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Research article

Phytostabilization of arsenic and manganese in mine tailings using *Pennisetum purpureum* cv. Mott supplemented with cow manure and acacia wood-derived biochar

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ABSTRACT

The purpose of this research was to investigate the effects of cow manure and acacia wood-derived biochar on the immobilization of arsenic (As) and manganese (Mn) in contaminated mine tailings using Mott dwarf Napier grass (*Pennisetum purpureum* cv. Mott). Cow manure or acacia wood-derived biochar was separately mixed with mine tailings at rates of 1, 3, and 5% (w/w). Samples of mine tailings and plants were collected every 30 d during the 120-d period. The total As and Mn accumulation amounts in the plants were analyzed in both the underground (roots) and aboveground (stems and leaves) parts of the plants. The results revealed that cow manure and acacia wood-derived biochar can reduce the mobilization of As and Mn in mine tailings and thus reduce their uptake and accumulation in *P. purpureum*. Acacia wood-derived biochar was able to stabilize and immobilize As and Mn in mine tailings, allowing the metals to be taken up for plant utilization despite the lower plant growth (biomass and relative growth rates) than that obtained with added cow manure. The accumulation amounts of As in the aboveground and underground parts of *P. purpureum* grown in mine tailings with 5% BC application were 0.52 ± 0.05 mg kg^{-1} and 1.57 ± 0.1 mg kg^{-1}, respectively, while the accumulation amounts of As in the aboveground and underground parts were 31 ± 1.08 mg kg^{-1} and 73.05 ± 2.60 mg kg^{-1}, respectively. In other words, the percentage reductions in As and Mn uptake and accumulation in the aboveground and underground parts were 78.6% and 63.9% for As and 72.5% and 69.3% for Mn, respectively. The results of this study can be applied for the remediation of heavy metal-contaminated areas, especially gold mines and surrounding areas, as well as in other areas.

1. Introduction

The development of economic status and industries through various human activities often creates problems from heavy metal contamination in many environmental media, such as air, soil, sediment, surface water, and groundwater. As a result of metal contamination, both direct and indirect effects on humans, plants, and animals have been found. Mining activities are a particularly important source of heavy metal contamination causing negative impacts on the surrounding environment. For example, processes in gold mines often produce waste in various forms, including metallurgical wastes or tailings, manufacturing wastewater, and mining ores. The mobilization of several heavy metals that are formed naturally in the mineralized areas of mines might be exacerbated through washout by wet deposition and leakage from mine tailings ponds. As a consequence, contamination by several heavy metals, such as arsenic (As), manganese (Mn), lead, and cadmium, has been observed in mine areas as well as adjacent areas (Sevilla-Perea et al., 2016). This would also lead to improper use of contaminated agricultural areas as well as heavy metal contamination in agricultural products from the areas surrounding the mines.

Humans may be exposed to these heavy metals via breathing, eating, drinking, and skin contact (Jaishankar et al., 2014). After exposure, heavy metals can cause various human health impacts; for example, prolonged As exposure can cause lung, bladder, and skin cancers, genetic mutations of the fetus and death (70–80 mg of As), and prolonged Mn exposure may affect the nervous system and lead to behavioral changes

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such as slow movements, spasms, and slurred speech; these symptoms can resemble Parkinsonism in severe stages (Pfeiffer et al., 2012).

Currently, several alternative technologies are used to treat environmental heavy metal/metalloid contamination, and these are comprised of chemical/physical treatments and biological treatments, especially the use of green plants, or phytoremediation. This technology is based upon the ability of plants to absorb and store contaminants to treat areas contaminated with heavy metals, thus reducing the distribution and toxicity of these pollutants in the environment and reducing their toxicity towards the environment and humans. Phytoremediation, in particular, is a treatment technology with uncomplicated treatment steps and relatively low treatment costs. This technique relies on the natural ability of plants to uptake contaminants, including heavy metals and other pollutants, from the environment. Phytostabilization is one of the phytoremediation processes that can be used to immobilize pollutants in soil, sediments, surface water, and groundwater, as well as contaminated mine tailings that are stored in tailing storage facilities (TSFs). In the phytostabilization process, pollutants are stabilized and absorbed at the root zone of plants, and thus, the uptake and translocation of the pollutants to the stems and leaves are retarded. In addition, pollutants can be changed into more stable and precipitated forms with the addition of certain substances (Bolan et al., 2011).

This study was conducted to evaluate the potential application of cow manure and acacia wood-derived biochar to immobilize As and Mn and inhibit translocation to plants. Cow manure was selected because it is composed of organic matter (OM), a material with a high ion exchange capacity, which not only favors heavy metal adsorption but also improves soil fertility (Li et al., 2017; He et al., 2016). Biochar is a carbon-rich material with a high ion exchange capacity, porosity, surface area, and alkalinity (Pituya et al., 2017).

The plant used in this study was Mott dwarf Napier grass (*Pennisetum purpureum* cv. Mott), which is a fast-growing plant that is durable against environmental conditions. Although it is classified as a forage plant, it is also an energy crop with a high calorific value, allowing it to be burned directly or to be used to produce biogas for electricity production, resulting in a lower environmental impact than direct fuel combustion (Ana et al., 2017; Deshmukh et al., 2015). When comparing biogas production from *P. purpureum* to that from other raw materials, such as palm oil and cassava, *P. purpureum* was reported to have lower production costs and provide products throughout the entire year (Sawasdee and Pisutpaisal, 2014; Ibrahim, 2019).

