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Effects on *Folsomia candida* Willem, 1902 of products resulting from anaerobic digestion of biomass tested at different soil pH

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Abstract

In recent years, it has become increasingly important to reduce the production and impact of wastes on the environment to save and restore natural resources. A way of disposing industrial, agro-industrial and urban wastes is their treatment by anaerobic digestion, with the production of biogas and residual organic material. The latter is commonly called digestate and can be used in agriculture as fertilizer, soil improver or adsorbent material, but only scarce or no evaluations of its biological effects on soil organisms are presently available. The aim of the present research was to study the effects of digestate, when added to soils with different pH values, on the survival and reproduction of the collembolan *Folsomia candida*. The digestate investigated in this study was obtained from agro-industrial wastes mixed with biological sludge. We exposed springtails to two different concentrations of digestate (2.5%, 5%) in two artificial soils with pH values of 6 and 4.5. The addition of digestate resulted in an increase of experimental-soil pH values, depending on the concentration and original pH value of the artificial soil used. The negative effects of digestate detected on the survival and reproduction of *F. candida* was mainly attributed to pH values > 6. The high pH value, however, does not explain by itself the strong decrease in number of juveniles in the experimental soil when the highest concentration of digestate was combined with the highest pH value reached. In this case it is possible to assume a combined effect of pH and other digestate characteristics, such as nitrogen content and salinity, currently under study.

Keywords Collembola | digestate | toxicity test | survival | reproduction

1. Introduction

In recent years waste disposal has significantly increased as a result of the current economic system, based on continued growth in the production and consumption of goods and services. By 2025 it is estimated that the urban population will reach 4.3 billion people, which will generate 2.2 billion tons of waste per year (Hoornweg & Bhada-Tata 2012). It is therefore important to reduce waste production and impact on the environment, and to save natural resources by waste recovery. A way of disposing waste is its treatment by anaerobic digestion, a promising technology enhancement to treat industrial, agro-industrial and urban wastes, obtaining two categories of products: biogas and the remaining digested substrate, commonly called digestate. The digestate can be further refined into concentrated fertilizer and organic amendment, all suitable for recycling (Tambone et al. 2009, Dolan et al. 2011).

The addition of digestate to soil could contribute to solving one of the most important problems of agricultural soils, the alteration of their structure, and could increase the retention of the nutrient levels and microbial activity, thus promoting soil fertility (Tambone et al. 2009, Makádi et al. 2012). However, the presence of digestates in soil could also alter physical-chemical properties, such as pH

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(Gómez et al. 2007, Pognani et al. 2009), or buffer capacity and, by modifying the environmental conditions, could cause ecotoxicological effects, leading to a decrease of soil-organism population fitness. Therefore, this practice could exacerbate the decline of biodiversity in agricultural soils rather than improving it. Chemical analyses seem inadequate to reveal the biological long-term effects of materials added to soil because of a lack of knowledge about potential interactions between contaminants and toxicity for soil organisms. Thus, chemical analysis has to be combined with ecotoxicological tests (Van Gestel 2012). Ecotoxicological studies have been conducted to test the effects of sewage sludge and organic waste compost on soil organisms (Domène et al. 2007, 2008, 2010, 2011, Fuchs et al. 2008, Moreira et al. 2008, Pivato et al. 2014). However, only scarce (Fuchs et al. 2008) or no evaluations of the biological effects of digestates are available.

The aim of present research was to study the effects of digestate, when added to soils with different pH values, on the survival and reproduction of the collembolan *Folsomia candida* Willem, 1902. This springtail is among the most widely used standard test organism for terrestrial ecotoxicology (Fountain & Hopkin 2005, Filser et al. 2014), due to its widespread distribution in different soil types, its role in the decomposition of organic matter, regulation of microbial activity and nutrient cycling (Luo et al. 2014) and its tolerance to a wide range of important soil properties (Amorim et al. 2005). A digestate obtained from the biomethanization of biomass, produced in the Emilia Romagna region in Italy in large amounts, was used.

