The effect of cow manure application on the Distribution Fractions of Fe, Mn and Zn in agricultural soils

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Abstract: *The speciation of metals in environmental samples is a vital factor in assessing the potential environmental impacts. Fractionation of heavy metals in soils is needed to predict elemental mobility in soil and phytoavailability to plants. A study was conducted to determine the effect of cow manure on Fe, Mn and Zn availability and their redistribution among soil fractions. Chemical properties such as pH, EC, CEC and Lime of concerned soils are also analyzed. A soil was selected from agricultural soil in sistan va baluchestan, southeast Iran and amended with 0, 1 and 5% of Cow Manure (CM) with three triplications and incubated at 60% of field moisture. The amounts of Fe, Mn and Zn were determined from the soil after 4 months of incubation time using sequential extraction procedures. Tessier method was exerted to decompose the metal content into exchangeable (EXC), bond to carbonates (CAR), bound to Fe-Mn oxides (Oxids), bound to organic matter (OM) fractions and Residual (RES) fraction was determined in aqua regia digest. Results of these analysis showed that the concentrations of Fe and Mn decreased mainly in the carbonate-bound and residual fractions but increased in the exchangeable, Fe-Mn oxide and organic matter-bound fractions with cow manure application. It was concluded that cow manure was the most effective in immobilizing Zn. The application of Cow Manure (CM) levels significantly decreases the exchangeable, carbonate-bound and residual fractions (P < 0.05) of Zn and increased in the Fe-Mn oxide and organic matter-bound fractions.*
Keywords*: Fractionation, micronutrients, Mobility, Organic matter, Sequential Extraction.*

I. Introduction

Plants require optimum concentrations of a number of essential nutrients, including heavy metals such as iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), boron (B), and molybdenum (Mo), to operatively complete their life cycle. For normal growth and metabolism of plants, these metal ions are required in trace quantities (Hansch and Mendel, 2009). Mineral elements can be present in the soil as free ions, or ions adsorbed onto mineral or organic ligands, as dissolved compounds or sediments, as part of lattice structures or contained within the soil biota. The most important soil properties governing mineral availability are soil pH, cation exchange capacity, redox conditions, soil structure, activity of microbes, organic matter and water content (Frossard et al., 2000).

Manganese (Mn), iron (Fe) and Zince (Zn) are essential micronutrients for almost all lifing organisms including plants. Mn is required as a cofactor or activator for enzymes belonging to different functional groups which perform various roles. For example, oxalate oxidase, RNA polymerase, Mn superoxide dismutase (MnSOD), isocitrate dehydrogenase, malic enzyme, and PEP carboxykinase use Mn as a cofactor (Alschera, 2002). Mn is also important in photosynthetic oxygen evolution in chloroplasts. Mn deficiency causes interveinal chlorosis and a reduction in the percentage of fructans and structural carbohydrates, resulting in lax leaves (Ishimaro, 2012). Extremitily, Mn-deficient plants are more sensitive to low-temperature stress and pathogen infection, leading to significant decreases in crop yield (Hebbern, 2005). Similarly, Fe servers as an integral cofactor for numerous proteins. In plants, Fe is essential for chlorophyll biosynthesis and the synthesis of heme and its deficiency significantly mars plant growth and development (Bashir, 2010). Zince is necessary for several physiological and metabolic processes of plants (Ramesh et al., 2004). It acts as a cofactor for more than 300 enzymes (Guerinot and Eide, 1999); Zn-dependent enzymes are functional in all major organelles of the cell and are involved in DNA replication, translation, transcription, and photosynthesis (Kramer, 2005a,b). Zinc deficiency results in leaf chlorosis, shortened internodes, stunted growth, and tiny leaves (Cakmak, 2002). The importance of organic manure is that it contains little or no soluble salt and it is applied in a large content without the risk of nocuous crop roots and also micro organisms in the soil help to break the organic materials into inorganic water soluble forms for plant use (Hignette, 1999). Many studies have shown that addition of inorganic and or organic fertilizers increases plant growth substantially because they contain considerable quantities of plant nutrient, including micronutrients which have high profits for plant growth (Ibeawuchi et al., 2006). Influence of organic materials on the availability of the heavy metals depends on the nature of these metgals, soil types, and the organic matter properties, specially the degree of humification (Walker et al., 2004)

