

Biosolids Soil Application: Why a New Special on an Old Issue?

Guest Editors: Silvana I. Torri, Rodrigo Studart Corrêa, Giancarlo Renella, Alejandro Valdecantos,
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Editorial

Biosolids Soil Application: Why a New Special on an Old Issue?

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1. Introduction

The term biosolids was introduced in the early 1990s by the Water Environment Federation to define the solid, semisolid, or liquid residues generated during primary, secondary, or advanced treatment of domestic and industrial sanitary sewage sludge through one or more controlled processes that reduce pathogens and attractiveness to vectors. Although the term biosolids is related to the definition of sewage sludge, it only refers to the portion of sewage sludge that has undergone adequate treatment to permit its land application, such as anaerobic and aerobic digestion, composting, thermal or air drying, and alkaline stabilization with additives such as lime or cement kiln dust.

An extensive body of scientific evidence has recognized that biosolids should not be seen just as a waste, but could be beneficially recycled as a soil conditioner because of its stable organic matter matrix. The fertilizer value of biosolids has also been extensively researched (e.g., [1, 2]). Consequently, a worldwide massive biosolids' use as soil conditioner and fertilizer arose in the early 1990s [3]. Whilst recognising its significant value as a resource, recycling sewage sludge to agricultural land requires a careful management to avoid potential negative impacts on the environment from chemical contaminants. Among them, potentially toxic elements (PTE) and organic chemicals are usually transferred to biosolids during waste water treatment process. As a consequence, in the 1990s there was pressure in some European countries to ban the use of biosolids for agricultural use.

Austria, Sweden, and Switzerland, among others, introduced the ban. Other countries developed strict sanitary and environmental regulations to ensure biosolids safe use for land application (e.g., European Directive 86/278/EEC, US EPA's Part 503). Very importantly, all the EU acts have been inspired by the fact that land application of biosolids improves soil fertility and has an important role in closing nutrient cycles, especially phosphorous (P). In addition to these benefits, land application of biosolids has the advantage of increasing carbon (C) sequestration and thus contributes to C mitigation measures to meet European climate change commitments under the Kyoto Protocol [4, 5]. For this reason, the restoration of degraded sites, commonly showing low soil fertility and poor physical properties, is a raising option as its final disposal [6].

2. Biosolids Soil Application: Agronomic and Environmental Implications

In the last decades, the cooperative activity of research institutions, industry, and authorities have led to the development of different technologies that dramatically improved biosolids quality to abate harmful organic and inorganic pollutants, together with pathogens control, so as to reach the highest certification degree for biosolids land application. The absence of manifest adverse effects on soils, crops, and humans is probably key point leading to a sustainable use of sludge and to its public acceptance. We believe that this

special issue, focused on the study of the potential benefits and risks related to land application of biosolids, though not exhaustive, sheds new light on this crucial topic.

The review made by Q. Lu et al. provides information on recent research progresses and regulation efforts regarding land application of biosolids in the USA, including forms and types, nutrient value, and environmental and health concerns.

S. Torri and R. S. Correa deeply reviewed the different aspects that control downward movement of biosolids-borne potentially toxic elements (PTEs) in soils. The paper describes the key issues of the possible problems generated by this practice related to cadmium, copper, chromium, lead, nickel, and zinc vertical mobilization. Soil pH, soil texture, and increased soil-dissolved organic matter concentration as a result of land application of biosolids may lead to the formation of organo-PTEs complexes that may result in a substantial movement of PTEs into subsoil horizons, with the risk of ground water contamination.

Controlling plant pests is a relevant issue of potential use of organic amendments. S. Kouki and colleagues tested, in vitro and in vivo, the effectiveness of a compost of vegetable waste and *Posidonia oceanica* mixture (VPC) in controlling *Fusarium* wilt in tomato. They found a significant reduction of damping-off incidence on tomato plants using the VPC amendment and it may be attributed to the effect of polyphenols and other chemical compounds. The study showed that microorganisms isolated from compost are able to suppress plant disease. Some VPC bacteria (*Bacillus*, *Burkholderia*, and *Pseudomonas*) play an antimicrobial activity against *Fusarium*, probably due to the antibiotics secreted by bacteria, which may confer fungicidal properties.

The paper of C. A. Christofolletti et al. evaluated the possible toxic effects of biosolids sample, under laboratory conditions, using diplopods (*Rhinocricus padbergi*) and plants (*Allium cepa*) as test organisms. The data obtained demonstrated that biosolids raw sample had genotoxic potential for *Allium cepa* root tip cells. In the diplopods exposed to biosolids sample, epithelium disorganization in the midgut and a reduction of the volume of the hepatic cells were observed after 7 days of exposure. After 30 days, the animals are still showing a reduction of the volume of the hepatic cells, but in minor intensity. *Allium cepa* analysis showed genotoxicity, but this effect was reduced after 30 days of bioprocessing by diplopods.

N. E. Hansen et al. compared composted municipal biosolids and dairy manure products to stabilize roadside soils. They reported that the greatest total and soluble phosphorus and total nitrogen losses in runoff water occurred from soils where the compost and woodchip mix were applied. In contrast, nitrate-nitrogen losses in runoff were similar when compost was incorporated in the soil or applied in the woodchip mix. Compost source affected the nutrient losses in runoff. While the composted municipal biosolids added greater nutrient loads to the soil, less nutrient loss in runoff occurred.

A. Branzini et al. studied the speciation of Cr, Cu, and Zn in soils amended or not with compost and highlighted how biosolids application was able to change metal

speciation. The complex soil-metal chemistry system suggests that the application of a single organic amendment may not effectively reduce the solubility of these elements because of dissimilar chemical properties. Therefore, different organic/inorganic combinations have to be considered.

S. Shaheen et al. examined the role of different organic and inorganic amendments for stabilizing industrial and domestic biosolids in two contrasted types of soils, as well as the effect of the time of incubation and mixing rate. In general, stabilized products increased soil Olsen-P and reduced the availability of copper and zinc, especially bentonite, sugar beet factory lime, coal fly ash, rice straw, and water hyacinth.

The responses of peanut plants to soil application of Pb-contaminated sludge in comparison to mineral fertilization were evaluated by F. Camilotti et al. The outcome from this greenhouse experiment showed that the used sludge worked as P source to young peanut plants and that high Pb concentrations in the sludge did affect the overall plant growth. However, the increased Pb solubilisation, likely induced by the plant root activity, along with the higher Pb content in plant shoots and pod husks, may pose risks to human health as peanut kernels and their products may mix up with husk fragments, the industrial processing of peanuts.

Taking into account the fact that land application of biosolids should nowadays meet the international protocols of pedosphere, atmosphere, hydrosphere, and biodiversity protection, there are a number of emerging issues that require consideration and attention.

3. Needs and Gaps

3.1. Needs

Wider Comparison of Effects of Sludge Treatments on Soil Properties and Functions. Composting and thermal drying are among the commonly used techniques for achieving concentration, sanitation, and stabilization of sludge. However, it is well established that different sludge after treatments will lead to different effects on soil properties and on soil microbial activity. Generally, composting has lower but more persistent stimulatory effects than raw or that thermally dried sludge [7, 8]. This is likely due to treatment-induced changes in the organic matter degradability, but it might also indicate the presence and release of toxic substances from the sludge matrix. Moreover, a subject still not fully investigated is the potential of selected biosolids for maintenance and protection of soils under short rotation forestry for biomass production, where fast nutrient removal needs to be balanced.

Monitoring Persistent Dangerous Substances Such as Potentially Trace Elements or Organic Pollutants, together with the Identification of New Classes of Micropollutants. For example, nanomaterials present in wastewater or intentionally used for biosolids stabilization [9] likely accumulate in biosolids at concentrations in the order of mg kg^{-1} . These nanomaterials may negatively impact plants, soil microorganisms or soil ecological functions. It is noteworthy to underline that

most of the use and release of nanoparticles are not fully regulated yet.

Monitoring Pathogens Such as Prions Which May Be Present in the Original Biosolids and Persist in Soils. While the biomolecular of methods for the screening of pathogens in wastewater, sludge, and soil are well established, a specific aspect of pathogens in soils is their viable but noncultivable (VBNC) status, induced by the paucity of nutrient and low water availability in soils. Because the VBNC phenomenon has been described also for pathogenic bacteria [10], more studies are surely needed to better understand the potential virulence of human pathogens during their transit from the sludge matrix to the soil environment. From this point of view, new potent bio-molecular approaches such as proteomics may be used for studying the functional structure of the different biosolids, following the example of the pioneering study of Ehlers and Cloete [11] and Wilmes and Bond [12].

3.2. Gaps

Effectiveness of Recovery of Degraded Marginal Lands through Biosolid Amendments, with Lower Nutrient Levels, and the Construction of Technosols from Mine Tailings through Biosolids amendment: from this point of view there is scarce published scientific evidence on the speed and direction of pedogenesis.

Improvement of Phosphorus Recycling and Availability on the Biosolids. Phosphate recycling is of great importance for primary productions not only because of its relatively low availability in soils, but also because at the current rate of extraction and use, the estimated P reserves may run out in 100–200 years [13].

Biosolids Mining. Biosolids incorporation into soils generally trigs a potent metabolic response of soil microbial communities, not fully explained by the nutrient loads. It has been reported that organic wastes may contain bioactive molecules such as phytohormone precursors [14], and that the presence of trace amounts of such substances stimulates soil microbial activity [15]. Therefore, more research is needed on biosolids biological and chemical prospection for the search of biologically active substances, which may act as extracellular signals for gene activation or signal molecules within the soil microbial communities, which might be concentrated and used for improving the beneficial effects of biosolids in agricultural production.

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Research Article

Influence of Stabilized Biosolids Application on Availability of Phosphorus, Copper, and Zinc

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The main aim of this study was to examine the influence of sewage sludge (SS) and stabilized SS application on Olsen-P and DTPA-extractable Cu and Zn in relation to soil type, sewage source, mixing rate and incubation time. Two different SS were mixed with amendments by mixing rates 10 and 25%. These amendments include coal fly ash (CFA), bentonite (B), sugar beet factory lime (SBFL), calcium carbonate, rice straw (RS), water hyacinth (WH), and cotton stalks (CS). Treated and untreated SS had been applied to fluvial and calcareous soil with application rate 2.5% and incubated for one and two month. After incubation, soil samples were analyzed for Olsen-P and DTPA-extractable Cu and Zn. Application of SS increased significantly Olsen-P and DTPA extractable Cu and Zn compared to control. Application of stabilized SS increased significantly Olsen-P, with high increasing rate with SBFL and WH-stabilized SS. Stabilized-SS decreased significantly Cu and Zn availability compared to mono SS treatment. Bentonite-, SBFL and CFA-stabilized SS were the highest among inorganic treatments for reducing available Cu and Zn either in fluvial or calcareous soil, while WH and RS-stabilized SS treatment were the most suitable organic ones for reducing DTPA-extractable Cu and Zn.

1. Introduction

Waste management is recognized as an important issue in modern societies, and waste recycling is encouraged as an alternative to stockpiling and incineration. The application of sewage sludge or composts of several origins as amendments to agricultural soils is an economically attractive waste management strategy promoted by scientists and regulating organisms and is a common practice since many years [1, 2]. Improvement of soil properties is among the benefits of sewage-sludge application to agricultural land. In association with its high organic matter content, sludge contains also appreciable amounts of macro- and micronutrients with significant fertilizer replacement value [1]. These beneficial effects make sludge application an attractive option for eroded soils of dry Mediterranean climates that usually have low organic matter content. However, land application of SS has been limited by its enriched pathogens and heavy metal contents. Excessive levels of trace elements introduced to soil by SS can lead to elevated uptake of trace elements by plants, which will cause damage to plants and effects on

human health upon consumption of crops grown on the soil [3]. So, the study of effective methods for trace element removal from sludge is very important in order to minimize prospective health risk after application.

Recently, increased attention was paid to the sludge stabilization/immobilization process aiming to minimize the mobility of trace elements by using various additives, due to compliance to more stringent regulations issue in USA and European Union. Stabilization/immobilization refers to techniques that chemically reduce the hazardous potential of a waste by converting the contaminants into less soluble, mobile, or toxic forms. Sewage sludge can be treated in a matrix of different organic and inorganic materials [4, 5].

Among the different macronutrients contained in sludge phosphorus is an essential element for plant metabolism since it is present in numerous molecules such as phospholipids or nucleotides. For this reason P is often considered one of the most limiting nutrients for plant productivity. Comparable quantities of sludge cakes are commonly applied to degraded soils in the Mediterranean areas. These rates, however, exceed crop nutrients requirements and may cause

undesirable changes in soil chemical properties, leading to environmental contamination. Such effects include accumulation of P which can impact water bodies through surface run-off leaching which in turn may threaten surface and ground waters through eutrophication [6]. So, the effective soil phosphorus management from both environmental and agronomic point of view requires the knowledge of P bioavailability [7–10].

Copper and zinc are essential elements for higher plants but at the same time potentially environmental contaminants. Copper and zinc may enter the soil through fertilizer, organic wastes, and pesticides applications. Sewage sludge usually contains significant amounts of Cu and Zn and can be a source of these two metals in the soil [11].

The availability of P and trace elements present in the sewage sludge depends on many factors such as the nature and amount of element, degree of element association in the sludge, sewage source, application rate, soil type, plant characteristics, and weather conditions [8, 10].

Although several studies have dealt with the possible increase of available P and trace elements arising through land application of SS [10, 12–15], little work has been done on the potential changes in their availability through its application with stabilized SS to soil differing widely in their properties. In Egypt, management of the large quantities of sewage sludge produced from sewage treatment plants and some environmental wastes, that is, sugar beet factory lime, brick factory fly ash, rice straw, and water hyacinth, is a serious problem since, so far, its agricultural use remains at very low levels. However, a sound alternative for these wastes would be their agronomic utilization. Although enough research has been carried out on the agricultural use of sewage sludge [16], relevant work on the agricultural utilization of sugar beet factory lime, fly ash, rice straw, and water hyacinth especially used together with SS as stabilized sewage sludge using these amendments is very limited. Therefore, the aim of this study was to examine the influence of SS from two different sources and stabilized sludge with different organic and inorganic amendments on Olsen-P and DTPA-extractable Cu and Zn in an incubation experiment in relation to soil type, sewage source, mixing rate, and incubation time.

2. Materials and Methods

2.1. Selection and Preparation of Soil, Sewage Sludge, and Amendments Samples

2.1.1. Soil Samples. Surface (0–30 cm) soil samples of cultivated Entisols (Typic Torrifluvents) developed on fluvial sediments and Aridisols (Typic Calcorthids) developed on calcareous sediments [17] were selected for this study. The fluvial soil was selected from the faculty of agriculture farm at Sakha city, Kafr el-Sheikh Governorate, and it was cultivated with wheat. The calcareous soil sample was collected from a farm in Ahmed Oraby village, Abo Elmatamer district, Elbehira Governorate, and it was cultivated with clover. The soils were air-dried and crushed to pass through a 2 mm sieve, eliminating stones, roots, and fragments. The dried

samples were stored at room temperature in plastic bags to be ready for carrying experiments and chemical analyses.

2.1.2. Sewage Sludge Samples. Two anaerobic digested sewage sludge samples were used; the first one (SS1) was collected from Kafr EL-Zayate wastewater treatment plant, which received sludge from both domestic and industrial sources. The second one (SS2) was collected from Messer wastewater treatment plant, Kafr el-Sheikh, which received sludge from both domestic and industrial sources with dominance of domestic. The sewage sludge samples were air-dried and passed through a 2 mm sieve.

2.1.3. Amendments Samples. Fly ash samples (CFA) collected from the electrostatic precipitator of a lignite-fired electric power plant in Northern Greece. Sugar beet factory lime (SBFL) was obtained from the sugar beet factory of the city of El Hamoul, Kafr el-Sheikh Governorate. Bentonite (B) is a calcium saturated clay mineral with a high sorption capacity was used in this study. Calcium carbonate (CaCO_3) is a chemical reagent with 90% purity. Rice straw (RS) is dried and grinded rice residual plants. Cotton sticks (CS) are residuals of cotton plants, dried and grinded. Water hyacinth (WH) is a vascular floating aquatic plant, fast growing with a well-developed fibrous root system and large biomass. These plants were collected from fresh water pathway, dried, and grinded.

2.2. Incubation Experiment. The influence of SS and the stabilized SS on soil properties, Olsen P and DTPA-extractable amounts of Cu and Zn in relation to soil types, sewage source, mixing rate, and incubation period was investigated in an incubation experiment in which alkaline fluvial and calcareous soils were used. An incubation experiment was conducted using the studied soils and sewage at room temperature and field capacity moisture content for 30 and 60 days as follows.

2.2.1. Stabilization of Sewage Sludge Using the Studied Amendments. Stabilized sewage sludge was prepared by mixing the sludge with the amendments at two rates (10% and 25%) as shown in Table 1. The SS and stabilized sewage sludge were applied to the studied soils by an application rate of 2.5% which is equivalent to 50 Mg per hectare. The treated soils were wetted with deionized water to field capacity and incubated at room temperature. All treatments included three replicated samples ($n = 3$). The samples were incubated for 30 and 60 days. After each incubation period, the samples were air-dried, grinded to pass through a 2 mm sieve, and analyzed for selected chemical characteristics, Olsen-P and DTPA-extractable copper and zinc.

2.3. Analyses of Soil, Sewage Sludge, and Amendments. The following chemical properties were determined in sieved samples: pH in deionized water in soil solution ratio of 1 : 1 for soil and inorganic amendments and 1 : 5 for sewage sludge and organic amendments according to [18]. Total calcium carbonate equivalent (TCCE) was determined by using

TABLE 1: Treatments of sewage sludge with the studied stabilized agents.

Codes	Treatments	Mixing rate	
		10%	25%
SS	Sewage sludge	100 g	100 g
SS + B	Sewage sludge + bentonite	90 g SS + 10 g B	75 g SS + 25 g B
SS + SBFL	Sewage sludge + sugar beet factory lime	90 g SS + 10 g SBFL	75 g SS + 25 g SBFL
SS + CFA	Sewage sludge + coal fly ash	90 g SS + 10 g CFA	75 g SS + 25 g CFA
SS + CaCO ₃	Sewage sludge + calcium carbonate	90 g SS + 10 g CaCO ₃	75 g SS + 25 g CaCO ₃
SS + RS	Sewage sludge + rice straw	90 g SS + 10 g RS	75 g SS + 25 g RS
SS + WH	Sewage sludge + water hyacinths	90 g SS + 10 g WH	75 g SS + 25 g WH
SS + CS	Sewage sludge + cotton stalks	90 g SS + 10 g CS	75 g SS + 25 g CS

a Collins calcimeter. Organic matter content was determined by the Walkley-Black method in soil samples [19] and by dry ashing method in sewage sludge sample. Available P was extracted by 0.5 M NaHCO₃ according to [20]. Phosphorus content in the solutions was determined colorimetrically by using the ammonium molybdate-ascorbic acid method as described by [21]. Total trace elements were extracted by a solution of concentrated nitric acid, concentrated hydrochloric acid, and 30% hydrogen peroxide according to [22]. Available trace elements were extracted by using diethylene triaminepentaacetic acid (DTPA) method according to [23]. The values of studied trace elements were determined by atomic absorption spectrometry (Varian, SpectrAA-400 Plus, Australia). Particle size analysis was performed according to [24].

3.1.1. Statistical Analyses. All results were analyzed statistically using one-way ANOVA to compare the means of different treatments. The individual means were compared by Duncan's test to a level of 5% using SPSS version 10.01. The results were subjected to multifactorial analysis. The considered variables were soil, sewage sludge, incubation period, maxing rate, and organic and inorganic amendments where *F*-test was significant at a 0.05 probability level using the JMP 10 software (SAS Institute Cary, NC, USA).

3. Results and Discussion

3.1. Characterization of Studied Soils, Sewage Sludge, and Amendments Samples

3.1.1. Soil Samples. Both studied soils showed a neutral pH. The soils exhibited quite different physical and chemical properties (Table 2). The diverse geological nature of these deposits is reflected in the wide variation of the different physicochemical properties and amounts of total and available trace elements [25]. In general, fluvial soil exhibited total amounts of all elements except for Cd higher than the calcareous one. Also, fluvial soil showed high concentrations of DTPA-extractable Cu, Ni, and Pb compared to fluvial soil, while the opposite trend was recorded for DTPA-Zn and Olsen-P (Table 2). However, the total concentrations of all the studied elements were found to be lower than the critical concentrations in soils as referred by [3] or the allowable

cumulative loading rates of European and US regulations [5, 26].

3.1.2. Sewage Sludge Samples. Kafr El-Ziat sludge (SS1) contains high amounts of all the studied metals except for Fe and Ni compared to Kafr el-Sheikh sludge (SS2). So, metal concentrations in sludge varied depending on several factors such as sludge origin, sludge pretreatment processes, organic matter content, and digestion process [27]. From data in Table 2, it is concluded that trace element concentrations in sewage sludge were relatively low, except for Fe, Zn and below the permissible upper limits set by EU (which are 2500–4000 for Zn, 1000–1750 for Cu, 20–40 for Cd, 750–1200 for Pb, and 300–400 mg kg⁻¹ for Ni) [26].

3.1.3. Amendments Samples. The pH of the organic amendments was lower than the inorganic amendments. Coal fly ash (CFA) and sugar beet factory lime (SBFL) recorded the highest pH values, that is, 12.50 and 12.59, respectively. Coal fly ash is an alkaline residue produced during the burning of coal for electricity generation usually containing appreciable amounts of ferroaluminum silicate minerals with Al, Ca, Mg, Fe, K, Na, and Si as predominant elements [28]. The alkalinity of FA is depending on its sources and composition [29]. The high alkalinity of the used coal fly ash was reported by [10, 30–32]. With respect to the high alkalinity of sugar beet factory lime, it may be due to its high content of calcium carbonate (81.6%, Table 3). Sugar beet factories have traditionally stockpiled factory lime near them which is produced during the sugar beet thin juice purification process. Milk of lime (Ca (OH)₂) and CO₂ are injected into the juice where it forms calcium carbonate and, along with many impurities [33], precipitates from the juice. The purified juice is further processed into crystal sugar, but the precipitated lime and impurities are expelled from the factory and hauled away. This factory lime meets the definition of a liming product and can be used on acidic soils to raise soil pH [10]. Bentonite and WH showed the highest concentrations of total Zn, while BFA showed the lowest concentrations. Total concentrations of Cu in the studied amendments were as follows: 60.8, 98.6, 30.1, 4.3, and 9.7 mg kg⁻¹ for WH, RS, B, CFA, and SBFL, respectively. Bentonite showed the highest total concentration of Fe (59750 mg kg⁻¹) and Mn (618.5 mg kg⁻¹) compared to all

TABLE 2: Selected characteristics and metal concentrations in the studied soils and sewage sludge.

Tested characteristics	Soil classification		Sewage sludge (SS1)	Sewage sludge (SS2)
	Calcareous soil Typic Torrifluvents	Fluvial soil Typic Calcorthids		
Tested basic characteristics				
pH	7.82	7.71	6.28	5.86
EC, dSm ⁻¹	0.94	0.55	3.90	3.80
OM, g kg ⁻¹	14.90	22.20	144.50	149.20
CEC, cmol(+)/kg	12.63	34.10	35.80	36.30
CaCO ₃ , %	28.50	5.30	15.20	9.88
Olsen-P, mg kg ⁻¹	8.70	7.70	53.90	57.30
Particle size distribution, %				
Sand	53.58	18.52	—	—
Silt	28.72	56.30	—	—
Clay	17.70	25.18	—	—
Texture class	Sandy clay loam	Silty loam	—	—
DTPA-extracted elements, mg kg ⁻¹				
Zn	2.18	1.21	—	—
Cu	3.10	4.26	—	—
Pb	1.25	2.18	—	—
Ni	0.24	0.45	—	—
Cd	0.02	0.02	—	—
Aqua-regia-extracted elements, mg kg ⁻¹				
Fe	18250.50	55724.00	30260.00	39907.50
Mn	331.50	779.00	723.00	251.00
Zn	57.20	111.80	1437.20	1218.10
Cu	24.50	56.10	158.80	148.90
Cd	1.50	1.15	1.45	1.40
Ni	23.10	60.00	34.10	41.90
Pb	46.50	63.50	193.00	120.50

EC: electric conductivity (dSm⁻¹); OM: organic matter; CEC: cation exchange capacity (cmol(+)/kg); (—): not measured; DTPA: diethylenetriaminepentaacetic acid.

the studied amendments, while SBFL showed the highest amounts of total Cd (3.45 mg kg⁻¹) and Pb (57.0 mg kg⁻¹). On the other hand RS showed the highest amounts of total Cu (98.6 mg kg⁻¹), CFA showed the highest amounts of Ni (170.9 mg kg⁻¹), and WH showed the highest amounts of total Zn (111.4 mg kg⁻¹) compared to all the studied amendments as shown in Table 3.

3.2. Effect of Sewage Sludge and Stabilized Sewage Sludge Application on P Availability in relation to Soil Type, Sewage Source, Mixing Rate, and Incubation Time. Sewage sludge application increased significantly Olsen-P concentrations from 7.7 to 21.0 mg kg⁻¹ in fluvial soil and from 8.8 to 25.7 mg kg⁻¹ in calcareous soil (Table 4). Moreover, it was observed that Olsen-P concentrations were high in the case of SS2 compared to SS1, in the second incubation period (60 day) compared to the first one (30 day), and in calcareous soil compared to fluvial soil. Also, data in Table 4 showed that mixing the studied amendments

with sewage sludge changed relatively the amounts of soil-available phosphorus. In the second incubation period, stabilized sewage sludge increased significantly available phosphorus compared with the mono-sewage sludge from 20.71 and 21.03 to 23.36 and 22.13 mg kg⁻¹ in fluvial soil with WH- and B-stabilized SS at mixing rate 10%, respectively. Also, available P increased significantly from 25.72 and 24.95 to 28.32 and 27.6 with SBFL-stabilized SS at mixing rate 10% and 25%, respectively. As indicated from one-way ANOVA (Table 5), there was a significant difference in P concentration as affected by soils ($P \leq 0.01$), sewages ($P = 0.04$), incubation periods ($P = 0.01$), or amendments ($P \leq 0.01$). However, there was insignificant difference in P concentration as affected by mixing rates ($P = 0.05$). On the other hand, according to a multifactor analysis, the interaction between the studied factors (i.e., soils*sewage sources*incubation periods*mixing rates*amendments) showed a significant difference ($P = 0.01$) in P concentration (Table 5).

TABLE 3: Selected characteristics and metal concentrations in the studied amendments.

Properties	Organic amendments				Inorganic amendments			
	WH	RS	CS	BFA	CFA	B	SBFL	CaCO ₃
pH	6.18	7.60	5.62	9.68	12.5	9.24	12.59	8.81
EC, dSm ⁻¹	6.91	2.83	2.01	0.98	4.48	1.13	1.55	0.17
OM, %	75.25	52.7	97.49	—	—	—	—	—
CaCO ₃ , %	—	—	—	—	12.4	—	81.6	—
Total elements, mg kg ⁻¹								
Fe	—	—	—	7590.0	21228.0	59750.0	651.5	—
Mn	—	—	—	78.35	202.9	618.5	18.9	—
Zn	111.4	28.6	65.0	5.55	55.1	108.25	26.40	37.0
Cu	60.8	98.6	47.1	4.30	30.1	33.70	9.70	41.1
Cd	nd	nd	nd	nd	2.15	1.35	3.45	nd
Ni	—	—	—	8.8	170.9	50.35	13.10	—
Pb	8.6	68.4	—	16.0	62.5	34.5	57.00	—

CFA: coal fly ash, B: bentonite, SBFL: sugar beet factory lime, RS: rice straw, WH: water hyacinth, CS: cotton stalks, nd: not detected, —: not measured.

TABLE 4: Effect of SS and stabilized SS on Olsen-P in relation to soil type, sewage source, mixing rate, and incubation period.

Treatments	Fluvial soil				Calcareous soil			
	SS1		SS2		SS1		SS2	
	30 day	60 day	30 day	60 day	30 day	60 day	30 day	60 day
	Mixing rate, 10%							
C	7.7 ^f	7.7 ^d	7.7 ^e	7.6 ^c	8.8 ^c	8.8 ^e	8.8 ^e	8.8 ^f
SS	19.4 ^c	20.7 ^b	20.7 ^b	21.0 ^a	25.2 ^b	25.7 ^b	24.1 ^{abc}	24.9 ^c
SS + CFA	17.0 ^d	17.8 ^c	18.6 ^c	19.9 ^a	20.4 ^d	18.1 ^d	24.5 ^{ab}	27.2 ^b
SS + B	21.8 ^a	18.0 ^c	23.6 ^a	22.1 ^a	22.1 ^c	25.2 ^b	26.1 ^a	25.9 ^c
SS + SBFL	21.2 ^{ab}	19.3 ^{bc}	21.2 ^b	20.8 ^a	31.3 ^a	28.3 ^a	21.9 ^{bcd}	29.2 ^a
SS + CaCO ₃	20.0 ^{bc}	18.7 ^{bc}	17.3 ^{cd}	15.3 ^b	26.1 ^b	27.3 ^{ab}	22.8 ^{abcd}	21.3 ^d
SS + WH	18.8 ^c	23.4 ^a	16.9 ^{cd}	15.5 ^b	20.7 ^d	22.5 ^c	20.7 ^{cd}	21.6 ^d
SS + RS	15.2 ^e	17.6 ^c	15.6 ^d	20.7 ^a	21.0 ^{cd}	20.0 ^{cd}	21.0 ^{bcd}	19.3 ^e
SS + CS	16.9 ^d	18.7 ^{bc}	16.1 ^d	19.1 ^{ab}	19.9 ^d	20.2 ^{cd}	19.8 ^d	22.3 ^d
	Mixing rate, 25%							
C	7.7 ^c	7.6 ^f	7.7 ^f	7.6 ^d	8.8 ^h	8.8 ^e	8.8 ^f	8.8 ^f
SS	19.4 ^a	20.7 ^{ab}	20.7 ^b	21.0 ^a	25.2 ^b	25.7 ^a	24.1 ^b	24.9 ^c
SS + CFA	16.6 ^b	16.3 ^{de}	19.8 ^{bc}	21.8 ^a	19.9 ^{ef}	20.9 ^{bc}	22.5 ^{bc}	25.4 ^{bc}
SS + B	21.2 ^a	18.6 ^{abcd}	23.3 ^a	21.2 ^a	21.8 ^{de}	22.6 ^b	24.5 ^b	29.7 ^a
SS + SBFL	19.7 ^a	21.0 ^a	18.2 ^{cd}	20.7 ^a	27.9 ^a	26.6 ^a	28.0 ^a	27.6 ^{ab}
SS + CaCO ₃	16.9 ^b	18.1 ^{bcd}	17.0 ^{de}	16.9 ^c	24.3 ^{bc}	25.8 ^a	21.8 ^{cd}	21.6 ^d
SS + WH	19.4 ^a	17.8 ^{cde}	16.3 ^e	16.5 ^c	18.5 ^{fg}	20.4 ^c	19.8 ^{de}	21.9 ^d
SS + RS	14.8 ^b	15.3 ^e	16.2 ^e	16.9 ^c	18.1 ^g	17.4 ^d	18.8 ^e	19.1 ^e
SS + CS	19.5 ^a	19.3 ^{abc}	19.5 ^{bc}	19.1 ^b	22.7 ^{cd}	21.3 ^{bc}	22.6 ^{bc}	21.9 ^d

Values accompanied by different letters are significantly different within column ($P < 0.05$).