Thus, this study was conducted to determine the suitable and efficient ratios of cow manure and acacia wood-derived biochar to immobilize As and Mn in contaminated mine tailings when coupled with phytoremediation using *P. purpureum*. It is expected that the results obtained from this study will be useful and can be applied to immobilize heavy metals, especially As and Mn, in other heavy metal-contaminated areas, including gold mines and surrounding areas, as well as TSFs.

2. Materials and methods

2.1. Plant preparation

The *P. purpureum* cv. Mott used in the study, was obtained from the Animal Nutrition Research and Development Center, Pak Chong District, Nakhon Ratchasima. Three plants were randomly cut and used to analyze the accumulation of total As and Mn in the plants prior to planting in the mine tailings. Sample preparation and extraction of total As and Mn using a microwave digestion system were conducted following USEPA method 3052 (USEPA, 1996). The total concentrations of As and Mn were measured using inductively coupled plasma-optical emission spectrometry (ICP–OES; model PQ 9000 Elite, Germany).

2.2. Biochar preparation

Biochar produced from acacia wood was obtained from the Huai Sai Royal Development and Study Centre, Cha-am District, Phetchaburi. The steps of biochar preparation included cutting the acacia wood stems (*Leucocephala glauca* Benth.) into several even segments, then pyrolyzing them under anaerobic conditions at 450 °C for 2 h, leaving the products at room temperature and crushing them into 0.5–1 mm sized particles. Prior to use, the basic properties of the biochar, including the pH, cation exchange capacity (CEC), Brunauer-Emmett-Teller (BET) surface area, pore size, pore volume, nutrient content as total elemental nitrogen (N), phosphorous (P), and potassium (K), total organic carbon (TOC), and total As and Mn concentrations were analyzed.

2.3. Cow manure preparation

The cow manure used in this study was obtained from the Huai Sai Royal Development and Study Centre, Cha-am District, Phetchaburi. Prior to use, the manure was heated at 105 °C for 24–48 h, cooled and crushed. The basic properties, including pH, CEC, OM, nutrient content (N, P, and K), and total As and Mn concentrations, were also analyzed.

2.4. Mine tailings preparation

As- and Mn-contaminated mine tailings were collected from a gold mine TSF at a depth of 0–30 cm from the surface layer. Prior to use, preliminary analyses of the characteristics of the mine tailings, including the pH, CEC, soil texture, particle size, nutrient content (N, P, and K), OM, and total As and Mn concentrations, were performed. The mine tailings were then mixed with the specified amount (0, 1, 3 or 5% by weight) of biochar or cow manure before being transferred into pots at a rate of approximately 5 kg per pot.

2.5. Experimental design

The experiment was separated into three groups. The first group was the control group, in which only *P. purpureum* was grown in the mine tailings with no added cow manure or biochar. The second group was as per the control but with the application of 1, 3, or 5% (w/w) cow manure (CM) to the mine tailings. The third group was as per the control but with the application of 1, 3, or 5% (w/w) biochar (BC) to the mine tailings.

2.6. Growth data collection

Biomass growth was recorded as the fresh (wet) weight (FW) and dry weight (DW) of *P. purpureum* after 30, 60, 90, and 120 d of cultivation. The relative growth rate (RGR) was calculated following Eq. (1) (Hunt et al., 2002);

$$\mathrm{RGR} = [\mathrm{Ln}(W_2) - \mathrm{Ln}(W_1)]/(t_2\text{-}t_1) \tag{1}$$

where RGR is in units of g/d, t_1 is the pre-experimentation time (d), t_2 is the postexperimentation time (d), W_1 and W_2 are the plant DW (g) at t_1 and t_2, respectively, and Ln is the natural logarithm.

2.7. Phytotoxicity

The phytotoxicity to *P. purpureum* grown in the mine tailings was observed by measuring the RGR and the chlorophyll content in the plant leaves after extraction with acetone and measuring the absorption values at 646.8 nm ($A_{646.8}$) and 663.2 nm ($A_{663.2}$) with a UV-visible spectrophotometer as previously reported (Wellburn, 1994). The levels of chlorophyll a and b and total chlorophyll (mg g^{-1} FW) were calculated following Eqs. (2), (3), and (4):

$$\text{Chlorophyll a (Chl a)} = 12.25\ A_{663.2} – 2.79\ A_{646.8} \tag{2}$$

Table 1. Physical and chemical properties of the mine tailings, manure and biochar.