The determination of chemical species is problem and sometimes impossible. Hence, the use of sequential extraction procedures for environmental studies provides an important implement for the determination of the different chemical forms or ways of binding between trace metals and soil components (Konradi, 2005). Both single and sequential extraction methods are used in the study of the fate of environmental pollutants. Tacitly, specificity of single extractants can be improved by combining them into a series of sequential extractions. Residue from a first section extraction is used for the second extraction and so on through a number of stages (Rao, 2008). Thus, solid materials can be successively discreted into specific fractions extracted selectively by using series of specific reagents (Tessier et al., 1979). Several procedures, varying in manipulative complexity, have been proposed for determining the speciation of metals (Rao, 2008). However, following increasing interest in an application of sequential extraction, several threats are distinguished: limited electivety of the extractants, redistribution of elements among phases during extraction, and limited inductivety of data obtained by several investigators (Gworek and Mocek, 2003). Also, the traditional system of the fractions classification may lead to some disarray, suggesting an unreal selectivity of the extractants used. The sequential separation of metal species is therefore denoted on an operational basis and as such has been widely used in various environmental studies (Bacon and Davidson, 2008). The objective of this study was to examine the concentration and chemical fractions of Fe, Zn and Mn in the surface layer of agriculture soils that have been threated with cow manure for 4 months.

II. Materials And Methods

The analyzed soil samples were taken from the 0- to 30-cm layer of agricultural soil in sistan va baluchestan, southeast Iran. The soil samples were packeted in pre-cleaned, airtight plastic bags and transferred to the laboratory for further processing and analyses. In the laboratory, the soil samples were air-dried at room temperature (Jackson, 1958), homogenized/calmly crushed using an agate pounder and pestle and sieved through a standard sieve of 2 mm mesh size (Tandon, 2001). The soil samples with particle size of <2 mm were stored in acid washed plastic dishs. Total Organic Carbon (TOC, %) in soil was appraised following the wet digestion method of Walkley & Black (1934) and the values were returned to the Total Organic Matter (TOM). The Carbonate Carbon (CO_3-C, %) content in soil was appraised following the rapid titration method of Allen, (1989). The chemical fractionation of metals was evaluated following Sequential Extraction Procedure based on Tessier et al., (1979). Some of the respective chemical and physical properties of soil and cow manure were given in Table 1.

The experiment totally contained 3 treatments with three triplications. The cow manure were added to the soil (3 replicates per treatment) at 1 and 5% w/w and the third part was kept as the control. the samples were incubated at 25^0C for 4 months at 60% of water holding capacity.

The samples was transported with an mass of 1.0 g oven dried sample. The selective extraction was carried out in polypropylene centrifuge tubes of 50 mL capacity. After each successive extraction, the supernatant liquid was removed after centrifugation at 4000 rpm for 15 min and diluted to volume. The residue was washed with 8ml of distilled water and again centrifugation. The extracts were stored in polythene bottles for metal content determination. The conventional method developed by Tessier et al., (1979) was followed for the sequential extraction (table 2). The residual and total metal contents were determined in aqua regia digest (Esakku et al., 2005). The total metal content in fresh samples were extracted with aqua regia.

The extraction procedure was as follows:

Step 1. 1 M Magnesium chloride adjusted to pH 7.0

Step 2. 1 M Sodium acetate adjusted to pH 5.0 with Acetic acid.

Step 3. 0.04 M Hydroxylamine hydrochloride in 25% Acetic acid.

Step 4. 30% Hydogen peroxide in 0.02 M Nitric acid.

Step 5. Aquaregia digestion.

Table 1. Characteristics of the initial soil, Cow Manure used for the pot experiment (dry weight basis)

Parameter	Soil	Cow Manure
Sand (%)	46	-
Silt (%)	38	-
Clay (%)	16	-
Teature	loam	-
pH	7.43	7.5
EC (dSm^{-1})	1.45	11
CEC (cmolc kg^{-1})	12.87	-
Parameter	Soil	Cow Manure
OM (%)	0.67	12.5
Total Fe (ppm)	21.58	21.62
Total Zn(ppm)	6.79	7.33
Total Mn (ppm)	20	19.8
Lime (%)	20	-

EC: electrical conductivity
CEC: cation exchange capacity

Table 2. Protocol for sequential extraction procedure

Step no.	Fractions	Reagents	pH	Temperature (^0C)	Time shaking
1	Exchangable	1 M $MgCl_2$	7	25	1h
2	Acid Extractable	1 M Na OAc	5	25	5h
3	Reducible	0.1 M $NH2OH\_HCl$	2	96	6h
4	Oxidisable	30% H_2O_2	2	85	3h
5	Residual	$HNO_3–HCl$	-	120	2h

F_1: exchangeable
F_2: acid extractible fraction: metals bound to carbonates
F_3: reducible fraction: metals bound to hydrated oxides of iron and manganese
F_4: oxidise fraction: metals bound to organic matter
F_5: residual fraction

Statistical analysis

Data were analyzed by one-way ANOVA procedure using SPSS software and standard deviation (SD) was used to indicate variability. Significances were based on probability (P ≤ 0.05) level for Least Significant Difference (LSD).

