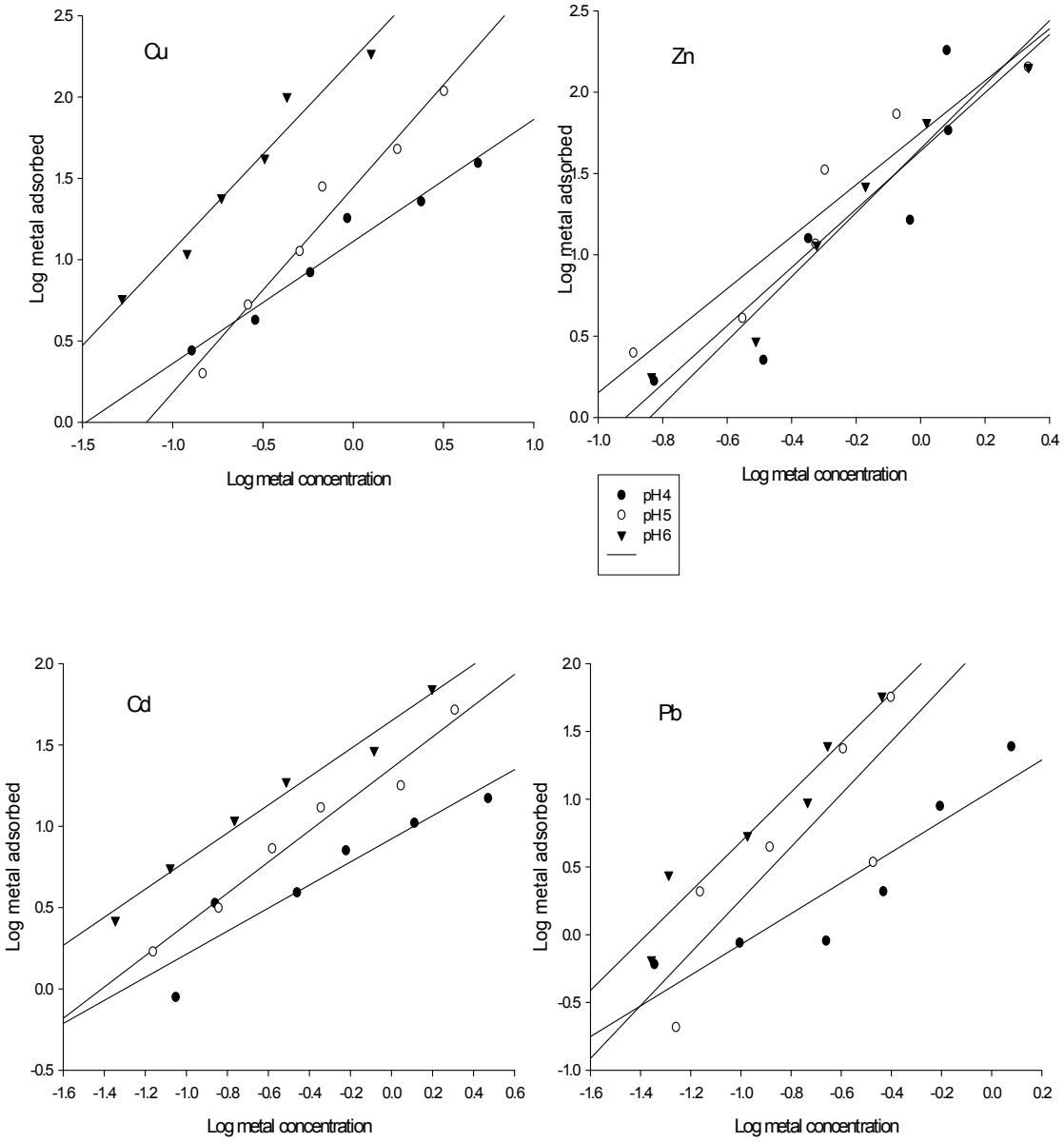


Figure 14. The adsorption of Copper (Cu), Zinc (Zn), Lead (Pb) and Cadmium (Cd) by Humic Acid (HA) extracted from SGV at pH 4-6 as described by the Freundlich adsorption model

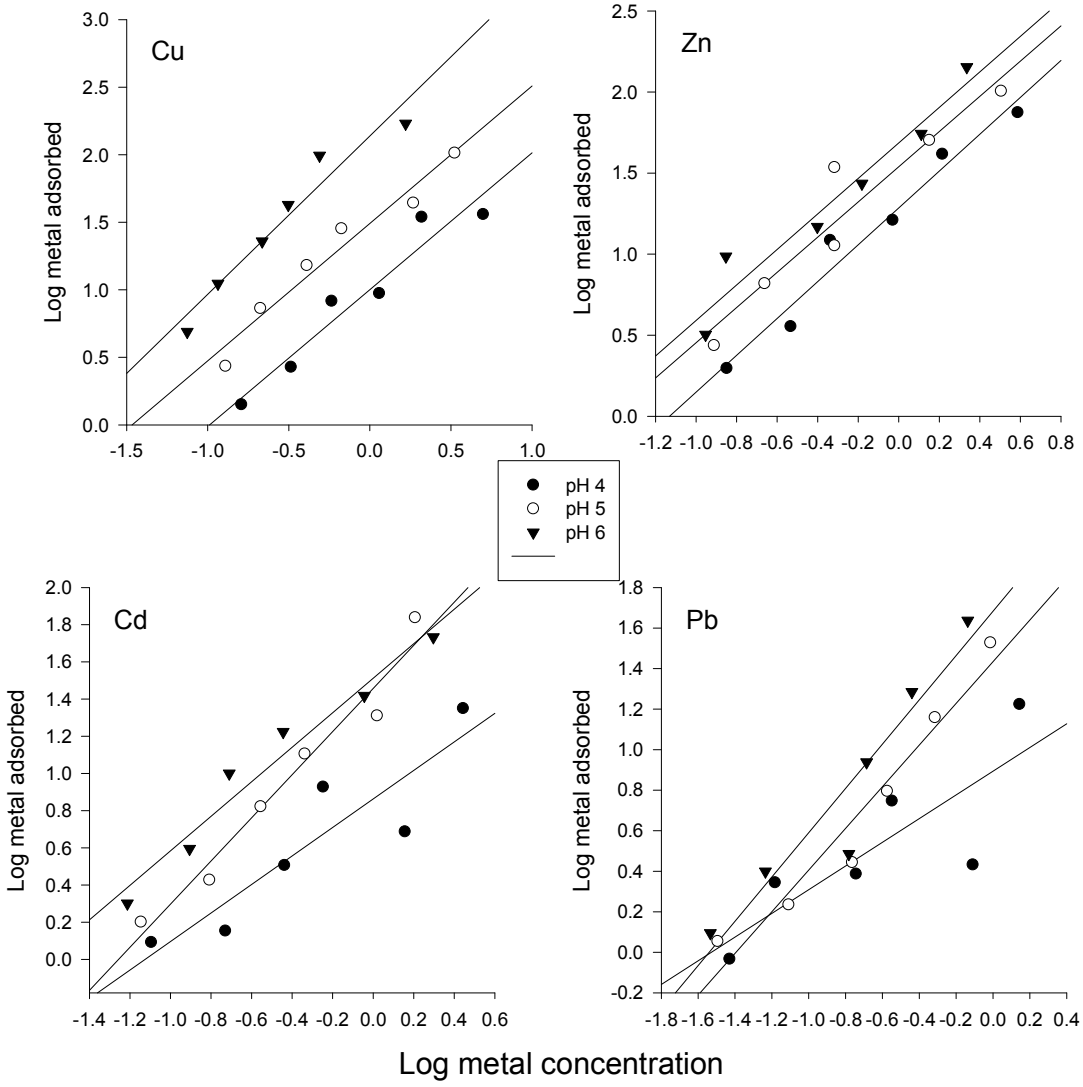


Figure 15. The adsorption of Copper (Cu), Zinc (Zn), Lead (Pb) and Cadmium (Cd) by Humic Acid (HA)extracted from SGF at pH 4-6 as described by the Freundlich adsorption model

