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Research article

Heavy metal availability and uptake by wheat crops cultivated in Tunisian field plots amended during five years with municipal solid waste compost and farmyard manure

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Abstract

Agricultural recycling of municipal solid waste compost provides a valuable source of plant nutrients and organic matter. However, these wastes could have negative effects due to their metal content. The aim of the study was to evaluate the influence of the addition of Tunisian municipal solid waste compost and farmyard manure on soil metal content, availability and uptake of heavy metals by wheat crops. A long-term field experiment in four blocks with wheat crops was carried out in the north of Tunisia. The following treatments were applied in five consecutive years: 40 and 80 t ha^{-1} of Tunisian municipal solid waste compost, 40 t ha^{-1} of farmyard manure, mineral fertilization and a control plot (without amendment).

In the last year of the experiment at harvest time, plant samples (grain, straw and root) and soils were collected for laboratory analyses. Results showed that the grain yields increased with the application of two organic amendments and mineral fertilization. The farmyard manure had no effects on the heavy metal contents in soil and in wheat plants. Municipal solid waste compost increased the concentration of Cd, Pb, Cu and Zn and had no effects on Ni and Cr concentrations in soils. In wheat plants, the roots accumulated and retained the heavy metals. Only small portions of Cu, Zn and Ni reached the straw, whereas only Cu and Zn reached the grain.

The availability of heavy metals in the soil was evaluated using an NH4OAc-EDTA pH 4.65 solution for metal extraction. Results showed that the long-term application of municipal solid waste compost increased the available heavy metals. The highest rate (80 t ha^{-1}) of compost caused a greater mobility compared to the farmyard manure amended soils and the control soil. Significant correlations were obtained between NH4OAc-EDTA extractable heavy metals on the one hand and the concentrations of Cu, Zn and Ni in the roots and Ni in the straw of the wheat plants on the other hand.

Key words: Municipal solid waste compost, farmyard manure, soil heavy metals, NH4OAc-EDTA, uptake, wheat crops.

1. Introduction

Agricultural recycling of municipal wastes provides an important source of plant nutrients and organic matter. Land application represents an economically desirable outlet for the producers of wastes by reducing its volumes and its disposal costs and a potential cheap source of organic matter and plant nutrients. In recent years, the application of municipal solid waste compost as fertilizer has increasingly become attractive due to its high content of plant essential nutrients such as N, P, K, Mg, Zn, Co, Mn, Fe and B. However, some waste materials may also contain toxic compounds and heavy metals, such as Cd and Pb that may be toxic to plants (Iwegbue et al., 2006; Munir Jamil and Athamneh, 2004).

The addition of heavy metals in long-term excessive compost amended soils raises a serious concern about the adverse environmental impact. These heavy metals can be accumulated in edible parts of plants and may eventually contaminate both the human and animal food chain (Gupta and Sinha, 2006; Michalska and Asp, 2001). The levels of heavy metals in composts widely depend on the sources, composting process and geographical location (He et al., 1992). Although heavy metals applied to soils as salts or in composted sludge are generally considered to be strongly immobilized in soils, numerous studies have shown that a suitable fraction of this metals are mobilized from the top soil over long-term of exposure to natural climatic conditions (McBride et al., 2004).

Introduced to the soil, heavy metals exist in several geochemical fractions which influence the solubility, mobility and availability of metals to plants. The total metal concentration in the soil is not a reasonable predictor of mobility, bioavailability or soluble concentration of the metals it also has limited significance in agronomic studies (Chaudri et al., 2000; Gupta and Sinha, 2006; Jordao et al., 2006). In the last two decades, particular attention has gone to the mobile and bioavailable heavy metals in biosolids-amended soils, since these fractions can possibly leach to pollute groundwater or enter food chains via plant uptake (Qiao et al., 2003). In order to predict the mobility and bioavailability of heavy metals introduced to agricultural soils, several methods have been developed. Single reagent extractions are now most commonly used in assessing the mobility and bioavailability of heavy metals in the biosolids-amended soils. Numerous reagents have already been used, such as $CaCl_2$, $NaNO_3$, NH_4NOAC, $MgCl_2$, HOAC, DTPA, EDTA,… (Meers et al., 2007).

