Phytotreatment of sludges (*Phragmites australis*) for their reuse in the environment

B. Ceccanti, V. Bianchi, R. Iannelli, E. Peruzzi and G. Masciandaro

ABSTRACT

The aim of this study is the evaluation of the agronomic characteristics acquired by a phytotreated sludge coming from a wastewater treatment plant (WWTP) located in Tuscany (central Italy). The chemical characterization showed values which are within the Italian legislation limits for mixed composts. From an agronomic point of view, the sludge did not present a level of phytotoxicity, as shown by the germination index (GI% = 77). Furthermore, pathogen compounds are inexistent (*Escherichia coli* <1,000 CFU/g). Different substrates (obtained by mixing the sludge with sandy agronomic soil – 0.5% w/w, 1% w/w, 2.5% w/w and 5% w/w) were prepared in order to evaluate the best mixture performance in terms of water retention capacity and plant growth. No significant differences were observed for all sludge mixtures. Different plants were tested in plots (*Lepidium sativum*, *Cucumis sativus* and *Avena sativa*). The best plant adaptation, measured as dry biomass production, was observed for *Avena sativa*. The results obtained underlined that the phytotreatment of sludge can bring about the transformation of sewage sludges into organic products that are reusable in agriculture, if previously mixed with other appropriate materials and taking into account their heavy metal content.

Key words | bulk density, organic amendment, phytotreatment, sludge treatment wetland, water retention capacity

B. Ceccanti
E. Peruzzi
G. Masciandaro
Institute of Ecosystem Study,
CNR,
Via Moruzzi 1,
56124 Pisa,
Italy

V. Bianchi (corresponding author)
R. Iannelli
Department of Civil Engineering,
University of Pisa,
Via Gabba 22,
56122 Pisa,
Italy
E-mail: *v.bianchi@ing.unipi.it*

INTRODUCTION

Given the need for sustainable development, the features and disposal situations of municipal sludge and its influence on agricultural and urban development is nowadays being seriously taken into consideration. It is widely recognized that the land application of municipal sludge is important and urgent for urban and agricultural sustainable development. When sewage sludge is applied to agricultural land, the effects of organic matter, nitrogen, and phosphorus are the characteristics that are of main interest (Bruinsma 2003). Organic matter can improve the structure and water-holding capacity of poor soils and the nitrogen and phosphorus in sludge have a fertilizing value. Furthermore, EU Directive (86/278/CEE) aims to establish certain initial community measures regarding soil protection and considering that sewage sludge can have valuable agronomic properties there is a clear justification in encouraging its application in agriculture. The key factor in sludge application to land often lies in the presence of non-stabilized organic substances, which can be the source of environmental health problems, or in the presence of toxic and non-degradable compounds (Basta *et al.* 2005).

The phytotreatment of sludge using wetland treatment represents an optimal and efficient solution in terms of volume reduction and rapid stabilization (Uggetti *et al.* 2010), obtaining a matrix similar to an agronomic amendment, from a chemical–physical and biological point of view. In the process of phytotreatment, plants play a key role in sludge consolidation due to the evapotranspiration process (Nielsen 2003), which decreases the residual water content of fresh sludge, and increases the biochemical activation of the microbial population in the root zone (Masciandaro *et al.* 2000). The phytotreated sludge is basically composed of stabilized organic compounds and some mineral elements that change the phytotreated sludge into a compost-like material, instead of a sewage sludge. Phytotreated sludge can be considered also less contaminated by heavy metals than a non-treated sludge, as demonstrated by some recent studies (Peruzzi *et al.* 2010).

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It has been demonstrated that the process of phytostabilization is able to transform sewage sludge into a substrate reusable in the environment by improving its chemical, biochemical and physical characteristics (Bianchi *et al.* 2010).

