

ORIGINAL ARTICLE

**MOBILIZATION OF IRON FROM SOIL
RECALCITRANT FRACTIONS BY USING MANGO
(*MANGIFERA INDICA*) PLANT LEAF EXTRACT**

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Abstract

*This study has been carried out to investigate the speciation of iron in the various, plant available and non-available, soil fractions and the efficiency of the Mango (*Mangifera indica*) plant leaf extract in mobilizing iron from the strongly-bound soil fractions of cultivated, forest and water logged soil samples which were collected around Jimma town. The soil samples were treated with the mango plant leaf extract and the level of iron the various fractions of the treated soil samples and untreated triplicates of each soil type (controls) was determined spectrophotometrically.*

*Results of the soil property studies revealed that, all the three soil types were moderately acidic. The percent organic matter analysis indicated that, the forest soil has the highest organic matter content (19%), followed by the cultivated soils (15%) and the water logged soil had the least content (7%). The speciation study indicated that, the *Mangifera indica* plant leaf powder extract is able to mobilize a substantial amount of iron from the strongly bound fractions of all the three soil types. The concentration of iron in the water soluble and exchangeable fractions of all the soil samples were found to increase while in the recalcitrant fractions it was found to decrease. In the water logged soil, the leaf powder extract was able to bring up to 7% more concentration of iron from the strongly bound fractions to the water soluble fraction of the water logged soil than the concentration extracted in the untreated soil (control). Similarly, the iron concentration in the water soluble fraction of the forest soil increased by 5% and in that of the cultivated soil by 4%. On the contrary, the concentration of iron in the strongly bound fractions of all the soil samples was found to decrease although the decrease varied with the strength of binding in each fraction.*

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INTRODUCTION

Fertilizers, natural or synthetic chemical substances or mixtures are used to enrich soil so as to promote plant growth. Plants require more than a dozen different chemical elements for their healthy growth (Microsoft Encarta, 2007). Some of these elements are required in large amounts and some in small amounts. Those elements which are needed in large amounts by plants for normal growth and development, e.g. nitrogen, carbon, or potassium are known as macronutrients and those which are needed by plants in small quantities e.g. iron, manganese, zinc, boron, copper, molybdenum, and chlorine are known as micronutrients. In modern agriculture, there is a widespread concern about the micronutrient level in soils. Following are some of the reasons for the concern:

- 1) Improved crop varieties and macronutrients fertilizer practices have greatly increased crop production and thereby depleting the soil micronutrient level.
- 2) The trend toward high-analysis fertilizers has reduced the use of impure salts, which formerly contained some micronutrients.
- 3) Increased knowledge of plant nutrition has helped in the diagnosis of trace-element deficiencies that formerly might have gone unnoticed.

The main source of the micronutrients for plants is the soil. Soils contain a

range of individual components, and each component can bind trace elements in more than one way (McBride, 1994).

Based on the degree of association with components of the soil the total content of a given element is sub-divided into: water soluble, exchangeable, bound to carbonates, bound to amorphous Fe-oxide phase, bound to crystalline Fe-oxide phase and bound to the organics and sulfides phase. Therefore, plant micronutrients are bound to soil components in a variety of ways ranging from loosely held, plant available (water soluble and exchangeable) form to firmly fixed components of mineral lattices (bound to: carbonates, amorphous Fe-oxide phase, crystalline Fe-oxide phase and, organics and sulfides phase). When the concentration of a given micro- or macro- nutrient in the mobile phases of the soil is extremely low, plants may face deficiencies of one or more of these elements.

Low concentrations of any of the nutrient elements may occur due to either the existence of extremely low concentrations of these elements in the soil, or because the elements are present in very unavailable (insoluble) forms

(Murray, 1994). Therefore, neither the total quantity of an element in the soil

nor the quantity extracted by aggressive reagents is closely correlated to the plant-available 'pool' of the element. A measure more useful than total elemental content for most purposes is an estimation of 'availability' or 'lability' of the element, since it is this property that can be related to mobility and uptake by plants and extractability by chemical treatments.

