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ORGINAL RESEARCH

Phytotoxicity of sewage sludge from selected wastewater treatment plants - new opportunities in sewage sludge treatment

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Abstract

Purpose The research was focused on evaluating phytotoxicity of sewage sludge from two selected wastewater treatment plants in Czech Republic.

Methods Laboratory experiments were carried out with samples of sewage sludge, which were focused on the evaluation of their phytotoxicity by using the Phytotoxkit^{TM} testing set. Specifically, the inhibitory/stimulating effect was established on the growth of roots from the seeds of white mustard (*Sinapis alba* L.). The chosen proportions of sewage sludge were 10%, 30% and 50%. The test was conducted in three repetitions. Additionally, the research also included a comparison of the effect of compost addition to the sewage sludge and its potentially increased stimulating effect on the growth of roots from the seeds of white mustard (*Sinapis alba* L.). Ratios of compost were 5%, 15%, 25% and 50%. The test was conducted in three repetitions.

Results Stimulating effect on the growth of white mustard (*Sinapis alba* L.) was demonstrated only in samples with the sludge proportion of 10%. The results also show that the addition of compost reduced phytotoxicity of sewage sludge in all three tested ratios (5%, 15%, 25% and 50%).

Conclusion It was found out that the addition of compost resulted in the decreased phytotoxicity of all tested sewage sludge samples, and hence in their suppressed inhibitory effect.

Keywords Sewage treatment plant, Sewage sludge, *Sinapis alba* L., Compost, Environmental problem

Introduction

World population shows an ever increasing trend and is expected to exceed nine billion people by 2050 (Buonocore et al. 2018). The world faces a water quality crisis following out of continual population growth, urbanization, land use changes, industrialization, rapid growth of organic solids waste (OSW) production that includes household solid waste, sewage sludge, green waste, animal manure, increased standard of living, non-sustainable procedures in water use and strategies of wastewater management (WWM) (Soudejani et al. 2019). Developed countries of the world produce more than 30 million Mg sewage sludge (SS) every year and exhibit an increasing trend, too (Herzel et al. 2016).

In the treatment of wastewater, undesirable components are removed in the form of SS as a side solid product, which forms 1–2% of polluted water content. Because SS absorbs approximately 50–80% of the initial pollution, it belongs in the category of hazardous wastes (HW) (Morgano et al. 2018). SS is a heterogeneous suspension of inorganic and organic substances; SS from wastewater treatment plants (WWTP) contains approximately 70% of organic substances and 30% of inorganic substances. The goal of SS treatment is mitigation of its risk to the environment and human health (Jin et al. 2014; Venkatesan and Halden 2014; Qian et al. 2016). Moreover, according to Soudejani et al. (2019) SS might be contaminated with antibiotic resistance genes. In the WWTP, a significant amount of SS is harvested by mechanical and biological treatment (MBT) of wastewater using activated sludge, which captures microorganisms and potentially harmful organic and inorganic substances (Maragkaki et al. 2018; Świerczek et al. 2018).

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In 2017, total production of SS in the Czech Republic (CR) amounted to 178,077 Mg dry matter (DM) (Czech Statistical Office CSO 2019). Average production of SS per capita was 16.8 kg. The production of SS is obviously continually increasing even under ever stricter legislation for SS management (Czech Statistical Office CSO 2019). SS is covered by numerous legal acts in the European Union (EU), such as those regarding water protection, soil fertilization, environmental protection and waste management (WM) (Grobelak et al. 2019).

According to Inglezakis et al. (2014), several Directives have an influence on SS management but the most significant are the Water Framework Directive 2000/60/EC on water protection, Directive 91/271/EEC on urban waste water treatment (WWT), Directive 96/61/EC concerning integrated pollution prevention and control, Directive 99/31/EC on the Landfill of Waste and Directive 86/278/EEC on the use of SS in agriculture (Inglezakis et al. 2014). Contemporary methods of SS disposal such as landfilling or direct application on agricultural soils will not comply with EU regulations in the future (Inglezakis et al. 2014). Moreover, landfilling cannot cover total volumes of produced SS and the low price of SS will not be able to compensate for environmental risks (Maragkaki et al. 2018; Grobelak et al. 2019). In general, it holds that SS handling and disposal represent up to 50% of total costs for the treatment of wastewater (Song et al. 2019).

