



## Effects of two composts and two grasses on microbial biomass and biological activity in a salt-affected soil



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### ARTICLE INFO

#### Article history:

Received 4 April 2013

Received in revised form 11 July 2013

Accepted 6 September 2013

Available online 10 October 2013

#### Keywords:

Composts

MSW compost

PW compost

Microbial biomass

Soil enzymes

Soil remediation

Saline soil

### ABSTRACT

The effectiveness of compost supply at several doses (0, 50, 100, and 150 t/ha) to a saline soil was studied using municipal solid waste (MSW) and palm waste (PW) composts. The experiment was carried out in pots under cultivation of *Polygomon monspeliensis* (halophyte forage species) and *Hordeum vulgare* (common forage species) and lasted three months. The investigation focused on some selected soil physico-chemical properties, soil microbial biomass, and ten soil enzymatic activities; Arylsulfatase (ARY), dehydrogenase (DEH),  $\beta$ -glycosidase ( $\beta$ -GLU), protease (PRO), urease (URE), invertase (INV), Fluorescein diacetate hydrolase (FDAH), catalase (CAT), acid and alkaline phosphatases (PHO). Both amendments improve markedly the saline soil quality. They ameliorate the physico-chemical properties. The increase of soil pH is regarded as an interesting fact and is usually proportional to the compost application rate. Electrical conductivity increased proportionally to the applied rates. Soil carbon and nitrogen amounts were also improved and the highest raise (7.5-folds) was noted for carbon. According to the substantial increase of the organic matter, levels of measured microbial biomass and several enzyme activities in saline soil were improved. DEH activity which proposed as a measure of overall microbial activity exhibited a significant increase only at dose 2 (100 t/ha). Consequently, One hundred tones of composts per hectare, under which some enzymes exhibited an optimal of activity and metal accumulation can be minimized, appeared an interesting rate for saline soil amendment.

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### 1. Introduction

Salt toxicity is one of three main effects of salt excess in the soil: osmotic effect, nutritional effect, and toxic (or specific) effect (Rafael, 2009). Apart from the natural salinization, human-induced secondary salinization occurs frequently mainly as a consequence of over irrigation caused by improper management of irrigation facilities, poor soil internal drainage condition. In general, two major approaches are used in reclaiming salinized soils: (i) accelerating soil desalinization process by leaching salts down the profiles, and (ii) enhancing the tolerance of the existing crop cultivars to salt stress coupled with breeding new salt-tolerant crop species (Liang et al., 1996). These measures are especially crucial to the sustainable agriculture in Tunisia.

The influence of salt as a major stress to soil microorganisms has been the subject of several studies (Mamilov et al., 2004; Pankhurst et al., 2001; Sardinha et al., 2003; Sarig and

Steinberger, 1994). A decrease in carbon dioxide (CO<sub>2</sub>) production, enzyme activities, or microbial biomass in soil has often been observed in the field (Pathak and Rao, 1998) and under laboratory incubations (Ghollarata and Raiesi, 2007; Rietz and Haynes, 2003; Wichern et al., 2006). Soil enzyme activities were found to decrease with increasing salinity but the degree of inhibition varied among the enzymes assayed and the amount of salt added (Frankenberger and Bingham, 1982; Sardinha et al., 2003; Wichern et al., 2006; Yuan et al., 2007). The same authors observed that dehydrogenase (oxydoreductase) activity was severely inhibited by salinity, whereas the hydrolases (amidase, urease, acid and alkaline phosphatase, phosphodiesterase, inorganic pyrophosphatase, rhodanase,  $\alpha$ -glucosidase, and  $\alpha$ -galactosidase) showed lower inhibition. In contrast, García and Hernández (1996) reported that the activity of hydrolases, such as protease,  $\beta$ -glucosidase, and phosphatase, were more negatively affected by salinity than oxydoreductases (dehydrogenase and catalase).

