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12 Accumulation and fractionation of trace metals in a Tunisian calcareous soil amended with
13 farmyard manure and municipal solid waste compost

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16 In: Journal of Hazardous Materials, 176, 99-108.

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18 **To refer to or to cite this work, please use the citation to the published version:**

19 Achiba, W.B., Lakhdar, A., Eturki, S., Gabteni, N., Du Laing, G., Verloo, M., Boeckx, P.,
20 Van Cleemput, O., Jedidi, N. & Gallali, T. (2010). Accumulation and fractionation of trace
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1 **Accumulation and fractionation of trace metals**
2 **in a Tunisian calcareous soil amended**
3 **with farmyard manure and municipal solid waste compost**
4
5

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24 **Abstract**

25 A field plots experiment was carried out to assess the effects of repeated application of
26 municipal solid waste compost in comparison to farmyard manure on the accumulation and
27 distribution of trace metals, as well as organic carbon and nitrogen in Tunisian calcareous
28 soil.

1 Compared with untreated soil, the application of the two organic amendments significantly
2 increased the organic carbon and nitrogen contents of the soil. Particle-size fractionations
3 showed that carbon and nitrogen were mainly found to occur in the macro-organic matter
4 fraction (80%). The two organic amendments significantly increased organic carbon in the
5 macro-organic and mineral >150 μm fraction and the 150-50 μm fraction, as well as the
6 organic nitrogen in 150-50 μm and macro-organic fraction. Compared with farmyard manure,
7 municipal solid waste compost significantly increased total Cd, Cu, Pb and Zn contents in the
8 topsoil. These trace metals were mainly present in the macro-organic matter fraction.
9 Significant increases of Cu, Zn and Pb were detected in the 150-50 μm , <50 μm and macro-
10 organic fractions after application of municipal solid waste compost. A significant increase of
11 Cd content was only observed in the 150-50 μm fraction. The trace metals also showed
12 different fractionation patterns when the BCR sequential extraction scheme was applied on
13 untreated and compost-treated soil. The residual fraction was found to be the major fraction,
14 especially for Cu, Cr, Ni and Zn. In contrast, Cd was mainly present in the acid-extractable
15 and reducible fraction, whereas Pb was mainly associated with the reducible fraction.

16

17 **Keywords:** municipal solid waste compost, organic carbon, nitrogen, trace metals, BCR
18 sequential extraction, particle-size fractionation

19

20 **1. Introduction**

21 In the Mediterranean region, difficult climate conditions and inadequate land management
22 have led to a reduction in the organic matter content of soils [1]. This effect is intensified by
23 the limited availability and high cost of manure and the popular use of mineral fertilizers.
24 However, maintenance of adequate organic matter levels in the soils is very necessary to

1 maintain soil fertility and sustainable crop production. Therefore, new sources of organic
2 matter have been tapped, such as municipal solid waste compost and sewage sludge [2,5].
3 In recent decades, the volume of municipal solid waste increased in Tunisia. Composting
4 these urban residues contributes to reducing the amount of waste, which makes its
5 management less difficult. Moreover, the produced low-cost compost could be used as
6 amendment in agriculture, meanwhile recycling its valuable components: organic matter, N, P
7 and other plant nutrients [2,6]. Application of the municipal solid waste compost to
8 agricultural soils was previously found to improve micronutrient complex formation, which
9 increases availability of micronutrients to plants, to stimulate the presence of beneficial soil
10 organisms and to reduce the presence of plant pathogens. Moreover, it seems to increase
11 water holding capacity, soil buffering and cation exchange capacity and it improves soil
12 porosity [4, 7-9]. The quality of such municipal solid waste compost was found to depend on
13 many factors, including the design of the composting process, feedstock source and
14 proportions used, composting procedure and length of maturation [9].
15 Despite the beneficial effects, high metal concentrations were observed in the compost due to
16 the use of urban wastes. These limit its utilization in agricultural soils as excessive loads of
17 contaminants could negatively affect soil fertility. Moreover, the presence of metals can
18 constitute a long-term environmental hazard as metals have very long residence times in soils.
19 In addition, transfer of toxic metals to the human food chain may occur since absorption by
20 plant roots is one of the main routes of entrance of trace metals in the food chain [8,10,11].
21 Trace metals are mainly associated with solid soil components and exist in various physico-
22 chemical forms. Total concentrations of trace metals in compost amended soils provide little
23 or no indication of their specific bioavailability, mobility and reactivity [12]. Consequently,
24 the knowledge of metal speciation and distribution between different size fractions in compost
25 treated soils may be useful to quantify potential environmental effects. Therefore, sequential

1 extraction procedures were developed in order to predict retention, mobility and
2 bioavailability of trace metals in soils, sediments and organic amendments [13-17]. A wide
3 variety of extraction procedures have been used [18] and results obtained from applying
4 chemical fractionation schemes are highly dependent on the extraction protocol used [19].
5 Besides the chemical speciation of trace metals in soils, size fractionation is used to predict
6 the distribution of these elements between different particle-size fractions of the soils. Despite
7 the great amount of work conducted on chemical speciation of trace metals in soils, little is
8 known about the effects of applying municipal solid waste compost on the distribution of
9 metals between different particle-size fractions in the soil.

10 The purpose of this study was to investigate the effect of seven successive years of municipal
11 solid waste compost and farmyard manure application on trace metal fractionation and
12 speciation in a Tunisian calcareous soil.

13

14

15 **2. Materials and methods**

16 **2.1. Experimental Design**

17

18 The field experiment was conducted in the experimental farm of the National Institute of
19 Agronomy of Tunis (INAT) in the north of Tunisia. This site is characterized by a semi-arid
20 climate. The total annual rainfall is approximately 500 mm, whereas the average air
21 temperature is 18 °C. The lowest air temperature is 10.7 °C in January and the highest air
22 temperature is 26.4 °C in July. The field experiment was designed as a randomized complete
23 block trial with a total number of 24 plots. The dimension of each plot was 1.5 x 1.5 m². Plots
24 were arranged in four repetitive blocks and were separated in all directions by a buffer zone of
25 1 m. The soil was loamy clay with low organic carbon content (Table 1) and was classified as

1 Xerofluvent. Six treatments were examined: (1) control without amendment (T); (2) 40 t ha⁻¹
2 year⁻¹ of farmyard manure (M40); (3) 120 t ha⁻¹ year⁻¹ of farmyard manure (M120); (4) 40 t
3 ha⁻¹ year⁻¹ of municipal solid waste compost (C40); (5) 80 t ha⁻¹ of municipal solid waste
4 compost (C80); (6) 120 t ha⁻¹ year⁻¹ of municipal solid waste compost (C120). The
5 amendments were applied each year from 1999 to 2005. They were applied to the soil surface
6 by hand in September/October and incorporated to a depth of 10-15 cm by manual hoeing.
7 Plots were manually weeded every month to avoid uptake of the amendments by plants.
8 Initial characteristics and trace metal concentrations of the municipal solid waste compost,
9 farmyard manure and soil used in this study are shown in Table 1.

