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Variation of soil properties with sampling depth in two different light-textured soils after repeated applications of urban sewage sludge

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ABSTRACT

Semi-arid agricultural soils have increasingly been subjected to urban sewage sludge (USS) applications due to accelerated soil depletion and shortages in manure supply. Research studies addressing USS reuse have mostly been conducted in cropping systems and focused on changes in topsoil properties of a given texture. Therefore, sludge-soil interactions could be largely influenced by the presence of plants, soil particle composition and depth. In this field study, two agricultural soils (sandy, S and sandy loam, SL) received simultaneously four annual USS applications of 40, 80, and 120 t ha^{-1} $year^{-1}$ in absence of vegetation. Outcomes showed the increase of carbon and macronutrients in both soils proportionally to USS dose especially in the topsoil profile (0–20 cm). Subsoil (20–40 cm) was similarly influenced by sludge rates, showing comparable variations of fertility parameters though at significant lower levels. The depth-dependent improvement of soil fertility in both layers enhanced the microbiological properties accordingly, with significant variations in soil SL characterized by a higher clay content than soil S. Besides, positive correlations between increases in sludge dose, salinity, trace metals, and enzyme activities in both soils indicate that excessive sludge doses did not cause soil degradation or biotoxic effects under the described experimental conditions. In particular and despite high geoaccumulation indices of Ni in both soils and profiles, the global concentrations of Cu, Ni, Pb, and Zn were still below threshold levels for contaminated soils. In addition, the maintenance of pH values within neutral range and the increase of organic matter content with respect to control would have further reduced metal availability in amended soils. Therefore, we could closely investigate the effects of texture and depth on the intrinsic resilience of each soil to cope with repetitive USS applications.

1. Introduction

Arid and semi-arid regions are particularly prone to a major environmental concern known as topsoil erosion and degradation (Jemai et al., 2013a; Zoghlami et al., 2020). By depleting the most fertile topsoil, erosion and crop intensification both reduce agricultural soil productivity and biodiversity. However, several studies have shown that organic materials increase the amount of soil organic matter (SOM), macro and micronutrients, which are essential for the improvement of soil physico-chemical properties and fertility (Börjesson and Kätterer, 2018; Hamdi et al., 2019; Hechmi et al., 2020). Without adequate chemical and biological fertility that keeps croplands functional and productive, the agricultural ecosystem cannot function (Melo et al., 2018). In semi-arid Mediterranean countries, a growing interest has been directed towards the reuse of urban sewage sludge (USS) as an organic amendment because it represents a cost-effective source of organic carbon and nutrients that can counteract soil depletion and shortages in conventional farm manures (Melo et al., 2018; Bastida et al., 2019). In spite of the benefits resulting from USS valorization in agriculture, long-term or mismanaged land application may lead to environmental hazards because of its complex composition as a by-product of wastewater treatment (Hamdi et al., 2007a; Healy et al.,

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2016; Lajayer et al., 2020). Detrimental effects include risks of soil acidification and salinization (Hamdi et al., 2007a; Tella et al., 2016) as well as chemical and biological contamination (Miranda et al., 2018; Zoghlami et al., 2018), which could negatively affect soil properties (Hamdi et al., 2007a; Shahid et al., 2018) and crop yield and quality (Mbarki et al., 2018). In this regard, one of the most addressed issues related to sludge agricultural reuse is the accumulation of heavy metals in amended soils and their possible transfer to humans via the food chain (Lajayer et al., 2019; Eid et al., 2019). Besides, changes of amended soil properties depend not only on sludge quality, dose and amendment frequency but also on pedo-environmental conditions (Hamdi et al., 2019; Tlili et al., 2019; Hechmi et al., 2020). For instance, Hoseini and Delbari (2015) explained that light-textured soils are generally well drained and allow for easy leaching of soluble salts to deeper profiles when enough water is available. Accordingly, Hamdi et al. (2007a) noticed that prolonged water stress conditions increases the risks of salinization in USS-amended soils to alarming levels.

Most of studies related to the agri-environmental impact of organic amendments including USS have focused on variation of topsoil properties (~0–20 cm) in cropping systems, probably because it corresponds to the incorporation depth of organic materials (Kończak and Oleszczuk, 2018; Hamdi et al., 2019), and the zone of high rhizosphere activity in water-saving irrigation systems (Mahgoub et al., 2017). However, changes in deeper profile should be considered for investigation purposes because it is also considered as a zone of activity for deep-rooted crops especially in light-textured soils (Gregory et al., 2016). Therefore, changes in subsoil properties are also important to estimate the vertical transfer of sludge-borne compounds and the available nutrient stock for plant growth and microbial activity (De Melo et al., 2018). Nevertheless, the variation of soil parameters with depth could be significantly influenced by the presence of plants, which may mask the intrinsic capacity of soils to interact with applied materials. In this regard, soil texture and climatic conditions are very important abiotic factors that control the vertical variation of soil parameters in a given region as well (Amlal et al., 2020; Nguyen et al., 2020). Accordingly, the current field study was conducted on two different light-textured soils (sandy and sandy loam) treated annually with USS for 4 years at the equivalent rates of 0, 40, 80 and 120 t ha^{-1} year^{-1} in absence of natural or cultivated plants. The accumulation of SOM, nitrogen (N), phosphorus (P), and potassium (K) was monitored in two soil profiles (0–20 and 20–40 cm) to gain a clear understanding of soil fertility variation with depth as influenced exclusively by soil texture under a semi-arid Mediterranean climate. To determine the extent of trace metal contamination in both layers, a geo-accumulation index was calculated for copper (Cu), zinc (Zn), lead (Pb), and nickel (Ni). This index compares actual concentrations to original contents in soils prior to any anthropogenic activity (Tendaupenyu and Magadza, 2019; Amaro-Espejo et al., 2020). Because of the sensitivity of microorganisms to soil contamination and/or physical degradation, we also investigated heterotrophs and enzyme activities in both soils. In general, the current study was based on the following hypotheses: i) the agricultural valorization of USS in semi-arid regions can alternatively improve depleted soil properties, ii) the sand fraction increases soil porosity and influences the accumulation and vertical partition of sludge-borne chemicals, iii) soil parameters are largely influenced by texture and climatic conditions especially in non-irrigated systems, and iv) maintaining amended soils consistently unvegetated would closely highlight intrinsic sludge-soil interactions by avoiding plant effects on soil properties.

2. Materials and methods

2.1. Soil and sludge characterization

The field study was carried out at an agricultural experiment station in the outskirts of the city of Nabeul, Tunisia (36° 27′ 15″ N, 10° 44′ 5″ E). The region is characterized by a south semi-arid Mediterranean climate with moderate annual rainfall (~370 mm), cool wet winters, and prolonged hot dry summers. Two close experimental plots of 400 m^{2} each were chosen according to differences in soil texture namely, a sandy soil (named S) and a sandy loam soil (named SL). Both soils are slightly alkaline and dominantly sandy but soil SL has higher specific surface area (27 m^{2} g^{-1}) and clay fraction (~11.7 %) composed mostly of illite and kaolinite (Hamdi et al., 2019). Besides, both soils are characterized by low organic matter and nitrogen contents typical for light-textured soils of the south Mediterranean region (Table 1).

