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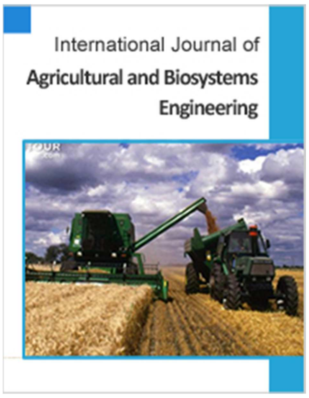

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Increasing Soil Nutrients Availability and Sustainability by Glomalin in Alkaline Soils

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Abstract
Alkaline soils in arid areas frequently have low-nutrient contents available for plant growth. Glomalin is a mycorrhizal glycoprotein produced in soil with the ability to sequester soil nutrients thereby increasing their availability according to the soil-ecological conditions. The study area has been selected to cover two different agro-ecological areas (coastal region: S1-S7 and S13-S16; Eastern Delta region: S8-S12). Within these areas, sixteen agricultural fields were selected with various soil textures, different water resources, appropriateness of the drainage system, manure addition, crop rotation and plant cover at sampling. Soil texture, pH, electrical conductivity (EC), soil organic carbon (SOC), easily extractable glomalin-related soil protein (EEGRSP) and total glomalin (TGRSP) contents were analysed. Soil micronutrients (Fe, Zn, Mn and Cu) and potentially toxic metals (Cd, Pb, Co, Ni and Cr) were measured in soil and in each extraction cycle of glomalin. Organic carbon in the GRSP extraction solutions amounted by 26-30% in all soils. One-way ANOVA showed significant differences ($p<0.05$) between the studied soils demonstrating the effects of agro-ecological differences on soil ecosystems. All soils showed wide range concentrations of metal ions bound to glomalin. The GRSP-bound metals were Fe_{GRSP} (0.04-1.16 mg kg^{-1}), Zn_{GRSP} (0.69 mg kg^{-1} only in S4), Mn_{GRSP} (9.52-105.16 mg kg^{-1}), Cu_{GRSP} (1.05-5.01 mg kg^{-1}) Ni_{GRSP} (0-0.23 mg kg^{-1}) and Pb_{GRSP} (2.70-3.26 mg kg^{-1}) and highly found in S4 and S8-S12 soils intercropped with legumes and annually received manure addition. The cumulative increase of these metals observed along the sequential extraction of GRSP may indicate the ability of glomalin for increasing their availability and sustainability during its persistence in soil. Factor analysis explained 41% of total variance in the 1st Factor with high positive loadings from silt, clay, SOC, TGRSP, Fe, Mn, Cu, Ni, Cr, Fe_{GRSP}, Mn_{GRSP} and Pb_{GRSP}. Factor 2 with 21% of total variance was positively correlated with EEGRSP, Zn, Cu, Cd, Pb, Fe_{GRSP}, Cu_{GRSP} and Pb_{GRSP}. These findings illustrate the capacity of glomalin to bind metals for increasing their availability to plant growth and, in addition, alleviate the effects of toxic metals depending on the appropriateness of drainage system, manure additions, crop rotation and changes in plant cover. As a result, glomalin can be used as a biofertilizer for sustainable agricultural management to help manage soil nutrients sustainability. It can be also used as a phytoremediator to recover toxic metals in polluted soils.

1. Introduction

Agricultural areas in Egypt are concentrated in the Nile delta and have recently been extended to marginal areas at the East and West of the Delta region and the Northern coastal region. All these regions are characterized by alluvial alkaline soils with high $CaCO_3$ and classified as salt-affected soils due to high salinity risks. These alluvial soils were formed from the deposition of organo-mineral materials during the flooding seasons for thousands of years before the construction of Aswan Dam [1]-[3]. The soils are extremely fertile with loamy texture due to the abundance of organic matter and minerals [4]. At the marginal areas and particularly in the northern coastal region, calcareous soils are predominated and affected by a Mediterranean climate. These areas often receive animal manure and are planted with legumes and Alfalfa to provide the soil with mycorrhizae and nitrogen-fixing bacteria [5]-[6]. This type of soil agricultural management allows these newly reclaimed areas to increase their fertility conditions within three to five years which cannot be achieved under any other system. It may also provide a complete nutrient source containing soil macro and micronutrients thus promoting soil biological activity and consequently improving soil structure.

In arid areas soils are characterized by low nutrients contents and the majority of plants tend to improve nutrient uptake through symbiotic associations with soil microbes, including fungi, that have the ability to resist desiccation and drought [7]. Thus plants develop strategies to grow under a variety of stressful conditions [8]. The major fungal group found in the roots of 80% of terrestrial plant species is the arbuscular mycorrhizal fungi (AMF). AMF live in mutualistic symbiotic associations with host plants, increasing nutrient uptake thus enhancing plant productivity. AMF produce a glycoprotein in soil known as ''glomalin''. Glomalin is operationally identified as glomalin-related soil protein (GRSP) totally extracted by sequential extractions using sodium citrate (pH 8.0) at 121°C [9], [10], [11]. It contributes to the formation of stable soil aggregates [12]. Increased soil aggregates stability may protect adsorbed nutrients within soil aggregates [13]. Moreover, soil aggregates increase the potential of metal nutrients sustainability and the exchangeable cations and micronutrients they contain may be good indicators of soil health. Therefore, glomalin can be considered as a stabilizing agent, in the formation of soil aggregates, and a binding agent of soil minerals promoting the formation of organo-mineral complexes [10], [14]. Many studies have shown high variability in cations bound to GRSP among different soils [15]-[16]. In addition, GRSP showed a high potential sequestration capacity for Pb, Cd, Zn, Cu, Fe and Mn thus influencing their mobility in the soil [16]-[18].

This work aimed to identify the capacity of GRSP to sequester metal ions in alkaline soils subjected to different agricultural practices. GRSP-bound metals were measured in the sequential extractants of GRSP to provide a direct evaluation of soil nutrients and toxic metals adsorbed to glomalin and their sustainability under different soil management practices.

2. Materials and Methods

2.1. Study Area and Sampling

The study area has an arid Mediterranean climate with temperature ranges from 8-25°C in winter and around 30-36°C in summer. The study area is including sixteen agricultural fields (S1-S16) of alkaline soils subjected to different agricultural practices. It covers two agro-ecological zones; Coastal region (S1-S7 and S13-S14) and Eastern Delta region (S8-S12). The study area is typical for the extensive flood plains in the Nile River Delta region. The potential evaporation rate is 1,500 mm year^{-1} with low precipitation (~120 mm year^{-1}). The studied soils have been selected to represent different sampling sites with various soil textures, water resources, appropriateness of drainage system, frequent manure applications, crop rotation system intercropped with legumes and current cultivation at sampling. The current cultivation of each site is recorded in Table 1. The major crops are rice, wheat, barley, corn, clover and cotton and sometimes intercropped with legumes and alfalfa. Surface irrigation is the common system used in all sites. The water table is shallow at 2.5-4 m depth thus producing salt-affected soils [19].

