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BARLEY SEED GERMINATION/ROOT ELONGATION TOXICITY TEST FOR EVALUATION OF SLUDGE PRE-TREATMENTS

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ABSTRACT

Application of sludge from wastewater treatment plants (WWTPs) on agricultural land is an approach for nutrient recycling that rise challenges due to recalcitrant and harmful pollutants. In this study we assessed the feasibility of a seed germination test to evaluate sludge ecotoxicity and compared germination responses from two test parameters, root elongation and seed germination (sprouts elongation) of the barley (*Hordeum vulgare*). 2nd objective was to evaluate sewage sludge pre-treatments at batch-scale of sludge samples from two WWTPs using anaerobic digestion, and thermal and ozonation pre-treatments. Glyphosate and eco-labelled soil were used as references. Inhibition of germination of seeds exposed to the glyphosate and sludge was registered and thus germination was successfully applied for sludge ecotoxicity assessment, and using the root elongation as the end-point was both faster and more precise than the sprout elongation. In comparison of pre-treated raw samples and pre-treated and subsequently digested sludge the effects of the pre-treatments were limited and hence, the anaerobic digestion in it-self gave the foremost detoxification.

Keywords: detoxification; growth inhibition; ozonation; sewage sludge; thermal treatment; toxic responses

INTRODUCTION

The medial sludge debate is on-going, with more or less well-founded arguments for or against the spreading of sewage sludge on agricultural land. At the same time, the heavy metal free and readily accessible reservoirs of the important nutrient phosphorus are running low. Digested sewage sludge from wastewater treatment plants (WWTPs) is an abundant and a abundantly available source of various macro nutrients and could thus be used to fertilize agricultural land, and with the implementation of the European Union urban wastewater directive (91/271/EEC) the loads of sludge are even increasing. For future sustainable production of both food and fodder, this source needs to be considered.

The existing EU sludge directive (278/86/EEC) is about to be updated/renewed and a new draft document (CIRCA, 2010) contains several tiers for the use of sludge and biowaste based on its origin according to the precautionary principle and risks assessment as well as practical experiences in EU Member States regarding to sewage sludge quality and outcome of studies of sludge application in agriculture. Thus guaranteeing human health, soil ecosystems and long-term agricultural soil use, and at the same time limiting unnecessary costs. Since then, in 2012 the pan-European screening of emerging pollutants in sludge was completed (Joint Research Center, 2012) calling for further studies on perfluorinated surfactants, and new test

end-points for polycyclic musks and siloxanes. Hence, there's a need for tools to evaluate the direct toxic effects of sludge pollutants to crops, if pollutants are taken up by plants and end up in food or fodder, and if recalcitrant pollutant levels can build up in the farm land and cause problems in the longer run. A combination of chemical and biological analytical methods is needed to assess these problems and protect the farm land, the food production and the crop.

Organic pollutants conveyed via wastewater to the WWTPs may undergo several processes that are important for how they are distributed between the sludge and water, and if they degrade or not, in the treatment plant. Biodegradation and sorption are the domination processes that determine the fate of chemical in treatment plants. Chemical or moieties of chemicals that do not degrade are subject to sorption and dependent of their properties and partly on the properties of the sludge the chemicals will distribute among the sludge and the water phase. Thus, the group of chemicals that are of interest in the case of sludge and sludge application on agricultural land are the chemicals that do not (bio)degrade and do sorb to sludge. Sludge ecotoxicity tests using barley was introduced by Wollan et al. (1978) who found that germination and initial growth was retarded especially by fresh sewage sludge and showed that the effect was connected to the organic part of the sludge rather than to metals.

The first objective was to assess the feasibility of a seed germination test for chemical testing of chemical substances and mixtures (US EPA, 1996) to evaluate sludge ecotoxicity. The second objective was to compare and evaluate efficiency of the pre-treatment methods, anaerobic digestion, thermal treatment and ozonation of the sludges as toxicity reduction methods. The amount of sludge added here is equivalent to 7 tons per hectare per year in the top 25 cm soil, which is the maximum limit for Danish farm land (Danish Ministry of the Environment, 2006).