The main objective presented in this paper was the evaluation of the agronomic characteristics acquired by a phytotreated sludge coming from a WWTP located in Tuscany (central Italy). With this aim, parameters usually involved in compost characterization (such as total and soluble elements, bulk density and biological tests) were taken in account. In order to ensure its suitability for use as an agronomic organic amendment, germination and growth tests on grass species were performed in the laboratory in order to evaluate the toxicity of the sludge, having previously mixed it with appropriate sandy soil.

MATERIALS AND METHODS

Materials

The phytotreated sludge comes from the Oratoio wetland treatment pilot plant (Pisa, central Italy). The plant treats 1,690 m^3/yr of sludge from an urban wastewater aerobic treatment plant (10,000 EI). The mean sludge load rate resulted in approximately 45 kg dry weight (dw) m^{-2} yr^{-1}. This paper reports results after 48 months of operation.

A sandy soil (agronomic soil, poor in organic compounds) was used for the preparation of the different mixtures for seedling growth tests and for the water retention curve. The sludge: soil ratio (w/w as dry weight) was chosen on the basis of nitrogen content. The mixtures chosen were 0, 0.5, 1, 2.5, 5 and 100% of sludge content, corresponding to a content of total nitrogen of 0.300, 0.320, 0.340, 0.400, 0.500 and 4%, respectively.

Chemical characterization

Total organic C (TOC) and total N (TN) were determined by dry combustion with a RC-412 multiphase carbon and an FP-528 protein/nitrogen determinator, respectively (LECO Corporation). Humic carbon was measured in pyrophosphate 0.1M pH 11 extract (1:10 w:v) and determined by dichromate oxidation (Yeomans & Bremner 1988).

Heavy metals were determined after acid digestion (HNO_3:$HClO_4$, 5:2) with atomic absorption spectrometry (Contraa HR-AAS, Analytical Jena).

Physical parameters

Soil bulk density (BD, g/cm^3) was measured on undisturbed cores (Blake & Hartge 1986). Particle density (PD, g/cm^3) was measured with the gas pycnometer method (Stolz *et al.* 2010). Porosity (%) values were calculated as follows P% = (1- bulk density/particle density)/100.

Germination test

A germination test was carried out using seeds of *Lepidium sativu*, a plant that is extremely sensitive to toxic substances. Ten seeds were tested in Petri dishes using 3 ml of 1: 10 (w/v) phytotreated sludge – water extract (controls were tested with distilled water). The Petri dishes were kept at 20 °C in the dark until germination of the seeds. The germination index (GI) was calculated after 72 h. The index is calculated as follows: GI% = P(T/C), where P is the mean percentage of seed germination, and T and C are mean lengths of the root in the treatment and in the control, respectively (Hoekstra *et al.* 2002).

Determination of *Escherichia coli* content

10 g of fresh samples were added to 90 ml of water. 1 ml of a serial dilution in water was transferred into a Petri dish and covered by tryptone bile X-gluconoride agar. After incubation at 44 °C for 24 h, typical blue-green colonies were transferred into a Petri dish and covered by tryptone soya agar and then incubated at 35 °C for 48 h. The colonies grown were transferred into tryptone water at 35 °C for 48 h. Then the presence of *E. coli* was confirmed by Kovac's reagent for indole. Concentrations were expressed as CFU/dry matter (ISO/DIS 16649-2 2001).

Seedling growth test

The different soil–sludge mixtures (100 g dry weight equivalent) were put into plastic pots. Ten seeds for each plant species (*Lepidium sativum*, *Cucumis sativus* and *Avena sativa*) were sown uniformly in each pot. After 21 days the seedling shoots were cut above the surface and the fresh biomass was immediately assessed. The dry biomass was measured after oven drying at 80 °C for 72 h. The treatment with 0% sludge was considered as a control thesis for each plant species. The biomass production was reported as a percentage (Wang *et al.* 2001).

