

# Behaviour of enzymatic activities and root elongation in Argiudoll soils from the Argentine Humid Pampa treated with biosolids

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## ABSTRACT

The incorporation of biosolids to soil is a strategy aiming at the re-location of these materials in the environment with a useful end: soil fertilization. In this work, the response of two Argiudoll soils (one with more than 100 years of agriculture and the other, a virgin one) to biosolid incorporation was studied under laboratory conditions. To measure this response, soil enzymatic biodescriptors, such as dehydrogenase and urease activities, and tests related to plant physiology (the root elongation test) were employed. The addition of the biosolid to both soils had a stimulating effect though different on each soil according to the added dose. Adjustment of the regression line for dehydrogenase activity with root elongation was positive and statistically significant ( $p < 0.001$ ). Results suggest that biodescriptors employed were suitable for studying the impact of amended biosolids on different soils.

**Key words:** biosolids, soil dehydrogenase activity, soil urease activity, root elongation test

## RESUMEN

**Comportamiento de actividades enzimáticas y elongación de raíces en suelos Argiudoles de la Pampa Húmeda, Argentina, tratados con biosólidos.** La incorporación de biosólidos al suelo es una estrategia que tiene como objetivo la reubicación de estos materiales en el ambiente con un fin útil, como es la fertilización del suelo. En este trabajo se estudió, en condiciones controladas de laboratorio, la respuesta de dos suelos Argiudoles (uno con más de 100 años de agricultura y otro virgen) frente a la perturbación físico-química y biótica que genera la incorporación de un biosólido. Para medir esta respuesta se emplearon dos biodescriptores edáficos (las actividades deshidrogenasa y ureasa) y un tercero referido a la fisiología vegetal, la prueba de elongación de raíces. La incorporación del biosólido en ambos suelos, en general no deprimió el funcionamiento de las actividades enzimáticas estudiadas; contrariamente, según la dosis aportada tuvo un efecto estimulante, aunque diferente, entre ambos suelos. El ajuste de la recta de regresión de la actividad deshidrogenasa con la elongación de las plántulas fue positivo y altamente significativo, lo que indica la complementariedad de ambos descriptores. Los resultados obtenidos sugieren que los biodescriptores empleados resultaron aptos para estudiar el impacto que produce la incorporación de biosólidos a suelos agrícolas.

**Palabras clave:** biosólidos, actividad deshidrogenasa del suelo, actividad ureasa del suelo, elongación radicular

Soils under intensive agricultural exploitation suffer an important loss in their natural fertility. In Argentina, the fertile soils of the Humid Pampa have suffered an important degradation since agricultural introduction. In 80 years of continuous farming, soils from the Pergamino region have lost almost 45% of their original macro and micro-nutrients (2). Given this condition, the addition of organic waste products—such as biosolids—could constitute a useful practice to stop soil degradation and/or to recover part of the original fertility of these systems (1). Biosolids are solids that have organic material partially transformed by the microbial activity, so that they contain as many microorganisms as their remaining organic substances do, de-

pending on their source. They can, for instance, be obtained from mud of drying beds sewage sludge produced by plants of urban wastewater, or from remains of animal-breeding places. The use of biosolids in agricultural soils is not new; biosolids addition to degraded soils could contribute to their recovery through the stimulation that these biosolids can exert on soil microbial activities (4), especially on those microorganisms that take part in biogeochemical cycles closely related to plant nutrition, such as the carbon and nitrogen cycles (14). The measures of the metabolic activities carried out by different functional microbial groups (directly involved in soil fertility) and the plant behaviour constitute good tools to moni-

for the characteristics of changes in soils treated with biosolids (11).

In this work, the impact that the addition of a biosolid has on the performance of two fertile soils was studied. The studied soils have similar origin, both are formed from sedimentary parent materials under a grassland cover, located in the Ondulated Humid Pampa in Argentina. One of them is a pristine soil, which has never been altered by anthropic action, and the other is a soil that has been exploited for over a century under intensive cultivation.

The hypothesis of this work is that the selected soils having different historical land-use, respond differently to the physical, chemical and biotic perturbation that the addition of a biosolid causes, and that this response could be detected through soil enzymatic biodescriptors: soil dehydrogenase activity and soil urease activity, and through another biodescriptor related to plant physiology: root elongation.

Soil dehydrogenase activity is quantified by the reduction of the electron acceptor 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT); it is reduced by the microbial activity to idonitro-tetrazolium formazan (INTF). The quantity of INTF expresses the capacity of microorganisms present in a given soil to produce electrons from the oxidation of organic substrates. Thus, soil dehydrogenase activity is an enzymatic activity closely associated with soil fertility (3). Soil urease activity takes part in the nitrogen cycle; it is mainly produced by microorganisms which hydrolyse the urea to form ammonia, thus influencing soil fertility. The root elongation test is a fast and easy parameter to estimate the plant's growth; it is performed on seedling grown on a layer of soil under controlled conditions of temperature, humidity and illumination (10).