In the coastal region, soils (S1-S7 and S13-S16) are generally irrigated by Nile water coming from El-Nasr canal supplied by El-Noubariya canal. The S13-S16 soils are occasionally irrigated by moderately saline water (groundwater). Soils are located in New Borg El-Arab city (Alexandria Governorate) and are colluvial calcareous alkaline soils classified as Typic Calciorthids according to the Soil Association Map of Egypt [20]-[21]. At the Eastern Delta region, soils (S8-S12) are located in El-Hawaber village, Diarb Nigm city (El-Sharkia governorate) and are alluvial alkaline soils classified as Vertic Torrifluvents, the prevalent soil characteristic of the Egyptian Nile River Delta. These soils are generally irrigated by Nile water comes from Damietta branch with a total dissolved salts (TDS) of 400-600 mg L^{-1}.

Soils were sampled in triplicate at each site and combined to obtain a representative soil sample. Coordinates of each site can be seen in Table 1. Samples collected from the upper 30 cm depth of the A-horizon. The first site (S1) represents a soil without any agricultural management (bare soil) with a loamy sand texture. Other soils at S2-S6 are sandy loam and soils at S7-S16 are sandy clay loam. Soils at S8-S12 receive a manure application annually and do not have an appropriate drainage system leading to salt accumulation in the upper surface layers.

2.2. Experimental Analyses

2.2.1. Soil Analysis

All soils were air-dried, ground and sieved at 2 mm.

Particle size analysis was determined using Hydrometer with Bouyoucos scale in g L^{-1}. Soil pH and electrical conductivity (EC) were measured in 1:1 (w/v) aqueous soil suspension. Soil organic carbon (SOC) was determined using dichromate oxidation method. Soil micronutrients such as Fe, Zn, Mn and Cu and potentially toxic metals including Cd, Pb, Cr, Ni and Co were extracted in diethylenetriaminpenta acetic acid (DTPA) solution [22] then quantified by the Agilent 4100 Microwave Plasma-Atomic Emission Spectrometer (MP-AES) (USA).

Glomalin was extracted in 20 mM trisodium citrate (pH 7.0) at 121°C for 30 min to represent the labile fraction (F1; first fraction) that is hereafter named the easily extractable glomalin-related soil protein (EEGRSP). Sequential extraction autoclave cycles were performed to obtain the other 10 fractions (F2-F11) using 50 mM trisodium citrate (pH 8.0) at 121°C for 30 min [10], [11], [13], [23]. The total extractable glomalin-related soil proteins (TGRSP) represented the sum of all fractions. All glomalin fractions were separately quantified by using the Bradford protein assay [11], [24]. Contents of micronutrients (Fe, Zn, Mn and Cu) and toxic metals (Cd, Pb, Co, Ni and Cr) in each fraction and in the TGRSP (representative sample) were quantified by the Agilent 4100 Microwave Plasma-Atomic Emission Spectrometer (MP-AES) (USA). Carbon in each glomalin fraction (G-C) was detected using the TOC Analyser (Torch Combustion TOC/TN Analyzer - Teledyne Tekmar, Ohio, USA) and expressed as a percentage.

2.2.2. Statistical Analysis

All data, previously standardized, were statistically analysed using STATISTICA 10 of StatSoft, Inc. [25] (Tulsa, Oklahoma, USA). One-way ANOVA was checked for the studied soil parameters. Pearson correlation was checked for all measured soil parameters. Factor analysis was run to reduce and classify variables (soil properties). The first three factor structures explained the most significant variance within the variables (soil properties). Moreover, factor analysis provided factor score values to explore the relationships between the variables (soil parameters) and the 16 soils to obtain a picture of the overall dynamics.

3. Results and Discussion

3.1. General Soil Physico-Chemical Characteristics

All soils were moderately alkaline conditions within a pH range of 7.5 to 8.5 (Table 1). Using the one-way ANOVA, high significant variability ($p<0.05$), indicated by letters from a-c, for clay, silt, sand, pH, EC and SOC was observed among the studied soils reflecting the variations in soil texture and ecological areas. For example, EC values showed significant data variability in soils with sandy loam (mean=1.075±0.81 dS m^{-1}), sandy clay loam (mean=4.44±2.31 dS m^{-1}) and loamy sand (mean=15.50±0.16 dS m^{-1}). Soils at S1 can be classified with very strong salinity risk [26] (>9.5 dS m^{-1}). Strong salinity risk (4.8-9.4 dS m^{-1}) was observed in S9, S11 and S13-S16 due to the inappropriate soil management and high salinity of water for irrigation in those soils (TDS 3000-4000 mg L^{-1}) while moderate salinity (2.5-4.4 dS m^{-1}) was observed in S4 and S8-S12. Slight salinity risk (1.3-2.4 dS m^{-1}) was observed in soil under Alfalfa (*Trifolium alexandrinum*) (S10). No salinity risk (<1.2 dS m^{-1}) was observed in S2-S3 and S5-S7 probably due to the low salt concentrations in the irrigation water (Table 1). The high SOC contents observed in S4 and S8-S12 were due to the frequent manure application. Soils in S8-S12 showed with high salinity and organic matter contents due to manure addition in soils with an inappropriate drainage system leading to salt accumulation and resulted in salt-affected soils. The highly significant negative correlation was found between soil pH and electrical conductivity ($r=-0.716$, $p<0.01$).

Table 1. *Locations Description, Current Plants and General Soil Characteristics of the Studied Soils.*