10

11 **2.2. Soil analysis**

12

13 2.2.1. General soil properties

14 Soil samples were collected in 2006, one year after ending the addition of organic
15 amendments by taking a composite of five cores using a hand auger (4 cm diameter) at a
16 depth of 0-20 cm for each plot. The soil was homogenized, air dried, crushed, and passed
17 through a 2-mm sieve for further analysis. The pH and electrical conductivity (EC) were
18 measured on a 1:5 sample/water extract after shaking during 2 hours for pH and during 1 hour
19 for EC [22]. Organic carbon was determined by dichromate oxidation and subsequent titration
20 with ferrous ammonium sulphate [20]. Total nitrogen was determined using the Kjeldhal
21 method. Total trace metals concentrations were determined by aqua-regia extraction: 7.5 ml
22 of concentrated hydrochloric acid and 2.5 ml of concentrated nitric acid were added
23 successively to one gram of soil. This suspension was allowed to react overnight and
24 subsequently heated during two hours. After cooling, the solution was filtered and diluted to
25 100 ml with nitric acid solution (2 M). The concentrations of trace metals were measured

1 using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer, Vista MPX,
2 Varian, Palo Alto, CA, USA). For quality control, a certified reference sediment of the
3 Scheldt estuary (CRM 277) was also analyzed in triplicate using the same method. Results
4 varied from 92.5 to 101.7% of the certified aqua regia extractable values. Moreover, standards
5 were always re-analyzed at the end of each batch of analyses. The analyses results were only
6 accepted when the measured standard concentrations were within 95 to 105% of the certified
7 value.

8

9 2.2.2. Particle-size fractionation

10 Fractionation of soil into different size fractions was performed by a method which was
11 slightly modified from Meijboom et al. [21]. The separation was carried out by wet sieving of
12 50 g soil sample over three sequential sieves (250 μm , 150 μm and 50 μm) with deionised
13 water. The soil was pushed through the sieves until the water passing the sieves became clear.
14 The suspension passing the bottom sieve was collected and left to settle for about 48 h at
15 room temperature. After settling, the clear supernatant was considered to contain only soluble
16 compounds and removed, whereas the residue was considered as size fraction $<50 \mu\text{m}$. Size
17 fractions 250-2000 μm and 150-250 μm were also collected, remixed, washed into buckets
18 and swirled with a jet of water. The macro-organic material was separated from the mineral
19 $>150 \mu\text{m}$ fraction by decantation. Swirling and decanting were repeated until no floating
20 organic matter appeared anymore. Consequently, four fractions (a macro-organic fraction and
21 mineral $>150 \mu\text{m}$ fraction, a 150-50 μm and a $<50 \mu\text{m}$ fraction) were obtained. Suspensions
22 were dried at 40°C for 48 h and stored for further analysis. Total metals were extracted from
23 the fractions $>150 \mu\text{m}$, 150-50 μm and $<50 \mu\text{m}$ by digestion with aqua regia and from the
24 macro-organic matter by digestion with nitric acid after ashing the organic matter at 450-
25 550°C for 2 h [22].

1 The concentrations of trace metals (Cd, Cu, Zn, Pb, Ni and Cr) were determined using ICP-
2 OES (Vista MPX, Varian, Palo Alto, CA, USA).

3 Total carbon and nitrogen (TC and TN) in the whole soil, in the different soil fractions and in
4 macro-organic matter fraction were analysed using a CNS analyser (Vario Max, Elementar,
5 Germany).

6

7 2.2.3. BCR Sequential extraction

8 Different types of sequential extraction procedures have been developed and applied to
9 fractionate trace metals in soils. We followed the standardized BCR sequential extraction
10 method [16]. The developers of this method justified the use of a very strict standardized
11 protocol as the only way to achieve comparability between data when using operationally
12 defined procedures [23]. It distinguishes between only three fractions (acid-extractable,
13 reducible and oxidizable) arguing that analysis of more fractions could increase error between
14 fractions [24].

15 One gram of soil sample (<2 mm) was weighed into a 100 ml glass centrifuge tube. To
16 extract the acid-extractable fraction (f_1) in the first step of the procedure, the soil sample was
17 shaken with 40 ml 0.01 M CH₃COOH at a room temperature for 16h (overnight). To extract
18 the reducible fraction (f_2) in the second step, the residue from the previous step was extracted
19 by shaking the sample with 40 ml of 0.1 M NH₂OH.HCl (adjusted to pH 2 with HNO₃) for 16
20 h at a room temperature. To extract the oxidizable fraction (f_3), 10 ml of H₂O₂ was added to
21 the residue from the second step and the mixture was digested at room temperature for 1 h. It
22 was then heated to near dryness in a water bath at 85°C. A second 10 ml of H₂O₂ was added
23 and heated to near dryness again. After cooling, 50 ml of 1 M ammonium acetate was added
24 to the moist residue and shaken for 16 h at room temperature. In a supplementary step, the

1 residual fraction (*f_r*) was determined in the residue of the third step by digestion with 3:1
2 hydrochloric: nitric acid, heated to 150°C (aqua-regia).
3 To separate the extraction solution from the solid residue after shaking in each extraction step,
4 the suspension was centrifuged at 3000g for 20 min. The supernatant was removed by
5 filtration into a polyethylene container and analyzed immediately or stored in a refrigerator at
6 about 4°C prior to analysis. Prior to each following step, the residue was washed with 20 ml
7 of deionised water, followed by mechanical agitation for 15 min in an end-over-end shaker
8 and removal of the supernatant after 20 min of centrifugation at 3000g. The concentrations of
9 trace metals (Cd, Cu, Zn, Pb, Ni and Cr) were determined in each extract using ICP-OES.

10

11

12 2.2.4. Statistical analysis

13 Statistical analysis was performed using SPSS 10.0. Significance of effects was evaluated by
14 one-way ANOVA. Duncan's test was used for comparison of means. Statistical significance
15 of hypotheses was assumed when $p < 0.05$.