Table 1
Major physico-chemical properties of experimental soils and urban sewage sludge.

	Soil S		Soil SL		Urban sewage sludge
Sampling depth (cm)	0–20	20–40	0–20	20–40	–
Sand (%)	83.3	78.5	70.9	71.7	–
Silt (%)	11.5	16.3	17.2	16.8	–
Clay (%)	5.2	5.2	11.9	11.5	–
SSA (m^{2} g^{-1})	13		27		–
pH	7.24	7.77	7.72	7.82	7.73
EC (μS cm^{-1})	119	119	155	167	992
TOC (%)	0.67	0.65	0.76	1.75	16.4
SOM (%)	1.15	1.12	1.31	3.01	28.2
N (%)	0.1	0.08	0.07	0.12	1.05
C:N	6.7	8.1	10.8	14.6	15.6
P total (mg kg^{-1})	275	150	315	250	2200
P inorganic (Pi) (mg kg^{-1})	17.5	16.1	14.1	8.3	537
K^{+} (mg kg^{-1})	84.4	44	58.8	18.1	750
Na^{+} (mg kg^{-1})	8.0	8.4	19.6	26.4	1010
Total Pb (mg kg^{-1})	16.2	14.3	16.5	14.5	22.2
Total Ni (mg kg^{-1})	0.58	0.3	0.44	0.17	22
Total Zn (mg kg^{-1})	5.9	2.6	2.5	1.6	348
Total Cu (mg kg^{-1})	1.4	0.5	1.0	0.7	130.2

All parameters are given on dry weight basis.

For amendement trials, the urban sewage sludge (USS) had annually been collected from the drying ponds of a wastewater treatment plant of the same region. Before soil application, it was further air-dried and crushed at the experimental site to reduce water content and ensure an adequate mixing with soil. According to Table 1, this aerobically digested sludge has relatively high OM (28.2 %) and macronutrient (N. P.K) contents but also significantly higher salinity levels (EC = 992 μS cm^{-1}; Na^{+} = 1010 mg kg) than both agricultural soils. The concentration of trace metals (Pb, Ni, Zn, and Cu) is characteristic of urban sludges but complies with Tunisian guidelines (NT 106.20) for agricultural reuse (INNORPI, 2002).

2.2. Experimental design and sample collection

Field trials were carried out between 2012 and 2018 based on repetitive USS amendments performed annually during the fall season (October–November). Four treatments were chosen according to sludge application rates and named as follows: C (control without sludge), USS-40 (40 t ha^{-1} year^{-1}), USS-80 (80 t ha^{-1} year^{-1}), and USS-120 (120 t ha^{-1} year^{-1}). For each soil type, treatments were conducted in quadruplicate on 4-m^{2} elementary plots separated from each other by a pathway of 2 m (Figs. S1 and S2, supplementary material). For each USS-amended soil, the corresponding sludge amount was first uniformly spread on soil surface then incorporated by hand hoeing up to 10 cm depth. Control soil surface was hand hoed similarly without sludge addition. Throughout this long-term field study, emerging weeds had been removed manually when required to keep plots consistently bare and avoid the effect of plant roots on soil conditions. Consequently, any changes in soil properties would exclusively highlight the combined effect of sludge dose and natural pedo-climatic conditions.

The outcomes described in this study represent the variation of soil properties after four annual sludge amendments. To this end, three soil

Table 2
Variation of physico-chemical properties with sampling depth in soil S and SL treatments.

Treatments	C			USS-40			USS-80			USS-120		
Depth (cm)	0–20	20–40	% R_{sub}[a]	0–20	20–40	% R_{sub}	0–20	20–40	% R_{sub}	0–20	20–40	% R_{sub}
Sandy soil (S)												
pH	7.8 d A	7.66 d A	*−1.8*	7.67 c A	7.57 c A	*−1.3*	7.56 b A	7.43 b A	−1.7	7.54 a A	7.42 a A	*−1.6*
EC (μS cm^{-1})	160 a A	179 a B	*+11.9*	212 b A	245 b B	*+15.6*	240 c A	268 c B	+11.7	315 d A	344 d B	*+9.21*
TOC (%)	0.84 a B	0.53 a A	*−36.9*	1.4 b B	1.07 b A	*−23.6*	2.03 c B	1.52 c A	−15.9	2.28 d B	1.73 d A	*−24.1*
N (%)	0.05 a B	0.041 a A	*−18*	0.063 b B	0.051 b A	*−19*	0.086 c B	0.062 c A	−27.9	0.12 d B	0.086 d A	*−28.3*
P (mg kg^{-1})	262 a B	181 a A	*−30.9*	299 b B	264 b A	*−11.7*	339 c B	301 c A	−11.2	359 d B	324 d A	*−9.7*
Pi (mg kg^{-1})	14.4 a B	13.3 a A	*−7.6*	15.1 b B	14.2 b A	*−6.0*	16.1 c B	15.1 c A	−6.2	17.02 d B	16 d A	*−5.6*
K (mg kg^{-1})	2.3 a B	1.37 a A	*−40.4*	4.8 b B	2.06 b A	*−57.1*	7.75 c B	4.05 c A	−47.7	10.4 d B	6.3 d A	*−39.4*
Sandy loam soil (SL)												
pH	7.42 d A	7.31 d A	*−1.48*	7.33 c A	7.26 c A	*−0.95*	7.26 b A	7.2 b A	*−0.82*	7.18 a A1	7.10 a A	*−1.4*
EC (μS cm^{-1})	276 a A	310 a B	*+12.3*	313 b A	352 b B	*+12.5*	358 c A	389 c B	*+8.7*	483 d A	508 d B	*+5.2*
TOC (%)	0.9 a B	0.6 a A	*−33.3*	1.58 b B	0.89 b A	*−43.6*	2.04 c B	1.2 c A	*−41.2*	2.32 d B	1.52 d A	*−34.5*
N (%)	0.07 a B	0.055 a A	*−21.4*	0.083 b B	0.062 b A	*−25.3*	0.1 c B	0.082 c A	*−18.0*	0.13 d B	0.11 d A	*−15.4*
P (mg kg^{-1})	313 a B	249 a A	*−20.4*	339 b B	281 b A	*−17.1*	361 c B	333 c A	*−7.7*	401 d B	354 d A	*−11.7*
Pi (mg kg^{-1})	12.6 a B	10.2 a A	*−19*	14 b B	11.7 b A	*−16.4*	14.7 c B	13.9 c A	*−5.4*	15.8 d B	14.8 d A	*−6.3*
K (mg kg^{-1})	4.55 a B	2.41 a A	*−47*	6.65 b B	3.65 b A	*−45.1*	8.85 c B	5.6 c A	*−58.0*	11.4 d B	7.9 d A	*−30.7*

Numbers associated to treatment names represent the dose of urban sewage sludge (USS) added annually to elementary plots in equivalent tons per ha per year. C: control (unamended) soil. For each soil type and depth, means of each soil parameter with the same lowercase letters are not statistically different at $P \leq 0.05$. For each soil type and treatment, parameter means for 0–20 and 20–40 cm with the same uppercase letters are not statistically different at $P \leq 0.05$.