Two experiments were carried out with the soil of upper horizon (A horizon, 0-15 cm depth) from two Argiudolls (silt-textured soils), sites located in the south of Santa Fe Province (Argentina). In Experiment I, an agricultural soil classified as vertic Argiudoll, having undergone conventional agricultural practices for 90 years and zero-tilled during the last 10 years, was used. In Experiment II, a natural grassland soil, a typic Argiudoll was employed. The main characteristics of the agricultural soil were as follows: oxidizable carbon (OC), 1.4%; total nitrogen (tN), 0.16%; pH 5.6; cationic exchangeable capacity (CEC), 21.7 meq/100 g; equivalent humidity (EH), 25.3%. The main characteristics of the grassland soil were: OC, 1.68%; tN, 0.21%; pH 5.9; CEC meq/100 g, 15.5; EH, 28.3%. In relation to the characteristics of both soils, it should be stated that OC and tN were higher in the grassland soil than in the agricultural soil. Nevertheless, both soils had a similar C/N ratio. The biosolid employed in both experiments was obtained from pools used for the desiccation of fluids from drying bed sewage sludge of urban origin (6). Their main characteristics are: OC, 9.01%; tN, 0.68%; pH 6.8; dehydrogenase activity 0.598

μmol INTF/g dry weight/h (g d.w./h); urease activity 4.01 μmol NH<sub>4</sub><sup>+</sup>-N/g d.w./h; total bacterial number (acridine orange stain) 3.4 × 10<sup>9</sup> cell/g d.w.; heavy metals were analyzed through atomic absorption spectrometry, of which only traces were found (data not shown).

In both experiments, soils and biosolids were sieved through an ASTM N° 5 mesh (4000 μm) and the natural plant remains were eliminated by hand. The mixtures of 30 g of each soil (agricultural soil, AS; or grassland soil, GS) with biosolids at rates of 0 (AS0 or GS0, respectively, control treatments), 3 (AS3 or GS3, respectively), 6 (AS6 or GS6, respectively) and 12 (AS12 and GS12, respectively) percent of biosolid. These quantities were based on the tN content added to soil (5). The mixtures were perfectly homogenised and placed in 100 ml pots and demineralized water was added in order to reach the EH. Then, pots were sealed with Parafilm® and placed in a dark chamber at 25 °C for 72 h. At this time, samples of mixtures were taken and the dehydrogenase and urease activities were quantified according to techniques described by Leirós *et al.* (8) and Trasar-Cepeda *et al.* (15), respectively, and the root elongation test (10) was performed.

Results of selected descriptors of both soils are related to the main characteristics of soil (tN and OC); thus, dehydrogenase and urease activities and root elongation were higher in GS0 than in AS0 (Table 1). The biosolid addition did not depress these biodescriptors; on the contrary, it had a stimulating effect, though different, in each soil. The dehydrogenase activities of both amended soils were higher than those of control soils (Table 1). While low doses added to agricultural soil (AS3 and AS6 treatments) did not substantially differ from the AS0 ( $p > 0.05$ ), AS12 had the highest dehydrogenase activity ( $p < 0.05$ ). In the grassland soil, between the lowest dose (GS3) and the control grassland soil without added (GS0), it had the most important increase ( $p < 0.05$ ), approximately 13 percent; while with major doses (GS3 and GS6 treatments) the increase of the dehydrogenase activity was near to 8 percent, and only an increase of 3 percent was observed between GS6 and GS12 treatments.

Effect of lower doses of biosolid added to both soils had different behaviours on the soil dehydrogenase activity, which were the lowest increases in the agricultural soil and the highest increases in grassland soil. These results lead us to think that both soils have different microbial population dynamics and could suggest that the different soil reactions to the biosolid addition were determined by differences in the functional microbial population of each studied soil (7). Results would have two possible interpretations: (i) Naturally, the system has an important numerical and functional microbial component (as suggested by the high values of dehydrogenase activity observed in GS0) that is stimulated by the biosolid (probably as organic carbon supply used for the synthesis of biomass and metabolic energy production); and (ii)