Parameters (units)	Materials		
	Mine tailings	Manure	Biochar
pH	7.68 ± 0.08	8.65 ± 0.05	9.13 ± 0.11
EC (mS cm^{-1})	1.65 ± 0.02	2.02 ± 0.04	0.68 ± 0.01
ORP (mV)	271 ± 3.4	186.43 ± 2.0	269.53 ± 2.9
CEC (cmol kg^{-1})	3 ± 0.09	36.9 ± 1.12	14.54 ± 0.18
OM (%w/w)	1.58 ± 0.05	28.8 ± 0.13	16.84 ± 0.15
TOC (%w/w)	0.92 ± 0.01	16.71 ± 0.14	9.77 ± 0.07
N (mg kg^{-1})	0.029 ± 0.005	1.2 ± 0.06	0.8 ± 0.02
P (mg kg^{-1})	35.1 ± 1.44	0.4 ± 0.03	0.13 ± 0.01
K (mg kg^{-1})	102 ± 3.25	1.34 ± 0.06	0.97 ± 0.05
As (mg kg^{-1})	68 ± 2.65	-	-
Mn (mg kg^{-1})	958.33 ± 9.58	0.29 ± 0.02	-
BET surface area (m^2 g^{-1})	-	-	90.51 ± 1.28
Pore size (nm)	-	-	2.56 ± 0.62
t-Plot micropore volume (cm^3 g^{-1})	-	-	0.0320 ± 0.003
Total pore volume (cm^3 g^{-1})	-	-	0.0357 ± 0.008

$$\text{Chlorophyll b (Chl b)} = 12.25\ A_{646.8} – 5.1\ A_{663.2} \quad (3)$$

$$\text{Total chlorophyll} = \text{Chl a} + \text{Chl b} \quad (4)$$

2.8. Sample collection and analysis

Plant samples and contaminated mine tailings were randomly collected every 30 d during the 120-d cultivation period. Collected plants were separated into aboveground (stems and leaves) and underground (roots) parts and analyzed separately. Mine tailings were collected at the same time as plant sample collection and were separated into two parts. The first part was dried at room temperature and analyzed for the pH, oxidation reduction potential (ORP), and electrical conductivity (EC) values. The second part of the mine tailings was heated at 105 °C for 24–48 h, crushed, and sieved to ≤2 mm sized particles for analyses of total As and Mn concentrations.

Plant samples were analyzed following USEPA method 3052 (USEPA, 1996), while the contaminated mine tailings were analyzed following USEPA method 3051A (USEPA, 2007). The As and Mn concentrations were then measured using ICP–OES.

2.9. Statistical data analysis

Numerical data are presented as the mean ± one standard deviation (SD). Analysis of the variation in the uptake and accumulation of As and Mn in *P. purpureum* grown in contaminated mine tailings was evaluated using ANOVA. The differences in means were compared using Duncan's new multiple range test (DMRT). $p < 0.05$ was used to identify significance. All analyses were performed using the software Statistical Package for the Social Sciences (SPSS).

3. Results and discussion

3.1. Properties of mine tailings, manure, and biochar

3.1.1. Physical and chemical properties of the mine tailings, manure, and biochar

The analyses of both the physical and chemical properties of the mine tailings revealed that the tailings were a sandy loam solid material containing 68 mg kg^{-1} As and 958.33 mg kg^{-1} Mn with a pH of 7.68, a CEC of 3 cmol kg^{-1}, and 1.58% (w/w) OM. The biochar had a higher pH, CEC and OM content than the mine tailings, while the manure had a higher OM and CEC than the biochar. The physical and chemical properties of the mine tailings, manure, and biochar are summarized in Table 1.

The physical properties and structures of the manure and biochar were examined using scanning electron microscopy (SEM; model TSM-IT300, Japan) at a magnification of 1000x in conjunction with energy dispersive X-ray spectroscopy (EDS) for elemental identification and location. The manure had a coarse surface (Figure 1a), in contrast to the smooth and porous surface of the biochar (Figure 1b). The structures of the manure and biochar are suitable for improving soil fertility, as their pores offer increased surface area, nutrition, and moisture retention and can act as beneficial sites for soil microorganisms, while their anionic surfaces can increase the CEC for the retention of positively charged elements (Mohamed et al., 2017; Edenborn et al., 2015; Ahmad et al., 2014). Furthermore, acacia wood-derived biochar has a high surface area and porosity, which would aid the adsorption of heavy metals (Rodríguez-Vila et al., 2016; Fellet et al., 2014). From the SEM-EDS analysis, the biochar surface had a higher composition of carbon and oxygen than that of the manure (Figure 1c, d). The plant micronutrients Mg, Cl, K, and Ca were found in differing amounts, while Al, Si, and Fe were found in the manure, which are major reserve micronutrients required for plant growth.

3.1.2. Changes in the properties of the mine tailings after manure or biochar addition

The addition of biochar at 1, 3, and 5% (w/w) to the mine tailings increased the soil pH, EC, and CEC according to the ratio of biochar addition. This result was in accordance with a previous study where biochar from coconut shells was reported to increase the soil pH, CEC, and OM content in proportion to the added biochar ratio (Brendova et al., 2016), while the addition of biochar increased the soil pH, CEC, dissolved organic carbon (DOC), and micronutrients for plant growth (Ibrahim et al., 2016). However, the efficiency of phytoremediation and biochar usage depends on the raw materials used to produce the biochar and the ratio of biochar addition.

