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Effects of municipal sewage sludge stabilized by fly ash on the growth of Manilagrass and transfer of heavy metals

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ABSTRACT

A greenhouse experiment was carried out to evaluate the feasibility of using an artificial soil for cultivation of Manilagrass. The transfer and transformation of heavy metals in the artificial soil–Manilagrass system were discussed at the same time. The results showed that fly ash–sludge indicated a positive effect on the growth of Manilagrass. The pots with 14% sludge and 6% fly ash mixture had the highest yield and nutrient concentrations of Manilagrass. With the increasing application of coal fly ash, the concentrations of Ni, Zn, Mn, Sb and Cu in Manilagrass decreased significantly, while Pb, V and Ti increased. Otherwise, the concentrations of Cd, As, Cr, Co, and Fe did not show a remarkable change. Except for Sb, the values of bio-concentration factor of heavy metals in Manilagrass were all below 1.0 after treated by the fly ash–sludge treatment, decreased as Sb > Ni > Zn > Cu > Pb > Mn > Co = Cr > Cd > Fe = V > Ti > As in an average for all treatments. Compared to the contrast check, the proportions of heavy metals in exchangeable, reducible and oxidizable fractions increased. Manilagrass could be used to reduce the eco-toxicity and bioavailability of Ti, V, Mn, Co, Cr and Cd in fly ash–sludge amended soil.

1. Introduction

It is well known that the wastewater treatment plants can produce a large quantity of municipal sewage [1]. The amount of sewage sludge is growing rapidly, which should be controlled in compliance with the government and law [2]. If not, the disposal of sewage sludge, containing heavy metals and pathogens, may present a potential health influence to human beings. This is a serious environmental problem in the world [3].

Sewage sludge, which contains a significant amount of nitrogen (N), phosphorus (P), magnesium (Mg), organic matter (OM) and other trace elements, is considered as a good source of nutrients for plant growth and a good soil conditioner to improve the physical and chemical properties of soil [4,5]. Many scientific groups have oriented their research to find a process to recycle and treat other wastes. Some researches were carried out to characterize and evaluate the effect of waste, both as solid and liquid, in the growth of cultures of commercial interest [6–9]. Therefore, land application, compared with landfilling and incinerating disposal, is an alternative and predominant method which shows a way to dispose of the sewage sludge and reutilize the nutrient value at the same time. However, the sewage sludge also contains a range of potentially toxic heavy metals, such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), zinc (Zn) and nickel (Ni), as well as high soluble salt [10]. Adequate quantity of toxic heavy metals can cause the damage to plants. But, if sewage sludge is applied in horticultural applications, the effect on human health upon consumption of plants grown on metal-enriched sludge can be avoided.

Coal fly ash is the alkaline residues produced during the burning of coal being enrich in calcium oxide (CaO), magnesium oxide (MgO) and silicon dioxide (SiO_2)[11]. It contains potassium (K), calcium (Ca), sulfur (S), boron (B), molybdenum (Mo) and a possible number of other micronutrients, such as Zn. The pH value of the coal fly ash is about 12 which can be used to neutralize soil acidity. According to these characteristics, coal fly ash can be used as a stabilization agent for municipal sewage sludge improvement by reducing heavy-metal availability and killing pathogens [12].

However, there are also some limitations. Coal fly ash contains variable amounts of certain toxic trace elements (e.g., Cd, Cr, Pb, Ni, B and Mo) and high soluble salt [13], and this property may affect the application of coal fly ash [14]. Therefore, reducing the availability of heavy metals in coal fly ash and sewage sludge is one of the major hot points in the research of land application.

The combined employment of coal fly ash and sewage sludge for land application, especially horticulture and landscaping, can provide a beneficial way for their disposal. Due to the contrasting physical and chemical properties and nutrient contents, land

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application of both wastes, as a mixture, can improve the soil quality and turf production [11].

The environmental behavior, migration and transformation capabilities, bioavailability and accumulation in organisms, and biological toxicity of heavy metals depend on their existing forms in the environment to a large extent. The bioavailability is the direct method to evaluate the toxicity of heavy metal. Therefore, the knowledge of heavy metal speciation in soil amended with the mixture of coal fly ash and sewage sludge is important for the understanding of the bioavailability and mobility of heavy metals in soils [15].

Zoysia matrella (L.) Merr., commonly known as Manilagrass, is a species of mat-forming, perennial grass native to temperate coastal southeastern Asia and northern Australasia, from southern Japan (Ryukyu Islands), Taiwan, and southern China (Fujian, Guangdong, Hainan) south through Thailand, Indonesia, Malaysia and the Philippines to northern Australia (northeast Queensland), and west to the Cocos Islands in the eastern Indian Ocean. Manilagrass is grown as an ornamental grass, and is used for turf on campus green and golf courses in Asia, Europe and the Americas. The variety of multi-purposes attests to the widespread occurrence in above-mentioned regions along with its usefulness as a cultivated grass in diverse areas. In addition to its ability to grow on sandy soils, it tolerates high salinity, making it ideal for erosion control and lawns in coastal areas [16].

To the authors' knowledge, there have been few studies on improving the soil quality by using a combination of fly ash and sludge. The horticultural plants have been rarely employed in cultivation trials as well. In this study, the feasibility and the appropriate application of sludge and fly ash, which was used to passivate the heavy metals' availability, might be confirmed to the cultivation of Manilagrass. The main physico-chemical properties and heavy metal contents of the tested soil, sewage sludge, fly ash and the composite soil as well as the transformation of heavy metals (before and after the cultivation) in composite soil and rhizosphere soil were studied. Moreover, the transfer and transformation of heavy metals in the artificial soil–Manilagrass system were also discussed in this study. The results could provide a new approach for the practice of municipal sewage sludge conditioning and coal fly ash utilization.