The Freundlich isotherm describes adsorption in which the metal affinity for HA decreases exponentially as the amount of the metal sorbed increases (Sposito, 1989; Barrow, 1978). Although, the parameters of the Freundlich equation may not have physical meaning for metal adsorption by HA in terms of defining sorption maximum and affinity parameters as is possible for Langmuir equation, the parameter *k* can be interpreted as the concentration of the metals sorbed that can sustain a unit change in soil solution concentration of the metal (Polyzopoulos et. al.,, 1985). Consequently, *k* can be used as a measure of the metal sorption capacity of the HA. On the basis of *k* values for the metals, the reactivity of HA extracted across the ecological zones with respect to the metal adsorption can be assessed.

Grouping the HA on the basis of the environmental conditions of the soil during sampling (either virgin or fallow land), it can be seen that under virgin conditions, HA extracted from the Forest, Northern and Southern Guinea savannah had a high capacity to adsorb Cu (Table 9), whereas adsorption capacity for Zn and Pb was greater in HA extracted from SGV than the other ecological zones. However, Cd adsorption was greater in NGV than in the other soil samples. Similarly, under fallow Cu and Pb adsorption capacities were greater for HA from HFF than the other ecological zones, Zn and Cd adsorption were greater for HA for SGF than the other ecologies (Table 9). The lack of clear cut consistency across metal in the reactivity of HA could probably be a result of anionic interferences.

Table 9. Relative adsorption capacities (K) of HA for Cu, Zn, Pb and Cd from various ecological zones under fallow or virgin condition indexed as k from the Freundlich equation.

	Virgin	Fallow
Metal	Relative adsorption capacities	Relative adsorption capacities
Cu	NGV ≥ SGV = HFV	HFF > SGF > NGF
Zn	SGV > HFV > NGV	SGF > HFF > NGF
Pb	SGV > NGV > HFV	HFF > SGF > NGF
Cd	NGV > SGV > HFV	SGF > HFF > NGF

HFF = High Forest Fallow

HFV = High Forest Virgin

NGF = Northern Guinea Savannah Virgin

NGF = Northern Guinea Savannah Fallow

SGV = Southern Guinea Savannah Virgin

SGF = Southern Guinea Savannah Fallow

The interpretation of the *n* value for the Freundlich model for ionic adsorption is not very clear. But Sposito (1980) indicated that *n* is a measure of the inflection of the adsorption isotherm of the experimental data. Hence, according to Sposito (1980), *n* can be mathematically demonstrated to describe the heterogeneity of the adsorption sites in the adsorbent surface. For sorption data adequately described by Freundlich *n* is constrained to lie between 0 and 1. As *n* approaches zero, the heterogeneity of the adsorption increases, while as *n* approaches unity there is an increasing homogeneity of adsorption sites (Sposito, 1980). As evident from Tables 5-8, adsorption sites for Cu and Zn could be said to be more heterogeneous than the adsorption sites for Cd and Pb. The *n* values for Cd and Pb adsorption, as described by the Freundlich model, were all within or slightly above unity whereas values of *n* for Cu and Zn adsorption were below unity indicating wide distribution of adsorption sites in contrast to the narrow or localized adsorption sites for Cd and Pb, This may explain why adsorption of Cu and Zn by the HS was greater than Cd and Pb.

The reasons for the variation in sorption capacities of HA from the different ecologies are not immediately apparent although the statistical significance of the variation in adsorption capacities between the ecological zones were not assessed because of the relatively few samples involved in this report. However, it may not be unconnected with variation in functional group activities and humification parameters of HA for the different ecological zones.

This seems to be supported by the correlation between *k* values and the properties of functional groups of the humic substances. Table 10 shows the correlation coefficients of k and *n* adjustable parameters of the Freundlich with properties and functional groups of the humic substances. Cu sorption capacity indexed by *k* value had no significant correlation with the properties of humic substances probably because Cu has great affinity for a wide range of

Table 10. The correlation coefficients[1] ®(r) of the adjustable coefficients k and n of the Freundlich absorption model with the properties of the Humic substances extracted from the soil for the absorption of the metals.

Metals	C_{hA}[2]	C_{FA}[3]	C_{HA}/C_{FA}	E_4/E_6	HD[4]	HR[5]
			r			
			k			
Cu	0.200	0.509	0.223	0.708	0.134	0.100
Cd	0.671	0.584	0.007	0.083	0.597	0.789*
Pb	0.124	0.378	0.383	0.655	0.152	0.300
Zn	0.361	0.782*	0.967**	0.583	0.316	0.296
			n			
Cu	0.843**	0.068	0.365	0.373	0.506	0.704
Cd	0.483	0.612	0.084	0.030	0.587	0.699
Pb	0.005	0.081	0.253	0.553	0.024	0.101
Zn	431	0.646	0.950**	0.712	0.243	0.192

Correlation coefficient greater than or equal to 0.699 is significant at 10 % probability level while * and ** indicate significance at 5 and 1 %

[1]Probability level respectively.

[2]Carbon concentration in humic acid fraction

[3]Carbon concentration in fulvic acid fraction

[4]Humufication degree (HD)

[5]Humification ratio (HR)

organic macromolecules. But at 10% probability level, Cu correlated with E_4/E_6 ratio of the humic substances. Cadmium sorption capacity correlated significantly with humification ratio. There was no correlation between Pb sorption and properties of the HS. However, Zn adsorption showed high significant correlation in the ratio of HA: FA (Table 10) and this single property of the HS accounted for over 94% of the variation in Zn sorption by HS from the various ecological zones (Fig. 16). Similar relation can be seen between *n* value for Zn and the HA: FA ratio (Fig. 16).

The significant correlation between C_{HA} and *n* value for Cu adsorption is suggestive that the variable sites for Cu adsorption were mostly located in the HA rather than the FA fraction. Whereas Pb sorption could not be related to the properties of HS, it seems that its sorption by HS was related to the concentration of phenolic functional group and total acidity of the HS as can be seen in Table 11. It would seem that the spatial distribution and variability of phenolic group and total acidity of the HS strongly affected both Pb and Zn sorption but more strongly so for Zn whose adsorption was 'predicted up to 58% by *k* value and about 89% for *n* value (Fig.17). Similar relationship was obtained between the correlations of phenolic groups and total acidity with Pb adsorption parameters (Table 11).

Table 11. The correlation coefficients[1] (r) of the adjustable coefficients *k* and *n* of the Freundlich absorption model with the properties of the Humic substances extracted from the soil for the absorption of the metals.