In the CR, SS (sludge, in which the content of heavy metals (HM), organic pollutants and pathogenic organisms, was significantly reduced by hygienic treatment) is most frequently applied on agricultural soils and composted. Direct application on agricultural soils represents a method of using stabilized SS in the soil either by direct application or in combination with synthetic or natural fertilizers (Górecki et al. 2019). SS contains a considerable amount of organic substances, minerals, trace elements and nutrients (e.g. N, P, K etc.), which act as fertilizers. Disadvantage is, however, also the content of hazardous components in the SS such as pathogenic organisms, HM, persistent organic pollutants (POP) and other substances (e.g. antibiotic resistance genes) (Soudejani et al. 2019), which represent a risk to the environment unless being duly treated or eliminated (Venkatesan and Halden 2014; Liu et al. 2019). Possible environmental risks and threat to human health connected with the presence of HM in the SS used for the direct application on agricultural soils raise a great concern. HM may adversely affect microbial communities and hence soil functionality (Urra et al. 2019).

The procedure of direct application of SS on agricultural soil is governed in the CR by Decree no. 437/2016 coll, on the conditions for using treated SS on agricultural soil issued to the Waste Law. Nevertheless, composting of SS and its subsequent use for reclamation purposes comes recently to the fore. Composting of SS from WWTP is considered environment-friendly technology, which can efficiently decompose organic matter (OM) to stable final product (Du et al. 2019; González et al. 2019). A limiting criterion for using such composts is their possible content of HM (Gondek et al. 2018), pathogenic organisms (Ozdemir et al. 2018) or antibiotic resistance genes (Soudejani et al. 2019). Another method of SS disposal is burning together with the municipal solid waste (MSW). However, this method has not reached much response so far in the CR, mainly due to economic requirements on SS drying. Yet, the method is used because of the primary reason of being phosphorus, which the SS is rich of. Ash from burnt SS serves as a secondary source of phosphates in the manufacture of fertilizers (Zhao et al. 2019). The goal of SS treatment should be the utilization of its beneficial components, e.g. good fertilization effects and the high share of OM, nitrogen (N) and phosphorus (P), or its influence in the improvement of physical, chemical and biological soil properties. A limiting factor, however, remains the phytotoxic influence of SS.

For the purposes of this research, we chose a biological test of phytotoxicity with the use of Phytotoxkit™. The plant material on which the phytotoxicity was evaluated was represented by seeds of white mustard (*Sinapis alba* L.) due to their high sensitivity and previous research (Brtnický et al. 2019; Adamcová et al. 2016).

The goal of this study was to evaluate phytotoxicity of SS from two WWTP, including the assessment of compost amendment effect on reducing the phytotoxicity. We assumed to demonstrate the toxic effect of hygienically treated SS. We also supposed reduced phytotoxicity and stimulating effect on the growth of white mustard (*Sinapis alba* L.) seed roots after the addition of compost.

Material and Methods

Site description and sewage sludge sampling

We chose two WWTP in the Vysočina Region, CR. The first one was in the town with the population of

4,840 inhabitants (all samples marked as A) and the second one with the population of 36,018 inhabitants (all samples marked as B). In both WWTPs, increased P degradation from wastewater is ensured through chemical precipitation with ferric sulphate. Sludge management (SM) is equipped with mechanical dewatering and hygienic treatment of the SS by liming. Assumed method of SS disposal is collaboration with an external composting plant. If the statutory limits are observed, the produced SS can be used also for direct application in agriculture. Each WWTP was subjected to sludge sampling (3 samples). Samples of SS were collected into low-density polyethylene (LDPE) bags by using a plastic scoop and labelled to ease further work with them. Then they were placed in a cooling box and brought to the laboratory of the Department of Applied and Landscape Ecology, Mendel University in Brno for the phytotoxicity tests. The collection of samples was carried out in compliance with Annex no. 6 Methods of sampling, analyses and methods for microbiological determinations to Decree no. 437/2016 Coll, on details in handling biodegradable wastes.