**Table 1.** Dehydrogenase activity, urease activity and root elongation in biosolid-treated Argiudoll soils.

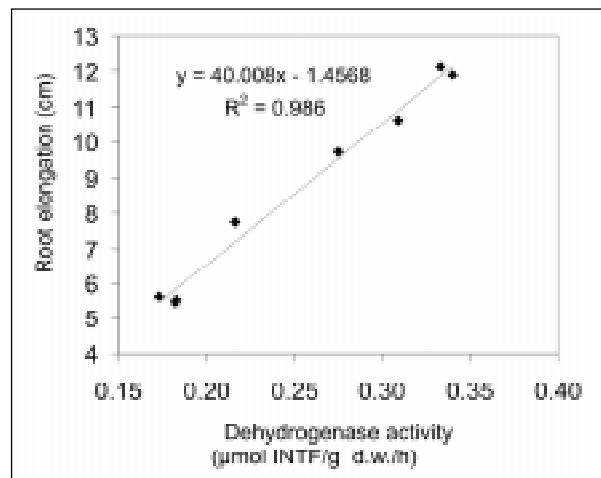
Biosolid (%)	Dehydrogenase activity ( $\mu\text{mol INTF/g d.w./h}$ )		Urease activity ( $\mu\text{mol N-NH}_4^+/\text{g d.w./h}$ )		Root elongation (cm)	
	AS <sup>(1)</sup>	GS <sup>(2)</sup>	AS	GS	AS	GS
0	0.173 a $\pm$ 0.007	0.275 a $\pm$ 0.009	0.793 a $\pm$ 0.015	4.218 a $\pm$ 0.154	5.67 a $\pm$ 0.56	9.73 a $\pm$ 0.42
3	0.182 a $\pm$ 0.002	0.310 b $\pm$ 0.023	0.949 b $\pm$ 0.012	4.523 b $\pm$ 0.143	5.35 a $\pm$ 0.65	10.75 ab $\pm$ 0.51
6	0.183 a $\pm$ 0.007	0.333 b $\pm$ 0.004	1.147 c $\pm$ 0.008	4.814 c $\pm$ 0.081	5.59 a $\pm$ 0.64	12.01 b $\pm$ 0.23
12	0.217 b $\pm$ 0.004	0.340 b $\pm$ 0.004	1.388 c $\pm$ 0.019	4.946 c $\pm$ 0.072	7.85 b $\pm$ 0.64	11.88 b $\pm$ 0.40

<sup>(1)</sup> AS: Agricultural soil; vertic Argiudoll, <sup>(2)</sup> GS: Grassland soil; typic Argiudoll. Values are means of three replications;  $\pm$  S.E. Significant differences between treatments (within a column) at  $p=0.05$  level (Tuckey's test) are indicated by different letters.

the biosolid adds a microbial load that is stimulated by the availability of nutrients present in the grassland soil (it is a pristine ecosystem, with high natural fertility). Nevertheless, through one of them or by both mechanisms simultaneously, this system reached equilibrium from biosolid additions higher than 6%.

In relation to soil urease activity, different doses of biosolid added to soils also caused an increase in this activity (Table 1). In both soils, the three biosolid doses produced an increase in urease activity compared to the unamended soils, respectively. While the highest increase of urease activity in the grassland soil was 14 percent (GS12 vs. GS0), in the agricultural soil it was nearly 46 percent (AS12 vs. AS0). The low biosolid doses added caused an increase in soil urease activity of about 14 percent (GS3 vs. GS0); the level of activity was similar between GS6 and GS12, the increase was only 3 percent ( $p>0.05$ ). The urease enzyme takes part in the important biogeochemical nitrogen cycle and through this participation, soil fertility can be significantly influenced. Furthermore, biosolids of urban origin have important urease activity (12) as had been informed at the beginning of this work. Soil urease activity reflects the presence of active microorganisms that have urease production but can also be originated by the presence of immobilized enzymes (13). For this reason, it is not possible to do the same analysis as the one done in relation to soil dehydrogenase activity. The different urease activity-behaviours of both biosolid-amended soils suggest that in the agricultural soil, the biosolid became mainly an enzyme addition (through the addition of active microorganisms or directly through extracellular enzyme), whereas in the case of the grassland soil, in addition to providing enzymes; it could have exerted a stimulation effect on the natural microbial activity of the soil. This interpretation is supported by the fact that the same addition of 6% biosolid to the grassland soil caused an increase in the activity 1.7 fold higher than in the agricultural soil.

Table 1 also shows that, in general, the test of root elongation in the grassland soil was higher than that in



**Figure 1.** Dehydrogenase activity and root elongation relationship in the experiments of different doses of biosolids added to the studied soils.

the agricultural soil (unamended soil). The root elongation of the AS12 and the GS12 treatments were significantly higher compared to their respective control soils ( $p<0.05$ ). The lower doses of biosolid only caused a significant increase in the root elongation in the grassland soil. The root elongation test turns out to be a useful indicator to study the soil response to the addition of biosolids to improve vegetable growth, not only for its simplicity, but also for being a parameter that expresses the capacity of soils to support vegetable growth. Furthermore, these parameters had a high significant correlation ( $r^2 = 0.986$ ;  $p<0.001$ ) with the dehydrogenase activity of the different treatments (Figure 1). This relationship is in agreement with the interpretation that root elongation is directly dependent on the rizospheric microbial activity (9).

In sum, it can be concluded that the impact of biosolid addition was detected by the bio-descriptors employed. In the agricultural soil, the biosolid addition increases the mentioned properties suggesting a direct effect of the addition. On the contrary, in the grassland soil, the prop-

erties undergo an intense increase at low doses, which suggests that, besides the direct effect, the biosolid addition has an indirect effect stimulating the microbial populations in soil.

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