Site	Plant	Coordinates		Sand%	Silt%	Clay%	pH	EC (dS m^{-1})	SOC (mg/kg)
S1	Bare soil	30°44'22.22"N	29°28'32.08"E	83.60 ^{b}	4.46 ^{ab}	11.94 ^{a}	7.56±0.03 ^{a}	15.50±0.16 ^{c}	2.79±0.30 ^{a}
S2	Bean	30°44'14.35"N	29°29'26.54"E	75.72 ^{b}	5.25 ^{a}	19.03 ^{a}	8.50±0.06 ^{b}	0.66±0.00 ^{a}	2.30±0.00 ^{a}
S3	Wheat	30°44'20.80"N	29°30'19.64"E	73.09 ^{b}	7.88 ^{a}	19.03 ^{a}	8.43±0.08 ^{b}	0.68±0.02 ^{a}	3.52±0.35 ^{a}
S4	Apple	30°45'30.04"N	29°32'10.67"E	75.72 ^{b}	6.56 ^{a}	17.72 ^{a}	8.04±0.02 ^{b}	2.53±0.01 ^{a}	10.08±0.35 ^{a}
S5	Artichoke	30°45'36.04"N	29°31'8.21"E	73.09 ^{b}	7.88 ^{a}	19.03 ^{a}	8.34±0.07 ^{b}	0.76±0.03 ^{a}	5.74±0.30 ^{a}
S6	Artichoke	30°46'4.86"N	29°30'58.86"E	70.47 ^{b}	10.50 ^{a}	19.03 ^{a}	8.06±0.02 ^{b}	0.75±0.00 ^{a}	10.33±0.00 ^{a}
S7	Alfalfa	30°46'4.99"N	29°32'34.51"E	73.09 ^{a}	5.25 ^{c}	21.66 ^{b}	8.44±0.05 ^{ab}	0.57±0.00 ^{b}	5.82±0.58 ^{b}
S8	Onion	30°43'13.67"N	31°23'45.11"E	57.34 ^{a}	18.38 ^{c}	24.28 ^{b}	7.87±0.04 ^{ab}	3.85±0.02 ^{b}	15.41±0.23 ^{b}
S9	Onion	30°42'58.77"N	31°23'39.50"E	52.09 ^{a}	21.00 ^{c}	26.91 ^{b}	7.85±0.06 ^{ab}	6.54±0.20 ^{b}	13.93±0.46 ^{b}
S10	Alfalfa	30°43'6.18"N	31°24'14.31"E	52.09 ^{a}	13.13 ^{c}	34.78 ^{b}	8.04±0.02 ^{ab}	1.73±0.12 ^{b}	14.43±0.70 ^{b}
S11	Garlic	30°43'0.57"N	31°23'7.02"E	52.09 ^{a}	21.00 ^{c}	26.91 ^{b}	7.76±0.01 ^{ab}	8.53±0.08 ^{b}	14.84±0.58 ^{b}
S12	Lettuce	30°43'22.82"N	31°23'11.40"E	57.34 ^{a}	13.13 ^{c}	29.53 ^{b}	8.02±0.03 ^{ab}	3.30±0.03 ^{b}	14.75±0.23 ^{b}
S13	Alfalfa	30°48'30.97"N	29°31'52.20"E	65.22 ^{a}	10.50 ^{bc}	24.28 ^{b}	7.68±0.06 ^{ab}	5.47±0.06 ^{b}	5.82±0.58 ^{a}
S14	Bean	30°47'5.26"N	29°37'42.13"E	59.96 ^{a}	13.13 ^{bc}	26.91 ^{b}	7.83±0.02 ^{ab}	5.03±0.23 ^{b}	6.15±0.12 ^{a}
S15	Zucchini	30°57'30.64"N	29°42'46.12"E	52.09 ^{a}	18.38 ^{bc}	29.53 ^{b}	8.38±0.04 ^{ab}	5.58±0.45 ^{b}	7.21±1.62 ^{a}
S16	Barley	30°57'3.05"N	29°46'7.43"E	49.46 ^{a}	18.38 ^{bc}	32.16 ^{b}	8.43±0.02 ^{ab}	3.77±0.04 ^{b}	5.25±0.23 ^{a}

EC: Electrical conductivity; $CaCO_3$: Calcium carbonate contents; SOC: Soil organic carbon.
Different letters (a-c) indicate the significant data variability at $p<0.05$ checked by one-way ANOVA.

3.2. Glomalin as a Reserve of Soil Organic Carbon

Glomalin is a glycoprotein containing about 15% carbon and has been shown by many authors to be positively correlated with SOC pools [10], [11], [13], [23], [27], [28]. Glomalin is considered to be a stabilizing agent binding soil nutrients and minerals with soil particles in the formation of organo-mineral complexes and the stabilization of soil aggregates. These complexes may contribute to soil carbon storage capacity via the assimilation of atmospheric CO_2 by mycorrhizal plant species. The current study showed a wide range of soil glomalin contents not only due to the significant differences in soil texture but also due to differences in plant species and manure application thus affecting the preservation of SOC pools [29]. In the 1st graph of Figure 2, the highest glomalin contents were generally found in the first fraction (F1) that represent the easily extractable glomalin fraction. Glomalin then decreased from F1 to F8. The F8 can be operationally considered as the final fraction containing glomalin molecules due to the disappearance of the brown colour in this fraction. The increasing of GRSP values in the F9 may indicate the reappear of glomalin-like compounds probably extracted with sodium citrate. In the 2nd graph of Figure 2, carbon contents in all glomalin fractions showed low concentrations (1-2%) in the first fraction (F1) in all soils. The carbon content in glomalin extraction fractions increased from F1 to the highest concentrations (4-5%) in the F6 and then decreased again <1% in the last fraction (F11). This means that different organic compounds can be found from each fraction of glomalin indicating the presence of other glomalin-like compounds probably subjected to different stabilization mechanisms of organic carbon (G-C) in all soils.

Accordingly, significant positive correlations ($p<0.01$) were obtained for the EEGRSP and TGRSP as a function of soil organic carbon (SOC) contents (Figure 1). The TGRSP contents were negatively correlated with sand ($r=-0.648$, $p<0.01$) while positively correlated with silt ($r=0.498$, $p<0.05$) and clay ($r=0.698$, $p<0.01$). SOC increased significantly with the EEGRSP ($r=0.658$, $p<0.01$) and TGRSP ($r=0.846$, $p<0.01$). The highest concentrations of the easily extractable glomalin fractions were found in soils under apple (S4) and under artichoke (S6) in agreement with the highest SOC contents among sandy loam soils. The highest concentrations of TGRSP were also found in soils under apple (S4) and soils of S8-S12 due to the high SOC contents because of the intense manure applications. In addition, intercropping with legumes and Alfalfa at these soils S8-S12, beside annual manure applications, promoting the potential preservation of SOC pools to be stabilized by the beneficial soil microbes such as mycorrhizae and nitrogen-fixing bacteria. This promotion in the preservation of organic compounds carried out by soil microbes is owing to the manure application that provides the soil with a complete nutrient source for promoting biological activities in the upper soil layers thus improving soil physical characteristics.

