

# Short-term effects of sewage sludge application on phosphatase activities and available P fractions in Mediterranean soils

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## Abstract

The aim of this study was to investigate factors regulating phosphatase activities in Mediterranean soils subjected to sewage sludge applications. Soils originating from calcareous and siliceous mineral parent materials were amended with aerobically digested sewage sludge, with or without physico-chemical treatment by ferric chloride. Sludge amendments, ranging from 6.2 to 10 g kg<sup>-1</sup> soil, were carried out in order to provide soil with a P total quantity equivalent to 0.5 g P<sub>2</sub>O<sub>5</sub> per kg of soil. Bacterial density, phosphatase activities (i.e. acid and alkaline phosphomonoesterases and phosphodiesterases) and available P (i.e. P Olsen and P water) were measured after 25 and 87 days of incubation. Results showed significant effects of sewage sludge application and incubation period. Sewage sludge effect resulted in an increase in phosphatase activities, microbial density and available P. Incubation period increased available P while decreasing phosphatase activities. This study also revealed that the origin of sludge and its chemical characteristics may show different effects on certain variables such as phosphodiesterases or bacterial density, whereas mineral parent materials of soils did not show any significant effects.

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## 1. Introduction

Many soils in the Mediterranean region have been progressively degraded resulting in a decrease of their fertility (Pascual et al., 1998). Organic wastes such as sewage sludge have been extensively used in order to improve soil quality. Indeed, sewage sludge can enhance plant productivity as it contains a considerable amount of nutrient such as C/N/P and trace elements. Among the different macronutrients contained in sludge, phosphorus is an essential element for plant metabolism since it is present in numerous molecules such as phospholipids or nucleotides. According to Lima et al. (1996), only a small portion of sludge total P is in the inorganic form and can be assimilated by plants or microorganisms, while approximately 70% of P is in the organic form. To be assimilated, organic P present in sludge must be previously mineralized

into inorganic orthophosphate (PO<sub>4</sub><sup>3-</sup>) ions. Only enzymes produced by plants and/or microorganisms are able to hydrolyze organic P into phosphates. This process is catalyzed by phosphatase enzymes, which are found in sewage sludge, soil microorganisms, roots and in extra-cellular forms in soils. Phosphatases catalyse reactions leading to the hydrolysis of both esters and anhydrides of H<sub>3</sub>PO<sub>4</sub>. Among these enzymes, acid and alkaline phosphomonoesterases (E.C. 3.1.3.) and phosphodiesterases (E.C. 3.1.4.) are considered as the predominant phosphatases in most types of soil and litter (Tabatabai, 1994; Criquet et al., 2004). The activities of these phosphatases are influenced by various soil properties, soil organism interactions, vegetation cover, leachate inputs and the presence of inhibitors or activators (Juma and Tabatabai, 1977). According to Turner and Haygarth (2005), a clear understanding of such factors remains difficult, despite numerous attempts to relate phosphatase activities to P pools in soils. Indeed, although numerous studies have described phosphatase activities in soils, data are still

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lacking regarding microorganisms and abiotic factors implicated in the production and the regulation of phosphatase activities in soils. In addition, the use of sewage sludge as organic fertilizer is common practice in agriculture but may also alter soil microbial processes. Studies about the effects of sludge addition on soil biological properties have been numerous. Nevertheless, they have generally focused on one type of sludge and soil, and it appeared difficult to generalize these results to different sludges and different soils. Thus, the originality of the present study was to test simultaneously the effects of three different sludges on P turn-over of two soils with distinct properties (i.e. calcareous and siliceous origin). Our study was focused on the response of P mineralizing enzymes (i.e. phosphomonoesterases and phosphodiesterases) in Mediterranean soils following sewage sludge application. We aimed to demonstrate the relationships between density of culturable bacteria, phosphatase activities, and available P (i.e. P Olsen and P water) in soils devoid of vegetation receiving different sewage sludges (SS).

## 2. Materials and methods

### 2.1. Origins and sampling of the soils

The experimental sites were located in the South-east of France, in the county of Var, and are representative of the French Mediterranean region characterized by a sub-humid bioclimate (Criquet et al., 2002). The soils were sampled in two vineyards located at the “The Domaine de Pourcieux” (Pourcieux, France) and “Château des Launes” (La Garde Freinet, France). These soils originated from calcareous and siliceous mineral parent material, respectively, and their characteristics are given in Table 1. The soils were sampled on 28 January 2003 between vine rows, from the 0 to 10 cm layer, which were devoid of vegetation at sampling.