[a] R_{sub} (%) represents the vertical variation of each soil parameter with respect to its topsoil value (0–20 cm).

subsamples were collected from each elementary plot at 0–20 cm and 20–40 cm just prior to the following USS addition. These sub-samples were composited to one single sample per replicate treatment for each depth then taken promptly for characterization. Therefore, each soil treatment had four replicate samples (repetitions) for each depth and soil type.

2.3. Physico-chemical analysis

Physical and chemical analyses were conducted using air-dried soil samples sieved through 2 mm mesh. Soil pH and EC were determined respectively in (1:2.5) and (1:5) slurry. Total organic carbon (TOC) was analyzed by dichromate oxidation method according to Walkley and Black (1934). Total N was measured by the Kjeldahl digestion-distillation method (Bremner and Mulvaney, 1982). The same method was applied to determine total phosphorus (P) as described by Taylor (2000). Inorganic (available) phosphorus (Pi) was measured by the molybdenum blue method after extraction with 0.5 M sodium bicarbonate at pH 8.5 (Olsen, 1954). Potassium (K^+) was analyzed by flame spectrophotometry after soil extraction according to Pauwels et al. (1992). Total concentrations of four heavy metals namely, Pb, Ni, Cu, and Zn were determined by atomic absorption spectrometry after extraction with concentrated HNO_3–HCl (1:3) (Ben Achiba et al., 2010).

2.4. Variation of soil properties

The effect of sampling depth on physico-chemical properties was estimated by taking the soil surface layer (0–20 cm) as a reference group with respect to subsoil. Hence, we propose a variation ratio (R_{sub}) to express how much a given soil parameter increased (+) or decreased (−) with depth in relation to the applied treatment as follows:

$$\%Rsub = \frac{Q2 - Q1}{Q1}\ \boldsymbol{x}\ 100$$

where, $\%Rsub$ = percent variation of a given parameter in subsoil layer compared to topsoil
$Q1$ = numerical value of the soil parameter in the surface soil layer (0–20 cm)
$Q2$ = numerical value of soil parameter in the subsurface soil layer (20–40 cm)

Besides, a geoaccumulation index (I_{geo}) was calculated for each trace element in all soil treatments and both depths as follows (Wei et al., 2011):

$$Igeo = log_2\ \boldsymbol{x}\ (\frac{Cn}{Bn*1.5})$$

where, $Igeo$ = geoaccumulation index of the metal for a given depth
Cn = actual concentration of the metal
Bn = background concentration of the metal

In the current study, background metal concentrations in both soils were those measured before the start of sludge treatments (Table 1). The constant (1.5) compensates for natural fluctuations of a given metal and for minor anthropogenic impacts (Lizárranga-Mendiola et al., 2008). The calculated $Igeo$ for a given metal reflects the degree of contamination with respect to its background level, which could be interpreted as follows: uncontaminated ($I_{geo} \leq 0$); uncontaminated to moderately contaminated ($0 < I_{geo} \leq 1$); moderately contaminated ($1 < I_{geo} \leq 2$); moderately to heavily contaminated ($2 < I_{geo} \leq 3$); heavily contaminated ($3 < I_{geo} \leq 4$); heavily to extremely contaminated ($4 < I_{geo} \leq 5$); or extremely contaminated ($I_{geo} > 5$) (Huang et al., 2020).

2.5. Total heterotrophs and enzyme activities

Soil microbial parameters were determined using fresh soil samples promptly stored at −20 °C after field collection. The enumeration of total heterotrophic microorganisms was carried out using the plate-counting technique as described by Hamdi et al. (2019). Accordingly, 10 g of fresh soil were first mixed with 90 mL of autoclaved water and shaken for 30 min to release microorganisms from the soil matrix. Then, a serial dilution was prepared and 0.1 mL of the suspension was spread on PCA (plate count agar) and Sabouraud agar for bacteria and fungi cultivation, respectively. After incubation at 30 °C for 72 h, bacterial and fungal counts were expressed as colony-forming units (CFU) per g dry soil.

Three important soil enzymes were also investigated for their respective role in cycling the organic forms of carbon, nitrogen and phosphorus after incorporation of organic materials (Sherene, 2017). Dehydrogenase activity (DHA) was evaluated by monitoring the reduction of iodonitetrazolium (INT) to iodonitetrazolium formazan (INTF) at 490 nm after soil incubation with 2 mL of Buffer Tris solution and 0.5 of INT (2 mM) (Kumar et al., 2013). Protease activity (PROT)

Table 3
Pearson correlation coefficient matrix for sandy soil (S) properties.

	pH	EC	TOC	N	P	Pi	K	Pb	Ni	Zn	Cu	Bact.	Fungi	DHA	PROT	PASE
pH	–	−0.64	−0.75	−0.64	−0.68	−0.68	−0.67	−0.69	−0.60	−0.61	−0.64	−0.63	−0.45	−0.54	−0.54	−0.68
EC		–	0.92	0.96	0.91	0.95	0.95	0.94	0.97	0.92	0.93	0.89	0.85	0.93	0.84	0.97
TOC			–	0.90	0.95	0.95	0.93	0.91	0.93	0.83	0.87	0.94	0.83	0.89	0.80	0.95
N				–	0.88	0.96	0.97	0.94	0.93	0.93	0.93	0.95	0.81	0.95	0.80	0.96
P					–	0.93	0.90	0.91	0.90	0.91	0.85	0.92	0.79	0.84	0.78	0.91
Pi						–	0.96	0.97	0.94	0.93	0.93	0.96	0.84	0.96	0.84	0.96
K							–	0.96	0.93	0.91	0.91	0.95	0.84	0.96	0.77	0.98
Pb								–	0.91	0.93	0.92	0.97	0.97	0.98	0.96	0.99
Ni									–	0.88	0.90	0.92	0.99	0.96	0.97	0.98
Zn										–	0.91	0.95	0.91	0.98	0.98	0.98
Cu											–	0.96	0.95	0.97	0.98	0.96
Bact.												–	0.82	0.94	0.97	0.94
Fungi													–	0.98	0.96	0.98
DHA														–	0.96	0.98
PROT															–	0.99
PASE																–

was assayed by the method described by Ladd and Butler (1972). This method involves the colorimetric determination of tyrosine at 660 nm, produced by the microbial reduction of casein. To determine alkaline phosphatase activity (PASE), 1 g of soil was mixed with 1 mL of disodium phenyl phosphate (0.025 M) in citrate buffer at pH 6.4 and 0.2 mL of toluene before incubation for 24 h at 37 °C. The phenol released during the reaction was measured at 420 nm (Tabatabai and Bremner, 1969).

2.6. Statistical analysis

Data were analyzed by ANOVA with *post hoc* Duncan's multiple range test at $P \leq 0.05$ for mean separation to highlight the effect of the four treatments on each soil type and for each depth separately (STATISTICA 8.0, StatSoft Inc., Tulsa, USA). Student's *t*-test was also applied to compare two by two the variation of each soil parameter with sampling depth for each soil type. A Pearson product–moment correlation matrix ($P \leq 0.05$) was constructed to measure the strength of relationship (r) between the measured parameters in each soil.