In the soil, iron exists in the reduced (+2) and oxidized (+3) valence states. The reduced valent state prevails under low oxygen supply and relatively higher moisture level conditions while the oxidized valent state prevails under oxidizing conditions. At pH values common in soils, the oxidized state of iron is known to form highly stable complexes with organic matter, bonding to two or more functional groups and resist extraction (Brady 2002). Plants may show iron deficiency under this situation. Some Fe^{3+} humic complexes are so stable (kinetically or thermodynamically) that they resist dissociation over the pH range of 3 – 10. In effect, then, Fe^{3+} blocks potential cation exchange sites in organic matter, reducing the cation exchange capacity

(Murray 1994). Application of commercial fertilizers, $FeSO_4 \cdot 7H_2O$ in

the case of iron deficiency, is a common management practice to overcome micronutrient deficiency. Recently, foliar sprays are used. Foliar sprays of dilute inorganic salts or organic chelates are more effective than soil treatments where high soil pH or other factors render the soil-applied nutrients unavailable. However, both practices are quite expensive (Nyle, 2002).

Plant tissues contain many organic compounds which are chemically varied. Leaching studies have shown that naturally occurring organic substances do promote metal mobilization in soils. For example, when soils were leached with overlying layers of forest litter from several sources, a correlation was observed between dissolved organic carbon and Al concentrations in the leachate (Janet 1995). The major leaching agents produced by plants include CO_2 , organic acids, and ligands. (Eugene et.al 1998). Among the organic acids, fulvic and low molecular weight humic acids are generally considered to be important chemical agents for the leaching of metals from soils (Fox and Comerford 1990). They are water soluble and can contribute significantly to the soil acidity by releasing protons into the soil solution upon dissociation. In

addition, they contribute to mineral dissolution by acting as ligands in the process of complexation, dissolution and metal mobility (Schnitzer & Kodama 1977). In contrast, Ochs demonstrated an inhibitory effect of humic acids on Al oxides at pH 4 – 4.5 (Ochs, 1996). These results suggest that it may be the low molecular weight acids and not humic and fulvic acids that enhance mineral dissolution.

All the organic compounds in plant tissues usually begin to decompose simultaneously when fresh plant tissue is added to a soil. The sugars and simple proteins decompose most readily. The plant proteins decay under microbial action yielding not only carbon dioxide and water but also amino acids such as glycine ($\text{CH}_2\text{NH}_2\text{COOH}$) and cysteine ($\text{CH}_2\text{HSCHNH}_2\text{COOH}$). Such organic acid decomposition products of plant tissues may be able to mobilize the micronutrients which are not plant available but exist in sufficiently large quantity in the soil. (Lundstrom & Ohman, 1990).

The facts mentioned above indicate that, application of plant tissues to a soil which is deficient in a given micronutrient while the soil contains sufficiently large amount of the element,

may improve element availability by bringing the fraction of the element which is contained in the strongly bound fractions of the soil to the plant available, water soluble and exchangeable fractions, through different chemical and physical processes. Based on this background, this research was designed to investigate the efficiency of the mango (*Mangifera indica*) plant leaf powder extract in mobilizing iron from three types of soil samples which were collected around Jimma town, in western Ethiopia. The mango plant leaf was chosen for the study based on the widespread availability of the plant in the area.

1. MATERIALS AND METHODS

1.1. Soil and Plant leaf Sampling

The upper 0 - 15 cm of cultivated and forest soil samples were collected from the Eladale farm site of the Jimma University College of Agriculture and Veterinary Medicine, JUCAVM, (about 5 km north of the town) and its surrounding respectively by using a stainless steel Auger. Similarly, water logged soil sample was collected from Boye area (about 5 km South of the town). The random sampling technique was employed in collecting all soil samples. Fresh mango leaves were also

collected from the upper parts of the branches of randomly selected mango trees.

The soil samples were oven dried at 120 °C for 24 hrs and allowed to pass through a 2mm mesh size sieve and the ≤ 2 mm fractions were used for the study. The leaf samples were air dried at room temperature. The air dry plant leaves were hand crushed and then powdered by using an electric grinder. A portion, 100g, of the powdered plant leaf was soaked in 1L distilled water for 24 hrs and the extract was used to treat the soil samples.

1.2. Determination of Soil Properties

1.2.1. Acidity

A) Active Acidity (pH(H₂O))

Ten gram from each of the soil types was transferred into each of 50ml beakers. Twenty five mL of distilled water was added into each beaker and stirred for 15 minutes. The solutions were then allowed to stand for 5 minutes, and pH measurement was carried out by using a model ATC 353 pH meter. The pH meter was calibrated with buffer solutions before use.