Table 1  
Physical and chemical characteristics of soils

| Characteristic <sup>a</sup>                   | Soil 1<br>(“Pourcieux”) | Soil 2 (“La Garde<br>Freinet”) |
|---|-------------------------|--------------------------------|
| Clay (%)                                      | 22.6                    | 12.9                           |
| Silt (%)                                      | 40.3                    | 20.0                           |
| Sand (%)                                      | 36.0                    | 67.0                           |
| pH (H <sub>2</sub> O)                         | 8.55                    | 6.9                            |
| Organic matter (g kg <sup>-1</sup> )          | 8.8                     | 11.83                          |
| Organic C (g kg <sup>-1</sup> )               | 5.12                    | 6.88                           |
| Total N (g kg <sup>-1</sup> )                 | 0.6                     | 2.31                           |
| P Olsen (g kg <sup>-1</sup> )                 | 0.18                    | 0.16                           |
| Total CaCO <sub>3</sub> (g kg <sup>-1</sup> ) | 319                     | 0                              |
| CEC Metson (cmol kg <sup>-1</sup> )           | 9.0                     | 7.5                            |
| Exchangeable K (mg kg <sup>-1</sup> )         | 208                     | 155                            |
| Exchangeable Mg (mg kg <sup>-1</sup> )        | 146                     | 135                            |

<sup>a</sup>Data refer to dry matter (105 °C) of surface soil (0–10 cm) sieved at <2 mm.

### 2.2. Origin and properties of SS

SS used during the experiment were purchased on 28 January 2003 from “Biotechna Company”, located at “Ensuès-la-Redonne” (Bouches-du-Rhône, France). Three types of SS (SS1–3) were collected: (SS1 and SS2) aerobically digested SS including anaerobic P and N treatments originating, respectively, from “Rognac” (Bouches-du-Rhône, France) and “La Palun” (Bouches-du-Rhône, France); (SS3) aerobically digested sewage sludge originating from Carry (Bouches-du-Rhône, France), including a physico-chemical treatment by ferric chloride in order to remove suspended materials and chemicals such as PO<sub>4</sub><sup>3-</sup> ions. None of these sludges were stabilized with lime. The characteristics of the different SS are given in Table 2.

### 2.3. Incubation experiment

For the incubation experiment, the soils were previously sieved (<2 mm) and thereafter mixed with the different SS. Organic amendments were carried out in order to provide to soil a P total quantity of 0.22 g kg<sup>-1</sup> by soil (equivalent to 0.5 g P<sub>2</sub>O<sub>5</sub>), a value which is coherent with that of Pote et al. (2003) and which represents a rate commonly used in the area. The different mixtures were distributed in 9 × 9 × 9 cm polypropylene pots and overlaid with perforated black plastic to avoid plant growth. Each pot contained 750 g of an individually prepared mixture and the experiments were carried out in triplicate. Distilled water was added to each soil mixture to bring it to 60% of its water holding capacity, and water content of each

Table 2  
Characteristics of sewage sludges

| Parameters <sup>a</sup>                                     | SS1                    | SS2                    | SS3                    |
|---|------------------------|------------------------|------------------------|
| pH (H <sub>2</sub> O)                                       | 6.8                    | 6.5                    | 6.7                    |
| Water content (g kg <sup>-1</sup> )                         | 855                    | 841                    | 765                    |
| Organic matter (g kg <sup>-1</sup> )                        | 717                    | 729                    | 612                    |
| Organic C (g kg <sup>-1</sup> )                             | 359                    | 364                    | 274                    |
| Organic N (g kg <sup>-1</sup> )                             | 54.4                   | 54.3                   | 16.2                   |
| C/N   | 6.6                    | 6.7                    | 16.9                   |
| Total P (g P <sub>2</sub> O <sub>5</sub> kg <sup>-1</sup> ) | 78.9                   | 80.2                   | 49.9                   |
| Cr (mg kg <sup>-1</sup> )                                   | 47.5                   | 29.4                   | 187.5                  |
| Cu (mg kg <sup>-1</sup> )                                   | 377.5                  | 227.8                  | 22.5                   |
| Pb (mg kg <sup>-1</sup> )                                   | 95.0                   | 35.6                   | 30.5                   |
| Zn (mg kg <sup>-1</sup> )                                   | 447.5                  | 301.1                  | n.d.                   |
| Pac activity (U g <sup>-1</sup> )                           | 2.1 × 10 <sup>-3</sup> | 1.7 × 10 <sup>-3</sup> | 9.6 × 10 <sup>-5</sup> |
| Pal activity (U g <sup>-1</sup> )                           | 1.3 × 10 <sup>-3</sup> | 8.4 × 10 <sup>-4</sup> | 1.4 × 10 <sup>-4</sup> |
| Pdi activity (U g <sup>-1</sup> )                           | n.d.                   | n.d.                   | n.d.                   |
| Quantity added (g kg <sup>-1</sup> soil) <sup>b</sup>       | 6.33                   | 6.23                   | 10.02                  |
| Equivalent quantity added (t ha <sup>-1</sup> )             | 28                     | 28                     | 45                     |

(SS) Sewage sludge: (1), (2), (3): aerobically digested; (3) physico-chemical treatment.

Pac: acid phosphatase; Pal: alkaline phosphatase; Pdi: phosphodiesterase; n.d.: not determined.

<sup>a</sup>Data refer to dry matter (105 °C).

<sup>b</sup>To obtain an equivalent amendment of 0.22 g P (0.5 g P<sub>2</sub>O<sub>5</sub>) kg<sup>-1</sup> of each soil.

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