The objective of this study was to evaluate the effects of a long-term field application of Tunisian municipal solid waste compost in comparison to farmyard manure on the amelioration of grain yield of wheat crops, on the soil metal content and the uptake of heavy metals by wheat plants in Tunisia. The available fraction of heavy metals was determined using single reagent extraction procedures and subsequently correlated with the uptake by the wheat crops.

2. Material and methods

2.1. Field experiment

The field experiments were carried out at the experimental farm of the Agronomic National Institute of Tunis (INAT) in the region of Mornag situated in the North of Tunisia. The used soil had a clayey-loamy texture and its other characteristics were: pH 8.4, organic carbon content 1.03 %, organic nitrogen 0.09 %, silts content 51 %, clay content 29 % and sand content 18.36 %.

Two types of organic amendments were used: livestock farm manure (MN) aged for six months and a Tunisian municipal solid waste compost. The compost was produced under aerobic conditions by mixing a municipal solid waste with garden waste and arranging the mixture into rows of long piles. The composting process was achieved in six months until the C/N ratio and the temperature became constant. The main characteristics of the soil, farmyard manure and compost used in this study are presented in Table 1.

The experiments were conducted in a randomized complete block design with four replications. Each block consisted of four plots, with each plot having a total area of 4×2 m^2. The plots were separated in all directions by a border space of 1 m. The following treatments were annually incorporated since 1999: control (Co, without amendment), two rates of municipal solid waste compost (C_1 = 40 t ha^{-1} and C_2 = 80 t ha^{-1}), 40 t ha^{-1} of cow manure (MN) and a mineral fertilization consisting of a combination of 100 kg ha^{-1} of P_2O_5 and 300 kg ha^{-1} of NH_4NO_3 (MF). All treatments were incorporated to 15-20 cm depth by chisel plowing and disking the day after application.

All plots were cultivated yearly with wheat (Karim: Tunisian variety). Irrigation was applied when rainfall was insufficient. Plants were sowed in October/November and harvested in July, leaving 20 cm stalks, which were tilled into the soil at the same time as the amendment application. At the harvest time, wheat plants were sampled from each plot to obtain grain yield and heavy metal contents (Cd, Cu, Zn, Pb, Ni and Cr) in grain, straw and roots.

2.2. Collection and preparation of soils and wheat samples

At the harvest time, plants and soils were sampled. Plants were entirely taken to the laboratory and washed with deionized water. The plants were then separated into grains, straw and roots, and subsequently dried at 70°C and ground in an agate mortar in order to pass a 1 mm sieve. Ground samples were stored at room temperature in polyethylene bags.

Topsoil samples were taken after the harvest. Five cores to a depth of 20 cm were taken from each plot using a hand auger (4 cm diameter). The collected soil samples were mixed, air dried, crushed to pass through a 2 mm sieve and stored in polyethylene bags for analysis.

2.3. Analysis

a) Soil

The pH and electrical conductivity (EC) were measured on a 1:2.5 soil/water extract and on a 1:5 organic amendment/water extract after shaking for 2 h. Organic carbon content was determined by dichromate oxidation and subsequent titration with ferrous ammonium sulphate (Walkley and Black, 1934).

Organic matter was calculated by multiplying total organic carbon by 1.72. Total nitrogen in all samples was determined by the kjeldahl method. The total contents of heavy metals in the soil samples (Cd, Cu, Pb, Zn, Ni and Cr) were determined by aqua regia extractions. Therefore, one gram of sample was digested overnight (minimum 12h) at room temperature with a mixture of 3:1 hydrochloric: nitric acid (7.5 ml: 2.5 ml). Subsequently, the suspensions were digested at 150 °C for 2 h, filtered and diluted to 100 ml with nitric acid (2 M) for further analysis. Available heavy metals were extracted in control and amended soils using an NH_4OAc-EDTA pH 4.65 solution at a soil solution ratio of 1:5, after a continuous agitation for 30 min at room temperature (Van Ranst et al., 1999). The concentrations of heavy metals in all extracts were determined using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer, type Varian Vista MPX, Varian, Palo Alto, CA).