B) Exchangeable Acidity (pH(KCl))

Ten gram from each of the soil types was transferred into each of 50ml capacity beakers. Twenty five mL of 1.0M potassium chloride was added into each soil sample and shaken for 15 min. The solutions were then allowed to stand for 5 minutes, and pH measurement was carried out by using a model ATC 353 pH meter.

1.2.2. Percent Organic Matter

All soil samples were oven-dried at 105°C and five gm from each of the oven-dry soil samples was transferred into clean tarred porcelain crucibles (in triplicates). Finally, the organic matter was removed by combustion of the soil sample at 600 °C for 3 hrs in a temperature regulated muffle furnace and the percent organic matter content was obtained by subtracting the percent mineral content from the total mass.

1.2.3. Soil texture

Soil texture were determined by using feel method (Gupta 2004)

1.3. Soil Treatment with Mango leaf

Extract.

A portion, 100g, of the powdered mango plant leaf was soaked in 1L distilled water for 24 hrs and filtered. Triplicates of 20 g portions of each soil sample were

soaked in 25 mL of the plant leaf extract for 24 hrs. Blank triplicate 20 g portions of samples were also soaked in 25 mL double distilled water. Then, each moist soil sample was spread on a watch glass and air-dried. These samples were used for the determination of iron in the various phases (speciation) of the treated soils.

1.4. Speciation of Iron in the soil samples

1.4.1. Water soluble

An aliquot, 3.0 g of each of the mango leaf extract treated and the untreated soil samples was transferred into 100 ml Erlenmeyer flasks in triplicates. Twenty mL distilled water was added into each flask and shaken overnight in a thermostated water bath set at 25 °C. The mixtures were then centrifuged for 15 min at 3000 rpm on a U18V-2 Centrifuge, and filtered by using a Black-ribbon S&S filter paper. The residue was used for the extraction of the exchangeable fraction and the filtrate was kept for analysis

1.4.2. Exchangeable

To each of the residues left after the extraction of the water soluble fraction, 40mL of 1M ammonium acetate, (pH=7) was added and shaken on a horizontal shaker for 5 hrs at 20 °C. Then the mixtures were transferred into a screw

cap centrifuge tube and centrifuged for 10 min at 2800 rpm, decanted and filtered. The residue was used for the

extraction of the carbonate bound fraction and the filtrate was kept for analysis

1.4.3. Bound to Carbonates

To each of the residues from the extraction of the Exchangeable fraction, 40 mL of 1M sodium acetate, (adjusted to pH=5 using acetic acid) was added and shaken on a horizontal shaker for 5 hrs at room temperature. Then the mixtures were centrifuged for 10 min at 2800 rpm, decanted and filtered. The residues were used for the extraction of the amorphous iron oxide fraction and the filtrate was kept for analysis

1.4.4. Bound to Crystalline Fe-oxide phase

To each of the residues from the extraction of the carbonate bound fraction, 30 mL of 1M hydroxyl amine hydro chloride solution in 25% acetic acid was added. Then the mixture was shaken and put in a thermostated water bath at 90 °C for 3 hrs (with the cup on tightly). Each mixture was then shaken for 20 min on a horizontal shaker, centrifuged, and filtered. Each of the residues was rinsed with 10 mL of 25% acetic acid twice and the supernatant was

added to the filtrates to make the final volume to 50 mL. The filtrates were then kept for analysis and the residues were used for the extraction of the fraction bound to organics and sulfides.

1.4.5. Bound to the Organics and sulfides phase

To each of the residues from the extraction of the crystalline Fe-oxide bound phase, 750 mg of potassium chlorate and 5ml of 12M hydrochloric acid were added. The mixture was capped and shaken carefully and additional 10 mL of hydrochloric acid was added. Each solution was then allowed to stand for 30 min and then, 15mL of distilled water was added and shaken for 5 min. The mixtures were then centrifuged for 10min, decanted and filtered. The filtrates were transferred into labelled test tubes. To each of the residues, 10mL of 4M nitric acid was added; the mixtures were shaken and placed in water bath at 90 °C for 20 min. Then the solution was shaken, centrifuged decanted and filtered. Each supernatant was added to its respective previous filtrate. The residue was finally rinsed twice with 5mL distilled water,

centrifuged, and filtrated. The filtrate was transferred into its respective test tube to make the final solution to 50mL.