16

17 **3. Results and discussion**

18

19 **3.1. Soil organic carbon, total nitrogen and trace metal contents**

20 Soil organic carbon, total nitrogen and trace metal contents are presented in Table 2. Addition
21 of both organic amendments during the seven successive years enhanced the total organic
22 carbon and total nitrogen content for all rates. Organic carbon increased from 11 g kg⁻¹ in the
23 control treatment to 21 g kg⁻¹ and 33 g kg⁻¹ in the 40 and 120 t ha⁻¹ farmyard manure
24 treatments and to 21 g kg⁻¹, 29 g kg⁻¹ and 35 g kg⁻¹ in the 40, 80 and 120 t ha⁻¹ municipal solid
25 waste treatments, respectively. The increase of organic carbon content seems to be related the

1 application dose. Similar results were obtained by Celik et al. [7] who stated that the soil
2 organic matter concentration at 0–15 cm depth in a clay loam soil was higher in plots
3 amended with manure and compost during 5 years, compared to control plots and plots
4 treated with mineral fertilizer.

5

6 N'Dayegamiye et al. [4] reported that the addition of municipal solid waste compost (20, 40
7 and 60 t ha⁻¹) increased the organic matter content of a sandy loamy soil. The total organic
8 carbon content of two sandy Malian agricultural soils also increased after application of
9 industrial Belgian compost [25]. Moreover, Weber et al. [26] showed that one year after the
10 application of two kinds of commercial composts in a loamy sand soil, the soil organic carbon
11 content increased significantly, but only when the composts were applied at medium (60 t ha⁻¹)
12 and high rates (120 t ha⁻¹).

13 For soil total nitrogen, a positive effect of both organic amendments can also be observed. In
14 fact, the addition of 40 and 120 t ha⁻¹ of farmyard manure significantly increased the total
15 nitrogen content from 1.1 g kg⁻¹ in the control soil to 1.7 g kg⁻¹ and 2.8 g kg⁻¹, respectively.
16 After application of 40, 80 and 120 t ha⁻¹ of municipal solid waste compost, the organic
17 nitrogen content increased to 1.5 g kg⁻¹, 1.8 g kg⁻¹ and 2.4 g kg⁻¹, respectively. Consequently,
18 farmyard manure enhanced organic nitrogen in the soil insignificantly compared to municipal
19 solid waste compost. This may be attributed to the organic nitrogen content being higher in
20 manure compared to compost. Soumaré et al. [25] reported that application of industrial
21 Belgian compost on two sandy Malian soils had variable effects on total nitrogen content. In
22 the first soil, total nitrogen increased significantly when compost was applied at a rate of 50 t
23 ha⁻¹ while even lower doses (25 t ha⁻¹) showed a significant effect in the second soil. After
24 one year of application of commercial compost on a loamy sandy soil, only the highest rate
25 (120 t ha⁻¹) significantly increased total soil nitrogen [26].

1
2 Our study confirms the results of a previous experiment conducted at the same plot which
3 revealed that the two organic amendments (manure and compost) applied during three
4 successive years were a good supplier of organic matter [27]. Increasing the soil organic
5 matter content improves the soil physical characteristics, such as soil water retention and
6 movement, soil structure and porosity, and favours the establishment of carbon cycling [28].
7 Moreover, mineralization of organic matter in added organic amendments leads to an
8 important release of nutrients for plants, in particular nitrogen. The kinetics of mineralization
9 in amended soils depend on soil texture, moisture regime and the nature of added organic
10 matter [29].

11
12 The application of municipal solid waste compost in order to ameliorate the soil fertility may
13 however cause the introduction of various contaminants in the soils. The increase of soil trace
14 metal concentrations is usually reported as one of the most common undesirable effects that
15 may result from application of composts [25,26,30].

16 The application of farmyard manure at 40 and 120 t ha⁻¹ during successive seven years had no
17 significant effect on the trace metal contents in the 0-20 cm layer of our soil (Table 2). Only a
18 slight insignificantly increase of Cu and Zn was observed after the addition the rate of 120 t
19 ha⁻¹. Manures contain plant functional nutrient metals such as copper, zinc, manganese, iron,
20 and may contain trace amounts of non-functional elements. These functional elements were
21 due to the natural presence of micronutrients in feed as well as their use as dietary
22 supplements [62]. This may be due to the low heavy metal concentrations in manure. Similar
23 results were found by Qian et al. [63] who stated that three to five years of annual swine and
24 cattle manure applications at low and high rates increase slightly the total and bioavailable
25 copper and zinc in surface soil. Gil et al. [28] announced that after application of composted

1 cow manure to sandy clay loam soil in Spain, Cr, Ni, Pb and Cd concentrations in the soil
2 were not significantly different from the initial values. Moreover, repeated applications of
3 farmyard manure for 20 years at $10 \text{ t ha}^{-1} \text{ y}^{-1}$ did not result in a significant accumulation of
4 trace metals in the surface layer of Fluvisoil [31].

5 The addition of Tunisian municipal solid waste compost at different rates however showed a
6 significant increment of Cd, Pb, Cu and Zn at 0-20 cm depth in our soil, whereas Ni and Cr
7 concentrations were not significantly affected (Table 2). Compared to the control soil, Cd
8 contents only increased significantly after application at the highest rate of 120 t ha^{-1} of
9 compost. In contrast, Pb, Zn and Cu concentrations showed a significant increment already
10 upon application of the lowest rate of 40 t ha^{-1} . The magnitude of increment seemed to depend
11 on the application rate.

12 Similar results were reported by Canet et al. [32]. They found that application of three organic
13 amendments during seven successive years to a clay-loam soil had different effects on metal
14 contents of the 0-20 cm soil layer. Sludge and municipal solid waste compost significantly
15 increased the trace metals contents in all cases, with the exception of Pb. This increment also
16 corresponded to the load of metals supplied by the two organic wastes and therefore depended
17 on the application rate. The use of ovine manure did not increase trace metal concentrations of
18 the soil due to its low metal content. Weber et al. [26] announced that the application of
19 commercial compost originating from a polluted region to a loamy sandy soil significantly
20 increased the Zn, Pb and Cu concentrations at all rates ($30, 60$ and 120 t ha^{-1}). Contrary, Cr
21 and Ni concentrations only increased significantly after compost supply at the highest rate
22 (120 t ha^{-1}). Upon application of urban waste compost to a clay-loam calcareous soil, soil Cu,
23 Zn and Pb contents were significantly greater after 6 years. The Cr concentration increased
24 only in the last two years of application, whereas no significant differences were found in Ni
25 and Cd concentrations between treated and untreated soil [33]. Results given by Walter et al.