b) Plant

One gram of wheat plant material (previously dried at 105 °C) was transferred in a porcelain crucible and ashed in a muffle furnace at a temperature of 450°C during 2 h. The crucible was transferred to a hot plate, 10 ml of 1 N HNO_3 was added and the mixture was heated at 150 °C during 30 min to obtain a volume of 5 ml. After cooling, the digested plant samples were filtered into a 50 ml volumetric flask and diluted to the mark with 1% NHO_3. The concentrations of heavy metals (Cd, Cu, Pb, Zn, Ni and Cr) were determined by atomic absorption spectrometry (Perkin Elmer Spectra AA 220 FS).

Statistical analysis

The statistical analyses were performed using SPSS Version 10 for Windows. The significance of differences between the means of treatments (three replicates) was evaluated using ANOVA, followed by Duncan's post hoc tests ($p < 0.05$). Correlations between total and NH_4OAc-EDTA extractable heavy metal concentrations in soils and the content of heavy metals in roots, straw and grains of wheat crops were evaluated using Pearson correlation analysis.

4. Results and Discussion

4.1. Grain yield

The five years of consecutive application of the two organic amendments (farmyard manure and municipal solid waste compost) and the mineral fertilizers (NH_4NO_3: 300 kg N ha^{-1} and calcium superphosphate: 100 kg P_2O_5 ha^{-1}) increased the wheat grain yield in all amended plots compared to the control plot (without amendment).

For example, the addition of municipal solid waste compost increased the grain yield from 15 q ha-1 in the control treatment to 25.3 and 34.6 q ha-1 when 40 and 80 t ha^{-1} of compost was applied, respectively. The wheat plants grown on the plots amended with a mixture of the highest rate of compost and the mineral fertilizer gave the highest grain yield (38.9 q ha-1). Wei and Lin, (2005) studied the use of composted sewage sludge consisting of wastewater and wood waste as amendment during a three years field study with a Chinese soil having a low inherent fertility. They stated that the application of a mixture of composted sewage sludge and mineral fertilizers combined the advantages of both mineral and organic fertilizers. The mineral fertilizers provide readily available nutrients for the crops, whereas the compost is a slow-releasing fertilizer which provides a whole array of nutrients to the soil. We, however, found no significant differences between the grain yield of plants grown on manure amended plots and of plants grown on the plots amended with a mixture of manure and mineral fertilizers. The yields on the farmyard manure and municipal solid waste compost amended soils were slightly better compared to the control areas which were fertilized by mineral fertilizers.

Table 1: Physical-chemical characteristics and heavy metal contents of soil, farmyard manure and municipal solid waste compost

	Soil	**Manure**	**Compost**
pH (H_2O)	8.4 ± 0.2	7.4 ± 0.6	7.2 ± 0.3
EC (mS/cm)	0.16 ± 0.03	2.5 ± 0.2	6.5 ± 0.7
CEC (meq/100g)	17.9 ± 2.0	24.6 ± 3.7	33.1 ± 4.1
C (g/kg)	10 ± 2	362 ± 32	202 ± 21
N (g/kg)	0.9 ± 0.1	13.0 ± 1.0	10.6 ± 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	-	-
Cu	50 ± 7	26 ± 3	278 ± 22
Zn	86 ± 8	120 ± 18	410 ± 26
Pb	29 ± 6	10 ± 1	325 ± 24
Cd	0.22 ± 0.02	0.7 ± 0.2	3.3 ± 0.4
Ni	20 ± 7	22 ± 4	34 ± 7
Cr	51 ± 6	24 ± 3	52 ± 9