Phytotoxicity assays

For the assessment of the influence of hazardous compounds on the vegetation, we used the Phytotoxkit™ test (Brtnický et al. 2019; Adamcová et al. 2016). The test is based on the cultivation of seeds and on the impact of toxic substance on the root growth. As the most suitable plant, we chose the white mustard (*Sinapis alba* L.). In the toxicity tests, the white mustard (*Sinapis alba* L.) represents cultural crops and higher plants, belonging in frequently used crops because of its high sensitivity to changed environmental conditions (Brtnický et al. 2019) to which it reacts by leaf necrosis or by retarded or even stopped growth (Brtnický et al. 2019; Adamcová et al. 2016; MicroBioTests Inc 2004). Designation of sludge samples from the two WWTPs (A and B) was as follows: lower index expresses the serial number according to the sampling date (samples A/B 1, A/B 2 and A/B 3). Roman numerals I–III express the order of repetition. Soudejani et al. (2019) states that, according to many studies conducted, additives (e.g. zeolite, Ca-bentonite and medical stone) in different ratios may significantly improve SS characteristic (biological parameters). Therefore, we decided to use compost as an additive in three different ratios (5%, 15% and 25%). Marking of test samplers is presented in Table 1. To the best of our knowledge, compost is not commonly used as an additive to the SS.

Table 1 Marking of samplers according to sludge samples collected for the Phytotoxkit™ test

Collected sample	Repeat order	Amount of added sludge in %
A_1 / B_1	I II III	10
A_2 / B_2	I II III	30
A_3 / B_3	I II III	50

The amount of water added to the respective amounts of sludge was calculated by using the method for the establishment of water holding capacity (WHC) of tested substrate according to instructions (MicroBioTests Inc 2004). The chosen proportions of SS were 10% sludge and 90% OECD soil, 30% sludge and 70% OECD soil and 50% sludge and 50% OECD soil. Standard soil (OECD) was used as a control. The test was conducted in three repetitions. A control sample (100% OECD soil) did not contain SS and served as a basis for the establishment of inhibition of white mustard (*Sinapis alba* L.) seed roots. OECD soil consisted of 85% dried quartz sand, 10% kaolin clay, 5% peat and calcium carbonate. In order to detect possible changes in the phytotoxic properties of SS – stimulation/inhibition of the growth of white mustard (*Sinapis alba* L.) seed roots, the test was repeated with the addition of compost.

Test of root growth inhibition/stimulation

Evenly spread and moistened substrate in the lower part of sampler was covered with filter paper. On the upper part of filter paper, ten seeds of white mustard (*Sinapis alba* L.) were placed in even arrangement.

The seeds were placed in a row, at the same distance from one another, approximately 1 cm from the centre of sampler top, always opposite to groove for plant growth (Fig. 1). After the placement of the tested plant seeds, the upper part of the sampler was closed. The samplers were duly labelled with the information on the collection date, percentage of sludge content or sludge and compost content, on the number of test repetition and then photographically documented. Subsequently, they were inserted in vertical position into a cardboard holder and placed for incubation in the Ecocell dryer for 72 hours at a temperature of 25 ±1°C and darkness.