1 [34] showed that after one and five years after cessation of biosolid application to agricultural
2 soil during eight years, the total concentrations of trace metals were significantly higher
3 compared to the control. This was observed for 50 and 100 t ha⁻¹ rates, with the exception of
4 Ni at low application rates. In sandy soil treated by three successive applications of municipal
5 solid waste compost at a rate of 1.8 and 2.1 kg m², Madrid et al. [1] found an increase of trace
6 metals contents in the 0-25 cm layer.

7 From the above, it should be clear that the addition of municipal solid waste compost to soil
8 can enhance its trace metals contents. However, the magnitude of increment depends on the
9 origin of the composts [8].

10

11 **3.2. Relative abundance of the different particle size-fractions within the soil**

12

13 The highest weight percentage of soil (80 %) was found in the size fraction <50 µm (Fig. 1).
14 The other size fractions formed smaller amounts of soil weight. Application of municipal
15 solid waste compost and farmyard manure had no clear effects on the particle size
16 fractionation. These two types of amendments however significantly increased the percentage
17 of macro-organic matter in the soil. This percentage varied from 1.35% in the control soil to
18 6.82% and 7.49% after addition of 120 t ha⁻¹ of farmyard manure and municipal solid waste
19 compost, respectively.

20

21 **3.3. Organic carbon and nitrogen concentrations in the different particle size-fractions**

22

23

24 The organic carbon (C) and organic nitrogen (N) contents in the different particle-size
25 fractions are shown in Fig. 2. In the control soil, the largest C and N contents were found in
26 the macro-organic matter fraction, followed by the size fraction <50 µm. In the soil amended

1 by 120 t ha⁻¹ of municipal solid waste compost and farmyard manure, the largest C and N
2 contents were observed in the macro-organic matter fraction, followed by the 50-150 µm
3 fraction. The application of both organic amendments significantly increased the C content in
4 all fractions, except in the <50 µm fraction where increments were not significant. In 50-150
5 µm fraction the C content increased from 8.8 g kg⁻¹ fraction in the untreated soil to 20.1 g kg⁻¹
6 fraction and 21.7 g kg⁻¹ fraction in the plots treated with 120 t ha⁻¹ of manure and compost,
7 respectively. In the macro-organic matter fraction, the amount of C increased from 105 g kg⁻¹
8 fraction in the control soil to 152.7 g kg⁻¹ fraction and 152.3 g kg⁻¹ fraction after application of
9 farmyard manure and municipal solid waste compost, respectively.

10 The N content only increased significantly in the macro-organic matter fraction and the
11 50-150 µm fraction after the application of the organic amendments. The N content increased
12 in 50-150 µm fraction from 0.8 g kg⁻¹ fraction to 1.9 g kg⁻¹ fraction and 2.2 g kg⁻¹ fraction and
13 in the macro-organic matter fraction from 3.4 g kg⁻¹ fraction to 9.3 g kg⁻¹ fraction and 10.4
14 g kg⁻¹ fraction after the application of farmyard manure and municipal solid waste compost,
15 respectively.

16 The results obtained in this study showed that the transfer of organic carbon and total
17 nitrogen, added with the application of the two organic amendments, into the different size-
18 fractions of soil starts from the coarser fractions towards the finest fractions. It presents a slow
19 process and depends on the mineralization of the organic matter. Since the finest fractions
20 (fine silt and clay) are not easily accessible, the transfer and accumulation of organic matter
21 are only detectable after a long term for these fractions.

22 According to Accoe et al. [35], the largest content of soil C was stored in the 50-150 µm and
23 macro-organic matter fractions of arable land with a sandy loam texture. According to
24 Clemente et al. [36], the finest fractions (fine silt and clay) in a control calcareous soil had the
25 highest concentrations of organic carbon but the coarsest (sand) fraction contained the highest

1 content (80% of the added carbon) of organic carbon in the same soil treated with humic acid
2 of commercial peat. Accoe et al. [37] stated that in 0-10 cm and 10-20 cm layers of grassland
3 soil, the largest C and N contents were found in the size fraction $<50\ \mu\text{m}$.
4 The C/N ratio fluctuated around 10 and did not vary strongly between fractions, except the
5 macro-organic matter fraction (Fig. 2). In the macro-organic matter fraction, C/N ratios
6 exceeded 30 in unamended soil, compared to about 15 in the treated soil. Christensen [38]
7 stated that the decrease of C-to-N ratios from the macro-organic matter fraction towards the
8 $<50\ \mu\text{m}$ fraction is due to the increasing degree of humification. The transfer of C and N from
9 the macro-organic matter fraction towards the 50-150 μm and $<50\ \mu\text{m}$ fractions is however a
10 slow process and that the soil organic matter accumulation in these two fractions is only
11 detectable after a long term [37].

12

13

14 **3.4. Presence of trace metals in the different particle size-fractions of the soil**

15 In the control soil, the total concentrations of Cu, Zn and Cr were higher in $<50\ \mu\text{m}$ fraction
16 than in the other fractions. The Cd concentration was higher in the macro-organic matter
17 fraction. There were no significant differences in the concentrations of Pb and Ni between the
18 different particle-size fractions. The addition of farmyard manure during seven successive
19 years only increased significantly the Zn concentration in the macro-organic matter fraction
20 ($249\ \text{mg kg}^{-1}$ fraction, compared to $109\ \text{mg kg}^{-1}$ fraction in the control soil) and the Cu
21 concentration in the 50-150 μm fraction ($60.2\ \text{mg kg}^{-1}$ fraction, compared to $38.5\ \text{mg kg}^{-1}$
22 fraction in the control soil) (Table 2).

23 Application of municipal solid waste compost significantly increased the concentrations of
24 Cu, Zn and Pb in the 50-150 μm , $<50\ \mu\text{m}$ and macro-organic matter fractions, and the

1 concentration of Cd in the 50-150 μm fraction. Municipal solid waste compost had no
2 significant effect on the concentration of Ni and Cr in all size fractions.

3

4 Venditti et al. [39] also found that the highest metal concentrations are found in the finer
5 particle-size fraction ($<50 \mu\text{m}$). They attributed this to the presence of clays, which have a
6 high density of adsorption sites, in this fraction, as well as to the higher efficiency of acid
7 digestion in this fraction, since the mineral components are more accessible to the reagents
8 used during digestion. The clay minerals and the so-called humic-clay complexes are indeed
9 mainly responsible for metal fixation in soils [40]. According to Qian et al. [41], trace metals
10 accumulate in the clay ($<2 \mu\text{m}$) as well as the sand fraction ($>125 \mu\text{m}$). The accumulation of
11 trace metals in the clay fraction is attributed to the high surface area and presence of clay
12 minerals, organic matter, Fe-Mn oxides and sulphides. The higher metal concentration in the
13 sand fraction results from the presence of heavy minerals which retain metals very strongly
14 [41].