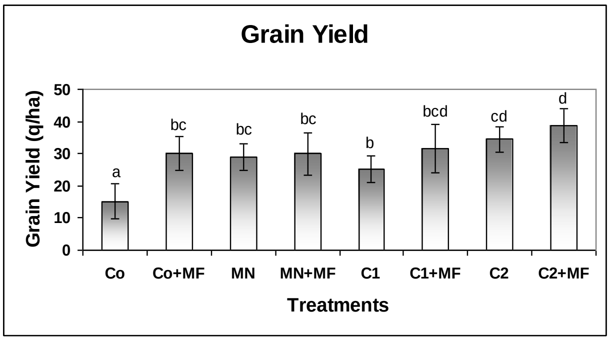

Fig. 1: Effects of different treatments on the wheat grain yield. Co: control, MF: mineral fertilizer, MN: Manure, C1: 40 t ha^{-1} of compost, C2: 80 t ha^{-1} of compost. (Values are expressed as means ± standard deviations of four replications after five years of field experiment). Bars with the same letters are not significantly different according to Duncan's multiple range test at ≤ p0.05.

Garcia-Gil et al., (2000) studied the long-term field addition of municipal solid waste compost (40 and 80 t ha^{-1}) and cow manure (20 t ha^{-1}) to a sandy soil with low organic matter content in Spain and also observed that the grain yield of barley increased significantly upon municipal solid waste compost and farmyard manure treatment. They found the highest grain yields with the highest application rate (80 t ha^{-1}) of compost and cow manure.

Wei and Lin, (2005) reported that the application of composted sewage sludge (120 t ha^{-1}) with supplementary addition of mineral N-P-K fertilizer (100 kg N ha^{-1} + 100 kg P_2O_5 ha^{-1} + 50 kg K_2O ha^{-1}) during a three years field study, increased the barley grain yield by 46 % as compared to plots which were only fertilized by the mineral N-P-K fertilizer (300kg N ha^{-1} + 300 kg P_2O_5 ha^{-1} + 150 kg K_2O ha^{-1}). Perez et al., (2007) stated that increasing application of municipal solid waste compost rates had no negative effects on the yield of four cultivated vegetables (carrot, cauliflower, sweet corn and radish). N'Dayegamiye et al., (2005) found that the addition of urban waste compost, during four repetitive years in sand loamy soil, increased the wheat grain yield from 2479 kg ha^{-1} in control soil to 4670 kg ha^{-1} in amended soil. The highest grain yield was obtained in the plots amended by a mixture of urban waste compost and mineral fertilizers and the yield was not proportional to the added rates of compost (20, 40 and 60 t ha^{-1}). Moreover the application of 20 to 40 t ha^{-1} of compost was found to reduce the need for the nitrogen fertilizer by about 30 kg ha^{-1} without affecting the grain yield.

4.2. Heavy metal accumulation in the soil

The heavy metal contents of the control soil (without treatment), the farmyard manure and the municipal solid waste compost are shown in Table 1. Heavy metal concentrations in the soil after five successive years of field experiment are illustrated in Table 2.

Farmyard manure application during each of the five seasons (1999 to 2004) had no effect on the concentration of heavy metals (Cd, Cu, Pb, Zn, Ni and Cr) in the 0-20 cm soil layer, compared to the control soil. On the other hand, the application of municipal solid waste compost during five successive years increased the heavy metal contents in the soil significantly. In fact, the concentrations of Cd, Pb, Cu and Zn respectively increased by 0.1, 16.4, 23.1 and 48.6 mg kg^{-1} (corresponding to: 37%, 55%, 46% and 54%, respectively) in the C_2 amended soils compared to the control soil, respectively. The concentrations of Ni and Cr were similar in the control, farmyard manure and municipal solid waste compost amended soils. However, total metal concentrations were still below the sanitation threshold value for the agricultural soils permitted by French legislation (Norm AFNOR NFU 44-041) and the legislation of the Flemish region in Belgium (Vlarebo).