2. Materials and methods

2.1. Collection and preparation of materials

Coal fly ash was obtained from the Nanpu Power Station of Quanzhou City, Fujian Province, China. Sewage sludge, demoistured by the frame type filter, was collected from Yundang Wastewater Treatment Plant in Xiamen City, Fujian Province, China. Before pressure filtration, the sewage sludge was added a large amount of CaO, which can be used as a stabilization agent to make the sludge cohesive and compact enough so that to be separated easily from water, and also can be used to reduce pathogens and heavy metal availability in the sludge. The red acidic soil from Xiamen campus, Huaqiao University was selected for the plant growth experiment. Manilagrass employed in this study was grown in the campus landscaping areas.

The coal fly ash was mixed with dewatered sludge at the volumetric proportion of 0% (F0), 10% (F10), 20% (F20), 30% (F30), 40% (F40) and 50% (F50), respectively. The coal fly ash–sludge mixtures were then mixed with the acidic soil at the volumetric proportion of 1:4. In order to obtain homogeneous composite samples of coal fly ash, sludge and soil, appropriate distilled water was poured into the mixtures, and then air-dried at room temperature. This process was carried out three times, which could make the mixtures mixed thoroughly. The mixture was air-dried and stabilized before further utilization and chemical analysis. Prior to cultivation, all of the treated samples of soil, sludge and fly ash were passed through an 8-mm sieve.

2.2. Greenhouse experiment

The prepared samples of soil, sludge and fly ash were filled into the earthen pots (15 cm diameter × 20 cm height). Seven treatments, including six different treatments of fly ash–sludge mixtures (i.e. F-series) plus one contrast pot of soil without fly ash and sludge (i.e. CK) were set. There were three replications for each treatment. The coal fly ash-stabilized sludge amendments used in the experiments were given in Table 1. No fertilization was done before and during the experiment.

The Manilagrass turfs, which were selected in good health and the same age, were collected from campus's lawn in the same size, including area, height and biomass. Pots were placed on a bench in a greenhouse, and permitted to grow for 67 days. Plants were grown in the glasshouse at 25–30 °C and 50–70% relative humidity in a 14 h photoperiod with natural daylight. The pots were watered daily to keep 70% of the field water holding capacity using about 100 mL distilled water every morning and evening.

At harvesting, the rhizosphere soil of each pot was separated from bulk soil. The whole soil and root system was carefully removed from the pot, bulk soil mass was gently crushed and loosely held soil separated by shaking the root system. The remaining tightly held soil (<5 mm from the root surface), considered as the rhizosphere soil, was obtained from the roots in a plastic bag after a brief period of air-drying. Simultaneously, the plants were washed with tap water to remove attached particles and then rinsed twice with deionized water. The shoot and root tissues, which were oven-dried at 60 °C for 72 h, were then ground to pass through a 1-mm sieve using a stainless-steel mill for chemical analysis.

2.3. Analytical methods

2.3.1. Determination of physico-chemical properties

The samples of soil, sludge and fly ash were sieved (<2 mm) before the determination for their physical and chemical properties. The pH values of soil, sludge and fly ash were measured in the 1:5 (w/v) suspension of solid sample and distilled water using a pH meter [4]. Organic matter, cation exchange capacity (CEC), moisture content and calcium carbonate ($CaCO_3$) were determined following standard laboratory procedures [17].

After digestion with nitric-perchloric acids, total N, total P and total K were measured by Micro-Kjeldahl [18], Vanado-Molybdate spectrophotometry and flame-photometry, respectively [19]. 0.5 mol L^{-1} sodium bicarbonate ($NaHCO_3$) solution was used to extract available P, and then determined colorimetrically using the molybdenum blue method [20]. The available K and Ca were measured by atomic absorption spectrophotometer (AAS) in an acetylene-air flame in extracts obtained by digestion with nitric acid (HNO_3) and ammonium acetate. Selected physico-chemical properties and heavy metal contents of the coal fly ash, sewage sludge and soil were listed in Table 2.

The plants were digested using sulfuric acid (H_2SO_4) for the determination of total N. A ternary mixture of HNO_3:H_2SO_4:$HClO_4$ in the ratio of 10:1:4 (v:v:v) was used for the determination of total P and K [21].

2.3.2. Extraction and determination methods for heavy metals in samples

0.5 g dry sample (soil, sludge and fly ash) was induced into a 50-mL plastic centrifuge tube and 7 mL concentrated hydrochloric acid and 2.3 mL concentrated HNO_3 was added into the same centrifuge

Table 1
Sludge and fly ash treatments used in the experiments.

Treatment	Soil (cm^3)	Sludge (cm^3)	Fly ash (cm^3)	Percentage of sludge (%)	Percentage of fly ash (%)
F0	2000	500	0	20	0
F10	2000	450	50	18	2
F20	2000	400	100	16	4
F30	2000	350	150	14	6
F40	2000	300	200	12	8
F50	2000	250	250	10	10
Contrast	2500	0	0	0	0

The fly ash amendment rates for sludge were as follows: F0: sludge + 0% fly ash, F10: sludge + 10% fly ash, F20: sludge + 20% fly ash, F30: sludge + 30% fly ash, F40: sludge + 40% fly ash, F50: sludge + 50% fly ash, and the total content of the six experimental groups were equal.

tube. After being deposited for 16 h at 22 ± 5 °C and 2 h boiling water bath, the mixture was centrifugated at 4000 rpm for 10 min. Then, the supernatant was transported into a 50-mL volumetric flask. The residue was washed and centrifugated twice with de-ionized water and the supernatants were decanted into the same flask, then diluted to 50 mL with 3% (v/v) HNO_3 and stored at 4 °C prior to analysis.

0.5 g ash of Manilagrass samples were added into a 50-mL plastic centrifuge tubes with 10 mL HNO_3 to digest at 80 °C for 24 h in water bath. After the tubes were removed from the water bath and cooled down, the solutions were filtered. The filtrate was transported into a 50-mL volumetric flask after the residue was washed and centrifuged three times, adding 3% HNO_3 (v/v) to 50 mL. The solutions were stored in refrigerator at 4 °C for determining [22].

The contents of heavy metals in the extraction solutions of soil, sludge, fly ash and Manilagrass above were determined by inductively coupled plasma-atom emission spectrometer (ICP-AES, Model 7510, Shimadzu, Japan).