The addition of cow manure at 1, 3, and 5% (w/w) initially increased the initial soil pH, EC, and CEC; however, after 30–120 d of culture, the pH, EC, and ORP values decreased with increasing cultivation time (Figure 2 a–f). This relationship is due to the decomposition of the OM in the mine tailings and manure into various organic acids, such as humic acids, which resulted in the mine tailings becoming more acidic. This process is also the reason for the reduction in the available adsorption area due to the displacement of OM by protons (H^+) (Shen et al., 2016). Furthermore, plant growth and the uptake of various charged elements and minerals can result in a

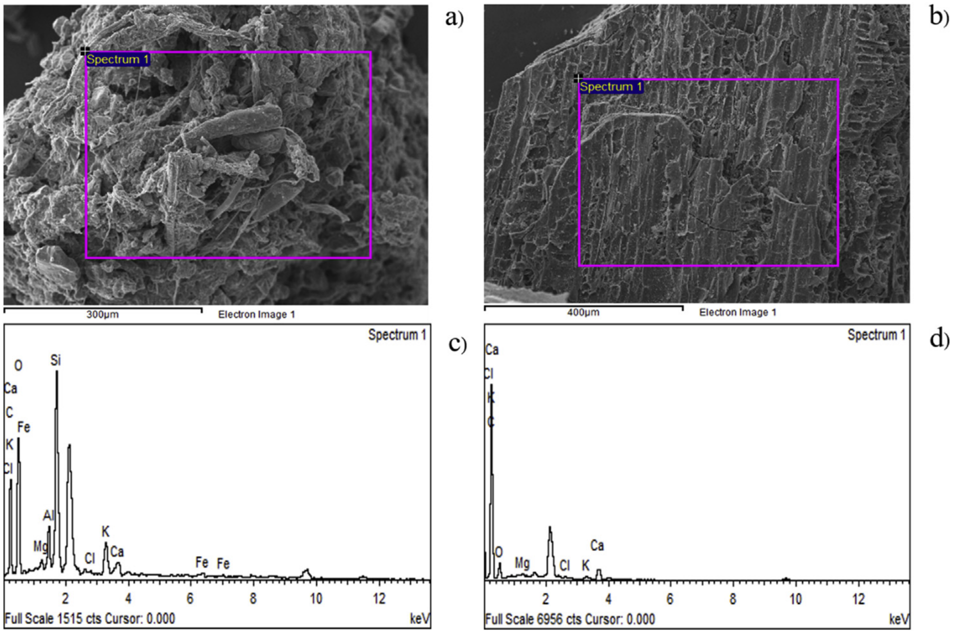

Figure 1. (a, b) SEM images showing the surface characteristics and (c, d) EDS images showing the elements found in the (a, c) manure and (b, d) biochar.

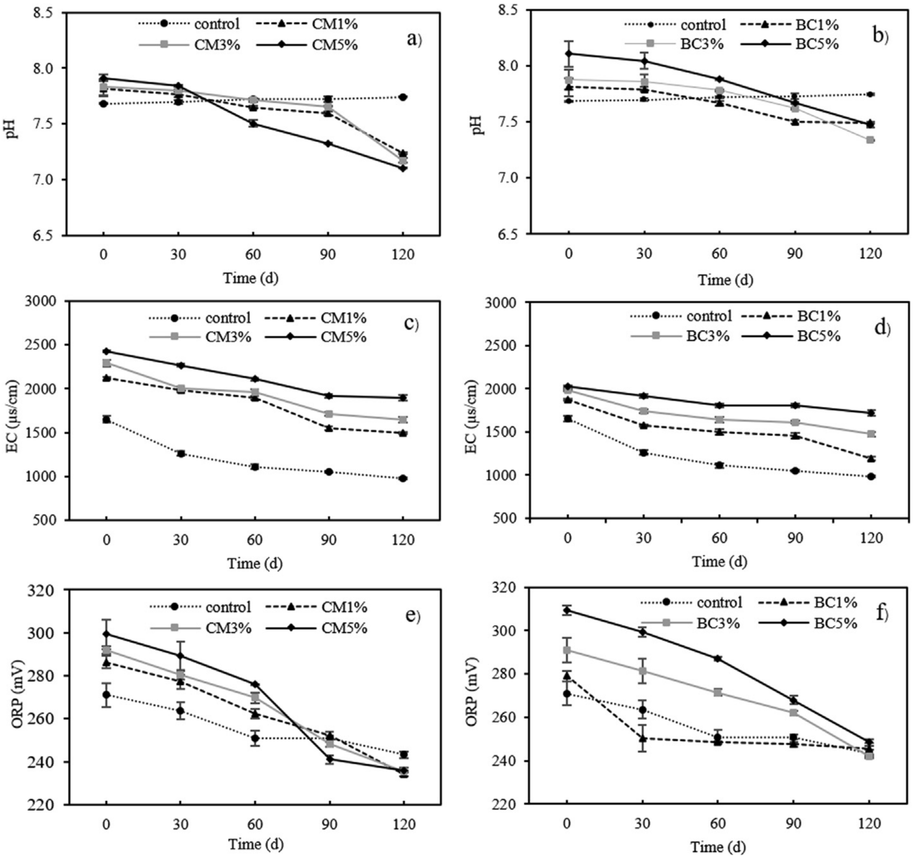

Figure 2. Changes in (a, b) pH (c, d) EC, and (e, f) ORP of the mine tailings over time with the addition of (a, c, e) manure or (b, d, f) biochar at 0, 1, 3, or 5% (w/w). Data are shown as the mean ±1 SD, derived from 3 replications.

reduced EC, where the aforementioned ions will be proportional to the conductivity values (Tananonchai and Sampanpanish, 2014). The addition of manure also increased the content of soil microorganisms, which require continuous redox reactions to generate energy for cellular use. Aerobic microorganisms require a positive redox potential (Eh), but their reduced oxidation capacity also reduces the Eh value of mine tailings. Furthermore, the substances that microorganisms produce, such as hydrogen sulfide (H_2S), also reduce Eh (Thangavel and Subbhuraam, 2004).