The highest municipal solid waste compost rate (80 t ha^{-1}) provokes the most important increase of the Cd, Pb, Cu and Zn concentrations. The heavy metal accumulation in the soil depends on the original contents of heavy metals in the compost and the compost application rates. These results were confirmed by Madrid et al., (2006). They found that the three successive applications of 30 t ha^{-1}of municipal solid waste compost, under intensive farming conditions, increased significantly the concentration of heavy metals in the surface layer (0-25 cm) of amended sandy soils. Wei and Lin, (2005) used composted sewage sludge as amendment and stated that three successive years of application increased the Zn and Cu concentrations at 0-10 cm and 10-20 cm depth significantly. El-Demerdashe et al., (1995) even stated that the long-term application of composted sewage sludge can gradually accumulate heavy metals in the soil to levels that may be toxic to some plant species.

4.3. Heavy metals contents in wheat plants

The mean contents of heavy metals in wheat plants (grain, straw and root) grown on control, farmyard manure and municipal solid waste compost amended soils were summarized in Table 3.

The long-term application of farmyard manure had no effect on the concentration of heavy metals in the root, straw and grain. This is due to the low original heavy metal contents in the manure. Compared to the control (1.08 mg kg^{-1}), the incorporation of 40 and 80 t ha^{-1} of municipal solid waste compost in the soil slightly increased the copper concentrations to 1.41 and 2.36

mg kg^{-1}, respectively. Moreover, an increase of the Zn concentrations in the grains was found, which was proportional to the incorporated amount of municipal solid waste compost. Consequently, the highest Zn concentration (9.66 mg kg^{-1}) was found in the C$_2$ treatment. The Cd, Pb and Ni contents were not detectable in the grains.

In straw, the addition of municipal solid waste compost increased the Zn, Cu and Ni concentrations, whereas the Cd and Pb concentrations were not detectable.

Table 2. Average total content and NH$_4$OAc-EDTA extractable heavy metals in the soils (mg kg^{-1}) after five years of successive amendments (mean of four repetitions ± standard deviation)

Heavy metals (mg kg^{-1})	**Control**	**MN**	**C$_1$**	**C$_2$**
Total Content HM in Soils				
Cd	0.25 ± 0.03 ^{a}	0.28 ±0.03 ^{ab}	0.36 ±0.05 ^{c}	0.35 ±0.03 ^{bc}
Cu	51.2 ±2.0 ^{ab}	53.7 ±2.0 ^{a}	60.9 ±3.3 ^{b}	74.3 ±15.8 ^{b}
Pb	30.4 ±1.4 ^{a}	31.3 ±1.4 ^{a}	39.8 ±2.3 ^{a}	46.8 ±8.6 ^{a}
Zn	90.4 ±1.4 ^{a}	94.7 ±3.7 ^{a}	113.9 ±1.9 ^{ab}	139.0 ±30.8 ^{b}
Ni	21.4 ±0.1 ^{a}	20.7 ±0.3 ^{a}	21.0 ±0.5 ^{a}	22.0 ±0.9 ^{a}
Cr	52.3 ±0.7 ^{b}	50.1 ±0.7 ^{a}	49.8 ±1.3 ^{a}	52.4 ±0.7 ^{b}
EDTA Extractable MH in soils				
Cd	0.23 ±0.01 ^{a}	0.25 ±0.02 ^{a}	0.30 ±0.01 ^{a}	0.30 ± 0.03^{a}
Cu	16.4 ±0.4 ^{a}	18.4 ±0.6 ^{ab}	21.0 ±1.2 ^{bc}	24.4 ±4.0 ^{c}
Pb	13.7 ±0.4 ^{a}	14.9 ±0.4 ^{a}	22.1 ±1.2 ^{b}	26.5 ±4.5 ^{b}
Zn	5.0 ± 0.04^{a}	10.3 ±1.7 ^{a}	23.6 ±1.5 ^{a}	40.2 ±19.3 ^{b}
Ni	0.90 ±0.9 ^{a}	0.91 ±0.03 ^{a}	1.1 ± 0.03^{a}	1.1 ±0.04 ^{a}
Cr	0.65 ±0.7 ^{a}	0.64 ±0.01 ^{a}	0.72 ±0.03 ^{a}	0.72 ±0.02 ^{a}

Values at the same line for the same extract followed by the same letter are not significantly different as determined by Duncan's test ($p<0.05$).