III.   Results And Discussion

Soil properties

The range of clay and organic matter contents was 16% and 0.67%, respectively (Table 1). Soil CEC ranged from 12.87 cmolc kg^{-1}. The soils have a almostly alkaline pH, CaCO3 concentrations (mean of 20 %), and EC 1.45 dS m^{-1}.

Heavy metal total content

Table 1 shows total metal concentrations of Fe, Mn and Zn in soil sample. The total concentrations of Fe, Mn and Zn were 21.58, 20 and 6.79 mg kg−1, respectively. Altogether, Fe was the most abundant metal; presumably due to the Fe-enriched parent materials. The mean values of heavy metal contents arranged in the following decreasing order: Fe>Mn> Zn.

Distribution of chemical fractions of Fe, Zn and Mn in the cow manure treatment

Bio-available fractions: The exchangeable and bond to carbonates fractions, relevanting to the first two fractions, are usually called "bio-available", as they show a mobility comparative to the environment and have rather leaching potential than the other fractions. However, Results indicate that the amounts of Fe and Mn in exchangeable fraction are very low, but along with the application of CM levels, the values of Fe and Mn in exchangeable fraction is partly impressive, namely along with increased of CM levels, increased Fe and Mn concentration significantly relative to the control in the exchangeable fraction and decreased in the carbonate-bound fraction (P < 0.05). This significantly increases Fe concentration relative to the control, at 1% and 5% levels, for exchangable section was 11.76% and 11.76% respectively (fig 1), and for Mn concentration, was 10% and 20%, respectively (fig 2). In carbonate section, significantly decreases Fe concentration relative to the control, at 1% and 5% levels was respectively 17.35% and 17.35% (fig 1) and for Mn concentration, was 30.33% and 16%, respectively (fig 2). However, for Fe concentration no significant difference was observed between the two CM at 1% and 5% levels in the these two sections. But, for Mn, compared with 1% CM application, higher level of CM resulted in less decline of carbonate-bound fraction. The reason should be remarked to three phases: (1) lager amount soluble organic ligands was derived from CM, therefore the complexation of Mn with the ligands was more competitive than the adsorption to the soil organic matter (Madrid et al., 2007); (2) the soluble organic matter could increase the Mn availability by providing a strong buffer for free Mn (Song et al., 2004; Businelli et al., 2009); (3) higher level of CM can significantly increase the biomass of soil microorganisms which would enhance organic matter mineralization, thus increasing the potential Mn mobility in soil (Lipoth and Schoenau, 2007; Marcato et al., 2009). For Zn, reductions in the percentage present in the exchangeable/ bond to carbonates fractions following additions of Cow Manure. This significantly decreases Zn concentration relative to the control, at 1% and 5% levels, for exchangable section was 21.42% and 67.85% respectively (fig 3), and for carbonate section, was 100% and 100%, respectively (fig 3). It is noteworthy that, for Zn concentration, no significant difference was observed between the two CM at 1% and 5% levels in the carbonate section (P < 0.05). The main reason of deficiency of plant-available Zn in soil is the sediment or adsorption of Zn with various soil components, depending on the pH and redox potential (Impa and Johnson-Beebout, 2012). Sabir et al., (2008), showed that the non significant variations in Zn amount of soil may be due to the low level of Zn present in soil as well as in water. For example, 25 years of annual applications of cattle manure to soil in Southern Alberta increased organic matter concentrations basically. Soil

experiment extractable Zn concentrations decreased significantly whereas extractable Cu concentrations increased (Benke et al. 2008). Moreover, Allaway, (2009), associations between low soil zince concentrations and micronutrient deficiencies in humans reported for iodine and selenium concentrations.