Determination of water retention curve, air capacity, available water and non-available water

The water retention curve (WCR) is the relationship between the water content and the soil water potential. It is used to predict the soil water storage, water supply to the plants (field capacity) and soil aggregate stability. WRCs were analyzed in the laboratory after an initial saturation of the different mixtures (pot of 5 cm diameter, 2.5 cm height) by a stepwise desorption using the standard suction plate apparatus and pressure chambers for pressure head values corresponding to values between pF 0 and pF 4 (Teepe *et al.* 2003). A pF equal to 2.5 represents field capacity and a pF value of 4.5 represents the wilting point. Pore space of the sludge was divided into air capacity (AC) ($0 < PF < 2.5$), available water (AW) ($2.5 < PF < 4.5$) and non-available water (NAW) ($pF > 4.5$). ACs, AWs and NAWs were calculated for each of the WCRs and subsequently stratified according to texture and bulk density classes (Teepe *et al.* 2003).

Statistical analysis

All results are the means of determinations made on three replicates. One-way analysis of variance (ANOVA) was calculated using Statistica (Statsoft, Inc) to test the effect of different sludge percentages on water retention capacity and biomass production. The significant levels reported ($p < 0.05$) are based on the student's t-distribution.

RESULTS AND DISCUSSION

Phytotreated sludge characterization

The phytotreated sludge was compared with a mixed compost, referring to the Italian limit values (D.lgs 75/2010, ex D.Lgs 217/2006). Values obtained for the sludge are satisfactory (Table 1), supporting the possible utilization of the phytotreated sludge, previously mixed with sandy soil, in agriculture, as far as pH, and nutrients (TOC, C/N, humic carbon) are concerned. The crucial point, however, which allows the safety of sludge utilization for agricultural purposes, is represented by heavy metals. In this study Cu (563 mg/kg) and Zn (1,532 mg/kg) resulted in much higher values than the legislative limit (D.lgs 75/2010: Cu 230 mg/kg; Zn 500 mg/kg). Several authors (Fuentes *et al.* 2006; Cai *et al.* 2007) have reported results on the bioavailability of heavy metals in sludges, while other studies (Peruzzi *et al.* 2010) regarding sludge treatment wetlands show, indeed, that the stabilization process in reed bed systems helps the immobilization of heavy metals in metal-organic fractions, making them unavailable for plant uptake in expectation of the land application of sludges for agricultural or environmental purposes. In fact, the percentages of heavy metals not bioavailable were 95, 92, 74, 67 88 and 59%, respectively for Cr, Cu, Ni, Cd, Pb and Zn (Peruzzi *et al.* 2011).

Table 1 | Phytotreated sludge characterization in comparison with a mixed compost (D.lgs 75/2010, ex D.Lgs 217/2006). Values are means of 3 replicates ($p < 0.05$)

		Phytotreated sludge	D.Lgs 75/2010
pH		5.95 ± 0.29	6–8.5
TOC	%	28.1 ± 0.98	>25
TN	%	4.16 ± 0.45	–
C/N	–	6.76	<25
Humic carbon	%	24 ± 4.1	>7
Cr	mg/kg dw	159 ± 13.4	–
Cr (VI)	mg/kg dw	Not determined	0.5
Cu	mg/kg dw	563 ± 33.5	230
Ni	mg/kg dw	30 ± 1.05	100
Cd	mg/kg dw	1.42 ± 0.19	1.5
Pb	mg/kg dw	30.1 ± 3.43	140
Zn	mg/kg dw	1,532 ± 264	500
Hg	mg/kg dw	Not determined	1.5
Germination Index	%	77 ± 4.38	>60
E. coli	CFU/g	<1,000	<1,000

The phytotreated sludge seemed to be non-toxic in terms of seed germination (GI% = 77 versus control with distilled water 100%); Sellami *et al.* (2008) reported that GI values, in compost samples, of 80% were phytotoxin-free and considered to be completely mature. In addition, with regard to the presence of pathogens, the absence of *E. coli*, as an indirect measure of pathogen organisms (<1,000 CFU/g), indicated that the phytotreated sludge could be considered sanitized. Moreover, throughout the experimentation period (48 months), no presence of *Salmonella* was observed in the wetland treatment (Personal communication by Acque S.p.A.).