Table 3. Heavy metal concentrations in roots, straw and grains of wheat crops (mg kg^{-1}) (Mean of four repetitions ± standard deviation after five successive years of field experiment)

Heavy metals (mg kg^{-1})	**Co**	**MN**	**C$_1$**	**C$_2$**
Roots				
Cd	0.20 ± 0.02 ^{a}	0.21 ±0.03 ^{a}	0.29 ±0.02 ^{b}	0.32 ±0.2 ^{b}
Cu	7.13 ±1.5 ^{a}	7.88 ±1.7 ^{a}	12.4 ±2.2 ^{b}	14.7±1.9 ^{b}
Pb	9.43 ± 5.8^{a}	10.93 ±9.9 ^{a}	14.7 ± 7.3 ^{a}	19.0 ±3.9 ^{a}
Zn	12.7 ± 1.6^{a}	16.1 ±1.2 ^{a}	24.7 ±2.5 ^{b}	29.7±3.8^{c}
Ni	4.93 ±1.4 ^{a}	5.01± 0.7^{ab}	6.73 ±0.5 ^{bc}	7.68 ±0.5 ^{c}
Straws				
Cd	ND	ND	ND	ND
Cu	1.45 ±1.0 ^{a}	2.58 ±1.3 ^{a}	3.68 ±1.5 ^{a}	3.66 ±1.8 ^{a}
Pb	ND	ND	ND	ND
Zn	9.78 ±3.8 ^{a}	13.6 ± 3.7^{a}	16.3 ±5.0 ^{a}	22.1 ±4.3^{b}
Ni	0.38 ±0.1 ^{a}	0.33 ±0.8 ^{a}	1.58 ±0.8 ^{a}	1.46 ±0.9 ^{a}
Grains				
Cd	ND	ND	ND	ND
Cu	1.08 ±0.1 ^{a}	1.15 ±0.2 ^{a}	1.41 ±0.8 ^{a}	2.36 ±0.3 ^{b}
Pb	ND	ND	ND	ND
Zn	6.65 ±1.4 ^{a}	8.03 ±1.1 ^{ab}	8.13 ±0.5 ^{ab}	9.66 ±0.9 ^{b}
Ni	ND	ND	ND	ND

ND: not detectable; Values at the same line followed by the same letter are not significantly different as determined by Duncan's test ($p<0.05$).

The concentration of Cu and Ni increased insignificantly with the added rates, whereas a significant increase of the Zn concentration was observed upon application of municipal solid waste compost. The addition of the two rates (C_1 and C_2) increased the Zn concentration to 16.33 and 22.11 mg kg^{-1}, respectively, from 9.7 mg kg^{-1} in the control soil.

In the roots, the application of municipal solid waste compost increased the concentrations of Cu, Zn, Cd and Ni significantly. Only, Pb increased insignificantly after the incorporation of compost. Compared to the control, application of the highest rate (C_2) increased the concentration of Cu, Zn, Cd, Pb and Ni by 60%, 106%, 101%, 136% and 55%, respectively. These results showed that the heavy metals were rather accumulated in the roots than in the straw or the grains.

Zhang et al., (2000) reported that the long-term application of municipal solid waste compost increased Cu and Zn concentrations in the wheat and barley crops cultivated in less productive farming soils. Walker et al., (2004) mentioned that the application of cow manure in soil contaminated by mine wastes reduced shoot concentrations *Chenopodium album* for all metals measured, especially Cu, Zn and Mn. Merrington et al., (1997) showed that the concentrations of Cd and Zn in the shoots and the concentration of Zn in the ears of wheat grown on the sewage sludge amended soils were dependent on sewage sludge application rates. The bioavailability and accumulation of Cd and Zn within the wheat plants depended upon many soil and plant factors, including the presence of other cations in soil solution, formation of organic chelates in root tissue, nutritional status of the phloem, genotypic differences and transpiration rates (Alloway, 1995; Merrington et al., 1997).