Metals	Carboxilic Group	Phenolic group	Total group	Percent group
	r			
	k			
Cu	0.313	0.435	0.538	0.358
Cd	0.602	0.424	0.270	0.276
Pb	0.077	0.793*	0.757*	0.093
Zn	0.346	0.740	0.761*	0.058
	n			
Cu	0.645	0.137	0.055	0.471
Cd	0.689	0.414	0.228	0.395
Pb	0.145	0.794*	0.699	0.247
Zn	0.345	0.813**	0.860**	0.173

Correlation coefficient greater than or equal to 0.699 is significant at 10 % probability level while * and ** indicate significance at 5 and 1 %
[5]Humufication degree

Note
Cu = Copper
Cd = Cadmium
Pb = Lead
Zn = Zinc

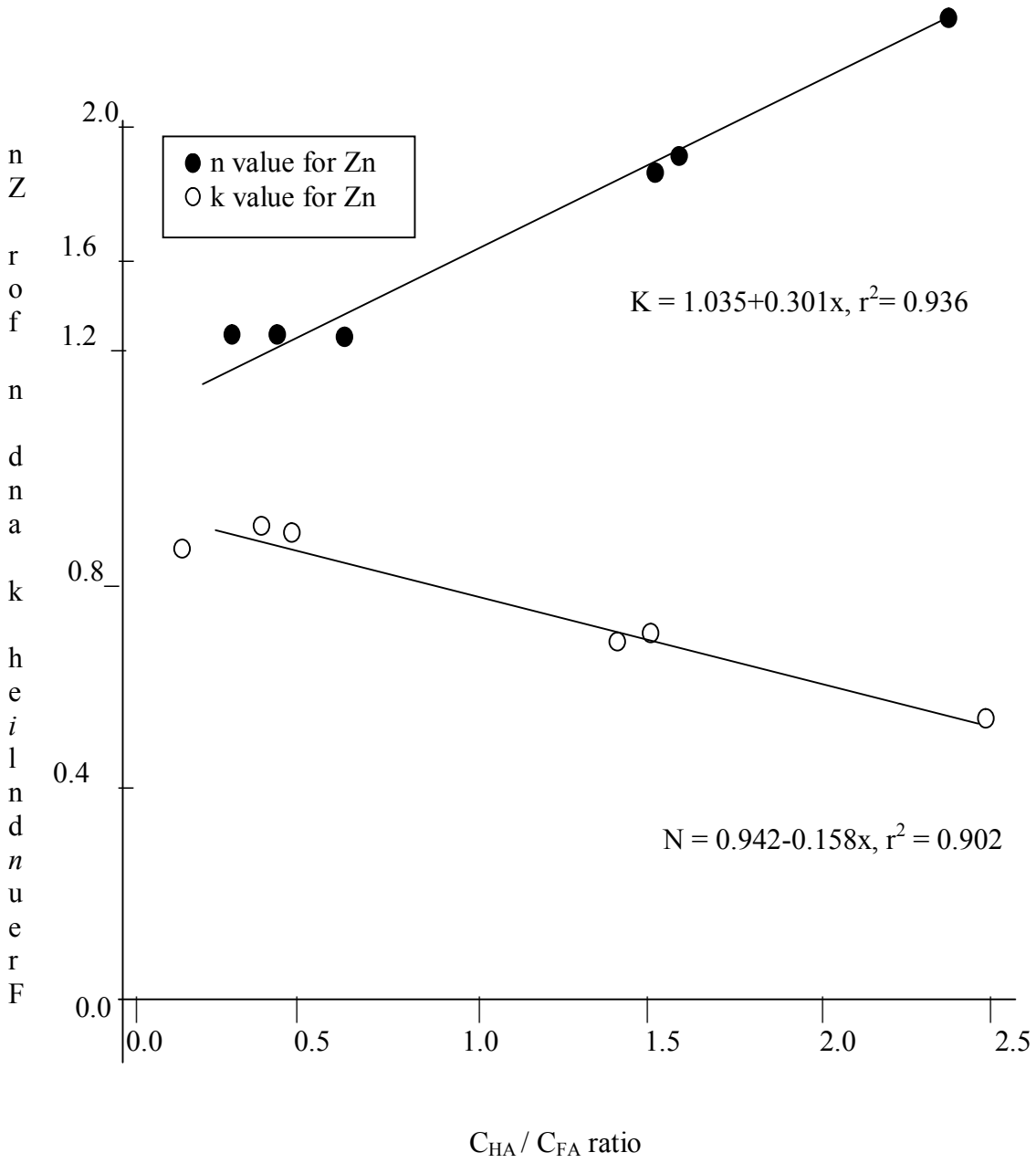


Figure 16**.** The relationship between Freundlich absorption parameters k and n of Zn and the ratio of humic acid to fulvic acid, indexed C_{HA} / C_{FA}, in humic substances extracted from the soils.

