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DIGESTATE AND FUGATE – FERTILIZERS WITH ECOTOXICOLOGICAL RISKS

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Abstract

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Increasing number of decentralised biogas plants increases not only the amount of biogas produced, but also the production of digestate. Digestate and fugate are believed to be good fertilizers. However, there is often a tradeoff between other environmental impacts linked to agricultural production like eutrophication or ecotoxicity. Only limited ecotoxicological information is known about the effects of digestate or fugate on terrestrial fauna and flora.
This is the first study comparing the survival and reproduction of collembolans as the representative soil fauna and the root growth and photosynthetic activity of *Sinapis alba* and *Panicum miliaceum* plants when exposed to digestate and fugate. Comparison of ecotoxicological results with chemical analysis of both digestate and fugate has led to the conclusions that application of digestate and fugate was beneficial neither for tested plants, nor for zooedaphon. Under practically used dosing both digestate and fugate can represent potential ecotoxicological problems, which can affect zooedaphon diversity and reproduction resulting in degradation of soil structure, reduction of microbial activity or water retention capacity of treated soils. That is why we do recommend at least simplified ecotoxicological testing of digestate as presented in this study. Ecotoxicity testing can support decision of direct application on soil, or mixing the digestate with other materials (like compost, manure, pond sediments, or biochar), what will help to utilize nutrients and consequently can prevent degradation of soil fertility.

Keywords: digestate, fugate, ecotoxicity, soil fertility

INTRODUCTION

Biogas plants utilize different organic materials like residual waste from livestock, food production, effluents from industry or sludge from wastewater treatment plants. As a result, biogas (a mixture of different gases produced by the breakdown of organic matter in the absence of oxygen) is produced as a renewable energy source with small ecological footprint. Biogas consists of methane, carbon dioxide and usually small amount of hydrogen sulphide and can be used e.g. for a production of CNG (compressed natural gas).

A material remaining after anaerobic digestion of waste is called digestate and consists of liquid phase (fugate) and solid phase (called digestate, as well). Digestate (solid phase) is believed to be a good fertilizer. On the other hand, it should be noted that it contains a lot of water, organic dry matter is reduced by 45–65% in comparison with material before digestion, and C_{tot}:N_{tot} ratio is usually 8:1. Around 90% of N-NH_4^+ changes into liquid form $NH_{3(aq)}$ and the residual organic matter consists of structural plant matter including lignin and cellulose (Kolář and Kužel 2009). Although a lot of attention is payed to nutritive properties of digestate and fugate, almost nothing is known

about ecotoxicity of these materials, despite it contains potential toxicants, especially toxic metals. Thus, the aim of this study is to assess the potential ecotoxicity of fugate and digestate towards two macrophytes (here represented by dicotyledonous *Sinapis alba* and monocotyledonous *Panicum miliaceum*) and invertebrate *Folsomia candida* as representatives of terrestrial organisms.

MATERIALS AND METHODS

Source of digestate and fugate

Digestate and fugate samples were obtained from a company which runs a biogas station. Waste consists mainly of corn silage and swine manure in approx. weight ratio 9:1.

Testing concentrations

For ecotoxicity testing, two different concentrations of the digestate and two concentrations of fugate plus control samples without waste content were used. Doses of the waste for trials were based on the maximum recommended values for biogas plant waste fertilization (revision process of the Fertiliser Regulation; Reg. EC No. 2003/2003), i.e. 167 tons of waste per hectare during three years. Because in many cases the waste is applied to agricultural land every year, we chose a concentration of 55.6 t/ha/year for our test. In conditions of ecotoxicological laboratory testing with invertebrates and plants, it corresponds to the concentration of 22.2 mg of digestate and/or fugate per gram of soil or water. For the second concentration, we chose 2 times higher concentration to simulate conditions of double application, which is commonly used in practise, i.e. 44.4 mg/g (corresponding to application of 111.2 tons of waste per hectare per year).