2. Material and methods

2.1. Digestate

The digestate used in this study was obtained from agro-industrial wastes mixed with biological sludge and it is characterized by a high pH value (pH = 8). The pH value was measured in a suspension 1:2.5 digestate:$CaCl_2$ following standard procedures (MiPAF 1999). The digestate was dried at 110°C for 24 hours, milled and used in the soil in particles of < 500 µm.

2.2. Test organisms

The utilised Collembola belonged to the species *Folsomia candida* Willem, 1902. The specimens were reared in the laboratory for several generations. They were maintained in 100 ml glass jars with a diameter of about 5 cm containing clay, mainly consisting of kaolinite and smectite, bottom saturated with deionized water and kept in a thermostatic chamber at 20±1°C with a light-dark cycle of 16:8 h. Animals were fed on Brewer's yeast.

2.3. Toxicity test

According to ISO guideline (ISO 11267: 2014), the artificial soil was composed of 69% quartz sand, 20% kaolinite clay and 10% *Sphagnum* sp. peat, air-dried, ground and sieved to 0.05 mm. Deionized water was added during mixing to reach about 60% of the maximum water-holding capacity (WHC measured using guideline ISO 11267). Following the ISO guideline, pH value was adjusted to 6.0 ± 0.5 by adding $CaCO_3$. The artificial soil was also used to prepare soils with different pH values (4.5 and 7.0) by adding adequate amounts of $CaCO_3$.

Four different experimental soils were obtained with the addition of the digestate at two different concentrations (2.5% and 5%), namely:

- 2.5% H: digestate 2.5% added to artificial soil with pH = 6 (High level pH)
- 5% H: digestate 5% added to artificial soil with pH = 6 (High level pH)
- 2.5% L: digestate 2.5% added to artificial soil with pH = 4.5 (Low level pH)
- 5% L: digestate 5% added to artificial soil with pH = 4.5 (Low level pH)

Experimental soil with pH = 6 without digestate (0 pH 6) was used as control soil. Experimental soil with pH = 7 without digestate (0 pH 7) was used for comparison, because preliminary studies revealed that the addition of digestate causes an increase in soil pH. The experimental soils were prepared at least three days prior to the start of the test in order to equilibrate acidity. The chosen concentrations of digestate of 2.5 and 5% correspond to 27 and 54 ton/ha (dry weight) respectively. The lower dose is in line with recommendations for Italian agricultural soils. The conversion was made using the density of artificial standard soil of 1.1 g/cm^3, measured according to ISO 11272 (1998) and assuming a mixing in the first 10 cm of soil.

The pH value of experimental soils was measured at the start of the toxicity test, in two replicate samples of each experimental soil, in accordance with standard procedures for soil characterization (MiPAF 1999). The soils were shaken with distilled water (1:2.5) for 2 h at 200 rpm. After settlement of the particles, the pH of the soil solution was measured using a Crison BASIC20 pH meter.

Eight replicates were prepared for each experimental soil utilizing glass containers (100 ml capacity, 5 cm diameter). According to ISO guideline 11267, ten 10-day-old springtails were placed in each tightly closed test container and were maintained at a temperature of

20±1°C with a light-dark cycle of 16:8 h for 28 days. The containers were opened twice a week for aeration and feeding with yeast (4 mg in 28 days). At the end of the experiment, water was added to each container: the animals sorted by floating were grouped into adults and juveniles and counted manually under a stereomicroscope.

2.4. Statistical analysis

All statistical analyses were carried out using SPSS software release 21.0. One-way Analysis of Variance (ANOVA) was performed to compare the survival and reproduction levels of *F. candida* among control soil, the four experimental soils with digestate and an experimental soil without digestate at pH = 7. When significant statistical differences were detected ($P < 0.05$), a post hoc Student-Newman-Keuls test (SNK) was used. Percentage survival data were arc-sin transformed before statistical analysis. In order to evidence possible interactions between digestate concentrations and soil pH values on survival and reproduction of *F. candida*, the Generalized Linear Model (GLM) procedure was used.