Effect of bulk density on the water retention curve and other physical parameters

The bulk density gives an indication of soil structure and quality (Hakansson & Lipiec 2000), extendible to sludge with the aim of knowing the degree of compactness.

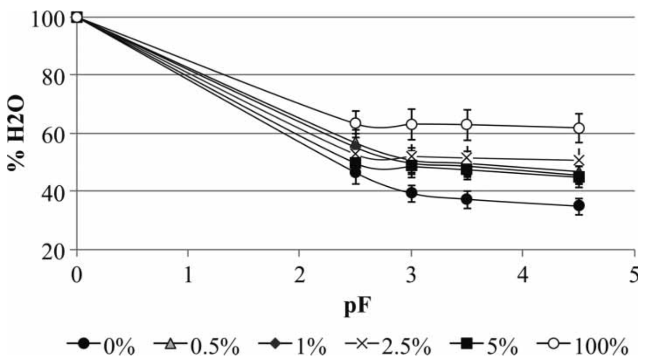

Figure 1 | Water retention curves for each sludge mixture (0, 0.5, 1, 2.5, 5 and 100% w/w). Values are means of 3 replicates ($p < 0.05$).

When using bulk density or porosity to characterize the state of compactness of soils with respect to crop growth, it is well known that the crop response curves may be very different for soils with different texture and water content. Extending this concept to the phytotreated sludge to be used in agriculture, the addition of different percentages of sludge did not seem to affect water retention capacity, as expressed by the water retention curve for the sludge–soil mixtures of 0.5, 1, 2.5 and 5% (Figure 1). This means that the possible water storage and water supply to plants is not affected by such small amounts of sludge added to the soil. A greater difference was observed instead comparing the sandy soil (0% w/w) with the phytotreated sludge (100% w/w). As expected, the sandy soil represents a good solution to be used in the field (Table 2), because a great amount of water (gravitational water) was lost through lixiviation (AC = 53.69%) for the sandy soil (0% w/w), while the plan-available water capacity was insufficient (AW = 1.35%) in the case of only sludge (100% w/w). Similar levels of AW were observed by Al Naddaf *et al.* (2011) for pig manure compost.

Several reports in the literature indicate that a porosity of less than 10% (v/v) represents a critical limit of soil aeration and rootability (Hakansson & Lipiec 2000). In the case of compost, total porosity range is usually 70–97% (Genevini 1998). Referring to the phytotreated sludge, the values obtained for the total porosity were similar to those usually found in good quality compost, usable in agriculture (Table 2). Starting from a sandy soil, a sludge addition of 5% (w/w) produced a good porosity value (70%). As found by other authors (Grossbellet *et al.* 2011), the bulk density decreased significantly, while the total porosity increased with increasing doses of sludge compost applied to the soil.

Agronomic properties of the phytotreated sludge

The seedling growth test was performed to detect the phytotoxicity and the biomass growth of selected plant species, planted on different substrates, as a function of sludge content (Figure 2). The phytotreated sludge (100% w/w) represented a suitable substrate for growth of *Avena sativa* (Gong *et al.* 2001), compared with the other different sludge mixtures. The higher concentration of sludge in the mixture (5 and 100% w/w) was however restrictive for *Lepidium*