McLaren and Crawford, (1973) and Williams et al., (1980 and 1985) stated that low mobility was due to specific adsorption of the metals. The tendency of heavy metals in the carbonate fraction might indicate a pH suitable for metal precipitation. Moreover, calcium carbonate may act as a strong absorbent for heavy metals and could complex as double salts like $CaCO_3.MCO_3$ (Ramos et al., 1994). Sabir et al., (2008) in their incubation studies reported reduction of Mn with addition of activated carbon, but DTPA extractable Mn increased with farm yard manure and poultry manure. Also, sally Brown et al., (2005) showed that extractable metals (Zn, Pb and Cd) concentrations were declined by amendment application. These results imply that in cases of superabundant contamination, soil extracts may not be an apposite predictor of plant response. Moreover discussed that, in soils irrigated with sludge for long periods, Zn is biologically available and mobile, which exhibits a menace to crops and water quality (Kabala et al., 2011).
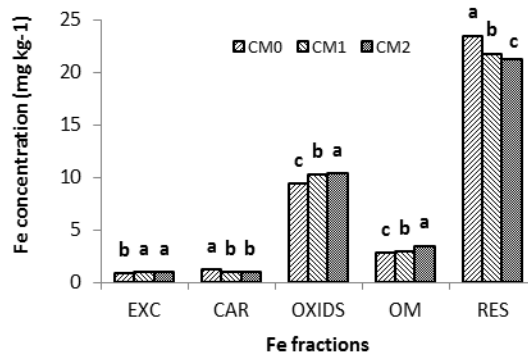

Fig 1. Effect cow manure on the Distribution of chemical fractions of Fe(Mean values followed by different letters are significantly different ($p \leq 0.05$) by Tukey test)
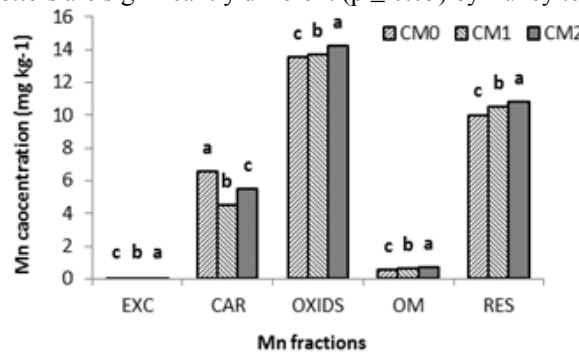

Fig 2. Effect cow manure on the Distribution of chemical fractions of Fe(Mean values followed by different letters are significantly different ($p \leq 0.05$) by Tukey test)
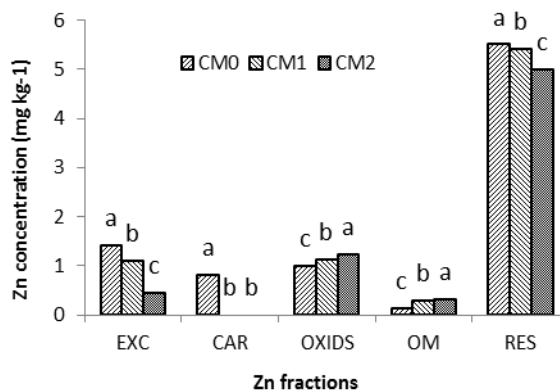

Fig 3. Effect cow manure on the Distribution of chemical fractions of Fe(Mean values followed by different letters are significantly different ($p \leq 0.05$) by Tukey test)

Species bound to Fe-Mn oxides: Fe-Mn oxids fraction exhibits the second most significant sink for Fe, Mn and Zn after residual fraction. Albeit the mobilitys of metal scavenging by Fe and Mn oxides is still trivially understood, assuming that the extraction using Hydroxylamine hydrochloride with acetic acid is an suitable indicator of metals associated with amorphous iron and manganese oxides, this process seems to indicate a significant control on the metals studied in this grassland system. Although the affinity of metals to Fe-Mn oxides indicates that metals are relatively immobilized, slight chemical changes in the ambient conditions could result in their likely mobility and easy absorption by plants. pH and redox changes are the two ambient factors in this portal.

It has been shown that the second extraction step (carbonate fraction) may not be drastic in removing all the carbonate minerals into solution. Metals extracted in step 3 (Fe-Mn oxids fraction) hence may comprise a percentage of the carbonate fraction in addition to those bound to Fe-Mn oxides (Maskall and Thornton, 1998).