C: control (un amended soil), SS: sewage sludge, CFA: coal fly ash, B: bentonite, SBFL: sugar beet factory lime, RS: rice straw, WH: water hyacinth, CS: cotton stalks, nd: not detected, —: not measured.

Our results were in consistency with [34] who reported that some amendments-stabilized sewage sludge increased significantly available phosphorus. The highest increase in available P was found in the treatment SBFL (29.68 mg kg⁻¹). This increase in available P because of the lime application may be attributed to the decomposition of organic P as it was stated by [35]. Also, this is probably due to the high available P content of this amendment (391 mg kg⁻¹) [10]. The enrichment of SBFL in P could be due to its formation

process. Also, it may be due to the increase of exchangeable Ca in lime treatment which increases the calcium phosphate. In this respect, [8, 36] reported that the increase in Olsen-P for treatments receiving alkaline materials compared to the control was likely related to an increase in Ca-associated P which is extractable with 0.5 M NaHCO₃. Also, [37] studied the crop availability of SBFL phosphorus and found that Olsen-P was increased with the application of factory lime. Additionally, the increased Olsen-P response to factory lime

TABLE 5: Multifactor analysis of statistical differences in P, Cu, and Zn concentrations as affected by soil types, sewage source, mixing rate, and incubation time.

	Source	Soils	Sewages	Rates	Incubation periods	Amendments	Soils*sewages*rates*incubation periods*amendments
P	DF	1	1	1	1	8	8
	F ratio	949.08	8.23	3.79	10.37	620.57	3.39
	Prob > F	<0.01*	0.04*	0.05	0.01*	<0.01*	0.01*
Zn	DF	1	1	1	1	8	8
	F ratio	278.49	90.99	3.49	216.33	894.80	1.15
	Prob > F	<0.01*	<0.01*	0.06	<0.01*	<0.01*	0.32
Cu	DF	1	1	1	1	8	8
	F ratio	1.23	1.01	1.01	0.96	0.99	0.99
	Prob > F	0.26	0.31	0.31	0.32	0.43	0.43

rates was probably due to increased solubilization of factory lime P as the factory lime reacted with the soil over the time [37].

3.3. Effect of Sewage Sludge and Stabilized Sewage Sludge Application on DTPA-Extractable Zn in relation to Soil Type, Sewage Source, Mixing Rate, and Incubation Time

3.3.1. Effect of Mono Sewage Sludge Treatment on DTPA-Extractable Zn. The application of SS increased significantly DTPA extractable Zn compared to untreated soil from 1.20 to 9.57 mg kg⁻¹ with increasing percent 684% and from 2.16 to 10.50 mg kg⁻¹ with increasing percent 367% in fluvial and calcareous soil, respectively. This increase could be explained by the following possible reasons: high content of Zn in sewage sludge (1437 mg kg⁻¹, Table 2); formation of soluble Zn-organic associations and the decrease in soil pH. These interpretations are in agreement with [38]. In this respect, [1, 39] reported that SSs exhibit a wide range of Zn concentrations which are generally higher than the background levels found in soils. Increasing of DTPA extractable Zn after biosolid application seems to agree with those reported in an early study by [12, 13, 15, 31].

Also, data indicated that the increasing rate was high with SS2 compared to SS1 in fluvial soil especially after the first incubation period. However, the opposite trend was recorded in calcareous soil, where SS1 application increased available Zn compared to SS2 especially in the first incubation period. Additionally, fluvial soil showed increasing rate higher than calcareous soil. Lower increase rate of DTPA-extractable Zn in calcareous soil compared to fluvial one after application of SS may be due to the precipitation and/or sorption of biosolid-born released Zn with calcium carbonate. Increasing Zn sorption in soils rich in CaCO₃ may be due to the formation of ZnCO₃ and/or Zn sorption by calcium and magnesium carbonate. In this respect, [40] reported that Zn can be strongly sorbed by magnesite (MgCO₃), to intermediate degree by dolomite [Ca Mg (CO₃)] and latest by calcite CaCO₃. In magnesite and dolomite, it appears that Zn is actually adsorbed into the crystal surface at the sites in lattice normally occupied by magnesium atoms. Also, in the presence of Ca⁺²,

a more or less fraction of Zn may be involved in specific adsorption reactions. Kiekens [40] studied the reversibility of the exchange reaction between soil Ca⁺² and solution Zn and found that an important fraction of Zn was irreversibly fixed by the soil. Additionally, under the high pH values of these alkaline soils, the increasing of Zn sorption could be explained by the adsorption of Zn in hydrolysed form and precipitation of Zn (OH₂). Similar results and interpretations about the high affinity of calcareous soil for Zn sorption were reported by [13].

3.3.2. Effect of Stabilized Sewage Sludge Treatment on DTPA-Extractable Zn. The data showed that mixing SS with the studied amendments decreased significantly the amounts of DTPA-extractable Zn in both fluvial and calcareous soil with SS1 and SS2 and after both mixing rates and incubation periods except for CS-stabilized SS in some cases (Table 6). However, the decreasing rate differed depending on type of amendments, soils, mixing rate, incubation period, and SS source.

Regarding the efficacy of tested amendments in reducing the Zn availability in biosolid amended soil, they differed widely depending on soil type and sewage source. In fluvial soil, in the case of SS1 and mixing rate 25% after 60-day incubation period, B- and CFA-stabilized SS treatment showed the highest decreasing rate of available Zn compared to the mono-SS treatment between the inorganic amendments and RS-stabilized SS between the organic ones. On the other hand, in calcareous soil and in the case of SS1 and mixing rate 25% after 60-day incubation period, B-stabilized SS treatment showed the highest decreasing percent of available Zn compared to the mono-SS treatment (9.6% reduction), while WH-stabilized SS treatment showed the highest decreasing percent between the organic amendments (35.2% reduction). These trends indicate that B was the best inorganic amendment for reducing DTPA-extractable Zn either in fluvial or calcareous soil. This result indicates that B has the ability to take up a significant concentration of Zn associated with the mobile forms. The fact that the bentonite binds the available element form had been reported before by [41].

TABLE 6: Effect of sewage sludge and stabilized sewage sludge on the DTPA-extractable zinc in relation to soil type, sewage source, mixing rate, and incubation period.

Treatments	Fluvial soil				Calcareous soil			
	SS1		SS2		SS1		SS2	
	30 day	60 day	30 day	60 day	30 day	60 day	30 day	60 day
Mixing rate, 10%								
C	1.21 ^f	1.20 ^e	1.21 ^d	1.20 ^e	2.18 ^e	2.16 ^e	2.18 ^f	2.16 ^e
SS	8.46 ^{ab}	8.11 ^a	9.57 ^a	7.80 ^b	10.50 ^a	8.84 ^a	10.10 ^a	9.15 ^a
SS + CFA	7.77 ^{bcd}	6.40 ^c	7.46 ^{bc}	6.39 ^{cd}	9.02 ^b	6.51 ^{cd}	9.46 ^{bc}	7.79 ^b
SS + B	7.49 ^{cde}	7.36 ^{ab}	7.56 ^{bc}	7.46 ^b	6.24 ^a	7.13 ^{bc}	8.63 ^d	7.60 ^{bc}
SS + SBFL	8.15 ^{abc}	6.49 ^c	7.59 ^{bc}	6.68 ^{cd}	7.57 ^c	8.10 ^a	8.78 ^d	7.81 ^b
SS + CaCO ₃	7.19 ^{de}	6.63 ^{bc}	7.57 ^{bc}	6.85 ^c	7.76 ^c	7.32 ^b	8.89 ^{cd}	6.72 ^{cd}
SS + WH	7.15 ^{de}	6.18 ^c	7.37 ^c	6.07 ^d	7.22 ^c	6.69 ^{bcd}	6.87 ^e	6.49 ^d
SS + RS	6.78 ^e	5.35 ^d	8.14 ^b	7.72 ^b	7.54 ^c	6.01 ^d	9.04 ^{bcd}	7.96 ^b
SS + CS	8.89 ^a	7.33 ^{ab}	7.85 ^{bc}	8.60 ^a	8.62 ^b	8.19 ^a	9.66 ^{ab}	9.70 ^a
Mixing rate, 25%								
C	1.21 ^c	1.20 ^e	1.21 ^e	1.20 ^f	2.18 ^e	2.16 ^f	2.18 ^d	2.16 ^g
SS	8.46 ^a	8.11 ^a	9.57 ^a	7.80 ^a	10.5 ^a	8.84 ^b	10.10 ^a	9.15 ^a
SS + CFA	7.24 ^{ab}	5.77 ^{cd}	7.50 ^{bc}	7.14 ^{bc}	8.36 ^{bc}	7.24 ^d	7.92 ^{bc}	7.39 ^{de}
SS + B	6.86 ^{ab}	5.64 ^{cd}	7.28 ^c	7.31 ^b	7.72 ^{bcd}	7.99 ^c	8.75 ^b	7.87 ^{bcd}
SS + SBFL	7.33 ^{ab}	6.70 ^b	8.00 ^b	7.34 ^b	7.12 ^{cd}	8.22 ^c	7.98 ^{bc}	7.54 ^{cde}
SS + CaCO ₃	6.12 ^b	8.00 ^a	7.37 ^{bc}	6.28 ^d	8.17 ^{bc}	7.34 ^d	8.64 ^b	6.00 ^f
SS + WH	6.66 ^b	6.29 ^{bc}	5.99 ^d	5.80 ^e	6.52 ^d	5.73 ^e	7.20 ^c	6.90 ^e
SS + RS	6.66 ^b	4.83 ^d	9.17 ^a	7.49 ^{ab}	7.17 ^{cd}	6.25 ^e	9.74 ^a	8.33 ^{abc}
SS + CS	7.56 ^{ab}	7.88 ^a	8.92 ^a	6.88 ^c	8.71 ^b	9.64 ^a	9.95 ^a	8.73 ^{ab}

Values accompanied by different letters are significantly different within column ($P < 0.05$).

One-way ANOVA showed a significant difference in Zn concentration as affected by sewage, soils, sewages, incubation periods, or amendments at the same significance level in each one of them ($P \leq 0.01$), whereas there was insignificant difference in Zn concentration in case of rates ($P = 0.06$). In addition, a multifactor analysis was performed to evaluate significant statistically differences in Zn concentration as affected by soils*sewage sources*mixing rates*incubation periods*amendments. There was insignificant difference in Zn concentration ($P = 0.32$) at interference between these factors as presented in Table 5.

The efficiency of CFA in reducing the availability of Zn in biosolid-amended soils may be due to its high alkalinity (pH = 12.5 Table 3) and its high ability to adsorb the released biosolid-born Zn. The high ability of CFA in sorption of Zn in biosolid-amended soil was studied by Tsadilas et al. [30] who reported that application of CFA increased sorption of Zn about 10 times greater than the control. In addition, Ciccu et al. [42] stated that FA may increase the surface area available for element adsorption, increase pH of soils, and render most cationic metals less mobile. Also, Tsadilas et al. [43] studied the influence of FA and sewage sludge application on the bioavailability of Zn and showed that fly ash application did not affect total concentration of soil Zn but decreased significantly their available forms in all cases from about 32 to 73% compared to the control treatment.

These results confirmed that alkaline fly ash can be used as an effective amendment for remediation of sewage sludge-amended soils.

Also, data in Table 6 showed that WH-stabilized SS was the best organic amendment in reducing DTPA-extractable Zn compared with other amendments. The high efficiency of WH in decreasing Zn availability was in agreement with [44, 45]. The increasing of available Zn in CS-stabilized SS treatment compared to the mono-SS treatment could be explained by its low pH (pH = 5.62, Table 3).

The results indicated that the decreasing rate of available Zn in stabilized SS treatments increased with increasing the mixing rate from 10 to 25% and with increasing the incubation period from 30 to 60 days. The 25% application rate was the best rate in reducing DTPA-extractable Zn either in the first or in the second incubation period. Also, data showed that the DTPA-extractable Zn decreased over incubation time. This result was in accordance with [46]. Also [47] reported that solution Zn concentrations slightly decreased during the incubation study after mixing FA with SS and achieved the stable level after 22 days.

In regards to the effect of soil types, results indicated that the fluvial showed the best response for the effect of the tested amendments in decreasing DTPA-extractable Zn compared to calcareous soil in both incubation periods. The differences between the studied soils were highly significant. With

TABLE 7: Effect of sewage sludge and stabilized sewage sludge on the DTPA-extractable copper in relation to soil type, sewage source, mixing rate, and incubation period.

Treatments	Fluvial soil				Calcareous soil			
	SS1		SS2		SS1		SS2	
	30 day	60 day	30 day	60 day	30 day	60 day	30 day	60 day
Mixing rate, 10%								
C	3.86 ^d	3.88 ^d	3.86 ^d	3.88 ^b	3.11 ^d	3.10 ^d	3.11 ^b	3.10 ^a
SS	7.01 ^a	6.98 ^a	6.95 ^a	6.90 ^a	6.17 ^a	4.68 ^a	4.39 ^a	3.64 ^a
SS + CFA	6.70 ^a	6.03 ^b	6.62 ^{ab}	6.62 ^a	4.11 ^c	3.19 ^d	3.39 ^b	3.29 ^a
SS + B	6.22 ^b	6.85 ^a	6.21 ^{bc}	5.98 ^a	2.88 ^e	3.57 ^{cd}	3.36 ^b	2.77 ^a
SS + SBFL	6.40 ^{ab}	6.80 ^a	6.90 ^{ab}	6.55 ^a	3.66 ^c	3.88 ^{bc}	4.25 ^a	3.62 ^a
SS + CaCO ₃	6.99 ^a	6.94 ^a	6.80 ^{ab}	6.30 ^a	3.96 ^c	4.35 ^{ab}	4.32 ^a	3.36 ^a
SS + WH	6.73 ^a	5.79 ^b	5.88 ^c	4.20 ^b	3.81 ^c	3.33 ^d	3.12 ^b	3.18 ^a
SS + RS	5.27 ^c	4.63 ^c	6.55 ^{ab}	6.89 ^a	3.89 ^c	3.36 ^d	4.34 ^a	3.55 ^a
SS + CS	6.69 ^a	6.95 ^a	6.71 ^{ab}	5.97 ^a	4.87 ^b	3.33 ^d	4.12 ^a	3.39 ^a
Mixing rate, 25%								
C	3.86 ^c	3.88 ^e	3.86 ^d	3.88 ^e	3.11 ^d	3.10 ^{cd}	3.11 ^c	3.10 ^{bc}
SS	7.01 ^{ab}	6.98 ^a	6.95 ^{ab}	6.90 ^a	6.17 ^a	4.68 ^a	4.39 ^a	3.64 ^a
SS + CFA	6.46 ^{ab}	5.18 ^{cd}	5.92 ^c	5.53 ^b	3.26 ^d	2.88 ^d	2.63 ^d	2.97 ^{cd}
SS + B	5.80 ^{ab}	5.59 ^{bcd}	5.72 ^c	5.25 ^{bc}	3.27 ^d	3.39 ^{bc}	3.88 ^b	2.62 ^d
SS + SBFL	6.37 ^{ab}	6.50 ^{ab}	6.89 ^{ab}	6.59 ^a	3.52 ^{cd}	3.60 ^b	3.78 ^b	3.50 ^a
SS + CaCO ₃	7.21 ^a	7.11 ^a	7.28 ^a	6.77 ^a	4.05 ^{bc}	4.59 ^a	4.47 ^a	3.44 ^{ab}
SS + WH	6.40 ^{ab}	5.98 ^{abc}	6.00 ^{bc}	4.02 ^{de}	3.54 ^{cd}	2.81 ^d	2.75 ^{cd}	2.99 ^{cd}
SS + RS	5.22 ^{bc}	4.59 ^{de}	6.51 ^{abc}	6.64 ^a	3.67 ^{bcd}	2.70 ^d	3.85 ^b	2.74 ^{cd}
SS + CS	6.31 ^{ab}	6.39 ^{ab}	6.12 ^{bc}	4.73 ^{cd}	4.32 ^b	3.67 ^b	4.19 ^{ab}	2.84 ^{cd}

Values accompanied by different letters are significantly different within column ($P < 0.05$).

respect to sewage sources, SS1 showed a positive significance response for all the studied amendments in reducing DTPA-Zn compared to SS2.

3.4. Effect of Sewage Sludge and Stabilized Sewage Sludge Application on DTPA-Extractable Copper in relation to Soil Type, Sewage Source, Mixing Rate, and Incubation Time

3.4.1. Effect of Sewage Sludge Treatment on DTPA-Extractable Copper. In fluvial soil the DTPA-extractable Cu increased from 3.86 to 7.01 and 6.95 mg kg⁻¹ with SS1 and SS2, respectively. In calcareous soil, the DTPA-extractable Cu increased from 3.10 to 6.17 and 4.39 mg kg⁻¹ with SS1 and SS2, respectively (Table 7). Enrichment of soil by Cu as a result of SS application could be explained by its relatively high concentration of total Cu (158.8 mg kg⁻¹, Table 2). This is in consistent with [1] who found that Cu concentration in soil increased in soil amended with sewage sludge as compared to unamended control soil. Also, these results were in agreement with [48] who noticed that at the end of incubation period (60 days), following biosolids application, DTPA-extractable Cu increased with increasing biosolids application.

Also, data indicated that the increasing rate of DTPA-extractable Cu was high with SS1 compared to SS2 either in fluvial or calcareous soil. Additionally, fluvial soil showed

increasing rate higher than calcareous soil. Decreasing rate of DTPA-extractable Cu in calcareous soil compared to fluvial one after application of SS may be due to the precipitation and/or sorption of biosolid-born released Cu with calcium carbonate. In this respect, [49] studied sorption of copper in different soils of Egypt and reported that Cu adsorption by the Egyptian Aridisol may be attributed to the presence of high amounts of total calcium carbonate in this soil compared to the clayey fluvial soils. This interpretation is in agreement with [50] who reported that calcareous soils may retain higher amounts of Cu than acidic and neutral soils because of the greater adsorption of Cu on CaCO₃ particles.

3.4.2. Effect of Stabilized Sewage Sludge Treatment on DTPA-Extractable Copper. Mixing SS with the studied amendments changed significantly the amounts of DTPA-extractable Cu depending on type of amendments, soils, mixing rate, incubation period, and SS source (Table 7). There was insignificant difference in Cu concentration when affected by any of the studied factors (i.e., soils ($P = 0.26$), sewages ($P = 0.31$), rates ($P = 0.31$), incubation periods ($P = 0.32$), or amendments ($P = 0.34$) (Table 5).

Regarding the efficacy of tested amendments in reducing the Cu availability in biosolid-amended soil, they differed widely depending on soil type and sewage source. In fluvial soil, in the case of SS1, B-, SBFL-, and CFA-stabilized SS treatment showed the highest significant decreasing rate of available Cu compared to the mono-SS treatment between

the inorganic amendments and RS- and WH-stabilized SS between the organic ones. On the other hand, in calcareous soil, and also in the case of SS1, B, SBFL-, and CFA-stabilized SS treatment showed the highest significant decreasing rate of available Cu compared to the mono-SS treatment, while WH-stabilized SS treatment showed the highest decreasing percent between the organic amendments.

These trends indicate that B, SBFL, and CFA were the best inorganic amendments in reducing DTPA-extractable Cu either in fluvial or calcareous soil. Bentonite-stabilized SS treatments showed a relatively high reduction of available Cu in both of the tested soils. This reduction may be due to the adsorption of Cu on the surface of bentonite or fixation of Cu as a result of precipitation, physical entrapment in clay lattice wedge zones, or strong adsorption at the exchange sites. The same conclusions are reported by Kabata-Pendias [3]. With respect to organic amendments, RS-stabilized SS treatment was the more suitable organic one for reducing DTPA-extractable Cu in fluvial soil. The increase of RS efficiency of decreasing the available Cu may be due to the presence of high silicate amounts in RS [51]. This explanation was in agreement with [52] who reported that the presence of organic matter mixed with silicate (Si-organic form) can play an effective role in reducing the availability of heavy metals in polluted soils. This effect may be as the reaction of soluble silicate ions with the heavy metal (M) formed M-SiO₂ less or insoluble form. Also, metals reaction with organic materials forming complexing bonds.

The high ability of CFA in sorption of Cu in biosolid-amended soil was studied by [30] who reported that application of CFA increased sorption of Cu about 7 times greater than the control. Water hyacinth was the best organic amendment for reducing DTPA-extractable Cu in calcareous soil. It may be due to its high content of organic matter. Copper is well known to be predominantly associated with soil organic matter (SOM) because of the high stability constants of its organic complexes [53].

Regarding the effect of incubation time, DTPA-extractable Cu decreased with increasing the incubation time. This trend was in agreement with [54] who found that concentrations of Cu and Zn declined progressively over the incubation; the decline was more pronounced for Zn than for Cu. In regards to the effect of soil types, data in Table 6 indicated that the calcareous showed the best response for the effect of the tested amendments in decreasing DTPA-extractable Zn compared to the fluvial soil.

4. Conclusion

The present study investigated the influence of sewage sludge and stabilized sewage sludge application on phosphorus availability and DTPA-extractable copper and zinc in relation to soil type, sewage source, mixing rate, and incubation time. Application of SS increased significantly Olsen-P compared to control. The increasing rates were higher in the case of domestic SS (SS2) compared to the industrial one (SS1), in the second incubation period (60 day) compared to the first one (30 day), and in calcareous soil compared to fluvial soil.

Mixing SS with the studied amendments increased Olsen-P especially SBFL- and WH-stabilized SS treatments.

Application of SS increased significantly DTPA-extractable Zn compared to control. Fluvial soil showed increasing rate higher than calcareous soil. Stabilization of SS by all the studied amendments reduced Zn availability except for cotton stalks treatment. Bentonite-, CFA- and RS-stabilized SS showed the highest decreasing rate of available Zn. The highest application rate (i.e., %25) was the best in reducing DTPA-extractable Zn either in the first or in the second incubation period. DTPA-extractable Zn decreased over incubation time. Fluvial soil showed the best response for the effect of the tested amendments on decreasing DTPA-extractable Zn compared to calcareous soil in both incubation periods.

Application of SS increased significantly DTPA-Cu rate with the industrial SS compared to the domestic one. Fluvial soil showed higher increasing rate than calcareous soil. Bentonite-, SBFL-, CFA RS, and WH-stabilized SS treatment showed the highest decreasing rate of available Cu in relation to SS. Stabilized sludge products reduced the availability of sludge trace elements, that is, copper and zinc, compared to the mono-SS treatments. Nevertheless, the long-term accumulation of trace elements following the application of stabilized sludge warrants further studies.

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Review Article

Land Application of Biosolids in the USA: A Review

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Land application of biosolids has proven a cost-effective method of waste disposal by beneficially recycling organic matter and nutrients and improving soil quality; however, it may also pose potential threat to the environment and human health. The purpose of this paper is to provide information on recent research progresses and regulation efforts regarding land application of biosolids, including forms and types and nutrient values of biosolids, environmental and health concerns, and related best management practices (BMPs) of biosolids application, with emphasis on its land application in agriculture. More research and regulations are expected to minimize potential risks of biosolids land application, especially its long-term impacts.

1. What Are Biosolids?

Fifty years ago, partially treated wastewaters from thousands of US cities were discharged into the nation's rivers, lakes, and bays. These receiving water bodies became heavily polluted [1]. Since 1950s, federal legislation has been strengthened to control water pollution. Municipal sewage treatment systems or publicly owned treatment works (POTWs) were built, although ocean disposal of residual solids was still permitted. The Federal Water Pollution Control Act Amendments of 1972 (PL 92-500, 1972) placed further restrictions on the discharge of wastewater to waterways and encouraged other disposal methods such as land applications. Recent restrictions on ocean disposal (Ocean Dumping Ban Act, 1988) and cost increase of incineration and landfill make land application an attractive option of disposal.

Wastewater usually undergoes two processes in treatment factories. The primary step is a physical screening or settling process to remove sediment but with all the dissolved minerals still in the effluent. The secondary step is a biological process where dissolved biological matter is progressively converted into a solid mass using a cultivated culture of indigenous, water-borne bacteria, thereby large amount of biological oxygen demand (BOD) is removed from the wastewater. A tertiary step, which is a chemical and/or biological process, may also be employed to remove nutrients especially N and P, which are the main concern of eutrophication of waters [2]. After the tertiary step, the

effluent may be suitable for discharge into a stream, river, lagoon, or wetland or used for irrigation of public area such as golf courses, greenways, or park or injection to groundwater.

To meet the regulatory requirements (40 CFR Part 503, described below) for land application, the wastewater treatment residuals (sewage sludge) have to undergo treatments to reduce pathogens and attractiveness to vectors. Treatments include aerobic digestion, anaerobic digestion, composting, alkaline stabilization, and thermal drying. They may be also used to control odor and inactivate heavy metals. Biosolids is the term created in 1991 by the *Name Change Task Force* at Water Environment Federation (WEF), the water and wastewater industry's main trade and lobby organization in the USA, to distinguish treated sewage sludge from raw sewage sludge and facilitate land application of processed sewage sludge that would be more acceptable to the public. Biosolids are sewage sludges that have undergone treatments in accord with prevailing federal, state, and local regulations to permit its land application and are defined by the US Environmental Protection Agency (EPA) as "the nutrient-rich organic materials resulting from the treatment of sewage sludge" [3].

2. Regulations

Regulations that ensure the safe and responsible management of sludge have been in effect since 1970s. As a result,

sludge quality has dramatically improved since then. The Clean Water Act amendments of 1977 and 1987 and the Ocean Dumping Ban Act of 1988 (P.L. 100-688) prohibit ocean dumping of sludge and require controls on sludge use and disposal. Regulations entitled “The Standards for the Use or Disposal of Sewage Sludge” were developed and published by the US EPA on Feb. 19, 1993 as the 40 CFR (Title 40, Code of Federal Regulations) Part 503 Biosolids Rule (Part 503 Rule) and became effective on March 22, 1993. The law has become known as the “503 Rule” [4]. The 503 Rule is based on the results of risk assessments for chemicals that began in the mid-1970s, was more extensive than any previous federal rulemaking effort for sludge, and established biosolids quality requirements for its land application. These requirements address pathogen and vector attractiveness reduction, metal loading and concentration limits, and nutrient limits [4].

2.1. Pathogens and Vector Attractiveness. As pathogens (e.g., bacteria, viruses, protozoa, helminths) can cause diseases, pathogens in biosolids are required by the 503 Rule to be reduced prior to land application to minimize potential for diseases. There are two classes of pathogen reduction: Class A and Class B. Class A pathogen reduction is necessary if biosolids are to be applied to lawns, home gardens, or other types of land, or bagged for sale, or land application and requires pathogen densities be reduced to below detection limits: less than 3 MPN per 4 grams total solids biosolids (dry weight basis) for density of *Salmonella* sp., less than 1 PFU per 4 grams total solids biosolids (dry weight basis) for enteric viruses, and less than 1 viable helminth ova per 4 gram total solids biosolids (dry weight basis) for viable helminth ova [5]. There are 6 alternative methods for meeting Class A pathogen reduction requirement: specific time-temperature regimes, high pH-high temperature process, other processes, unknown processes, processes to further reduce pathogens (PFRPs), and a process equivalent to PFRP [5].

Class B pathogen reduction is necessary for any other application and requires a fecal coliform density in the treated sewage sludge (biosolids) of 2 million MPN or CFU per gram total solids biosolids (dry weight basis) [5]. Viable helminth ova are not necessarily reduced in Class B biosolids. There are 3 alternatives for achieving Class B pathogen reduction: the geometric mean fecal coliform density of 7 treated samples must be less than 2 million CFU or MPN per gram of biosolids (dry weight basis), use of a process of PSRPs, and use of processes equivalent to PSRP [5].

Public access is not restricted for biosolids that meet Class A requirements. Since Class B sewage sludge still contains considerable pathogens, site restrictions that limit crop harvesting, animal grazing, and public access for a certain period of time are required.

As vectors (e.g., rodents, birds, insects) can spread diseases by harboring and transferring pathogens, reducing the attractiveness of biosolids to vectors reduces the potential for transmitting diseases from pathogens in biosolids. Part 503 regulation contains 12 options for demonstrating

TABLE 1: Pollutant ceiling concentrations and cumulative loading amounts for biosolids (adapted from [6]) and mean concentrations measured in biosolids from the National Sewage Sludge Survey [7].

Trace metal	Ceiling concentration limit (ppm) ^a	Cumulative pollutant limit loading (kg ha ⁻¹)	Mean (ppm)
Arsenic (As)	75	42	10
Cadmium (Cd)	85	39	7
Copper (Cu)	4300	1503	741
Lead (Pb)	840	301	134
Mercury (Hg)	57	17	5
Molybdenum (Mo)	75	— ^b	9
Nickel (Ni)	420	421	43
Selenium (Se)	100	100	5
Zinc (Zn)	7500	2805	1202

^aDry weight basis.

^bThe February 25, 1994 Part 503 Rule amendment deleted the molybdenum cumulative limit loading for sewage sludge applied to agricultural land but retained the molybdenum ceiling concentration.

a reduction in vector attraction of sewage sludge: reduction in volatile solids content, additional digestion of anaerobically digested sewage sludge, additional digestion of aerobically digested sewage sludge, specific oxygen uptake rate (SOUR) for aerobically digested sewage sludge, aerobic processes at higher than 40°C, addition of alkali, moisture reduction of sewage sludge containing no unstabilized solids, moisture reduction of sewage sludge containing unstabilized solids, injection, incorporation of sewage sludge into the soil, covering sewage sludge, and raising the pH of domestic septage [5]. Options 1 through 8 and Option 12 are designed to reduce the attractiveness of sewage sludge to vectors, while Options 9 through 11 are to prevent the vectors from coming in contact with the sewage sludge.

2.2. Trace Elements. Trace elements in biosolids are of particular concern in regard to their effects on human and animal health. The US EPA [8] analyzed their risks to humans, animals, plants, and soil organisms from exposure to pollutants in biosolids via 14 different pathways for land-applied biosolids. Nine trace elements: arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn), were deemed to be of sufficient risk to regulate. Land application of biosolids must meet the ceiling concentrations and cumulative loading rates for these 9 trace elements set in the 503 Rule (Table 1). If the concentration limit of any one of these elements is exceeded, the biosolids cannot be land-applied. The application of biosolids will also be required to cease, if it is estimated that the cumulative loading limit is being approached.

Biosolids that meet Class A pathogen reduction requirements, metals limits (pollutant concentrations), and vector control requirements are considered to be “Exceptional Quality” (EQ) biosolids. Exceptional quality biosolids can be used with few site restrictions except following normal agronomic practices.

For biosolids that do not meet EQ standards, certain management practices are required: for example, do not apply to flooded or frozen land; apply at agronomic rates for N; stay 10 meters from waterways; must not harm endangered species.

2.3. Nutrients. Although maximum nutrient application rates in federal biosolids regulations are not well defined, the 503 Rule stipulates that agronomic rates cannot be exceeded. To protect groundwater or surface water quality, nitrogen is regulated through an agronomic rate approach, requiring an estimate of crop N need and biosolids N availability [8]. Biosolids P applications are not regulated by the US EPA, but increasing numbers of states are introducing regulations, because of concerns about the effects of repeated manure or biosolids applications on soil P and risk of P loss to surface water. Maryland's Water Quality Improvement Act is the first state law in the Mid-Atlantic region to regulate land applications of biosolids P in the same manner as fertilizer and manure P [9]. And Shober and Sims in 2003 [10] recommended a national biosolids P risk assessment be conducted to develop scientifically based national standards for P management.