Test with terrestrial plants

Seeds of *Sinapis alba* and *Panicum miliaceum* were placed in Petri dishes (10 seeds per dish) with diameters of 10 cm and filter paper on the bottom. Aqueous suspensions of fugate and digestate were added to the diluting water (according to ISO 11269-2, 2012) to achieve final concentrations of 22.2 and 44.4 mg.L^{-1}. Suspensions were stirred rapidly for 30 s and then added (volume of 10 ml) into Petri dishes with seeds and left for 72 h at 25 ± 1°C. All experiments were performed in five replicates. The root length and photosynthetic activity (induced chlorophyll fluorescence) after 72 h were used as the endpoint when determining the inhibition values.

Measurements of induced chlorophyll fluorescence were performed using PAM-based planar chlorophyll fluorescence imaging system FluorCam MF700 (PSI, Brno, Czech Republic). Prior to the measurement, seedlings placed on a Petri dish were dark-adapted for 10 minutes. After that, they were exposed first to weak red irradiation (up to 5 µmol.m^{-2}.s^{-1} for 30 s) for the determination of basal chlorophyll fluorescence (F_0), followed by a saturating flash of white light (1500 µmol.m^{-2}.s^{-1}) with the duration of 1.6 s for the determination of maximal fluorescence value (F_M) and 15 seconds relaxation in the dark. Average F_V/F_M value of each seedling was calculated automatically according to an equation $F_V/F_M = (F_M - F_0)/F_M$. Measurements were done in 5 replicates from each treatment. Received data were processed by means of FluorCam software (PSI, Brno, Czech Republic).

Tests with invertebrates

Examination of reproduction inhibition of collembolan *Folsomia candida* was performed according to DIN EN ISO 11267 Soil quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil contaminants (ISO 11267, 2014). The tested concentrations of fugate and digestate were consistent with experiments on plants i.e. 22.2 and 44.4 mg.g^{-1} of the artificial soil. Control was represented by artificial soil without the presence of tested wastes. After 28 days the test was terminated and the total number of living adult

I: *List of methods used for analysis of digestate and fugate.*

Parameter	Method
TSS	EN 872
Loss on ignition	EN 872
CODCr	ISO 6060
BOD5	EN 1899-1, EN 1899-2
P-PO4	EN ISO 6878
NH4-N	ISO 7150-1
NO3--N	ISO 7890-3
NO2--N	ISO 26777
NTot	EN 13342
PTot	EN ISO 17294
As,Zn,Cd,Cr,Cu,Mo,Ni,Pb,	EN ISO 17294

TSS = Total suspended solids, CODCr = Chemical oxygen demand by Chromium method, BOD = Biological oxygen demand after 5 days, PTot = total phosphorus, NTot = total nitrogen.

organisms and new-born juveniles was examined in all treatments.

Chemical analyses

Chemical analysis of wastes was provided by accredited laboratory Labtech s.r.o. (Czech Republic). Identification of methods used for analyses is listed in Tab. I.

Statistical analysis

One-way-ANOVA followed by a Tukey HSD post hoc test was conducted to analyse differences between particular treatments and control of collembolans adult and juvenile number, length of macrophyte roots and photosynthetic activity related to two different concentrations of each waste type. Statistical analyses were performed using statistical software Statistica (StatSoft Inc., Tulsa, OK, USA), and $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Chemical analysis of waste from biogas station

Results of chemical analysis clearly show a substantial difference between composition of digestate and fugate (see Tab. II). Digestate contained 33 times more of total suspended solids and the loss of ignition was 30 times higher. Also, in digestate, there was 35.9 times more of organic material (measured as chemical oxygen demand). If we consider the dry matter of digestate and fugate, it is obvious that fugate contains higher concentration of toxic metals per dry matter when compared with digestate, because the dry matter of digestate is 5.5 times higher than dry matter of fugate.

On the other hand, at the moment when these wastes are applied on the field their amount is calculated according to their fresh weight, not by the dry matter content. Thus, in reality, when 1 tone of digestate is applied, it contains more toxic metals then fugate. For example, regarding application of fresh weight, waste contains 1.9 (0.48) g/t of Cr, 36.51 (6.01) mg/t of Cd, 389.44 (88.83) mg/t of Pb, and 1.97 (3.99) mg/t of Hg in digestate (fugate), respectively.