15 Maisonnave et al. [42] stated that the $<20 \mu\text{m}$ fraction contains the highest concentrations of
16 trace metals (mg kg^{-1} dry weight of soil), which was attributed to the presence of clay and Fe
17 and Mn oxides. Some metals however tended to accumulate also in other fractions ($>20 \mu\text{m}$):
18 Cd in the 20-50 μm fraction, Cu in the 50-100 μm fraction and Zn in the 100-500 μm fraction.

19 Iwegbue et al. [43] stated that the fine fraction had the highest trace metal content and the
20 coarse fraction contained the least amount of each trace metal. Next to clay and Fe and Mn
21 oxides, organic matter also contributes to the retention of trace metals in soils by the
22 formation of stable complexes with the active sites of the soil's organic ligands [44, 45].

23 Balabane and Van Oort, [46] stated that metal concentrations in particulate organic matter
24 fractions were higher than that in the bulk soil samples and that they increased with
25 decreasing particulate organic matter size. Besnard et al. [47] found heterogeneity in the

1 distribution of Cu among particle-size fractions of soil. The highest Cu contents were detected
2 in particulate organic matter fractions. Thus, in non-amended and urban compost soil, the
3 total particulate organic matter fraction was nearly 6 times richer in Cu than the bulk soil.

4 A real environmental hazard may exist at long-term when the trace metals would be released
5 slowly over time as the added organic matter is decomposed [12]. Several authors claim that
6 upon mineralization, the metals previously bound to the organic matter will indeed be
7 remobilised into the environment. Others however claim that metals will be transferred from
8 the more available fractions to e.g. highly insoluble organic complexes with strongly humified
9 organic matter and thus sequestered in long-term sinks [29].

10

11 **3.5. Chemical fractionation of trace metals in the soil**

12

13 Figure 4 shows results of the application of the BCR sequential extraction scheme for the
14 control, farmyard manure and municipal solid waste compost amended soil, together with
15 results of the fourth, aqua regia extraction step, which determines the residual fraction. The
16 recovery was calculated for all metals as the sum of all BCR fractions divided by the amount
17 of metals released by aqua regia extraction, expressed as percentage. Recoveries for most of
18 metals ranged from 80 % to 120 %. The recovery for Cd and Pb however ranged from 120 %
19 to 130 %, which is explained by the very low concentrations of these metals.

20 Cadmium was mainly extracted in the first and second extraction step (acid-extractable and
21 reducible fraction), both for the control and amended soils. The application of farmyard
22 manure and municipal solid waste compost increased insignificantly the Cd concentration in
23 the acid-extractable fraction. This concentration increased from 0.093 mg kg⁻¹ in the control
24 soil to 0.121 mg kg⁻¹, 0.122 mg kg⁻¹ and 0.116 mg kg⁻¹ after the application of 120 t ha⁻¹ of
25 farmyard manure, 80 t ha⁻¹ and 120 t ha⁻¹ of municipal solid waste compost, respectively.

1 Furthermore, the main increment was observed in the reducible fraction, which the
2 concentration of Cd increased insignificantly from 0.100 mg kg⁻¹ in control soil to 0.356 mg
3 kg⁻¹ after application of 120 t ha⁻¹ of municipal solid waste compost. The two organic
4 amendments had no effect on the Cd concentrations in the oxidisable and residual fraction.
5 These results correspond well with those reported by Qiao et al. [48], who stated that the acid-
6 extractable and reducible fraction retained a large proportion of Cd in clay soil amended with
7 different rates of biosolids.

8 In the control soil, Cu seemed to be mainly present in the residual (60 %) and reducible
9 fraction (30 %). Application of farmyard manure had no effect on the Cu distribution.
10 Application of municipal solid waste compost however significantly increased the Cu
11 concentration in all soil fractions, except the reducible fraction. Upon addition of 120 t ha⁻¹ of
12 municipal solid waste compost, the Cu concentration increased from 0.053 mg kg⁻¹ in the acid
13 extractable fraction of control soil to 0.558 mg kg⁻¹ in amended soil and from 2.2 mg kg⁻¹ in
14 the oxidisable fraction of control soil to 13.3 mg kg⁻¹ in amended soil. The latter indicates that
15 Cu has a very high affinity to organic compounds, as also previously reported [49,51]. In the
16 residual fraction, the Cu concentration increased from 25.4 mg kg⁻¹ in the control soil to 51
17 mg kg⁻¹ in amended soil. Qiao et al. [48] however stated that reducible Cu in red clay soil
18 increased drastically with increasing biosolid application rates. Zheljzkov and Warman, [52]
19 reported that application of municipal solid waste compost increased the Cu concentration in
20 the oxidisable fraction, as well as exchangeable Cu concentrations. According to Kidd et al.
21 [53], the concentration of Cu in the oxidisable fraction increased from 15% in a control soil to
22 53% after long-term application of sewage sludge to a sandy loamy soil.

23 The largest portion of Pb was found in the reducible fraction, both in the unamended and
24 amended soils. Compared to the control soil, the farmyard manure had no effect on the
25 distribution of Pb. Similar results were found by Parat et al. [31], who observed no differences

1 between control and manure-treated soil during 20 years at $10 \text{ t ha}^{-1} \text{ y}^{-1}$ for Cu, Zn and Pb
2 concentrations. Municipal solid waste compost tended to increase the Pb concentration in all
3 fractions, except the acid-extractable fraction. Zhejzakov and Warman [52] reported that
4 municipal solid waste compost contributes to an important extent to the increment of the
5 presence of Pb in the reducible fraction. Lead moreover forms strong complexes with organic
6 matter in the soil. These complexes obtain a high degree of irreversibility, which hampers any
7 future attempt to remediate Pb-polluted soils [54].

8 Zinc was principally distributed between the reducible and residual fractions. Farmyard
9 manure increased insignificantly the Zn concentration in different fractions of soil. Municipal
10 solid waste compost increased significantly the Zn concentrations in all soil fractions,
11 proportional to the application rates. The highest increase was observed in the reducible and
12 residual fractions. Indeed, the Zn concentration passed from 15.2 mg kg^{-1} in reducible fraction
13 of unamended soil to 58.5 mg kg^{-1} and 96.5 mg kg^{-1} after the application of 80 t ha^{-1} and 120 t
14 ha^{-1} of municipal solid waste compost, respectively. The long-term application of composted
15 sewage sludge in a sandy loam soil increased the Zn bound to organic matter fraction from
16 $<5\%$ to 44% [53]. Furthermore, the long-term application of municipal solid waste compost
17 increased the concentration of Zn in acid-extractable, Fe and Mn oxides and organic matter
18 fractions [52]. Qiao et al. [48] showed that the application of biosolids increased the Zn
19 concentration in the reducible fraction of a Taihu paddy soil (Agri-Udic Ferrosol), and this
20 may have been due in part of differences in soil pH. Planquart et al. [55] reported that the
21 application of sewage sludge compost to three soils from France under greenhouse conditions
22 increased the proportion of Zn bound to acid extractable fraction.

