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Phytoattenuation of lead-contaminated agricultural land using *Miscanthus floridulus*—an *in situ* case study

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ABSTRACT

Phytoremediation is the most environmental friendly and economical technology for restoring agricultural land contaminated by heavy metals. However, it is a slow process, requiring hundreds to thousands of years to reduce pollutant level to meet soil environmental quality standards for highly contaminated soils. Such a long period makes the practice of phytoremediation nearly impossible without economic revenue from crop production. *Miscanthus floridulus* (*M. floridulus*) is characterized by its high dry matter yield and strong vitality. This case study planted *M. floridulus* on fallow land that was contaminated by 6,000 mg/kg of lead to investigate the feasibility of phytoremediation. The case study results show that lead accumulated primarily in the roots of the *M. floridulus*. After one year of growing, the average lead content in the roots and shoots was 806.7 and 50.3 mg/kg, respectively. *M. floridulus* was effective for the phytostabilization of lead-contaminated soil and was a lead-tolerant plant. The cropping produced 22.4 ton/ha/year dry matter weight (shoot part) and removed 1.13 kg/ha/year of lead from the soil. The *M. floridulus* grown on contaminated fields could be used as biofuels, and each hectare of *M. floridulus* dry matter could generate 365.1 GJ of thermal energy per year, which is equivalent to the heat energy from combustion of 13.4 tons of hard coal. Furthermore, replacing hard coal with *M. floridulus* would reduce CO_2 emissions by 33.1 ton/ha per year. The yields, Pb-absorption concentrations, and carbon mitigation of *M. floridulus* may change in subsequent years for different environmental conditions; thus, it needs further planting trials for regionalization.

Keywords: Energy crop; Lead; Phytoremediation; Bioenergy

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1. Introduction

Because lead has low mobility in soil, remediation techniques such as acid extraction and electrokinetics can be employed to restore the soil by using chemicals with low pH levels or by using chelating agents. However, these chemicals can cause considerable damage to agricultural soil; thus, effective restoration procedures are needed to restore the soil quality for agricultural productivity. Moreover, because the cost of physical and chemical remediation is high, remediating large areas of contaminated agricultural land may be economically unfeasible. Phytoremediation is a low-cost method that impairs neither the physical nor chemical properties of soil. The technique is applicable for remediating large areas of agricultural land contamination and it can be employed to maintain the soil quality, and has been commended as the most environmentally friendly and economically feasible soil remediation technique [1–3]. The mechanisms involved in phytoremediation include phytoextraction, phytostabilization, and phytostimulation. Currently, phytoremediation techniques for removing heavy metal pollutants typically involve the mechanism of phytoextraction. Specifically, the uptake of heavy metals by plants occurs through metabolic processes during plant growth. Thus, the heavy metals are removed when the plants are harvested.

The bioconcentration factor (BCF) and translocation factor (TF) are used to evaluate the potential heavy metal accumulation by plants [4]. A hyperaccumulator is a plant with BCF and TF values greater than 1 or with more than 1,000 mg/kg Pb content in its biomass [5,6]. Even with hyperaccumulators, the rate of phytoextraction is low. For soils contaminated with high concentrations of heavy metals, phytoextraction can take up to hundred or even thousands of years to reduce the heavy metal concentrations to regulatory standards [6,7]. Thus, phytoremediation is difficult to implement.

Numerous researchers have argued that phytoremediation is only sustainable and feasible when the planted crops can generate stable economic benefits. Meers et al. [2] proposed the concept of phytoattenuation, asserting that the ability for plants to uptake heavy metals should not be the primary focus of research and development in the field of phytoremediation. Rather, through appropriate agronomic management, crops that meet health and safety standards can be planted to generate economic benefits at an acceptable level of risk. Thus, through agricultural production, pollution levels can be reduced and sustainable phytoremediation practices can be realized.