3. Forms and Types

There are a large number of different forms of biosolids products including liquid, cake, and pellet form. Liquid biosolids may come directly from the digester without going through any dewatering/drying process, thus usually having high water content (94–97%) and a low dry solid content (3–6%) [11]. Cake biosolids with the texture of a wet sponge can be created with digested liquid sludge or undigested liquid sludge alkali-stabilized with quicklime (calcium oxide) or hydrated lime (calcium hydroxide). Cake biosolids usually have a solids content of 11–40%. Pellet biosolids are produced by heating and drying to a solid content of more than 90%.

From liquid to cake to pellet form, both volume and weight of biosolids are subsequently reduced, increasing economic value by reducing transportation or storage costs. Pellet biosolids can be easily handled and spread with conventional agricultural equipments.

Since soluble anions and cations are left in the liquid phase, nutrient composition can be changed in the dewatering processes during the production of cake and pellet biosolids. For instance, ammonium dominates the inorganic N in dewatered biosolids. Many nutrients may be dominated by less bioavailable forms in dewatered biosolids therefore, the loss of nutrients would be less from a dewatered biosolids than from a liquid biosolids when applied to soils [12]. Dewatered biosolids are more persistent but slower in nutrient release, while liquid biosolids are faster in providing nutrients for plant uptake.

4. Nutrient Value of Biosolids

Biosolids are a valuable source of nutrients although it contains lower N (2–8%), P (1.5–3%), and K (0.1–0.6%)

TABLE 2: Means and variability of nutrient concentrations^a in biosolids collected and analyzed in Pennsylvania between 1993 and 1997 [12].

Nutrient	Total Kjeldahl N (%)	NH ₄ -N (%)	Organic N (%)	Total P (%)	Total K (%)
Mean	4.74	0.57	4.13	2.27	0.31
Standard deviation	1.08	0.30	1.03	0.89	0.27

^aConcentrations are on a dried solids basis.

compared to commercial fertilizers, especially high-grade ones [13, 14]. A survey of nutrient levels in biosolids conducted by Stehouwer et al. [15] using more than 240 samples collected and analyzed from 12 POTWs in Pennsylvania between 1993 and 1997 showed average N, P, and K contents of 4.74%, 2.27%, and 0.31%, respectively (Table 2). These biosolids were aerobically digested, anaerobically digested, or alkali-treated.

Around 50–90% of N in biosolids is in organic compounds [16]. Processes such as digestion or composting result in the loss of organic N because the readily degradable organic matter undergoes mineralization during digestion or composting. For instance, a loss of N by 15.6% via ammonia volatilization was observed in biosolids composting [17].

Phosphorus is largely present as inorganic phosphates of Fe, Al, and Ca. For example, in the biosolids used by Shepherd and Withers [18], 72% of total P was HCl-extractable, 33% was NaHCO₃-extractable, 23% was NaOH-extractable, and 18% was water soluble. Hydrochloric acid-extractable P was mainly associated with Ca, while NaHCO₃- and NaOH-extractable P were mainly associated with Al and Fe [19]. The relatively small content of H₂O-extractable P may be due to the Fe, Al, and Ca in biosolids which are added during the treatment processes as metal salts and lime [20]. Compared to manures, biosolids have a lower N-to-P ratio, around 3.1–3.4 [18]. Biosolids have limited amount of K [21], which is partitioned into the aqueous fraction or effluent at the wastewater treatment plant.

Biosolids contain several essential micronutrients for plants (e.g., B, Cl, Cu, Fe, Mn, Mo, and Zn) which are not provided by most conventional chemical fertilizers [22, 23]. Therefore, biosolids can be applied on micronutrient-deficient soils (e.g., alkaline soils [24], sandy soil [25]).

Nutrient values of biosolids vary with sources of wastewater and wastewater treatment processes. Processes such as digestion or composting result in the loss of organic matter through decomposition, increase concentrations of P and trace metals, decrease ammonia-N by volatilization, and decrease K by leaching. Lime-stabilized biosolids contain lower N, P, and metal concentrations, but higher Ca concentration than digested biosolids, due to the large amount of lime added to the material.

However, nutrient composition of biosolids is significantly altered by stabilization processes. The rate of nutrient release (or mineralization) is also affected by the processes. Mineralization of N from aerobically digested biosolids (e.g., 32.1%) was reported to be significantly higher than that from anaerobically digested biosolids (e.g., 15.2%) [26, 27].

Besides, soil type [26], temperature [28], soil moisture content, aeration, and species and number of soil microorganisms play a role in organic matter mineralization in biosolids. More N in municipal biosolids was mineralized in a Dystrochrept soil, whereas more N in pulp and paper industrial biosolids mineralized in a Typic Udivitrant soil [27]. Mineralization of N was significantly higher at 20°C (average 22.8%) than at 10°C (average 9.7%) [27]. Mineralization rate is also closely related to C:N ratio. The higher the C:N ratio, the lower the N mineralization rate. In some cases, the mineralization process was more influenced by soil type than by rate and type of sludge applied [26].

The primary nutrients in biosolids are in organic forms, not as soluble as those in chemical fertilizers, and released more slowly. Therefore biosolids can nourish the plants at a slower rate over a longer period of time with higher use efficiency and a lower likelihood of polluting groundwater when applied rate is appropriate.

Although maximum nutrient application rates in federal biosolids regulations are not well defined, the 503 Rule stipulates that agronomic rates cannot be exceeded. Also groundwater or surface water is not permitted to be contaminated by biosolids applications.

5. Land Application of Biosolids: Benefits

Due to population increase and urbanization, biosolids production has been on the rise. And land application is generally considered the most economical and beneficial way of biosolids disposal [29]. The most recent national biosolids survey indicated that about 6.5 million dry metric tons of biosolids were produced in the US and approximately 60% of the total (i.e., 4 million dry tons) were land-applied to soils in the US in the year 2004 alone [30]. US EPA estimates that biosolids are applied to approximately 0.1% of available agricultural land in the United States on an annual basis [31].

Biosolids are nutrient-rich organic materials with an organic matter content of up to 50%. Therefore, biosolids can be utilized as a soil conditioner to improve physical, chemical, and biological properties of soils, especially those degraded or disturbed soils. Besides acting as a food source for microorganisms, organic materials are the major binding agents for aggregate formation and stabilization [32]. Optimum soil structure, in turn, improves many other important soil physical and chemical properties such as bulk density, porosity, water and cation exchange capacity, aeration and drainage, microbial communities and soil fauna, thus contributing to disease suppression, and reduced soil erosion [33].

In a 4-year trial with a sandy loamy silt soil by Krause [34], aggregate size and stability increased with increased soil organic matter (OM) after biosolids (2–4% DM content) incorporation. Similar results were also reported by Lindsay and Logan [35] in a 4-year trial where anaerobically digested biosolids are applied to a silt loam soil at 11 rates ranging from 0 to 300 Mg ha⁻¹. Bulk density significantly decreased, and porosity, moisture retention, percentage of water-stable aggregates, mean weight diameter of aggregates, and liquid

and plastic limits increased in the surface soils (0–15 cm) with biosolids application. Increase in aggregate stability was related to the increased organic C in the soil incorporated with biosolids [36].

Reduced bulk density and improved water holding capacity in the short-term [37] may be mainly due to biosolids' direct dilution effect, its high organic matter content, and partly due to its effect on aggregate formation and stabilization. Improved pore size distribution by long term application of biosolids in soil may be linked to the increased volume of macropores or micropores, depending on the texture of the soil [38, 39]. Biosolids-treated soil was less sensitive to compaction than untreated soil due to the improved pore volume [34].

Improvements in soil physical properties after biosolids application coincided with an increase in soil organic C [34, 35, 38]. Many of the soil physical properties differences were associated with addition of organic matter, and these effects persisted for at least 4 years according to Lindsay and Logan [35]. Regression analysis indicated that approximately 80% of the observed variations in the percent water holding capacity increases, at both field capacity and wilting point, could be attributed to variations in soil texture and soil organic C increases [40]. In a sandy and poorly buffered soil, 10 t ha⁻¹ y⁻¹ or 100 t ha⁻¹ of urban biosolids incorporated every 2 years for 20 years resulted in a 2.5-fold increase in organic C content [41].

Biosolids have the advantage of high organic matter content and have been used to remediate sites previously contaminated with trace metals by binding and converting the metals to less soluble fractions [42, 43].

Soil degradation mainly involves damage to soil structure [44]. Due to the beneficial effects of biosolids on soil structure, interest in using biosolids on degraded soils, such as metal-contaminated mine tailings, disturbed urban soils, landfill cover soils, and eroded land to reestablish the vegetation and the ecosystem, is on the rise. Turf grass establishment on a disturbed urban soil with low organic matter and nutrient contents was significantly ($P < 0.05$) enhanced by biosolids application [45]. Other similarly beneficial effects of biosolids application on degraded soils, such as increased aboveground plant biomass yield [46], restored vegetative cover [37, 43], and reestablished ecosystem viability with active microbial communities [47] were also reported.

Besides improving soil quality, biosolids application can supplement or replace commercial fertilizer. Biosolids addition increased total soil N concentration and extractable P in comparison with fertilizers with the additional benefit that a fraction of the N added in the biosolids had been conserved in the systems through partitioning to soil organic matter [48]. Composted biosolids supply plant available Fe and may represent a sustainable alternative to more costly chelated Fe fertilizers used in soils with elevated pH [49].

An advantage of using biosolids to supplement or replace fertilizer is its ability to slow-release nutrients. Biosolids release N and other nutrients over several growing seasons as soil bacteria slowly process through decomposition

(i.e., residual effect). Binder et al. [50] reported that approximately 40, 20, 10, and 5% of the total biosolids-N were recovered by the crops in the 1st, 2nd, 3rd, and 4th year, respectively, after a single biosolids application. The relative yield increase was 33%, 21%, 14%, and 9% in the 1st, 2nd, 3rd, and 4th year, respectively, after application. Slow release of nutrients is more beneficial to crops as the nutrients become available when needed, while most nutrients in commercial fertilizers are water soluble, readily subjected to leaching losses if they are not rapidly taken up by the crops, especially N. Therefore, the use of biosolids as an N source is being considered as one of the best management practices (BMPs) for N management in Florida citrus groves [51].

Studies have shown that application of biosolids resulted in similar or higher yields than inorganic fertilizers [52], especially when applied for multiple years [53]. In a 4-year study, biosolids and urea were applied for the first 2 years, and prairie grass (*Bromus willdenowii* Kunth) in the biosolids-applied plots began to produce higher yields than that in the urea-applied plots from the 2nd year [53]. Higher grain and straw yield of wheat (*Triticum aestivum*) and higher concentrations of elements (N, P, K, Fe, Zn, Mn, Cu, Co, Cd, and Pb) in wheat plant were observed with biosolids treatment as compared to control [54]. Nutrient uptake and crop growth enhancement with biosolids were also reported by Brown et al. [48]. Biosolids composts as horticultural substrates have been widely used in vegetable crop transplant production. The benefits have been reported by several researchers (Table 3).

Alkali-stabilized biosolids can be used as liming material to alleviate soil acidity and Al toxicity. pH was increased from 5.7 to 6.9 for an acid clay sandy loam and from 4.5 to 6.0 for a strongly acid sandy loam, while potassium chloride-extractable Al was decreased from 0.1 to 0.0 cmol kg⁻¹ for the former soil and from 4.0 to 0.1 cmol kg⁻¹ for the latter soil [55]. As a result, barley (*Hordeum vulgare*) showed better growth and higher grain yield in the biosolids-amended plots as compared to the unamended control [55]. Alkaline-stabilized dewatered biosolids were observed to have a liming value of 300 g kg⁻¹ DM CaCO₃ equivalent on average [56].

In addition to increasing yield, biosolids application improves forage quality by correcting trace metal imbalances. Native grass species in Colorado rangelands had low Cu-to-Mo ratios (<1.2:1) before biosolids application [57], which induced Cu deficiencies in cattle and sheep [58]. After receiving a one-time application of biosolids from 5 to 40 Mg ha⁻¹, Cu-to-Mo ratios in the forage from the biosolids-treated plots increased above the 2:1 ratio recommended by Miltimore and Mason [58], which corrected Cu deficiency in cattle and sheep [57].

6. Land Application of Biosolids: Precautions

Concerns and negative reports on biosolids application are related not only to that sludge contains heavy metals or organic compounds found in wastewaters from domestic and industrial sources but also to that excessive nutrients cause eutrophication of waters. And more than often, odors

TABLE 3: Benefits of biosolids composts used in growing media for vegetable transplants (modified from [59]).

Compost ^a	Growth response	Reference
BS	Tomato and cabbage dry wt. and stem diameter = peat-lite	[60]
BS	Broccoli, cabbage, eggplant, lettuce, pepper, and tomato dry wt. increased linearly over 8-week period	[61]
BS	% emergence slightly < peat-lite, plant height > peat-lite	[62]
BS/MSW	Lettuce and cabbage dry wt. = peat-lite	[63]
YT + BS	Tomato dry wt., leaf area, and stem diameter > peat-lite	[64]

^aBS: biosolids; MSW: municipal solid waste; YT: yard trimmings.

and pathogens are the concerns preventing the public from accepting biosolids land application. Therefore, precautions need to be taken in these aspects.

6.1. *Odors.* For some reason, federal biosolids regulations do not regulate odors. Unfortunately, odor is one of the reasons for lack of public acceptance and complaints of biosolids when land-applied. And odor complaints among other concerns have led to a dramatic increase in local ordinances that ban or restrict the use of biosolids in recent years. Although more research is needed to identify potential health effects of biosolids odors, nuisance odors can have detrimental effects on aesthetics, property values, and the quality of life in communities subjected to them. So, eliminating or mitigating odors is among the great challenges in gaining public acceptance of biosolids. Although many of the odor-causing bacteria in biosolids have been destroyed in the biosolids stabilization process, some actions can be taken to control odors at land application sites. Some of these steps could include actions such as minimizing the length of time biosolids are stored selecting remote sites and fields away from neighbors, directly covering or incorporating biosolids into the soil, cleaning tanks, trucks, and equipment daily, and avoiding land application when wind conditions favor transport of odors to residential areas [65].

6.2. *Excess Loading of P and Other Nutrients.* Because of a relatively low N:P ratio (about 3.1–3.4) [18], which is also lower than optimal for crop growth, application of biosolids based on crop N requirement can result in build-up of P in soil and subsequent P transport to surface or subsurface waters as dissolved and particulate P, causing eutrophication [66]. Therefore, an optimum P management practice is to apply biosolids based on crop P requirement. According to the laboratory characterization by Ajiboye et al. [20] and He et al. [21], labile P fractions in biosolids are significantly smaller than other P sources (e.g., fertilizers, manure, and yard waste). Furthermore, labile P fractions are different in biosolids stabilized by different processes. Biosolids produced via biological P removal (BPR) processes have higher labile P fractions than those produced by digestion or composting [67]. In biosolids, Fe-associated P

is less susceptible to loss than Al-associated P, which is less susceptible than Ca-associated P. Iron- and Al-bound P can shift to Ca-P and soluble P when sludge is treated with lime [68]. Most of the P in biosolids is in a recalcitrant form, suggesting that biosolids P may be less subjected to runoff loss as compared with other amendments when applied to agricultural lands as runoff P is positively related to the amounts of P extracted by either water or NaHCO_3 [69]. Phosphorus loss from soil also depends on the degree of P saturation (DPS) in soil or, more importantly, the soil P storage capacity (SPSC). If the soil is more saturated with P or has a low SPSC, it will have less capacity to retain added P and more susceptible to losses of P in runoff and leachate [70–73]. Coarse-textured soils have a low P adsorption capacity due to low contents of clay and reactive Fe and Al oxide in the soil [74]. Application of Fe- or Al-biosolids can increase the SPSC to provide additional sites for P adsorption [68, 75].

Nitrate N concentration higher than the maximum contaminant limit (MCL, $10 \text{ mg NO}_3\text{-N L}^{-1}$) for drinking water established by US EPA [76] is detected when high application rate of biosolids is used especially on coarse-textured soil and mine reclamation [77–79].

As mentioned previously, biosolids application can increase Cu-to-Mo ratio and correct Cu deficiency in ruminant animals [57], but often Mo availability is also increased by biosolids application, and its increase outcompetes Cu and suppresses Cu uptake by the animals, especially from the alkaline-stabilized biosolids applications [80].

6.3. Heavy Metals. There is public concern about the heavy metal pathways after entering the soil with biosolids land application: they may accumulate to hazardous levels in the soil after repeated application of biosolids, and they may migrate from the site to surface or subsurface waters.

Regarding metal behavior and fate in soils, its uptake by plants, and transfer along the food chain, there are plateau, time bomb, and soil-plant barrier hypotheses. The plateau hypothesis considers that the trace metals are so tightly held by the organic matter in biosolids and hydrous oxides of Fe and Mn and clays in the soil that their bioavailability or toxicity is greatly decreased and that they are retained in the soil's surface horizon, or in the plow layer instead of being taken up by plants or leaching down the soil profile. Therefore, the metal concentrations of plant tissue will reach a plateau as biosolids mass loading increases and remain at this plateau after termination of sludge application [81]. Reports both for and against this theory have been published. In a field trial reporting the results of 14 years of continuous, heavy biosolids applications, Dowdy et al. [82] reported that more than 50% of trace metals were so strongly adsorbed that they could be extracted only with a very strong nitric acid digest. At application rates up to $476 \text{ dry tons ha}^{-1}$, trace metals remained mainly in the zone of incorporation, even during long-term application. At application rates less than $100 \text{ dry tons ha}^{-1}$, essentially no movement of heavy metals was observed [83]. However, McBride et al. [84] reported that the high-Fe and high-Al sludge amendments did not

improve the soils' affinity for Cd. Subsurface transport of Cd, Cr, and Mo can be mediated by biosolids colloids with average metal elution in association with biosolids colloids ranging from 65 to 80% of the input metal concentration [85, 86].

Some scientists support the "time bomb model", arguing that these metals could be released and become available and toxic to plants (thus a time bomb) with time following termination of biosolids application and the organic matter undergoes biodegradation [87, 88].

Data obtained from repeated applications of biosolids over 10 years did not indicate an increase in Cd concentration in plant tissue or from organic matter breakdown over time even at loading doses reaching $1,080 \text{ t ha}^{-1}$ [89]. These results support the soil-plant barrier theory and indicate that plants play an important role in protecting the general food chain since they tend to provide an effective barrier against the uptake of most trace metals. But biological activities in the soil may be negatively impacted by biosolids. McGrath and coworker [90, 91] have published a series of papers on this topic. The amount of both free-living and symbiotic N_2 fixing organisms was decreased [92]. In contrast, Zerzghi et al. [93] reported that 20 consecutive years of Class B biosolids application to arid southwestern desert soils in Arizona had no deleterious effect on soil microbial diversity.

6.4. Organic Pollutants. Synthetic organic compounds used in food production, personal care products, plastics manufacturing, and other industrial processes such as flame retardants (e.g., TBB, TBPH), dioxins, and steroid hormones may end up in biosolids [94, 95] and migrate to the natural environment [96, 97]. Many of these compounds are toxic or carcinogenic to organisms exposed to critical concentrations over certain periods of time, and their presence in biosolids causes great concern also because they are persistent, difficult to degrade, and they bioaccumulate. But no organic chemicals are currently regulated under Part 503 just because the chemical has been banned for use and manufacturing in the USA, or it has been detected in 5% or fewer of the sludges tested in the National Sewage Survey, or the 1-in-10,000 cancer risk limit was less than the concentration measured in 99% of the sludges tested [94].

6.5. Pathogens. In biosolids, the primary source of pathogens is human wastes. Pathogens are generally classified as primary (e.g., bacteria, viruses, protozoa, and helminths) or secondary (e.g., fungi). A primary pathogen can invade and infect a healthy person, whereas a secondary, or opportunistic, pathogen invades and infects highly debilitated or immunosuppressed individuals. Composting, if conducted properly, is a very effective method for disinfection and destruction of pathogens primarily by exposing pathogens to high temperatures (e.g., 55°C) for a prolonged period of time [98]. This temperature-time relationship in composting biosolids is the basis of the US EPA biosolids PFRPs (processes to further reduce pathogens), as stated in 40 CFR Part 503 [4]. The specific pathogen reduction requirement for both Class A and Class B biosolids in Rule 503 is technical

requirement not based on risk assessment, and Class B biosolids still contain significant levels of pathogens. Incomplete destruction, contamination from external sources, and changes in environmental factors during storage can lead to recurrence of pathogens [99]. Regrowth or reactivation of fecal coliform was observed during incubation and storage of dewatered biosolids by Qi et al. [100]. But the soil environment is generally very hostile to pathogen survival. When biosolids is applied to the soil surface, desiccation and ultraviolet light will destroy the pathogens. If the biosolids are incorporated into the soil, the pathogens' survival will be affected by factors such as pH, organic matter, soil colloidal matter, temperature, and competitive organisms. With proper processes and precaution, the risks from pathogens in biosolids should be minimal.

7. Best Management Practices (BMPs) for Biosolids

Best management practices (BMPs) are operating methods that ensure the proper land application of biosolids for protection of the environment and human health. The BMPs include agronomic loading rates, slope limitations, soil pH limitations, buffer zones, public access restrictions, grazing deferments, soil conservation practices, restrictions for saturated and frozen soils, protection of endangered species, and other site restrictions.

Mismanagement of biosolids can result in NO_3^- leaching into groundwater [77–79]. Repeated application at high rates can cause accumulation of P in surface soils, which can increase the risk of runoff/erosion losses of P to surface water, resulting in eutrophication [76]. Therefore, the Phosphorus Index (P Index) should be taken into consideration when land-applying biosolids. The P Index was developed by the US Department of Agriculture (USDA), Natural Resource Conservation Service (NRCS), as a field-scale tool to assess the potential for off-site P migration [101]. The primary aspects of P Index include consideration of transport factors such as soil erosion, soil runoff class, leaching potential, and distance from a water body along with management factors such as soil test P, P application method, and source and rate of P application [102]. Biosolids application rate should be based on N application rates if the local P index is low or medium but should be based on matching P uptake if the P index is high and should take account of the nutrient content in the soil, the nutrient requirements of the crop, and how the nutrients will be provided by the biosolids. Taking account of both the initial content of trace elements in the biosolids and in the field is also one of the BMPs requirements on the use of biosolids.

Besides application rate, placement methods can have large effects on nutrient availability and water quality. Redeposition of ammonia volatilized from surface applied biosolids may contaminate surface water and sandy soils. Surface-applying biosolids without incorporation will maximize ammonia volatilization, whereas soil incorporation immediately after application can minimize N losses. The amounts of $\text{NH}_3\text{-N}$ volatilized during a 180 d incubation

period accounted for 18% of the total mineralized N for the surface-applied biosolids. Soil incorporation not only increased N mineralization by more than 60% in the biosolids but reduced NH_3 volatilization by 5-fold [103]. Also, it is likely advantageous to apply biosolids near the area of maximum root growth.

Timing of application should be based on the local weather and soil type so that nutrient release matches plant need instead of being leached by heavy rain. For instance, in the mid-Atlantic region of the US, loss of N by leaching was greater, and plant available N and crop yields were lower when biosolids were applied in mid-December to early January than in March [104]. When the ground is still covered with snow or when rainfall is likely, avoid applications [11].

Studies indicated that pH is a controlling variable for the redistribution of water-soluble, exchangeable, and nonexchangeable heavy metals in biosolids-applied soils. Zinc solubility increased by biosolids amendment in soils with $\text{pH} < 5.0$, while in soils with $\text{pH} > 5.8$, biosolids addition, compared with no biosolids addition, lowered the exchangeability of Zn and favored Zn partitioning into the nonexchangeable forms [105]. Soil pH should be taken into consideration when planning biosolids land application to minimize heavy metal pollution risk.

Other BMPs include, but not limited to the following: that microbial analysis of Class A material should be done at the last possible time before application or after storage in order to address the potential repopulation of pathogenic bacteria [106]; that coapplication with water treatment plant residuals (WTRs) can reduce P availability [107]; that nonapplication or buffer zones should be established around surface water bodies, wells, and wetlands; that those sites with slopes in excess of 8–12% or close to groundwater should be avoided to minimize runoff or leaching, respectively, loss of nutrients and water pollution. Leaving a time period for reaction of the biosolid-borne metals with the soil would be desirable prior to growing a crop [108].

8. Conclusions and Perspectives

Land application of biosolids is a beneficial way to recycle organic matter and nutrients, to improve physical, chemical, and biological properties of soils, and to re-establish vegetation and restoration of degraded ecosystem. However, caution needs to be exercised when biosolids are repeatedly applied or at heavy application rates as heavy metals, organic pollutants, and pathogens in biosolids, though at low concentration, may pose a threat to the environment and animal and human health with time. In the future, long-term monitoring is still needed to evaluate the potential impacts of biosolids land application on soil quality and the environment including surface and ground water, air, and plant/animal health as well as food quality. More protective regulations are expected based on scientific monitoring and assessment data. Community-specific outreach programs addressing public risk perceptions to assist solid waste managers in developing and implementing successful biosolids land application systems that are acceptable to the public

are also needed as a recent descriptive-correlational study conducted in the southeastern United States revealed that the public was inadequately informed about biosolids land application [109].

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Research Article

Biosolid Soil Application: Toxicity Tests under Laboratory Conditions

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A large volume of generated sewage sludge makes its disposal a problem. The usage of sludge in agriculture is highlighted by a number of advantages. However, heavy metals and other toxic compounds may exercise harmful effects to soil organisms. This study evaluated the possible toxic effects of a biosolid sample, under laboratory conditions, for 30 days, using diplopods *Rhinocricus padbergi* and plants *Allium cepa* (onion) as test organisms. The data obtained demonstrated that the biosolid raw sample had genotoxic potential for *Allium cepa* root tip cells. In the diplopods exposed to biosolid sample, epithelium disorganization in the midgut and a reduction of the volume of the hepatic cells were observed after 7 days of exposure. After 30 days, the animals still showed a reduction of the volume of the hepatic cells, but in minor intensity. *Allium cepa* analysis showed genotoxicity, but this effect was reduced after 30 days of bioprocessing by diplopods. This study was important to know the effects as well as to determine how this waste could be applied concerning the soil living organisms and plants.

1. Introduction

In sewage treatment plants (STP), after the sewage had been treated, a sludge rich in organic matter and nutrients is generated as a waste, known as sewage sludge. The composition of this sludge is very variable since it depends on the source of the sewage treatment process and the seasonality [1]. Generally, the sewage sludge presents around 40% to 60% of organic matter, 4% nitrogen, 2% phosphorus, and other macro- and micronutrients, besides potentially toxic elements [2].

The generated sewage sludge still can go through processes in order to increase the solids and reduce the number of pathogenic organisms, generating a residue called biosolid, which is considered most innocuous than the sewage sludge itself [3]. Good quality fertilizers can be generated with the sludge stabilization, reducing its volume through the use of “sludge thickeners drying beds,” filter presses, band presser, vacuum filters, and centrifugation [4]. According to Lambais and Do Carmo [5], chemical composition of the sludge depends on the origin of the wastewater. This way, the material is variable, but generally it

is a compound rich in organic matter and essential nutrients for plants and microorganisms.

Currently, sewage treatment plants in different Brazilian cities are facing the problem of sludge disposal. The alternatives to the sewage sludge usual fate are landfill disposal, reuse in industry (light-weight aggregate production, bricks and ceramics manufacturing, and cement production), incineration, conversion into fuel oil, ocean disposal, recovery degraded soils, and agricultural use [2, 6].

In Brazil, there is a preference for the use of sludge in agriculture, since there is a considerable land availability and the costs would be relatively low. However, this practice is still incipient, so that the application is made without an adequate management [4]. According to Melo et al. [7], when incorporated into the soil, sewage sludge provides changes in physical properties such as density, aggregates size, and water retention capacity; on chemical properties such as pH, electrical conductivity, CEC, and increased levels of P and N and biological properties, usually by increasing soil microbial activity.

However, the main limitation observed during the evaluation of possible utilization of sewage sludge in agriculture

refers to the presence of metals and other persistent pollutants [8], which may be toxic to plants [9], microorganisms [10], and soil invertebrates [11]. Although other work related the phytoavailability of these metals to a variety of cultures, few studies relate the sewage sludge to genotoxic and mutagenic potential. The application of metal-rich biosolids in clay and sandy soils, compared with biosolids with low-metal concentration, causes a transient soil microbial community increase in mass and activity, with reduced carbon immobilization [5]. Many field studies, based on biosolids agronomic doses, reported soil biota stimulation, probably due to the addition of organic matter, which causes an increase in fertility. The application of this kind of compound, however, has shown inhibitory effect on soil invertebrates [12].

Thus, the aim of this study was to investigate the effects of a biosolid sample according to Brazilian standards for its application on soils, under laboratory conditions, using *Allium cepa* (plant) and *Rhinocricus padbergi* (terrestrial invertebrate) as test organisms.

2. Materials and Methods

2.1. *Rhinocricus padbergi*. Adult specimens of *R. padbergi* with a mean size of 5.0 cm were collected at the campus of the São Paulo State University (UNESP), Rio Claro, in order to avoid intraspecific differences related to either diplopod size or age. After collection, the specimens were maintained in the laboratory for 3 weeks for acclimation, in a terrarium containing soil, tubercles, and decomposing pieces of tree trunks from the capture area. The experimental temperature was $21 \pm 2^\circ\text{C}$ and the photoperiod was a 12 : 12-h light/dark cycle.

2.2. *Allium cepa*. All assays were carried out with only one kind of seeds of *A. cepa* (variety Baía Piriforme) to avoid different responses in the several stages of the process.

2.3. Control Soil. The control soil was obtained from the site where diplopods were collected at a depth of 0–20 cm, in the UNESP Campus of Rio Claro, SP. Soil samples were homogenized, dried at room temperature, and sieved with 4 mm mesh.

2.4. Biosolid Sample. The wastewater treatment plant where the biosolid was collected occupies an area of 20 hectares. The facility serves approximately 80% of the 318.785 inhabitants [13] of a city in São Paulo state where sewage is treated by conventional activated sludge process. In October 1999, the plant received the license of Producer of Agricultural Amendments, by the Ministry of Agriculture. The product produced in the facility is a biosolid classified as soil conditioner. The brand name is Sabesfertil (SP-09599 00001-0). Biosolid samples were collected and stored in plastic boxes wrapped with dark plastic bags and maintained in a cold room (4°C), until use.

2.5. Chemical Analysis of Samples. The concentration of trace elements (As, Ba, Cd, Cu, Cr, Hg, Mo, Ni, Pb, Se, and Zn) and the 16 priority organic compounds (PAHs) in the biosolid

and control soil was determined followed the Standard Methods for the Examination of Water and Wastewater 21th Edition 2005 (SM21) and USEPA. The characterization of samples was measured by TASQA Laboratory (Paulínia, São Paulo, Brazil). Analyses of trace elements were performed by inductively coupled plasma emission spectrometry (ICP-AES). The PAHs analyses were performed by atomic absorption spectrometry. Chemical and physicochemical analyses, as well as a characterization of control soil sample based on macro- and micronutrients (N, Ca, Mg, P, K, S, Fe, Mn, Cu, and Zn), C/N ratio, organic matter, cation exchange capacity (CEC), and base-saturation percentage, were carried out by the Instituto Campineiro de Análise de Solo e Adubo (ICASA), Campinas, São Paulo, Brazil.