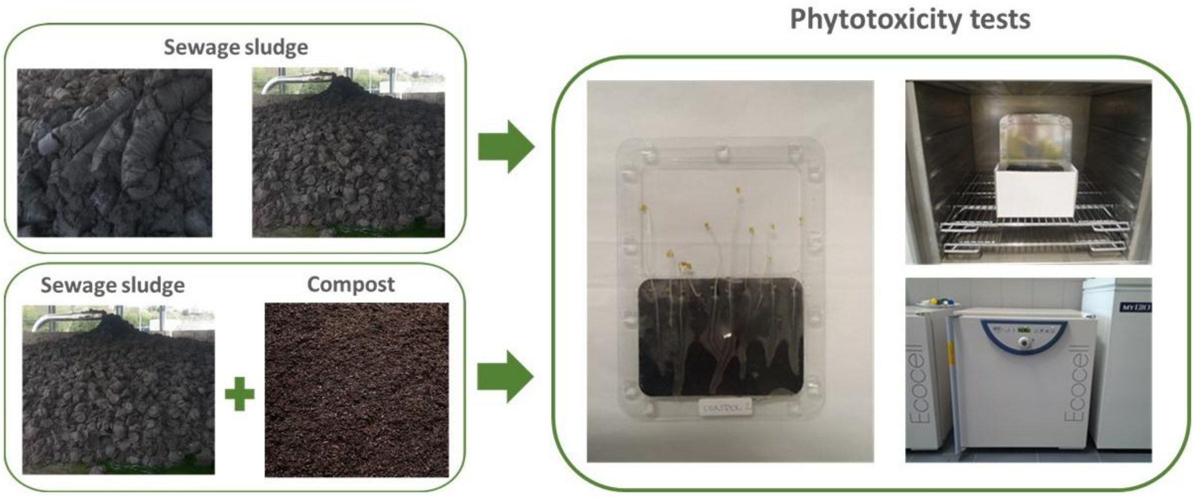

Fig. 1 Establishment of the experiment using the Phytotoxkit™

After the lapse of the set-up incubation time, the samplers were taken out from the Ecocell dryer, photographically documented, and lengths of white mustard (*Sinapis alba* L.) seed roots in the samplers were measured in millimetres. The data were subsequently used to calculate growth inhibition (GI) of tested seeds on the explored samples of sludge and sludge with the addition of compost according to the following equation (1):

$$(A - B) / A \times 100 = GI\ (\%) \qquad (1)$$

A: mean root length in the reference substrate
B: mean root length on the tested substrate (MicroBioTests Inc 2004).

Results and Discussion

GI is given by the following criterion: if GI > 0, we can speak of inhibitory effect. The higher is the number, the more phytotoxic the sample is, and thus inappropriate for being applied on agricultural soils. If GI < 0, we can speak of simulating effect. The lower is the number, the less phytotoxic the sample is, and thus suitable for being applied on agricultural soils.

Samples A_1, A_2 and A_3 exhibited growth inhibiting effect for the sludge proportions of 10%, 30% and 50%. In Samples A_1, the inhibitory effect ranged from 65.24% to 98.95%. Similar values were found also in Samples A_2 (10%, 30% and 50% of sludge), where the inhibitory effect ranged from 62.47% to 99.37%. Samples A_3 exhibited a stimulating effect for the sludge proportion of 10% (-8%), but the sludge proportions of 30% and 50% showed again a strong inhibitory effect 95.81% at a sludge ratio of 30% and nearly 100% inhibition at a sludge ratio of 50% (99.54%). Results of growth inhibition for Samples A_1, A_2 and A_3 are presented in Fig. 2.