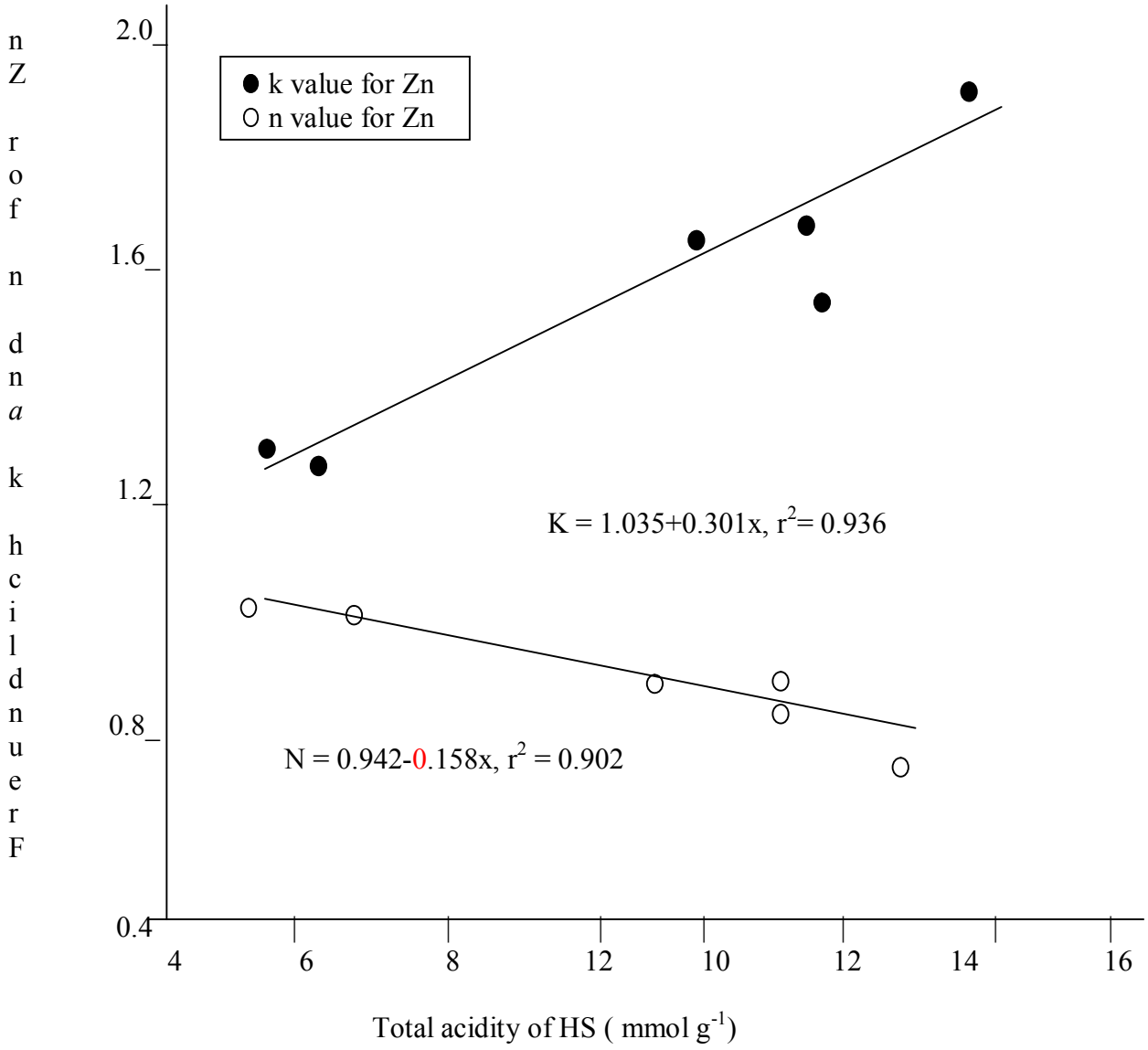


Figure 17. The relationship between Freundlich absorption parameters k and n of Zn and total acidity of humic substances extracted from the soils.

4.6 INFRA-RED SPECTRA OF HA AND METAL-HA INTERACTIONS FROM FOREST SOILS

The infra-red spectra produced from untreated HA extracted from forest soils under fallow showed a relatively strong absorption in the region of 2924cm^{-1} and 2854 cm^{-1} at both pH 4 and 6 respectively. Infra-red absorptions at this region are usually ascribed to C-H stretching vibration of aliphatic compounds. At pH 4 and 6 there was a strong absorption band at between 1461 and 1337 cm^{-1} with a weak absorption shoulder at 1598 cm^{-1} at pH 4 (Fig.18) and relatively strong absorption at 1634 cm^{-1} at pH 6 (Fig. 19). The absorption at between 1461 cm^{-1} and 1337 cm^{-1} at this region of infra-red spectra is due to OH bond and C-O stretching vibrations of phenolic group, whereas the absorption band for HA occurring at 1634 cm^{-1} at pH 6 (Fig. 19) is a consequence of C = C vibrations of aromatic compounds probably conjugated to C = O vibrations of other groups such as the C = O stretching vibrations of amide groups, ketones, quinones and COO^- symmetric stretching. There were no other distortion bands of HA at pH 4 (Fig. 18) whereas, at pH 6 (Fig 19) relatively strong absorption bands occurred at 1113 cm^{-1} due possibly to alcoholic groups and at 1018 cm^{-1} attributed to C-OH stretching of diproteic OH and CO stretching vibrations of polysaccharide-like substances (Fig. 19). The absorption bands occurring at 721 cm^{-1} was ascribed to Si-O stretching of silicate impurities (Senesi and Loffredo, 1999).

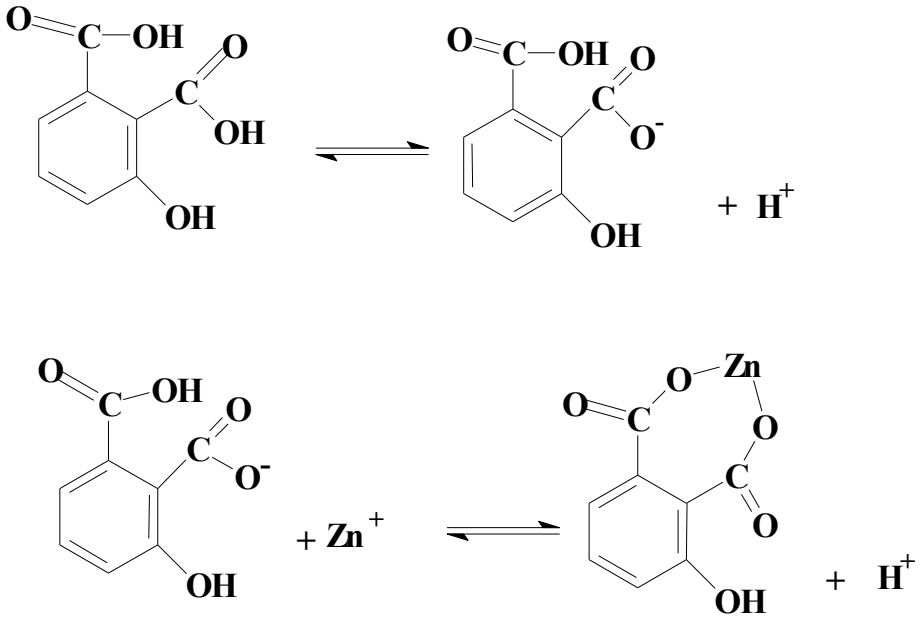
Additions of Cu, Cd, Pb and Zn either singly or together to produce HA-metal complex at pH 4 and 6 altered the absorption band of the HA either by broadening or by sharpening the absorption band or diminishing the intensity of the band (Figs.18 and 19). For instance, at pH 6, addition of Cu, Zn, Pb and Cd led to the appearance of absorption

Figure 18: Infra-red Spectra of HA-heavy metal complexes

Figure 19. Infra-red spectra of HA-heavy metal complexes from Forest fallow soil with an initial pH 6.

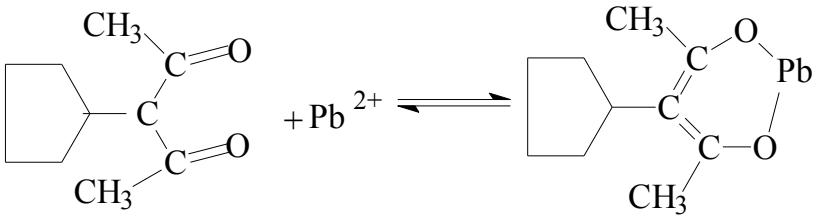
band at 1622 cm^{-1} for HA-Cu, 1620 cm^{-1} for HA-Zn, 1636 cm^{-1} for HA-Pb, 1644 cm^{-1} for HA Cd and 1597 cm^{-1} for HA-mixed metal complexes while also the absorption band between 1460 and 1337 cm^{-1} were broadened and sharpened (Fig. 19). Similar changes in the absorption of HA metal complexes at pH 4 were observed for the IR spectra for HFF (Fig. 18). At pH 6, a band, albeit weak, occurred at 1732 cm^{-1} for HA-Zn complex which is attributable to carbonyl group.

In the humic substances the most widely distributed functional groups that have strong metal complexing ability are –COOH, phenolic OH and possibly C = O and NH_2 groups (Schnitzer and Khan, 1972). The main changes occurring in the regions of 1600 cm^{-1} and 1400 cm^{-1} spectra of the HA-metal complex at pH 4 and 6 can be attributed to metal interaction with the carboxylic and the carbonyl groups that seemed to be quite reactive. The changes in absorption band of the HA-metal complexes at 1460 cm^{-1} in the soil maybe indicative of the conversion of COOH to COO^- with the metal ion reacting as follows:



On the other hand, the appearance of absorption band at 1600 cm^{-1} after metal adsorption to HA from the soils will support the notion of the interaction of the metal with carboxyl groups,

consisting of the C = O stretching vibrations of either the amide groups or the C = O of conjugated ketones and quinones. The reactivity of the carbonyl group is due to the polarity of the bond between C and oxygen as indicated by the large dipole moments of ketones and aldehydes. Because of unequal sharing of the electron cloud between C and O, the oxygen assumes net negative charge in addition to having two loan pairs of electrons to which metals can interact to form metal - organic complex:



Carbonyl group in HA HA-Pb complex

Infra-red spectroscopy of the native HA extracted from forest virgin (HFV) at pH 4 and 6 (Figs. 20 and 21) showed a strong absorption at 2922 cm^{-1} and 2853 cm^{-1} for the C – H aliphatic stretching. Another strong absorption band occurred in the region of 1660 and 1380 cm^{-1} and in the region of 1030 and 770 $cm^{-1.}$

The HA-metal complexes showed greater intensity of absorption especially at the frequency range of 1660 and 1350 cm^{-1} .As already alluded to, the changes in the IR spectra of the HA metal complexes compared to HA alone occurred in the region of 1600 and 1370 cm^{-1} in the forest HA.