3. Results

The pH values of experimental soils with and without digestate are reported in Figs 1 and 2. The number of

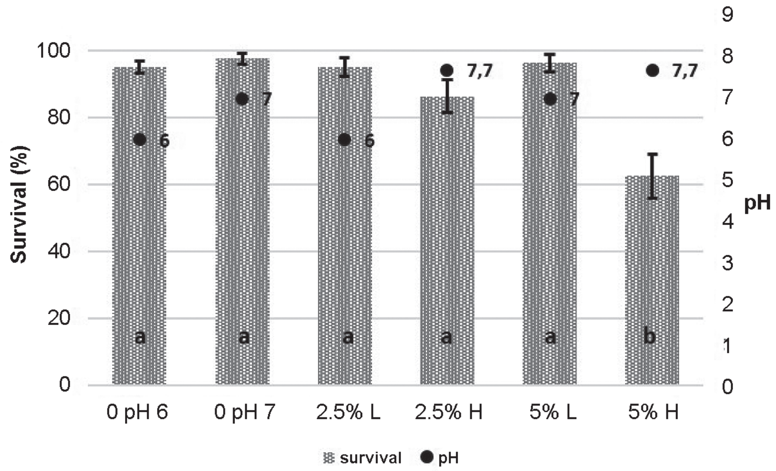

Figure 1. Percentage survival of *Folsomia candida* exposed for 28 days to different concentrations of digestate. pH values of experimental soils are also indicated. Survival values represent the mean number of eight replicates ± SE. Survival data were arc-sin transformed before statistical analysis. Different letters indicate significantly different means at $P < 0.05$ according to the Student-Newman-Keuls' test. H: artificial soil with pH = 6; L artificial soil with pH = 4.5.

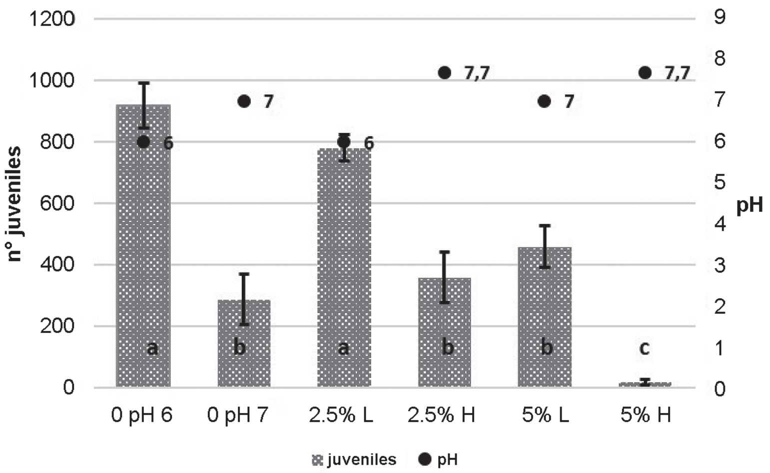

Figure 2. Reproduction, shown as number of juveniles, of *Folsomia candida* exposed for 28 days to different concentrations of digestate. pH values of experimental soils are also indicated. Numbers of juveniles are indicated as a mean number of eight replicates ± SE. Different letters indicate significantly different means at $P < 0.05$ according to the Student-Newman-Keuls' test. H: artificial soil with pH = 6; L artificial soil with pH = 4.5.

surviving adults of all experimental groups at the end of the test is reported in Fig. 1. The number of surviving adults of the experimental group with 5% digestate and pH = 7.7 (5% H) was statistically lower than the number of surviving adults of all other experimental groups (SNK; $P < 0.05$), while the numbers of surviving adults of the other experimental groups did not significantly differ among themselves (SNK; $P > 0.05$). The GLM analysis highlighted a significant effect of pH and digestate on survival, but no interactions between digestate and pH (pH, $P < 0.001$; digestate, $P < 0.05$; digestate*pH, $P = 0.088$).