2.3.3. BCR sequential extraction

Different types of sequential extraction procedures have been developed and applied to fractionate trace metals in soils, sediments, sewage sludge and so on. For the determination of heavy metals availability, the four-stage Community Bureau of Reference (BCR) sequential procedure was used as shown in Table 3 [23]. The BCR procedure is basically a revised and stripped-down version of the five-stage sequential extraction procedure originally proposed by Tessier et al. [24], and has been widely applied for the evaluation of metal availability from various matrices, e.g., sediment, soil, sludge, ash and other industrial residues [25,26].

The extracts were then separated from the solid phase by centrifugation at 4000 rpm for 8 min after every step of the extraction. The supernatant liquid was decanted into a 50-mL flask. The residue was washed twice with de-ionized water, and the supernatant liquid was decanted into the same flask, and then diluted with 3% (v/v) HNO_3. The supernatant was decanted and removed carefully to avoid loss of the solid residue. The extract solutions were stored at 4 °C prior to metal analysis.

For each extraction program, a blank was prepared with an equal amount of different reagents. All the glassware in experiments were previously soaked overnight with 20% (v/v) HNO_3 and then rinsed with ultra-pure water. Background correction and matrix interference were monitored throughout the analyses. All reagents were of analytical grade and contained very low concentrations of trace metals. Normal precautions for trace metals analysis were observed throughout.

2.4. Quality assurance

All the experiments were conducted in triplicate and the results were the average values. The recovery of heavy metals during the sequential extraction procedure which was essentially quantitative within the precision of the method [26], could be investigated by comparing the sum of each fraction's concentrations with the total heavy metal concentrations. A check about the results of BCR sequential extraction procedure was performed by comparing the sum of the four fractions (exchangeable and reducible, oxidizable and residual fractions) with the total concentrations of heavy metal from aqua regia digestion procedure.

The results showed that the sum of the four steps was in good agreement with the total heavy metal concentration, which indicated that this modified four-step BCR sequential extraction method used in detecting the speciation of heavy metals in fly ash–sludge and soil was reliable.

2.5. Statistical analysis

Data were statistically analyzed by ANOVA test using a statistical package, IBM SPSS version 19.0 (SPSS, China) programs for Windows 7. Duncan's multiple-range test was performed to test the significance of difference between the treatments.

3. Results and discussion

3.1. Physico-chemical properties of fly ash-stabilized sewage sludge

The positive effects of fly ash-stabilized sludge on the physical and chemical properties of soil had been reported [27]. Table 4 shows the selected physico-chemical properties and heavy metal contents of the artificial soil which was prepared by soil, coal fly ash and sewage sludge. With the increasing application of fly ash, pH value raised significantly. This was resulted from the high content of CaO and MgO in coal fly ash [15]. CEC decreased with the increasing of coal fly ash. The increase in pH values after ash application might cause the precipitation of exchangeable cations in the ash–sludge mixture, which caused the reduction in CEC of the fly ash–sludge mixture.

As seen in Table 4, the moisture content, $CaCO_3$ and OM of the artificial soil had been significantly reduced with the increasing application of fly ash. The contents of moisture, $CaCO_3$ and OM in F0 treatment were 12.6, 2.1 and 1.3 times, respectively, of those in the contrast check (CK) soil (Table 2). Similarly, soil total N, total P and total K concentrations significantly decreased with the increasing of coal fly ash amendment. There was no suspense that the content of available K was decreased with the increment of the coal fly ash because of the reduction of sludge content which had higher content of available K. On the contrary, due to the high content in coal fly ash, the content of available P was increased.

These above benefits came directly from the high concentration of nutrients in the dewatered sludge and a part of coal fly ash, as shown in Table 2. Organic matter played a major role in maintaining soil quality. Improvement in soil structure could enhance infiltration rate and reduce soil erosion [28]. The nutrient concentrations were extremely high, which were very significant for barren soil. In

Table 2
Selected physico-chemical properties and heavy metal contents of soil, sewage sludge and coal fly ash.

	pH	CEC (mmol kg^{-1})	$CaCO_3$ (%)	Moisture content (%)	OM (%)	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Ca (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
Soil	6.46a	5.36a	15.05a	1.44a	10.21a	0.07a	0.11a	2.42a	0.5a	1.53a	30.11a
Sludge	8.17b	369b	56.54b	9.06b	27.65b	8.14b	7.99b	86.04b	163b	160b	1440b
Fly ash	11.4c	36.9c	3.71c	0.1c	0.48c	0.007c	1.51c	7.34c	22.8c	234c	78.71c

	Cd	As	Ni	Pb	Cr	Cu	Zn	Mn	Co	Fe	V	Ti	Sb
	(mg kg^{-1})												
Soil	3.17a	5.00a	6.83c	31.8a	49.3b	15.7c	49.5b	251c	3.50c	45000a	132a	532.8b	5.83a
GB15618-1995	0.3	40	40	250	150	50	200	–	–	–	–	–	–
Sewage sludge	2.83b	5.00a	90.3a	21.7b	74.8a	190a	408a	495a	5.67b	43333b	14.8b	120.2c	5.33b
GB/T23486-2009	5	75	100	300	600	800	2000	–	–	–	–	–	–
Fly ash	2.83b	3.33b	33.0b	14.5c	33.5c	31.0b	56.0b	362b	21.7a	31667c	131a	1456.5a	0.16c
GB8173-87	10	75	300	500	500	500	–	–	–	–	–	–	–

All values based on dry weight. Values followed by the same letter within the same row do not differ significantly at 5% level according to the Duncan's multiple-range test. All the values are mean of three replicates.
Chinese quality standards: Environmental quality standard for soils in China (second standard, dry land, pH < 6.5) (GB15618-1995); Disposal of sludge from municipal wastewater treatment plant—quality of sludge used in gardens or parks (GB/T23486-2009); Control standards of pollutants in fly ash for agricultural use (GB8173-87).

this experiment, the fly ash-stabilized sludge application improved the soil properties.