MATERIALS AND METHODS

Sewage sludges and pre-treatment

The sewage sludge originated from two WWTPs in Southern Sweden, namely Öresundsverket (ORE) in Helsingborg and Sjölanda (SJO) in Malmö. The raw sludges were a mixture of primary sludge and excess activated sludge; 30:70 for ORE and 90:10 for SJO.

Sludge samples were subjected to batch-scale pre-treatment (thermal or ozonation). Thermal treatment was conducted in 0.7 L batch reactors immersed in water baths, where the temperature was raised to 55°C or 70°C within 30 min. The temperature of 55°C was kept for 6 hours and the one of 70°C was kept for 1 hour. Ozonation was done in batch-reactors by bubbling ozone gas through the sludge until the desired concentrations of 20 and 100 mg O₃/g dry matter were attained. Half of the batches were subsequently treated by mesophilic (35°C) anaerobic digestion. Three replicate batches of each sludge type and each pre-treatment were pooled into composite samples. Composite samples were frozen (-18°C) until preparation and testing.

Sample preparation

Pooled samples were thawed, homogenized with a propeller mixer at 1000 rpm for 10 min before sample aliquots were taken out. Samples for barley seed germination tests were lyophilized, whereas for chemical analyses samples were oven-dried.

Seed germination test

Tests were done according to US EPA (1996) using the responses (length) of the roots (plant number $n = 15$) after 72 hours and of sprouts ($n = 21$) after 168 h in beach sand. Beach sand (grain size 0.32-0.71 mm and bulk density 1507 kg/m^3) was washed 3 times in demineralized water and oven-dried at $105 \text{ }^\circ\text{C}$ prior to testing. 300 g sand was mixed with 0.56 g freeze-dried sludge and moisturized by 57 mL demineralized water and divided into three Petri dishes. Five (root elongation) or seven (sprout growth) barley seeds were placed with a forceps on a straight line in each of the dishes and the dishes were capped and sealed with tape and placed vertically with the seed line being horizontal. Germination was facilitated in a dark room with a temperature of $20 \text{ }^\circ\text{C}$. At the end of the test, the length of all roots (3-7 per seed) or sprout was measured with a ruler marked millimetres. The average root length was used for further calculation.

Six or 9 control replicates, (sand without sludge addition), were included in each test series. The herbicide Glyphosate (Riedel-de Haën, 98.4%) (CAS no. 1071-83-6) was used as positive reference substance. Based on the result of a test using a dilution series of glyphosate a 50 mg/l solution was prepared in demineralized water and added as the moistening liquid. Three replicates of the reference substance were included in all test series. Eco-labelled soil for domestic horticulture was used as negative reference.

Physical and chemical analyses

Dry matter (dm) and ignition residue were analysed in duplicates by DS/EN 14346, and metals and inorganic trace elements, including elemental phosphorus (P) and cadmium (Cd) were analysed by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-EOS) in duplicates after drying at $105 \text{ }^\circ\text{C}$ and acid digestion.

Statistical methods

F-test and two-tailed T-test were performed in Microsoft[®] Excel 2010.

RESULTS AND DISCUSSION

Chemical analyses

During the anaerobic digestion organic matter is consumed and therefore the dry matter decreased from an average 5.8 mg/l to 2.7 mg/l for both types of sludges. Analogue, the amount of phosphorous increased from 21 g P/kg dm to 37 g/kg (ORE) and 12 g/kg to 35 g/kg (SJO). The ratio between cadmium and phosphorus changed during the anaerobic treatment yielding an increase from 24 to 31 mg Cd/kg P (ORE) whereas SJO decreased from 97 to 37 g Cd/kg P. These values are both below the limit value of 100 mg Cd/ kg P as stipulated in the Danish Statutory Order (Danish Ministry of the Environment, 2006).