The number of juveniles of all experimental groups at the end of the test is reported in Fig. 2. The number of juveniles of the experimental group with 2.5% digestate and pH = 6 (2.5% L) was not statistically different from the number of juveniles of the control group 0 pH 6 (SNK; $P > 0.05$). The numbers of juveniles of the experimental groups with 2.5% digestate and pH = 7.7 (2.5% H), with 5% digestate and pH=7.0 (5% L), without digestate and pH = 7.0 (0 pH 7) are not different among themselves (SNK; $P > 0.05$), but are significantly lower than the number of juveniles of the control group 0 pH 6 (SNK; $P < 0.05$). The number of juveniles of the experimental group with 5% digestate and pH 7.7 (5% H) is significantly lower compared with the numbers of all other experimental groups ($P < 0.05$). In particular the numbers of juveniles of this group (5% H) is significantly lower compared to the numbers of juveniles of the group of experimental soil with the same concentration of digestate, but with pH = 7.0 (5% L) and of the group of experimental soil with the pH = 7.7, but a lower digestate concentration (2.5% H).

The GLM analysis highlighted a significant effect of pH on reproduction and interactions between digestate and pH (pH, $P < 0.001$; digestate, $P = 0.257$; digestate*pH, $P < 0.001$).

4. Discussion

The addition of the studied digestate resulted in an increase of experimental-soil pH values depending on digestate concentration and original pH values of the artificial soil. Reproduction seems to be the collembolan population characteristic mainly affected by the treatments.

The data obtained indicate that the negative effects of digestate on survival and reproduction of *F. candida* can be mainly attributed to the pH increase of experimental soils to a value above 6. In fact, a negative impact on reproduction was found in all experimental soils with pH > 6, with and without digestate. The high pH value, however, does not explain by itself the strong decrease in the number of juveniles in the experimental soil where the highest digestate concentration is associated with the highest pH value: in this case it is possible to assume a combined effect of pH and other digestate characteristics, such as nitrogen content and salinity, currently under study. The experimental soil characterized by the highest digestate concentration and the highest pH value is also the only one that showed a significant decrease of springtail survival compared to all the other experimental soils. Our results are in line with data on the sensitivity of *F. candida* to pH reported by other authors. Fountain & Hopkin (2005) report for this species a mild preference to settle on weakly acidic soils (pH = 5.6), whereas Greenslade & Vaughan (2003) affirm that the highest rates of reproduction were established at pH = 5.4 and a sharp decline was found at higher pH with few, if any, offspring being produced above pH = 7.0.

Our study pointed out that the biological effects and possible ecological sustainability of materials used for the improvement of soil structure and/or fertility, such as the digestate in study, do not depend only on the characteristics of the material itself, but also on the results of its interaction with the characteristics of the soil to which it is added. Preliminary studies of the soil characteristics are therefore needed before a widespread use of organic fertilizers or soil improvers, in order to avoid or limit disturbance on the soil populations and, in the long term, a decrease in biodiversity. The addition of digestate in soils should in particular be discouraged when this practice strongly increases soil pH values. On the other hand, soil acidification, which can cause degradation with an increase in the toxicity of some metals and deficiency of nutrients for the plants reducing crop yields (Russell et al. 2006, Zhang et al. 2008), could be countered by using digestates. Soils differ greatly in pH according to the geographical location and thus the pH ecological optimum of their communities varies. In Emilia Romagna (Italy), soil pH values generally range from 7.3 to 8.4 (ARPA Emilia Romagna, 2010), and ecotoxicological tests useful for regulatory decisions regarding acidic soils may not be appropriate for these types of soils.

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