1.5. Spectrophotometer Determination of Iron

Fifty mL of each filtrate was transferred into a 125 mL Erlenmeyer flask. Two mL concentrated hydrochloric acid and 1ml of hydroxyl amine hydro chloride solutions were added to each flask. Few boiling cheeps were added into each mixture and boiled until its volume reduced to 20 mL. Then, it was cooled and transferred into 100 mL volumetric flask. Ten mL of ammonium acetate buffer and 4mL of phenantroline solutions were added. The solution was homogenized thoroughly and allowed to stand for 15 minute for maximum color development. Finally, the concentration of iron in each of the solutions was determined by using a Modal 6300 UV-Vis spectrophotometer.

2. RESULT AND DISCUSSION

2.1. Soil Properties

Properties of the soils such as pH, texture, and percent organic matter content have been determined and mean values of a triplicate analysis for each of the properties are given in Table 1.

Table 1. Properties of the three soil types.

Soil type	pH			Texture	% Organic matter
	In H ₂ O	In 1M KCl	pH _(H₂O) - pH _(KCl)		
Cultivated	5.79±0.15	4.53±0.10	1.26±0.04	Sandy Clay Loam	15±0.55
Forest	5.29±0.18	4.65±0.14	0.64±0.02	Clay Loam	19±0.62
Water logged	5.01±0.2	4.82±0.17	0.19±0.01	Silty Clay Loam	7±0.32

2.1.1. Acidity

The data given in Table 1 indicate that, all the three soil types collected around Jimma were moderately acidic. However, the trend in the values of the active acidities (pH(H₂O)) and the salt-replaceable or exchangeable acidities (pH(KCl)) of the three types of soils were found to be different. The water logged soil was found to be the most acidic and the cultivated soil the least acidic. However, from the exchangeable acidity values we can see that, the cultivated soil is the most acidic and the water logged soil is least acidic. This fact clearly indicates that the water logged soil has a relatively small reservoir of H⁺ ion (pH(H₂O) - pH(KCl) = 0.19) while the cultivated soil, has a relatively large reservoir of H⁺ ions (pH(H₂O) - pH(KCl) = 1.26). The forest soil had an intermediate value.

The active acidity of a soil is a measure of the H⁺ ion activity in the soil solution at any given time, while, the exchangeable acidity is the acidity which is primarily associated with the aluminium and hydrogen ions that can be released into the soil solution by the unbuffered potassium chloride solution. Therefore, the relatively smaller difference between the active and exchangeable acidity in the water logged soil and larger difference in the cultivated soil indicates that, the water logged soil contains smaller concentrations of these ions in the exchangeable soil fraction while the cultivated soil has a relatively larger concentration. However, the total concentration of aluminium and total acidity (active + exchangeable + residual) of the cultivated soil is not

necessarily larger than that of the other soil types.

2.1.2. Percent Organic Matter

The data given in Table 1 also shows that, the percent organic matter content of the forest soil (19%) is greater than that of the water logged (7%) and cultivated soils (15%). The water logged soil contains the smallest percent organic matter content.

Soil organic matter comprises an accumulation of partially disintegrated and decomposed plant and animal residues and other organic compounds synthesized by the soil microbes as the decay occurs. The original source of the soil organic matter is plant tissue. As these organic materials are decomposed and digested by soil organisms, they become part of the underlying soil by infiltration or by actual physical incorporation. Accordingly, forest soils covered by higher plants, receive large quantities of organic matter from the tops and roots of trees, shrubs, grasses, and other native plants under natural conditions. As a result, the percent organic matter contents of such soils will be larger than that of soils that receive smaller or no plant organic matter. Similarly, in cultivated soils, one tenth to

one third of the plant tops commonly fall to the soil surface and remain there or are incorporated into the soil (Brady, 2002). Naturally, all of the roots remain in the soil. As these organic materials are decomposed and digested by soil organisms, they become part of the underlying soil by infiltration or by actual physical incorporation. Therefore, cultivated soils will also have higher percent organic matter content. However, the percent organic matter content of the forest soil sample was found to be greater than that of the cultivated soil. This can be accounted by the textural differences of the two soils types. The texture analysis data in Table 1 indicates that the cultivated soil has the highest sand content relative to the forest and water logged soil. Normally, soils with high sand content tend to drain quickly and have lower fertility. The organic matter in water logged soils is leached away from the soil and is not replenished by plants as in the case of the forest and cultivated soils. This accounts for the lower percent organic matter content in this sample.