3. Results and discussion

3.1. Soil fertility

Analytical results showed that the repeated applications of USS changed significantly soil fertility for both soils in a horizontal and vertical manner as illustrated in Table 2. More precisely, four annual amendments with increasing sludge rates significantly improved TOC and N.P.K contents in a dose-dependent manner (Börjesson and Kätterer, 2018; Hamdi et al., 2019). Consequently, the highest fertility level was observed in soils treated with the equivalent sludge rate of 120 t ha^{-1} year^{-1} while the unamended control treatment was the most deficient. For instance, TOC contents in the 0–20 cm profile reached 2.28 % in soil S and 2.32 % in soil SL compared to control soils (0.84 % and 0.90 %, respectively). Those observed in sub-soil samples showed similar proportionality with USS dose but with lower levels (Table 2). Accordingly, organic carbon accumulation in 20–40 cm reached significantly the highest concentrations of 1.73 % (soil S) and 1.52 % (soil SL) for the treatment USS-120.

Similarly, N, Pi, P, and K contents in topsoil S increased significantly with USS application rates from 0.063 %, 15.1, 299 and 4.8 mg kg^{-1} in USS-40 to 0.12 %, 17.02, 359, and 10.4 mg kg^{-1} in USS-120, respectively. The same trend of dose-dependent increase of macroelements was noticed in soil SL though with higher concentrations than soil S (Table 2). As observed for TOC, there was a significant vertical decrease of these elements in both soils. Fertility enhancement with sludge dose and repeated applications has previously been reported for the same field experiment after the second and the third USS application (Zoghlami et al., 2016; Hamdi et al., 2019). This is mainly attributed to the high and constant content of TOC in the used USS (16.4 %–18.5 %) as well as macronutrients. In addition, the moderate carbon-to-nitrogen ratio of the sludge (~15) indicates on its stability and allows for a rapid mineralization in both light-textured soils under the semi-arid conditions of the study region (Brust, 2019; Zouidi et al., 2019). This fact highlights the direct relationship between USS dose and macronutrient release, which was evidenced by strong correlations between TOC content and N, P, Pi, and K in both soils ($r \geq 0.90$) (Tables 3 and 4).

Rumpel and Kögel-Knabner (2011) pointed out that the topsoil

Table 4
Pearson correlation coefficient matrix for sandy loam soil (SL) properties.

	pH	EC	TOC	N	P	Pi	K	Pb	Ni	Zn	Cu	Bact.	Fungi	DHA	PROT	PASE
pH	–	−0.61	−0.89	−0.90	−0.87	−0.89	−0.94	−0.85	−0.92	−0.95	−0.87	−0.93	−0.86	−0.97	−0.98	−0.91
EC		–	0.91	0.95	0.91	0.85	0.95	0.91	0.94	0.94	0.93	0.95	0.96	0.97	0.97	0.96
TOC			–	0.93	0.93	0.93	0.96	0.91	0.93	0.90	0.92	0.93	0.84	0.99	0.99	0.96
N				–	0.92	0.88	0.94	0.90	0.91	0.91	0.94	0.93	0.92	0.97	0.98	0.98
P					–	0.92	0.94	0.91	0.92	0.87	0.95	0.92	0.87	0.97	0.99	0.97
Pi						–	0.92	0.89	0.90	0.87	0.89	0.88	0.81	0.98	0.99	0.96
K							–	0.89	0.98	0.93	0.95	0.96	0.91	0.99	0.99	0.99
Pb								–	0.87	0.87	0.90	0.92	0.88	0.97	0.99	0.99
Ni									–	0.91	0.94	0.93	0.92	0.96	0.94	0.87
Zn										–	0.87	0.94	0.91	0.94	0.97	0.87
Cu											–	0.94	0.92	0.99	0.98	0.85
Bact.												–	0.93	0.97	0.99	0.95
Fungi													–	0.88	0.94	0.97
DHA														–	0.97	0.88
PROT															–	0.95
PASE																–