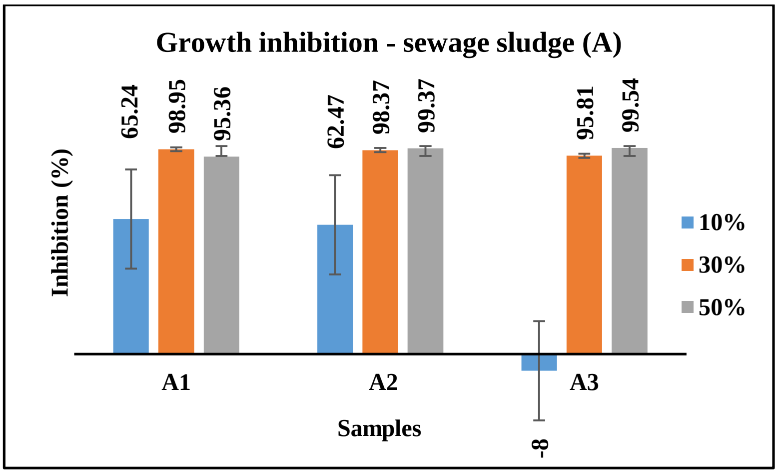

Fig. 2 Growth Inhibition of SS from WWTP – A

Samples B_1, B_2 and B_3 exhibited a similar inhibitory effect for the sludge proportions of 30% and 50% as Samples A_1, A_2 and A_3. However, for the sludge proportion of 10%, they exhibited lower phytotoxicity, which reached stimulating effect in Samples B_2 and B_3 (-21.54% and -31.05%, resp.). Inhibition in Samples B_1 ranged from 3.79% to 97.9%. Samples B_2 exhibited again a strong inhibitory effect for the sludge proportions of 30% and 50% (24.17% and 77.58%, resp.). Samples B_3 exhibited stimulating effect for the sludge proportion of 10% (-31.05%) but for the sludge proportions of 30% and 50%, they exhibited again a strong inhibitory effect (80.81% and 97.68%, resp.). Results of growth inhibition for Samples B_1, B_2 and B_3 are presented in Fig. 3.

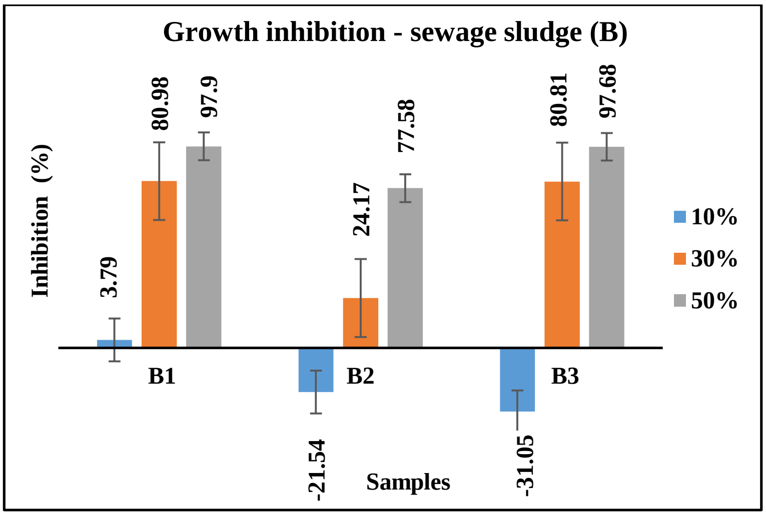

Fig. 3 Growth Inhibition of SS from WWTP – B

The results indicate that using hygienically treated SS from WWTP in direct application on agricultural soil far from being appropriate even at a sludge proportion of 10% due to the inhibitory effect of the SS. Stimulating effect on the growth of white mustard (*Sinapis alba* L.) was demonstrated only in three tested samples with the sludge proportion of 10% (A_1 10%, B_2 10% and B_3 10%).

The research also included a similar test for phytotoxicity determination by using the Phytotoxkit^{TM} with the addition of compost. Samples with 10% (5% sludge and 5% compost) exhibited stimulating effect in all Samples A_1, A_2 and A_3, the values ranging from -14.05% to -27.84%. Sample A_1 10% had stimulating effect after the addition of compost (-17.33%) and exhibited growth inhibiting effect (65.24%). Sample A_2 10% had stimulating effect after the addition of compost (-14.05%) and exhibited GI without the addition of compost (62.47%). Sample A_3 10% had also growth stimulating effect after the addition of compost (-27.84%) and exhibited only low stimulating effect on the growth of roots from the tested plant seeds (-8%); thus, the stimulating effect was improved also in this case. Samples A_1, A_2 and A_3 for the sludge proportion of 30% (15% sludge and 15% compost) exhibited GI effect ranging from 30.03% to 47.50%. These values were once again lower than in Samples A_1, A_2 a A_3 30% (30% sludge), in which the growth inhibition ranged from 95.81% to 98.95%. Similar results were recorded in Samples A_1, A_2 and A_3 50% (25% sludge and 25% compost), in which the inhibitory effect ranged from 44.85% to 53.12%. These values were again lower than those of Samples A_1, A_2 and A_3 50% (50% sludge), in which the inhibitory effect ranged from 95.36% to 99.54% (see Fig. 2 and Fig. 4).