The concentrations of the analysed sludge pass the Danish requirements of sludge quality for four of the metals; chromium, copper, lead and zinc (Cr 100, Cu 1000, Pb, 120, Zn 4000 mg/kg dm), but exceed the limits for cadmium, and nickel (Danish Ministry of the Environment, 2006). For cadmium (Cd 0.8 mg/kg dm) the majority of the samples exceed the limit, while for nickel a few sludges exceed the limit value (Ni 30 mg/kg dm). Neither the content of individual metals nor the total metal content in the sludges were statically correlated with the observed toxic effects.

Seed germination/root elongation test

The result of a preliminary root elongation test showed that the root elongation was inhibited about 50 % at a concentration of glyphosate in the moistening liquid of about 50 mg/L. In 16 later test runs the inhibition relative to the control root elongation was 42% on average.

The seed germination test intended for chemical substance testing responded to sludge samples and the positive reference substance glyphosate, and gave results that could be quantified and repeated. In the eco- labelled soil used as negative control the root elongation was slightly lower than the control and the sprout elongation slightly higher than the control, but none of the responses were significantly different from the control responses (Figure 1).

The precision of the test, based on relative standard deviation (RSD) was 11% for the root elongation controls and 21% sprout elongation controls, whereas for the positive reference the RSD for root elongation was 13%. For the negative control the RSD for root elongation was 16% and 19% for sprout elongation. The roots subjected to sludge exposure had an RSD of 26% whereas the deviation for the sprouts were 89%. Data shows that the method is relatively precise but it should be noted that it was challenging to obtain a uniform mixture of sand and sludge.

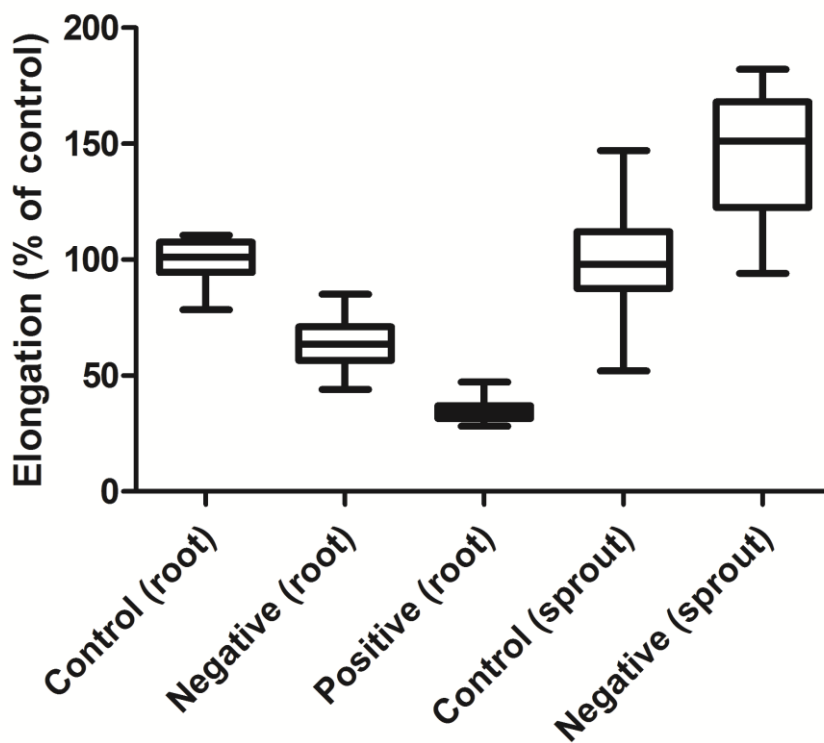


Figure 1. Controls and references, beach sand, glyphosate and eco-labelled soil (whiskers set to min and max, and n = 9 to 72)

As seen for sludge exposed seed the sprout elongation responded more sensitively than the root elongation, although the deviation on results was also higher making it impossible to identify any difference between the different treatment methods. The root elongation endpoint was more precise and more robust than the sprouts elongation, seen by lower variation

(Fig. 1). The growths (cm) was however in favour for the sprouts as control roots grew an average of 3.7 cm and control sprouts grew on average 6.4 cm.