The artificial soil contained abundant organic substance and nutrients, such as N, P and K as well as some microelements that are required for the growth of Manilagrass. However, heavy metals in the compost were a big risk due to their high concentrations. The concentration of heavy metals in soil amended with fly ash-stabilized sludge was measured and listed in Table 4. As the content of fly ash increased and sludge decreased from F0 to F50, there was no regular change pattern of the contents of heavy metals. Nevertheless, the concentration of heavy metals in all treatment was far below the second limited level (pH < 6.5) of environmental quality standard for soils in China (GB15618-1995). Therefore, the risks of amended soils for ecological and environmental pollution risk could be controlled by adding proper amount of compost.

3.2. Effects of fly ash-stabilized sludge on growth of Manilagrass

The effects of fly ash-stabilized sludge on the growth and biomass of Manilagrass were shown in Fig. 1. The highest mean leaf length and width of Manilagrass increase ratio was found in treatment F30. The Manilagrass plants cultivated in F-series treatments grew better than those in the CK treatments. The addition of a certain range (10–20%) of coal fly ash-stabilized sludge significantly stimulated the yield of Manilagrass compared with the CK. Fresh and dry weight yields of Manilagrass in F30 treatment were significantly higher than those in the other treatments.

Improvement of biomass growth with fly ash–sludge amendment could be explained by an increase in nutrients which improved soil physical properties as compared with the CK [29]. However, increasing sewage sludge amendment level of >14% in the composites caused a decrease in biomass of plant growth. On the other hand, the higher proportion (>6%) of fly-ash increased soil pH above 8.5, which would suppress the availability of nutrients for plant growth.

These results showed that the appropriate application of fly ash–sludge could increase plant growth in comparison with the CK. When the contents of sludge and fly ash in the composites were 14% and 6%, respectively, the best growth occurred in terms of leaf length and leaf width. The addition of fly ash–sludge also increased the fresh and dry weights of plant significantly. The maximum of weights appeared in the F30 treatment, while the lowest level occurred in the CK which was attributed to inadequate nutrient content in the soil. In other words, since P could enhance the growth of Manilagrass, the high P concentration of the fly ash–sludge resulted in an increase of plant weights. Additionally, N was important for leaves, thus sufficient N content of the fly ash–sludge caused the higher leaf-growth [30].

To sum up, the results indicated that the fly ash–sludge could be used as fertilizer to accelerate the growth of Manilagrass. When the application rate was 20% (fly ash and sludge were 14% and 6%, respectively), better beneficial effects could be obtained.

3.3. Effects of fly ash-stabilized sludge on nutrient of Manilagrass

The nutrient concentrations in Manilagrass were shown in Fig. 2. The fly ash–sludge affected plant nutrient significantly in Manilagrass during the cultivation experiment. Nutrient concentrations (N, P and K) in Manilagrass increased from CK to F30, while decreased from F30 to F50. Compared with CK, the increase of total N, total P and total K in Manilagrass cultivated in F30 were 63.3%, 19.0% and 11.9%, respectively. Higher absorption and translocation of N and P to Manilagrass might lead to a higher N, P concentration in Manilagrass at appropriate range content of sludge and fly ash. Increase of K levels in plant at increasing sewage sludge rates from CK to F30 clearly suggested that K absorption and

Table 3
Chemical reagents, analytical conditions and the relation among fraction of heavy metals, eco-toxicity and bioavailability of the four-stage sequential BCR extraction procedure.

Step	Fraction	Extraction procedure	Nominal target phases	Eco-toxicity	Bioavailability
F1	Exchangeable	40 mL of 0.1 mol L^{-1} CH_3COOH per 1 g of dry sample was shaken for 16 h at 22 ± 5 °C.	Water and acid-soluble	Direct toxicity	Direct effect faction
F2	Reducible	40 mL of 0.1 mol L^{-1} $NH_2OH·HCl$ (adjusted to pH of around 1.5 by adding of 2 mol L^{-1} HNO_3) was admixed with the residue and shaken for 16 h at 22 ± 5 °C	Fe/Mn oxides	Direct toxicity	Direct effect faction
F3	Oxidizable	10 mL of 30% H_2O_2 was admixed with the residue and digested at 85 °C for 1 h. Subsequently, the treatment with H_2O_2 was repeated with a new aliquot. After 50 mL of 1 mol L^{-1} CH_3COONH_4 (adjusted to pH = 2.0 with HNO_3) was admixed with the residue and shaken for 16 h at 22 ± 5 °C	Organic matter and sulfides	Potential toxicity	Potential effect fraction
F4	Residual	14 mL of concentrated HCl and 4.6 mL HNO_3 was admixed with the residue and deposition for 16 h at 22 ± 5 °C	Crystalline structures of the minerals	No toxicity	Stable fraction

translocation enhanced due to sewage sludge amendment, which has 8.6% total K content. When the proportion of sludge in the composites was more than 14%, the content of N, P and K decreased because of the hazardous substances like heavy metal, and the low proportion (<6%) of coal fly ash might be one of the causes.

3.4. Effects of fly ash-stabilized sludge on heavy metal's absorption in Manilagrass

Heavy metal accumulated in plants had been investigated because it affected the horticultural and agricultural application of fly ash–sludge. Some researchers found the significant heavy metal accumulation in plants growing in soil amended with sludge [31]. In this study, however, as shown in Table 5, the fly ash–sludge application did not significantly increase the heavy metal concentrations in Manilagrass plants, which might be caused by the high $CaCO_3$ content and CEC in the sludge.

Absorption of heavy metals by plants were not only influenced by their concentrations and chemical forms in soil and physicochemical properties of the soil, but also by plant nutrition, stage of growth, and other factors [32]. The concentrations of heavy metals in Manilagrass plants of all the treatments were given in Table 5. With the increasing proportion of coal fly ash in the composites, the concentrations of Ni, Zn, Mn, Sb and Cu in Manilagrass decreased significantly, while the concentrations of Pb, V and Ti increased. This indicated that the fly ash amendment could reduce the phytoavailability of Ni, Zn, Mn, Sb and Cu in the sludge. The concentrations of Cd, arsenic (As), Cr, cobalt (Co) and iron (Fe), however, showed little changes and fluctuations. Heavy metal accumulation in plants had been shown to damage plants by inducing crop micronutrient deficiencies and phytotoxicity [1].