2.6. Calculating Biosolid Quantities for Application

2.6.1. Application of Sewage Sludge. According to the law 375/2006 of the Environmental National Council (Conselho Nacional do Meio Ambiente, CONAMA) [14], the maximum annual application of sewage sludge and derivatives in tons per hectare shall not exceed the quotient between the quantity of nitrogen recommended for the crop (in kg/ha), following the official recommendation for São Paulo State and the nitrogen content available in the sewage sludge or derivatives (in kg/t), calculated as N recommended (kg/ha)/N available (kg/t).

To determine the nitrogen available in the sewage sludge and/or biosolid, mineralization fractions were calculated. According to CONAMA [14], this fraction represents 40% of undigested and 20% of digested sewage sludge.

2.6.2. Preparation of Soil and Residue Sample for the Bioassays with *R. padbergi*. Two glass terraria with capacity for 22.5 L were filled with 5 Kg of control soil each. After physicochemical analysis of soil samples and biosolid, the following bioassays were set up with control soil (CS) and soil + biosolid (SB):

- (1) CS: 5 Kg of control soil;
- (2) SB: 5 Kg of control soil + 234.4 g of biosolid.

Twenty specimens of *R. padbergi* were then placed in each terrarium, where they remained for 30 days to assess the toxicity of contaminants present in biosolid. The animals were monitored for 90 days. Six animals per bioassay were dissected for histological analyses, three animals on the seventh day, and three diplopods in 30th day of exposure.

2.6.3. Histology of the Midgut. The diplopods were anesthetized with chloroform, placed in Petri dishes containing isotonic salt solution, and dissected under the dissecting scope. The midgut was removed and fixed in paraformaldehyde 4%. Following that, the organ was dehydrated in increasing concentrations of ethanol (70%, 80%, 90%, and 95%), embedded in resin (Leica historesin), and kept in the refrigerator for 24 h. Later, the material was transferred to plastic moulds containing inclusion resin. After polymerization, $6\ \mu\text{m}$ slices were obtained with the help of a Leica

RM2245 microtome. For histological analyses, sections were stained with hematoxylin and eosin.

2.7. Germination of *A. cepa* Seeds in Residue Samples at Time 0 (t_0) and after Exposure to Diplopods after 30 Days (t_{30}). Approximately 100 seeds of *A. cepa* were allowed to germinate at 22°C in Petri dishes containing raw biosolid sample (B) and soil from each terrarium: CS and SB samples were collected and placed in Petri dishes for the germination of *A. cepa* seeds, at time 0 (t_0). Positive controls were made with the aneugenic herbicide trifluralin (TRIF) at the concentration of 0.019 ppm [15] and methyl methanesulfonate (MMS), a clastogenic agent at the concentration of 10 mg/mL [16]. Negative control (NC) consisted of seeds allowed to germinate in ultrapure water and the environmental control (CS) consisted of seeds allowed to germinate in the control soil.

After 30 days of exposure by diplopods, soil samples from each terrarium were collected for the tests with onion seeds.

2.7.1. Preparation of Slides of *A. cepa*. After germinating and reaching 2 cm in length, root tips were collected and fixed with Carnoy (3:1 ethanol/acetic acid). Samples were then stained with the Feulgen reaction [17], with acid hydrolysis for 11 minutes. Root tips were sectioned to remove the meristem and region F_1 . To intensify the staining and spread cells, one drop of 2% acetic carmine was added. All samples were lightly pressed between slide and coverslip. Coverslips were removed with liquid nitrogen and slides were mounted with Enthelan. The material was analyzed under light microscope, at magnification of 400x.

2.7.2. Evaluation of the Cytotoxic, Genotoxic, and Mutagenic Effects on Meristematic Cells of *A. cepa*. A total of 5000 cells were examined for each treatment at t_0 and t_{30} and for the negative and positive controls. Cytotoxicity was assessed based on morphological alterations indicating cell death, and the mitotic index (MI) calculated as $MI = (\text{number of dividing cells} / \text{total number of observed cells}) \times 100$. The cells in death process present a vacuolated cytoplasm, which is outcome of the cytoplasmatic organelles digestion by lysosomal enzymes [18]. They can still present enhanced cytoplasmatic volume, which can lead to a rupture of the plasmatic membrane exposing the cell content to the outer media [19].

Genotoxicity was evaluated based on the number of cells with chromosome aberration (CA). For the CA analyses, several aberrations within different cell divisions (metaphase, anaphase, and telophase) were considered such as C-metaphase, chromosomal adherence, multipolar anaphase and telophase, and chromosome bridge and loss [20]. The frequency of CA was calculated as $CAI = (\text{number of cells with chromosome aberrations} / \text{total number of observed cells}) \times 100$. Mutagenicity index (IMt) was determined based on the occurrence of cells with micronuclei (MN) and chromosome breaks, calculated as $IMt = (\text{total number of cells with MN and breaks} / \text{total number of observed cells}) \times 100$. The results obtained in all treatments at the different periods were compared with the negative control and soil control at

their corresponding times with the Mann-Whitney test, with significance set at 0.05.

2.7.3. Evaluation of Micronuclei in Cells of the F_1 Region of *A. cepa*. The damage to meristematic cells was assessed based on the number of micronuclei in cells of the F_1 region, which is composed by differentiated cells and is located about 1 mm above the meristematic region [21]. A total of 5000 cells were examined per treatment. The results obtained for all samples were compared with the negative control and soil control at their corresponding times with the Mann-Whitney test, with significance set at 0.05.

3. Results

3.1. Chemical Characterization of Samples. To follow a Brazilian standard for application of biosolids on soil, data regarding the agronomic potential and fertility of control soil became necessary. The values obtained are presented in Table 1. The results obtained by physical-chemical and trace elements analysis of control soil and biosolid sample are presented in Table 2. The concentrations of arsenic and copper found in the control soil were above the limits determined by CETESB-195/2005-E [22], but below the limits for intervention in agricultural areas. Levels of barium, lead, copper, chromium, molybdenum, nickel, and zinc found in the biosolid were high, but below the maximum level allowed by CONAMA (Table 2). None of the 16 PAHs, priority by EPA, were detected by analyses.

3.2. Histology of the Midgut of Diplopods. Animals from control soil presented the midgut (Figure 1(a)) as the histological pattern described for the species [23], being, therefore constituted by a pseudostratified epithelium with brush border (arrow head in Figure 1(a)). The epithelium showed principal cells with nuclei of round to oval morphology, located in middle apical region and regenerative cells in the basal portion; the epithelium is followed by a muscular layer and hepatic cells layer covered by an external membrane. The hepatic cells had an irregular morphology, spherical nucleus, and cytoplasm with cytoplasmic granules of varied content. Among the hepatic cells, some hemocytes were observed, generally isolated.

The group exposed for one week to SB showed epithelium disorganization (arrow in Figure 1(b)) with disruption in several places, indicating an epithelium renewal. The hepatic cells layer showed disorganization with volume reduction in some cells (Figure 1(c)).

After 30th day of exposure, animals from SB sample showed the minor disorganization of the hepatic cells layer when compared to the midgut of the animals exposed for 7 days (Figure 1(d)).

3.3. Cytotoxic, Genotoxic, and Mutagenic Effects on *A. cepa*. The mitotic index of cells examined for B, SB, TRIF, MMS, CS, and NC at 0 and after 30 days of exposure to diplopods is presented in Table 3. Seeds exposed to B and SB samples presented the highest mitotic index in both periods of exposure. However, no sample was statistically significant

TABLE 1: Fertility parameters of the control soil.

Sample	pH	g/dm ³	mg/dm ³	mmol/dm ³ TFSA					%	Ratio		
	CaCl ₂	OM	P res	K	Ca	Mg	H + Al	SB	CEC	V	Ca/Mg	Mg/K
Soil	6.20	18	3.0	0.8	2	1	88	3.9	91.9	4.2	2.0	1.25

OM: organic matter; CEC: cation-exchange capacity; V: base saturation.

TABLE 2: Physicochemical and metal analysis of the control soil and biosolid sample.

Parameter	Samples		Method	G (mg/kg)	MCA (mg/kg)
	CS (mg/kg)	B (mg/kg)			
Arsenic	16.8	<LQ	SM21 3120B	3.5	41
Barium	5.91	158	SM21 3120B	75	1300
Cadmium	<LQ	<LQ	SM21 3120B	<0.5	39
Total calcium	25.4	3939	SM21 3120B	—	—
Organic carbon (g/kg)	12.6	279	SSSA Cap40	—	—
Lead	49.3	174	SM21 3120B	17	300
Copper	37.2	276	SM21 3120B	35	1500
Electric conductivity ($\mu\text{s}/\text{cm}$)	115	5389	SM21 3120B	—	—
Chromium	31.2	224	SM21 3120B	40	1000
Total sulfur	151	11864	SM21 3120B	—	—
Total phosphorus	182	17027	SM21 3120B	—	—
Total magnesium	<LQ	358	SM21 3120B	—	—
Mercury	<LQ	1.08	EPA 7470A	0.05	17
Molybdenum	3.64	9.55	SM21 3120B	<4	50
Nickel	13.0	82.3	SM21 3120B	13	420
Nitrate (mg/Kg)	4.40	6.79	SM21 4500-NO ⁻³ E	—	—
Nitrite (mg/Kg)	0.06	1.39	SM21 4500-NO ⁻² B	—	—
Ammoniacal nitrogen (mg/kg)	31.8	167	SM21 4500-NH ₃ E	—	—
Kjeldahl nitrogen (mg/Kg)	476	21620	SM21 4500-Norg B	—	—
pH	6.20	8.01	EPA 4095 C	—	—
Total potassium	406	2152	SM21 3120B	—	—
Selenium	<LQ	<LQ	SM21 3120B	0.25	100
Total sodium	<LQ	<LQ	SM21 3120B	—	—
Solid content	0.86	0.24	SM21 2540B	—	—
Moisture (g/g)	0.14	0.76	SM21 2540B	—	—
Zinc	23.2	825	SM21 3120B	60	2800

CS: control soil; B: biosolid; LQ: limits of quantification; IV: inconsistent value; G: guidelines of quality for soil (mg/kg) and groundwater in São Paulo State, according to CETESB (195/2005-E); MCA: maximum concentration allowed in sewage sludge or derivative product, according to CONAMA (375/2006).

TABLE 3: Mean and standard deviation of the mitotic (MI), the chromosome aberration (CA), and mutagenicity indexes (IMt) in meristematic cells of *Allium cepa* after exposure to control soil, negative and positive controls, and biosolid samples.

Samples		MI		ICA		IMt	
		t_0	t_{30}	t_0	t_{30}	t_0	t_{30}
Controls	NC	13.66 ± 1.37	11.2 ± 1.78	1.2 ± 0.83	2 ± 1	0.2 ± 0.44	0.6 ± 0.54
	CS	14.21 ± 0.90	9.48 ± 1.07 ²	2 ± 0.70	1.6 ± 1.14	0.6 ± 0.54	0
	MMS	11.53 ± 0.88	5.73 ± 1.15	8.6 ± 3.04* ¹	14.2 ± 2.94* ¹	25.6 ± 8.5* ¹	24.8 ± 7.29* ¹
	TRIF	9.47 ± 0.54	9.31 ± 1.35	51.4 ± 8.79* ¹	23.6 ± 4.82* ¹	12.2 ± 3.42* ¹	3.6 ± 0.89* ¹
Raw	B	15.36 ± 0.99	NA	2.15 ± 1.12	NA	0.16 ± 0.18	NA
Combination	SB	15.07 ± 0.56	15.03 ± 0.79	21 ± 5.83* ¹	10.6 ± 3.33* ^{1,2}	1.2 ± 1.3	0.4 ± 0.54

NC: negative control; CS: control soil; MMS: positive control; TRIF: positive control; B: raw biosolid sample; SB: soil + biosolid.

t_0 : time of mixing and t_{30} : after 30 days of exposure to the diploids.

*Statistically significant values when compared to the negative control, by Mann-Whitney test, $P < 0.05$.

¹Statistically significant values when compared to the control soil, by Mann-Whitney test, $P < 0.01$.

²Statistically significant values when compared to the same treatments at 0 and 30 days.

NA: not available.

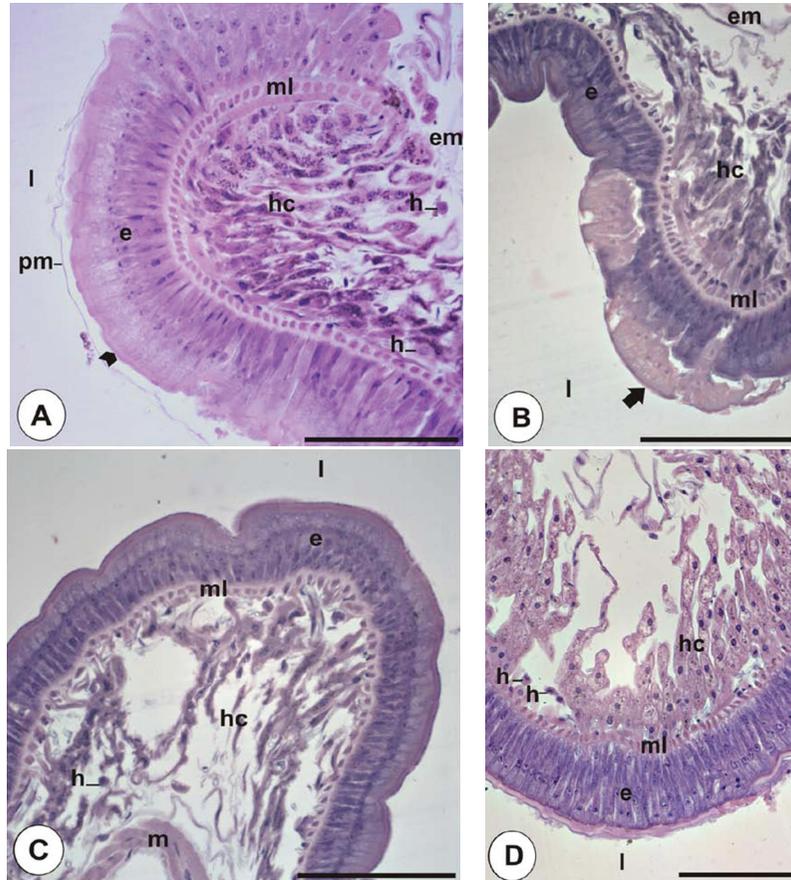


FIGURE 1: Micrographs of the *R. padbergi* midgut stained with HE. (A) Animals exposed for 7 days to the control soil; (B, C) animals exposed for 7 days to soil + biosolid; (D) animals exposed for 30 days to soil + biosolid. e: epithelium; em: external membrane; h: hemocyte; hc: hepatic cells; l: lumen; m: muscle fibers; ml: muscular layer; pm: peritrophic membrane; arrow head in (A) brush border; arrow in (B) epithelium renewal. Scale bars = 100 μ m.

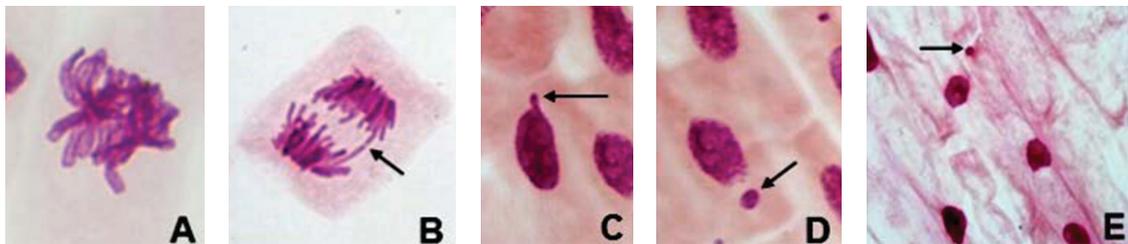


FIGURE 2: Chromosomal aberrations in *Allium cepa* exposed to raw biosolid (B) and control soil + biosolid (SB); (A) chromosomal adherence; (B) anaphase with polyploidy and chromosome bridge (arrow); (C) nuclear bud (arrow); (D) meristematic cell carrying micronuclei (arrow); (E) micronuclei in cells of the F₁ region (arrow).

when compared to the negative control. In comparison to the values obtained between the CS and B samples there was a significant difference. There were no cells in the cell death process for all samples.

B and SB samples induced genotoxic effects (Table 3), at t_0 and t_{30} ($P < 0.05$), characterized by chromosome aberrations, compared to the negative control and/or control soil. However, the genotoxic effect was significantly reduced after the bioprocessing by diplopods in the SB group. The most frequently observed alterations in the present study were cells

in metaphase with chromosomal adherence (Figure 2(a)), polyploidy (Figure 2(b)), anaphase with chromosomal bridge (Figure 2(b)), and nuclear bud (Figure 2(c)).

Meristematic cells with micronuclei (Figure 2(d)) and chromosome breaks were examined to assess the mutagenicity. Statistical analysis revealed no mutagenic effect of the samples examined at both times of exposure compared to the negative control and/or control soil (Table 3). Although micronuclei has been observed in meristematic and F₁ region (Figure 2(e)), the values were not statistically significant

TABLE 4: Mean and standard deviation for micronuclei in cells of the meristematic region (M) and F₁ of *Allium cepa* after exposure to control soil, negative and positive controls, and biosolid samples.

Samples		t_0		t_{30}	
		M	F ₁	M	F ₁
Controls	NC	0.2 ± 0.44	0.2 ± 0.44	0.6 ± 0.54	0.2 ± 0.44
	CS	0.6 ± 0.54	0	0	0.2 ± 0.44
	MMS	21.2 ± 7.08* ¹	4.6 ± 2.07* ¹	20.6 ± 5.12* ¹	4.6 ± 0.89* ¹
	TRIF	12.2 ± 3.42* ¹	2.6 ± 1.14*	3.6 ± 0.89* ¹	2.6 ± 0.89* ¹
Raw	B	0.14 ± 0.14	0.05 ± 0.04	NA	NA
Combination	SB	1 ± 1	0.6 ± 0.54	0.4 ± 0.54	0

NC: negative control; CS: control soil; MMS: positive control; TRIF: positive control; B: raw biosolid sample; SB: soil + biosolid.

t_0 : time of mixing and t_{30} : after 30 days of exposure to the diplopods.

*Statistically significant values when compared to the negative control, by Mann-Whitney test, $P < 0.05$.

¹Statistically significant values when compared to the control soil, by Mann-Whitney test, $P < 0.01$.

²Statistically significant values when compared to the same treatments at 0 and 30 days.

NA: not available.

(Table 4) and a decrease for these values was observed after 30 days of exposure to diplopods.

4. Discussion

Researches about sewage sludge disposal in soil are focused on its effects on soil fertility, plant development, and contamination by heavy metals and organic compounds [24]. However, few studies have been conducted to evaluate the toxic, genotoxic, and mutagenic potentials of the sewage sludge disposal on exposed plants and animals.

Chemical analysis of biosolid sample showed the presence of trace elements. According to the literature, these elements tend to induce genotoxic and/or mutagenic effects on plants [25, 26] and tend to concentrate in the terrestrial invertebrates tissues because their rate of absorption frequently surpasses their rate of elimination [27].

Several authors have used higher plants to diagnose and monitor the action of chemicals and environmental pollution. Among these, *Allium cepa* (onion) has been used in determination of cytotoxic, genotoxic, and mutagenic effects of substances [15, 28] and complex environmental samples [29–36].

In this study, *A. cepa* assays were carried out to assess abnormalities in dividing cells and to estimate the potential of B and SB samples to induct chromosome aberrations. The mitotic index of B was statistically significant compared to control soil. In our study, the mitosis stimulation observed in B and SB treatments may be due to the phosphorus and nitrogen presence and abundant elements in domestic sewage [37, 38].

Chromosomal aberrations are recognized as important consequences of the genotoxic environmental chemicals actions [39], to which many organisms, including humans, are exposed. Epidemiological studies have linked high chromosomal aberrations frequencies at significant cancer developing risk [40]. For this reason, numerous biological tests for chromosomal aberrations evaluation have been developed, in order to ensure the environmental quality [15, 31].

The most common aberrations observed in this study were metaphase with chromosomal adherence, polyploidy anaphase, and anaphase with chromosomal bridge and nuclear bud. According to the literature, all aberrations may be caused by the action of some trace elements/metals. Some metals are potentially genotoxic/mutagenic and are strongly related to environmental pollution. Several studies with plants have shown that the genotoxic effects of metals can cause changes in chromosome structure, chromosome number, and also the disturbances in the mitotic apparatus [25, 26]; they have the ability to inhibit mitotic spindle formation, leading to an abnormal chromosomes distribution and polyploidy [25, 41–43].

In this study was observed the presence of some elements such as barium, lead, copper, chromium, mercury, molybdenum, nickel, and zinc. Although their concentrations are within the standards established by CONAMA [14], according to De Godoy and Fontanetti [44], these standards do not take into account aspects such as the possible interaction of toxic metals and plants, as well as the lack of information regarding the influence of sludge on the soil fauna, including animals that promote humification, aeration, and enrichment of the same.

Like earthworms and collembola [8, 12, 45, 46], the diplopods are considered excellent test organisms for studying the organic amendment effects in the soil ecosystem due to their direct exposure and their sensibility to pollutants; therefore, the diplopods have been used as bioindicators of soil pollution and ecotoxicological assessment [27, 44, 47, 48].

The stimulation of soil biota revealed in some field studies using agronomic dosage of biosolids [49] is probably linked to the soil fertility enhancement, especially due to the contribution of the organic matter. However, in some laboratory investigations, the biosolid and sewage sludge application has caused inhibitory effects [50] and tissue damage on soil invertebrates [44, 47, 48].

Morphological changes can be employed in investigations of chemicals toxicity and in monitoring of acute and chronic effects, in impacted environments [51]. Analyses

involving morphology and histology of tissues in invertebrates have been widely used to identify different damage types caused by harmful substances to animals [52–55].

Preliminary histological analysis of the midgut of diplopods, exposed for 7 and 30 days to the SB sample, according to sewage sludge disposal for Brazilian law may help clarify the mechanisms used by animals in an attempt to detoxify the contaminants present in the biosolid when applied to the soil. It is inferred that the high rate of epithelium renewal of the midgut of these animals may be to maintain the integrity of the organ and an attempt by the body to compensate the damage suffered after the ingestion of contaminated soil [27]. Similar results were obtained by other authors in response to acute diplopods exposure to industrial soil contaminated with polycyclic aromatic hydrocarbons [27] and sewage sludge samples from different sewage treatment plants [47, 48].

Tissue changes related to defense and detoxification may be reversible, being present only as a result of an altered metabolic status of the organism [56]. In invertebrates, the intracellular accumulation of potentially toxic compounds in insoluble forms and in physiologically inactive ones is an efficient mechanism for detoxification of these elements [27, 57]. In diplopods, the midgut and the hepatic cells work actively in this process [47, 57].

The biotransformation of toxic compounds requires the consumption of energy reserves [27]. In the present study, after 7 days of exposure, the reduction of the volume of hepatic cells of the animals exposed to SB sample could be due to the large energy demand for detoxification of the toxic compounds found in the soil. However, after 30 days of exposure, a decrease in the aggression intensity in hepatic cells of the diplopods' midgut was observed, probably due to SB sample stabilization.

At the same time, there was a reduction of SB genotoxicity in onion seeds exposed to this sample. It is inferred that the genotoxicity reduction was obtained again due to SB sample stabilization for bioprocessing of this by diplopods and subsequent immobilization of trace elements. However, under field conditions, the animals would be chronically exposed to this residue, given the frequent application of this. In this sense, it is indispensable to develop studies that evaluate the subchronic exposure effects in an attempt to measure whether responses are sufficient to maintain the organ integrity and/or if will elapse in more severe cellular/tissue damage.

5. Conclusion

There is evidence that biosolids use will lead to increased soil fertility and trace elements levels; therefore, the environmental significance of such increases needs to be examined.

The results of this study reinforce the need for more research to evaluate the biological effects of waste to be discharged into the environment in different ecosystems compartments, as well as different levels of biological organization, even when toxic agents are present at low concentrations, since the sample studied in accordance with Brazilian standards for sewage sludge disposal has shown toxic and

genotoxic potential to onion seeds and terrestrial invertebrates.

In this sense, studies that evaluate the subchronic exposure effects in an attempt to measure how this provision can be harmful or not to the environment compartments and receptor organisms are necessary.

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Research Article

Biomass and Yield of Peanut Grown on Tropical Soil Amended with Sewage Sludge Contaminated with Lead

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Application of sewage sludge with high lead (Pb) contents may pollute soils and contaminate crops. The objective of this work was to evaluate peanut responses to application of sewage sludge with varying Pb contents in order to supply phosphorus (P) to the plant. A greenhouse experiment was carried out with peanut grown on soil sample from a medium-textured Haplustox. Treatments were arranged in $3 \times 2 + 2$ factorial scheme, replicated three times, distributed in randomized block design, and consisted of: three Pb rates applied to soil with sewage sludge (3, 21, and 42 mg kg^{-1}) \times two times of sewage sludge application (30 days before peanut sowing and at the day of the sowing) + mineral fertilization + control (without sewage sludge and mineral fertilization). Sewage sludge was efficient to supply P to peanut. Sewage sludge containing high rates of Pb, when applied, did not harm biomass and yield of the plant, but increased HCl-extractable Pb in soil and Pb content in shoot, roots, and pod husks. Increase of Pb content in pod husks may represent contamination risk of kernels and their products with fragments from husks detached during manipulation or industrial processing of peanuts.

1. Introduction

Lead is a naturally occurring trace element in soils with variable total concentrations depending on the parent material and prevalent pedogenetic processes [1], whereas its total content is far higher than the background concentrations in contaminated soils. Elevated Pb concentrations in soil may impact plants and soil microorganisms and cause serious effects in humans such as neurological problems and damages to the central nervous system [2].

Sewage sludge contains Pb and other heavy metals and its application on agricultural soils has been a way sought for disposal of high amounts produced of this waste particularly in large urban areas [3, 4]. While solving the problem of disposal repeated applications of sludge may increase Pb content in soils. Crops grown on these soils can uptake large Pb amounts and transfer it to edible parts [5]. Intake of these parts increases risks to human health.

Sewage sludge has been used in agriculture with the purpose of improving soil properties and to supply nutrients to crops [6]. In São Paulo state, Brazil, research results show the possibility of using the sludge to supply nutrients to sugarcane without impairing yield and quality of stalks [7] and with low potential for contamination of soil-plant system by heavy metals, including Pb [8]. However, it is essential to know the responses of other crops which might eventually be planted in areas of sugarcane grown on soils amended with sewage sludge. Peanut crop has been the preferred crop to grow in many traditional areas of sugarcane production in São Paulo state. In these areas, it is planted during reform of sugarcane plantation in order to establish a crop rotation. Responses of peanut to sewage sludge application are not known in Brazil.

Sewage sludge can increase peanut yield since it is applied in high rates because its content of macronutrients is relatively low [9]. As a phosphorus (P) source to the crop,

TABLE 1: Selected chemical characteristics of soil samples used in this study and interpretation of values[†].

pHCaCl ₂	OM g dm ⁻³	Resin P mg dm ⁻³	K	Ca	Mg mmol _c dm ⁻³	SB [‡]	H+Al	CEC [§]	BS [¶] %
5.6	17.8	1.0	5.2	18.2	4.7	28.1	11.0	39.1	71.9
Low acidity	—	Very low	High	High	Low	—	—	—	High

[†] According to Raij et. al. [10].

[‡] Sum of bases (K + Ca + Mg).

[§] Cation exchange capacity (SB + H+Al).

[¶] Base saturation ((SB/CEC) × 100).

TABLE 2: Contents of nutrients and Pb in original sewage sludge used in this study.

C	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Mo	Zn	Pb
		g kg ⁻¹							mg kg ⁻¹			
390.0	79.1	10.6	0.63	22.1	2.1	118.0	98.0	42224.0	242.0	9.8	1868.0	127.0

TABLE 3: Nutrients applied with sewage sludge and mineral fertilization to soil samples before peanut sowing.

Nutrient	Sewage sludge	Mineral fertilizer mg kg ⁻¹
N	1990.0	100.0
P	267.0	200.0
K	16.0	150.0
Ca	556.0	
Mg	53.0	
S		
B	3.0	5.0
Cu	2.5	15.5
Fe	1062.3	
Mn	6.1	22.2
Mo	0.2	3.1
Zn	47.0	50.0

it has been more efficient than other sources such as vermicompost, farmyard manure, poultry manure, and single superphosphate [11]. Furthermore, King and Hajjar [12] have found that sewage sludge applications to previous crops did not significantly increase Pb content in peanut plant. However, since sludge has a very varied composition in Brazil [13], it can contain high Pb contents and its agricultural use can contaminate soil and harm the peanut crop.

The objective of this work was to evaluate peanut responses to application of sewage sludge with varying Pb contents in order to supply P to the plant.

2. Material and Methods

A greenhouse experiment was carried out from November 2000 to March 2001 in Jaboticabal, São Paulo state, Brazil. Soil samples from 0–20 cm layer of a medium-textured Haplustox were used in this study. The samples were air-dried, sieved, homogenized, prepared, and chemically analyzed. Selected chemical characteristics of these samples are given in Table 1. Soil pH was measured in 0.01 M CaCl₂ (1:2.5 soil:solution ratio). Organic matter was obtained

by Walkley-Black method [14]. Phosphorus, K, Ca, and Mg were extracted by an ion-exchange resin procedure [15]. Potential acidity (H+Al) was estimated by SMP-buffer alternative method [16].

Sewage sludge used in this research was obtained from a sewage treatment plant in Franca, São Paulo state, Brazil. Sludge air-dried revealed the composition shown in Table 2. The Pb content was below from maximum limit allowed in São Paulo state (840 mg kg⁻¹) [17]. To increase it, PbCl₂ was added and well-mixed to the sludge, and the mixture was incubated for 30 d. The reagent was applied to raise the Pb content to 840 and 1680 mg kg⁻¹, that is, 1 × and 2 × the maximum allowed, respectively. Thus, sewage sludges used in this work had three Pb contents: 127 (original content; see Table 2), 840, and 1680 mg kg⁻¹.

Soil samples were placed in plastic pots with capacity to 8 L. Each pot was filled with 8 kg of soil sample. In part of the pots, sewage sludges with varying Pb contents were applied and well-mixed to the soil 30 d before peanut sowing or at the day of the sowing. The rate of sludge was fixed and defined in order to supply the peanut P requirement, since soil had low availability of P (Table 1). It was applied equivalent to 25.2 g kg⁻¹ of sewage sludge. In this application, the following Pb rates were added to soil by sludge: 3, 21, and 42 mg kg⁻¹. In another part of the pots, mineral fertilizers were applied and well-mixed to soil at the day of the sowing. Fertilizers were the following reagents: CO(NH₂)₂, Ca(H₂PO₄)₂, KCl, H₃BO₃, CuSO₄·5H₂O, MnSO₄·4H₂O, NaMoO₄·2H₂O, and ZnSO₄·7H₂O. The rates of nutrients applied with sewage sludge and mineral fertilizers are presented in Table 3. Finally, some pots did not receive both products. These treatments were arranged in 3 × 2 + 2 factorial scheme [three Pb rates applied with sewage sludge (3, 21, and 42 mg kg⁻¹) × two times of sewage sludge application (30 d before peanut sowing and at the day of the sowing) + mineral fertilization (reagents) + control (without sewage sludge and mineral fertilization)] and replicated three times.