Effect of anaerobic digestion

The raw undigested sludge and the raw anaerobically digested sludge, i.e., not subjected to any pre-treatment, were compared for both root elongation and sprout growth, Figures 2 and 3. It was evident that anaerobic digestion in itself is beneficial for root elongation. ORE had the most toxic raw undigested sludge, resulting in an average root length of 18 % of the control. Raw undigested SJO-sludge had an average root-length of 48 % of the control. Digested sludge from ORE yielded a root length of 79 % of the control whereas SJO reached 91 %. Hence, the raw sludge from ORE is more toxic than the sludge from SJO before digestion but degrades to a relative greater extent after digestion.

For sprout elongation both of the raw undigested WWTP sludges completely hampered the growth whereas ORE after anaerobic digestion showed a growth of <20% of the controls whereas SJOs sludge still hampered sprouting after digestion. Also the pre-treated and digested sludges generally were less toxic than their pre-treated but undigested counterparts, Figures 2 and 3.

The increase in root lengths suggests that significant parts of the pollutants exerting toxic effects towards the barley seeds are degraded when the sludge is digested and this process has thereby reduced the toxicity. This was to be expected, since the beneficial effect of anaerobic digestion has been documented before. Roig et al. (2012) reported that the reduction in toxicity might not be linked to metal content or polycyclic aromatic hydrocarbons (PAHs) but to the content of phenolic substances and/or the stabilization degree of the sludge. This might also be the case here, but in order to determine an eventual linkage, chemical analysis of the sludge is necessary to direct in this direction.

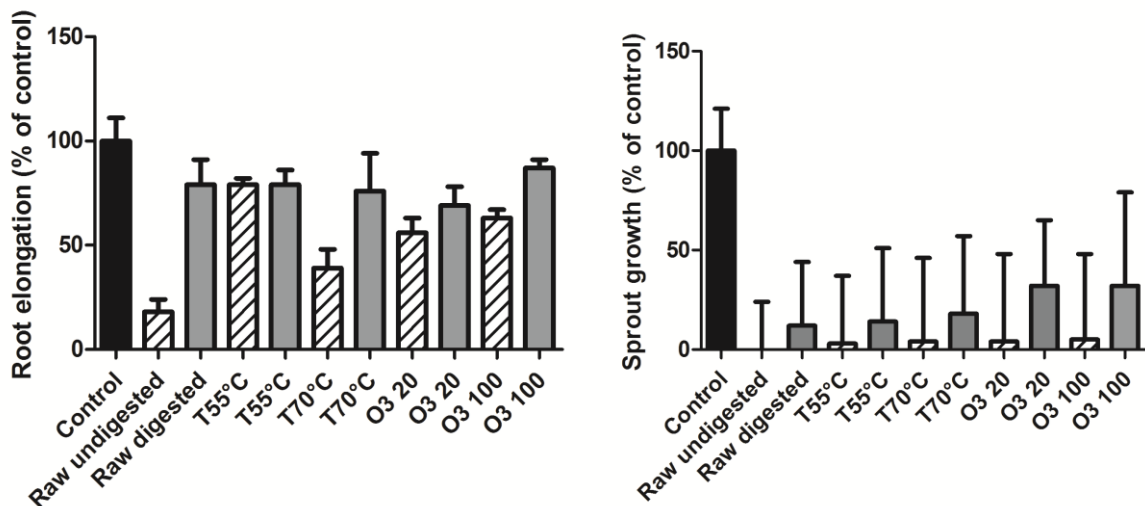


Figure 2. Root elongation and sprout growth for ORE, grey bars showing pre-treated samples and striped bars showing pre-treated and digested samples where the whiskers refers to RSD (%)

Effect of pre-treatments

For the root elongation there is an improvement from raw undigested samples compared to thermally pre-treated undigested samples but the observed detoxification is not better than the

one obtained from merely mesophilic digestion. For sprout growth no statically difference could be seen for the thermal pre-treatment of undigested and digested sludge, beyond a substantial difference between the 70 °C sludges for SJO, where the thermal treatment of undigested sludge seemingly decreased toxicity but this was probably attributed to an outlier, Figure 3.