3.5. Transfer and accumulation of heavy metals in tested soil–Manilagrass systems

Heavy metals can be transported by plants from belowground tissues to above tissues and then may accumulate in leaves and stems. The degree of upward translation depends on not only the species of plant and the nature of metal but also a number of environment conditions [33]. The ability of plant accumulating heavy metals can be expressed by bio-concentration factor (BCF), which is defined as the ratio of heavy metal concentration in plant to heavy metal concentration in the rhizosphere soil. The average BCF values of heavy metals in the cultivated Manilagrass plants were listed in Table 6.

Table 6 shows, obviously, that the BCFs of most heavy metals in the contrast soil (CK) were higher than those in F-series. Except for Sb, the BCFs of other heavy metals in the F-series treatments were quite low (from 0.13 to 0.77). Even in the CK treatments, the BCFs of most heavy metals were below 1.0 except for Mn, Ni, Cu and Zn. The average BCFs of heavy metals in Manilagrass plant decreased in the order of Sb (1.20) > Ni (0.67) > Zn (0.65) > Cu (0.63) > Pb (0.54) > Mn (0.52) > Co (0.31) = Cr (0.31) > Cd (0.24) > Fe (0.20) = V (0.20) > Ti (0.19) > As (0.17) for all treatments (both F-series and CK). Among these heavy metals, the BCFs of essential elements of life, such as Ni, Zn, Cu and Mn were slightly larger than

Table 4
Physico-chemical properties and heavy metal contents of soil amended with fly ash-stabilized sludge.

Treatment	pH	CEC (mmol kg^{-1})	$CaCO_3$ (%)	Moisture content (%)	OM (%)	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
F0	8.11d	34.41a	31.79a	18.19a	13.41a	0.74a	1.89a	22.56a	101.91c	394.51a
F10	8.19cd	32.61b	30.03ab	15.89b	13.18a	0.71a	1.57b	19.13b	103.65bc	358.85b
F20	8.26cd	31.85b	29.09bc	15.54bc	12.54a	0.66ab	1.25c	16.36c	104.55bc	333.76c
F30	8.43c	28.91c	28.08bc	13.52d	12.00a	0.60bc	1.01d	13.51d	106.38ab	302.35d
F40	8.67b	27.41d	26.81 cd	14.72c	11.78a	0.57bc	0.87e	11.55e	106.98ab	243.23e
F50	9.06a	26.39d	24.59d	12.78d	11.60a	0.55c	0.68f	8.39f	108.49a	216.90f

Treatment	Cd	As	Ni	Pb	Cr	Cu	Zn	Mn	Co	Fe	V	Ti	Sb
	(mg kg^{-1})												
F0	2.33ab	5.50ab	12.67b	19.50c	36.83a	25.83d	46.67d	206.33e	3.00a	41667a	92.67c	371.67d	3.67b
F10	2.50a	5.20ab	18.67a	22.83a	31.67b	40.50a	82.67a	249.17a	4.83a	41667a	80.00d	343.33e	6.50a
F20	2.50a	5.10b	11.67b	11.50e	28.33c	28.33c	55.00b	220.00d	3.33a	40833ab	93.33c	411.67c	1.67c
F30	2.33ab	5.60ab	13.17b	21.17b	29.17c	27.50cd	50.67c	243.67b	5.17a	40633b	105.00a	463.33b	4.17b
F40	2.50a	5.00b	16.00ab	17.33d	28.00c	30.83b	58.67b	248.50a	6.00a	40333b	97.50b	517.17a	0.17d
F50	2.00b	5.80a	14.67ab	21.17b	28.67c	26.83cd	50.83c	228.17c	5.17a	39167c	92.50c	465.00b	6.50a

All values based on dry weight. Values followed by the same letter within the same row do not differ significantly at 5% level according to the Duncan's multiple-range test. All the values are mean of three replicates.

Table 5
The concentration of heavy metals in Manilagrass (mg kg^{-1}).

Treatment	Cd	As	Ni	Pb	Cr	Cu	Zn	Mn	Co	Fe	V	Ti	Sb
F0	0.5d	0.3c	20.0b	12.2d	8.4ef	54.7a	68.7b	169.9b	0.9c	4620e	10.5bc	17.2c	5.2a
F10	0.4e	0.4b	18.1b	13.5d	17.5a	48.9b	68.0b	161.9c	1.1c	4750e	10.4c	17.9c	4.4b
F20	0.6c	0.5a	15.2c	19.0b	15.8b	41.1c	57.2c	154.9d	1.8a	8010b	11.4abc	20.9b	3.8c
F30	0.9a	0.4b	14.6c	21.6a	9.8de	40.7c	56.5c	147.5e	1.1c	8500a	11.6abc	21.1b	2.5d
F40	0.8b	0.3c	13.6c	21.8a	10.4 cd	39.4c	55.5c	147.1e	1.5b	5840d	12.6a	24.2a	2.3d
F50	0.4e	0.3c	13.1c	22.5a	7.9f	34.4d	54.9c	146.3e	1.0c	4640e	12.8a	24.4a	2.0d
CK	0.6c	0.5a	35.4a	17.5c	11.5c	55.7a	77.3a	213.4a	1.8a	6450c	12.0ab	21.0b	0.1e

All values based on dry weight. Values followed by the same letter within the same row do not differ significantly at 5% level according to the Duncan's multiple-range test. All the values are mean of three replicates.

those of most other metals except Sb. The BCF values indicated that heavy metals could accumulate in plant from the rhizosphere soil, but they had a certain limitation to transport to plant. The roots of Manilagrass could excrete several kinds of organic matters to accumulate and fix the heavy metals in the rhizosphere soil.

The results presented in Table 6 illustrated that the discrepancy of BCFs and the absorbency of heavy metals in Manilagrass plants were linked to variation in: (a) fly ash–sludge application in soils; (b) concentrations of available heavy metals in fly ash–sludge amended soils; (c) interferences of other physico-chemical parameters in the tested soils.