Figure 20. Infra-red spectra of HA-heavy metal complexes from Forest virgin soil with an initial pH 4.

Figure 21. Infra-red spectra of HA-heavy metal complexes from Forest virgin soil with an initial pH 6.

This region of the IR spectra showing either a positive or negative shift in the absorption band is indicative of metal complexation by carbonyl and carboxylic acids groups at the region of 1600 and 1370 cm^{-1} respectively.

4.7 INFRA-RED SPECTRA OF HA AND HA-METAL INTERACTIONS FROM SAVANNA SOILS

One notable feature of IR-spectra of HA and HA-complexes from virgin plots of the Southern guinea savanna soils (Figs 22-25) is the broad absorption band at frequency of 3400 and 3800 cm^{-1}. The IR curves of HA at pH 4 and 6 from uncultivated soils (virgin) from SGV (Figs 22 and 23) revealed a wide but relatively weak absorption at 3416 cm^{-1} representing OH stretching and H bonded OH. The absorption also produced two weak bands at 2724 and 2357 cm^{-1} in the aliphatic stretching regions (Figs. 22 and 23). There was a weak band at between 1622 and 1598 cm^{-1} representing symmetric COO-stretching and C=C aromatic stretching vibrations. The spectrogram showed sharp absorption band at 1461 and 1370 cm^{-1} representing aliphatic CH deformations and OH deformations and CO stretching of phenolic OH.

On metal addition to the HA to produce HA-metal complexes the spectra of HA-metal from virgin plots (Figs 22 and 23) showed changes in the adsorption band particularly at the region of 1700 cm^{-1} to 1600 cm^{-1} and between 1400 cm^{-1} to 1600 cm^{-1} .The HA-Cu complex at pH4 (Fig22) produced a characteristic sharp band at 3477 cm^{-1} and at 1750 cm^{-1} for C = O stretching of COOH, aldehydes and ketones and increased vibration frequency at 1646 cm^{-1} representing amides, quinones and H-bonded conjugated ketones. Similar changes are observed for the spectra of HA-Zn and HA-Pb complexes.

Figure 22: Infra-red spectra of HA-heavy metal

Figure 23. Infra-red spectra of HA-heavy metal complexes from Southern Guinea savanna soil with an initial pH 6.

However at pH 6 (Fig23) similar changes at the IR spectra of the HA-metal complexes were observed except that the absorption bands were more intense than absorption of the HA-metal complexes at pH 4. One major inference to be drawn from the IR spectra of HA and HA-metal complexes is that the carboxylic acid and the carbonyl groups of the aldehydes/ketones were involved in the metal complex reactions evidenced by the changes in vibration frequency in the region of 1700 to 1400 cm^{-1} .

For the SGS soils under fallow (SGF), the IR spectra of HA and HA-metal complexes were quite similar to those produced for the uncultivated soils (Figs. 24 and 25). The native HA at pH 4 (Fig 24) produced weak absorption at 3400 cm^{-1} and 3140 cm^{-1} representing O-H stretching and aromatic C-H stretching respectively. The sharp absorption frequency at 1729 cm^{-1} and 1599 cm^{-1} for this HA at pH 6 represented C=O of the carboxylic and of the carbonyl respectively. HA-Cu at pH 4 produced a frequency shift in the C=O stretching of the carbonyl from 1645 cm^{-1} to a lower frequency vibration at 1631 cm^{-1}, 1630 cm^{-1} for Zn-HA, 1613 cm^{-1} for HA-Pb, 1632 cm^{-1} for HA-Cd and 1618 cm^{-1} for HA-MX (Fig. 24). Similar changes were also observed at pH 6 except for the appearance of weak absorption band at 1727 cm^{-1} for HA-Cu and 1746 cm^{-1} for HA-Pb (Fig25) probably representing increased C=O stretching vibration of COOH, aldehydes and ketones.

The native humic acid extracted from virgin plot of NGS (NGV) samples at pH 4 (Fig 26) and pH 6 (Fig 27) produced infra-red spectograms with a weak aromatic C-H stretching at 3140 cm^{-1} and 3160 cm^{-1} but with a strong absorption band at 2923 cm^{-1} and 2853 cm^{-1} at pH 4 and 6 representing C-H aliphatic stretching. The spectograms showed adsorption at 1622 cm^{-1} representing COO- symmetric stretchings.

Figure 24. Infra-red spectra of HA-heavy metal complexes from SGF Southern Guinea Savanna fallow @ initial pH 4.

Figure 25. Infra-red spectra of HA-heavy metal complexes from SGF Southern Guinea savanna soil with an initial pH 6.

Fig 26 and 27 to follow immediately

Furthermore, sharp absorption at 1461 cm^{-1} and 1377 cm^{-1} for C-H deformation and of phenolic groups for HA at pH 6 (Fig 27) but not at pH 4 (Fig26) were also observed. A weak absorption at 1020 cm^{-1} representing C=O of polysaccharides was also noted.

The HA-metal complexes produced changes in the COO^- and $C\text{-}O^-$ vibration frequency at the region of 1600 $cm^{-1.}$ The vibration frequency of the COO^- either decreased or increased as inferred from the wave numbers. For instance, addition of Cu to HA increased the absorption frequency from 1618 cm^{-1} to 1634 cm^{-1} and from 1400 cm^{-1} to 1460 cm^{-1} with the appearance of a new peak at 1378 cm^{-1}. Similar changes can be seen in HA-Zn, HA-Pb and HA-Cd with sharper absorption bands at 1600 cm^{-1} to 1300 cm^{-1} (Figs. 24 and 25). The fact that changes in the IR spectra of the HA-metal complexes were mainly restricted to the 1600 cm^{-1} to 1300 cm^{-1} band regions is consistent with the notion that the carboxylic groups and the carbonyl groups were the most active sites for metal complexation HS from the uncultivated or virgin NGS soil.

The spectrogram of the infra-red analyses of the untreated humic acid and metal -humates at pH 4 and 6 from NGF are shown in figures 28 and 29. The spectrograms were not much different from those of HA and HA-metal complexes of NGV (Figs. 26 and 27) There was a weak absorption at 3177 cm^{-1} representing the N–H stretching and two relatively strong absorptions at 2924 cm^{-1} and 2853 cm^{-1} for aliphatic C – H stretching. A broad shoulder was also noted which culminated in a weak absorption at 1618 cm^{-1} representing the COO^- stretching. The spectrogram was characterized by two sharp absorption peaks at 1460 cm^{-1} and 1377 cm^{-1} representing OH bending and C-O of phenolic groups.