Given the scarcity of fossil fuels, a crucial development direction for energy and environmental conservation is to replace fossil fuels with bioenergy to reduce carbon dioxide (CO_2) emissions and help meet targets for mitigating climate change [8,9]. Energy crops are feasible for phytoremediation if they can grow productively in contaminated soil [1–3]. *Miscanthus* is a genus of C4 perennial grasses that originates from East Asia and it is characterized by its ability to grow in adverse weather condition, resist against insects and diseases, and strong vitality. *Miscanthus* can grow in various harsh environments, including the shore areas of water bodies or in mountainous regions, as well as in arid areas, saline coastal, snow-prone, and heavy metal-contaminated soil [10–13]. According to Jiang [10], *Miscanthus floridulus* that was grown in the lead-contaminated areas exhibited high tolerance to lead because of the high activity of superoxide dismutase in the plant. *M. floridulus* exhibits high photosynthetic efficiency, carbon sequestration capacity, and dry biomass yield [14,15]. Qin et al. [16] estimated the annual net primary production of food crops and biofuel crops of this species. The results showed that the annual net primary production yield of *Miscanthus* more than double that of switchgrass and food crops, and almost equals to that of productive sugarcane. Lewandowski et al. [17] substituted coal energy with biomass energy derived from *Miscanthus* and successfully generated power. The thermal energy yielded from burning of 20 tons of dry *Miscanthus* was equal to that from burning 12 tons of hard coal. In addition, CO_2 emission was only 10% of that emitted through hard coal burning. These results indicate that *Miscanthus* is advantageous because of its high biomass energy potential and low carbon emissions [18,19]. Currently, numerous studies have developed low-cost high-efficiency technologies that can be used to convert *Miscanthus* into bioethanol or to produce bio-oil through the pyrolysis of *Miscanthus* [8,9,14,20–24]. Accordingly, *Miscanthus* has been considered as a novel biomass energy crop that has a high potential for development [13,25].

In the Niuyunjue region of Nantou City, Taiwan, the soil is of a silt loam texture, which is suitable for producing ceramic products. During the past 200 years, people in this area have used local soil materials for manufacturing ceramic products. Ceramic glazing waste, confounded by improper disposal of waste and wastewater, has resulted in lead contamination in many patches of agricultural land, with maximal concentrations of 6,000 mg/kg. Many technologies, such as dilution method, electrokinetic treatment, and acid washing, were studied and tried to remove heavy metals; however, these methods did not yield excellent removal efficiency. Soil solidification for soil remediation is expensive in cost outlay and loss of use of the land. To avoid harm to consumers, these lands were in fallow over the past 10 years. For recovery and sustainable use of this contaminated land, present study evaluated the biofuel yield and lead contamination attenuation effects of planting *M. floridulus* on-site.