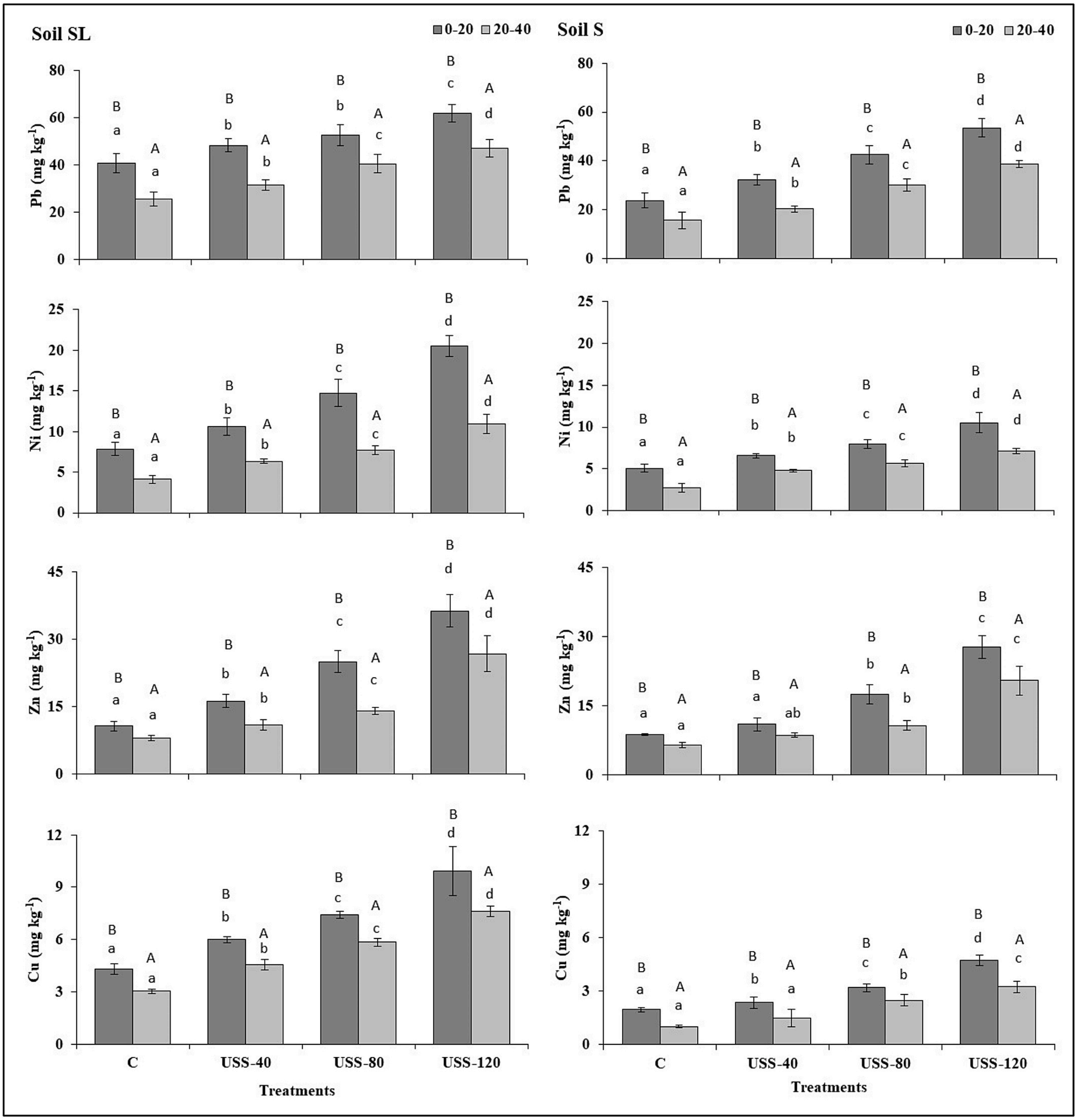

Fig. 1. Concentrations of Pb, Ni, Zn, and Cu in topsoil (0–20 cm) and subsoil (20–40) samples taken from sandy (S) and sandy loam (SL) soils. Numbers associated to treatment names represent the dose of urban sewage sludge (USS) added annually in equivalent tons per ha per year. C: control (unamended) soil. For each soil type and each depth, treatment means with the same lowercase letters are not statistically different at $P \leq 0.05$. For each treatment, depth means with the same uppercase letters are not statistically different at $P \leq 0.05$.

(0–20 cm) is generally believed to be the most active layer in terms of fertility-related parameters compared to deeper soil profiles. In this study, the consistent higher content of TOC and macroelements in the topsoil layer of both soils could be explained by the incorporation zone of sludge, which had repeatedly occurred in the upper 0–10 cm profile. Our results support earlier findings by Jobbágy and Jackson (2001) and Zhang et al. (2016) who observed an increase of C, N, P, and K in the surface layer (0–20 cm) and related it to the usual practice of adding organic/mineral fertilizers into the topsoil and/or to nutrient cycling by plants when present. Consequently, subsoil samples from both experimental plots had lower fertility potential reflected by negative R_{sub} ratios for all addressed parameters regardless to soil treatment. For instance, TOC variation ratios for C, USS-40, USS-80, and USS-120 were respectively −36.9 %, −23.6 %, −15.9 %, and −24.1 % for soil S; −33.3 %, −43.6 %, −41.2 %, and −34.5 % for soil SL (Table 2). Nutrient leaching to deeper profiles could be limited by the soil adsorption capacity in the accumulation zone and the dry semi-arid conditions deficient in rainfall (McLay et al., 1991; Moreno-Jiménez et al., 2020). Besides, the lack of vegetation cover throughout the experimental period would have accentuated evaporation rates from bare soil treatments (Meyer et al., 2019) and reduced soil macroporosity for nutrient leaching owing to the absence of root channels (Bodner et al., 2014; Wu et al., 2017).

The effect of soil texture on fertility levels was also obvious throughout this field study despite the little variation of particle composition between the two light-textured soils (Table 1). Börjesson et al. (2018) and Tlili et al. (2019) reported that a finer soil texture plays an important role in carbon storage and nutrient retention. In particular, the presence of clay minerals has been shown to increase crop production and organic carbon retention (Tahir and Marschner, 2016; Hamdi et al., 2019). Accordingly, higher TOC and macronutrient contents were consistently observed in the sandy loam soil treatments characterized by larger clay fraction and SSA than soil S (11.7 % and 27 m^2 g^{-1} against 5.2 % and 13 m^2 g^{-1}, respectively). Besides, the original soils had already different soil organic matter (SOM) content at the start of the experiment (1.13 % and 2.16 % for S and SL in 0–40 cm, respectively as shown in Table 1). As both experimental plots had lengthily been kept fallow before the implementation of the current experiment, this original SOM was likely to be humified (mature and stable) and slowly decomposing. Such properties could be also involved in nutrient retention and the increase of cationic exchange capacity by providing an extra negatively charged surface to the soil (Oorts et al., 2000; Paul, 2016). In particular, the higher content of clay particles in soil SL results in more clay-humic complexes than in soil S, which play very important roles in regulating the transport and retention of chemicals (Wang and Xing, 2005).

3.2. Soil pH and salinity

According to Table 2, a dose-dependent decrease of soil pH with USS was observed for both soils and sampling depths in parallel. This was reflected by negative correlations between pH and TOC content in soils S ($r = -0.75$) and SL ($r = -0.89$) as illustrated in Tables 3 and 4 Accordingly, the pH of USS-120 topsoils lost approximately 0.25 units and showed values as low as 7.54 in soil S and 7.18 in soil SL with respect to soil controls (7.8 and 7.42, respectively) or USS-40 (7.67 and 7.33, respectively). Subsoil samples showed the same gradual decrease with sludge dose reaching the lowest values of 7.42 and 7.10 for soils S and SL amended repetitively with the highest sludge rate equivalent to 120 t ha^{-1} $year^{-1}$, respectively (Table 2). Vašák et al. (2015), Tella et al. (2016), and Hamdi et al. (2019) also reported decreases in soil pH after sludge application with intensities depending largely on the experimental pedo-environmental conditions and management practices. Decreases of pH in amended soils could be mainly explained by the aerobic decomposition of the added organic materials and the subsequent release of byproducts having acidification effects such as H^+, CO_2, H_2CO_3, and NO_3^- (Stevenson, 1994; Hamdi et al., 2007a; Akbari et al., 2020). In particular, a significant dose-dependent increase of nitrate levels was also observed in both soils and depths (data not shown). Van Miegroet and Cole (1984) pointed out the occurrence of soil acidification resulting from the release and combination of H^+ and NO_3^- to form HNO_3 in N-enriched soils. Despite this significant dose-dependent decrease of soil pH on the long-term, pH values were still maintained within a neutral range for all treatments in both soils (Table 2). In addition to the slow acidification effect of the weak acids formed during SOM mineralization, the buffering capacity of soils could also play an important role in stabilizing soil pH and maintaining their structure from degradation (Hajnos, 2011).

In contrast to the observed pH-lowering effect of sludge addition, there was a significant increase of salinity levels in both soils with application rates (Table 2). Indeed, EC reached respectively the highest values of 315 μS cm^{-1} in soil S and 483 μS cm^{-1} in soil SL for treatment USS-120 at the surface layer (0–20 cm). The same significant dose-dependent increase of EC was observed at the subsoil level as well reaching 344 and 508 μS cm^{-1} in soils S and SL, respectively (Table 2). In this regard, the consistent higher EC levels in soil SL with respect to soil S for comparable treatments reflects a strong textural effect on salinity distribution (Setia et al., 2011). More precisely, it highlights a greater retention capacity of ions responsible for salinity owing to the presence of a higher clay fraction in soil SL compared to S (11.7 % vs 5.2 %) as illustrated in Table 1. Besides, strong correlation coefficients ($r >$ 0.90) were observed between EC and TOC for both soils indicating the direct involvement of sludge dose and repetitive applications in soil salinity increase (Tables 3 and 4). Urban sewage sludge has always been reported to affect soil salinity with salinization rates depending on sludge quality and experimental conditions (Cantrell and Linderman, 2001; Hamdi et al., 2007a; Hechmi et al., 2020). Soil salinity is mainly attributed to the presence of water-soluble ions in the soil solution namely, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, and SO_4^{2-} (Hernández Bastida et al., 2004; Shahid et al., 2018). On the other hand, positive variation ratios (R_{sub}) of CE in both soils indicate that there had been a vertical leaching of USS-borne salts and accumulation in deeper horizons over time (Table 2). Several studies confirmed that salt leaching might be accentuated in soils with a dominant sandy fraction and high porosities (Shaygan et al., 2018; Li et al., 2019). Light-textured soils are generally well drained and allow for easy vertical leaching of soluble salts after irrigation or under the action of rain as is the case for the current study (Li et al., 2018; Gelaye et al., 2019). As a matter of fact, a previous investigation on soils of the same agricultural experiment station showed an important mean surface infiltration rate of 15.5 mm h^{-1} at soil saturation level (Jemai et al., 2013b).