The pots were placed in a greenhouse and were organized in a randomized complete block design. In each pot, 9 seeds of peanut (*Arachis hypogaea* L. cv. Tatu) were sown, and 20 d after sowing, plants were thinned out, leaving 2 plants per

TABLE 4: Dry matter of peanut as a function of Pb rates applied to soil with sewage sludge, time of sewage sludge application, mineral fertilization, and control.

Treatment	Dry matter (g per pot)				
	Shoot	Roots	Pods	Pod husks	Kernels
Additional treatments					
Control	54.76a [†]	3.59a	32.61a	13.91a	18.10a
Mineral fertilization	46.31a	3.06a	41.22a	15.73a	25.49a
Sewage sludge					
Pb rate (mg kg ⁻¹)					
3	67.99a	5.28a	44.23a	17.02a	27.39a
21	66.81a	4.90a	47.50a	18.11a	29.44a
42	74.65a	5.42a	42.04a	16.98a	25.03a
Time of application					
30 d before sowing	77.14a	5.66a	41.18a	16.87a	24.40b
At the day of sowing	62.49a	4.73a	48.00a	17.87a	30.16a
Analysis of variance					
Source of variation	<i>F</i> test [‡]				
Additional treatments	NS	NS	NS	NS	NS
Pb rate	NS	NS	NS	NS	NS
Time of application	NS	NS	NS	NS	*
Pb rate × time of application	NS	NS	NS	NS	NS
Sewage sludge × additional treatments	*	**	*	*	NS
CV (%)	29.46	23.67	17.66	12.16	22.45

[†] Means followed by same letter in column within additional treatments and each factor of factorial do not differ significantly by Tukey test at $P < 0.05$.

[‡] NS: not significant. *, ** significant at $P < 0.05$ and $P < 0.01$, respectively.

pot. In all pots, except those which did not receive sewage sludge and mineral fertilizers, potassium was applied as KCl at the sowing and at 30 and 60 d after sowing. At each application, 50 mg kg⁻¹ of K were added.

At 110 d after sowing, the plants were harvested, and a soil sample was collected from each pot. The plants were separated into shoot, root, pod, pod husk, and kernel, washed with distilled water, and dried in a forced-air oven at 65°C. After drying, all plant parts were weighed for determining dry matter. Plant material was digested in an acid mixture (HNO₃ + HClO₄) [18], and Pb was determined in extracts by atomic absorption spectrophotometry (AAS). Soil samples were air-dried, passed through 2 mm sieve, and Pb was extracted with 0.1 M HCl [19]. Lead concentration in soil extracts was determined by AAS.

Results were submitted to analysis of variance. When there was significance, means were compared by Tukey test at $P < 0.05$. Linear regression was adjusted to data of Pb rates and HCl-extractable Pb contents.

3. Results and Discussion

No significant differences in dry matter of shoot, roots, pods, pod husks, and kernels of the peanut between control and mineral fertilization were observed (Table 4). Phosphorus fertilization did not increase the plant dry matter but only increased the P content in the shoot (Table 5), probably due to the low P requirement of the peanut [20], confirmed by field studies [21, 22]. Another explanation for this lack

of response to P fertilization is a possible Mg deficiency in the peanut (Table 5), which may have been induced by K application. Interactions between Mg and K have been previously reported [23]. In this case, as the soil used had high K content and low Mg content (Table 1), the applied K may have rapidly saturated the rhizosphere and restricted the Mg uptake, causing reduction in the Mg content in the shoot (Table 5). This probable deficiency induced by K application may therefore have limited the crop response to P fertilization.

When sewage sludge with increased Pb rate was added to soil, it did not significantly affect the dry matter of shoot, roots, pods, pod husks, and kernels of the peanut (Table 4). Time of Pb application also did not affect the dry matters of these plant parts, except for kernels, which were minor in Pb application 30 d before planting than at the day of planting (Table 4). This reduction was probably not directly related to Pb, because there was no rate effect of this heavy metal in kernel dry matter. Additional treatments significantly differed from Pb treatments in dry matter of shoot, roots, pods, and pod husks (Table 4). On average, dry matters of these parts were higher in Pb treatments than mineral fertilization and control together (in g per pot, 69.8 × 50.5 for shoot, 5.2 × 3.3 for roots, 44.6 × 36.9 for pods, and 17.4 × 14.8 for pod husks). Since Pb was added to soil with sewage sludge and Pb is not a nutrient, this positive effect of Pb treatments is due to the sludge and not to Pb. Although sewage sludge increased dry matter of non-kernel parts, there was no significant difference in kernel

TABLE 5: Phosphorus and Mg contents in peanut shoot as a function of Pb rates applied to soil with sewage sludge, time of sewage sludge application, mineral fertilization, and control.

Treatment	Nutrient content (g kg ⁻¹)	
	P	Mg
Additional treatments		
Control	0.8a [†]	4.1a
Mineral fertilization	1.7a	2.3b
Sewage sludge		
Pb rate (mg kg ⁻¹)		
3	1.8a	3.1a
21	1.9a	3.1a
42	1.8a	3.0a
Time of application		
30 d before sowing	1.9a	2.8a
At the day of sowing	1.8a	3.3a
Analysis of variance		
Source of variation	F test [‡]	
Additional treatments	**	**
Pb rate	NS	NS
Time of application	NS	NS
Pb rate × time of application	NS	NS
Sewage sludge × additional treatments	**	*
CV (%)	11.97	16.16

[†] Means followed by same letter in column within additional treatments and each factor of factorial do not differ significantly by Tukey test at $P < 0.05$.

[‡] NS: not significant. *, ** significant at $P < 0.05$ and $P < 0.01$, respectively.

production among sewage sludge treatments and mineral fertilization and control together (Table 4).

Sewage sludge treatment increased the P content in the shoot at the same level as mineral fertilization (Table 5), thus confirming its potential as fertilizer. Mohanty et al. [11] reported that sewage sludge was more efficient than single superphosphate to supply P to the peanut using a ³²P probing technique. Sewage sludge also increased the Mg content in the shoot as compared to mineral fertilization (Table 5), probably due to the Mg release from the sludge (Table 2), suggesting that the sludge minimized possible Mg deficiency induced by K application.

Lead content in the shoot was influenced by Pb rates applied with sewage sludge (Table 6). The highest Pb concentration in the shoot was 20.0 mg kg⁻¹, which is below the hypothetical reported toxicity threshold of 35 mg kg⁻¹ in plant tissues [24], although the Pb concentrations in plants may vary in the range from 30 to 300 mg kg⁻¹ [25]. The highest Pb content in the peanut shoot was not associated to reduction of the plant dry matter (Tables 5 and 6), and therefore 20 mg kg⁻¹ in this case could be considered as nontoxic.

In the sludge treatments, the Pb contents in shoot, roots, and pod husks (Table 6) increased by 33%, 118%, and 130%, respectively. The pronounced increase in the roots as compared to the shoot suggests a limited translocation of Pb from root to shoot. Wierzbicka [26] and Verma and Dubey

[27] have shown data that confirm this tendency for several other plant species. Studies have indicated that the roots act as a barrier for the Pb transport to shoot. In these studies, it was found that the Pb enters the roots and accumulates in the cell wall of endodermis cells, possibly due to the Pb movement restriction by Casparian strips [28–30]. Jarvis and Leung [31] have suggested that the Pb accumulation in the root is based on (i) binding of Pb²⁺ to ion exchangeable sites on the cell wall and (ii) extracellular precipitation, mainly in the form of Pb-carbonates deposited on the cell wall. The Pb retention in the root endodermis limits the Pb transport to the central vascular tissue, restricting the Pb translocation to shoot [32]. Thus, plants tend to prevent excessive accumulation of Pb in the shoot, and subsequent Pb redistribution to grains. King and Hajjar [12] reported no detection of Pb in peanut kernels grown on soil after repeated applications of metal-contaminated sewage sludge, including Pb. In the present work, kernels were not analyzed for Pb, and although it was observed limited Pb translocation, further studies are needed to assess whether kernels are not directly contaminated with Pb.

Contamination can also occur indirectly. In fact, it has been reported that the peanut husk has an ability to adsorb heavy metals in solution including Pb [33–35]. Based on this ability, it is hypothesized that Pb released from the sewage sludge passed to soil solution and a considerable part of it was adsorbed on the pod husk. This would explain the large increase of Pb content in the pod husk with increasing Pb rate. High Pb contents in the pod husk may contaminate the final products with fragments detached of the husk during manipulation or industrial processing. Therefore, the risk of Pb contamination of products derived from pods harvested from peanut crops grown on soil receiving elevated rates of sewage sludge with high Pb contents cannot be excluded by the presented data.

The HCl-extractable Pb significantly increased ($P < 0.01$) in soils amended with increasing Pb rates, with mean concentrations ranging from 0.94 to 6.89 mg kg⁻¹ at Pb rates of 3 and 42 mg kg⁻¹, respectively (Figure 1). Borges and Coutinho [36] reported lower range (0.37 to 1.27 mg kg⁻¹) for Brazilian soils amended with no metal spiked sludge. The observed increase of Pb content in soil (Figure 1) was consistent with the increase of Pb in the plant (Table 6). Lead in soil was higher ($P < 0.05$) with Pb applied 30 d before sowing than in Pb applied in the day of sowing, probably due to longer time taken by Pb to be released from the sludge.

4. Conclusion

Sewage sludge with twice the maximum allowed content of Pb applied to soil to supply P to peanut did not harm biomass and yield of the plant. However, pod husks had their Pb contents increased, what may represent contamination risk of kernels and their products with fragments from husks detached during manipulation or industrial processing of peanuts.

TABLE 6: Lead content in peanut as a function of Pb rates applied to soil with sewage sludge, time of sewage sludge application, mineral fertilization, and control.

Treatment	Pb content (mg kg ⁻¹)		
	Shoot	Roots	Pod husks
Additional treatments			
Control	15.66a [†]	13.99a	10.66a
Mineral fertilization	15.32a	12.65a	9.66a
Sewage sludge			
Pb rate (mg kg ⁻¹)			
3	14.99b	15.99b	12.16b
21	18.99ab	24.49ab	16.50b
42	19.99a	34.85a	28.00a
Time of application			
30 d before sowing	17.88a	25.11a	17.44a
At the day of sowing	18.10a	23.10a	20.33a
Analysis of variance			
Source of variation		<i>F</i> test [‡]	
Additional treatments	NS	NS	NS
Pb rate	*	*	**
Time of application	NS	NS	NS
Pb rate × time of application	NS	NS	NS
Sewage sludge × additional treatments	NS	**	*
CV (%)	16.26	31.40	24.10

[†] Means followed by same letter in column within additional treatments and each factor of factorial do not differ significantly by Tukey test at $P < 0.05$.

[‡] NS: not significant. *, ** significant at $P < 0.05$ and $P < 0.01$, respectively.

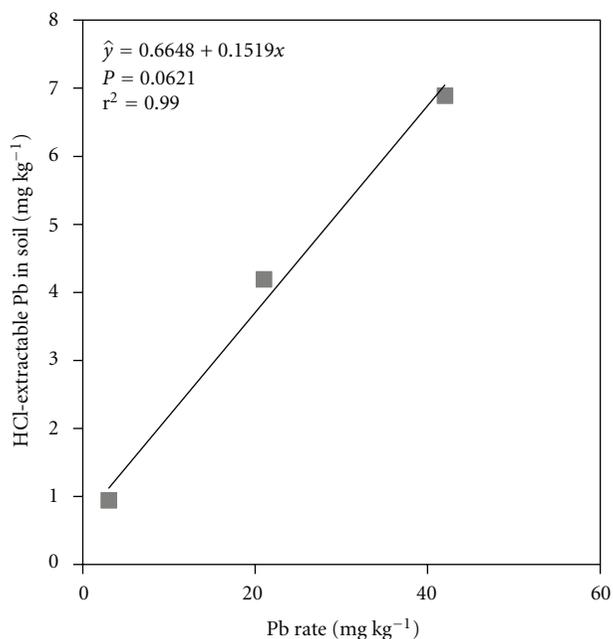


FIGURE 1: HCl-extractable Pb in soil as a function of Pb rates applied with sewage sludge varying content Pb.

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Research Article

Control of *Fusarium* Wilt of Tomato Caused by *Fusarium oxysporum* F. Sp. *Radicis-Lycopersici* Using Mixture of Vegetable and *Posidonia oceanica* Compost

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A compost of vegetable waste and *Posidonia oceanica* mixture (70:30% vol:vol) was tested *in vitro* and *in vivo* for its efficacy against *Fusarium oxysporum* f.sp *radicis-lycopersici* (Forl), the causal agent of Fusarium wilt of Tomato (*Lycopersicon esculentum* cv. chourouk). The incorporation of non-sterilized VPC in the culture medium showed potent antifungal activity against Forl and complete inhibition of mycelium growth was observed for all the tested compost rates (0.5, 1, 2, 4, 6, 8, 10, 15 and 20%). However, only the highest rates (15 and 20%) of a sterilized suspension of VPC were effective in preventing mycelial growth. Nine indigenous bacterial strains isolated from VPC exhibited antagonism against Forl. Based on 16S rDNA sequence analysis, the isolates were assigned to *Bacillus sphaericus* (B12 and BS2), *Pseudomonas putida* PPS7 and *Burkholderia gladioli* BuC16. Under green house condition, seed inoculation by B12, BS2, PP7 and BuC16 strains protected significantly tomato against *Fusarium oxysporum* f.sp *radicis-lycopersici* (Forl) attacks.

1. Introduction

During the last decades, various studies in Tunisia investigated the effects of organic composts as nutrient-rich amendments to correct mineral deficiencies of soils in a semiarid climate [1]. In this context, we have conducted some studies, aimed to exploit the ability of Municipal Solid Waste (MSW) as an organic feedstock to be transformed into compost as well as the mineralization of MSW when added to soil [1–3]. The evolution of microbial biomass was studied by [4, 5]. Application of composted Municipal Solid Waste Compost (MSWC) can lead to addition of potentially toxic heavy metals to Tunisian soils [6]. Some compost may also contaminate the soil with aflatoxins [7]. Other sources of organic matter could be used as alternatives to MSWC. In Tunisia vegetable garden waste was not considered for compost production.

Also, the use of compost as a biocontrol agent able to limit some plant disease has not been reported from Tunisia.

It is well known that compost offers a disease control alternative to fungicides [8]. The use of composts to suppress soil-borne plant pathogens has been extensively reviewed by several authors [9]. Different mechanisms have been postulated to control plant diseases by compost application such as competition for nutrients, antibiotic production by beneficial micro-organisms, activation of disease-resistance genes in plants [10], triggering systemically acquired resistance mechanisms [11] and compost obtained from heterogeneous vegetable wastes [12]. Pascual et al. [13] showed important suppressive effects against disease caused by several plant pathogens such as *Phytophthora* spp. [10, 14], *Rhizoctonia* spp. [15], and *Fusarium* spp. [16]. Also, *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Streptomyces* spp., and other

bacterial genera, as well as *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., *Glocladium virens*, and other fungi have been identified as biocontrol agents contained in compost. Hardy and Sivasithamparam [17] showed important suppressive effects against diseases caused by several plant pathogens such as *Pythium* spp. Moreover, compost is biodegradable, safe to apply, and less expensive to develop than fungicides.

Soil sterilization destroys the most heat-labile part of compost microbial communities and temporarily reduces microbial activity [18]. Manipulation of agricultural systems, through additions of composts, green manures, and cover crops is aimed at improving endogenous levels of general suppression [19]. This generalized improvement in microbial activity due to the addition of green composts may be another limiting factor to the establishment of plant pathogens into soils [20]. In addition, individual species of bacteria or fungi antagonisms could be related to extracellular metabolites released in the culture medium [21, 22], which act as the functional elements of the autochthonous microflora in suppressive composts. A key role in the suppressive effect of composts against root pathogens has also been attributed to the microbial activity of composts [23].

Tomato plants treated by *Pseudomonas putida* PPS7 were characterized by a percentage of healthy plants ranging between 78 and 83.6%. *P. putida* is well known BCA for their suppressive effects; this may be due to various mechanisms such as the chelation of iron by siderophore [24, 25]. De Boer and Kindt (2006) [26] found that the role of siderophores was associated with the antagonistic properties of *Pseudomonas putida* WCS358 in suppressing Fusarium wilt of radish.

Bacillus spp. are attractive candidates for use as BCA, because of their capacity to produce a wide variety of active metabolites, their abundance in soil, and their ability to form endospores [27]. Parasitism is expressed through degradation of the cell walls of pathogenic fungi and relies on production of extracellular lytic enzymes. For example, several *Bacillus* species such as *B. circulans* [28]; *B. licheniformis* and *B. cereus* [29]; *B. pabuli* [30] and *B. pumilis* [31] produce enzymes that degrade chitin, the insoluble linear polymer of β -1,4-N-acetylglucosamine (GlcNAc), which is the second most abundant polysaccharide in nature and a major component of most fungal cell walls.

Fusarium crown and root rot is an important soil-borne disease, with the potential to limit productivity in glasshouse and field tomato crops. The causal agent, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Forl*) race 0, was found in Japan, but was subsequently identified in many other regions, including USA, Canada, Europe, and Israel [32].

Increased early injury to the roots and collar of tomato plants caused by *Forl* was also observed in Tunisia [33], where yield losses were reported to range between 20 and 60%.

Fungicides are of little use on most Fusarium diseases [34]. *Forl* widely distributed in soil is known as a plant pathogen. It is also known to be the first eukaryotic denitrifier that catalyzes the reduction of nitrate into gaseous nitrous oxide (N₂O) [35]. In fact, *Forl* contributed to denitrifying soil.

Biological control of Fusarium wilts, in the form of natural microbial populations in soils, has been recognized for over 70 years [36]. The potential of compost for the control of crown and root rot CCR of green house-grown tomato caused by *Forl* was investigated by [37] who showed the suppressive effect on Fusarium wilt using various composts not involving *Posidonia* as organic matrix in tomato cv. *Marmande*.

The overuse of chemical fertilisers and excessive disturbance often leads to soils low in soil organic matter (SOM). The levels of SOM in Tunisian soils have been declining sharply in the last decades, which increased the soil degradation. Municipal solid compost could bring some pollutants like heavy metals into soil [1]. As alternative to municipal solid compost, we propose the use of wastes or *Posidonia* residues.

Posidonia oceanica is the main sea grass in the Mediterranean countries, as such Tunisia and provides substrates to a species-rich epiphytic community, which achieves maximum biomass between the end of spring and the end of summer [38], covering over 50000 km² [39]. As [40] indicated, the disposal of the annual accumulation of *P. oceanica* on the beaches of the Mediterranean causes a series of economic and environmental problems. In this particular situation, the leafy deposits of *P. oceanica* on the beach can be considered refuse. At present, they are dumped as waste, which result in the loss of enormous mass of organic material.

The agronomic reuse of *P. oceanica* refuse may be an interesting way to provide high-quality organic matter to soils. The dead sheets of *P. oceanica* were traditionally used as compost by the farmers of the coasts Mediterranean [41]. This sea plant is known by its high content in C, N, and P. The desalination of *P. oceanica* did not present a technical problem since *P. oceanica* was a plant with a smooth surface, impermeable to salt existing in its natural environment and a simple rinsing eliminates the quasi totality of chlorides.

It is estimated that the quantity of composted vegetable residues and market wastes produced by Tunis City is greater than 17 t/d, which is a sizable volume of substrate for a biological treatment [42]. Nevertheless more than 90% of vegetable residues (VRs) are dense material with low water content that requires mixing with other wastes or bulking materials.

In this project, we studied the biofungicide effect of VPC against *Forl*. We analysed the direct effect of VPC on mycelial growth of *Forl* *in vitro*. The inhibitory effect of VPC was verified in container experiments using sterile soil inoculated with *Forl* and amended with three rates of VPC. Isolates from VPC were used in order to verify their possible antagonism effect against *Forl*.

2. Materials and Methods

2.1. Composting Materials

2.1.1. Composting Materials. The initial compost material consisted of 70% of vegetable residues (VR), and 30% of *P. oceanica* residues (PoRs). The materials were stacked in

an uncovered pile and followed by a composting cycle as described by [3].

2.1.2. On Site Sampling. The windrow was sampled during each turning. Four samples were taken at the start of the composting process and samples were collected every 5 days for 150 days. Samples of 5 kg taken from various composts were subdivided into three subsamples introduced by [43]. The first subsample was stored at -20°C for enzyme analysis; the second was used for the physicochemical analyses, and the third was used for microbiological analyses. The subsample for physicochemical analysis was dried at 70°C for 2 days and crushed.

2.1.3. Chemical Analysis. Each fraction obtained was characterised by measuring the following parameters: CO_2 released, pH, Kjeldahl N and inorganic N concentrations. Oxidable-C was determined by dichromate oxidation according to the procedure described in norm NF T 90–101 (October, 1998). Total organic N was measured using the Kjeldahl procedure and the inorganic N content was determined in a 1 mol/L KCl extract (1 : 10, W/V) by steam distillation in the presence of MgO ($\text{NH}_4^{++}\text{-N}$) or MgO + Devarda's alloy ($\text{NH}_4^{++}\text{-N} + \text{NO}_2\text{-N} + \text{NO}_3\text{-N}$) [44].

The CO_2 evolution was measured according to the incubation method of [45]. Previously screened sample (25 g) at 60% (*w/w*) moisture content was sealed in 0.5 L respirometer flasks along with a beaker containing 5 mL of 0.5 mol/L NaOH solution. The samples were incubated at room temperature ($25 \pm 2^{\circ}\text{C}$). During the incubation, the released CO_2 was captured by the NaOH solution, which was then analyzed titrimetrically with 0.2 mol/L. HCl in an excess of BaCl_2 at regular intervals.

2.1.4. Evaluation of the Compost Toxicity Using Seed Germination and Root Elongation (GI Index). Germination tests were performed with wheat (Karim, var) provided by the gene bank of National Agronomic Institute Tunisia. Eight seeds, three replicates for each sample of the compost, were left to germinate in the water extract of the compost at 25°C for 72 h. The germination index (GI) was computed by the formula [46]

$$GI = nVSS \times \frac{RLS}{nVSC} \times RLC \times 100\%, \quad (1)$$

where, *nVSS* and *nVSC* express the number of viable seeds in the sample and in the control, respectively (extract compost was replaced by distilled water); RLS and RLC expressed the root length in the sample and in the control, respectively.

VPC samples used in studies on biocontrol of *Fusarium* were kept at -20°C until use.

2.2. In Vitro Antagonistic Activity of VPC

2.2.1. Effect of VPC on Mycelial Growth of Forl. To study the activity of VPC against mycelial growth of Forl, the potato dextrose agar PDA medium was autoclaved for 15 min at 100 kPa. Then, different concentrations (0.5, 1, 2, 4, 6, 8, 10,

15, and 20%) of VPC (sterilized or not) were incorporated in the potato dextrose agar (PDA) medium. VPC was sterilized during 1 hour at 100 kPa.

The production of Antifungal Volatile (AFV) compounds, by compost were assayed by the sealed plate method as described by [47]. From each compost a 200 μL suspension was spread on trypticase soy agar (TSA, Difco Laboratories, Detroit, M) medium. After incubation at 37°C , a second Petri dish (containing PDA) was inoculated with 6 mm plug of the test fungus in the centre of the plate, inverted and placed over the compost culture. The two plates were sealed together with parafilm (Pechiney Parafilm M PM996 SKU: PH-LF) and further incubated at 25°C . This ensured that the both (compost and Forl) were growing in the same atmosphere though physically separated. As a control, a Petri dish containing TSA medium without compost extract was placed over a plate containing a culture of Forl. Fungal growth was measured as increases in colony diameter of the test fungus at 24 h intervals for a period of 5 days. Each test was replicated 3 times.

The cyanide production was detected using the assay method of [48], where 10% TSA containing 4.4 g glycine liter 1 was inoculated with the compost extract (0.5, 1, 2, 4, 6, 8, 10, 15 and 20%). The lid of each Petri dish contained filter paper impregnated with a picric acid solution (0.5% picric acid and 2% sodium carbonate) was sealed to the bottom Petri dish with Parafilm (Pechiney Parafilm M PM996), and incubated at 28°C for 3 to 5 days. A change in color from yellow to orange-brown of the impregnated filter paper indicated cyanide production.

For detection of antibiotic production, suspensions of VPC were incubated for 60 h in an incubator shaker maintained at 30°C and 170 rpm. The compost extract was centrifuged at $10000 \times g$ at 4°C for 10 min. Each supernatant was filtered through a sterile 0.45 μm filter membrane. The cell filtrates were assayed for their ability to inhibit mycelia growth of *F. oxysporum* strain Fo2 by using an agar well diffusion method [49] (Tagg and McGiven, 1971). Molten PDA kept at 45°C was seeded with conidia of *F. oxysporum* and a 5 mm portion spread uniformly over nutrient agar medium (NA, Oxoid). After the seeded layer solidified, three wells were aseptically made using a cork borer, and filled with 100 μL of the test filtrate. The control consisted of 50 μL filter, sterilized distilled water. The samples were allowed to diffuse into the agar and the plate was inverted and incubated at 28°C for 24 h. The plates were examined for halos of inhibition around the wells [49].

2.2.2. Isolation of Bacteria from VPC. For isolation of bacterial strains, 10 g of VPC was suspended in 90 mL of sterile distilled water and shaken for 10 min at 250 rpm. One millilitre of this suspension was used to prepare serial 10-fold dilutions in 0.9% NaCl. Aliquots (100 μL) of an extract of each suspension were spread on Lauria-Bertani agar (LBA: tryptone: 10 g, yeast extract: 5 g, NaCl: 5 g, agar: 15 g and distilled water: 1 L). Representative colonies, that differed morphologically, were selected from the countable plates and restreaked on a new plate of the same media to obtain pure

colonies. Bacterial isolates were stored in 30% glycerol at -20°C .

2.2.3. In Vitro Antagonistic Activity of Bacteria Isolated from VPC In Vitro. Antagonism tests were performed on Potato Dextrose Agar (PDA) in 10 cm Petri plates using a dual culture technique [50]. Bacterial isolates were streaked across the center of the plate, with a second streak made at right angles to the first. Four discs (5 mm in diameter) cut from the edge of 7-day-old cultures of Forl were placed at each side of the antagonist. The distance between the two microorganisms was 2.5 cm. Plates were then incubated at 25°C for one week. Percent growth inhibition of Forl was calculated by the method of [51]. The following formula was used: $(R1-R2) R1 \times 100$, where R1 is the farthest radial distance (measured in mm) grown by Forl, after 7 days of incubation, in the direction of the antagonist (the control value), and R2 is the distance of fungal growth from the point of inoculation to the colony margin in the direction of the antagonist. Three replications of each treatment were used and the experiment was conducted for a week.

Antibiotic Substances Produced by Bacteria Isolated from VPC. Bacterial isolates were streaked on TSB medium and then incubated at 30°C for 24 h. A loop of inoculum from a 12 h culture was introduced into 100 mL of the production medium as per [52]. (consisting of 20 g dextrose, 5 g DL-glutamic acid, 1.02 g $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$; 1.0 g K_2HPO_4 ; 1.5 g KCl and 1 mL of trace element solution (0.5 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$; 0.16 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.015 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 100 mL of water). The pH of the medium was adjusted to 6.0–6.2 with 5 N NaOH. The inoculated media were then incubated for 60 h in an incubator shaker maintained at 30°C and 170 rpm. The bacterial suspensions were centrifuged at 10,000 g for 10 min at 4°C . The supernatants were filtered through sterile $0.45 \mu\text{m}$ disc filters. The cell filtrates were assayed for their ability to inhibit mycelial growth of Forl strain Fo2 by using an agar well-diffusion method [49]. Five mL of molten potato dextrose agar kept at 45°C were seeded with conidia of Forl and spread uniformly over the NA medium. After the solidification of the seeded layer, 3 wells were made using a cork borer and filled with $100 \mu\text{L}$ of the test fluid. The control consisted of $50 \mu\text{L}$ of filter sterilized distilled water. The samples were allowed to diffuse into the agar, and the plate was inverted and incubated at 28°C for 24 h. The plates were examined for halos of inhibition around the wells.

Identification of the Potential Antagonistic Bacteria. The identification of bacterial strains was achieved by sequencing the 16S rRNA gene (rrs). Amplification was carried out by PCR with primers F667-pA-rrs AGAGTTTGATCCTG-GCTCAG and F668-pH-rrs AAGGAGGTGATCCAGCCGCA designed by [53]. Standard PCR conditions were 1 min DNA denaturation at 94°C , 1 min annealing at 57°C , and 1 min extension at 72°C for 35 cycles. The 16S rDNA sequences were compared with sequences in the GenBank database with the Basic Alignment Search Tool [54].

Effect of VPC in Suppressing the Fusarium Attack in Container Experiments. An agricultural Soil Vertic Xero Fluvent (clay: 27%, silt: 62%, sand: 11%, $\text{pH}_{[\text{water}]}$: 7, C (0.87%), N: 0.095% C/N: 9.15). was obtained from a field located in the region of Mornag (southwest of Tunis). The soil was moistened with distilled water to 60% of its water-holding capacity and autoclaved for 1 h at 121°C twice, on 2 successive days. The soil was maintained at 60% of its water-holding capacity for a week under sterile conditions. The soil was again autoclaved twice for 20 min at 121°C . After cooling, it was placed in plastic pots (dimensions: 10 cm \times 10 cm \times 2.5 cm).

This part of experience is divided in two parts: the first one consists of testing the effect of VPC on the fusariose development. The second part concerns the effect of bacteria isolated from VPC on the studied disease. To study the activity of VPC against the development of mycelial of Forl, under pot experience. Different concentrations (0, 10, and 20%) of VPC (sterilized or not) were incorporated and homogenised in the soil. VPC was sterilized during 1 hour at 100 kPa.

Tomato seeds (*Lycopersiconm esculentum cv chourouk*) were surface-sterilized by immersion in 2.5% sodium hypochlorite for 2–3 min and washed thoroughly in 3 changes of sterile distilled water. The seeds were pre-germinated for three days in Petri dishes containing sterile distilled water and the seeds were transplanted into plastic pots (250 cm^3 , 4 seeds per pot, and 3 pots per treatment) containing Forl-inoculated soil mixture (positive control). Negative controls were grown in the uninoculated soil mixture.

This part of experience is divided in two parts, the first one consists of testing the effect of VPC on the fusariose development. The second part concerns the effect of bacteria isolated from VPC on the studied disease. To study the VPC effect against the development of mycelial of Forl, under pot experience. Different concentrations (0, 10, and 20%) of VPC (sterilized or not) were incorporated and homogenised in the soil. VPC was sterilized during 1 hour at 100 kPa.

The second part of the pot experience was undertaken in the same pot condition as in the first experience at the difference the seeds were inoculated with bacteria isolated from VPC by using a liquid suspension (\approx approximately 10^7 colony-forming units (CFU) mL^{-1} of the appropriate isolate), at the rate of 2 mL bacterial suspension per seedling at sowing time. The plants were maintained in greenhouse conditions at $25 \pm 5^{\circ}\text{C}$ and 60 to 90% relative humidity for a period of 6 weeks. Plants were watered as needed and fertilized weekly with 100 mL of Hoagland's nutrient solution. Three replications of each treatment were provided. All values given are averages of three samples for each treatment.