3.6. Chemical speciation transformation of heavy metals in the rhizosphere soil

The bioavailability and eco-toxicity of metals mainly depend on their speciation in sludge [34]. Heavy metals distribute in acid soluble/exchangeable fraction (F1) and reducible fraction (F2) can be readily absorbed by organisms. Hence, these two types of fractions should be identified as the direct effect fractions. The oxidizable fraction (F3), which is identified as potential effect fraction, is easily mobilized and transformed into exchangeable fraction or reducible fraction under oxidizing conditions. The tested red soil, which is the dominated type soil in Xiamen – a region located in acid rain area, is acidic and always possesses highly oxidizing property. So, the potential eco-toxicity of the oxidizable fraction should not be ignored in this region. The residual fraction (F4) of heavy metals is often considered as the "unreactive" and stable fraction, which is hardly affected by environment changes.

The speciation of heavy metals determined by BCR procedure in the composite soils and the rhizosphere soils was shown in Fig. 3, which showed the results of the chemical speciation transformation of heavy metals in the rhizosphere soils of CK and F-series before and after the cultivation experiment.

It was worth noting that the concentrations of Ti, V and Cd in exchangeable fraction, reducible fraction and oxidizable fraction were very low both in the composite soils before the experiment and the rhizosphere soils after the cultivation experiment. Ti, V and Cd were primarily present in the residual fraction (74.6–99.3%, 68.5–80.6%, 60.0–82.4%, respectively). These could indicate the low direct and potential bioavailability to environment if the fly ash–sludge was used for organic amendment in soil. Considerable part of oxidizable fraction of Ti was changed into residual fraction after the cultivation experiment, which illustrated that the planting of Manilagrass could decline the bioavailability of Ti. Part of reducible and oxidizable fractions of V was transformed into residual fraction after the cultivation. The same tendency was found for Cd except in the F0 group.

The percentages of exchangeable fraction and oxidizable fraction of Fe were very low. Especially, the exchangeable fraction was lower than 0.5%, showing the less direct toxicity to environment. 78.1–97.9% of total Fe presented in residual fraction in all experimental groups. Before cultivation, Fe had higher concentration in residual fraction which had low bioavailability and no eco-toxicity. However, the residual fraction had a little addition in rhizosphere after cultivation, and the growth of Manilagrass added an increment into the exchangeable fraction.

Mn and Co predominantly existed in residual fraction in the CK groups, while decreased significantly in the F-series groups. Compared with CK, increment of oxidizable fraction of Mn and Co was higher because of the fly ash–sludge amendment. Exchangeable fraction of Mn and Co decreased while residual fraction increased in F-series experimental groups after the cultivation of Manilagrass. Reducible and oxidizable fraction of Mn and Co changed a little.

Cr mainly distributed in oxidizable and residual fraction with 75–97% of Cr in these fractions (Fig. 3). This indicated that Cr would not be easily released in the environment during utilization of fly ash–sludge. Application of coal fly ash–sludge resulted in an increase of Cr concentration in the exchangeable and oxidizable fraction. A pot experiment carried out by Bose et al. [35] showed that upon addition of industrial waste, a maximum level of Cr was bound with Fe and Mn oxides. Due to the high affinity of Cr with organic matter, the solubility of this metal was seemed to be limited by the formation of insoluble chromium-organic complexes [6–9,36]. Consequently, application of fly ash–sludge increased the Cr concentration in the organically bound fraction and decreased the proportion of residual fraction. The cultivation of Manilagrass led to an increase of Cr concentration in the exchangeable fraction while a decrease in the oxidizable fraction.

Ni, Cu and Zn were largely present in the residual fraction in the CK. The results were in accordance with those found by other researchers [37]. However, the application of fly ash–sludge had an effect on the distribution of Ni, Cu and Zn in the tested soils. The non-residual fractions of Ni, Cu and Zn increased after the

Table 6
BCFs of heavy metals in Manilagrass in different amendments of fly ash–sludge.

Treatment	Cd	As	Ni	Pb	Cr	Cu	Zn	Mn	Co	Fe	V	Ti	Sb
F0	0.19	0.14	0.56	0.28	0.19	0.57	0.42	0.43	0.22	0.13	0.15	0.17	1.64
F10	0.17	0.17	0.43	0.44	0.38	0.47	0.42	0.41	0.15	0.14	0.15	0.13	1.39
F20	0.24	0.23	0.74	0.52	0.45	0.73	0.41	0.41	0.37	0.24	0.17	0.18	2.85
F30	0.36	0.17	0.48	0.64	0.24	0.54	0.46	0.39	0.19	0.28	0.21	0.17	0.77
F40	0.28	0.14	0.53	0.77	0.21	0.59	0.57	0.41	0.26	0.22	0.21	0.18	0.55
F50	0.17	0.13	0.55	0.75	0.19	0.42	0.43	0.48	0.24	0.14	0.15	0.11	1.20
CK	0.26	0.23	1.39	0.35	0.54	1.09	1.86	1.08	0.72	0.26	0.33	0.36	0.02