Root elongation for ORE sludge showed a statistically significant improvement from low (20 mg O₃/mg dry matter) to high ozone dosing (100 mg O₃/mg dry matter) both for the undigested and digested sludges, whereas for SJO the increased ozone dosing hampered the root elongation. The subsequent anaerobic digestion levelled out the observed effects and no statistical difference could be seen from the other digested samples, Figures 2 and 3. The increased effect at higher ozone dosing could indicate that the ozonation chemically degrade the sludge yielding toxic degradation products, these are however seemingly readily biodegradable during mesophilic degradation.

For sprout growth no statistically significant difference could be seen for the low and high ozone dosing, but results for ORE indicate that the combination of ozonation and mesophilic anaerobic degradation is favourable. For SJO root elongation the high ozone dose even seems to provoke the toxicity of the undigested sample, compared to the low ozone dose.

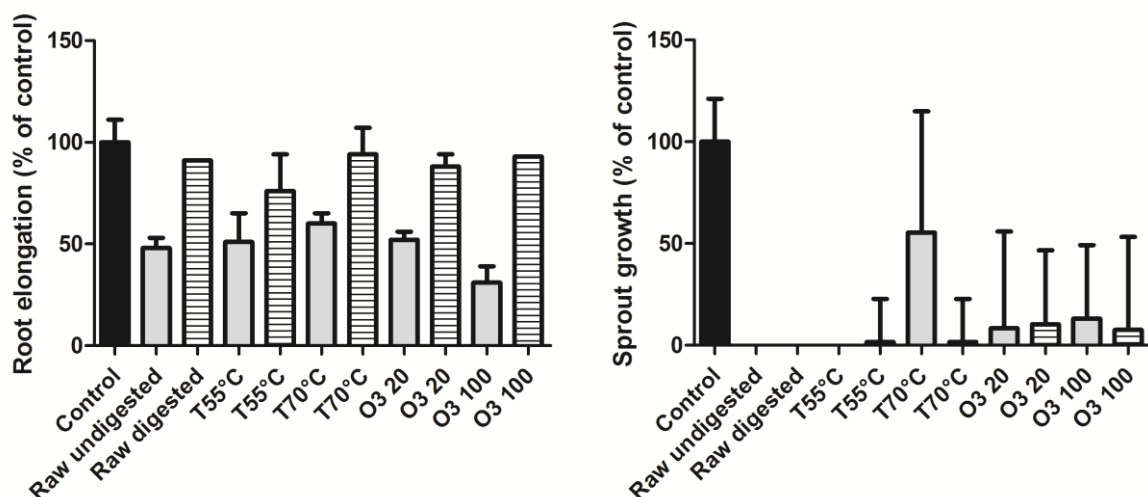


Figure 3. Root elongation and sprout growth for SJO, light grey bars showing pre-treated samples and striped bars showing pre-treated and digested samples where the whiskers refers to RSD (%)

All the sludge samples have shown to have a toxic effect on root and sprout elongation of barley seeds, however in order to fully understand the total ecotoxicological effects of sewage sludge, higher trophic plants have to be tested. The root elongation test does not investigate the potential beneficial effect of the sludge, since in the early stages of plant development the plants do not take up nutrients. The plants receive all the necessary nutrients from the seed material. The germination test will therefore only display the negative effects, and not beneficial which could be seen for the negative sprout control. The inhibition of early root formation, does not necessarily mean that the plant will have a decreased growth altogether.

Renoux et al. (2001) tested a battery of ecotoxicity tests (barely, lettuce, worm, Microtox, cladoceran mortality and SOS Chromotest) for evaluation of a bioleaching technology for

reducing metal content in sludge, and found out that treated sludge was less inhibiting for barley than untreated sludge. The sludge was however added as wet weight and the sprout measured by weight (g) thus hampering the comparability of the approaches. That the source and pre-treatment of the sewage sludge prior to testing were of importance to the outcome was also concluded by Zapusek and Lestan (2011) who assessed the effects of sludge addition on artificial soil. Thus, standardized methods are needed for assessment of potential short-term and long-term effects of sewage sludge spread on fields. As sludge is a complex inhomogeneous matrix of matter that contains natural as well as anthropogenic organic chemicals used or produced in the catchment area of the WWTP. The contents will vary with place as well as time, and basing the tests on dry and homogenized samples are needed to obtain reliable results. Since the toxicity in this study alerted during ozonation and anaerobic digestion it is thought that the majority of the toxic effects are exerted by organic pollutants, which was not included in the chemical analyses. PAHs would have been relevant to include in further studies, as well as other chemical which have an affinity for advanced oxidation.