23 Nickel was largely present in the residual fraction in unamended and amended soil. These
24 results are in accordance with those found by other authors [56]. While studying two
25 untreated calcareous soils, Rajaie et al. [57] found that Ni was predominantly bound to oxides

1 and in the residual fraction (99.1% in clay loam and 98.7% in sandy loam respectively). The
2 application of farmyard manure had no effects on the distribution of Ni in our soil. Municipal
3 solid waste compost increased significantly the Ni concentration in reducible fraction, but
4 only after the addition of 120 t ha⁻¹. The Ni concentration passed from 3.47 mg kg⁻¹ in control
5 soil to 5.06 mg kg⁻¹ in amended soil. In the oxidisable fraction, the Ni concentration increased
6 insignificantly after the application of both organic amendments. Contrary, Businelli et al.
7 [58] found upon application of municipal waste compost to the superficial layer of a landfill-
8 covering soil, the percentage of Ni in all fractions of the A horizon remained relatively
9 constant. This is possibly due to the limited contribution of compost to the concentration of Ni
10 in the A horizon.

11 Chromium was mainly distributed between the third and the fourth fraction: more than 95%
12 of Cr in the soil was found in these fractions (Fig. 3). This indicates that under natural
13 conditions Cr would not be easily released. When applying a modified BCR sequential
14 extraction procedure, Zemberyov et al. [59] found that chromium was found exclusively in
15 the residual fraction (47–65%) bound in mineral lattice. Application of municipal solid waste
16 compost resulted in a significant increase of the Cr concentration in the reducible and
17 oxidisable fraction. A pot experiment of Bose et al. [60] showed that upon addition of
18 industrial waste, a maximum level of Cr was bound with Fe and Mn oxides. Due to the high
19 affinity of Cr to organic matter, the solubility of this metal seemed to be limited by the
20 formation of insoluble chromium-organic complexes [61]. Consequently, application of
21 municipal waste compost during 10 years increased the Cr concentration in the organically-
22 bound fraction of the superficial layer (A horizon) of a landfill-covering soil [58].

23

24

25 **4. Conclusions**

1 The application of Tunisian municipal solid waste compost instead of farmyard manure to a
2 Tunisian calcareous soil during seven successive years significantly ameliorated the soil
3 fertility under field conditions. Consequently, this amendment can be used to increase the
4 organic matter content of Tunisian soil. However, the compost seems to be a source of
5 accumulation of trace metals in the soil which could cause a real environmental hazard on the
6 longer term. The distribution of trace metals between different particle-size fractions of soil
7 showed the highest dominance of the macro-organic matter fraction in accumulating trace
8 metals, both in untreated and in treated soil. The results of the sequential extraction indicate
9 that all trace metals were initially mainly found in the residual fraction, except Cd and Pb in
10 reducible fraction. Municipal solid waste compost and farmyard manure had no effects on the
11 exchangeable metal fraction within the soil. Since a redistribution of trace metals in the
12 treated soils may however occur upon decomposition of organic matter, especially the
13 mobility and bioavailability of trace metals might increase on longer term and finally cause an
14 environmental hazard. Longer-term field experiments are needed to completely assess the
15 environmental risks of these metals accumulating in the soils upon municipal solid waste
16 compost application.

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1 **Table 1.** Characteristics of soil, manure and compost used in the experiment
 2 (mean \pm Standard deviation, n =3). Results are expressed on dry weight basis.

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	Soil	Manure	Compost
pH (H₂O)	8.4 \pm 0.2	7.4 \pm 0.6	7.2 \pm 0.3
EC (mS/cm)	0.16 \pm 0.03	2.5 \pm 0.2	6.5 \pm 0.7
CEC (meq/100g)	17.9 \pm 2.0	24.6 \pm 3.7	33.1 \pm 4.1
C (g/kg)	10 \pm 2	362 \pm 32	202 \pm 21
N (g/kg)	0.9 \pm 0.1	13.0 \pm 1.0	10.6 \pm 2.0
C/N	11.4	27.8	19.1
OM (g/kg)	17.5	724	404
HR (%)	8.2	71.6	25.8
Clay (%)	29.1	-	-
Silt (%)	51.9	-	-
Sand (%)	18.4	-	-
Texture	Loamy - Clayey	-	-
Trace metals (mg/kg)			
Cu	50 \pm 7	26 \pm 3	278 \pm 22
Zn	86 \pm 8	120 \pm 18	410 \pm 26
Pb	47 \pm 6	10 \pm 1	325 \pm 24
Cd	1.0 \pm 0.2	0.7 \pm 0.2	3.3 \pm 0.4
Ni	31 \pm 7	22 \pm 4	44 \pm 7
Cr	54 \pm 6	24 \pm 3	52 \pm 9

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HR: relative humidity

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1 **Table 2.** Soil organic carbon, nitrogen and trace metals contents in the 0-20 cm soil layer after
 2 a 7-year application of manure (M) and compost (C) at different rates (40, 80 and 120 t ha⁻¹)
 3 (mean ± Standard deviation, n=4). Results are expressed on soil dry weight basis.

	T	M 40	M 120	C 40	C 80	C 120	
7	C (g kg ⁻¹)	11 ±0.3 ^a	21±0.6 ^b	33±0.5 ^c	21±0.6 ^b	29±0.4 ^{ab}	35 ±0.3 ^c
8	N (g kg ⁻¹)	1.1 ±0.01 ^a	1.7 ± 0.04 ^b	2.8±0.03 ^c	1.5±0.04 ^{ab}	1.8 ±0.02 ^b	2.4± 0.04 ^c
9	Cu (mg kg ⁻¹)	42.8± 2.7 ^a	43.3 ± 7.0 ^a	50.5±2.0 ^{ab}	60.3±6.3 ^{bc}	67.0±3.9 ^{cd}	82.13±18.8 ^d
10	Zn (mg kg ⁻¹)	88 ± 4 ^a	85±2 ^a	117±24 ^{ab}	121± 11 ^{ab}	135± 12 ^b	212±41 ^c
11	Pb (mg kg ⁻¹)	27.1 ±2.3 ^a	28.7±6.2 ^{ab}	29.8± 1.4 ^{ab}	38.3±5.3 ^{ab}	50.0±8.2 ^b	83.5±25.6 ^c
12	Cd (mg kg ⁻¹)	0.28 ±0.03 ^a	0.27 ±0.08 ^a	0.32±0.01 ^a	0.38±0.04 ^a	0.40±0.03 ^a	0.64±0.29 ^b
13	Ni (mg kg ⁻¹)	20.9±0.3 ^c	20.1 ±0.4 ^b	19.6±0.6 ^a	22.0±0.4 ^d	21.4±0.6 ^{cd}	21.3±0.5 ^{cd}
14	Cr (mg kg ⁻¹)	51.5 ±1.7 ^c	45.4±1.6 ^a	48.3±3.3 ^{ab}	54.3±1.6 ^c	50.1 ±1.4 ^{bc}	50.3± 3.1 ^{bc}