2. Material and methods

2.1. Site location and soil properties

The study site is located in the Niuyunjue region of Nantou City, Taiwan (23˚ 54´ 59.17224´´ N, 120˚ 41´ 12.92383´´ E). About 10 years ago, this study site was investigated for lead contamination. Before the discovery of Pb contamination, the site had been for planting rice. For the past 10 years, it has been in fallow, growing weeds. This study conducted *M. floridulus* planting test on an area of 30 m^2. Before the planting test, 30 soil samples were collected from 1 × 1 m grid at a depth of 0–30 cm. After the soils were dried, a 2-mm sieve was used to remove impurities. X-ray fluorescence (XRF, Innov-X Systems Inc., α-4000) was used to measure the lead content to determine the distribution of lead content. From these 30 samples, 10 samples were randomly selected for the determination of lead concentration by the aqua regia extraction method. Specifically, 3.0 g of dry soil from a sample was extracted by using 21 mL of concentrated hydrochloric acid and 7 mL of concentrated nitric acid. The lead content was determined by atomic absorption spectroscopy (Perkin Elmer, AA400). In accordance with Tessier et al. [26] and the European Union Community Bureau of Reference extraction method [27], the lead content in soil was divided into five chemical bond fractions: exchangeable, bound to carbonates, bound to iron and manganese oxides, bound to organic matter, and residual. Lead of the exchangeable and bound to carbonate fractions belongs to the phytoavailable parts. The exchangeable fraction was extracted using 1.0 M $MgCl_2$, and carbonates bound fraction was extracted using 1.0 M NaOAc. Lead bound to iron and manganese oxides was extracted using 0.04 M $NH_2OH{\cdot}HCl$. Lead bound to organic matter was extracted using 0.02 M HNO_3 and 30% H_2O_2 mixture. The residual fraction was extracted using the aqua regia method. The soil samples were analyzed for soil texture, pH, cation-exchange capacity (CEC), and organic matter content. The soil texture was determined by combining the pipette and sieving methods. A pH meter was used to measure the pH value of the mixture with 20-g soil samples and 20 mL of deionized water. CEC was measured by sodium acetate method, using 33 mL of sodium acetate (pH 8.2, 1.0 M) combined with 4 g of dried soil for ion exchange reaction. Subsequently, 33 mL of ammonium acetate (pH 7.0, 1.0 M) was used to replace the absorbed sodium ion. The sodium ion concentration was measured using an atomic absorption spectrometer (Perkin Elmer, AA400) to calculate the CEC value. The organic matter content was measured using the dry combustion method at 540℃ [28].

2.2. Method for planting M. floridulus

Miscanthus species are widely planted in Taiwan, and there are five species being considered as native. Among the five Taiwan native species, *M. floridulus* has the highest biomass yield, and widely spread from low to high elevation land [18]. Therefore, this study selected the species of Taiwanese native *M. floridulus* from Chia-Yi Agriculture Experiment Station in Taiwan for the *in situ* experiment that started from July, 2012. *M. floridulus* originally planted in the pots. The diameter of each pot was about 10 cm; the heights of *M. floridulus* were about 80 cm. The roots of *M. floridulus* were collected and planted directly into soil at a density of one pot per square meter. During experiment period, this study performed no field management in irrigation and fertilization to simulate the natural growing conditions.

2.3. Plant sampling and analytical methods

The heights of *M. floridulus* were measured every 3 months. Five *M. floridulus* plants were selected randomly to measure the biomass and lead content every 3 months during the growing period. The shoots were washed with tap water and deionized water. The roots were rinsed and brushed under running tap water until no soil traces were detected by naked eyes, and then washed three times using deionized water. Before drying, plant parts were measured for their fresh weight. Then, the plant parts were exposed to the sun for 3 d and dried in an oven at 105℃ until a constant weight was obtained. Finally, the dry weights of the plant parts were measured to calculate the water content. In lead content analysis, each plant part was subject to three-sample replication analysis. For 0.5 g of dried plant part, 21 mL of concentrated hydrochloric acid and 7 mL of concentrated nitric acid were added to conduct extractions, and an atomic absorption spectrophotometer (Perkin Elmer, AA400) was applied to perform quantitative analysis. To measure the combustion heats for shoot part of *M. floridulus* plant, 1.0 g dry biomass was used in a bomb calorimeter (Isoperibol Bomb Calorimeter, Parr 1356).

3. Results and discussion

3.1. Properties of the Pb-contaminated soil site

Before planting trials, a soil sample was taken from every 1 m^2 of the experimental area for a total of 30 samples. The Pb content of each sample was measured using an XRF analyzer to assess the lead content distribution at the site. The lead concentration ranged from 6,220 to 6,870 mg/kg; the mean concentration was 6,630 ± 434 mg/kg. These data show, within planting plot, Pb concentration varies within 10%, indicating rather even distribution among the soil samples. Aqua regia was used to extract Pb from 10 soil samples and the results demonstrated that the average Pb concentration in the soil samples was 5,844.2 ± 433.9 mg/kg. The correlation coefficient between aqua regia data and XRF analysis results was 0.88, which was a respectable value, indicating the agreement between the results of these measurement methods.