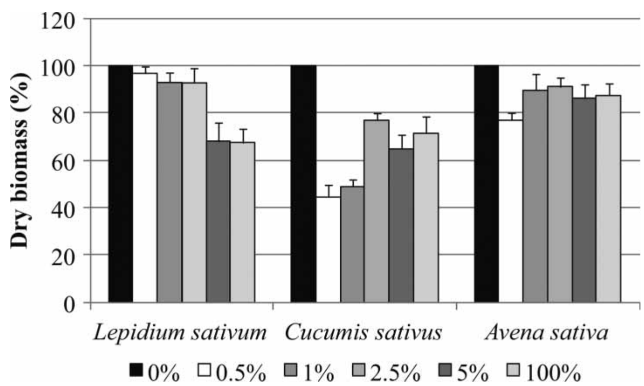

Figure 2 | Seedling growth test repeated for each sludge mixture (0, 0.5, 1, 2.5, 5 and 100% w/w). Values are means of 3 replicates ($p < 0.05$).

Table 2 | Physical parameters of sludge and sludge air capacity available water and non-available water. Values are means of 3 replicates ($p < 0.05$). AC = Air capacity ($0 < pF < 2.5$); AW = available water ($2.5 < pF < 4.5$); NAW = not available water ($pF > 4.5$)

Mixture (w/w)	Particle density (g/cm^3)	Bulk density (g/cm^3)	Total porosity (%)	AC (%)	AW (%)	NAW (%)
0%	1.39 ± 0.03	0.68 ± 0.02	51	53.69 ± 3.71	11.53 ± 0.92	34.78 ± 2.99
0.5%	1.30 ± 0.08	0.57 ± 0.04	56	43.32 ± 4.54	9.80 ± 0.78	46.88 ± 3.76
1%	1.33 ± 0.09	0.60 ± 0.03	54	44.68 ± 4.43	9.50 ± 0.76	45.81 ± 3.67
2.5%	1.35 ± 0.08	0.47 ± 0.02	65	47.25 ± 4.23	1.98 ± 0.15	50.78 ± 4.08
5%	1.90 ± 0.02	0.57 ± 0.03	70	50.22 ± 3.99	4.76 ± 0.38	45.02 ± 3.61
100%	1.06 ± 0.05	0.13 ± 0.01	88	36.86 ± 5.06	1.35 ± 0.10	61.79 ± 4.96

sativum growth. *Cucumis sativus* dry biomass production, even if resulting lower than in the control soil, seemed to be stimulated by an increasing concentration of stabilized sludge. A similar result has also been reported by Oleszczuk (2008) for sludge compost and *Lepidium sativum*. There is very little information in the literature about the influence of phytotreatment on the toxicity of sludge. The decrease observed in *Lepidium sativum* and *Cucumis sativus* could be the result of the presence of toxic organic compounds or heavy metals (Oleszczuk 2008). However, previous studies (Nielsen 2005; Peruzzi *et al*. 2011) have highlighted that the stabilization process occurring in sludge treatment wetland decreases significantly the level of organic pollutants. In fact, after 48 months the content of linear alkyl benzene sulfonates (LAS = 78 mg/kg), nonylphenol ethoxylates (NPE = 48 mg/kg), and di(2-ethylhexyl)phthalate (DEHP = 4 mg/kg) decreased respectively by 95, 60 and 90% with respect to the activated conventional sludge.

CONCLUSIONS

The different laboratory experiments and characterization performed on the phytotreated sludge coming from the treatment wetland plant of Oratoio (Pisa, Italy), were carried out to test the acquired agronomic characteristics of the sludge. The aim was to consider the possible utilization of the phytotreated sludge in agriculture as an organic amendment. With this purpose, a comparison was made with legal requirements for mixed compost. Results showed that the values detected for chemical and physical parameters fell within the range required by Italian legislation, with the exception of Cu and Zn content. Furthermore, the water retention capacity curves successfully confirm the possible utilization of the phytotreated sludge in agriculture as an organic agronomic amendment, if previously mixed with other materials (e.g. soils, lignocellulosic compounds, etc.). However, from a practical point of view, the phytotreated sludge still needs to be previously tested in order to evaluate which vegetal species could be inhibited by this material.

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