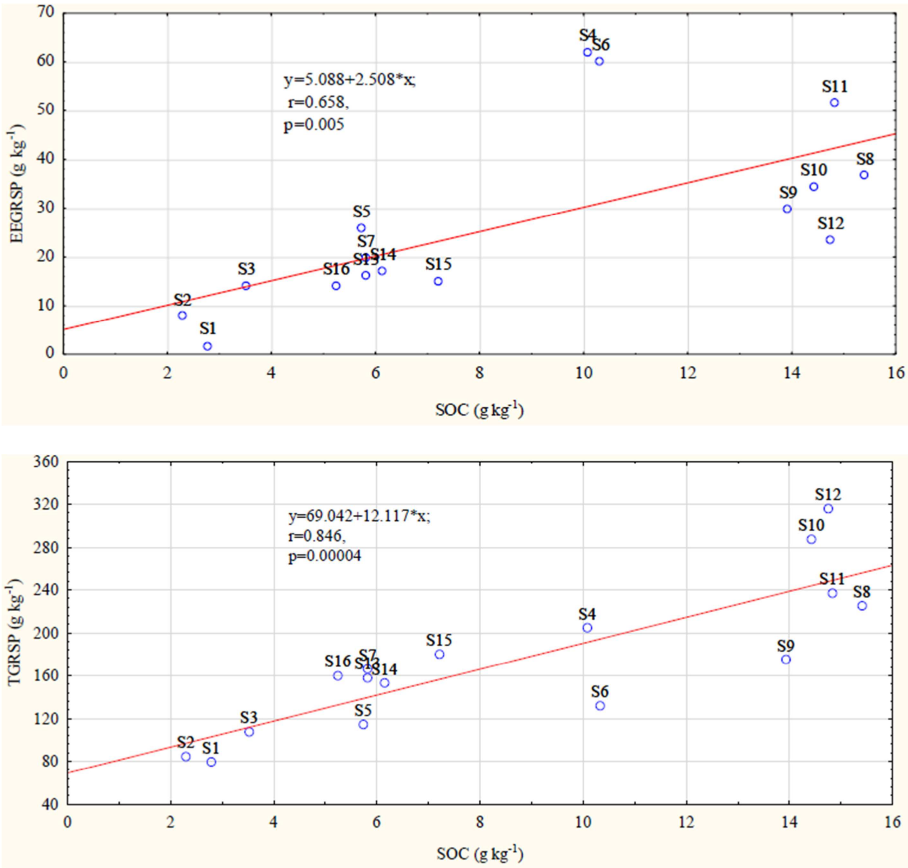

Figure 1. *Linear Correlations Between the Easily Extractable (EEGRSP) and Total (TGRSP) Glomalin-Related Soil Protein and Soil Organic Carbon (SOC) Contents.*

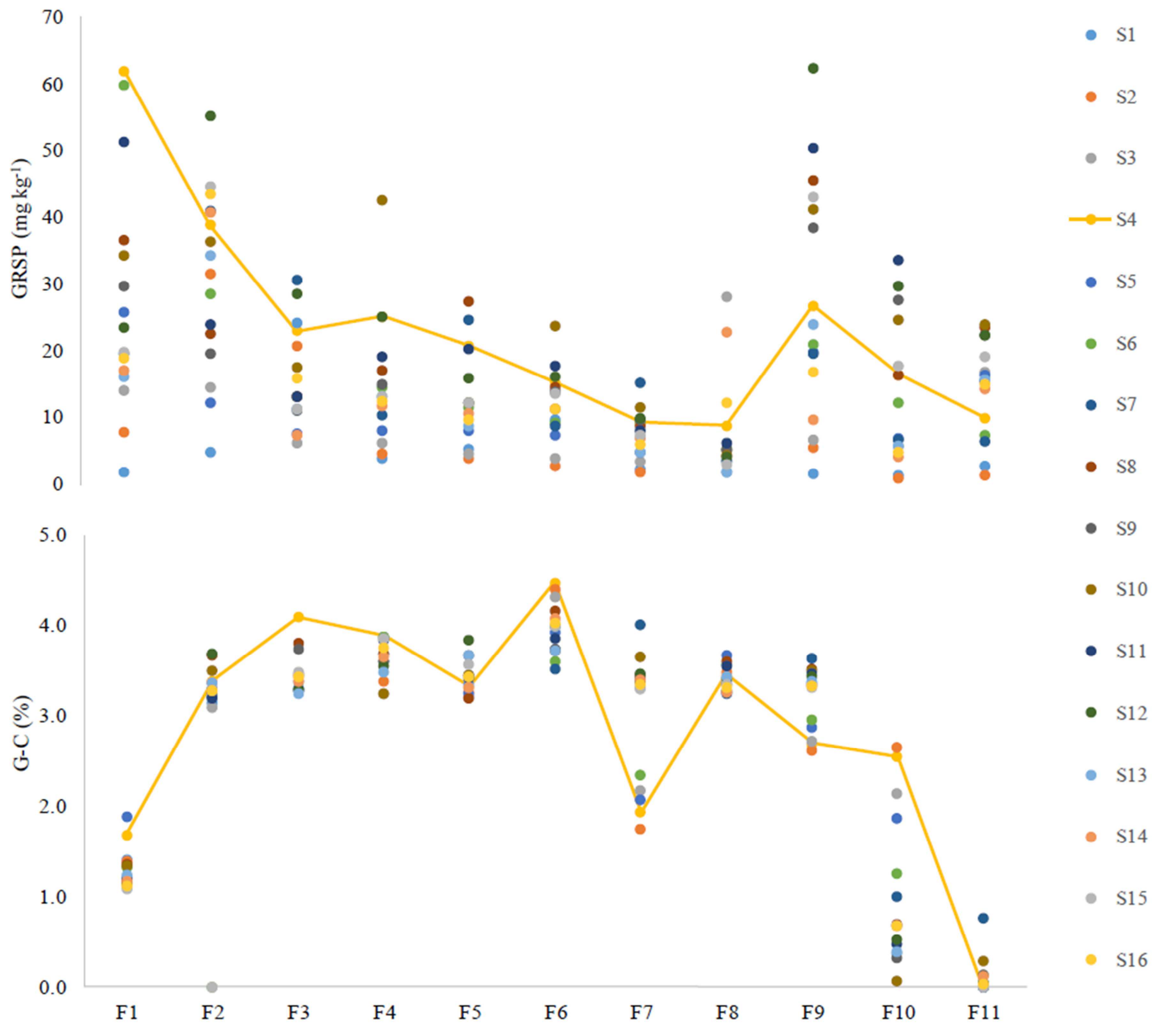

Figure 2. *Glomalin-Related Soil Protein (GRSP) Concentrations and Glomalin-Carbon (G-C) for Each Extraction Fraction (F1-F11) in All Soils.*

3.3. Increasing the Availability of Soil Micronutrients in Soil

Soil micronutrients varied widely among the studied soils probably due to manure addition, plant cover and soil texture (Table 2). Iron (Fe) varied from 0.12 mg kg^{-1} in the bare soil to 18.08 mg kg^{-1} in S10 under Alfalfa. Zinc showed very low values varied within 0.02-7.38 mg kg^{-1} and notable below the recommended limit of 50 mg kg^{-1} as proposed by the WHO 1996 and under 300 mg kg^{-1} at pH more than 7.0 as proposed by the Department of Environment [30]. The highest value was found in S4 under Apple trees probably comes from agrochemical additions. Zinc was positively correlated with the easily extracted glomalin fraction ($r=0.557$, $p<0.05$). Manganese also showed a wide range of concentrations among the studied soils varied from 2.43 mg kg^{-1} in bare soil (S1) to the highest value in S10 under Alfalfa. The higher values (18.18-26.21 mg kg^{-1}) were found in soils of S8-S12 due to manure addition. Manganese was positively correlated with silt ($r=0.546$, $p<0.05$) and clay ($r=0.674$, $p<0.01$) while negatively correlated with sand fraction ($r=-0.659$, $p<0.01$). Highly significant correlations were also found between manganese in soil and SOC ($r=0.866$, $p<0.01$) and TGRSP ($r=0.836$, $p<0.01$) contents. Copper (Cu) contents were very low (0.11-2.77 mg kg^{-1}), with high concentrations in soils at S8-S12, but contents remained below the recommended maximum limit of 200 mg kg^{-1} at pH >7.0 [30]. Copper concentrations in DTPA extraction solutions were significantly correlated with SOC ($r=0.768$, $p<0.01$), EEGRSP ($r=0.561$, $p<0.05$) and TGRSP ($r=0.807$, $p<0.01$) contents. Nickel, recently recognized to be a soil micronutrient, was present in low amounts between 0.02-0.24 mg kg^{-1} and far below the recommended maximum limit of 110 mg kg^{-1} [30] (pH >7.0). Soil micronutrients increased in soils after manure applications [31]. Nitrogen addition generally increased the availability of soil micronutrients such as Fe, Mn and Cu and their availability were pH dependent.