2.2. Speciation of Iron in the Untreated Soil Fractions

The speciation results of the untreated soil samples depicted in Fig.1 indicates that, the total concentration of iron in the

six soil fractions of the cultivated soil was found to be 214 ± 8.5 ppm, in that of the forest soil 208 ± 6.9 ppm and that of water logged soil 257 ± 9.8 ppm.

And, the percent concentration of iron extracted from the various soil phases relative to the total iron concentration extracted in all the phases is given in Table 2.

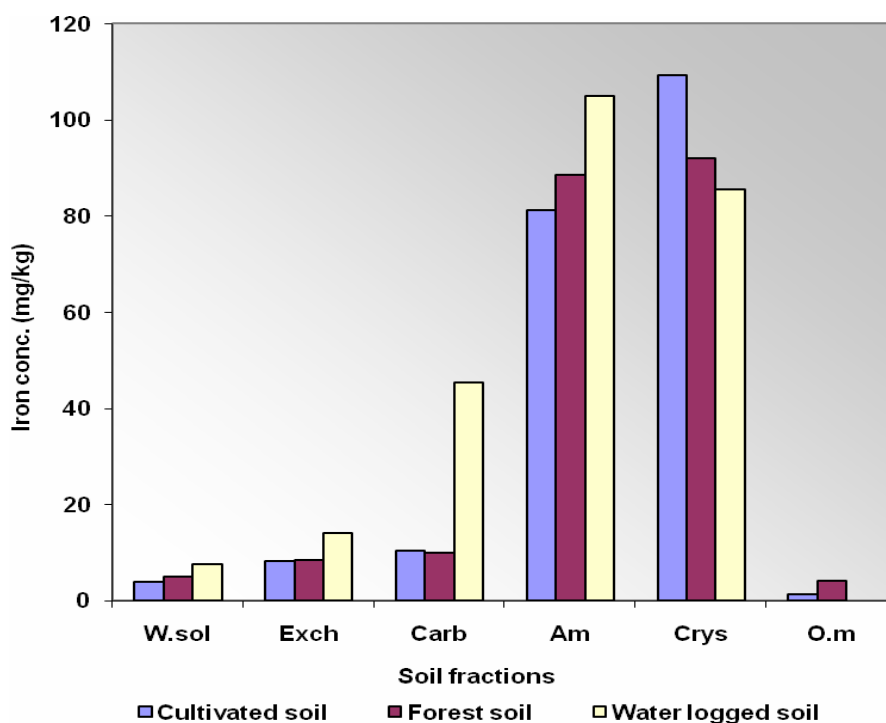


Fig. 1 Concentration of iron in the various soil fractions in non-treated soils.

The data shows that nearly 90% of the iron in the cultivated and forest soils is bound to the amorphous iron oxide, and crystalline iron oxide phases. This quantity reduces to about 80% in the

water logged soil. The concentration in the carbonate bound phase of the water logged soil is greater than in the same phases of the other soil types by more than 10%.

Table 2. Percent of the concentration of iron in each of the phases relative to the total concentration in the six soil phases.

Soil fraction	% iron (relative to total extracted Fe)		
	Cult	For	W L
W.sol	1.83±0.048	2.35±0.10	2.95±0.11
Exch	3.81±0.10	4.07±0.16	5.46±0.14
Carb	4.84±0.18	4.76±0.20	17.58±0.59
Am	37.85±1.25	42.62±1.90	40.76±2.10
Crys	51.02±1.98	44.23±1.80	33.25±1.32
O.m	0.65±0.02	1.96±0.07	nd

W.sol= water soluble, Exch= exchangeable, Carb=Bound to carbonate, Am = amorphous iron oxide, Crys= crystalline iron oxide, O.m= organic matter bound, nd = not detectable

The concentration of iron in the water soluble fractions of all the three soil types is less than their respective iron concentrations in all the other fractions. However, the relative water soluble iron concentration extracted from the water logged soil was found to be greater than that of the other two soil samples. The least water soluble iron concentration was found in that of the forest soil. The plant available fraction (water soluble + exchangeable) in the water logged soil was about 8.5 ppm that of forest soil was 6.5 ppm and that of the cultivated soil 5.5 ppm.

Iron is found in soils in more than one valence state. And, its lower valent state (+2) is prevalent under low oxygen supply and relatively higher moisture level. In addition to this, at pH values common in soils, the oxidized state (+3) of iron, is generally much less soluble than are the reduced states. The hydroxide of the high-valence form of iron is known to precipitate even at low pH values, reaching 3 to 4, and are extremely insoluble. Whereas ferrous hydroxide does not precipitate until a pH of 6.0 or higher is reached. This fact accounts, the higher concentration of mobile iron in the water logged soil.