3.2. The effect of As and Mn on the RGR of *P. purpureum*

3.2.1. Effects of manure and biochar on the RGR of *P. purpureum*

After 120 d, the biomass of the plants grown in the control was 17 ± 0.39 g DW. The *P. purpureum* grown in the mine tailings with 5% (w/w) manure had the highest biomass (40.39 ± 2.05 g DW), followed by those from the 3% and 1% (w/w) manure applications, respectively (Figure 3a). For the biochar, the highest biomass was found after the addition of 3% (w/w) biochar (24.06 ± 0.40 g), and the biomass content decreased with higher (5%) or lower (0 and 1%) biochar levels (Figure 3b).

The greater increase in plant biomass with the addition of manure is consistent with the high content in manure of OM and nutrients, which are beneficial to plant growth. When decomposed, manure creates humus, which is negatively charged with a surface composed of carboxylic (COO^-) and hydroxyl (OH^-) groups, giving it a high CEC; thus, such decomposition supports plant growth and releases nutrients that plants can use (Kim et al., 2017). *P. purpureum* in the control treatment had the lowest biomass since the mine tailings had unsuitable properties for plant growth.

With respect to biochar application, the greatest biomass was achieved when 3% (w/w) biochar was added and was then significantly reduced with the addition of a higher biochar application rate. This result might be caused by the increase in pH to a level unsuitable for plant growth. Nutrients beneficial for plants may be transformed into insoluble precipitates or other forms that are difficult for plants to uptake, leading to nutrient-deficient plants that are dwarfed with a reduced biomass.

This result was also in accordance with a previous report on the effects of Mott Dwarf Napier grass-derived biochar on the immobilization of Cd-contaminated soil using Chinese cabbage (*Brassica parachinensis*) (He et al., 2017). In that study, the biomass of *B. parachinensis* increased with prolonged duration of the experiment, and the biomass increases were smallest when 5% (w/w) biochar was added. Likewise, biochar was reported to reduce the mobilization of Cd in contaminated soil, although it did not stimulate plant growth (Zhang et al., 2013). In contrast, the addition of biochar at 10% (w/w) gave the highest growth of *Vigna radata*, while biochar addition at 15% (w/w) also affected the plants (Prapagdee et al., 2014). Indeed, the effects of biochar on plant biomass also depend on other factors, such as the plant species, type of biochar, rate of application and type and fertility of the soil (Lebrun et al., 2018).

Regarding the RGR of *P. purpureum*, a reduction in RGR with increasing experimental duration was observed, where biochar addition at 5% (w/w) had the lowest RGR at day 120 (0.027 g d^{-1}), while for the addition of manure, the lowest reduction in RGR (0.035 g d^{-1}) was obtained with 5% (w/w) manure after 120 d (Figure 4a).

3.2.2. Effects of manure and biochar on *P. purpureum* phytotoxicity

The phytotoxicity to *P. purpureum* was evaluated in terms of the leaf chlorophyll content, where an inverse relationship exists such that increasing toxicity reduces the chlorophyll content in plants (Pavlovic et al., 2014). This relationship occurs because chlorophyll creates complex compounds with some heavy metal ions that then change the leaf color (Yilmaz and Gokmen, 2016). In addition, stress due to heavy metal accumulation in plants leads to a reduction in chlorophyll content (Li et al., 2015).

In this study, the chlorophyll content in *P. purpureum* grown in mine tailings tended to decrease with time down to a chlorophyll content of 0.58 mg g^{-1} FW after 120 d (Figure 4b). The addition of manure tended to increase the chlorophyll content, especially at 5% (w/w), where the chlorophyll content reached the highest level of 3.34 mg g^{-1} FW at 120 d. However, with biochar addition, the chlorophyll content still tended to decrease, but not as much as without biochar addition, and the lowest chlorophyll content (0.97 mg g^{-1}) was found with 5% (w/w) biochar after 120 d.

The reduction in chlorophyll content may be due to the accumulation of As in the plants, which is not a micronutrient for plants, as at a plant As concentration of 5 mg kg^{-1}, toxicity will clearly be displayed (Virender and Mary, 2009). Plants uptake As from contaminated soil and translocate it within the plant, where it accumulates in various parts, but the plants have no mechanism to process and decompose As. Thus, the accumulated As can interrupt metabolic processes, resulting in reduced biomass, root and shoot length and mass, stem height, and rate of germination, ultimately resulting in plant death (Sakakibara et al., 2010).