Table 1 shows the soil properties of the site. Regarding to soil composition, silt particles accounted for the highest proportion of soil (68%), followed by sand (26%). According to the United States Department of Agriculture texture triangle [29], the soil texture of the study site was silt loam. The pH value of the site soil was 6.35. The CEC and the organic matter content of the soil were 41.2 cmol kg^{-1} and 5.47%, respectively.

In terms of lead bonding fractions in the contaminated soil, the iron–manganese oxides bond fraction accounted for 38.3%, followed by the organic bond fraction (24.5%), carbonate bond fraction (19.6%), exchangeable fraction (9.0%), and residual fraction (8.6%). According to sequence extraction theory [26], exchangeable and carbonate bond fractions are phytoavailable, accounting for approximately 28.6% of the total lead content. The mean lead content of the site is 5,844.2 mg/kg (if the soil density and contaminated soil depth were 1.3 ton/m^3 and 0.3 m, the Pb content is 22,792 kg/ha), of which approximately 1,671 mg/kg (6,518 kg/ha) is phytoavailable.

Table 1
Characteristics of soils in Pb-contaminated site

Properties	Contaminated	Unit
Soil texture	Silt loam	–
Sand	26 ± 2	%
Silt	68 ± 2	%
Clay	6 ± 1	%
pH	6.35 ± 0.21	–
CEC	41.2 ± 1.6	cmol/kg
OM	5.47 ± 0.21	%
Pb conc.	5,844.2 ± 433.9	mg/kg

Notes: Sample size *n* = 3 for soil texture, pH, CEC, and OM analysis; *n* = 10 for soil Pb concentration analysis; average ± standard deviation.

3.2. Biomass yields of M. floridulus

Fig. 1 shows the variations in height and shoot fresh biomass yield of the *M. floridulus* planted in Pb-contaminated site. The average heights of the plants at 3, 6, 9, and 12 months were 105, 191, 249, and 286 cm; the average fresh biomass yields of the shoot part of *M. floridulus* plants were 0.5, 1.4, 3.2, and 5.4 kg/m^2, respectively.

Huang et al. [13] planted *M. floridulus* in an uncontaminated area located at an altitude of 260 m in Taiwan, with one experiment spanned a 7-month period from March 2009 to October 2009 and the other from April 2010 to November 2010. The average heights in the two experiments were 230 and 160 cm, and the fresh biomass yields were 66.4 and 33.38 ton/ha, respectively. In this study, the average height of the grasses was 191 cm at 6 months, which was within the range of the height of *M. floridulus* measured by Huang et al. [13] at 7 months after plantation. This indicates that the presence of lead had little significant influences on the height of the *M. floridulus*. The fresh yields of the shoot part of *M. floridulus* at 6, 9, and 12 months were 14, 32, and 54 ton/ha, respectively. Compared with the fresh yields at 7 months reported by Huang et al. [13], the fresh yields were slightly less at 6 and 9 months in this study. However, the experiments by Huang et al. [13] were conducted from March to November, which

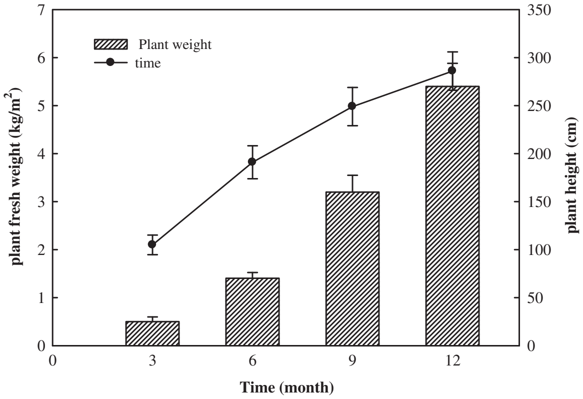

Fig. 1. The variations in height and shoot fresh weight of *M. floridulus* planted in Pb-contaminated site.