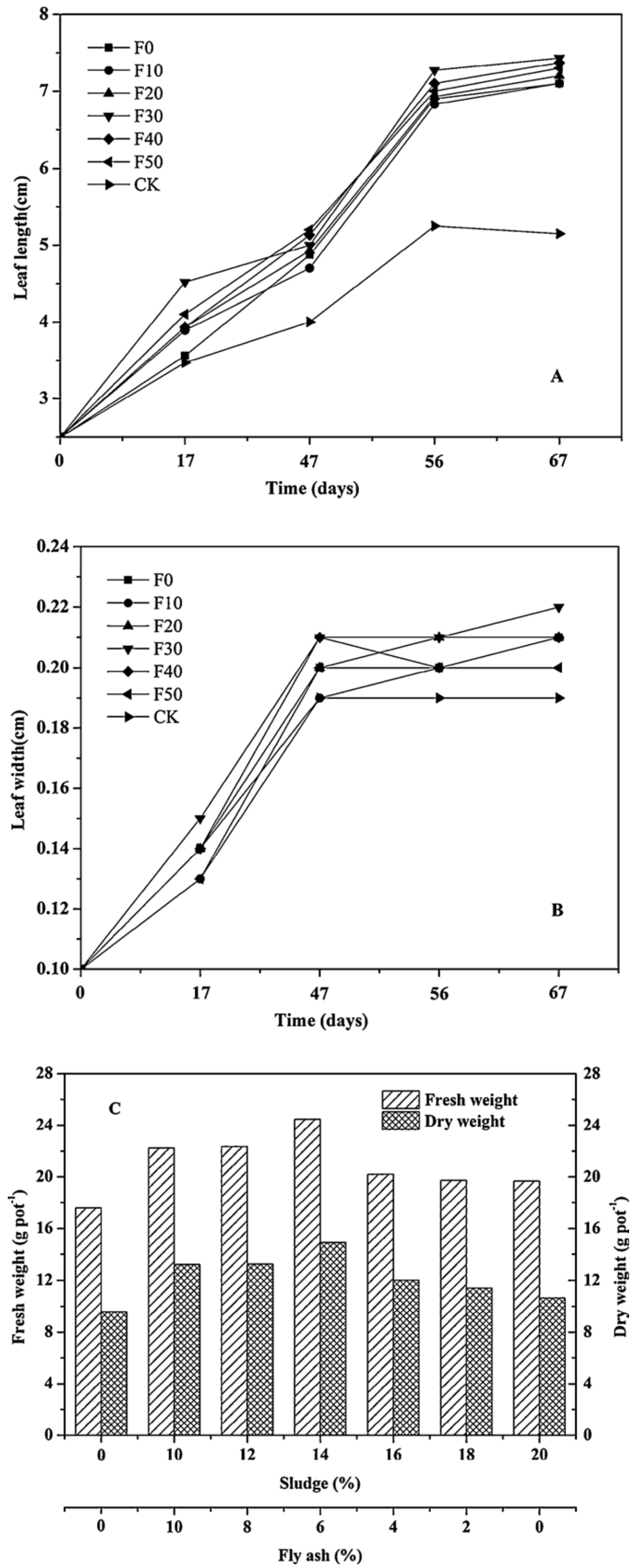

Fig. 1. Effect of sludge and fly ash content on the growth of Manilagrass (A: leaf length; B: leaf width; C: fresh and dry weight).

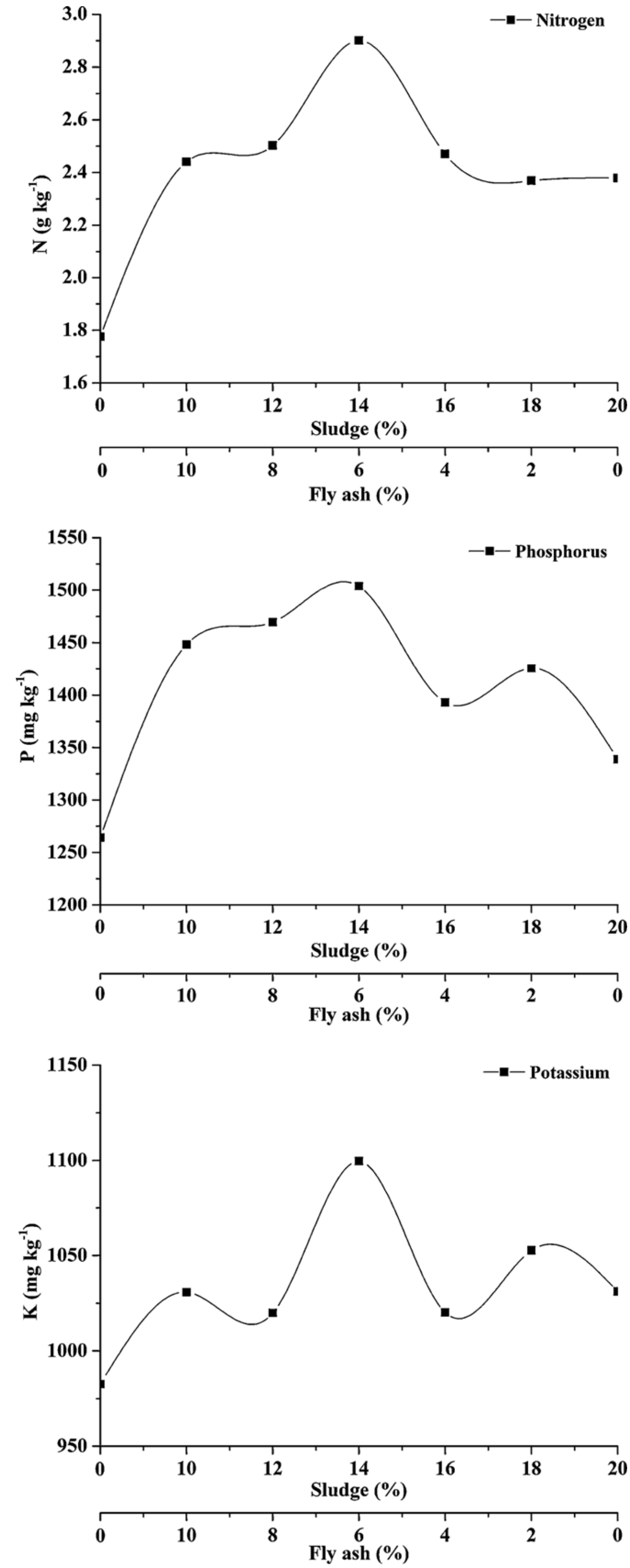

Fig. 2. The concentration of N, P and K in Manilagrass.

