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Is digestate safe? A study on its ecotoxicity and environmental risk on a pig manure

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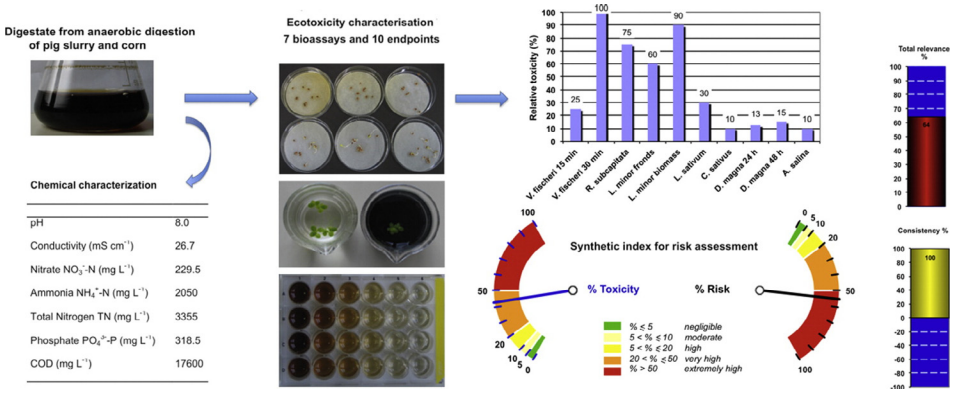
HIGHLIGHTS

- Digestate and its impact on the environment and human health are still unexplored.
- Ecotoxicity tests on digestate can predict its impact and the need of pretreatments.
- The outputs of 7 ecotoxicity tests were summarised with a synthetic index.
- Algae were the most sensitive among all tested organisms.
- Extremely high environmental risk was due to high battery consistency and relevance.

GRAPHICAL ABSTRACT



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ABSTRACT

Digestate represents a precious by-product in particular in agriculture, however its impact on the environment and human health is still unexplored. In this work, the toxicity of a pig slurry digestate was assessed through 7 ecotoxicity tests and considering 10 different endpoints. Besides, a synthetic index was applied to the outputs of the battery of tests for the environmental risk assessment, in order to evaluate the opportunity to use directly this kind of digestate in agriculture or to introduce an additional treatment. All the organisms were sensitive to digestate toxicity (EC_{50} ranged from 14.22% for *Cucumis sativus* to 0.77% for *Raphidocelis subcapitata*). The physical–chemical features at the base of this toxicity seem to be the high content of ammonium, salinity, COD, phosphate and colour. The synthetic index showed that the digestate was very toxic and associated to an extremely high environmental risk. Thus, a pre-treatment is needed to reduce its toxicity and environmental impact, whatever could be its exploitation.

1. Introduction

Intensive livestock breeding produces a large quantity of biodegradable wastes that have to be managed adequately. EU Landfill Directive (1999/31/EC) has underlined the importance of waste reduction and

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management with sustainable methods such as recycling and composting. Since the anaerobic digestion of agriculture and zootechnical wastes is of great value both for livestock waste management and biogas production, the number of composting and anaerobic digestion plants increased in all the Europe Countries (Holm-Nielsen et al., 2009). Italy is the third country in the world for biogas production, after Germany and China, with approximately 1300 plants and 7400 GWh produced in 2013 (Baronchelli, 2015). Benefits of anaerobic digestion basically consist in the production of biogas, and the reduction of both greenhouse gas emissions and water pollution (Möller and Stinner, 2010). On the other hand, anaerobic digestion produces the digestate, a residual material that is rich in recalcitrant organic molecules and nutrients, thus it has to be adequately managed and disposed (Provenzano et al., 2011).

In the light of Directive 2008/98/EC, which gives an adding value to wastes by means of their integrated management, digestate addition to soil is considered an appropriate option, with multiple benefits for agriculture and environment by reducing the use of mineral fertilisers (Zhang et al., 2015). However, applications of biogas digestates and their impacts on the environment and human health are still unexplored and the effectiveness of digestate as organic amendment and fertiliser is still under debate (Nkoa, 2014).

Ecotoxicity analyses of digestates before their exploitation in agriculture can predict their environmental impact and the necessity for additional treatments. Nevertheless, the few studies that have been done on this kind of samples used a limited number of bioassays and did not calculate a risk for the environment (Chen et al., 2014; Różyło et al., 2015). In ecotoxicity studies, indeed, the application of a battery of bioassays with organisms representing different positions in the food chain is essential, in order to obtain results that may realistically represent the impact on the environment. Moreover, the outputs of a battery should be summarised in a single datum, with the aim to give information about the environmental risk associated to the tested samples. This elaboration could allow to take decision for the digestate manage and use (Costan et al., 1992; Persoone et al., 2003; Canna-Michaelidou and Christodoulidou, 2008).

In the present study, the toxicity of a pig slurry digestate was assessed through 7 ecotoxicity tests and considering 10 different endpoints. Besides, the synthetic index developed by UNICHIM Water Quality Commission was used to for the environmental risk assessment, in order to evaluate the opportunity to use directly this kind of digestates in agriculture or to introduce an additional treatment.