Relative to the cultivated and forest soils, the water logged soil has the lowest oxygen supply and highest moisture level since it is covered with water for more than half of the year and its texture allows it to retain water. Therefore, this soil should have more concentration of the lower oxidation state iron; the iron species which is more mobile than the higher oxidation state under the prevailing mildly acidic soil pH in the area. The slightly greater concentration of iron in the water soluble fraction of the forest soil as compared to the cultivated soil could be due to the mobilization of iron by the organic compounds in the

forest soil which are greater in concentration than in the cultivated soil.

2.3. Speciation of Iron in the Mango (*Mangifera indica*) Plant Leaf Powder Extract Treated Soils

The iron speciation data obtained from the three soil samples which were treated with *Mangifera indica* leaf powder extract are given in Fig. 2.

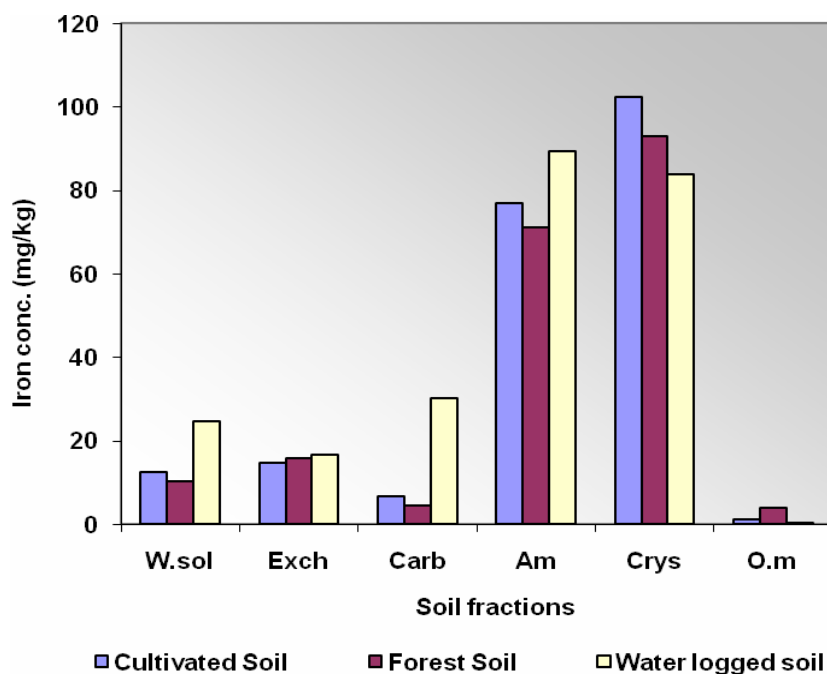


Fig. 2. Concentration of iron in the various soil fractions in *Mangifera indica* treated soils.

The results show that, the *Mangifera indica* plant leaf powder extract can mobilize a substantial amount of iron from the strongly bound soil fractions. The concentration of iron in the water soluble and exchangeable fractions of all the soil samples were found to increase while in the other fractions it was found

to decrease. However, the decrease in all the strongly bound fractions was not similar. The relative increase in the water soluble and exchangeable soil fractions and the decrease in the other fractions are given in Fig. 3. The figure depicts the percent iron concentrations relative to the total extracted iron before and after treatment with the *Mangifera indica* leaf powder in the various soil fractions side-by-side.