For *P. purpureum* grown in mine tailings with biochar application, the biochar may reduce the amount of micronutrients available to plants as it is able to adsorb and retain elements. With respect to the mine tailings with manure application, the chlorophyll content in the plants tended to increase. This decrease may be due to the manure being composed of nutrients beneficial to plants that were released as the OM decomposed, as seen from the increased RGR. That is, the plants show toxicity by a reduced chlorophyll content and thus a reduced RGR.

3.3. Effects of manure and biochar on As and Mn accumulation in mine tailings

The As and Mn concentrations in the mine tailings over the 120-d period were reduced over time, as derived from the analysis of the As and Mn uptake and accumulation in the plants with increasing cultivation time (Table 2). This reduction is due to the plants' mechanisms for taking up minerals and heavy metals, along with other pollutants, in soil and water through their roots for storage in other parts of the plant. However, the addition of manure or biochar slightly reduced the uptake and accumulation of As and Mn in the plants compared to the control treatment, and these reductions occurred to unequal degrees at the end of the experiment (based on the ratios of manure and biochar added), as summarized in Table 2.

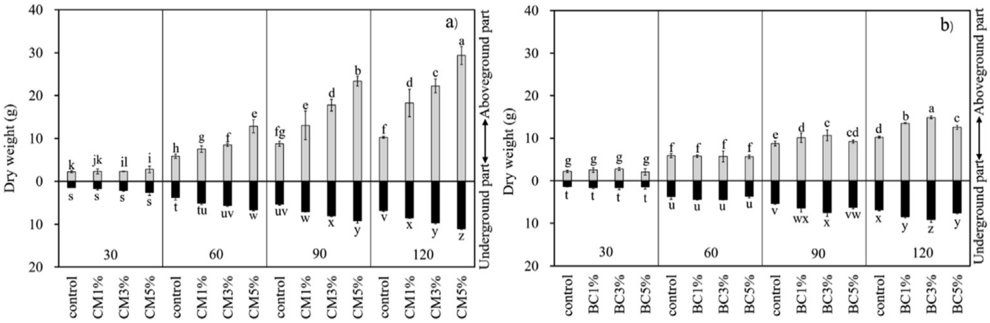

Figure 3. Biomass (g DW) of *P. purpureum* grown in mine tailings supplemented with (a) manure or (b) biochar at 0, 1, 3 or 5% (w/w) for 30, 60, 90, and 120 d. Data are shown as the mean ±1 SD from 3 replications. Means with a different letter indicate significant differences in values.

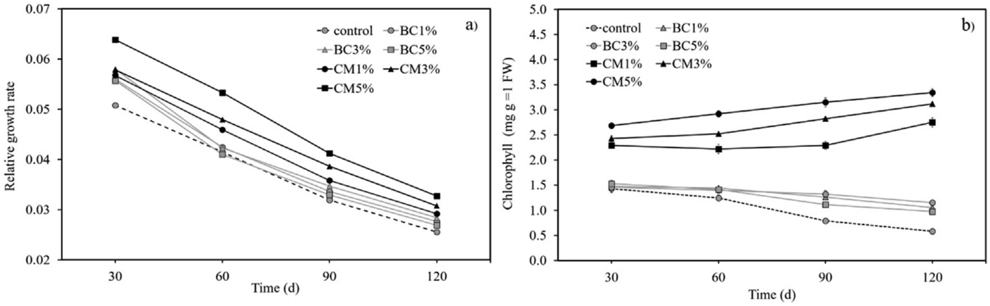

Figure 4. The (a) RGR (g d^{-1} DW) and (b) chlorophyll content in *P. purpureum* grown in mine tailings supplemented with manure or biochar at 0, 1, 3 or 5% (w/w) for 30, 60, 90, and 120 d. Data are shown as the mean ±1 SD from 3 replications. Means with a different letter indicate significant differences in values.

Table 2. Total As and Mn concentrations in the mine tailings.

Experimental conditions	Total As and Mn concentrations (mg kg^{-1})			
	30 d	60 d	90 d	120 d
As:				
Control	61.60 ± 0.81^{d}	59.74 ± 1.24^{b}	59.00 ± 0.23^{d}	58.02 ± 0.34^{d}
BC1%	64.72 ± 64.72 ± 0.24ab	62.22 ± 0.71^{ab}	61.30 ± 0.37^{bc}	60.51 ± 0.89^{bc}
BC3%	65.73 ± 0.03^{a}	64.20 ± 0.46^{a}	63.30 ± 1.00^{b}	62.09 ± 0.30^{ab}
BC5%	64.10 ± 0.40^{b}	63.61 ± 1.10^{a}	64.03 ± 0.78^{a}	63.71 ± 0.71^{a}
CM1%	61.68 ± 1.29^{d}	60.05 ± 2.52^{b}	59.86 ± 1.35^{cd}	59.00 ± 0.81^{d}
CM3%	62.92 ± 00.62^{d}	61.80 ± 1.19^{ab}	61.01 ± 0.34^{c}	60.77 ± 1.82^{bc}
CM5%	64.16 ± 0.20^{b}	63.31 ± 1.47^{a}	62.61 ± 1.20^{ab}	62.50 ± 2.01^{ab}
Mn:				
Control	891.00 ± 4.36^{ab}	859.33 ± 9.02^{b}	832.67 ± 16.62^{b}	815.67 ± 7.64^{e}
BC1%	890.33 ± 2.52^{ab}	882.33 ± 4.04^{a}	865.33 ± 3.51^{a}	847.00 ± 9.64^{bc}
BC3%	907.67 ± 22.19^{ab}	894.67 ± 3.51^{a}	860.00 ± 7.94^{a}	857.00 ± 4.58^{ab}
BC5%	877.33 ± 53.13^{b}	874.33 ± 6.66^{ab}	870.67 ± 7.77^{a}	861.67 ± 3.79^{a}
CM1%	922.00 ± 11.00^{ab}	895.33 ± 11.15^{a}	876.33 ± 11.59^{a}	827.33 ± 2.52^{de}
CM3%	926.67 ± 5.77^{a}	892.00 ± 29.91^{a}	862.00 ± 14.73^{a}	821.33 ± 13.32^{e}
CM5%	896.67 ± 28.85^{ab}	885.33 ± 5.13^{a}	855.00 ± 23.39^{b}	836.67 ± 4.16^{cd}