3.3. Trace metal accumulation

Four annual repetitive applications of USS increased the concentrations of Cu, Ni, Pb, and Zn proportionally to the applied dose, reaching therefore the highest levels in USS-120 treatments (Fig. 1). This significant dose-dependent increase was observed simultaneously for both soil types at 0–20 and 20–40 cm depths. However, these four trace metals had consistently higher concentrations in the topsoil layer, reaching respectively 4.7, 10.5, 53.5, and 27.7 mg kg^{-1} for soil S; 9.9, 20.5, 62, and 36.2 mg kg^{-1} for soil SL. Metals have historically been the most addressed contaminants in USS and the major concern that restricted the agricultural valorization of sludge (McGrath et al., 1994; Geng et al., 2020). Accordingly, guidelines on the safe reuse of USS include ubiquitously limits of heavy metal concentrations (Chang et al., 2002; Hudcová et al., 2019), and sometimes estimate the sludge dose that could be incorporated based on the maximum permissible metal concentrations in the receiving soil (USEPA, 1994; Hudcová et al., 2019). On the other hand, the textural effect was obvious as greater concentrations of these four heavy metals were consistently observed in soil SL compared to soil S (Fig. 1). The presence of a higher clay fraction composed of kaolinite and illite combined to negatively charged SOM chains and neutral soil pH are all conditions that increased the retention of sludge-borne metals in soil SL (He et al., 2000; Miranda-Trevino and Coles, 2003; Hamdi et al., 2019). As shown in Fig. 1, higher metal concentrations in S and SL topsoil samples indicate that Cu, Ni, Pb, and Zn were much less leachable than soluble salts due to stronger retention by the clay and SOM fractions (Correia et al., 2020). In any case, the highest measured concentrations of these metals after four sludge applications at 120 t ha^{-1} $year^{-1}$ remain below the regulatory limits for light-textured agricultural soils as stipulated by the European Council Directive 86/278/CEE (EUR-Lex, 2021). In addition, neutral soil pH values in all soil treatments would further reduce trace metal release in the soil solution. Consequently, immediate risks of metal bioavailability and toxic effects on biota are unlikely (Hamdi et al., 2007a; Król et al., 2020).

The geoaccumulation index (*Igeo*) was also calculated to estimate the actual degree of contamination by each trace metal with respect to its background level before treatments. In this regard, average *Igeo* calculations were made for the whole 0–40 cm layer as future risks of metal

Table 5
Variation of geoaccumulation index (*Igeo**) for Pb, Ni, Zn, and Cu in soil S and SL treatments.

Treatments	C	USS-40	USS-80	USS-120
		Sandy soil (S)		
$\boldsymbol{Igeo_{Pb}}$	−0.25 a	0.16 b	0.64 c	0.99 d
$\boldsymbol{Igeo_{Ni}}$	2.57 a	3.16 b	3.43 bc	3.79 c
$\boldsymbol{Igeo_{Zn}}$	0.36 a	0.73 b	1.22 c	2.02 d
$\boldsymbol{Igeo_{Cu}}$	0.15 a	0.57 b	1.16 c	1.64 d
		Sandy loam soil (SL)		
$\boldsymbol{Igeo_{Pb}}$	0.47 a	0.74 b	0.99 c	1.22 d
$\boldsymbol{Igeo_{Ni}}$	3.78 a	4.32 b	4.69 c	5.19 d
$\boldsymbol{Igeo_{Zn}}$	1.62 a	2.15 b	2.64 c	3.37 d
$\boldsymbol{Igeo_{Cu}}$	1.53 a	2.05 b	2.39 c	2.79 d

**Igeo* $\leq$ 0: uncontaminated; 0 < *Igeo* $\leq$ 1: uncontaminated to moderately contaminated; 1 < *Igeo* $\leq$ 2: moderately contaminated; 2 < *Igeo* $\leq$ 3: moderately to heavily contaminated; 3 < *Igeo* $\leq$ 4: heavily contaminated; 4 < *Igeo* $\leq$ 5: heavily to extremely contaminated; *Igeo* > 5: extremely contaminated. For each soil type and each trace metal, *Igeo* values of a given trace metal with the same lowercase letters are not statistically different at $P \leq 0.05$. Numbers associated to treatment names represent the dose of urban sewage sludge (USS) added annually to elementary plots in equivalent tons per ha per year. C: control (unamended) soil.

contamination could affect crops that are allowed to grow on sludge-amended soils (except leafy vegetables), and whose roots generally extend in this profile (USEPA, 1994; INNORPI, 2002). As shown in Table 5, geoaccumulation indices for each metal were proportional to USS dose and higher in SL treatments when compared to their analogs in soil S. These outcomes are in line with the variation of heavy metal concentrations illustrated in Table 2. According to the classification scale of geoaccumulation index, a soil is considered heavily polluted by a given metal X when $Igeo_X > 3$ (Huang et al., 2020). Consequently, Ni is likely to be the most contaminating element in both sludge-treated soils with higher risks in soil SL ($Igeo_{Ni} > 5$ in USS-120, Table 5). This shows that Ni accumulation is favored in both soils under the current experimental conditions even though its highest observed concentration in USS-120 of soil SL (20.5 mg kg^{-1}, Fig. 1) is below maximum permitted levels in soils (USEPA, 1994; EUR-Lex, 2021). In addition to very low solubility at pH > 6.7, Ni is an essential microelement for plants at very low concentrations (Chen et al., 2009), and its major routes for human toxicity have been revealed to be contaminated food (mainly vegetables), tobacco smoking, and to a lesser extent airborne particles (Das et al., 2019). Consequently, risks of future Ni bioaccumulation and food chain transfer remain low in this study due to unfavorable soil conditions but principally to the ban of vegetable cultivation on sludge-amended soils (INNORPI, 2002).