15 Values at the same line followed by the same letter are not significantly different at p<0.05.

16 M and C: rates (t ha⁻¹) of manure and compost, respectively. T: untreated soil.

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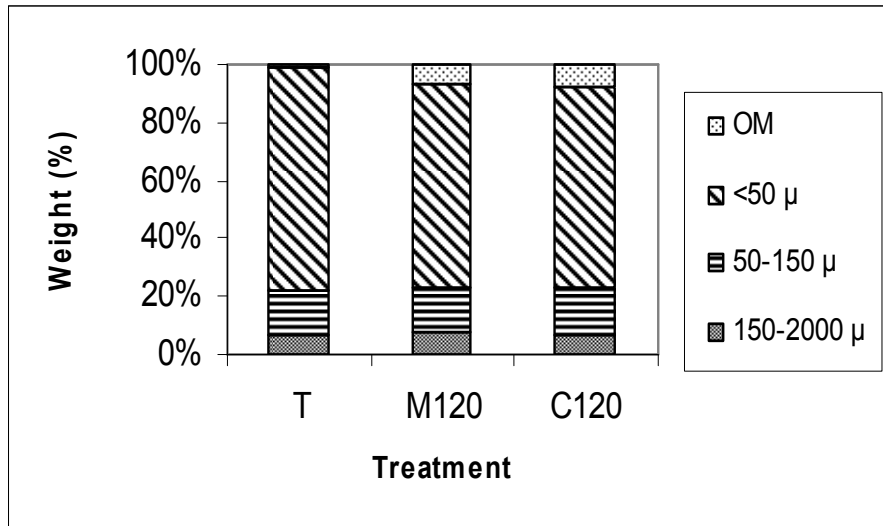


Fig. 1. Relative abundance of the different particle-size fractions within the soil (weight percentage).

T: unamended soil, M120: 120 t/ha of manure, C120: 120 t/ha of compost and OM: organic matter.

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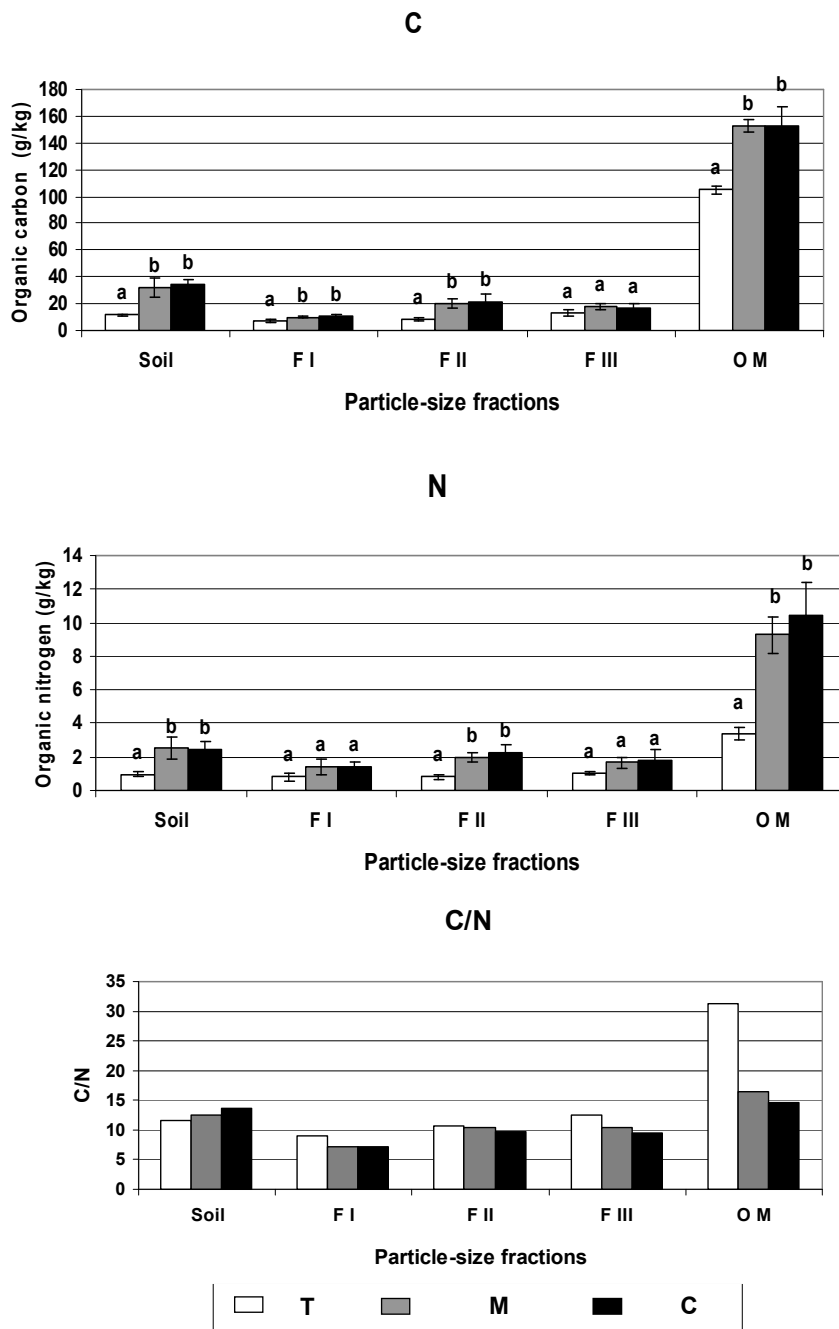
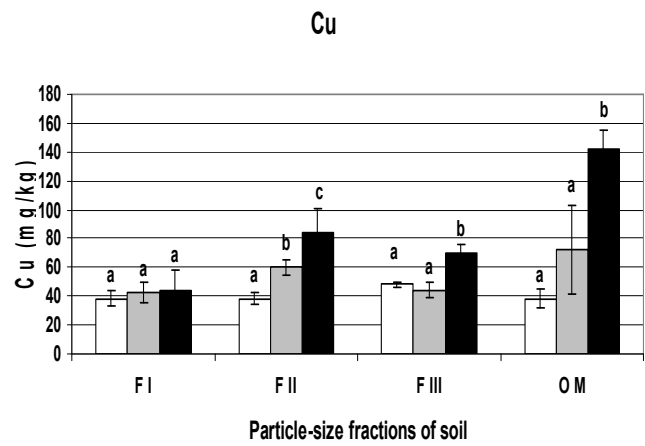
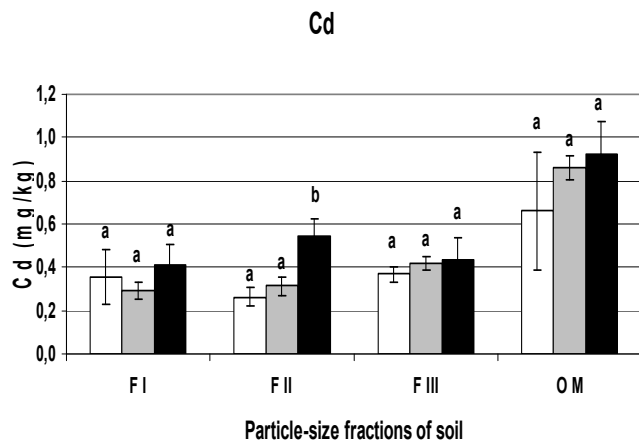
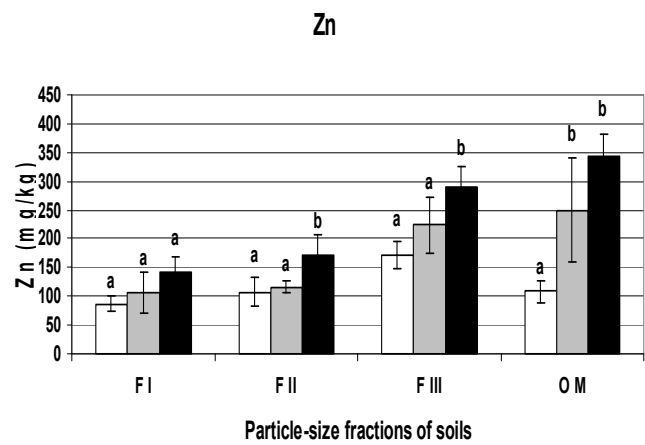
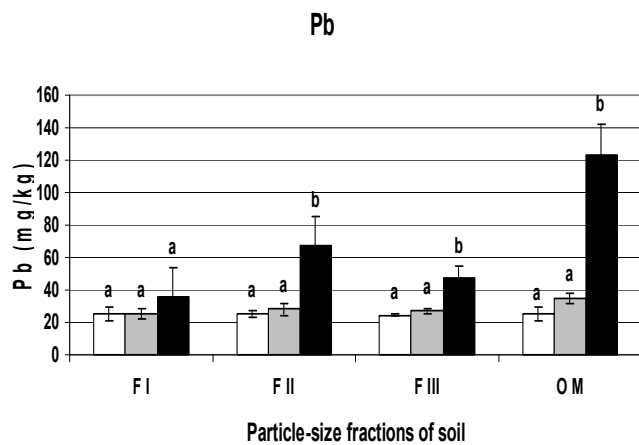