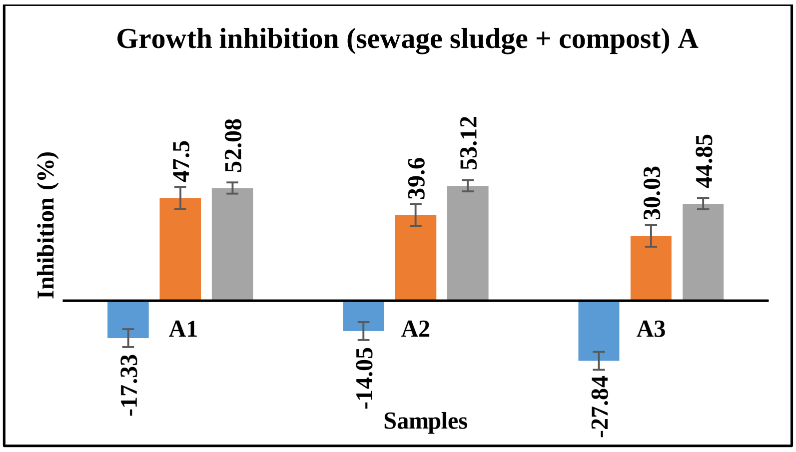

Fig. 4 Growth Inhibition of SS + compost from WWTP – A

The proportion of 10% (5% sludge and 5% compost) in Samples B_1, B_2 and B_3 exhibited again a stimulating effect after the addition of compost with values ranging from -19.1% to -24.11%. Sample B_1 10% had a stimulating effect after the addition of compost (-19.27%) and a growth inhibiting effect without the addition of compost 3.79%. Sample B_2 10% exhibited a stimulating effect after the addition of compost (-24.11%) and without the addition of compost, it exhibited growth stimulation of -21,54%. Similarly, Sample B_3 10% had a stimulating effect after the addition of compost (-19.1%), but exibited higher stimulation without compost (-31.05%). Samples B_1, B_2 and B_3 for 30% (15% sludge and 15% compost) exhibited an inhibitory effect ranging from 31.96% to 43.42%. As compared with Samples B_1, B_2 and B_3 with 30% sludge content, the inhibitory effect was decreased with the exception of Sample B_2 30%, which showed a lower inhibitory effect without the addition of compost (24.17%). Similar results were measured also in Samples B_1, B_2 and B_3 50% (25% sludge and 25% compost). Although these samples exhibited the inhibitory effect too (ranging from 73.42% to 78.85%), the values were lower than those in Samples B_1, B_2 and B_3 50% (50% sludge), where the range of the growth inhibiting effect was from 77.58% to 97.90% (see Fig. 5 and Fig. 3).

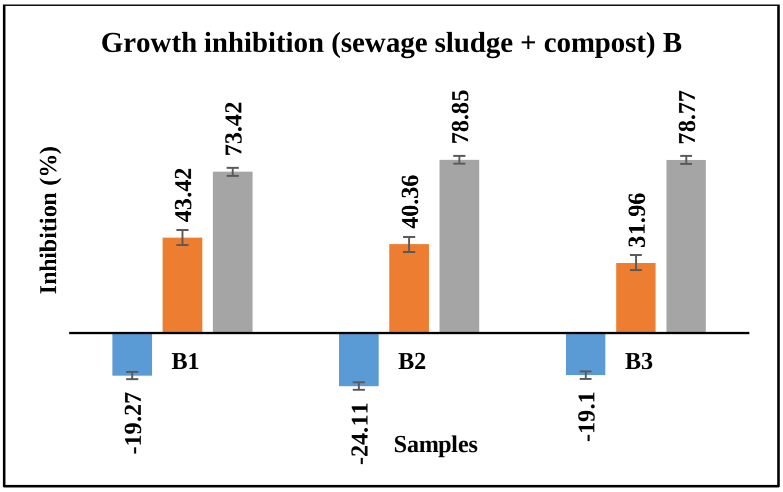

Fig. 5 Growth Inhibition of SS + compost from WWTP – B

The results show that the addition of compost reduced phytotoxicity of SS samples in all three tested ratios and hence suppressed the GI effect of SS both from WWTP A and B.