Data are shown as the mean ± 1 SD, derived from 3 replications. Means with a different letter indicate significant differences in values ($p < 0.05$; DMRT).

3.4. Effects of manure and biochar on As and Mn uptake and accumulation in P. purpureum

3.4.1. Effect of manure on phytostabilization of As and Mn in P. purpureum

Analysis of the As and Mn concentrations in the aboveground (stems and leaves) and underground (roots) parts of *P. purpureum* grown in the manure-added mine tailings revealed that after 120 d of plant cultivation, CM5% had the lowest As uptake and accumulation in the aboveground and underground parts at 0.74 ± 0.07 mg kg^{-1} and 2.11 ± 0.15 mg kg^{-1}, respectively, followed by those from CM3% (0.97 ± 0.13 mg kg^{-1} and 2.43 ± 0.08 mg kg^{-1}, respectively) and then CM1% (1.27 ± 0.05 mg kg^{-1} and 2.85 ± 0.16 mg kg^{-1}, respectively), as shown in Figure 5a.

Regarding the accumulation of Mn at 120 d, the plants grown in CM5% also took up and accumulated Mn in both aboveground and underground parts (65.50 ± 3.94 mg kg^{-1} and 110.99 ± 1.43 mg kg^{-1}, respectively). After 120 d, the plants grown in CM3% took up and accumulated Mn in the aboveground and underground parts, with levels of 80.89 ± 4.41 mg kg^{-1} and 137.38 ± 2.01 mg kg^{-1}, respectively (Figure 5b). This result indicated that manure can immobilize As and Mn in contaminated mine tailings and thus reduce their uptake by plants. OM can absorb 2- to 30-fold more positively charged particles, including positively charged heavy metal particles, than other colloids (Beckett and Ranville, 2006). Even so, acidic conditions can make heavy metals less stable and able to be taken up by plants despite a high OM content in the soil. Furthermore, manure will decompose over time, releasing various elements (Igalavithana et al., 2017). After 120 d of culture, *P. purpureum* had increased As and Mn uptake, in accordance with a previous report (Elouear et al., 2016), where sheep manure-derived biochar and potassium chloride fertilizer (KCl)-supplemented soil with 970 mg kg^{-1} Pb, 9, 641 mg kg^{-1} Zn, and 53 mg kg^{-1} Cd was used to grow alfalfa grass (*Medicago sativa* L.). *M. sativa* was resistant to high concentrations of Pb, Cd, and Zn, and the addition of KCl fertilizer had no significant effect on plant biomass. Furthermore, the EC of the sheep manure-derived biochar increased that of the soil and reduced the concentration of heavy metals that could be extracted with DTPA. Overall, in that study (Elouear et al., 2016), KCl was able to increase the accumulation of heavy metals in plants and improve soil fertility as well as phytoremediation, while sheep manure-derived biochar could also be used for phytostabilization of heavy metals at the root zone.

3.4.2. Effects of biochar on phytostabilization of As and Mn uptake and accumulation in P. purpureum

With respect to the accumulation of As and Mn in the aboveground and underground parts of *P. purpureum* in the biochar-added mine tailings, the ability of *P. purpureum* to uptake and accumulate As and Mn in both the aboveground and underground parts was decreased relative to the control. After 120 d, plants grown in BC5% had the lowest As uptake and accumulation in the aboveground and underground parts (0.52 ± 0.05 mg kg^{-1} and 1.57 ± 0.13 mg kg^{-1}, respectively), and the values increased in BC3% and BC1% in a biochar dose-dependent manner (Figure 5c).