Disease Assessment and Data Analysis. Disease incidence was assessed at 6 weeks by counting number of healthy plants. Forl was reisolated from wilted plants by plating stem pieces from the crown region onto PDA. All treatments were replicated in completely randomised blocks using three replications. Analysis of variance was carried out using SPSS software (SPSS for Windows, version 10; SPSS Inc., Chicago, IL, USA), and means were separated by the least

significant difference according to the Student-Newman-Keuls test. Dendrogram were prepared using cluster analysis and average linkage between groups. Sampling unit for statistical analysis is the pot, not the plant.

3. Results

3.1. Stability and Maturity Indexes of VPC. The carbon-to-nitrogen ratio ranged from 30 at the beginning of composting and decreased notably through the process to reach values around 12 (Figure 1). Dehydrogenase activity reached $6 \text{ mg TPF g}^{-1} \text{ DM}$ during the thermophilic phase of the composting cycle. At the end of composting (maturity phase), the values observed were negligible indicating a high degree of maturity. *Salmonella* was isolated only at the beginning of composting. After 40 days, these bacteria were not detected. The mature compost was relatively rich in N (13.0 g kg^{-1}), P (9.48 g kg^{-1}) and Mg (15.80 g kg^{-1}). At the end of composting cycle we obtained mature stable compost; The chemical characteristics of the mature compost expressed in g kg^{-1} are as follow: N: 13, P: 9.48, K: 7.48, Ca: 37.14 and Mg: 15.18.

3.2. In Vitro Effect of VPC against Forl. The incorporation of VPC in the culture medium revealed potent antifungal activity against Forl and complete inhibition of mycelium growth at all the tested concentrations of unsterilized compost extract (Figure 2(a)). However, for sterilized compost extract, only the highest concentrations (15 and 20%) prevented mycelial growth.

3.2.1. In-Vitro Anti-Fungal Volatile (AFV) Produced by VPC Against Mycelium Growth. Different rates of VPC produced AFV's able to inhibit mycelial growth (Figure 2(b)). AFV's became more inhibitory at higher concentrations of VPC. However, we showed that there was no significant difference in inhibition when we concentrated compost over 6% VPC in the media suspension (Figure 2(b)). Cyanide was produced by compost extracts over 2% concentrations. Antibiotics were detected at the lowest concentrations. At 0.5% VPC, the compost extract was able to limit fungal growth (wells method AWDM) (Table 1).

In Vitro Effect of the Indigenous Bacterial Strains of VPC. Nine bacterial strains were isolated from VPC that exhibited antifungal activity towards Forl in agar well-diffusion assays and in dual culture (Table 2). Based on 16S rRNA sequences analysis, the strains B6, B10, B12, BS2, and B17, were identified as *Bacillus sphaericus*, BuC16 as *Burkholderia gladioli*, PPS7 as *Pseudomonas putida*, and the other isolates, BS1 and BS3, were associated with the genus *Bacillus*.

3.3. Suppression of the Fusarium Attack by VPC in Pot Experiments. VPC application on *Fusarium* infected seeds showed that Forl incidence was reduced by unsterilized VPC. The percentage of tomato diseased plants was reduced by both concentrations of unsterilized VPC (10 and 20%) (Figure 3) and suppressive effects were observed against Forl.

However, the highest concentration (20% VPC) reduced more the percentage of infected tomato seeds compared with the lower concentrations. This could be related to the strong concentration of nitrate in the 20% VPC. On the other hand, the sterilisation of compost suppress natural microflora able to limit Fo2 strain growth. However, a low quantity of sterilized VPC was showed to slightly reduce the percentage of infected tomato compared to 10 and 20%.

3.4. Effect of Indigenous Bacteria in Container Experiments. Inoculation of seeds with the nine bacterial isolates significantly increased the percentage of healthy plants (Table 2). The percentage of healthy plants ranged from 33 to 96.8%. The best disease control was obtained with isolate B4, B5, B17, and B18, that reduced the wilt incidence to less than 20%. These strains were grouped in the same portion of the dendrogram established by cluster analysis. Cluster analysis determined that the bacteria fell into three phylogenetic groups (Figure 4). In the first group (top of the dendrogram), point B6 and B17, tomatoes treated with this group (GI) were able to reach a percentage of healthy plants ranging between 54.4 and 70%.

Strain *B. sphaericus* BS2 showed the highest level of protection and were grouped with the control test GII, (treatment without pathogenic fungi). Indeed, 96.8% of plants treated with this strain were healthy. The third group GIII (in the centre of dendrogram), composed by B12, PPS7 and Bu C16, was characterized by a percentage of healthy plants ranging between 78 and 83.6%. All the efficient strains studied *in vitro* presented the same trends *in vivo* under greenhouse conditions.

4. Discussion

The VPC can be an interesting biodegradable organic material for compost production. Since both vegetable waste and *Posodonia* pose an ecological problem, their stabilization can help to preserve the environment. Furthermore, the addition of VPC on soil can correct the amount of organic matter on local soils. It is well known that during composting, the majority of fungal pathogens will be eradicated by maintaining a compost temperature above 55°C for 21 days [55]. Moreover, in previous study we showed that during composting, VPC reached a temperature higher than 70°C . This temperature had a selective effect on the microbial community that is of great importance for the suppression in *Fusarium* wilts [3].

The vast majority of studies on compost suppressiveness demonstrate a relationship between disease suppression and microbial activity. Suppression of *Fusarium* wilt of tomato using VPC could have been caused by compounds such as cyanide, as well as to some of VPC's indigenous bacterial strains that act as antagonists. After sterilization, VPC lost the ability to suppress *Fusarium*. The inability of sterilized VPC to suppress *Fusarium* wilt indicates the importance of compost microflora after biological control. Reference [56] showed that compost microflora had no significant effect

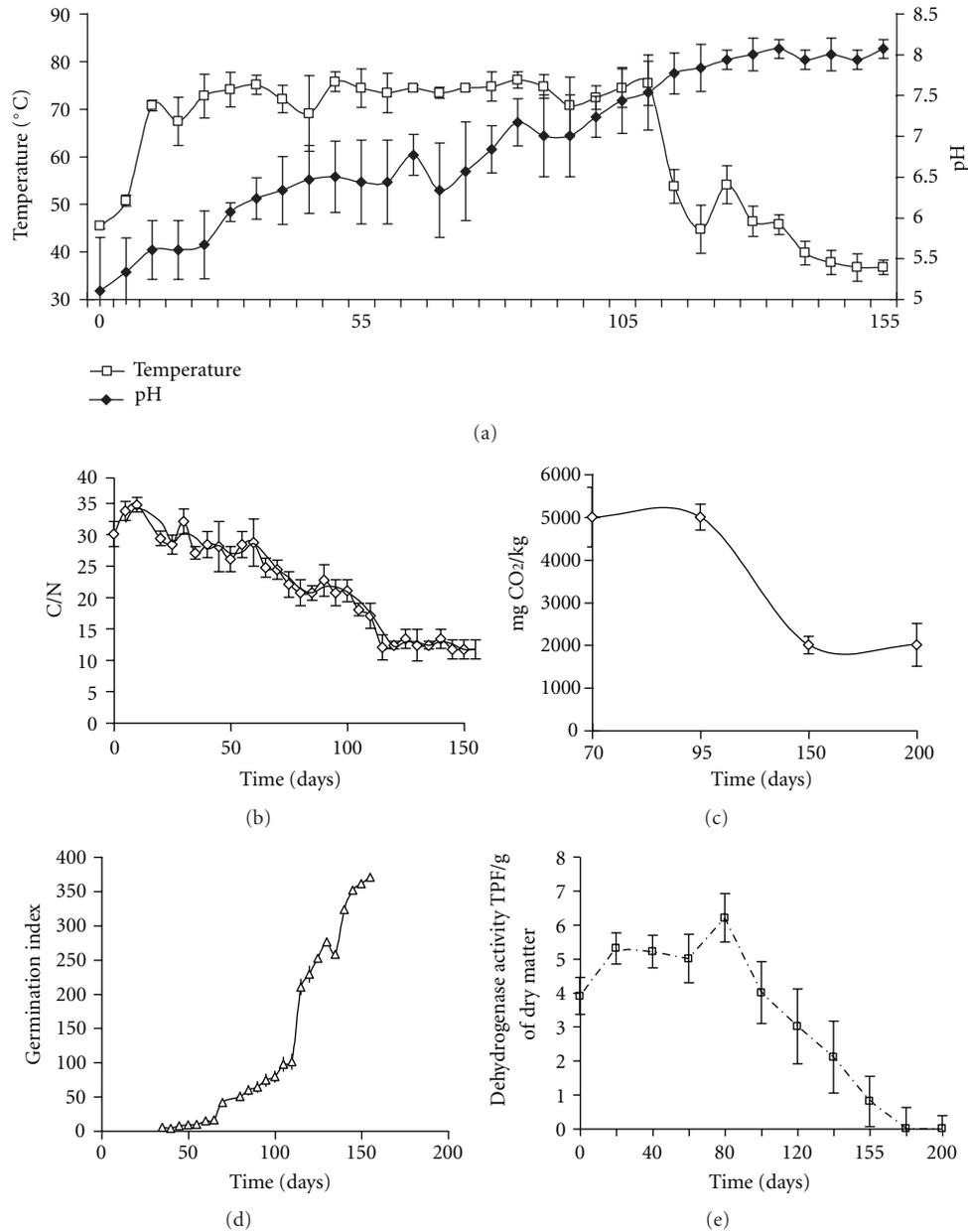


FIGURE 1: Trends of biochemical and microbiological parameters during composting cycle of mixed vegetable waste (70%) and dead *P. oceanica* (30%) (vol; vol).

on the fungus *Fusarium oxysporum* which could account for the less-efficient suppression of the pathogen with the nonsterilized compost. However, our results were consistent with those obtained by [57], who showed that when the compost was sterilized, it did not control disease, and that compost microflora induced host resistance to the pathogen.

The complete growth inhibition of Forl demonstrated that *Fusarium oxysporum* cannot grow on media containing unsterilized VPC at high rates. This result was not consistent with that reported by [58], who demonstrated that pathogenic fungi were able to grow on solid media containing compost. Total inhibition in our study could be due to effect of Anti-Fungal Volatiles of VPC and/or to the

chelation of transition metals by cyanide. [59] reported that cyanide compounds are able to chelate transition metals and also lower the reactivity of metallic iron by forming an inert metal-ligand complex. Chelating of transition metals, such as iron and copper, reduces their bioavailability for fungal growth. Thus the growth inhibition of mycelia could be due to cyanide compounds that can play a critical role in inhibition of fungal growth [60]. Cyanide salts of potassium produce a strong inhibitory effect on the terminal step of the cytochrome-mediated respiratory pathway in fungi. Indeed, [61] showed that the addition of 0.5 mM potassium cyanide reduced the oxygen consumption rate over 70% of biomass content.

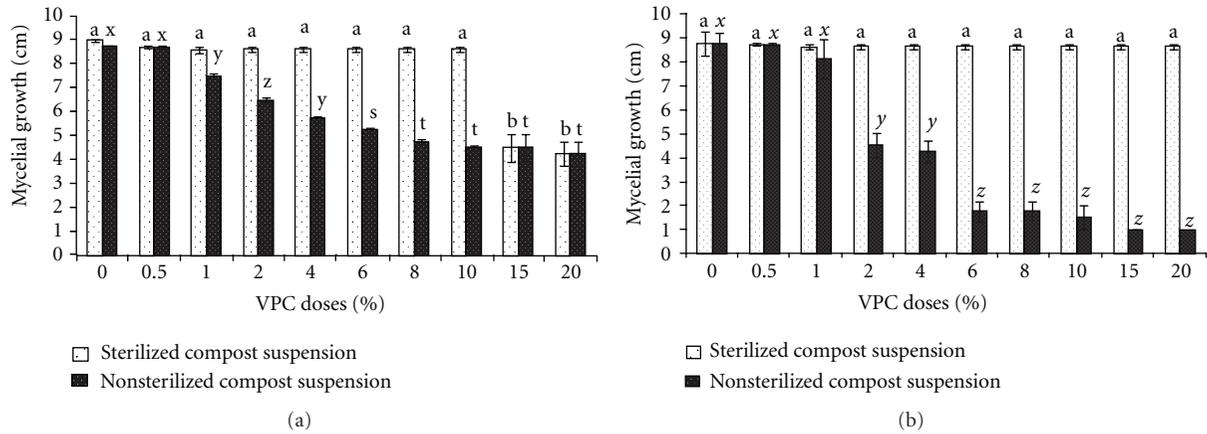


FIGURE 2: Effect of VPC extract (a) and volatiles produced by VPC extract (b) against *Forl* on mycelial growth of *Forl*.

TABLE 1: Effect of doses compost in volatiles, antibiotic, cyanide production, and growth of fungi in sterilized extract compost and non sterilized compost.

	Compost extract (%)										
	0	0.5	1	2	4	6	8	10	15	20	
Volatiles	-	-	-	-	+	+	+	+	+	+	+
Cyanide	-	-	-	-	+	+	+	+	+	+	+
Antibiotic	-	+	+	+	+	+	+	+	+	+	+
Growth of fungi in SEC	-	+	+	+	+	+	+	+	+	+	+
Growth of fungi in NSEC	-	-	-	-	-	-	-	-	-	-	-

SEC: sterilized extract compost; NSEC: nonsterilized extract compost (+): positive production, (-): negative production. Cyanide production was detected as described by [48],

TABLE 2: Effect of *Bacillus* isolates obtained from the compost materials on *in vitro* growth of *Fusarium oxysporum* f.sp. *radicis-lycopersici* and determination of the ability of the most effective bacterial isolates in dual culture on producing cyanide, volatiles and antibiotics.

Isolates	Identify of the selected isolates	Dual culture assay			
		% mycelial inhibition	% fungal inhibition by volatiles	Antibiotic production	Cyanide production
B6	<i>B. sphaericus</i>	42.30 ⁱ ± 3	8.99 ^b ± 0.89	+	-
B10	<i>B. sphaericus</i>	66.33 ^g ± 0.89	52.78 ^l ± 0.89	+	+
B12	<i>B. sphaericus</i>	56.36 ^k ± 0.89	23.89 ^j ± 0.89	+	-
B17	<i>B. sphaericus</i>	46.80 ^l ± 0.89	11.55 ⁱ ± 0.89	-	+
BuC16	<i>Burkholderia gladioli</i>	41.00 ⁱ ± 0.89	7.07 ^k ± 0.89	-	-
PPS7	<i>Pseudomonas putida</i>	66.33 ^g ± 0.89	44.77 ^h ± 0.89	+	+
BS1	<i>Bacillus</i>	7.06 ^m ± 0.89	1.08 ^m ± 0.89	+	-
BS2	<i>B. sphaericus</i>	41.00 ⁱ ± 0.89	6.67 ⁿ ± 0.89	+	-
BS3	<i>Bacillus</i>	15.56 ⁿ ± 0.89	4.79 ^e ± 0.89	+	-

Percent growth inhibition was determined after days of incubation using [51] formula. Values followed by the same letter were not significant ($P \leq 0.05$) by student's Newmans Keuls test. (+) production, (-): nonproduction, ±: indicate standard error of the mean.

Cyanide produced by antagonistic bacteria results in a natural mechanism of plant defence. The forms of cyanide most often discussed from a monitoring viewpoint were free cyanide, weak acid dissolvable (WAD) cyanide, and total free cyanide consisting of HCN and CN. We noted that WAD

cyanides were the form required for plant defence. Some authors established that when compost was added to soil, it enhanced the growth of fungi compared to a control (soil not amended with compost) and the WAD cyanide quantities were concentrated in plant shoots [62].

could be due to the antibiotics secreted by bacteria, which may confer fungicidal properties. This finding confirms the hypothesis of [67], who considered that the control of soil-borne plant pathogenic fungi by using organic amendments is due to a specific suppression, which is related to an increase in the population of specific groups of microorganisms that act as antagonists to the plant pathogens.

The inhibition effect of sterilized VPC at high rates may be due to the chelating of nutrients (from the environmental medium) necessary for fungal growth. High nitrogen content could also inhibit the fungal growth. Indeed, Forl was found to be more sensitive to unsterilized VPC. This result seems to be evident due to the antagonistic bacterial effect. In the present study *Burkholderia gladioli* strain BuC16 exhibited strong biocontrol activity and increased the percentage of healthy inoculated plants.

Abbreviations

VR:	Vegetable residues
PoR:	<i>P. oceanica</i> residues
VPC:	Vegetable <i>Posidonia oceanica</i> compost
MSW:	Municipal solid waste
CCR:	Control of crown and root rot
AWDM:	Agar well diffusion method
PDA:	Potato dextrose agar
WAD:	Weak acid dissolvable
AFV:	Antifungal volatile
t/d:	Tons per day
CO ₂ :	Carbon dioxide.

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Review Article

Downward Movement of Potentially Toxic Elements in Biosolids Amended Soils

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Potentially toxic elements (PTEs) in soils are mainly associated with the solid phase, bound to the surface of solid components, or precipitated as minerals. For most PTEs, only a small portion is dissolved in the soil solution. However, there is an interest in following the fate of mobile PTEs in the environment, for a growing amount of evidence indicates that downward movement of PTEs may occur in biosolids amended soils, leading to groundwater contamination. Therefore, it is crucial to understand the factors that control the release of these elements after land application of biosolids, in order to overcome problems related to downward movement of PTEs in the soil profile.

1. Introduction

The treatment of municipal wastewater produces huge amounts of sludge. This material consists of the solids that were originally present in the wastewater and/or new suspended materials originated as the result of wastewater treatment processes [1]. The term biosolid was officially recognized in 1991 by the Water Environment Federation (WEF) and refers to the organic solids that have received a biological stabilization treatment at the municipal wastewater treatment plant, to make a distinction from other types of sludges that cannot be beneficially recycled as a soil amendment.

In recent years, the quantity of biosolids generated all over the world has increased dramatically, and this trend is expected to increase many folds in the years to come. The safe disposal of biosolids is a major environmental concern. Since biosolids contain significant amounts of macro- and micronutrients [2–4], land application of this waste is an economically attractive management strategy. Biosolids contain a high concentration of organic matter, which can ameliorate soil quality by improving soil structure, water-holding capacity, air, and water transport [5]. Nonetheless,

the presence of biosolids-borne potentially toxic elements (PTEs) such as cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), nickel (Ni), and zinc (Zn) is the most critical long-term hazard when this amendment is land applied. Elevated levels of PTEs in agricultural soils may adversely affect soil's quality and may represent an ecological and human health risk if they enter the food chain or leach into ground waters, ultimately causing metabolic disorder and chronic diseases in humans. Potentially toxic elements accumulation in soils and, in some cases, in crops has been reported when biosolids have been applied for a long time [6–8].

When PTEs are introduced into the soil, they may be subjected to a series of chemical and biochemical processes, such as adsorption/desorption, precipitation/dissolution, complexation/dissociation, and oxidation/reduction [9, 10]. Speciation of heavy metals in soils is generally related to their existing physicochemical forms: simple and complex ions in interstitial solution; exchangeable ions; associated with soil organic fractions; occluded or coprecipitated with metal oxides, carbonates or phosphates and other secondary minerals; ions in the crystal lattice of primary minerals. Among the different geochemical phases, simple and complex ions in interstitial solution and exchangeable ions have a major

effect on metal solubility [11]. However, only a small portion of PTEs in soil is soluble and mobile and has the potential to leach, be taken up by plants or enter the food chain.

Most soil profile studies from short- and long-term sludge applications concluded that biosolids-borne PTEs were largely retained in the topsoil or the zone of biosolids incorporation [12]. McBride et al. [13] and Richards et al. [14] indicated that downward mobility might occur in the field without a substantial increase in PTEs concentrations in the subsoil. These conclusions were consistent with later research, which did not show significant increases in total PTEs concentrations below 30-cm depth in soil profiles despite the differences in biosolids application methods or soil properties [12, 15, 16].

In recent years, there has been concern that biosolids-borne PTEs or other contaminants might be more mobile in soil than previously thought. Various authors reported increase in the concentrations of PTEs in leachates after biosolids' soil applications [17]. Grolimund et al. [18] proposed that mobile, colloidal particles could act as pollutant carriers and thus provide a rapid transport pathway, even for strongly sorbing PTEs. Although the annual export of trace metals from the surface layer usually represents a small fraction of the total amount added, the cumulative transport of these elements over a prolonged period of time may result in a substantial redistribution into the subsoil, with the risk of ground water contamination [19].

However, metal movement occurs and metal leaching through the soil profile may contaminate groundwater [20]. Some soil properties like pH or dissolved organic matter may contribute to increase the migration of trace elements to the subsurface in biosolids-amended soils. The aim of this review is to analyze the effect of different soil factors that determine the mobility of potentially trace elements into the subsoil in biosolids-amended soils.

2. Texture

The most important chemical process that affects PTEs availability and mobility in soils is sorption onto solid phases. Many authors have described the influence of clay size fraction in trace elements retention. For example, McGrath and Loveland [21] observed that the retention of Cr and Ni in topsoils increased with the increment of the clay size fraction; Richards et al. [22] reported that mobility of Ni, Cd, and Zn was greater in fine sandy loam soil than in a silt loam soil at similar pH levels; Egiarte et al. [23] stated that exchange reactions are the main way of retention for sludge-borne Zn compounds in soils.

The negative charge of 2:1 layer silicates, as measured by cation-exchange capacity (CEC), is essentially independent of pH and ionic strength. The anion-exchange capacity (AEC), which is due to protonation and incomplete hydroxylation at clay plate fringes, is negligible. Trace elements in cationic form show typical ion exchange behavior on layer silicate clays with permanent charge, having the same affinity for exchange sites as alkaline earth metals with the same charge and similar ionic radius. The affinity of these soil clays

with PTEs usually results in the formation of outersphere complexes. Studies on Cu adsorption by individual soil components have indicated relatively strong bonding and high capacity of silicate minerals to adsorb Cu, whereas the amounts of Cu that can be readily desorbed are very small [24]. Nevertheless, PTEs are vastly outnumbered by other cations with which they compete. For instance, Cd and Zn effectively compete with dominant electrolyte ions in the soil solution like Ca and Mg. Besides, trace elements adsorption does not only depend on their competition for soil sorption sites, but also on the nature of PTEs involved. More strongly sorbed PTEs, such as Pb and Cu, are less affected by competition than mobile PTEs, such as Cd, Ni, and Zn [25].

In a soil column study in which biosolids was applied on surface, Gascó et al. [26] reported a relatively strong negative relationship between clay content and total percentage of leached PTEs, in agreement with the results reported by other authors who have described the influence of clay size fraction in metals retention [27, 28]. These authors concluded that sandy textured soils have relatively few adsorption sites if low in organic matter, phyllosilicate mineral, and hydrous oxide content.

3. pH

It is generally viewed that soil pH is the main soil variable controlling PTEs speciation, solubility, mobility and transport [29, 30]. With the exception of arsenic (As), molybdenum (Mo), and selenium (Se), trace elements usually increase solubility and become more available for leaching and plant uptake as soil pH decreases. This effect is mainly due to the influence of pH on PTE's binding state in the soil (i.e., complexed by organic or inorganic agents; precipitated with primary or secondary minerals, among others). Stehouwer et al. [31] reported that increased acidity originated the mobilization of Cu, Ni, Pb, and Zn. The important role of pH has been recognised by policy makers, who have developed legislations concerning maximum permissible heavy metal loads in sludge-amended soils that depend on soil pH [32].

Organic matter and metal oxides are much more effective sorbents for PTEs in cationic form than phyllosilicates. It has been widely reported that Al, Fe, and Mn oxides, allophanes and imogolite retain PTEs more strongly than alkaline earth cations [33]. Direct spectroscopic investigations of Cu(II)-iron oxyhydroxide adsorption or the strong affinity of Ni for Mn has been interpreted by the formation of pH-dependent innersphere sorption complexes [34].

The type of surface complex formed has an important effect on the mobility of PTEs. Some PTEs ions are directly bound to mineral surfaces due to the absence of the hydration sphere, forming innersphere complexes. Such ions are relatively difficult to desorb, except for large pH changes; thus, they are relatively immobile. On the contrary, if PTEs ions form a weakly bound outersphere complex in which the ion is surrounded by hydration water and no direct chemical

bonds to the surface are formed, trace elements can be easily desorbed when pH changes.

The inorganic component of biosolids mainly consists of Si, Al, and Fe oxides [3, 35] which have the ability to immobilise trace element's cation. As a result, much of the PTEs are bound to the biosolids' solid phase. However, a dynamic equilibrium of biosolids-borne PTEs following soil application occurs, and many authors concluded that soil pH is a controlling variable for this redistribution.

Sequential extraction is a useful technique for predicting PTEs bioavailability, leaching rates, or transformation between chemical forms in agricultural and polluted soils [36]. In most protocols, PTEs are divided into the following fractions: (1) soluble and exchangeable ions, (2) bound to organic matter, (3) bound to carbonates, (4) bound to iron and manganese oxides and hydroxides, and (5) in the mineral lattice of silicates or residual fraction. This technique assumes that mobility and bioavailability decrease in the order of extraction. Thus, metals in the exchangeable fractions are most mobile and bioavailable, whereas metals in the residual fraction are tightly bound and least mobile under natural environmental conditions. Although some PTEs may be initially sorbed to the iron oxide surface sites at the solid-water interface, they may afterwards diffuse to internal sorption sites, which are not readily accessible by the bulk solution [37]. In other cases, PTEs may react with the oxide surface, penetrating the coordination shell of the Fe atom, and forming covalent bonds [38]. This specific sorption or chemisorption is influenced by pH and PTEs concentration in the soil solution.

Torri [39] reported that the soil factor most closely associated with the distribution of Cu and Zn among soil fractions in biosolids amended soils was soil pH. Tsadilas [40] found a negative correlation between exchangeable Cu and soil pH, reporting that the drop in this fraction was mainly from pH 4.4 to 6.6. Conversely, other authors reported an increase in Cu availability with increasing pH and a concurrent increase in DOM [41]. They attributed these results to the irreversible dissolution of organic matter with organically bound Cu at high pH during natural weathering of the sludge in the soil. Shuman [42] indicated that organic Cu was mainly associated with soil organic matter. A negative correlation was reported between organic Cu and soil pH a year after biosolids application [3] indicating that organic Cu increased as soil pH decreased. On the other hand, a positive correlation was observed between carbonate Cu and soil pH [3]. Similar results were reported by Tsadilas [40], concluding that an increase in soil pH caused a shift of exchangeable, organic, and residual Cu into the carbonate fraction.

On the other hand, biosolids-borne Zn was completely adsorbed by the soil-solid phase [43]. The concentration of exchangeable Zn in biosolids amended soils was not related to clay content but with soil pH [4]. Similar results were found in other studies [40, 44]. As soil pH increases above pH 5.5, Zn was adsorbed on hydrous oxides of Al, Fe, and Mn [9]. However, the extent to which Zn was retained on Fe and Al hydrous oxides was also influenced

by the nature of clay minerals and surface conditions [45]. Redistribution of Zn towards the carbonate fraction was observed, representing the most abundant fraction a year after biosolids application [4]. The carbonate Zn fraction was positively and significantly correlated with soil pH [4, 40]. The role of carbonates on Cu and Zn retention has been pointed out by other authors and has been partly attributed to the formation of metal carbonates in soils [46].

Neutralization of acidic or alkaline soils is one of the most simple and inexpensive methods used for immobilization of trace elements [40]. Alkaline amendments reduce the concentration of PTEs in the soil solution by allowing the formation of insoluble PTEs precipitates, complexes, and secondary minerals. Numerous research has reported that calcium carbonate may be the dominant sorbent for a variety of metals in alkaline environments, involving reactions with CaCO_3 surfaces, with adsorption occurring at low PTEs concentration in soil solution, and precipitation dominating at high concentrations. In oxidizing environments and at soil pH values greater than 7, Cd precipitates to minerals such as octavite (CdCO_3), CdO, and $\text{Cd}(\text{OH})_2$ [47]. The mobility of As in soil is also mostly controlled by adsorption/desorption processes and coprecipitation with metal oxides [48].

4. Organic Matter

Organic matter dynamics and its role on PTEs availability has been extensively investigated. However, there is still continuing controversy over the likely long-term effects of sludge applications on PTEs mobility. It has been argued that, in temperate climates, where organic matter decomposition is not particularly fast, the protective role of organic matter remains unaltered decades after sludge application, in good agreement with the "sludge protection hypothesis" or "plateau theory" [49].

Except for some noncrystalline minerals that have very high specific surface charge density with highly reactive sites, organic matter appears to have a great capacity for sorption of trace elements in cationic form. Depending on the solubility of the organic ligand, soil organic matter plays a dual role on PTEs availability; solid organic matter immobilizes PTEs by the formation of insoluble complexes, whereas dissolved organic matter (DOM) promotes PTEs solubility by forming strong soluble complexes [50]. Although the composition of DOM in biosolids-amended soils is variable and complex, a large portion is composed of fulvic and humic acids. Minor components include macromolecular hydrophilic acids and low molecular weight substances, like carboxylic and amino acids, carbohydrates and microbial degradation products [51, 52]. Digested biosolids contain a significant portion of DOM, making up 2-3% of total organic carbon content [53].

The high degree of selectivity shown by DOM to form stable complexes with certain PTEs (e.g., Cd, Cu, Ni, Pb, and Zn) suggests that these elements form inner-sphere complexes with the acid functional groups, often forming an internal five- or six-member ring on structures [54]. Because of the many variables involved, there are many inconsistencies in reported selectivity orders of PTEs with organic matter.

A generalized order is $\text{Cr}^{3+} > \text{Pb}^{2+} = \text{Hg}^{2+} > \text{Cu}^{2+} > \text{Cd}^{2+} > \text{Zn}^{2+} = \text{Co}^{2+} > \text{Ni}^{2+}$ [55, 56]. In general, the more electronegative the metal ion, the stronger the bond formed with organic matter. Copper is commonly found strongly complexed by organic matter. Different studies suggested that Cu^{2+} is bonded rigidly as an innersphere complex. Copper may be coordinated either with O atoms or with N atoms [57]. On the contrary, most of the first-row transition PTEs form outersphere complexes with organic matter [58].

The composition of organic amendments may change with time due to decomposition of organic matter by soil microorganisms. Torri et al. [59] reported that sludge-borne carbon consisted of two fractions of different degrees of biodegradability: a labile fraction (53–71%) that mineralized quickly following a first-order kinetic process and a resistant fraction (29–45%), apparently not available to microorganisms. Following application of biosolids to soils, there is a rapid phase of decomposition as the easily decomposable organic fractions are degraded, with soluble organo-PTEs complexes being released into soil solution [60, 61] that demonstrated that land application of biosolids can lead to a dramatic increase in the amount of DOM during the first few weeks following biosolids application. In a 14-year study of biosolids-amended soil, van Erp and van Lune [62] reported that concentrations of Cd and Zn in the leachate decreased over time, whereas Cu and Pb concentrations increased. This was explained by the fact that Pb and Cu are strongly bound to biosolids organic matter and would be released slowly over time as the organic matter decomposed. On the contrary, Cd and Zn are not strongly bound to organic matter and, therefore, would not be so affected by biosolids mineralization [62].