Figure 26. Infra-red spectra of HA-heavy metal complexes from Northern Guinea Savanna fallow @ initial pH 4

Figure 27. Infra-red spectra of HA-heavy metal complexes from Northern guinea savanna fallow @ initial pH 6

Figure 28. Infra-red spectra of HA-heavy metal complexes from northern guinea savanna fallow @ initial pH 4

Figure 29. Infra-red spectra of HA-heavy metal complexes from northern guinea savanna fallow @ initial pH 6

There was a relatively weak absorption at 1100 cm^{-1} probably due to lower vibration frequency of alcoholic groups distorted by impurities.

The spectrogram of the HA-metal complex revealed vibration of N–H stretching at a higher frequency 3182 cm^{-1} and the disappearance of the aliphatic C–H stretching as well as collapse of the broad shoulder giving way to short, but weak absorption bands at 2726 cm^{-1} and 2031 cm^{-1} for the HA-Cu. The COO^- symmetric stretching at 1600 cm^{-1} vibrated at a higher frequency of 1622 cm^{-1}. However, the phenolic OH absorption band at 1376 cm^{-1} vibration frequency became sharper.

The mixed metal HA complex showed a spectrogram not very different from its Cu–HA complex counterpart. The main difference was the vibration at a higher frequency of 1034 cm^{-1} in the mixed metal – HA complex as compared to the Cu-HA complex 1018 cm^{-1}. This indicated that Cu^{2+} has a stronger affinity for humic acid than the other metals.

As has been alluded to, the IR spectra of the HA-metal complex showed that the major changes were in the region of 1700 $cm^{-1}$to 1300cm^{-1}frequency where the C=O stretching vibration due to –COOH or carbonyl groups associated with aldehydes and ketones are the active functional groups responsible for metal adsorption and complexation.

4.8 METAL-HUMIC SUBSTANCES INTERACTIONS AS REVEALED BY INFRA-RED SPECTRA

The infra-red spectra obtained in this study revealed strong interactions between metals and oxygen-containing functional groups. Several workers have documented reactions between metals and humic acids (Dai et. al.,, 2001; Chen, 2003). Strong absorption band in the regions of 3450 cm^{-1} to 3400 cm^{-1} observed in all the spectra in this study are attributed to hydroxyl

stretching and or N-H stretching (Chien et. al.,, 2003). The aliphatic C-H stretching between 2830 cm^{-1} to 2965 cm^{-1} (Smith, 1999) are very evident on each of the spectrum. In almost all the spectra there was no significant change in absorption level in this region, indicating absence of metal complexation. Absorptions in the regions of 1740 cm^{-1} to 1640 cm^{-1} were prominent in the humic acid sample in this study. These are the regions of C=O indicative of metal bond to aldehyde group (Chang-Chien et. al.,, 2003b), ketone, carbonyl (Ouatmane et. al.,, 2000), or even carboxylic acid (Smith, 1999; Tan, 1993). Spectra of HA-metal complexes at pH of 6 revealed this clearly. The shift in the vibration band of certain functional groups to lower wavelength numbers after metal adsorption indicates reduction in the strength of the functional group (Ouatmane et. al.,, 2000). This is due to a strong interaction of the functional group with the metal ions (Cruz-Guzman et. al.,, 2003). The increase in absorption band of some functional groups is as a result of HA-metal group vibrating at a different frequency compared to HA without metal complexation (Chien et. al.,, 2003; Stevenson, 1997).

The broad bands at about 1430cm^{-1} are attributed to COO- stretch of carboxylic acid (Hesse et. al.,, 1995; Smith, 1999). These bands and those of C-O stretchings of phenolic OH at 1400cm^{-1} to 1380 cm^{-1} were expectedly the most prominent bands and also the most varied in the HS samples analyzed in this study. In all the spectra obtained, the phenolic-OH was more prominent than the carboxylate (COO^-) consistent with the higher concentration of phenolic acid over that of carboxylic acid as determined by potentiometric titrations (Table 4). The near absence of COOH for NGV and NGF samples as revealed by potentiometric titrations is also confirmed by the sharp absorption bands of phenolic OH in the native HA spectra. But the sharper absorption of the HS at pH 6 than at pH 4 after reacting with the metals indicates greater affinity of HS for the metals at higher pH.

Tan (1993) reported that polysaccharides absorb between 1170 cm^{-1} and 950 cm^{-1}. Absorption bands in these regions as obtained in the spectra of this study can be attributed to polysaccharides as reported by Grube *et. al.*,(1999). Smidt *et. al.*, (2005) obtained similar results in their study of biowaste materials.

Infrared analysis of the various metal-humic complexes suggests that strong metal binding was associated with carboxyl group and phenolic-OH (Merit and Erich, 2003) but in this study the carbonyl group was greatly involved with metal complexation.

The greater affinity of HS for Pb over Cd and differences in the affinity for Cu and Zn of the various humic acids observed in the sorption isotherms could not be explicitly detected in the IR spectra of HA-metal complexes at different pH. However, Appel and Ma (2002) attributed this behavior to a greater tendency of Pb to undergo both inner and outer surface complexation than Cd over a wide range of pH. Hubeey (1983) reported that Cd^{2+} is a closed shell cation with a valence orbital that favours coulombic type reactions as opposed to electron sharing. Furthermore, the stability of the HS-metal complex formed between metals and humic substances depends on the ionic radii, radius: charge ratio as well as its elecrtonegativity (Lake, 1987). On this basis Cu is more reactive than Zn in terms of their ionic radii, radius: charge ratio as well as its elecronegativity. Cu is a softer Lewis acid compared to Zn (Huang et. al.,, 2005) which accounts for the preferential formation of inner sphere complexes of copper with HS as compared to Zn (Huang et. al.,, 2005). In addition, phenols are considered important agents for complexing of Cu by soil organic acid than of Zn (Senesi, 1992). The prevalence of phenol groups as obtained in this study may well contribute to greater values of *b* for Cu than Zn (as observed in SGF, NGV, and NGF) obtained in the Langmuir isotherms. Huang et. al., (2005) obtained results indicating preference of Cu-HA complex formation over that of Zn-HA.

CHAPTER FIVE

5.0. SUMMARY AND CONCLUSION

This study attempted to determine the characteristic and reactivity of humic acids extracted from soils across ecological gradient in Nigeria. Soils samples from the various ecological zones were assayed using various chemical and spectroscopic methods. Total organic carbon was significantly higher in fallow plots in both forest and savanna ecological zones than the corresponding virgin plots. Labile organic carbon was significantly higher in virgin than fallow plots.

Extracted humic acids from the soil samples were also subjected to different chemical analysis including functional groups and their reactivity with trace metals (Cu, Zn, Pb and Cd) at varying concentrations and pH of the reaction medium. Concentrations of phenolic groups were significantly higher than concentrations of carboxylic groups in both virgin and fallow plots. The adsorption isotherms varied widely in this study. Competitive adsorption of the metals onto humic acids at varying pH observed across the ecological zones showed increasing metal adsorption with increasing pH, but the isotherms depended on the metal. For instance, whereas Cu adsorption followed the H-type isotherm, Pb, Cd, and Zn tend to follow either the S- or L-type isotherm. Spectroscopy analyses of the various HA-metal carried out to determine the various functional groups of the humic substances that were reactive with the metals showed strong absorption bands associated with the carbonyl groups.

The extracted humic acid from various ecological zone varied widely both in character (properties) and reactivity. C_{HA} and humification degree, (HD) increased from forest to savanna, while colour quotient (E_4/E_6 ratio) and total acidity increased from savanna to forest.