3.4. Microbiological parameters

Soil fertility enhancement with USS application rates had positive effects on the biological properties in both soils, especially in the surface profile (0–20 cm) (Figs. 2 and 3). For instance, total numbers of heterotrophic bacteria and fungi in soil S reached respectively 1.5 10^6 and 9.6 10^4 in USS-120 compared to soil control (0.65 10^6 and 4 10^4) (Fig. 2). Those in soil SL followed the same trend but with higher counts in all soil treatments. Mohammad (2015) related higher bacterial populations in finer soil particles (<2 μm) to nutrient availability and to the role of the clay fraction in protecting them against predation. Improvement of fertility parameters such as TOC, N, P, and K is a good indicator of soil quality and productivity because of their favorable effects on the physical, chemical and biological properties of soils (Miranda et al., 2018; Zoghlami et al., 2020). This fact was proved by strong correlations between the increase of microbial populations and the trophic levels in both soils (Tables 3 and 4). In addition, this dose-dependent improvement of microbial growth in both soils confirms

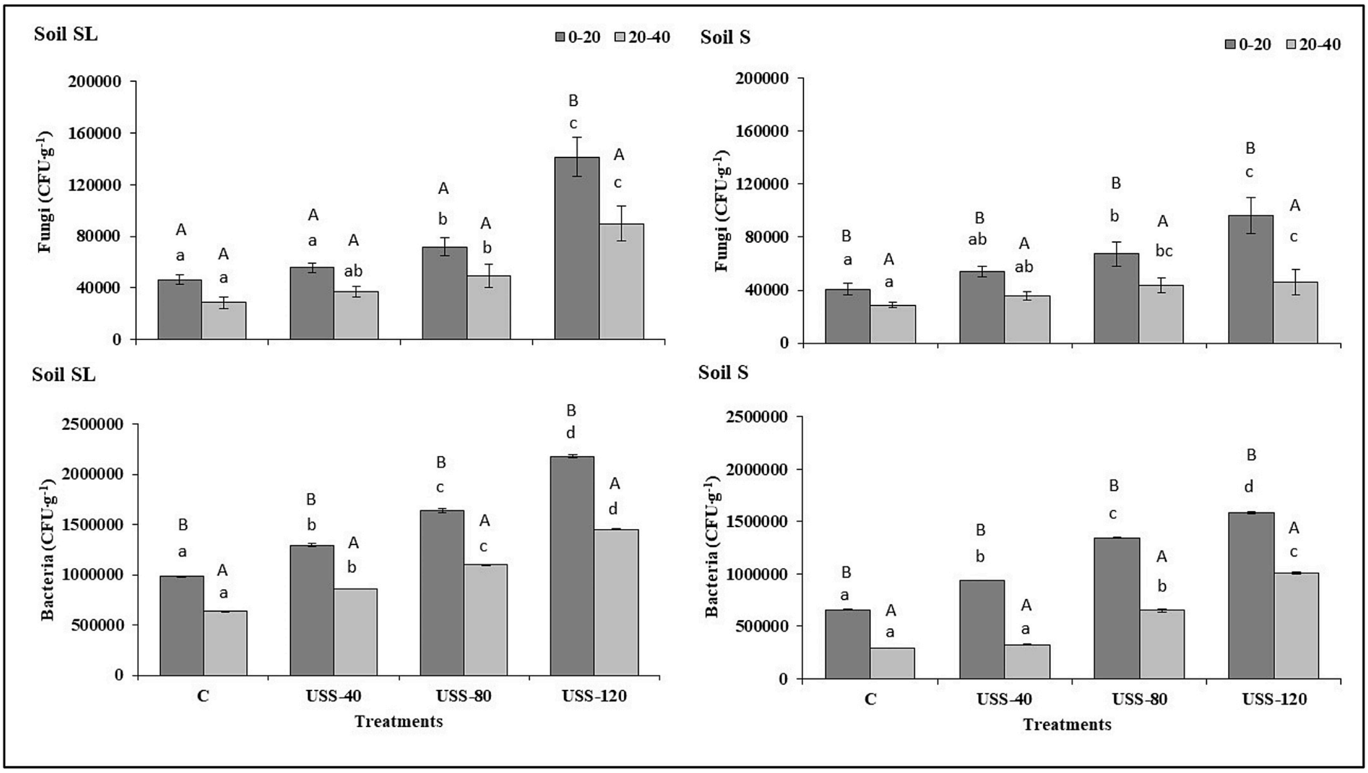

Fig. 2. Bacterial and fungal counts in topsoil (0–20 cm) and subsoil (20–40) samples taken from sandy (S) and sandy loam (SL) soils. Numbers associated to treatment names represent the dose of urban sewage sludge (USS) added annually in equivalent tons per ha per year. C: control (unamended) soil. For each soil type and each depth, treatment means with the same lowercase letters are not statistically different at $P \leq 0.05$. For each treatment, depth means with the same uppercase letters are not statistically different at $P \leq 0.05$.