2. Materials and methods

2.1. Origin of samples and chemical analyses

Digestate was obtained from the effluent of an anaerobic digester, which treats pig slurry and corn, located in North West Italy. Samples of digestate liquid phase were stored at 4 °C after collection and analysed periodically to check its stability for two months during which all the experiments were carried out. Parameters measured for the chemical characterisation of the digestate were: ammonium, nitrate, total nitrogen, phosphate and COD. They were selected on account of their usual abundance and potential impact on the environment. All of them were spectrophotometrically estimated (LASA 100-HACH LANGE) according to APAT-IRSA CNR Standard Methods 2003 for nutrients and ISPRA Metodo 5135 — 2014 for COD. Moreover, pH and conductivity were measured by using the probe WTW Multi340i.

2.2. Ecotoxicity tests

Seven ecotoxicity tests were selected on account of data in literature about their sensitivity to toxic substances and their low cost and easy availability also for a private company. Moreover, some of them were selected on account of their recommendation in the European legislations (i.e. Italian law Dlg 152/2006).

Vibrio fischeri strain NRRL B-11,177 was bought at Ramcon A/S (Birkeroed, Denmark) and used for the test of luminescence inhibition (UNI EN ISO 11348-3) with Microtox® toxicity system (Microtox Model 500; Microbics Corp., USA) as described by Tigini et al. (2011). The luminescence intensity in all cuvettes was measured before the addition of the wastewaters and after 15 and 30 min exposition and automatic colour correction was performed. A computer programme for Microtox Acute Toxicity Test (Azur Environmental Ltd., UK) was used for the data elaboration.

Raphidocelis subcapitata (Korshikov) Nygaard et al., originating from Agenzia Regionale per la Protezione dell'Ambiente (ARPA Piemonte, Grugliasco, TO), was used for the algal growth inhibition (UNI EN ISO 8692:2005). The tests were performed as described by Tigini et al. (2011), and data were elaborated using ToxCalc™ 5.0.

The aquatic plant *Lemna minor* L. was used for the assessment inhibition of both biomass dry weight and frond number (ISO SO/WD 20079). The test was performed as described by Casieri et al. (2008).

Cucumis sativus L. and *Lepidium sativum* L. were used for phytotoxicity tests (UNICHIM N. 1651, 2003). Seeds were purchased from Blumen Group S.p.A. (Piacenza) and the test was performed as described by Tigini et al. (2011).

Daphnia magna Straus, cultured at ARPA Piemonte, was used for the immobilisation test (UNI EN ISO 6341:99). The tests were performed as described by Tigini et al. (2011), and immobile animals were counted after both 24 h and 48 h.

In the *Artemia franciscana* L. bioassay, after a preliminary test, 3 dilutions were chosen with 3 replicates each and 3 repetitions were used for the control. Three dilutions of 100 mg *A. franciscana* cysts were placed in a Petri dish (5 cm diameter) for hatching, containing 12 mL of saltwater and incubating for 48 h at 25 °C in the dark (changing saltwater after 24 h). After the incubation, 10 instar I and II nauplii were inoculated in 1 mL of sample, or saltwater for the control, for each replicate. Nauplii were incubated for 24 h at 25 °C in the dark, after that the nauplii mortality was assessed.

The sensitivity of the test organisms cultivated in directly in laboratory (*D. magna*, *L. minor*, *R. subcapitata*) was periodically assessed with a potassium dichromate solution ($K_2Cr_2O_7$).

Results of ecotoxicity tests were plotted on a dose-effect chart; the EC_{50} and its confidence limits ($p = 0.05$) and toxic units ($100/EC_{50}$) were estimated using standard procedures.

2.3. Synthetic index and ecotoxicological risk assessment

The synthetic index was developed by the Associazione per l'unificazione nel settore dell'industria chimica (UNICHIM) Commissione Qualità dell'Acqua, Gruppo di Lavoro Metodi Biologici, Sottogruppo Acque salate/salmastre e Sedimenti, Gruppo ad hoc Batterie, scale di tossicità e indici integrati. It is a modification of the model proposed by Hartwell (1997), and described by Baudo et al. (2011). This synthetic index allows to compare the results of ecotoxicity test batteries through a toxicity score (BTS), that represents the mean of the relative toxicity of each test ($RT_{endpoint}$). This last parameter is calculated as follows:

$$RT_{endpoint} = 100 - 100 \cdot \frac{[\log(C \cdot EC_x) \cdot R \cdot S]_{max} - [\log(C \cdot EC_x) R \cdot S]_{endpoint}}{[\log(C \cdot EC_x) R \cdot S]_{max}} \quad (1)$$

where C is a statistical corrective ($C = 2$ if the EC_x is higher than 100%; $C = 1$ if the EC_x and its 95% confidence limits are lower than 100%); S is a score depending on the considered endpoint (mortality = 8; bioluminescence = 7; development = 6; reproduction = 5; growth = 4; genotoxicity = 3; mutagenicity = 2, behaviour = 1); R is the rank of toxic concentrations and it is assigned from the lowest concentration to the highest one.