Increasing salinity thus has detrimental effects on biologically mediated processes in the soil, such as soil respiration (Ghollarata and Raiesi, 2007; Pathak and Rao, 1998). Despite, soil microorganisms have the ability to adapt or tolerate osmotic stress caused

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by salinity (Sparling et al., 1989; Wichern et al., 2006; Yuan et al., 2007).

Numerous physical, chemical, and biological approaches were established to reclaim salt-affected soils (Qadir et al., 2007; Wong et al., 2009; Rabhi et al., 2010; Feizi et al., 2010; Mokoï and Verplancke, 2010). The application of organic matter increases soil microbial biomass and some soil enzymatic activities such as urease, alkaline phosphatase and  $\beta$ -glucosidase (Lakhdar et al., 2008). Tejada and Gonzalez (2005) demonstrated that an increase in the organic matter content of saline soils increases soil microbial biomass. The role of compost in salt-affected soils is very vital because the organic source is ultimate opportunity to improve the physical properties of such soils which have been deteriorated to the extent that water and air passage become extremely difficult in such soils. Resultantly, the water stands on the surface of these soils for weeks long. The plants when grown under these conditions often die due to deficiency of root respiration. The compost can be a very good organic amendment in saline agriculture as well as for reclamation of salt-affected soils (Zaka et al., 2003).

The aim of this work was to study the effects of two different composts (municipal solid waste compost and palm waste compost) on saline soil reclamation. Soil physical, chemical and biological properties during a period of three months under cultivation of *Polypogon monspeliensis* (halophyte forage species) and *Hordeum vulgare* (common forage species) were emphasized.

## 2. Materials and methods

### 2.1. Saline soil, and municipal solid waste (MSW) and palm waste (PW) composts sources

The current investigation was conducted in pots and under green house conditions. The used saline soil is a loam-silt one (20% clay, 58% silt, and 22% sand), collected from salt-affected soil of Soliman (North-East Tunisia, 36°41'16"), dried and sieved at 2 mm.

Palm waste compost (PW) was obtained from composting station of Gabes (South Tunisia). Municipal solid waste (MSW) compost was collected from composting station of Beja (North Tunisia). It was mechanically produced by mixing weekly the waste heap under aerobic conditions by fast fermentation and aged 8 months before use. The main analytical characterization of the saline soil, PW and MSW were initially determined (Table 1).

### 2.2. Soil amendment and culture conditions

The saline soil was prior amended with different doses of MSW and PW compost (0, 50, 100 and 150 t/ha), and putted in 5 kg pots. After that, *P. monspeliensis* and *H. vulgare* seeds were sown. The pots were placed in a randomized complete block design with four treatments and three replicates, with minimum–maximum temperatures of 21–25 °C, 40–60% relative humidity and natural daylight with minimum–maximum light flux of 200–1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . All pots were irrigated when necessary with tap water to saturation and then drained freely to field capacity. Hand weeding was done to manage the weeds and no pesticides were applied. After 90 days, plants were harvested and fresh soil samples were collected for biochemical assays. Physico-chemical parameters were determined on an oven dried soil.

### 2.3. Physico-chemical properties determination

Physico-chemical analyses were performed on air-dried and sieved (<2 mm) soil samples according to standard techniques (Sparks, 1996), and they are shown in Table 1. According to USDA

(Soil Survey Staff, 1975), the soil was classified as a sandy clay loam soil (clay 20%, sand 45%, and silt 58%). Soil and organic residues pH were determined with a glass electrode pH meter in 1:2.5 soils to water ratio. Total nitrogen was determined by the Kjeldahl method as recommended by Brookes et al. (1985). Organic C content was determined by dry combustion (Walkley and Black, 1934). Heavy metal contents (Cu, Zn, Pb, and Cd) were determined by atomic absorption spectrophotometer after acid digestion (nitric acid and chloridric acid, 3:1 ratio) according to Pauwels et al. (1992). Three independent replicates were performed for each sample and blanks were measured in parallel.