Digestion temperature may also have an influence on the toxicity of the sludge. Switching temperature from mesophilic to thermophilic anaerobic degradation was found to increase the biological removal of the heaviest PAHs (Trably et al., 2003). And methanogenic degradation was shown to remove 40% of polychlorinated biphenyl's (PCB), where the heaviest PCBs were removed in a relative higher fraction as stated due to higher dechlorination rates (Patureau and Trably, 2006).

CONCLUSION

Barley seed germination/root elongation tests were successfully applied for sludge ecotoxicity assessment: Using the root elongation as the end-point was both faster and more precise than the sprout elongation. The germination tests were able to show a statistically significant difference between raw untreated sludge and digested sludge, but did not show any clear tendencies with respect to thermal treatment or ozonation used as pre-treatment methods. Based on the results described above we conclude that the seed germination/root elongation test can be successfully used for evaluation of sludge ecotoxicity with root elongation being the optimal response parameter to use.

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REFERENCES

- CIRCA (2010) WORKING DOCUMENT SLUDGE AND BIOWASTE 21 SEPTEMBER 2010, BRUSSELS, retrieved from http://circa.europa.eu/Public/irc/env/rev_sewage/library (2012-03-01)
- Danish Ministry of the Environment (2006) Statutory Order on application of waste for agricultural purposes (Bekendtgørelse om anvendelse af affald til jordbrugsformål, Slambekendtgørelsen), BEK nr 1650 af 13/12/2006, retrieved from <https://www.retsinformation.dk/forms/R0710.aspx?id=13056>
- DS/EN 14346:2007. Characterization of waste - Calculation of dry matter by determination of dry residue or water content, Dansk Standard.

- European Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. OJ L 181, 4.7.1986, p. 6–12.
- European Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment, OJ L 135, 30.5.1991, p. 40–52.
- European Standard DS/EN 14346: 2007. Characterization of waste - Calculation of dry matter by determination of dry residue or water content.
- Joint Research Center (2012) FATE SEES Results of a Pan-European Snapshot of randomly taken sewage sludge sample, PowerPoint
http://ec.europa.eu/environment/waste/sludge/pdf/FATE_SEES_pres.pdf, retrieved Oct 2012.
- Patureau, D. & Trably, E. (2006). Impact of Anaerobic and Aerobic Processes on PolyChloroBiphenyl Removal in Contaminated Sewage Sludge. *Biodegradation*, **17**(1), 9-17.
- Renoux, AY., Tyagi, RD., and Samson R. (2001) Assessment of toxicity reduction after metal removal in bioleached sewage sludge *Water Research*, **35**(6): 1415-24.
- Roig N., Sierra J., Nadal M., Martí E., Navalón-Madrigal P. 2012 Relationship between pollutant content and ecotoxicity of sewage sludges from Spanish wastewater treatment plants, *Science of the Total Environment* **425**: 99–109.
- Trably, E., Patureau, D. & Delgenes, J.P. (2003). Enhancement of polycyclic aromatic hydrocarbons removal during anaerobic treatment of urban sludge. *Water Science & Technology*. **48**(4), 53–60.
- US Environmental Protection Agency 1996. Ecological Effects Test Guidelines. OPPTS 850.4200. Seed Germination/Root Elongation Toxicity Test. EPA 712–C–96–154
- Wollan, E., Davis, R.D., and Jenner, S. (1978) Effects of sewage sludge on seed germination. *Environ. Pollut.* **17**: 195-205.
- Zapusek, U. and Lestan, D. (2011) Functioning and toxicity of artificial soil mixtures with metal-bearing sewage sludge. *Ecological Engineering*, 37(12): 1977-1982.