The application of CM treatment in both levels, increase bound to Fe-Mn oxides fraction to all heavy metals, which had significant difference relative to the control treatment ($P < 0.05$). Increase concentrations of Fe, Mn and Zn in 1% and 5% levels at the end of 4 months of incubation time was measured which presented in figures 1 to 3. dialectically, Mn occur mainly in the oxide fraction, at CM levels (significantly increases Fe concentration relative to the control, at 1% and 5% levels was respectively 9.3 % and 10.69% (fig 1), 1.4 % and 4.94% Mn (fig 2). Zn bond to oxides occur in the low proportion in samples, around 12 % and 24% respectively (fig 3). The fraction of Fe, Mn and Zn associated with oxides is much higher in the CM at 5% level, especially in the case of Mn. The fraction of elements bound to Fe/Mn oxides in soil 2 is the most important. The high amount of Zn associated with Fe-Mn oxides may comprise metals present in other poorly crystalline compounds (Kuo et al, 1985; Ramos et al, 1994). The attendance of Fe and Mn in Fe-Mn oxides and residual fractions agrees with many studies (Ehsan et al. 2006; Guo et al. 2006) that have shown that Fe and Mn has high contiguity for Fe–Mn oxides and rsidual fractions. Guo et al. (2006) in Songhua river sediments showed that Fe and Mn display the strongest contiguity to Fe–Mn oxides and residual fractions.

Species bound to organic matter: The amount Fe, Mn and Zn concentration in bound to Fe-Mn oxides fraction, increased with increase of cow manure levels. The lowest of amount concentration of Fe, Mn and Zn related to control treatment and utmost of amount concentrations related to CM at 5% level. The significantly increases ($P < 0.05$) Fe, Mn and Zn concentration relative to the control, at 1% level was respectively 4.28%, 11.66% and 107% and at 5% level was respectively 23.57%, 23.33% and 135.7% (fig 1, 2, 3). Fe has a much more contiguity for organic soil colloids (humic material) than Zn and Mn.Thus, acetic acid (which extracts metals mainly from inorganic fractions) extracted a more percentage of Zn and Mn than Fe. The organic complexes associated with Mn are relatively impermanent, because the complex associated with humic acid has an utterly electrostatic quality, and fulvic acids have a limited digit of complexing sites that are specific to the element (McBride, 1994).

Karbassi and Shankar, (2005) related that in the coastal sediment cores Cu, Zn and Fe are associated with organic matter and detrital particles, whereas Ni and Co are tyupically associated with the insoluble fraction. While profusion of calcareous shells in some zones has lead to the dilution of most of the metals, it demonstrators that Pb and Mn are associated with this phase.

Species in residual fraction: The proportion of Fe and Zn (above 50 %) in the residual form of is much higher than the other fractions, while the weight of Mn in this fraction was less than bound to Fe-Mn oxides fraction in treatments. The residual fractions of Fe and Zn are higher in control treatment than in cow manure treatments. Namely, the residual fraction reduced slightly after CM application at 1% and 5% levels. But, The residual fraction of Mn is lower in control treatment than in cow manure treatments. The significantly decreases ($P < 0.05$) Fe and Zn concentration relative to the control, at 1% level was respectively 7.28% and 2.16% and at 5% level was respectively 9.42% and 9.74% (fig 1, 3). And significantly increases ($P < 0.05$) Mn concentration relative to the control, at 1% and 5% levels was respectively 5.6% and 8.2% (fig 2). The observed higher relationship of Fe and Zn to the residual fractione is compatible with observations of Li et al., (2001). The highest attachment of Zn against residual fraction was also related by Svete et al., (2001) in a study on the chemical partitioning of Zn from a mine area. Zn, in general, rather exists in drastic associations with soil silicates in the short time, and the transformation of residual Zn in impermanents fractions depends on soil management (Lucho-Constantino et al., 2005). Ehsan et al., (2006) derived that Fe and Mn had strongest relationship to residual fraction. These findings implied that Fe and Mn were held in a more strict fraction in which the movement of these heavy metals in the soil profile would be trivial.

IV. Conculsion

In interpreting the results of this study it is important to note that after CM application at 1% and 5% levels, Fe, Mn and Zn is sorbed to inorganic soil colloids (Fe and Al oxides) by specific adsorption more strongly and, in addition, the application of CM levels, significantly declined the carbonate of Fe, Mn and Zn.

And significantly declined the residual fractions of Fe and Zn, and significantly increased the residual fraction of Mn. Also declined the exchangable fractions of Zn and inceased the exchangable fractions of Fe and Mn. Generally, the results indicate two major points: (1) rsidual fraction is the major binding site for Fe, Mn and Zn, indicating that the major proportion of the metal is associated in the silicate mineral matrix. This may indicate that this element was branched from natural geological sources. (2) Fe-Mn oxids fraction is the important binding site for Fe, Mn and Zn and the reducible Fe and Mn plays a important role in binding these metals.

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