is the optimal growth period for *M. floridulus.* In contrast, the experiment in this study began in July and lasted over a winter, a period less optimal for *M. floridulus* to grow, and the *M. floridulus* grew slowly. The annual fresh yield in this study was 54 ton/ha, comparable to that of Huang et al. [13]. The average moisture content of the *M. floridulus* measured in this study was 58.5%, which would yield 22.4 tons of dry biomass per hectare. Lewandowski et al. [11] reported that the annual dry matter yield of *Miscanthus* without irrigation was approximately 10–25 ton/ha. This suggests that the yield of *M. floridulus* planted in the lead-contaminated field in this study reached the level of yield in the uncontaminated soil. The yields of *M. floridulus* may change in subsequent years for different environmental conditions; thus, it needs further planting trials for regionalization.

3.3. *Lead uptake by* M. floridulus

Fig. 2 shows lead content in roots and shoots of the *M. floridulus* based on the dry weight through the growth time. At 3, 6, 9, and 12 months, the lead contents in shoot part were 94.5, 78.6, 56.5, and 50.3 mg/kg, respectively, indicating that the lead content in the *M. floridulus* decreased over time. The lead contents in root show little changes along growing time, with an average of 806.7 mg/kg.

The BCF and TF values of lead were shown in Table 2. For the evaluation of BCF, the concentration of lead in the soil was set equal to the concentration of phytoavailable lead (i.e. 1,671 mg/kg). At 12 months, the BCF of lead in the roots and shoot of the *M. floridulus* was 0.483 and 0.031, respectively. The TF of lead in the shoot part of the *M. floridulus* was 0.062.

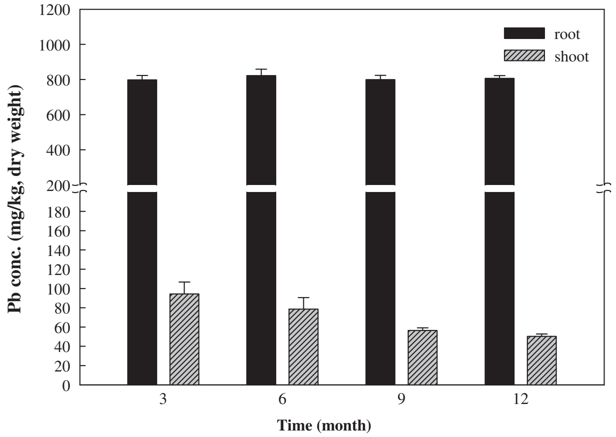

Fig. 2. Lead contents (dry matter) in root and shoot parts of *M. floridulus* by growing time.

Table 2
BCF and TF values of lead in *M. floridulus* at various planting times

	BCF value		TF value
Time (mon)	Root	Shoot	Shoot
3	0.478	0.058	0.118
6	0.492	0.049	0.096
9	0.478	0.035	0.071
12	0.483	0.031	0.062

Notes: BCF (bioconcentration factor) = plant Pb concentration/soil phytoavailable-Pb concentration; TF (translocation factor) = shoot Pb concentration/root Pb concentration.

Because the BCF and TF were both smaller than 1.0 and the Pb concentration in the shoot part was less than 1,000 mg/kg, it indicated that *M. floridulus* was not a hyperaccumulator for lead. Additionally, the TF values less than 1.0 indicate that roots act as a barrier for the translocation of metals and protect the aerial parts of the plant from toxic heavy metal contamination, thus indicating that *M. floridulus* is a heavy metal-tolerant species. The roots of *M. floridulus* can exert the effect of phytostabilization of lead [6,30]. The Pb-absorption concentrations of *M. floridulus* may change in subsequent years for varying environmental conditions.