Lavado et al., (2007) reported that the application of a biosolid caused a significant accumulation of Pb and Zn in wheat shoots and Cu and Zn in grains of wheat grown on plots receiving massive doses. Jin Qian et al., (1996) reported that the roots of wheat accumulated and retained Pb, Ni and Co, but that only small portions reach the shoots. However, the concentration of Cu in the roots did not seem to be much different from those in shoots. The transfer of Cu to shoots was not due to the particularly weak binding of Cu in the soil, since Cu was bound strongly to soil organic matter, but rather to the function of the roots. In fact, Cu is an essential trace element and plays a key role in several metabolic processes for the plants (Clemens, 2001). Moreover, Greger and Löfstedt, (2004) found that wheat roots had 8 to 36 times higher Cd level, compared to the grains. No correlation was found between the uptake of Cd via the roots and the Cd concentration in grains on a short and longer term.

4.4. EDTA-extractable concentrations of heavy metals in the soils

The fraction of heavy metals extracted by EDTA solutions provides an estimate of the total pool of potentially available species (Lund, 1990). EDTA is a strong chelator which is able to compete with most inorganic and organic ligands contained in samples (Aballino et al., 2002). As a result, it can extract exchangeable metals, adsorbed metals and metals precipitated as carbonates (Singh et al., 1996). The EDTA-extractable metal content in a soil has been reported to be the principal factor predicting its concentration in plants (Rattan et al., 2005).

The addition of municipal solid waste compost increased insignificantly the EDTA extractable Cd concentration compared to the control and manure amended soil (Table 2). The EDTA-extractable Cd concentration increased from 0.238 mg kg^{-1} in the control treatment to 0.305 and 0.309 mg kg^{-1} on 40 and 80 t ha^{-1} amended plots, respectively. Similar results were reported by Lavado et al., (2007). The Cu concentration increased significantly after the application of the municipal solid waste compost. It ranged from 16.4 mg kg^{-1} in the control treatment to 24.4 mg kg^{-1} in the 80 t ha^{-1} municipal solid waste compost amendment, while there was no significant ($p<0.05$) difference between the C_1 treatment (21.0 mg kg^{-1}) and the C_2 treatment (24.4 mg kg^{-1}). Mohammad and Athamneh, (2004) mentioned that the allocation of composted sewage sludge up to 80 t ha^{-1} significantly increased the EDTA-extractable Cu concentration, but they did not observe a significant difference between rates of 80 t ha^{-1} and 160 t ha^{-1}. Jordao et al., (2006) reported that a mixture of urban solid waste composts (70 t ha^{-1}) from Rio de Janeiro and Coimbra in Brazil with a clayish oxisol increased the available Cu concentration almost three times in the substrate as compared to the control soil, after 30 days of incubation in a pot.

The repetitive addition of municipal solid waste compost since 1999 also increased the EDTA-extractable Pb concentration compared to the control treatment. EDTA extractable Pb increased from 13.7 mg kg^{-1} in the control treatment to 22.1 and 26.5 mg kg^{-1} at the C_1 and C_2 rates, respectively. There was no significant difference between C_1 and C_2. Jordao et al., (2006) reported that the application of the compost of Rio de Janeiro increased the available Pb concentrations in the soil by six times, as compared to the control.

The available Zn concentration increased from 5.0 mg kg^{-1} in the control soil to 23.6 and 40.2 mg kg^{-1} in the C_1 and C_2 treatments, respectively, while there was only a significant difference between C_2 and the other treatments (Table 2). Lavado et al., (2007) mentioned that the application of biosolids only significantly ($p<0.05$) increased the EDTA-extractable Cu and Zn concentrations. Mohammad and Athamneh, (2004) stated that the application of 60 t ha^{-1} of composted sewage sludge on a clay loam soil increased the soil extractable Zn concentration by 27%.

The addition of municipal solid waste compost slightly increased the EDTA-extractable Ni and Cr. The concentration of Ni increased from 0.889 mg kg^{-1} in the control soil to 1.07 mg kg^{-1} in the C_2 treatment. The EDTA-extractable concentration of Cr increased from 0.649 mg kg^{-1} in the control soil to 0.717 mg kg^{-1} after the application of 80 t ha^{-1} of municipal solid waste compost.