Toxicity against plants

For testing, terrestrial plants *Sinapis alba* (dicotyledonous) and *Panicum miliaceum* (monocotyledonous) were chosen. Germination of seeds and subsequent measurement of seedling root length plus determination of photosynthetic activity were used as endpoints. Results of trials indicated that digestate was more toxic when compared with fugate (see Fig. 1, section A and B). Digestate concentration of 22 mg/L inhibited the growth of roots by 87.4 %. Double dosage caused 100% inhibition and since no seeds germinated after the exposure by this concentration, measurement of photosynthetic activity was not provided in this particular case. On the other hand, growth of seedlings was not affected by fugate. We assume that it was caused by higher content of toxic metals in digestate as discussed in the paragraph of chemical analysis of waste from biogas station section.

II: *Values of key parameters in digestate and fugate*

Parameter	Unit	Digestate	Fugate
TSS	mg/l	128,000	3,850
Loss on ignition	mg/l	74,100	2,450
CODCr	mg/l	76,200	2,120
BOD5	mg/l	8,280	217
P-PO4	mg/l	1,350	24.4
Dry matter	%	8.17	1.47
NH4-N	mg/kg of dry matter	41,600	35,500
NO3-N	mg/kg of dry matter	411	328
NO2 -N	mg/kg of dry matter	12.1	10.9
NTot	% of dry matter	11.8	8.51
PTot	mg/kg of dry matter	15,610	10,060
As	mg/kg of dry matter	1.24	0.78
Cd	mg/kg of dry matter	0.3	0.2
Cr	mg/kg of dry matter	11.4	9.2
Cu	mg/kg of dry matter	70.1	60.3
Mo	mg/kg of dry matter	5.69	1,6
Ni	mg/kg of dry matter	11.3	7.7
Pb	mg/kg of dry matter	3.7	1.3
Zn	mg/kg of dry matter	360	323
Hg	mg/kg of dry matter	0.016	0.013