The stability of PTEs organic complexes is strongly influenced by the pH range. Generally at low pH, most metals are in the cationic form, but as pH increases, humate complexes are formed. For fulvic acids, the complex stability increases from pH 3.5 to 5.0 [63]. Many authors found PTEs stability constant in the range of 1.2–7.2 for different organic acids. Generally, the lowest pH values were found for Cd^{2+} , Zn^{2+} , and Ni^{2+} while Cr^{3+} , Cu^{2+} , and Pb^{2+} had the highest values [64, 65]. The stability constant of complexes between PTEs and fulvic acids may also decrease with increasing ionic strength in the soil solution.

The transport of soluble PTEs-organic complexes in soils also depends on their concentration gradient and the mass flow of water. The latter is a function of the soil's matric water potential and porosity. In addition, preferential flow through continuous biopores or cracks has been shown to greatly increase the mobility and velocity of solute movement to the groundwater [66]. Several studies, employing measurements of soil pore water using lysimeters or drainage tiles, have found that PTEs concentrations are elevated below the zone of incorporation of biosolids [22, 67]. Leachate sampling below field plots and/or undisturbed monolith lysimeters amended with biosolids has also revealed elevated PTEs concentrations [68, 69]. Elevated concentrations of both PTEs and DOM are frequently found together in leachates below biosolids-treated soils [25, 46]. Due to its negative charge at typical soil pH, DOM is very mobile in soil, promoting PTEs

mobility [43]. Among PTEs, Pb is known to be immobile in soils due to its association with soil components [60]. However, the known affinity of Pb for organic matter ligands may lead to its mobilization through soil horizons over the 10-y period, as reported by Businelli et al. [70].

High temperatures, elevated pH, high water content, and low ionic strength may enhance the increase in DOM's concentration in biosolids amended soils [71]. Therefore, land application of biosolids may not only introduce potentially trace metals but also mobile organic compounds into soils as well [72]. Although much of the potentially mobile organic material in biosolids-amended soils undergoes microbial transformation near the soil surface, some fraction of it may move to shallow horizons due to its net-negative charge at typical soil pH.

The contribution of DOM to metal transport is expected to be magnified as pH increases [73]. For example, at $\text{pH} > 6$, almost all Cu in soil solution is complexed by DOM [74]. This is due to the increased solubility of DOM and the very low solubility of metal ions in neutral to alkaline pH soils [22]. Moreover, when soil pH increases, protons dissociate from organic functional groups such as carboxyl, phenolic, hydroxyl, or carbonyl, thereby increasing the affinity for metal cations [75].

5. Chemical Immobilization

Chemical immobilization may reduce the environmental risk of downward movement of trace elements. Chemical amendments such as organic matter, alkaline material, and phosphate fertilizer have been applied to reduce the availability of PTEs. Immobilizing amendments induce sorption processes like adsorption to mineral surfaces, formation of stable complexes with stable organic matter, and surface precipitation, limiting PTEs solubility. Precipitation as salts and coprecipitation of PTEs may easily occur onto other soil components such as hydroxides, phosphates, oxides of Fe, Mn, and Al, and others [48, 76]. These reactions are considered the major mechanisms to sequester PTEs. A single mechanism rarely accounts for the mobility and immobilization of elements in soil [48].

Sorption/dissolution processes are influenced by many factors, such as pH, redox potential, type of soil constituents, cation exchange capacity, synergy among elements, and others. For example, the mobility of Zn is affected by the presence of P, Ca, Al, Mn, and Fe oxides and organic matter. Chromium mobility in soil depends on its oxidation state, and phosphates can reduce Pb mobility by ionic exchange and precipitation of pyromorphite-type minerals. Zn can precipitate with hydroxides, carbonates, phosphates, sulfides, molybdates and several other anions as well as form complexes with organic ligands. Although biosolids may be a source of soil and crop metal contamination, it is also applied for immobilization of Pb, Cd, and Zn in contaminated soils [76]. It has been found that composted biosolids reduce the bioavailability of soil Pb by converting it from exchangeable forms to Fe and Mn oxides and organic bound forms [47]. The mechanisms of elemental immobilization by organic

amendments are still not quite clear, but studies suggest chelation as the main responsible process [76].

6. Conclusions

Increased use of biosolids on agricultural soils needs a better understanding of the benefits and the associated environmental risks of this practice. Although the availability of PTEs has been reported to decrease over time, many studies indicate that a small portion of PTEs is dissolved in the soil solution and may move to subsoil horizons. The most important chemical process that affects PTEs solubility and mobility is sorption onto soil solid phases. Metal sorption, among many factors, depends on the nature of organic and inorganic soil constituents, as well as soil pH. Land application of biosolids may significantly modify soil pH and increase soil DOM concentration, leading to the formation of organo-PTEs complexes which may enhance the downward movement of potentially toxic elements in the soil profile.

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Research Article

Comparative Use of Soil Organic and Inorganic Amendments in Heavy Metals Stabilization

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Remediation strategies are capable to mitigate negative effects of heavy metals (HMs) on soils. The distribution of copper (Cu), zinc (Zn), and chromium (Cr) was evaluated in a contaminated soil after adding biosolid compost (BC) and phosphate fertilizer (PF). A greenhouse assay and sequential extraction procedure were performed to determine the fractionation of HM in contaminated and remediated soil. In BC treatment, among 4 to 6% of Cu was associated with soil humic substances. Without amendments and with fertilizer application, Zn solubility increased by 15.4 and 8.4%, respectively, with experiment time. Although Cr was significantly adsorbed to the inorganic fraction, with compost application there was a transfer to organic fraction. A single amendment application is not suitable for immobilizing all metals of concern, because there are diverse union's behaviors between HM and soil matrix. As the organic matter and phosphate fertilizer were effective in reducing mobility of Cu, the organic matter was more effective in the immobilization of Cr, and inorganic amendment induced the Zn precipitation, results from this pilot study suggest a combined use of these two amendments for soil remediation strategies. However, liming may be further needed to prevent soil acidification on longer time scales. Also, we propose the use of chemical and biological remediation strategies for potential improvement of effectiveness.

1. Introduction

Point source and diffuse contamination of soils by heavy metals (HMs) is an environmental problem worldwide. An important input pathway is anthropogenic activities, such as disposal of industrial and hazardous residues, mining activities, incidental accumulations, industries atmospheric deposition, energy generation, and agricultural chemicals [2, 3]. High proportion of the contamination is retained by soil particles, so that is why this system is considered a vulnerable environment to being contaminated [4]. In addition, there is an increasing interest in HM contamination as a result of a continuous accumulation on top soil, with elevated availability to organisms [5]. Therefore, high concentrations of HM and their potential toxicity may affect soil ecology, agricultural production, and water quality, implicating risks for environment and human health [6].

In Argentina, soil contamination has been mostly recorded in urban and periurban areas. Reference [7] indicated

that concentrations of Cd, Cu, Pb, and Zn in urban soils exceeded the international standards referred to the human health, and according to [8], elevated levels of Cu, Pb, Cd, and Zn were found in horticultural soils of Buenos Aires. Contamination with Cu, Cr, Zn, As, Cd, Hg, and Pb occurred near the Ecological Reserve area of Buenos Aires, close to an automobile cemetery. Moreover, industrialized areas are affected by high Cu, Zn, and Cr concentrations in industrial effluents [9].

Heavy metals partition in contaminated soils is influenced by several chemical and biological reactions [10, 11]. Soils retain HM by sorption, precipitation, and complexation; such reactions reduce the metal mobility and bioavailability [2, 12, 13]. However, this natural attenuation process can be complemented by *in situ* application of chemical technologies, as organic and inorganic amendments [14]. *In situ* chemical immobilization is a cost-effective remediation approach that stabilizes HM in contaminated soil [15, 16]. Generally, these technologies reduce HM availability,

TABLE 1: Physicochemical characteristics of the Typic Hapludoll soil. All data are expressed as dry basis (DM).

Clay	Silt	Sand	Texture	CEC	pH	EC	OM _{total}	C _{soluble}	N _{total}
	%			cmol·kg ⁻¹	1 : 2.5; H ₂ O	dS m ⁻¹		%	g kg Soil ⁻¹
19.2	23.2	57.6	Sandy loam	22.1	5.19	0.57	1.92	1.45	2.2

OM_{total}: total organic matter; C_{soluble}: soluble carbon; N_{total}: total nitrogen.

TABLE 2: Physical and chemical properties of the Biosolid Compost used as organic amendment. (DM: dry mater).

CEC	pH	EC	OM _{total}	C _{soluble}	N _{total}	C/N
(cmol·kg ⁻¹)	(1 : 2.5; H ₂ O)	dS m ⁻¹	(%)		(g kg Soil ⁻¹)	
14.3	7.5	1.29	4.25	0.031	0.32	13.2

OM_{total}: total organic matter; C_{soluble}: soluble carbon; N_{total}: total nitrogen; C/N: carbon-nitrogen coefficient.

improve soil fertility, and increase plant growth [17, 18]. On one hand, organic amendments (compost) contain a high proportion of humified organic matter and could decrease HM bioavailability in soil, even though temporally and thus permitting the reestablishment of vegetation [19]. On the other hand, within the inorganic amendments, phosphate fertilizers are also effective in HM immobilization through formation of stable mineral HM phosphate [20]. The formation of these insoluble metal compounds, reduce their mobility through the soil profile and the pool available for biota [21]. In addition, the application of amendments simultaneously, could promote plant growth and biological activity of degraded soil [22].

The objective of this study has been to assess the distribution of three heavy metals (Cu, Zn, and Cr) as a mixture in contaminated soil, after applying biosolid compost (BC) and phosphate fertilizer (PF), both independently and in combination.

2. Materials and Methods

2.1. Soil Sampling. Typic Hapludoll soil representative from sandy Pampas (35°37'S, 61°22'O) (Tables 1 and 3) was chosen for the experiment. Five composite topsoil samples (0–20 cm depth) were randomly collected (10 individual samples). The sampled soil was air-dried and passed through a 2 mm sieve to obtain homogeneous particle size. Soil samples were enriched by adding metal solutions (CuCl₂·2H₂O, ZnSO₄·7H₂O, and H₂CrO₄), achieving final concentrations of 350 μg g⁻¹ copper (Cu), 700 μg g⁻¹ zinc (Zn), and 750 μg g⁻¹ chromium (Cr). This enrichment overcame the maximum levels of total HM established in agricultural soils by Argentinean legislation (Table 3). Wetting cycles (at field capacity) and air-drying every five days were performed, during a period of three months.

2.2. Greenhouse Experiment. The experiment consisted in 1 kg pots containing HM-enriched soil and two different amendments: Biosolid Compost (BC) (Tables 2 and 3) as organic amendment and superphosphate triples (PF) as inorganic amendment (100 Mg ha⁻¹ and 100 kg ha⁻¹ rate, resp.). The compost was enriched with sawdust as bulking agents and biosolids from sewage sludge treatment plant

located in the north of Buenos Aires City (1 : 1, biosolid: sawdust ratio by volume). Samples of enriched soil were thoroughly mixed with the amendments resulting in four treatments: (i) Contaminated Soil (CSC) as control, (ii) Contaminated Soil + Phosphate Fertilizer (CSC + PF), (iii) Contaminated Soil + Biosolid Compost (CSC + BC), and (iv) Contaminated Soil + Phosphate Fertilizer + Biosolid Compost (CSC + PF + BC). The experiment was carried out in a completely randomized design with three replications per treatment. Destructive samplings were performed at two time points of the experiment: beginning (0 days) and end (190 days).

2.3. Soil Chemical Analysis. Sequential extraction procedure was performed on all samples from each pot (3 g in 50 cm³ polypropylene centrifuge tubes) to measure Cu, Zn, and Cr in different fractions after the application of BC and PF, separately and simultaneously, according to [23] methodology. This type of procedure has been successfully used for sludge-treated soils [24–26]. The procedure separates metals into four operationally defined fractions: soluble and exchangeable (EX), organic matter bound (OC), carbonate bound (CB), and residual (RES) (Table 4). After each extraction, the solution was separated from the solid by centrifugation at 3000 rpm for 45 min. The supernatant was decanted and filtered through Whatman N. 42 filter paper, and the wet residue in the tube was recorded. The pseudototal HM concentrations were determined by acid digestion with mixture of HCl, H₂SO₄, and HNO₃ [1]. Heavy metals contents of each supernatant were measured at two time points by flame atomic absorption spectrophotometer (AAS). Soil pH and electricity conductivity (EC) was measured using a mixture of soil and deionized water (1 : 2.5 m/v) with a glass electrode [27] in each time point.

2.4. Statistical Analysis. Treatments effects were determined by factorial arrangement of 4 × 2 (amendment levels and time points), according to the general linear model procedure of Statistics, version 8.0. Differences between means were separated using least significant differences (LSDs). Variances were stabilized when necessary by a logarithmic transformation of some data set. In addition, a simple linear correlation between these variables was performed.

TABLE 3: Heavy metals content (in ppm) of soil samples and composted biosolids, and the maximum levels of total heavy metals established by Argentinean law (Hazardous wastes regime: soil quality guide).

	Heavy metals (mg kg DM ⁻¹)				
	Typical Hapludoll		Biosolid Compost		Law 24051 ^a
	Available	Total	Available	Total	Total
Cu	0.051	17.0	1.52	726.7	150–500
Zn	0.040	38.2	0.65	1183.3	600–1500
Cr	<0.001	14.9	13.2	230.0	750

^a Levels for soils with agricultural and industrial uses.

TABLE 4: Sequential extraction procedure of heavy metals from soil samples and operationally defined fractions.

Phase/association	Abbr.	Step	Operational procedure
Soluble—exchangeable	EX	1	30 mL 0.1 M CaCl ₂ , shaken 16 h at room temperature
Organic matter—bound	OB	2	30 mL 0.5 M NaOH, shaken 16 h at room temperature
Carbonates—bound	CB	3	30 mL 0.5 M Na ₂ EDTA, shaken 1h
Residual	RES	4	Estimated by summation of three fractions and the total content (acid digestion with hydrochloric, sulfuric, and nitric acids) [1]

3. Results and Discussion

3.1. Distribution of HM in Soil Fractions. The distribution of Cu, Zn, and Cr in contaminated and remediated soils for both sampling dates is reported in Table 5. Heavy metals in contaminated and amended soils showed similar pattern of distribution between fractions. Furthermore, each metal was associated in a different way with soil components, as was expected. These differential trends are in agreement with other studies, which demonstrate that the adsorption process is metal specific [28, 29].

In particular, copper was mainly associated with soil organic matter (Table 5(b)) ($P < 0.001$), forming that make metals less available for plants [30]. Copper-organic matter association was slightly higher at the end of the experiment, especially with biosolid compost application (among 4 to 6%) in comparison with the other treatments. In agreement with [31], extraction procedure showed that in contaminated soils Cu was mostly present in the acid-soluble and reducible fractions, and in soil amendment with organic matter the soluble and exchangeable Cu fraction was reduced. A possible explanation for this result is the precipitation and complexation by the presence of stabilized organic matter in the compost, such as humic substances (Table 2) [16, 32]. These results corroborate other investigator findings which indicated that organic matter plays a fundamental role in controlling soil HM adsorption, being Cu the preferentially associated with the organic fraction [33]. On the other hand, [34] reported that extractable amounts of Cu were always very small, indicating a low contribute from soluble organic Cu complexes. However, in this pilot study the amount of Cu-EX was lower than 1% of total heavy metal content, and at the end of the experiment, compost and BC + PF treatments had more Cu in residual fraction, indicating greater Cu immobilization.

In all treatments, Zn was remained mostly in soluble and exchangeable fraction of soil as mobile Zn ($P < 0.001$).

In contaminated soil without amendments (CSC) and with fertilizer application, Zn solubility increased by 15.4 and 8.4%, respectively, with experiment time. These results differ with [35], who demonstrated that leached Zn concentration decreased 5.92 ± 2.74 mg kg⁻¹ with phosphate amendment, as a result of immobilization by metal-phosphate precipitates. However, our results are in agreement with those of other studies, which confirm that this increase was possible associated with soil acidification [16, 36, 37]. It cannot be excluded that the simultaneous adding of metal cations could affect Zn adsorption, in agreement with [38], due to ionic competition between Zn, Cu, and Cr [8]. Metals with high water solubility pose higher risk of leaching into groundwater. In fact, [39] established that, according to maximum leached concentrations (as a fraction of the total concentration in soil), Zn was the most mobile element in a sequence composite by Zn, Cu, As, and Cr. As a consequence, Zn may offer major environmental risk compared to other metals, since mobile Zn is immediately available for absorption by plants and soil biota in accordance [40, 41].

Chromium was initially adsorbed by soil carbonates as compared with soil organic matter ($P < 0.001$) [42, 43]. In contrast, at the end of the experiment, a Cr redistribution among fractions was observed and the Cr fraction organic associated to the soil organic matter increased by 10% in the BC treatment, as compared to the beginning of the experiment. This suggests that the addition of organic amendments could enhance the reduction rate of Cr (VI) to Cr (III) increasing its temporal immobilization in soil [44]. According to [34] Cr amounts associated with organic matter increase with the increasing extent of soil contamination, thereby suggesting high constants values of organic-Cr complexes. Moreover, these authors demonstrated that Cr undergoes a progressive insolubilization (organically bound Cr into insoluble Cr) in about 20 years. In this pilot experiment we cannot corroborate this ageing process due to the soil metal spiking approach and the relatively short incubation time.

TABLE 5: Distribution of copper (a), zinc (b), and chromium (c) in soil chemical fractions at two time points of the experiment: beginning (A) and end (B). Treatments: CSC: Contaminated Soil, CSC + PF: Contaminated Soil + Phosphate Fertilizer, CSC + BC: Contaminated Soil + Biosolid Compost, and CSC + PF + BC: Contaminated Soil + Phosphate Fertilizer + Biosolid Compost (Mean \pm S.D., $n = 3$).

(a)			
Soluble and exchangeable	Heavy metals concentration (mg kg ⁻¹)		
	Cu	Zn	Cr
CSC (A)	2.56 \pm 0.8	553.00 \pm 2.6	0.27 \pm 0.0
CSC (B)	4.25 \pm 0.5	457.00 \pm 47.6	0.54 \pm 0.1
CSC + PF (A)	4.70 \pm 2.4	569.33 \pm 77.5	0.53 \pm 0.0
CSC + PF (B)	5.14 \pm 2.7	438.17 \pm 77.9	0.64 \pm 0.2
CSC + BC (A)	3.72 \pm 0.3	547.67 \pm 67.2	0.43 \pm 0.0
CSC + BC (B)	3.75 \pm 1.0	426.17 \pm 68.0	0.50 \pm 0.5
CSC + PF + BC (A)	4.76 \pm 0.7	557.33 \pm 19.0	0.62 \pm 0.1
CSC + PF + BC (B)	4.95 \pm 0.4	422.67 \pm 23.1	0.79 \pm 0.2
ANOVA			
Amendments			
Moment		*	
Amendments \times moment	ns	ns	ns
(b)			
Organic matter bound	Heavy metals concentration (mg kg ⁻¹)		
	Cu	Zn	Cr
CSC (A)	183.27 \pm 16.3	195.17 \pm 6.7	32.63 \pm 6.6
CSC (B)	200.73 \pm 7.2	115.73 \pm 0.6	57.48 \pm 2.9
CSC + PF (A)	186.20 \pm 12.6	141.33 \pm 25.0	32.20 \pm 2.7
CSC + PF (B)	199.93 \pm 18.7	110.80 \pm 26.0	52.15 \pm 10.6
CSC + BC (A)	196.93 \pm 13.4	193.33 \pm 63.1	34.20 \pm 7.4
CSC + BC (B)	206.30 \pm 16.3	140.20 \pm 31.5	45.57 \pm 6.9
CSC + PF + BC (A)	202.00 \pm 10.1	172.50 \pm 13.5	28.87 \pm 2.8
CSC + PF + BC (B)	189.80 \pm 3.7	126.20 \pm 20.9	39.70 \pm 0.8
ANOVA			
Amendments			*
Moment		*	*
Amendments \times moment	ns	ns	ns
(c)			
Carbonate bound	Heavy metals concentration (mg kg ⁻¹)		
	Cu	Zn	Cr
CSC (A)	189.20 \pm 12.6	330.33 \pm 29.5	139.47 \pm 5.1
CSC (B)	183.97 \pm 17.1	199.83 \pm 19.3	152.13 \pm 2.8
CSC + PF (A)	183.90 \pm 2.5	340.67 \pm 111.6	138.20 \pm 9.0
CSC + PF (B)	166.73 \pm 10.8	197.17 \pm 29.5	143.83 \pm 14.3
CSC + BC (A)	190.53 \pm 11.3	334.00 \pm 84.3	123.73 \pm 14.6
CSC + BC (B)	150.85 \pm 11.6	234.67 \pm 26.3	145.95 \pm 0.9
CSC + PF + BC (A)	187.20 \pm 9.8	317.67 \pm 9.7	115.93 \pm 11.6
CSC + PF + BC (B)	162.30 \pm 8.9	242.33 \pm 44.7	143.47 \pm 4.3
ANOVA			
Amendments			*
Moment	*	*	*
Amendments \times moment	ns	ns	ns

(d)

Residual	Heavy metals concentration (mg kg ⁻¹)		
	Cu	Zn	Cr
CSC (A)	29.25 ± 2.5	39.6 ± 12.2	446.8 ± 1.0
CSC (B)	20.76 ± 3.2	30.2 ± 5.8	466.28 ± 2.6
CSC + PF (A)	20.1 ± 8.3	123.4 ± 39.6	469.5 ± 6.0
CSC + PF (B)	39.52 ± 17.1	28.52 ± 5.6	616.02 ± 14.4
CSC + BC (A)	32.8 ± 14.4	113.2 ± 38.6	476.3 ± 12.4
CSC + BC (B)	50.18 ± 0.8	63.21 ± 2.6	496.43 ± 4.1
CSC + PF + BC (A)	31.2 ± 5.5	55.0 ± 4.4	431.5 ± 6.0
CSC + PF + BC (B)	45.67 ± 4.3	39.99 ± 28.5	543.13 ± 2.4
ANOVA			
Amendments	*		*
Moment	*		*
Amendments × moment	ns	ns	*

Significance levels: $P < 0.05$; ns: not significant.

According to [45] and assuming that mobility and availability of Cu, Zn, and Cr are associated with their solubility and geochemical forms and that these HMs reduce in the order of extraction sequence, the apparent mobility and potential availability in the soil were $Zn > Cu > Cr$. In addition, [46] reported that in carbonate soils Cr, Cu, and Pb had higher affinity for the reactive solid phases than Ni, Zn and Cd. The active role of amorphous Al silicates and interaction between clays and organic matter from compost, with formation of organoclays, would have improved the soil adsorbing capacity and enhanced this HM competition [34].

3.2. Effect of Amendments Application on Soil Chemical Characteristics. Application of amendments improved the soil chemical characteristics (Figure 1). Initially, soil pH increased with the addition of both amendments, compared to untreated soil. Since these results were in agreement with those found in a previous study [47], this soil pH modification was slight (2.87% = BC and 2.87% = PF), and there was no correlation with HM bioavailability [48]. Soil acidification observed upon increasing of the experiment time for all treatments was possibly due to soil organic matter mineralization [49] and/or to H⁺ formation by the fertilizer solubilization [50]. Therefore, liming may be necessary to compensate potential soil acidification when using PF and BC application to reduce HM-contaminated soil [51]. Contrary to [52], we found that soil EC decreased significantly when organic amendment was added ($P = 0.044$), at the end of the experiment, and no correlation was found between the EC and heavy metals availability.

4. Conclusions

Results from this pilot experiment, showed that application of BC and PF in combination affected the partition of Cr, Cu, and Zn added to soil. In particular, BC and PF in combination reduced the solubility and mobility of Cu

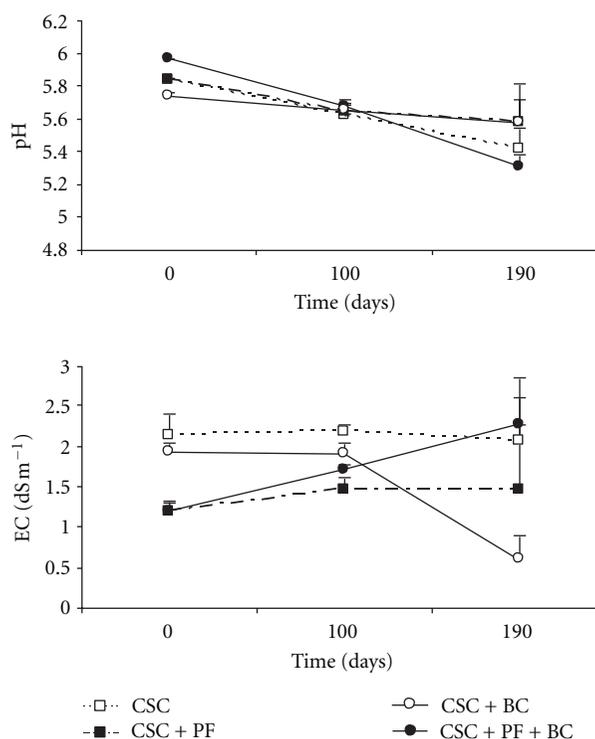


FIGURE 1: Changes in chemical characteristics of contaminated and organic and inorganic remediated soil, respect to assay time. Treatments: CSC = Contaminated Soil, CSC + PF = Contaminated Soil + Phosphate Fertilizer, CSC + BC = Contaminated Soil + Biosolid Compost, and CSC + PF + BC = Contaminated Soil + Phosphate Fertilizer + Biosolid Compost. The bars indicate Standard Error of the data.

and Cr whereas Zn stabilization was increased through compost with or without PF application. The use of PF alone increased the available Zn. These results show that combination of organic and inorganic amendments are needed for immobilizing different metals of concern and

could provide a basis for future research about the existence of interactions between metals added simultaneously into the soil. However, the use of liming agents may be needed to buffer soil acidification on prolonged time scales.

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Research Article

Runoff and Nutrient Losses from Constructed Soils Amended with Compost

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Composted organic materials used to stabilize roadside embankments in Texas promote rapid revegetation of soils disturbed by construction activities. Yet, adding compost to soil may increase total and soluble plant nutrients available for loss in runoff water. Composted municipal biosolids and dairy manure products were applied to soils in Texas according to prescribed Texas Department of Transportation specifications for stabilizing roadside soils. The specifications included a method for incorporating compost into soils prior to seeding or applying a compost and woodchip mix over a disturbed soil and then seeding. Applying compost and woodchips over the soil surface limited sediment losses (14 to 32 fold decrease) compared to incorporating compost into the soil. Yet, the greatest total phosphorus and nitrogen losses in runoff water occurred from soils where the compost and woodchip mix was applied. The greatest losses of soluble phosphorus also occurred when the compost and woodchip mix was applied. In contrast, nitrate-nitrogen losses in runoff were similar when compost was incorporated in the soil or applied in the woodchip mix. Compost source affected the nutrient losses in runoff. While the composted municipal biosolids added greater nutrient loads to the soil, less nutrient loss in runoff occurred.

1. Introduction

State Departments of Transportation (SDOT) are mandated to manage highway construction sites as potential nonpoint pollution sources. Soil particulate loads are often the greatest fraction of soil components in runoff from highway construction sites [1]. Soil erosion can occur when disturbed soils are unprotected from rainfall and flowing water. Silt fences, straw mulch, and material blankets are among several practices used to control erosion [2]. Additionally, composted biosolids and blends of biosolids with yard waste are among materials top-dressed or incorporated on constructed soil slopes to control erosion and enhance vegetation establishment [3–5]. Persyn et al. [5] reported 5 or 10 cm blankets of composted biosolids, yard waste, or industrial waste reduced runoff water and sediment compared to exposed subsoil or imported topsoil to high-way construction sites. In a complementary report, Glanville et al. [3] reported top-dressing composted materials decreased nutrient loss in

runoff water during a simulated 30 min rain event compared to excavated soil alone.

Specifications for the composition and application of composted materials to soil on construction sites vary among SDOTs [6]. Generally, application rates are depth- or volume-based and include limits on the maximum rate which can be applied. Composted materials are required to meet standards for Class A biosolid, but requirements or limits on mineral nutrient concentrations or rates in applied materials are not often specified [7]. Large volume-based rates may have the potential to increase total nutrient concentrations in amended soil above requirements for establishment and maintenance of vegetation and contribute to nonpoint source losses in surface runoff [8].

In Texas, the Department of Transportation (TxDOT) developed three specifications for top-dressed and incorporated amendments on high-way construction sites. A top-dressed layer of compost on excavated soil, termed erosion control compost (ECC), is comprised of a 5 cm depth of

a blend of equal volumes of compost and woodchips [7]. Top dressing of a 0.6 cm depth of 100% compost on existing vegetation was defined as general use compost. In contrast to top-dressed treatments, compost-manufactured topsoil (CMT) was composed of a mixture of 0.25 m³ compost m⁻³ soil. The compost is incorporated with soil on-site or premixed and applied at a depth specified by engineers after excavation of a site.

The CMT and ECC specifications were developed to enable construction contractors a method to control sediment loss in runoff water and minimize the duration of vegetation reestablishment. Both composted dairy manure (CDM) and blends of municipal biosolids and yard waste (CMB) were among materials construction contractors used to comply with the TxDOT specifications [9]. Systems integrating compost use with soil stabilization of roadsides offer an option for recycling composts, but total nutrient rates can exceed agronomic recommendations for crops or establishing vegetation [10]. Specifications for volume-based rates typically do not change with respect to compost source and composition.

Similar to concerns about CDM applications to agricultural fields, volume-based rates specified of ECC or CMT could increase soil nutrient concentrations and contribute to nonpoint-source runoff losses. Previous reports indicated runoff concentrations and losses of dissolved P were directly related to concentrations in top-dressed amendments [11, 12]. Incorporation of CDM or CMB at volume-based rates increased concentrations of extractable soil P and dissolved P in runoff [13–15]. Variation of nutrient concentration and form among composted materials, including CDM and CMB, and amended soils needs to be evaluated in relation to nonpoint-source losses [12, 16]. In some cases, manure applications increase dissolved P loss more than inorganic fertilizers [17, 18]. Yet a dense layer of crop residues or established vegetation on amended surfaces can limit runoff loss of P from applied manure or compost [18, 19].

The goal of this research was to evaluate TxDOT specifications for amending roadside soils with CDM and CMB. The specific objectives were (1) quantify nutrient loading rates in soil amended according to CMT and ECC specifications, (2) compare runoff concentration and losses of sediment P and N among CMT and ECC treatments during natural storm events, and (3) relate P loss in runoff water to soil P concentrations.

2. Materials and Methods

2.1. Experimental Design. A randomized complete block design was comprised of three replications of seven treatments to compare and contrast treatment effects. Six treatments represented TxDOT specifications for application of CDM or CMB on roadsides [7]. Compost sources were incorporated at a rate of 0.125 and 0.25 m³ m⁻³ into a 50 mm depth of a sandy clay loam soil (Table 1) for four CMT treatments. A compost/woodchip blend (1:1) utilizing both compost sources was top-dressed onto an excavated soil for two ECC treatments. An established stand of common bermudagrass (*Cynodon dactylon* L.) was used as the one control treatment.

TABLE 1: Characteristics of two compost sources used to amend soils according to TxDOT specifications for stabilizing soils affected by highway construction ($n = 2$ for each compost source).