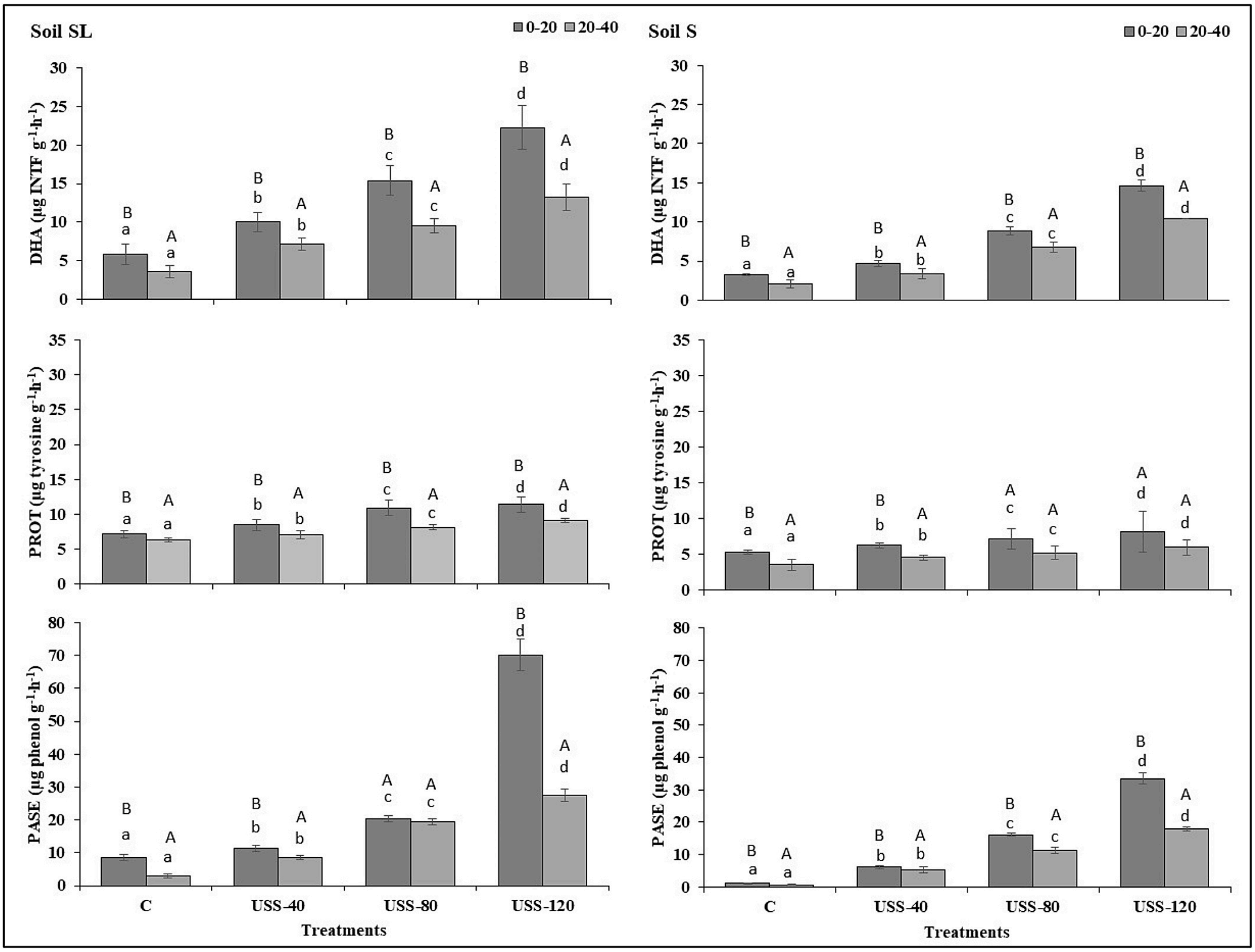

Fig. 3. Activities of dehydrogenase (DHA), protease (PROT) and phosphatase (PASE) in topsoil (0–20 cm) and subsoil (20–40) samples taken from sandy (S) and sandy loam (SL) soils. Numbers associated to treatment names represent the dose of urban sewage sludge (USS) added annually in equivalent tons per ha per year. C: control (unamended) soil. For each soil type and each depth, treatment means with the same lowercase letters are not statistically different at $P \leq 0.05$. For each treatment, depth means with the same uppercase letters are not statistically different at $P \leq 0.05$.

the absence of any unfavorable conditions after four successive sludge applications even at excessive rates of 80 and 120 t ha^{-1} $year^{-1}$. This was previously confirmed by similar variations of the same biological parameters after the second and the third application (Zoghlami et al., 2016; Hamdi et al., 2019). Consequently, the observed increases of soil salinity and heavy metal contents in both soils were still below levels that could hinder microbial proliferation under the described experimental conditions. In particular, previous studies have confirmed that metal toxicity to microorganisms is related to the concentration of the bioavailable fraction rather than the total concentration, which in turn can be controlled by soil physico-chemical properties as previously discussed (Gao et al., 2018; Hamdi et al., 2019). Due to the mineralization of the added sludge, these pollutants can be subjected to continuous processes of remobilization and repeated binding by newly formed organic structures, which could have affected their bioavailability and, at the same time, their toxicity (Hechmi et al., 2020). Moreover, Miranda et al. (2018) explained that the inhibitory effects of soil contaminants on soil biological activities could be also masked by the enhancement effects of the added organic carbon, which stimulates the microbial growth and enzyme activities. It is likely that the risks of sludge biotoxic effects in amended soils were significantly reduced by a combination of favorable pedological factors and sludge-borne growth promoters.

Numerous studies have suggested that enzyme activities are valuable indicators of soil "health" and quality (Alkorta et al., 2003; Sherene, 2017; Acosta-Martinez et al., 2018). In this study, soil enzyme activities were positively influenced by the addition of USS as well showing consistent increases with the applied dose (Fig. 3). As such, DHA, PROT and PASE increased significantly in topsoil S up to the highest activities of 14.6 µg INTF g^{-1} h^{-1}, 8.2 µg Tyr g^{-1} h^{-1} and 33.4 µg *p*-NP g^{-1} h^{-1}, respectively in USS-120 compared to unamended control (3.3 µg INTF g^{-1} h^{-1}, 5.4 µg Tyr g^{-1} h^{-1}, and 1.2 µg *p*-NP g^{-1} h^{-1} respectively). Following the same variation patterns observed for microbial counts and fertility levels, activities of the three enzymes were also higher in soil SL reflecting the significant role of soil texture. The incorporation of organic materials activates soil microorganisms to produce enzymes involved in the decomposition of organic matter and nutrient cycling (Hamdi et al., 2007b; Sherene, 2017). This fact was evidenced by strong correlations between DHA, PROT and PASE and microbial counts in both soils (r = 0.88–099) as shown in Tables 3 and 4 Moreover, the strong correlations between the PROT activity and nitrogen (r = 0.80 and 0.98 in soils S and SL, respectively) reflects generally an enhancement of the biological mineralization of N organic forms provided by USS applications (Zoghlami et al., 2016; Hamdi et al., 2019). In addition, the activity of PASE was significantly correlated to P content in soil, suggesting that sludge-borne organic P stimulates its biotransformation to more available forms (Sardans et al., 2006).

Because the top surface layer has been reported to have the highest concentration of organic matter, nutrients and microorganisms and is the zone where most of soil biological activity occurs, deeper soil horizons have received much less attention in biowaste reuse studies (Rumpel and Kögel-Knabner, 2011Hamdi et al., 2019). Zhang et al. (2016) connected these higher microbial activities to the surface activation effect that consists of favorable abiotic conditions such as nutrients, temperature, moisture, and aeration. Therefore, it appears rationale that lower activities in the 20–40 cm profile are in direct relationship with the attenuation of all these favorable factors. Nevertheless, subsoil microorganisms were still active and variations of DHA, PROT and PASE activities with respect to topsoil were not generally large (Fig. 3). This fact was probably explained by the importance of the trophic level in subsoil as much as in the topsoil. Liu et al. (2011) showed that nutrients increase in the upper layer (0–20 cm) can result in their translocation into subsoils (20–40 cm) and even into deeper profiles (up to 120 cm), confirming that nutrient availability and distribution features within a soil profile can have a strong control action on soil microbial and plant growth. In particular, light-textured soils with a large sand fraction like S and SL (> 70 %) facilitate nutrients vertical leaching that boosts microbial activities in depth when compared to clayey soils (Tahir and Marschner, 2017). For instance, the addition of inorganic phosphorus to activated sludge was shown to enhance enzyme activities responsible for the biodegradation of organic carbon and nitrogen (Zheng et al., 2019). In the current study, Pi distribution between 0-20 and 20–40 cm was generally characterized by low variations ratios ($\%R_{sub}$) especially in soil S (as low as −5.6 %), which may explain the important microbial activities in subsoil samples as well (Table 2).

4. Conclusions

Four annual urban sewage sludge applications improved organic matter contents in two semi-arid agricultural soils, and enriched surface and subsoil profiles with macronutrients in a dose-dependent manner. Despite the dominant sand fraction in both soils, a difference as little as 7 % of clay minerals content influenced significantly the variation of addressed parameters. In particular, the sandy loam texture with a higher adsorption potential resulted in greater organic matter and nutrient accumulation. Accordingly, soil biological activities were more significant with respect to the sandy texture. With exception made for soil salinity, the rest of soil properties showed negative variation ratios with depth testifying to the importance of topsoils as primary receiving environment for organic amendments. In addition, the dose-dependent accumulation of soluble salts and heavy metals in both soils did not inhibit essential microbial activities. As such, 4 annual applications of excessive sludge doses of 80 and 120 t ha^{-1} $year^{-1}$ were likely to enhance the quality of semi-arid agricultural soils without significant environmental impacts. However, it is worth mentioning that these observations should be interpreted in the pedo-climatic context of the study region, which have influences on future soil management and productivity. Consequently, the current project is still underway to monitor the long-term impact of repetitive USS applications and provide updated guidelines on the safe reuse of sludge in a more comprehensive way.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.113355.

Credit author statement

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