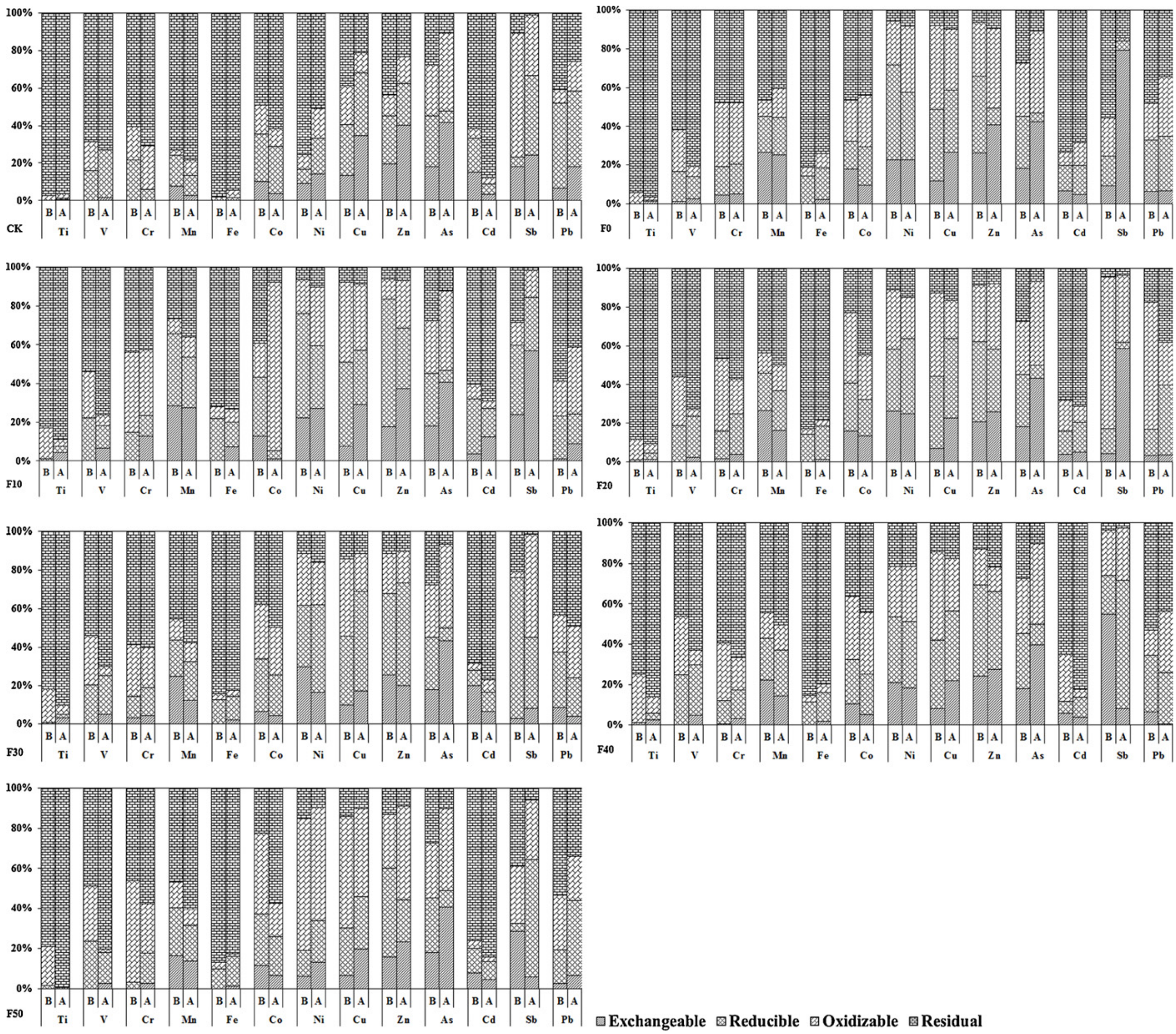

Fig. 3. Chemical speciation transformation of heavy metals in the rhizosphere soil (B = before planting and A = after plantin).

application of fly ash–sludge, while the residual fraction decreased significantly. In the F-series groups, Cu seemed to be mainly present in reducible and oxidizable fractions. The exchangeable fraction of Cu increased significantly after the cultivation of Manilagrass, while the oxidizable fraction decreased a lot.

Zn principally distributed in exchangeable fraction and reducible fraction in F-series. The cultivation trial significantly increased the exchangeable and oxidizable fractions (except F30). Planquart et al. [38] reported that the application of sewage sludge compost to three soils from France under greenhouse conditions increased the proportion of Zn bound to acid-extractable fraction. The high proportion of non-residual fractions of Ni, Cu and Zn in F-series indicated the high bioavailability and eco-toxicity to environment.

The concentrations of exchangeable, reducible, oxidizable and residual fractions of As were almost similar between the CK and the F-series. The amendment of fly ash and sewage sludge had little effect on the speciation of As. The highest increase was observed in the exchangeable and oxidizable fractions while significant reduction was found in the reducible and residual fractions after the cultivation of Manilagrass. This indicated the increase of direct ecotoxicity and potential bioavailability to environment due to the cultivation of Manilagrass.

The various experimental groups did not show a uniform pattern of distribution for Sb and Pb in the various chemical phases. Application of fly ash–sludge had almost no effect on the four forms of Sb and Pb. The only regular pattern that could be seen was the reduction of the residual fractions after the cultivation of Manilagrass.

It could be seen that the residual fraction of most heavy metals was high in many groups. Moreover, an increase in the exchangeable fraction could also be seen. This was very important bearing in mind that the exchangeable fraction was usually considered as the maximum amount which might be mobilized within the soil–plant system and, therefore, liable to pollute the agricultural or horticultural environment [39].

Since plants differed in their ability to take up, accumulate and tolerate heavy metals, selection of plant species was also important for non-agricultural application of sludge. For example, chrysanthemum could accumulate more Pb from soil amended with sludge

[40], while ryegrass could accumulate more Zn [28]. Therefore, in order to deal with metal-contaminated fly ash–sludge, the metals could be reduced by growing some ornamental plants before recycling of fly ash–sludge for agricultural or horticultural purposes. As shown in this study, Manilagrass could be used to reduce the eco-toxicity and bioavailability of Ti, V, Mn, Co, Cr and Cd in fly ash–sludge amended soil.

4. Conclusions

The present experimental results demonstrated the beneficial effects of fly ash–sludge amendment which had a certain degree of fertilizer and soil-conditioner on the growth of Manilagrass. The yield of Manilagrass cultivated in F30, which was the optimum application rate, was the highest (significantly about 39% higher than that of the CK treatment). The results suggested that an economic alternative for the disposal of fly ash and sludge through the reutilization of valuable resources for horticultural use. Nevertheless, the long-term effects of repeated applications of the ash-sludge still required further in-depth investigations on these issues.

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