3.4. *Benefit assessment of planting* M. floridulus *in a fallow, lead-contaminated site*

The benefit of *M. floridulus* planting is estimated as shown in Table 3. The potential dry matter yield of *M. floridulus* was 22.4 ton/ha per year. The average heat of combustion yielded from the dry *M. floridulus* was 16.3 kJ/g. The annual yield of bioenergy produced by *M. floridulus* was approximately 365.1 GJ/ha, which is equivalent to the thermal energy produced by 13.4 tons of hard coal with a heating value of 6,500 kcal/kg. In addition, with Pb content of 50.3 mg/kg in dry yield, annual harvest of 22.4 ton, *M. floridulus* can remove 1.13 kg of lead per hectare per year. Moreover, cultivating *M. floridulus* can provide other environmental benefits, such as reducing CO_2 emissions. According to Lewandowski et al. [17], CO_2 emissions by coal production and combustion is 96.6 kg/GJ; by comparison, the amount of CO_2 emissions by *Miscanthus* production and combustion is only 6.1 kg/GJ. The experimental results of this study indicate that 22.4 ton/ha of dry *M. floridulus* can be produced per year, which could generate 365.1 GJ of thermal energy and only 2.2 tons of CO_2 emissions. By comparison, generating the same amount of thermal

Table 3
Benefit assessment of planting *M. floridulus* in lead-contaminated soil

Item	Fresh yield	Dry yield	Pb content	Pb removal	Unit heat value	Bioenergy yield	Biofuel CO_2 emission
Value	54.0	22.4	50.3	1.13	16.3	365.1	2.2
Unit	ton/ha/year	ton/ha/year	mg/kg	kg/ha/year	kJ/g	GJ/ha/year	ton/ha/year

Notes: Pb removal = dry yield × Pb content; unit heat value was the actual measured results in this study; bioenergy yield = unit heat value × dry yield.

energy by burning hard coal would produce 35.3 tons of CO_2. Thus, substituting hard coal with *M. floridulus* could reduce CO_2 emissions greatly.

Because of lead contamination, the experiment site in this study has been in fallow for a decade. During the fallow period, various remediation techniques including acid extraction, electrokinetics, and overturn dilution were studied. These methods are inefficient, costly, and failed to meet the goal of remediation. Excavation combined with soil solidification can achieve remediation goals, but the method does not result in the restoration of sustainable agriculture use. Fallow strategy is incapable to restore land productivity or reduce lead contamination. However, *M. floridulus* has strong vitality and requires little management; after planting, the aerial parts of the *M. floridulus* need to be harvested once a year. In the Pb-contamination farmland, planting *M. floridulus* alternative fallow is feasibility.

4. Conclusion

In cases where heavy metal-contaminated soil cannot be remediated through cost-effective approaches, a fallow strategy is typically applied. However, during the fallow period, the land provides no productive benefits and the contamination level does not reduce. *M. floridulus* is a sturdy perennial grass, full of bioenergy potential. This case study planted *M. floridulus* in a lead-contaminated field and observed the roots of the *M. floridulus* acted as a translocation barrier, accumulating lead contamination in roots, and preventing high concentration of lead in the shoot part. *M. floridulus* is a lead-tolerant species. In a contaminated site where the lead content was 6,000 mg/kg, no land management efforts (e.g. irrigation or fertilization) were required in *M. floridulus* planting. The annual dry matter yield was 22.4 ton/ha, which can generate 365.1 GJ of thermal energy from combustion and remove 1.13 kg of lead from the soil. Although *M. floridulus* cannot rapidly and effectively remove lead from the soil, it functions well in the phytoattenuation and phytostabilization of lead with biomass for bioenergy use. Furthermore, the biomass of *M. floridulus* is less CO_2 polluting in comparison with hard coal. This study provides experimental data to support that planting *M. floridulus* in fallowed Pb-contaminated land can reactivate the fallow land for bioenergy production while achieving the phytoattenuation and phytostabilization of heavy metals. The yields and Pb uptake of *M. floridulus* may change in subsequent years due to differences in environmental conditions. The speciation, planting procedure, and analytics of this study are commendable for future and other studies pertaining to lead-contaminated agricultural land.

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