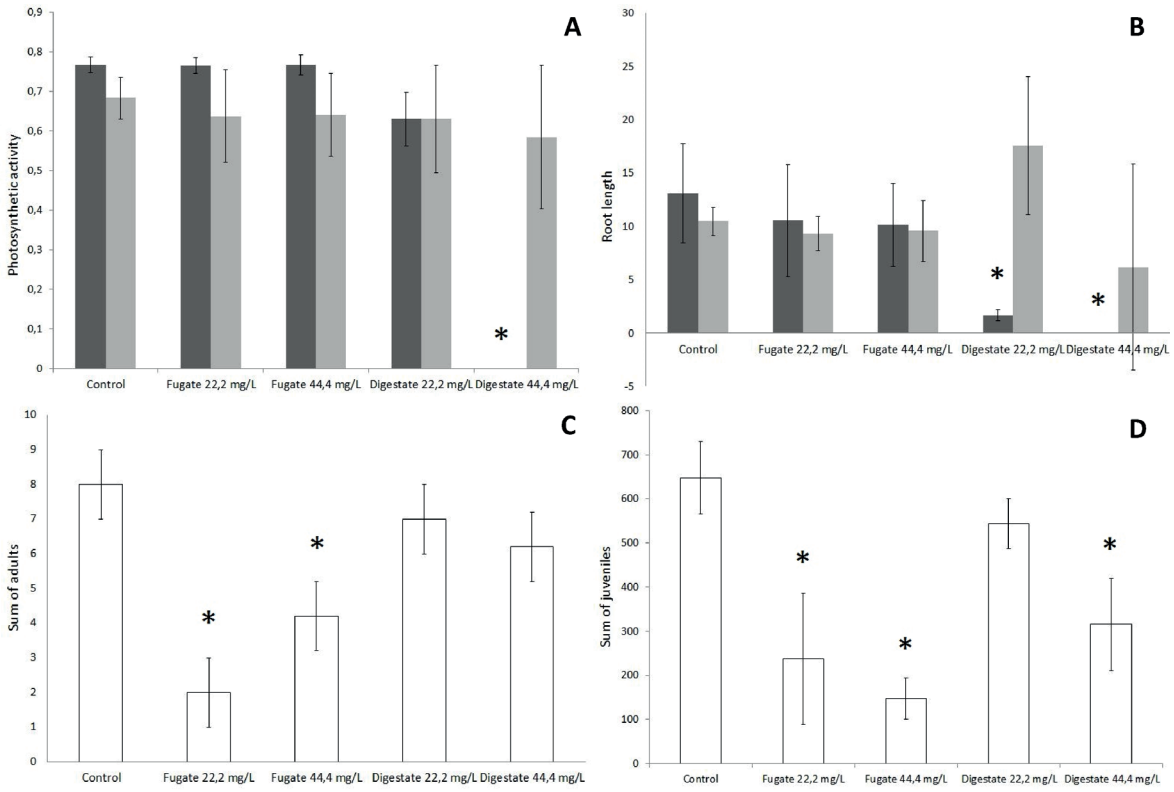
Results also showed stronger toxic effect of especially digestate towards *S. alba* when compared with *P. miliaceum*. This could mean higher sensitivity of dicotyledonous plants contrary to monocotyledonous plants but we realize that such generalization may be misleading and will be probably case-specific. On the other hand, differences between dicotyledonous and monocotyledonous species and their sensitivity towards toxic metals are well described in literature. For example, cadmium was found to be accumulated in dicotyledonous plants 10 times more than in monocotyledonous plants and similar results were obtained in case of Pb and Zn (Tlustoš *et al.* 2007). Similarly Baderna *et al.* (2015), when comparing effects of some toxic metals to two dicots (cucumber and cress) and one monocot (sorghum) as models of crop plants, described toxic effects of arsenic and nickel on both dicot and monocot plants, while chromium and mercury were toxic only in dicots and inversely lead induced toxic effects in sorghum.

Toxicity to invertebrates

For testing, invertebrate *Folsomia candida* (Collembola) was used as relevant and sensitive terrestrial organism. As endpoints, number of adults and juveniles after 28 days of testing were chosen. Number of adults was decreased in both treatments containing fugate, whereas digestate did not show any fundamental effect. Inhibition was found to be 75.0 and 47.5 % for concentration of 22.2 and 44.4 mg.g^{-1} of fugate, respectively. On the other hand juveniles were inhibited by both fugate samples as well as by high concentration of digestate. From this point of view, reproduction endpoint seems to be more sensitive parameter than adult survival. Inhibition of juvenile numbers was as follows 36.3, 77.2, and 51.2 % for 22.2 mg.g^{-1} and 44.4 mg.g^{-1} of fugate and 44.4 mg.g^{-1} of digestate, respectively.

Concentrations of toxic metals present in the waste did not exceed safe concentrations presented by other authors. For example, in acute toxicity tests with Cd, *F. candida* exhibited paralysis at 10–20 µg Cd.g^{-1} (Trublaevich and Semenova 1997). In another study, toxicity signs became evident in concentrations above 1000 mg Cu. kg^{-1} (Scott-Fordsmann *et al.* 1997). Regarding Zn toxicity, EC50 value found in soil toxicity test with *F. candida* was found to be 526 mg Zn.kg^{-1} (Smit and Van Gestel 1997). Bur *et al.* (2012) defined LOEC for reproduction of F. candida after metal addition which was found to be 100–2400 µg Pb.g^{-1} for different soils. On the other hand, toxicity can be enhanced by a mixture of toxic metals in matrix as well as content of other chemicals which were not analysed and can be present in samples.