. Complexation of metals by various humic acids was a function of pH and metal loadings. Increasing the initial pH of the reaction medium resulted in significant increase in sorption of Zn, Cu, Cd and Pb by the various humic acids.

. Adsorption of Cu, Zn, Pb and Cd on the different humic acids was better described by linearized Freundlich sorption equations than Langmuir adsorption model.

. Binding capacity *k* of Freundlich parameter was in the order $Zn^{2+} > Cu^{2+} > Pb^{2+} > Cd^{2+}$ for samples HFF, HFV and SGV. The relative adsorption capacity of Cu and Pb on to HA from fallow plots increased across the ecological zones.

. Adsorption surfaces for Pb and Cd were less heterogeneous than for Cu and Zn as indexed by adsorption isotherm models. Differences in the infra-red absorption bands of HS depended on the metal and pH. Increased complexation of Cu, Zn, Pb and Cd by HS was observed as pH increased from 4 to 6.

. Metal complexation across the different HA occurred mostly at carbonyl (ketones & aldehydes) as well as phenolic group. Significant shifts in spectra absorption in the regions between 1170 cm^{-1} and 1030 cm^{-1} is probably an indication of strong participation of polysaccharides especially the conjugated OH groups in metal complexation.

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Table 1. Some selected physico-chemical characteristics of the soils from different ecological zones used for the study

Ecological Zones	Clay	pH Exch. Acid H_2O	pH CEC $CaCl_2$	Sand	Silt
	---	--------- mmol kg^{-1} ----------		---------- (g kg^{-1}) ----------	
Virgin					
HFV		7.3	6.3	443	321
	266	53	257		
SGV		6.6	5.3	418	418
	164	73	200		
NGV		5.3	4.0	472	414
	159	22	84		

Fallow

HFF		5.9	4.5	406	456
	138	102	238		
SGF		5.3	4.2	430	461
	109	9	90		
NGF		5.1	4.2	496	432
	162	2	110		

Table 2. The influence of environmental gradient on the total organic carbon (TOC) and its characteristics in soils from the different ecological zones from Nigeria

Ecological Zones	TOC[1]		MBOC[2]	WSOC[3]	LOC[4]
	Total N	C: N			
	g kg^{-1}	ratio	g kg^{-1}		mg kg^{-1}
Virgin					
HFV	28.8		0.80	1.50	96.8
	2.7	11.1			
SGV	28.0		1.44	0.10	58.1
	1.3	21.1			

NGV	15.7		1.28	0.50	77.5	
	0.6	28.1				
Fallow						
HFF	40.5		1.22	1.58	19.4	
	2.1	19.1				
SGF	19.2		2.92	0.10	58.1	
	13.0	15.1				
NGF	33.2		1.44	0.10	19.4	
	1.4	23.1				

[1]Total organic carbon (TOC)

[2]Metal-binding organic carbon (MBOC),

[3]Water soluble organic carbon (WSOC) [4]Labile organic carbon (LOC)

Table 3. The influence of environmental gradient on the total organic carbon (TOC) and its characteristics in soils from the different ecological zones from Nigeria

Ecological	C_{HA}[1]	C_{FA}[2]	C_{HA}:C_{FA}			
Zones	----------g kg^{-1}----------		ratio	HR[3]	HD[4]	E_4
	E_6	E_4 / E_6				
Virgin						
HFV	380	247	1.54	0.22	2.61	0.41
	0.07	5.55				

SGV		351		147	2.39	0.18	3.11	0.21
	0.05		5.85				
NGV		327		568	0.58	0.57	4.97	0.10
	0.03		3.57				
Fallow								
HFF		181		554	0.33	0.18	2.61	0.37
	0.06		6.11				
SGF		683		482	1.42	0.61	3.79	0.22
	0.04		4.89				
NGF		250		529	0.47	0.23	4.84	0.05
	0.02		2.50				

[1]Carbon concentration in humic acid fraction

[2]Carbon concentration in fulvic acid fraction

[3]Humification ratio (HR) [4]Humification degree (HD)

Table 4. The influence of environmental gradient on the concentrations of some functional groups in the humic acid at pH 4.0

Ecological Zones	Percent phenol	Carboxylic group	Phenolic group	Total acidity
Virgin				

HFV		3.33	6.86	10.2
	67.2			
SGV		2.17	11.5	13.7
	84.1			
NGV		1.42	4.59	6.01
	76.4			
Fallow				
HFF		2.33	9.52	11.9
	80.3			
SGF		1.10	10.8	11.9
	90.2			
NGF		NA	5.01	5.01
	100			

Table 5. The range and means of Langmuir and Freundlich adsorption parameters for Cu-humic acid adsorption at pH 4-6 from soils of different ecological zones from Nigeria

	Langmuir[a]	
	Freundlich[b]	

Ecological Zones	*k*	*b*	r^2	log *k* *n*	r^2
Virgin					
HFV	0.009 – 0.067 (0.043)	0.002 – 0.015 (0.009)	0.259 – **0.440** (0.361)	0.79 – 1.27 (1.06)	0.46 – 0.94 (0.70)
					0.87 – 0.99 (0.94)
SGV	0.009 – 0.054 (0.031)	0.002 – 0.016 (0.009)	0.178 – **0.819** (0.446)	0.75 – 1.26 (1.06)	0.45 – 0.90 (0.68)
					0.96 – 0.97 (0.96)
NGV	0.008 – 0.061 (0.046)	0.001 – 0.015 (0.008)	0.032 – **0.443** (0.036)	1.01 – 1.32 (1.07)	0.46 – 1.00 (0.68)
					0.93 – 0.96 (0.95)
Fallow					
HFF	0.009 – 0.157 (0.061)	0.002 – 0.019 (0.011)	0.071 – 0.188 (0.111)	1.16 – 1.32 (1.23)	1.04 – 0.70 (0.73)
					0.84 – 0.96 (0.89)
SGF	0.025 – 0.059 (0.040)	0.002 – 0.016 (0.008)	0.050 – 0.218 (0.161)	0.95 – 1.20 (1.08)	0.62 – 0.67 (0.64)
					0.82 – 0.97 (0.90)
NGF	0.010 – 0.096 (0.034)	0.003 – 0.026 (0.005)	0.007 – 0.084 (0.170)	0.77 – 1.16 (0.97)	0.46– 0.91 (0.68)
					0.87 – 0.98 (0.93)

[a] *k* is affinity term L mol^{-1}); *b* is adsorption maximum (mmol kg^{-1}), r^2 is the coefficient of determination.
[b] log *k* and *n* are adjustable coefficients in the Freundlich equation.