Cadmium content in these soils was very low (0.0004-0.08 mg kg^{-1}) (Table 2). Lead (Pb) varied from 0.24 mg kg^{-1} to 4.74 mg kg^{-1}. These values are very low and did not exceed the limit of 200 mg kg^{-1} [30] in soils with a pH >7.0. Cobalt (Co) and chromium (Cr) concentrations in these soils were very low and less than 0.06 mg kg^{-1} and 0.97 mg kg^{-1}, respectively.

Table 2. Soil Micronutrients (Fe, Zn, Mn and Cu) and Toxic Metals (Cd, Pb, Co, Ni and Cr) in the Studied Soils.

	S1	S2	S3	S4	S5	S6	S7	S8
Fe	0.1170±0.0070	4.8276±0.0162	4.2069±0.0115	5.8842±0.0197	4.0677±0.0070	3.9586±0.0088	4.3982±0.0085	9.4152±0.0435
Zn	0.0245±0.0012	0.2774±0.0019	0.5704±0.0008	7.3774±0.0201	0.5756±0.0026	0.4946±0.0026	0.3113±0.0011	0.9448±0.0029
Mn	2.4322±0.0095	8.6438±0.0300	8.2171±0.0440	10.4976±0.0132	9.0625±0.0187	9.0858±0.0577	9.0622±0.0092	20.1162±0.0231
Cu	0.1106±0.0013	0.2219±0.0012	0.3605±0.0020	2.7683±0.0222	0.3645±0.0029	0.2831±0.0013	0.3566±0.0025	1.9732±0.0206
Cd	0.0017±0.0001	0.0007±0.0012	0.0006±0.0002	0.0846±0.0007	0.0007±0.0006	0.0009±0.0005	0.0004±0.0008	0.0081±0.0004
Pb	0.2431±0.0011	0.6914±0.0011	1.0263±0.0008	4.7367±0.0012	0.6345±0.0005	0.6707±0.0004	0.8363±0.0010	1.6459±0.0014
Co	0.0030±0.0008	0.0391±0.0010	0.0139±0.0008	0.0337±0.0002	0.0020±0.0008	0.0560±0.0007	0.0037±0.0005	0.0002±0.0004
Ni	0.0205±0.0001	0.1489±0.0002	0.1199±0.0005	0.1072±0.0002	0.0944±0.0004	0.1132±0.0006	0.1251±0.0010	0.2437±0.0004
Cr	0.0161±0.0002	0.0115±0.0004	0.0184±0.0001	0.0144±0.0001	0.0071±0.0001	0.0041±0.0004	0.0048±0.0003	0.0838±0.0004

Table 2. Continued.

	S9	S10	S11	S12	S13	S14	S15	S16
Fe	0.5857±0.0013	18.0750±0.0943	0.7482±0.0164	12.8909±0.0141	2.3078±0.0243	2.4335±0.0206	2.7508±0.0153	3.3194±0.0192
Zn	0.3905±0.0033	0.4913±0.0018	0.4640±0.0042	0.8621±0.0058	0.4182±0.0009	0.6067±0.0006	0.4448±0.0030	0.4868±0.0017
Mn	16.9731±0.0422	26.2145±0.0309	16.5329±0.0874	18.1809±0.0340	8.0748±0.0052	8.4367±0.0029	8.7072±0.0078	10.2277±0.0050
Cu	1.2732±0.0060	2.5093±0.0182	1.0979±0.0132	2.2152±0.0204	0.3143±0.0019	0.3063±0.0021	0.3415±0.0020	0.3127±0.0021
Cd	0.0130±0.0002	0.0067±0.0008	0.0009±0.0008	0.0037±0.0004	0.0018±0.0005	0.0015±0.0008	0.0012±0.0005	0.0030±0.0007
Pb	0.3392±0.0008	0.5562±0.0005	1.0010±0.0003	2.1031±0.0018	0.9623±0.0011	3.8674±0.0011	1.1095±0.0011	1.0169±0.0011
Co	0.0001±0.0002	0.0002±0.0005	0.0001±0.0001	0.0002±0.0003	0.0008±0.0003	0.0019±0.0006	0.0008±0.0002	0.0002±0.0006
Ni	0.1367±0.0004	0.2353±0.0003	0.1132±0.0003	0.2418±0.0009	0.0851±0.0003	0.0948±0.0006	0.0985±0.0005	0.1403±0.0003
Cr	0.2953±0.0006	0.9722±0.0044	0.0097±0.0002	0.6473±0.0007	0.0091±0.0001	0.0079±0.0001	0.0205±0.0001	0.0182±0.0001

3.4. GRSP-Bound Metals

GRSP has been recently considered as the most important fraction of SOM for its contribution to soil macro nutrients such as soil organic carbon, nitrogen and phosphorus. It also has the important ability to bind metals and so to sequester soil micronutrients such as Fe, Zn, Mn and Cu thus increasing their availability in soil [15], [17], [18]. In addition, glomalin tends to bind with toxic metals thus may alleviate their adverse effects in soils depending on the differences in metal chemistry [32]. Our results showed high significant variability in the metal sequestration capacity of glomalin among the studied soils. The highest values of iron (Fe_{GRSP}), manganese (Mn_{GRSP}), copper (Cu_{GRSP}) and lead (Pb_{GRSP}) in GRSP extraction solutions were observed in S4 and S8-S12 (Figure 3). It can be also observed that the higher values were generally found in the first extraction fraction (F1) that represented the easily extractable glomalin fraction. In Figure 3, the Fe, Mn and Cu showed almost the same trend along the sequential extraction fractions from F1 to F11. The total sum of metal ions for all fractions (F1-F11) extracted by sodium citrate along the sequential extractions may have released more ions bound to other soil fractions such as glomalin-like compounds. These other soil fractions resulted in the ninth fraction (F9) when the GRSP values increased again in all soils despite the absence of brown colour in the extraction solution. Lead showed the same trend in the first 6 fractions despite its absence in the F7. The one-way ANOVA showed significant variations among all fractions from F1 to F11 for iron, manganese and copper (Table 3).