In contrast with effects on vascular plants, fugate was more toxic in trials with collembolans. The explanation for this phenomenon can be



1: *Effect of fugate and digestate on photosynthetic activity (F_V/F_M; A) and the root length (cm; B) of two plant species Sinapis alba (dark grey) and Panicum miliaceum (light grey) and on the survival of Collembola adults (C) and their juveniles (D). Columns represent means, standard deviations are indicated by error bars. Asterisk (*) indicate statistically significant change in comparison with control ($p < 0.05$).*

substantially higher content of organic matter in digestate which can be attached on soil particles and therefore less bioavailable in tests with *F. candida*. Effect of tested media on ecotoxicological properties of chemical compounds have been described thoroughly in many publications. Another explanation could be different routes of exposure in both tested types of organisms. Similar results were obtained by Natal-da-Luz *et al.* (2011) who found out that metal mixture-contaminated sludge was less toxic than the soil spiked with same metal mixture for *Eisenia andrei* and *F. candida*. Results obtained for the earthworms suggested a decrease in metal bioavailability promoted by the high organic matter content of the sludge. Similar results were obtained also in the study by Fountain and Hopkins (2004) who pointed out that care should be taken in extrapolating the results of laboratory toxicity tests on metals in OECD soil to field soils, in which the biological availability of contaminants is likely to be lower.

Šimon *et al.* (2015) stated, that the fertilization with digestate brings an effect on crop yield increase of winter wheat, but does not improve significantly the level of organic matter in the soil, so in longer-term it is necessary to add organic matter from other sources. Fertilisation should keep fertile soil, including active soil microflora and zooedaphon as the ideal substrate for agricultural plants. Adverse effects of digestate are studied dominantly as the reduction of water infiltration and water retention capacity of soils as the result of dispersive effect on soil structure (Voelkner *et al.* 2015). Research focused on the effects of digestate on soil microbial community structure and activity also proved that direct application of digestate on soil (in contrast to compost or manure) did not stimulate microbial activity with exception of nitrification (Gomez-Brandon *et al.* 2016). The toxicity of digestate to bacteria was also proved in the comparative study of seven ecotoxicity tests (Tigini *et al.* 2016), where bioassay with *Vibrio fisheri* was the most sensitive bioassay. This recent study as probably the first scientific paper in this field clearly concludes, that the digestate represents extremely high environmental risk and that is why a pre-treatment of digestate is needed to reduce toxicity and negative environmental impact. The fact, that there is only limited scientific literature on the ecotoxicity of digestate and, moreover, the fact, that available data represent predominantly only acute bioassays signalises the potential gaps of knowledge in this field. Currently probably the only chronic ecotoxicity data for digestate were published by Pivato *et al.* (2016) from the experiments on the earthworm *Eisenia foetida*. In this recent study the matrix-based approach is used with conclusion, that the direct-contact bioassays bring more realistic results. We can fully agree with these conclusions because our data presented in this study are conducted on direct contact bioassays which proved to be sensitive for ecotoxicity testing of digestate.

CONCLUSION

It is remarkable, that ecotoxicity of digestate is so rare topic for scientific papers, even the practical agriculture sees it as real hot topic. Our results confirmed an assumption that residual waste from biogas station may contain a mixture of toxic compounds which can be toxic to plants and invertebrates. Application of digestate showed significant decrease of root growth and photosynthetic activity in dicot plant *S. alba* and fugate had a significant negative effect on survival and reproduction of collembolans. In general, it can be assumed that application of digestate and fugate was not beneficial for plants or zooedaphon.

Moreover, looking for the ecosystem services of agroecosystems, the soil structure, erosion resistance and water retention capacity are important parameters, which are seriously modified by fertilisers like digestate and that is why we conclude, that ecotoxicological properties of digestate should be studied regularly.

Negative effects on survival and reproduction of collembolans as reported in this study could increase the research interest focused on the zooedaphon diversity, reproduction and metabolic activity.

These findings highlighted the ecotoxicological consequences of the application of digestate in agriculture. That is why we do recommend the use ecotoxicological testing in all new anaerobic digestion plants or after changes in technology or material sources for anaerobic digestion process. Ecotoxicological results would be useful in the decision process (dosing, direct use or mixing with other materials, composting etc.) and this decision support will help to both sites – advanced use of this source of nutrients as well as to prevent the degradation of soil structure and fertility.

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