The problem of the effect of SS on GI and plant growth has been addressed by numerous researchers (MicroBioTests Inc 2004; Oleszczuk 2010; Hu and Yuan 2012; Oleszczuk et al. 2012; Křivánková et al. 2016). Křivánková et al. (2016) dealt with the assessment of toxicity on dewatered and anaerobically stabilized SS with ca. 24% DM and dewatered sludge with ca. 92% DM by using the Phytotoxkit™ testing kit. The tested plant was *Sorghum saccharatum* L. at the ratios of added SS 10%, 25%, 50% and 100%. GI observed in the studied samples ranged from 94.97% to 100%. The results showed that the tested samples were toxic. Adamcová et al. (2016) tested stabilized SS with ca. 24% DM and dewatered SS with ca. 92% DM by using the Phytotoxkit™ testing kit. The tested plant was white mustard (*Sinapis alba* L.) at the ratios of added SS 10%, 25%, 50% and 100%. GI of studied samples ranged from 70.45% to 100%.

According to the above presented results of the assessment of phytotoxicity of SS from the selected two WWTP and in comparison with expert papers tackling this issue, we can state that the phytotoxic effects of SS in all tested samples apparently cause inhibition of seed germination. According to Oleszczuk (Oleszczuk et al. 2012), the addition of SS to soil was shown to reduce the plant seed germination and growth, though phytotoxicity decreased with aging.

Studies have confirmed that the addition of organic amendments, such as agroindustrial wastes and composts from different origins can act on a great variety of processes, leading to improvements in physico-chemical properties and fertility status (Kubana et al. 2015).

SS is a source of not only organic substances but also of minerals, nutrients and trace elements. In the CR, the most commonly used method is exactly its composting and direct application on the soil followed by reclamation. This was the situation in 2017 and also in preceding years. These two methods of SS management totalled 76.6% in managing total production of SS in 2017 (Czech Statistical Office CSO 2019).

The most important criterion in selecting a suitable method of SS handling consists in the costs of its processing. In economic terms, direct application onto the agricultural soil is the most advantageous. Disadvantageous remain high costs of analyses that have to be carried out not only prior to the application but also after the application of treated SS on the soil. Certain problems might be requirements to observe limits for microorganisms and limits for the content of hazardous substances for SS originating from WWTP, which are many a time much stricter than those for classic manures.

Inconvenience of both methods could be also possible lack of interest on the part of owners or land tenants in SS, expressing not only fear of possibly negative consequences of SS application but also of increased administrative burden accompanying this method. More complicated in this case is also the selection of subsequent crop rotation.

Conclusion

In this research, we conducted an experiment by using the biological Phytotoxkit™ kit aimed at the assessment of phytotoxicity of sewage sludge from the selected waste water treatment plants in the Czech Republic and a test focused on the suppression of the phytotoxic effect of sewage sludge through the addition of compost. Based on the above research results, we can state that compost has a capacity for suppressing phytotoxicity of sewage sludge.

The results demonstrate that the hygienically treated sewage sludge has inhibitory effect on the growth of white mustard (*Sinapis alba* L.) seeds already at a ratios of 10%. This is why the sludge is not suitable for direct application on agricultural soils. Stimulating growth was exhibited only by three sludge samples for the proportion of 10%. The addition of compost resulted in the suppressed phytotoxicity of sludge in all tested ratios and hence in the reduced inhibitory effect.

A contribution of this research should be seen in deeper insight into the importance of hygienically treated sewage sludge – not from the perspective of waste but rather from the perspective of raw material. Sludge represents a possibility for improvement of soil quality by adding organic matter, necessary nitrogen, phosphorus and potassium. Advantage is also the capacity of sewage sludge to retain water in the soil as well as the general improvement of physical, chemical and biological properties of soils. This study opens new opportunities in sewage sludge treatment and can be considered one of possible alternatives to various other methods of using sewage sludge.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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