In Table 4, the highly significant positive correlation found between Fe in soil and TGRSP ($r=0.692$, $p<0.01$) probably indicating the stabilization of glomalin by binding with iron in soils with high iron contents [16], [27]. This hypothesis was confirmed through the significant positive correlations between iron in GRSP extraction solution (Fe_{GRSP}) and EEGRSP ($r=0.588$, $p<0.05$), TGRSP ($r=0.778$, $p<0.01$) and soil organic matter (SOM) ($r=0.697$, $p<0.01$) contents. Accordingly, iron can bind with the easily glomalin fraction (EEGRSP) and its recalcitrance/stable form increased relatively with the stabilization of organic compounds in soil. Manganese in GRSP extraction solutions found to be positively correlated with silt ($r=0.546$, $p<0.05$) and clay ($r=0.530$, $p<0.05$) while negatively correlated with the sand fraction ($r=-0.581$, $p<0.05$). Highly significant positive correlations were also found between manganese (Mn_{GRSP}) and SOC ($r=0.854$, $p<0.01$) and TGRSP ($r=0.728$, $p<0.01$) contents (Table 4). Copper (Cu_{GRSP}) was positively correlated with the easily glomalin fraction ($r=0.666$, $p<0.01$) and total glomalin ($r=0.559$, $p<0.05$) (Table 4). Nickel is recently added to leguminous crops in the soil as a micronutrient for its role in nitrogen fixation and nodulations. The excessive addition of Zn and Cu in soil with alkaline conditions may cause a Ni deficiency [33]. Consequently, the highest Ni concentration observed in GRSP extraction solution was found in S2 under bean because Ni deficiency was treated by adding Ni in its water-soluble form as a foliar spraying (water-soluble Ni fertilizer). Lead showed high values in S4 and S8-S12 corroborating the role of glomalin in the immobilization of Pb thus lowering its toxicity in soils with high pH values [17]. A significant positive correlation was found between TGRSP and Pb_{GRSP} ($r=0.739$, $p<0.01$).

The GRSP-bound metals (Fe_{GRSP}, Mn_{GRSP}, Cu_{GRSP} and Pb_{GRSP}) showed high ratios when compared to their contents in soil. Each metal showed a wide range ratio as iron (Fe_{GRSP}/Fe_{soil}) ranged from zero to one ratio, Mn (Mn_{GRSP}/Mn_{soil}) from 1.0 to 4.4 ratio, Cu (Cu_{GRSP}/Cu_{soil}) from 0.8 to 10.5 ratio, Pb (Pb_{GRSP}/Pb_{soil}) from 0.7 to 12.0 ratio and Ni (Ni_{GRSP}/Ni_{soil}) from zero to 1.5 ratio. These metals decreased significantly with their contents in soil as can be indicated by the following negative power equations: Fe

($y=0.18x^{-0.46}$, r=-0.65, p<0.01), Mn ($y=9.85x^{-0.91}$, r=-0.88, p<0.01), Cu ($y=1.85x^{-0.63}$, r=-0.94, p<0.01) and Pb ($y=3.04x^{-0.99}$, r=-0.99, p<0.01). These correlations may indicate the potential sequestration capacity of glomalin to bind metals. The higher adsorption capacity was found in soils at S4 and S8-S12 than other soils because of the high SOM and glomalin contents. Nickel did not show any significant relationships because of the very low quantities in soil and therefore in GRSP extracts.

After logarithmic transformation of the ratio of metal content in GRSP and in soil, clear trends were observed for iron, manganese and copper in some soils such as S4 and S8-S12 indicating their capacity for sequestering these nutrients. Highly significant correlations of logarithm transformed were ratios of iron (Fe_{GRSP}), manganese (Mn_{GRSP}), copper (Cu_{GRSP}) and lead (Pb_{GRSP}) in GRSP extraction solutions as a function of their soil contents (y-axis) against the logarithmic transformation of their contents in soil (x-axis) (Figure 4). No significant correlation was found for Ni_{GRSP}.

Table 3. *One-way ANOVA of Fe, Mn and Cu in all Glomalin Fractions (F1-F11) Extracted from the Studied Soils.*

Nutrient	Source of Variation	SS	df	MS	F	P-value
	Between Groups	5826	15	388.40	2.28	0.00638700
Fe	Within Groups	24537	144	170.40		
	Total	30363	159			
	Between Groups	12536	15	835.75	5.43	0.00000001
Mn	Within Groups	24314	158	153.89		
	Total	36850	173			
	Between Groups	7	15	0.46	3.36	0.00006343
Cu	Within Groups	22	159	0.14		
	Total	28	174			