Table 6. The range and means of Langmuir and Freundlich adsorption parameters for Zn-humic acid adsorption at pH 4-6 from soils of different ecological zones from Nigeria

Ecological Zones[c]	Langmuir[a]			Freundlich[b]	
	k	*b*	R^2	log *k*	*n* R^2
Virgin					
HFV	0.040 – 0.082 (0.054)	0.006 – 0.055 (0.025)	**0.451 – 0.775** (**0.624**)	1.16 – 1.90 (1.47)	0.53 – 0.86 (0.71) 0.94 – 0.99 (0.96)
SGV	0.013 – 0.076 (0.046)	0.020 – 0.082 (0.046)	**0.372 – 0.572** (**0.482**)	1.60 – 1.97 (1.79)	0.51 – 0.63 (0.56) 0.83 – 0.96 (0.90)
NGV	0.038 – 0.054 (0.041)	0.001 – 0.005 (0.004)	0.012 – 0.230 (0.126)	1.09 – 1.14 (1.11)	0.88 – 0.92 (0.89) 0.91 – 0.96 (0.94)
Fallow					
HFF	0.031– 0.094 (0.053)	0.002 – 0.030 (0.017)	0.094 – 0.424 (0.205)	1.16 – 1.26 (1.22)	0.79 – 0.86 (0.82) 0.67 – 0.94 (0.78)
SGF	0.036 – 0.109 (0.084)	0.009 – 0.035 (0.021)	0.199 – 0.291 (0.244)	1.31 – 1.70 (1.45)	0.59 – 0.77 (0.70) 0.84 – 0.93 (0.87)
NGF	0.027 – 0.062 (0.047)	0.001 – 0.015 (0.009)	0.001 – **0.477** (0.382)	0.93– 1.41 (1.20)	0.71 – 1.08 (0.91) 0.90 – 0.96 (0.90)

[a]*k* is affinity term L mol^{-1}); *b* is adsorption maximum (mmol kg^{-1}), r^2 is the coefficient of determination.
[b]log *k* and *n* are adjustable coefficients in the Freundlich equation.

Table 7. The range and means of Langmuir and Freundlich adsorption parameters for Pb-humic acid adsorption at pH 4-6 from soils of different ecological zones from Nigeria

Ecological Zones[c]	Langmuir[a] *k*	*b*	R^2	Freundlich[b] log *k*	*n*	R^2
Virgin						
HFV	0.015 – 0.058 (0.043)	0.011 – 0.146 (0.088)	**0.484 – 0.559 (0.520)**	0.45 – 0.88 (0.72)	1.13 – 2.22 (1.52)	0.52 – 0.99 (0.80)
SGV	0.014 – 0.155 (0.113)	0.079 – 0.317 (0.011)	0.210 – 0.439 (0.286)	1.13 – 1.95 (1.64)	0.51 – 0.88 (0.65)	0.69 – 0.93 (0.82)
NGV	0.032 – 0.058 (0.045)	0.015 – 0.080 (0.039)	0.032 – 0.191 (0.165)	0.59 – 1.09 (0.90)	0.92 – 1.71 (1.20)	0.69 – 0.95 (0.85)
Fallow						
HFF	0.043 – 0.124 (0.094)	0.019 – 0.182 (0.083)	0.023 – **0.551 (0.308)**	1.11 – 1.78 (1.44)	0.56 – 0.90 (0.72)	0.75 – 0.95 (0.85)

SGF 0.032 – 0.048 (0.039) 0.006 – 0.019 (0.172) 0.020 – 0.115 (0.071) 1.00 – 1.12 (1.06) 0.89 – 1.01 (0.95) 0.87 – 0.92 (0.89)

NGF 0.029 – 0.057 (0.047) 0.015 – 0.080 (0.038) 0.238 – **0.827** (**0.474**) 0.61 – 0.85 (0.75) 1.17 – 1.64 (1.35) 0.94 – 0.97 (0.96)

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[a]*k* is affinity term L mol^{-1}); *b* is adsorption maximum (mmol kg^{-1}), r^2 is the coefficient of determination.
[b]log *k* and *n* are adjustable coefficients in the Freundlich equation.

Table 8. The range and means of Langmuir and Freundlich adsorption parameters for Cd-humic acid adsorption at pH 4-6 from soils of different ecological zones from Nigeria

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Ecological	Langmuir[a]			Freundlich[b]	
Zones[c]	*k*	*b*	R^2	log *k*	*n* R^2

Virgin

HFV 0.008 – 0.016 (0.013) 0.006 – 0.019 (0.013) 0.086 – **0.972** (**0.630**) 0.53 – 1.00 (0.71) 1.89 – 1.72 (1.53) 0.77 – 0.93 (0.86)

SGV 0.016 – 0.066 (0.041) 0.003 – 0.044 (0.018) 0.036 – **0.857** (**0.451**) 0.71 – 0.96 (0.85) 1.04 – 1.60 (1.20) 0.88 – 0.98 (0.95)

NGV 0.026 – 0.106 (0.061) 0.006 – 0.300 (0.106)0.132 – 0.394 (0.295) 0.77 – 1.16 (0.95) 1.04 – 1.30 (1.08) 0.82 – 0.96 (0.92)

Fallow

HFF 0.023 – 0.112 (0.062) 0.000 – 0.036 (0.013)0.000 – 0.322 (0.110) 0.76 – 0.97 (0.89) 1.03 – 1.31 (1.13) 0.84 – 0.94 (0.89)

SGF 0.041 – 0.079 (0.064) 0.003 – 0.041 (0.021)0.018 – 0.238 (0.123) 1.04 – 1.23 (1.13) 0.82 – 0.97 (0.89) 0.63 – 0.93 (0.82)

NGF 0.017 – 0.077 (0.041) 0.012 – 0.067 (0.032)**0.824 – 0.893** (**0.865**) 0.72 – 0.77 (0.75) 1.30 – 1.38 (1.34) 0.89 – 0.97 (0.94)

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[a]*k* is affinity term L mol^{-1}); *b* is adsorption maximum (mmol kg^{-1}), r^2 is the coefficient of determination.
[b]log *k* and *n* are adjustable coefficients in the Freundlich equation.

Table 10. The correlation coefficients[1] (r) of the adjustable coefficients k and n of the Freundlich absorption model with the properties of the Humic substances extracted from the soil for the absorption of the metals.

Metals	C_{hA}[2]	C_{FA}[3]	C_{HA}/C_{FA}	E_4/E_6	HD[4]	HR[5]
	r					
	k					
Cu	0.200	0.509	0.223	0.708	0.134	0.100
Cd	0.671	0.584	0.007	0.083	0.597	0.789*
Pb	0.124	0.378	0.383	0.655	0.152	0.300
Zn	0.361	0.782*	0.967**	0.583	0.316	0.296
	n					
Cu	0.843**	0.068	0.365	0.373	0.506	0.704
Cd	0.483	0.612	0.084	0.030	0.587	0.699
Pb	0.005	0.081	0.253	0.553	0.024	0.101
Zn	431	0.646	0.950**	0.712	0.243	0.192

~~Correlation coefficient greater than or equal to 0.699 is significant at 10 % probability level~~ while * and ** indicate significance at 5 and 1 %
[1]Probability level respectively.
[2]Carbon concentration in humic acid fraction
[3]Carbon concentration in fulvic acid fraction
[4]Humification ration (HR)
[5]Humufication degree

Table 11. The correlation coefficients[1] (r) of the adjustable coefficients *k* and *n* of the Freundlich absorption model with the properties of the Humic substances extracted from the soil for the absorption of the metals.

Percent	Carboxilic Group	Phenolic group	Total group

Metals	r		
	k		
Cu	0.313	0.435	0.538
0.358			
Cd	0.602	0.424	0.270
0.276			
Pb	0.077	0.793*	0.757*
0.093			
Zn	0.346	0.740	0.761*
0.058			
	n		
Cu	0.645	0.137	0.055
0.471			
Cd	0.689	0.414	0.228
0.395			
Pb	0.145	0.794*	0.699
0.247			
Zn	0.345	0.813**	0.860**
0.173			

~~Correlation coefficient greater than or equal to 0.699 is significant at 10 % probability level~~
while * and ** indicate significance at 5 and 1 %
[5]Humufication degree

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