### 2.4. Biological activity assays

Microbial biomass (MB) was determined by the chloroform fumigation–extraction method (Vance et al., 1987). The enzyme activities were determined on fresh, moist and sieved (<2 mm) soil. Substrates: *p*-nitrophenyl- $\beta$ -deglucoside and *p*-nitrophenyl-phosphate were used for determination of  $\beta$ -glucosidase (E.C. 3.2.1.21 [ $\beta$ -GLU]) and phosphatase (E.C.3.1.3.2 [PHO]), respectively. An aliquot (1 g) of soil was incubated with 5 ml of buffered substrate in reaction flasks for 1 h at 30 °C, under continuous stirring. Specific buffers and pH were used as reported in Sannino and Gianfreda (2001) and Eivazi and Tabatabai (1990). Enzymatic reactions were stopped by rapidly transferring the mixtures to a freezer and holding them there for 10 min. Concentrations of *p*-nitrophenol were determined at 400 nm after addition of NaOH and  $\text{CaCl}_2$  for PHO and Tris/NaOH buffer (pH 10.0) and  $\text{CaCl}_2$  for  $\beta$ -GLU.

For the determination of protease (E.C. 3.2.1.26 [PRO]) activity, 1 g of soil was treated with 1 ml Tris buffer (pH 8.0) and 0.5 ml toluene for 15 min, then 2 ml 1% (w/v) casein were added to, and the soil was placed in an incubator at 37 °C for 24 h. The aromatic amino acids (product) released were extracted with 3 ml trichloroacetic acid 15% (w/v). Five milliliters 0.4 M  $\text{NaCO}_3$  and 1 ml Folin–Ciocalteu reagent were added to 1 ml filtrate. After incubation at 37 °C for 15 min, the products were measured calorimetrically at 680 nm (Institute of Soil Science, Chinese Academy of Science, 1985; Xu and Zheng, 1986).

Catalase (E.C. 1.11.1.6 [CAT]) activity was measured using the titration method. Fresh soil (5 g) was placed at 0–4 °C for 30 min, with 25 ml 3%  $\text{H}_2\text{O}_2$  added, the samples were placed at 0–4 °C for 30 min again, before terminating the reaction with the addition of 25 ml (1 M)  $\text{H}_2\text{SO}_4$ . After filtration, 4 ml (0.5 M)  $\text{H}_2\text{SO}_4$  was added to 1 ml filtrate, using 20 mM  $\text{KMnO}_4$  to measure the  $\text{O}_2$  absorbed (Institute of Soil Science, Chinese Academy of Science, 1985; Xu and Zheng, 1986).

Urease (E.C. 3.5.1.5 [URE]) activity was measured using urea, and the ammonium released from soil was assayed calorimetrically at 460 nm (Kandeler and Gerber, 1988). Arylsulfatase (E.C. 3.1.6.1 [ARY]) activity was determined by the method of Tabatabai and Bremner (1970). Dehydrogenase (E.C. 1.1 [DEH]) activity was measured by mixing 1 g of soil with 1 ml of buffered tetrazolium salts (TTC) solution, according to Trevors (1984). Invertase (INV) (E.C. 3.2.1.26) activity was determined using the Stemmer et al. (1999) method. Fluorescein diacetate hydrolase (FDAH) was measured by the method of Greena et al., 2006. A fumigation–extraction method was used to estimate microbial biomass C (MB-C) with extractable C converted to microbial C using standard factor (Vance et al., 1987). Soil was fumigated with ethanol-free chloroform for 24 h. Fumigated and non-fumigated soil samples were then extracted with 0.5 M  $\text{K}_2\text{SO}_4$  for 30 min in an oscillating plane at room temperature, after filtration soil extracts were stored at 2 °C prior to analysis. Sub-samples of filtrates from both fumigated and non-fumigated

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