	N (%)	P (%)	Dry mass (%)
Composted dairy manure	0.54	0.32	77.2
Composted municipal biosolids	1.73	1.66	65.5

Treatments were imposed on a Boonville fine sandy loam previously graded to an 8.5% slope [18, 20]. The excavated slope was located at the Texas A&M University Turfgrass Field Laboratory, College Station, TX. The 4 m by 1.5 m plots were oriented parallel to the slope. A 100 mm width of 1.9 mm sheet metal was inserted 25 mm into soil along the perimeter of each plot to channel runoff through an H-flume into an uncovered 311 L holding tank.

The compost products used in the study are characterized in Table 1. Both products were aerobically composted and produced in Texas. The mass of soil and compost or woodchips and compost mixes were measured, and sampled prior to application (Table 2). A seed mixture of 50 g common bermudagrass and 70 g T-587 Bluestem (*Andropogon gerardii*, Vitman) was broadcast over each plot area on 22 April 2003. The soil and compost and the woodchip and compost mixes were smoothed and packed to optimize seed to soil contact. The plots were irrigated daily to minimize water stress during seed germination and establishment. Care was taken to not produce runoff when irrigation water was applied. The bluestem/bermudagrass mixture was clipped on 26 June and 29 July. The mass of freshly clipped grass was measured and samples were collected to measure dry matter yields. Soil core samples were collected after the final rain event. A 20 mm diameter soil probe was used to sample soil to a 50 mm depth at eight randomly selected points within each plot. Soil cores were homogenized for each plot and prepared for analysis.

2.2. Runoff Sampling and Analysis. Captured runoff was measured and sampled after each of 10 natural rainfall events that produced measurable runoff from all plots. Rain depth was measured using an onsite rain gauge. Rainfall depth was subtracted from water depths in the uncovered tanks before runoff volumes were calculated. Tank volumes were mixed thoroughly before collecting 500 mL samples at the conclusion of rainfall events or before tanks overflowed. After collection, water samples were stored at 4°C and filtered through glass fiber filters (<0.7 μm) under vacuum within a 24 hr period. Samples were frozen if stored for more than 24 hr before filtering. The mass of the sediment was calculated by subtracting the filter mass from the filter plus sediment mass after filtering was complete. Filters and sediment were ground and digested for analysis of total N and P after selected rain events [21].

Total and dissolved forms of P and N in the filtrate were analyzed for all runoff events. Inductively coupled plasma optical emission spectroscopy (ICP) was used to measure total TDP in digests of filtrate and total P (TP) in digests of sediment [22]. Total Kjeldahl nitrogen (TKN) in digests

TABLE 2: Effect of rates of compost and associated total N (TN) and P (TP) on TN, TP, soil-test P (STP), and water-extractable P (WEP) concentrations in soil on an 8.5% slope. Composted dairy manure (CDM) and municipal biosolids (CMB) were incorporated at volume-based rates in compost-manufactured topsoil (CMT) or mixed with wood chips in erosion-control compost (ECC) as specified by the Texas Department of Transportation. The control was established perennial grass.

Treatment	Compost	Rate ($\text{m}^3 \text{m}^{-3}$)	Rates applied as compost				Concentration within 5 cm sampling depth									
			Mass (Mg ha^{-1})	TP (kg ha^{-1})	TN (kg ha^{-1})	TP (mg kg^{-1})	TN (mg kg^{-1})	STP (mg kg^{-1})	WEP (mg kg^{-1})							
Control	NA	NA					254	e	1188	cd	80	e	17	d		
CMT	CDM	0.125	62	d [†]	199	f	320	f	537	de	725	e	264	de	34	cd
CMT	CMB	0.125	28	f	408	d	468	e	875	cd	1035	de	439	cd	29	d
CMT	CDM	0.25	109	b	337	e	566	d	761	d	1059	de	496	cd	50	bc
CMT	CMB	0.25	50	e	810	b	865	c	1201	c	1515	c	623	c	33	cd
ECC	CDM	0.50	185	a	611	c	944	b	1773	b	2759	b	1115	b	77	a
ECC	CMB	0.50	100	c	1773	a	1712	a	5121	a	5212	a	2203	a	55	b

[†] Different letters in columns represent significant differences using Fisher's LSD means separation test.

of filtrate and sediment were measured colorimetrically [23, 24], and NO_3^- -N in filtrate was quantified through cadmium reduction in an autoanalyzer [25]. As filtrate concentrations of TKN dropped below 10 mg L^{-1} , Kjeldahl digestions of filtrate were discontinued and the ICP was used to measure TDP in filtrate. Nutrient concentrations in filtrate were adjusted to account for dilution from rainfall in the uncovered tanks. A malachite-green assay was used to quantify dissolved reactive phosphorus (DRP) concentrations in filtrate within 72 hr after filtering and storage at 4°C [26].

Concentrations of total and extractable forms of P and N in soil samples were analyzed in Texas AgriLife Extension's Soil, Water, and Forage Testing Laboratory, College Station, TX. An acidified NH_4OAc -EDTA extraction method was used to measure soil-test P (STP) [27]. In addition, 1 g soil was extracted in 10 mL distilled water for 1 hr on an orbital shaker to determine water extractable P concentration. The ICP was used to measure STP and WEP. Soil nitrate was extracted and analyzed as described by Dorich and Nelson [23].

2.3. Statistical Analysis. The General Linear Model (GLM) procedure of SAS [28] was used for analysis of variance of runoff depth, nutrient concentration in soil and runoff, and mass loss of sediment and nutrients among treatments over ten runoff events. Fisher's least significant difference (LSD) was used to compare treatment means [28]. Treatment means were determined to be significantly different at the $\alpha = 0.05$ level. Regression analysis was used to relate variation of total and extractable P within CMT and ECC to mass loss in runoff over 10 runoff events during grass establishment. A *t*-test was performed to detect significant differences between CMB sources by comparing the slopes of the regression lines [29].

3. Results and Discussion

3.1. Soil. The mass of the compost and soil or woodchip mixes was different due to the differences in bulk density

of the compost sources. The bulk density of CDM was 1.34 Mg m^{-3} , and the bulk density of CMB was 0.79 Mg m^{-3} which contributed to a greater mass of CDM than CMT in the volume-based rates applied as CMT and ECC (Table 2). The comparatively greater bulk density of CDM was attributed to soil scraped and hauled with raw manure from confined animal dry lots [30]. Yet the physical difference in mass did not translate into greater nutrient loading from the CDM since the nutrient concentrations were lower relative to CMB (Table 1). For each treatment with contrasting compost sources and application rates, the TP applied was over two times greater for the CMB than the CDM (Table 2). The TN application rate was also greater when CMB was used to amend soils rather than CDM (Table 2). In general, adding CDM or CMB as a soil amendment increased total P and N, STP, and WEP compared to the control. However, applying CDM at $0.125 \text{ m}^3 \text{m}^{-3}$ did not increase the nutrient concentration above the control for any soil nutrient characteristic tested (Table 2). Additionally, nutrient concentrations in the blended soil or woodchip mixes at the $0.125 \text{ m}^3 \text{m}^{-3}$ rate were not considered different between contrasting compost sources. Soil nutrient concentrations in the surface 50 mm layer were different for TP and TN when compost was blended at the $0.25 \text{ m}^3 \text{m}^{-3}$ volume-based rate. A large difference in concentration of soil nutrients became apparent when the compost sources were blended with woodchips for the ECC treatment. Total P and STP concentrations were nearly twice as great when CMB was used in the blend rather than CDM. The concentration of STP exceeded the concentration required to supply sufficient P for grass growth for each treatment included in the soil of the control plots. Grass plants used for turf are not expected to increase yield due to added P when STP concentrations are greater than 45 mg kg^{-1} [31]. Increasing STP concentrations relative to the critical concentration for plant response may relate to elevated P in runoff [32].

3.2. Grass Response. Bluestem seedlings emerged quickly and composed most of the biomass clipped from all treatments on two harvest dates. At 63 d after seeding, dry biomass yields

TABLE 3: Above-ground dry-mass yield (g m^{-2}) of a bermudagrass, *Cynodon dactylon* L., and big bluestem, *Andropogon gerardii*, Vitman grass mix grown on soils amended with composted dairy manure (CDM) or composted municipal biosolids (CMB) using TxDOT specifications for stabilizing soils affected by highway construction. See Table 2 caption for description of treatments.

Treatment	Compost	Rate ($\text{m}^3 \text{m}^{-3}$)	1st harvest (g m^{-2})		2nd harvest (g m^{-2})	
Control	NA	NA	35	c	58	c
CMT	CDM	0.125	56	bc	244	ab
CMT	CMB	0.125	103	abc	272	ab
CMT	CDM	0.25	42	bc	284	ab
CMT	CMB	0.25	113	abc	211	bc
ECC	CDM	0.50	184	a	215	bc
ECC	CMB	0.50	130	ab	382	a

[†] Different letters in columns represent significant differences using Fisher's LSD means separation test.

were similar between the established grass control and CMT comprising either CDM or CMB (Table 3). In contrast, the yield of dry biomass was greater for ECC composed of either CDM or CMB than for the established grass control. At 98 d after seeding, comparative biomass yields between the control and CMT were dependent on the compost source. Clipping yields of CMT amended with CDM were greater than the control for both of the volume-based rates (0.125 and $0.25 \text{ m}^3 \text{ m}^{-3}$). Similarly, clipping yields from the CMT composed of $0.125 \text{ m}^3 \text{ CMB m}^{-3}$ soil were greater than the established grass control, but yields were comparable between the higher CMB rate and control. In addition, yields were similar between ECC amended with either CDM or CMB and the established grass control on the second harvest. Developing deficiencies of available N or other mineral nutrients could have limited grass growth and differences in biomass yield between ECC and the established grass control at the second harvest date [33].

In a previous study of grass establishment after mixing CMB with soil at $0.25 \text{ m}^3 \text{ m}^{-3}$, grass coverage was 14% greater with compared to without a top-dressing of fertilizer N (50 kg ha^{-1}) at 56 d after sprigging [34]. Over a similar phase of wheat (*Triticum aestivum* L.) establishment, N deficiency symptoms and reduced dry matter production occurred for soil with up to 44 Mg ha^{-1} CMB compared to fertilized soil without CMB [33]. Wheat establishment was deterred even though 100 mg kg^{-1} of fertilizer N was applied to supplement the total N applied as CMB. Incubation studies with and without CMB in the same study indicated N immobilization could limit availability to crops established in soils amended with CMB at rates comparable to the CMT and ECC in the present study.

3.3. Runoff Loss of Sediment and Total N and P. The cumulative depth of rainfall for the ten recorded events was 281 mm. Total runoff loss for the ten events ranged between 137 mm for the ECC using CMB to 206 mm for the $0.25 \text{ m}^3 \text{ m}^{-3}$ CMT using the CDM (Table 4). Only the runoff loss from the latter

treatment was greater than runoff loss from the established grass control. Generally, each specification for amending constructed soils was equally effective at controlling runoff water loss; however, compost source did result in differences in runoff. Runoff water losses were lower when CMB was incorporated into soil or mixed with woodchips and topdressed. The difference was most apparent for the ECC-treated plots (Table 4). The variation of runoff depth among treatments under the 10 natural rain events was substantially less than observed previously under 30 min of simulated rain. Persyn et al. [5] reported that 50 or 100 mm depths of biosolids mixed with yard waste achieved major delays and volume reductions in runoff compared to exposed subsoil under the brief simulated rain event.

Similar to evaluations of ECC comprising a 3 : 1 mixture of CDM and wood chips [10], sediment loss from ECC in the present study was comparable to established perennial grass (Table 4). On a 33% roadside embankment without vegetation, a 50 or 100 mm depth of biosolids mixed with yard waste reduced interrill erosion 77% compared to exposed subsoil [35]. Seeding and establishment of vegetation reduced interrill erosion from the 33% slope, but erosion remained 70% less for biosolid mixed with yard waste than for exposed subsoil. Over the period of grass establishment in the present study, incorporation of CDM or CMB in soil controlled sediment loss less effectively than ECC. In addition, sediment loss from CMT amended with $0.25 \text{ m}^3 \text{ m}^{-3}$ of compost was greater for CDM than for CMB (Table 4). As the greater weight of applied CDM indicated (Table 1), soil made up a larger portion of CDM than CMB, which could have diminished CMT effects on sediment loss. In the previous study on a steep slope (33%), interrill erosion was comparable between exposed subsoil and a 150 mm depth of topsoil applied over subsoil with or without seeding [5].

Treatment effects on sediment loss were reflected in variation of runoff loss of TKN in sediment. Runoff loss of sediment TKN was low and similar between ECC and established grass (Table 4). In addition, runoff loss of sediment TKN from ECC was less than two of the four CMT treatments even though TN concentrations within the top-dressed ECC layer were greater than CMT or established grass (Tables 2 and 4). On a steep embankment (33%), low runoff volume prevented sediment TN loss from top-dressed biosolids and yard waste during a 30 min. simulated rain event despite a doubling of sediment TN concentration [3].

Although ECC prevented loss of sediment TKN similar to established grass, sediment TP loss was greater for ECC than established grass and the lower rate ($0.125 \text{ m}^3 \text{ m}^{-3}$) of CDM in CMT (Table 4). In addition, runoff loss of sediment TP from ECC composed of CDM was greater than all other treatments, including ECC derived from CMB. This result is likely due to the relatively higher TP concentration in the ECC when CDM was used in the compost/woodchip mix (Tables 2 and 4).

3.4. TDP and NO_3^- -N Concentrations in Runoff. Runoff events were analyzed separately to accommodate significant ($\alpha = 0.001$) interactions between treatment and rain event for variation of TDP and NO_3^- -N concentrations in filtrate

TABLE 4: Total runoff and the sum of mass loss of runoff sediment and associated total P and N over 10 rain events during grass establishment on roadside slope amended with composted dairy manure (CDM) and composted municipal biosolids (CMB). See Table 2 caption for description of treatments.

Treatment	Compost	Rate ($\text{m}^3 \text{m}^{-3}$)	Runoff (mm)		Sediment (g m^{-2})		Total P (mg m^{-2})		TKN (mg m^{-2})	
Control	NA	NA	156	bc	111	cd	328	c	115	b
CMT	CDM	0.125	194	ab	525	b	361	c	554	ab
CMT	CMB	0.125	178	ab	398	b	439	bc	1373	a
CMT	CDM	0.25	206	a	872	a	646	b	1380	a
CMT	CMB	0.25	168	abc	469	b	454	bc	664	ab
ECC	CDM	0.50	178	ab	44	d	965	a	60	b
ECC	CMB	0.50	137	c	11	d	681	b	16	b

[†] Different letters in columns represent significant differences using Fisher's LSD means separation test.

TABLE 5: Comparison of runoff concentration of total dissolved P (TDP) among contrasting roadside treatments for selected rain events on an 8.5% slope. Rain amounts for the respective events were 23, 5, 18, 29, and 50 mm. See Table 2 caption for description of treatments and compost sources.

Treatment	Compost	Rate ($\text{m}^3 \text{m}^{-3}$)	Rain event									
			1 (5 Jun) (mg L^{-1})		3 (14 Jun) (mg L^{-1})		6 (4 Jul) (mg L^{-1})		8 (11 Aug) (mg L^{-1})		10 (31 Aug) (mg L^{-1})	
Control	NA	NA	1.9	d	2.2	d	1.8	c	3.2	b	2.6	c
CMT	CDM	0.125	2.7	d	3.2	cd	2.4	c	3.4	b	2.6	c
CMT	CMB	0.125	2.6	d	2.8	d	2.1	c	3.8	b	3.3	c
CMT	CDM	0.25	5.2	c	6.0	c	2.8	c	3.9	b	3.3	c
CMT	CMB	0.25	3.2	cd	3.4	cd	2.7	c	4.2	b	3.2	c
ECC	CDM	0.50	17.1	a	17.3	a	11.4	a	11.0	a	11.2	a
ECC	CMB	0.50	9.6	b	13.0	b	8.0	b	11.4	a	8.3	b

[†] Different letters in columns represent significant differences using Fisher's LSD means separation test.

of runoff. Five of the 10 runoff events were selected to illustrate variation of runoff concentrations of TDP and NO_3^- -N among treatments during the period of bluestem establishment (Tables 5 and 6). As anticipated from variation of runoff loss of sediment TP among treatments (Table 4), runoff concentrations of TDP were consistently greater for ECC than CMT or the established grass control (Table 5). Similarly, incorporation reduced runoff concentrations of dissolved P compared to top-dressed application of livestock manures [11, 36]. In contrast, a previous study indicated TDP concentration in runoff from ECC was less or similar to that from CMT during the first two rain events after these CDM-amended treatments were applied and seeded [10].

In the present study, incorporation of CDM at $0.25 \text{m}^3 \text{m}^{-3}$ with soil did increase TDP concentrations in runoff compared to established grass during rain events 1 and 3 (Table 5). In addition, CDM contributed to greater TDP concentrations in runoff than the CMB mixed with wood chips in ECC on four of the five selected rain events. Yet TDP concentrations in runoff were similar among three of the four CMT treatments during events 1 and 3. In addition, runoff concentrations of TDP were similar among

all four CMT treatments and the grass control as bluestem establishment progressed during rain events 6 through 10 (Table 5).

After the initial rain event, filtrate concentration of NO_3^- -N declined and remained low and similar between CMT and the established grass control over the remaining events (Table 6). Comparable results were observed previously using two soil types and CDM to apply CMT amendments at $0.25 \text{m}^3 \text{m}^{-3}$ [10]. In the previous and present study, runoff NO_3^- -N concentration was greater for CMT amended with CDM or CMB at $0.25 \text{m}^3 \text{m}^{-3}$ of soil than established grass during the initial rain event (Table 6). After the first rain event, NO_3^- -N concentrations in runoff were similar (0.57mg L^{-1}) to those reported for grassland soils mixed with poultry litter [28]. Runoff concentrations of NO_3^- -N were greater for ECC than CMT on rain events 8 and 10, but runoff concentrations were well below the EPA drinking water standard [37]. Both bluestem uptake and slow mineralization from compost could have minimized NO_3^- -N concentrations in amended soil and runoff [33]. Glanville et al. [3] similarly reported low NO_3^- -N concentrations in runoff (0.2mg L^{-1}) from a surface application of biosolids mixed with yard waste on a roadside embankment.

TABLE 6: Comparison of runoff concentration of $\text{NO}_3\text{-N}$ among contrasting roadside treatments for selected rain events on an 8.5% slope. Rain amounts for the respective events were 23, 5, 18, 29, and 50 mm. See Table 2 caption for description of treatments and compost sources.

Treatment	Compost	Rate ($\text{m}^3 \text{m}^{-3}$)	Rain event									
			1 (5 Jun) (mg L^{-1})		3 (14 Jun) (mg L^{-1})		6 (4 Jul) (mg L^{-1})		8 (11 Aug) (mg L^{-1})		10 (31 Aug) (mg L^{-1})	
Control	NA	NA	0.59	d	0.22	a	0.25	a	0.55	b	0.25	bc
CMT	CDM	0.125	1.20	bcd	0.48	a	0.51	a	0.64	b	0.23	c
CMT	CMB	0.125	1.10	cd	0.32	a	0.17	a	0.41	b	0.21	c
CMT	CDM	0.25	2.38	a	0.69	a	0.40	a	0.41	b	0.23	c
CMT	CMB	0.25	1.65	abc	0.47	a	0.25	a	0.40	b	0.21	c
ECC	CDM	0.50	1.41	bcd	0.28	a	0.46	a	1.15	a	0.30	ab
ECC	CMB	0.50	2.01	ab	0.45	a	0.45	a	1.42	a	0.34	a

[†] Different letters in columns represent significant differences using Fisher's LSD means separation test.

TABLE 7: Mass runoff loss of total dissolved P (TDP), dissolved reactive P (DRP), $\text{NO}_3\text{-N}$, and dissolved total Kjeldahl N (TKN) during grass establishment on roadside soils amended with composted dairy manure (CDM) and composted municipal biosolids (CMB) over 10 natural rainfall events. See Table 2 caption for description of treatments.

Treatment	Compost	Rate ($\text{m}^3 \text{m}^{-3}$)	TDP (mg m^{-2})		DRP (mg m^{-2})		$\text{NO}_3\text{-N}$ (mg m^{-2})		TKN (mg m^{-2})	
Control	NA	NA	301	d	165	e	45	b	775	b
CMT	CDM	0.125	450	cd	303	d	86	b	786	b
CMT	CMB	0.125	428	cd	272	de	59	b	815	b
CMT	CDM	0.25	637	c	450	c	140	a	1025	b
CMT	CMB	0.25	440	cd	308	d	78	b	770	b
ECC	CDM	0.50	1845	a	1321	a	75	b	1756	a
ECC	CMB	0.50	1050	b	841	b	71	b	1549	a

[†] Different letters in columns represent significant differences using Fisher's LSD means separation test.

3.5. Nutrient Losses. Variation of cumulative runoff loss of TDP among treatments over the 10 rainfall events reflected variation of runoff concentrations of TDP for events 1 and 3 (Tables 5 and 7). Similar to benefits reported for agricultural soils, incorporating CDM or CMB into the 0 to 50 mm soil depth (CMT) reduced TDP losses in runoff compared to top-dressed ECC [17]. Except for CDM incorporated at $0.25 \text{ m}^3 \text{ m}^{-3}$ soil, CMT limited cumulative runoff loss of TDP during grass establishment as effectively as the established grass control (Table 7). In contrast, cumulative mass loss of TDP during grass establishment was greater for ECC than for CMT or the established grass control. In addition, cumulative TDP loss from ECC was greater for CDM than for CMB even though TP concentration was nearly 3 times greater in CMB than CDM incorporated with soil (Tables 2 and 7). Greater cumulative TDP loss from ECC amended with CDM did reflect the greater total runoff depth over 10 rainfall events for CDM-compared to CMB-amended ECC (Tables 4 and 7). Glanville et al. [3] reported runoff volume effects on runoff loss of soluble and adsorbed P forms were even greater between top-dressed biosolids and excavated soil during a simulated 30 min storm. Runoff loss of soluble plus adsorbed P was 8 times greater for exposed soil than for the top-dressed biosolids layer. Conversely, respective concentrations were 81 and 20 times greater for soluble P (3.1 mg L^{-1}) and sediment P (17.3 g L^{-1}) in

runoff from top-dressed biosolids than from exposed soil [3].

The dissolved reactive fraction of P (DRP) contributed 64% or more of the runoff loss of TDP over the 10 runoff events during establishment of the seeded bluestem (Table 7). Similarly, DRP accounted for 64% of total P loss in simulated runoff from soils amended with manure sources of P [11]. For both simulated and natural rainfall in the previous and present study, runoff loss of DRP was lower for incorporated compared to top-dressed raw manure, CDM, or CMB (Table 5). In addition, incorporation of the lower rate of CMB ($0.125 \text{ m}^3 \text{ m}^{-3}$) reduced DRP runoff loss to amounts comparable to the grass control. Similar to TDP losses in runoff from ECC over 10 runoff events, greater runoff loss of DRP from CDM than from CMB was attributed to differences in cumulative runoff depth and WEP concentration (Tables 2 and 7). Importantly, DRP is the fraction of P in surface water considered most available to aquatic plants and could contribute to accelerated eutrophication at concentrations orders of magnitude less than observed in this study [8].

High runoff volume contributed to relatively high runoff loss of $\text{NO}_3\text{-N}$ from CMT amended with CDM at $0.25 \text{ m}^3 \text{ m}^{-3}$ of soil, but losses from ECC and other CMT treatments reflected low $\text{NO}_3\text{-N}$ concentrations in runoff (Tables 6 and 7). Although ECC prevented sediment and

associated TKN loss similar to the grass control, ECC was a major nonpoint source of dissolved TKN. In contrast to treatment differences in runoff loss of sediment TKN, loss of dissolved TKN was greater for ECC than for CMT or established perennial grass over the 10 runoff events (Tables 4 and 7). Conversely, runoff loss of dissolved TKN from CMT was comparable to the established grass control (Table 7). The ECC did not lower runoff volume sufficiently to reduce dissolved TKN in runoff compared to CMT or the grass control during the 10 natural rainfall events in the present study. In contrast, Glanville et al. [3] reported lower runoff volume during a 30 min simulated rain reduced total N in runoff from top-dressed biosolid and yard waste to 0.1% of that from the exposed subsoil.

3.6. Relationship between Soil and Runoff P. Similar runoff depths among five of the seven treatments indicated variation of nutrient concentrations in soil, and runoff contributed a major portion of variation of mass loss of nutrients among treatments [13, 14, 17, 18, 35]. Regression analysis was used to relate variation of soil P concentration to variation of P runoff losses among roadside amendments and between compost sources. Relationships between soil and runoff P, though soil specific, enable evaluation of ECC and CMT effects on water quality for both site and watershed scales [14, 15, 38, 39]. In the present study, the sum of TDP and sediment P losses for each treatment was accumulated over 10 rainfall events and related to P concentrations in soil sampled after the final event. Linear relationships were observed between mass runoff losses of TP for the control, CMT, and ECC treatments of each compost source and mean WEP, STP, and TP in the 0 to 50 mm soil depth (Figures 1, 2, and 3). Slopes of regression relationships between soil WEP and TP runoff loss were similar between CDM and CMB (Figure 1). In contrast, slopes of relationships between STP (Figure 2) or soil TP (Figure 3) and runoff TP varied markedly between CDM and CMB sources used to amend soil. Although the volume-based rates of CMB increased soil total P and STP concentrations compared to respective CDM rates, soil WEP concentrations were greater for CDM-amended treatments. Similar to previous comparisons among varied sources of livestock manure [16], WEP was the most effective environmental indicator of nonpoint-source runoff loss of P from the roadside amendments.

4. Conclusions

Runoff and nutrient losses during grass establishment on an excavated slope revealed similarities and differences between ECC and CMT treatments. The ECC enhanced early establishment of bluestem compared to CMT, but both ECC and CMT yielded biomass comparable to or greater than established grass after 98 d. Although ECC more consistently limited runoff loss of sediment and associated TKN, loss of dissolved TKN was greater for ECC than CMT or established grass. Incorporation of CDM or CMB in CMT reduced loss of TDP and sediment and dissolved TKN to levels comparable to established grass. However, the interaction between the

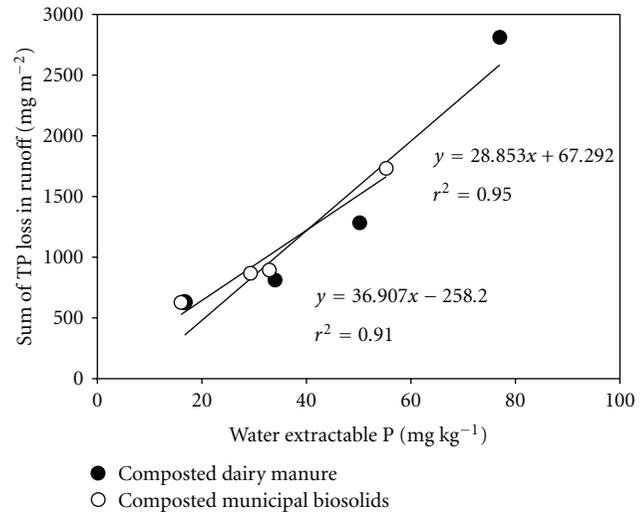


FIGURE 1: The mean sums of total P (total P) loss in runoff water over 10 rain events compared to mean water-extractable P concentration in 0 to 50 mm depth of contrasting roadside treatments on an 8.5% slope. Soil was sampled from established perennial grass, compost-manufactured topsoil, and erosion-control compost treatments after the final rain event.

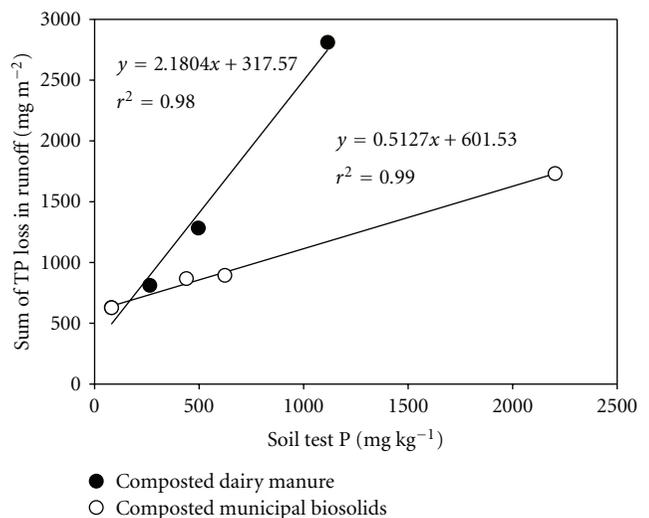


FIGURE 2: Relationship between the mean sum of total P (TP) loss in runoff water over 10 rain events and mean soil-test P within 0 to 5 mm depth of contrasting roadside treatments on an 8.5% slope. Soil was sampled from established perennial grass, compost-manufactured topsoil, and erosion-control compost after the final rain event.

compost products used and soil properties affecting nutrient losses in runoff is likely influenced by the soil type used in this study. Variation of nutrient concentration in amended depths of ECC and CMT and in runoff contributed to a major portion of variation of P loss in runoff. Variation of TP loss in runoff was directly related to variation of WEP, STP, and TP within the 0 to 5 cm depth of treatments. For the CDM and CMB sources used in ECC and CMT,

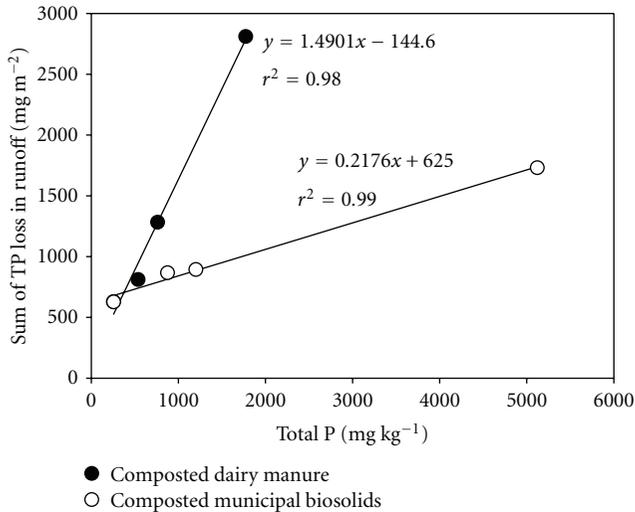


FIGURE 3: The mean sums of total P (TP) loss in runoff water over 10 rain events compared to mean total P concentration in 0 to 50 mm depth of contrasting roadside treatments on an 8.5% slope. Soil was sampled from established perennial grass, compost-manufactured topsoil, and erosion-control compost treatments after the final rain event.

variation of WEP within amended soil depths was the best indicator of variation of TP runoff loss. Compost needs to be analyzed and rates and application method managed to keep WEP and other water-soluble nutrient concentrations in CMT at levels that prevent nonpoint-source losses in runoff. Additional research is needed to quantify long-term water quality impacts of CMT and ECC composed of CDM or CMB and the interaction differing soil types may have on those impacts.

Abbreviations

ECC:	Erosion control compost
CMT:	Compost manufactured topsoil
N:	Nitrogen
P:	Phosphorus
CDM:	Composted dairy manure
CMB:	Composted municipal biosolids
TKN:	Total Kjeldahl N
TDP:	Total dissolved phosphorus
WEP:	Water extractable phosphorus
TP:	Total phosphorus
TxDOT:	Texas Department of Transportation.

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