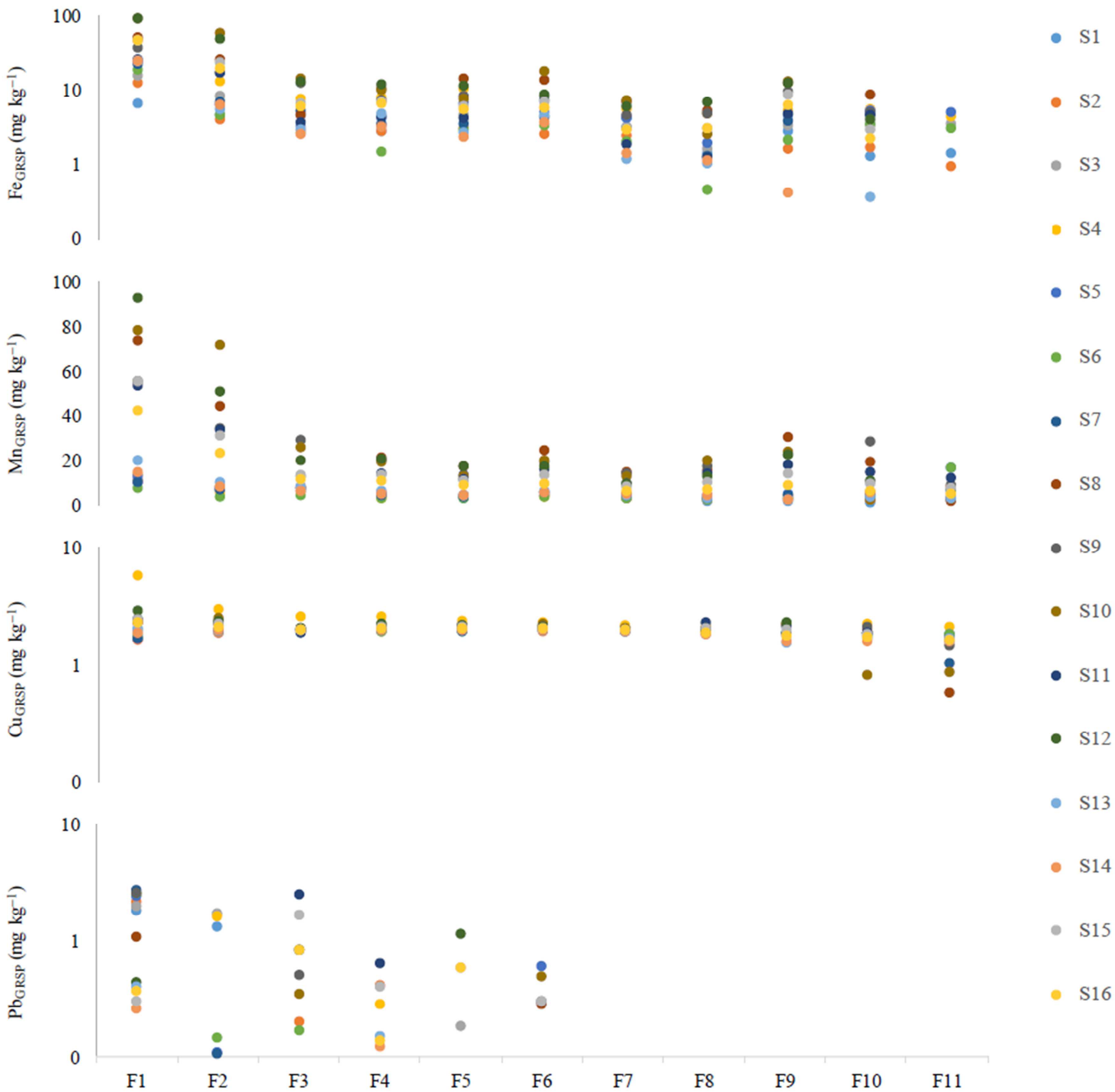

Figure 3. *Concentrations of Fe, Mn, Cu and Pb in each Operational Fraction of GRSP.*

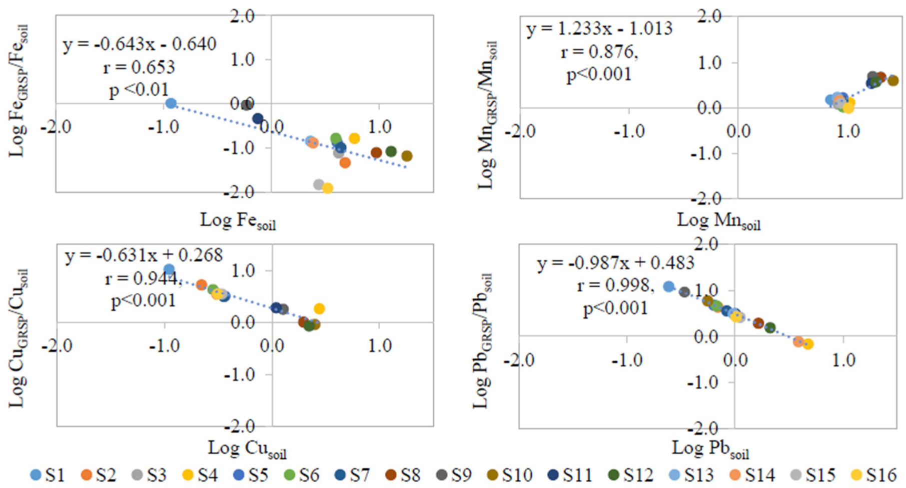

Figure 4. *Linear Correlations Between Log Transformations of GRSP-Bound Metals (Fe_{GRSP}/Fe_{soil}, Mn_{GRSP}/Mn_{soil}, Cu_{GRSP}/Cu_{soil} and Pb_{GRSP}/Pb_{soil}) Against the Log of their Contents in Soil.*

Table 4. *Correlation Matrix Among all Soil Parameters Obtained from all the Studied Soils.*

	Sand	Silt	Clay	pH	EC	SOC	EEGRSP	TGRSP	Fe_{GRSP}	Cu_{GRSP}
Silt	-0.925**									
Clay	-0.933**	0.726**								
pH	0.074	-0.210	0.066							
EC	-0.019	0.194	-0.151	-0.716**						
SOC	-0.613*	0.633**	0.508*	-0.389	-0.010					
EEGRSP	-0.130	0.221	0.025	-0.200	-0.242	0.658**				
TGRSP	-0.648**	0.498*	0.698**	-0.217	-0.093	0.846**	0.421			
Fe_{GRSP}	-0.096	-0.032	0.201	-0.165	-0.371	0.697**	0.588*	0.778**		
Cu_{GRSP}	-0.009	0.038	-0.021	-0.205	-0.044	0.486	0.666**	0.559*	0.606*	
Ni_{GRSP}	-0.228	0.274	0.151	0.084	-0.251	0.141	0.224	0.062	-0.017	0.268
Mn_{GRSP}	-0.581*	0.546*	0.530*	-0.344	0.041	0.854**	0.288	0.728**	0.632**	0.303
Pb_{GRSP}	-0.041	0.029	0.043	-0.419	-0.095	0.750**	0.616*	0.739**	0.895**	0.679**
Fe	-0.246	-0.009	0.451	0.123	-0.442	0.491	0.188	0.692**	0.794**	0.267
Zn	0.228	-0.206	-0.218	-0.031	-0.158	0.151	0.557*	0.194	0.420	0.900**
Mn	-0.659**	0.546*	0.674**	-0.141	-0.211	0.866**	0.408	0.836**	0.711**	0.360
Cu	-0.296	0.217	0.327	-0.231	-0.135	0.768**	0.561*	0.807**	0.861**	0.816**
Cd	0.198	-0.164	-0.203	-0.090	-0.085	0.186	0.543*	0.190	0.425	0.933**
Pb	0.036	-0.069	0.000	-0.126	-0.128	0.117	0.339	0.261	0.298	0.602*
Co	0.502*	-0.438	-0.492	0.240	-0.396	-0.176	0.425	-0.349	0.108	0.199
Ni	-0.504*	0.346	0.582*	0.148	-0.455	0.662**	0.209	0.734**	0.662**	0.197
Cr	-0.421	0.186	0.582*	-0.114	-0.121	0.574*	0.083	0.719**	0.714**	0.214

Table 4. *Continued.*

	Ni_{GRSP}	Mn_{GRSP}	Pb_{GRSP}	Fe	Zn	Mn	Cu	Cd	Pb	Co	Ni
Mn_{GRSP}	0.100										
Pb_{GRSP}	0.120	0.681**									
Fe	-0.079	0.612*	0.585*								
Zn	0.215	-0.096	0.435	0.113							
Mn	0.171	0.940**	0.661**	0.740**	0.006						
Cu	0.131	0.712**	0.848**	0.706**	0.588*	0.768**					
Cd	0.233	-0.019	0.462	0.095	0.984**	0.048	0.615*				
Pb	0.205	-0.127	0.316	0.101	0.764**	-0.021	0.435	0.700**			
Co	0.379	-0.377	0.095	-0.056	0.347	-0.279	-0.068	0.315	0.138		
Ni	0.182	0.763**	0.513*	0.839**	-0.023	0.856**	0.668**	-0.026	0.036	-0.124	
Cr	-0.103	0.742**	0.574*	0.832**	-0.080	0.784**	0.649**	-0.029	-0.100	-0.260	0.694**