Fig. 2. Organic carbon (C) and nitrogen (N) concentrations and carbon/nitrogen ratio (C/N) in the different particle-size fractions of the soil (g kg^{-1} fraction).

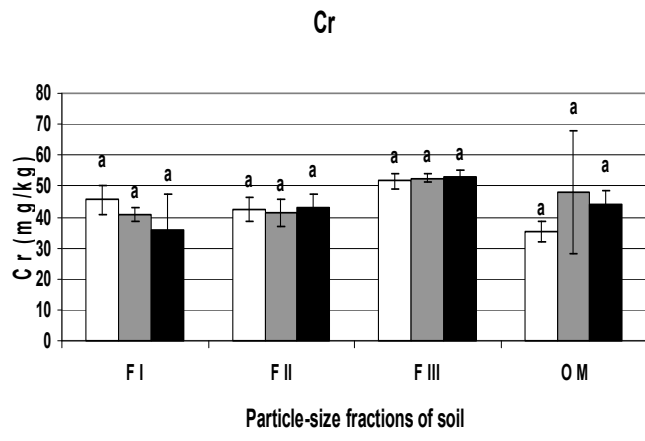
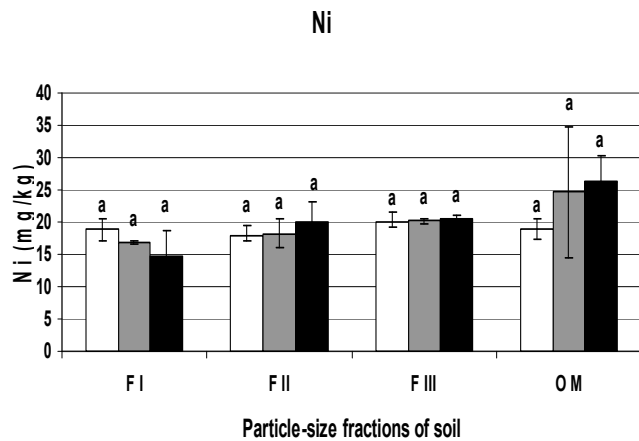
Bars showing the same letter at the same soil fraction were not significantly different, according to Duncan's test at 5% probability level ($n = 3$). T: unamended soil, M: 120 t/ha of manure, C: 120 t/ha of compost. FI: $>150 \mu\text{m}$, FII: $150\text{-}50 \mu\text{m}$ and FIII: $<50 \mu\text{m}$ fractions; OM: macro-organic matter fraction. (Mean \pm Standard deviation, $n=4$).



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43 Fig.3. Cd, Cu, Pb, Zn, Ni and Cr contents in the different particle-size fractions of the soil
44 (mg kg⁻¹ fraction).

45 Bars showing the same letter at the same soil fraction were not significantly different, according to Duncan's test
46 at 5% probability level (n = 4). T: unamended soil, M: 120 t/ha of manure, C: 120 t/ha of compost. FI: >150
47 μm, FII: 150-50 μm and FIII: <50 μm fractions; OM: macro-organic matter fraction. (Mean ± Standard
48 deviation, n=4).

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