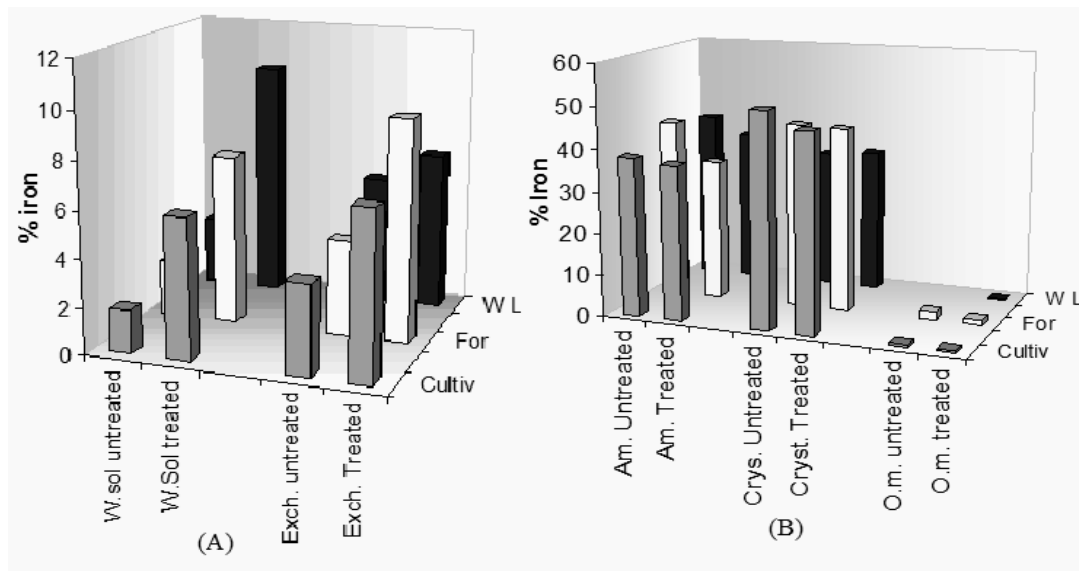


Fig. 3. Percent concentration (relative to total extracted in all phases) in the various phases of the soil extracts in the untreated and *Mangifera indica* leaf powder extract treated soil samples.(A) Relative concentrations in the water soluble and exchangeable soil fractions of the untreated and treated soils (B) Relative concentrations in the carbonate bound, amorphous iron oxide, crystalline iron oxide and organic matter bound iron fractions in **the untreated and *Mangifera indica* leaf powder extract treated soils.**

The results indicate that, the percent iron concentration in the water soluble fraction of the W.L soil showed a 7% increase while the forest soil increased by 5% and the cultivated soil by 4%. The trend in the percent concentration increase was not similar in the exchangeable phases of the three soil samples. The forest soil showed a 5% increase in its exchangeable fraction, the cultivated soil 3% and the water logged soil about 1.5%. On the other hand the percent iron concentration decrease observed in carbonate bound phase is highest for the forest soil (9%), followed by the water logged soil (4%) and the cultivated soil (1%). This fact indicates that the organic compounds originating from the plant leaf powder were able to release more iron from the carbonate bound fractions of the water logged and forest soils, as compared to that of the cultivated soil. The relatively greater release of iron from the carbonate bound fractions of the forest and water logged soils could be due to the oxidation state of iron in the soils. The two soil types are less oxidizing than the cultivated soil which is relatively aerated. The reduced and more mobile form of iron dominates in the soil fractions of less oxidizing soils. Therefore, this mobile iron could be easily brought into the water soluble and exchangeable fractions.

3. CONCLUSION AND RECOMMENDATION

Previous studies (Drever, 1994, Garrison, 1989, Schnitzer & Kodama 1977), have indicated that, organic substances, synthetic or natural, can mobilize essential plant micronutrients. It is also evident that, Plant tissues contain many organic compounds which are chemically varied. With these points in mind, the central goal of this study was to investigate the efficiency of the mango (*Mangifera indica*) plant leaf powder extract in mobilizing iron from three different types of soil samples which were collected around Jimma town, in western Ethiopia.

The findings of the study have shown that, treatment of the soil samples with the *Mangifera indica* plant leaf powder extract has substantially increased the concentration of iron in their water soluble and exchangeable fractions while decreasing the iron concentrations in the other more strongly bound fractions. In the water logged soil, the mango leaf powder extract treated soil had 7% more iron in its water soluble fraction as compared to that of the untreated soil (control). Similarly, the iron concentration in the water soluble fraction of the forest soil showed a 5%

increase and in that of the cultivated soil a 4% increase. On the contrary, the concentration of iron in the strongly bound fractions of all the soil samples was found to decrease although the decrease in the fractions varies with the strength of binding. This shows that the mango plant leaf contains chemical substances (possibly organic acids) that can form stable and soluble compounds with iron.

The fact that the mango plant leaf extract is able to mobilize iron from its strongly bound forms in the soil implies that, the plant leaf powder could be applied to soils which are deficient in plant available iron while containing sufficiently large total concentration. This can enable us to overcome iron deficiency in plants. In addition to iron the potential that the mango or other plant leaves have to mobilize other plant micro-nutrients need to be investigated.

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