4.5. Correlation

Pearson correlation analysis was performed in order to investigate the relationships between the EDTA extractable metals, the total metal contents in the soils and the concentration of the heavy metals in the different wheat plant parts. Table 5 shows the correlations between the total and EDTA extractable heavy metal concentrations in the soils on the one hand and the content of heavy metals in the roots, straw and grains of the wheat plants on the other hand.

The total content of the heavy metals in the soils did not correlate significantly with the concentration of the heavy metals in the different plant parts, except for Cu. Similar results were obtained by Jin Qian et al., (1995) and Mc Bride et al., (1997), who indicated that the total metal content in soils is a poor indicator of bioavailability. Lavado et al., (2007) however found some strong correlations ($p<0.001$) between the soil and heavy metal concentrations in maize and wheat, but only for the micronutrients (Mn, Cu and Zn).

Significant correlations ($p<0.05$) were however found between EDTA extractable heavy metals and the concentrations of Cu, Zn and Ni in the wheat roots. A positive correlation existed between the EDTA extractable concentrations of Cu and Zn in the soil and the Cu and Zn concentrations in the roots (r = 0.978* and r = 0.983*, respectively), whereas a negative correlation was found for Ni (r = -0.956*).

In turn, a strongly significant, positive correlation was obtained between the EDTA extractable Ni concentration in the soil and the Ni concentration in the straw (r = 0.994*). The correlation between EDTA extractable Cu and Zn contents and the concentration of Cu and Zn in the wheat grains were high, but not significant at $p <0.05$.

Table 4. Correlations between the total and EDTA extractable heavy metal concentrations in the soils on the one hand and the concentrations in the wheat plants on the other hand

Pearson correlation coefficient (r)	Roots	Straws	Grains
EDTA extractable HM in soils			
Cd	-0.116	ND	ND
Cu	0.978*	0.899	0.939*
Zn	0.983*	0.928	0.933
Ni	-0.956*	0.994**	ND
Pb	-0.890	ND	ND
Total HM content in soils			
Cd	0.464	ND	ND
Cu	0.974*	0.782	0.955*
Zn	-0.007	-0.118	-0.419
Ni	-0.782	0.604	ND
Pb	-0.434	ND	ND

ND*: not detectable*

Walker et al., (2004) observed significant correlations between Cu, Zn and Mn concentrations in the shoots and the $CaCl_2$-extractable concentrations of Cu, Zn and Mn in the soil. This $CaCl_2$-extractable fraction is considered to represent the soluble and easily exchangeable metals in the soil. Gupta and Sinha, (2006) reported that EDTA extraction was a better predictor for the availability of metals in sewage sludge amended soils, compared to DPTA, $CaCl_2$, NH_4NO_3 and $NaNO_3$ extraction. Contrary, Pneyo et al., (2004) found that $CaCl_2$ extractions procedures were the most suitable for assessing the plant availability of Cd, Zn, Cu and Pb in contaminated soils. Nyamangara and Mzezewa, (1999) stated that the use of EDTA extractable heavy metals gives the best indication for the plant available fraction in a long term sewage sludge amended clay loam soils.

5. Conclusion

The present study focused on investigating the effects of long-term supply of Tunisian municipal solid waste compost, farmyard manure and mineral fertilizers to a Tunisian agricultural soil under natural conditions, on the grain yield of wheat and the distribution of heavy metals in the soils and plants. All amendments added yearly during five consecutive years ameliorated significantly the grain yield. However, only the municipal solid waste compost caused an increase of the heavy metal accumulation in the wheat crops and its EDTA-extractable contents in the soil, especially when applied in high doses.

This illustrates that the municipal solid waste compost can be used in agriculture without risks for animal and human health. Nevertheless, a strict regular monitoring of these organic amendments is needed in order to minimize the risk of environmental accumulation of the heavy metals.

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