** significant p-level <0.01, * significant p-level <0.05.

3.5. Factor Analysis

In Table 5, the first three factor structures, obtained from Factor Analysis, explained 76% of total variance to represent the most effective correlations among the analysed soil properties (variables). Factor 1 explained 41% of total variance with high positive loadings >0.60 from silt, clay, SOC, TGRSP, Fe, Mn, Cu, Ni, Cr, Fe_{GRSP}, Mn_{GRSP} and Pb_{GRSP} but negative loadings >0.70 only from sand. It is very interesting to note that iron (Fe) and manganese (Mn) appear to be sequestered by glomalin and potentially biostabilized because of their significant positive correlation with TGRSP (containing the more stable glomalin form) and SOC in the soil. Factor 2 explained 21% of total variance with high positive loadings from EEGRSP, Zn, Cu, Cd, Pb, Fe_{GRSP}, Cu_{GRSP} and Pb_{GRSP}. The second factor showed that the labile glomalin fraction (EEGRSP) was positively correlated with Cu in soil and Cu_{GRSP} indicating its high potential sequestration capacity with this metal. Despite that Zn in soil was positively correlated with EEGRSP but the lower concentrations found in all soils were not enough to represent a clear trend of glomalin to sequester Zn from the soil. High positive loadings from TGRSP and Pb_{GRSP} in Factor 1 and from EEGRSP and Pb_{GRSP} in Factor 2 may indicate that glomalin is predominantly bound to lead with a capacity to reduce its bioavailability in soil [16]. The third factor explained 14% of the total variance with high positive loadings from pH against electrical conductivity. The total communality values emphasized that Fe, Cu and Mn are the main soil micronutrients predominantly bind to glomalin with a potential of biostabilisation in the soil.

The factor score values were calculated to detect which soil may positively or negatively contribute to each factor (Figure 5). Score values relevant to Factor 1 showed a positive contribution from S8-S12 soils while the negative contribution from other soils because of the large manure addition in the former soils. Score values of Factor 2 showed the positive contribution from S4, S6 and S8-S12 soils relatively associated with the high positive loadings found in Factor 1 and 2. The plot diagram in Figure 5 may summarize the relationships among soils and variables loadings in the orthogonal space defined by the first two Factors (1 and 2) in order to better explain the dynamics of SOM and glomalin to bind metals. The progressive shift from S1 to S16 along Factor 1 represents a valuable indication of glomalin (TGRSP) to sequester Mn, Fe and Cu, respectively, depending on their availability in soil. This trend was clearly observed in S8-S12 soils. Delimited squares may display the distribution of each metal along the orthogonal space defined by the two Factors.

Table 5. *Variable Loadings from the First Three Factor Structures with the Communality Values Obtained by Factor Analysis.*

Variables	Factor 1	Factor 2	Factor 3	Communalities
Sand	-0.79			0.70
Silt	0.65			0.58
Clay	0.81			0.71
pH			0.80	0.74
EC			-0.95	0.93
SOC	0.83			0.87
EEGRSP		0.71		0.57
TGRSP	0.87			0.87
Fe	0.69			0.76
Zn		0.92		0.88
Mn	0.95			0.96
Cu	0.65	0.72		0.95
Cd		0.92		0.86
Pb		0.69		0.48
Co				0.58
Ni	0.83			0.87
Cr	0.81			0.68
GRSP-bound metals				
Fe_{GRSP}	0.59	0.64		0.84
Cu_{GRSP}		0.93		0.91
Ni_{GRSP}				0.08
Mn_{GRSP}	0.91			0.86
Pb_{GRSP}	0.62	0.72		0.79
Explained Variance (%)	41	21	14	
Cumulative variance (%)	41	62	76	76

EC: Electrical conductivity; SOC: Soil organic carbon; EEGRSP: Easily extractable glomalin-related soil protein; TGRSP: Total glomalin-related soil protein. Values below 0.60 are deleted.

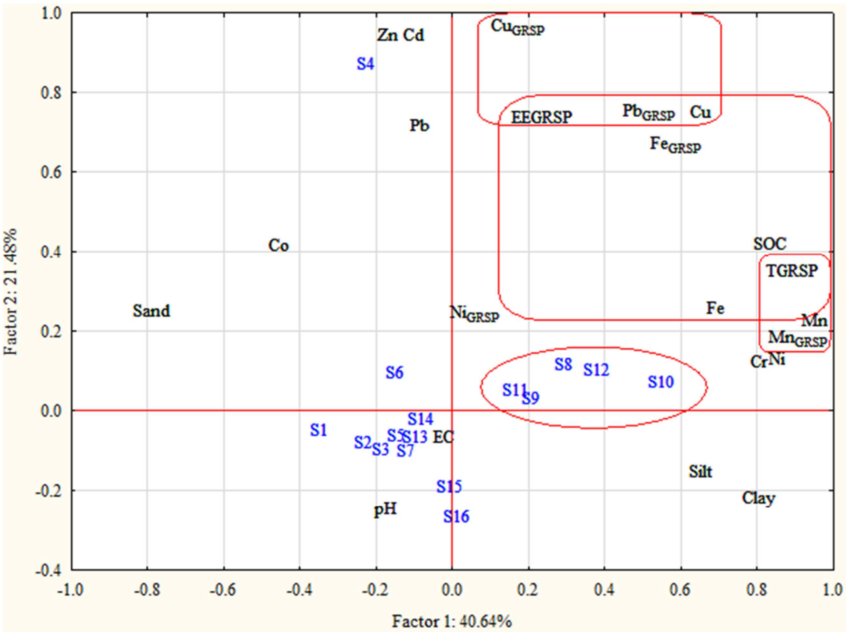

Figure 5. *Plotting the Factor Loadings and Score Values of Factor 1 and Factor 2.*

4. Conclusion

The GRSP-bound metals have been identified in this study as Fe_{GRSP}, Zn_{GRSP}, Mn_{GRSP}, Cu_{GRSP}, Ni_{GRSP}, and Pb_{GRSP} for their abundance in the glomalin extracts and were significantly correlated with their availability in soil. Iron, manganese, copper and nickel were highly sequestered by glomalin in those salt-affected soils (S4 and S8-S12) with moderately alkaline conditions due to the intercropping with legumes and receiving annual manure additions. Zinc was highly sequestered by glomalin in S4 soil under apple trees that sometimes receive Zn in its soluble form for promoting plant growth. Despite that, glomalin resulted in the ability to lower the toxicity of lead in soils. As a result, glomalin can be used as a biotechnological tool of phytoremediation to recover polluted soils. Further studies became paramount to identify the structural components in each GRSP fraction to better understand the glomalin sustainability under different agricultural management.

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