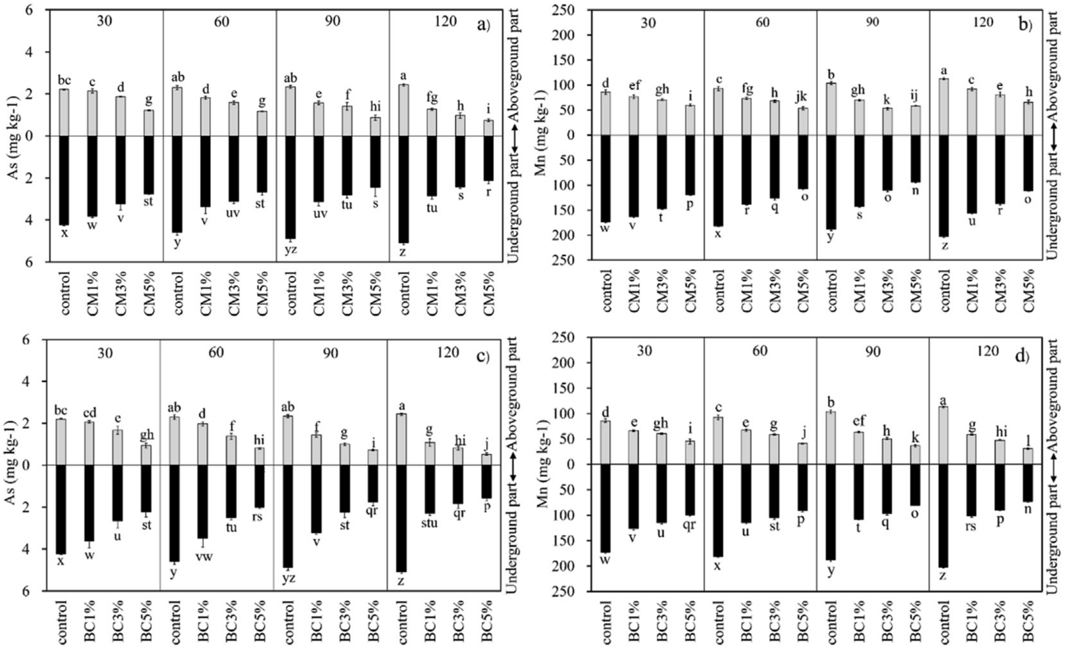

Figure 5. Effects of (a, b) manure and (c, d) biochar application to mine tailings on (a, c) As and (b, d) Mn uptake and accumulation in *P. purpureum* after culture for 30, 60, 90, and 120 d. Data are shown as the mean ±1 SD, derived from 3 replications. Means with a different letter indicate significant differences in values ($p < 0.05$; DMRT).

Regarding Mn uptake and accumulation in *P. purpureum*, as with As in the plant grown in BC5%, the lowest Mn uptake and accumulation in the aboveground and underground parts (31 ± 1.08 mg kg^{-1} and 73.05 ± 2.60 mg kg^{-1}, respectively) was observed in BC5%, and the values then increased in BC3% and BC1% in a biochar dose-dependent manner (Figure 5d).

Thus, biochar was able to reduce both As and Mn uptake from the mine tailings by *P. purpureum* in a biochar dose-dependent manner, and the highest uptake and accumulation of As and Mn was in the underground parts (roots). This finding corresponds to observations reported by Park et al. (2011), who found that biochar was able to reduce the uptake and accumulation of Cu, Pb, and Cd in Chinese mustard from Cd-contaminated soil and that the accumulation was highest in the roots. Likewise, Souza et al. (2019) discovered that heavy metal uptake and accumulation values were reduced with increasing biochar ratios in the soil, as the biochar had a porous structure with a high surface area of carbon, high CEC and pH, giving the biochar the ability to adsorb heavy metals on its surface. In addition, as already mentioned, increased pH may change heavy metals to forms that are less easily displaced or dissolved, making it difficult for plants to uptake these heavy metals, while biochar has several functional groups, including carboxylic, carbonyl, and hydroxyl groups, that can adsorb heavy metals and reduce their mobilization and distribution in the environment (Kiran et al., 2017; Hossain et al., 2014).

When comparing the results of As and Mn uptake and accumulation in *P. purpureum* grown in the manure- and biochar-amended tailings at the end of the experiment (120 d), lower As and Mn uptake and accumulation were found in the plants grown in the biochar-added mine tailings. However, similar significant decreasing trends in As and Mn uptake and accumulation were observed as the application rates of both biochar and manure increased. Plants grown in CM5% accumulated 0.74 ± 0.07 mg kg^{-1} and 2.11 ± 0.15 mg kg^{-1} As in the aboveground and underground parts, respectively. The accumulation amounts of Mn in the aboveground and underground parts were 65.50 ± 3.94 mg kg^{-1} and 110.99 ± 1.43 mg kg^{-1}, respectively. For the plants grown in BC5%, the As and Mn accumulation amounts in the aboveground and underground parts were equal to 0.52 ± 0.05 mg kg^{-1} and 1.57 ± 0.13 mg kg^{-1} for As and 31 ± 1.08 mg kg^{-1} and 73.05 ± 2.60 mg kg^{-1} for Mn, respectively. These results indicated the potential better immobilization ability of biochar for As and Mn than that of cow manure. The results are in good agreement with those reported by Kiran et al. (2017), in which 0, 3 and 6% cow manure and biochar were used to amend soil and reduce the accumulation of Cd in *Brassica juncea*. Kiran et al. (2017) found that biochar produced from cow manure was more efficient in reducing the bioavailability of Cd than common cow manure because the biochar had a higher carbon content and negative charges, which could exchange with positively charged pollutants in the soil. Furthermore, biochar has shown an ability to remove heavy metals from soils via physical and chemical adsorption processes (Zhu et al., 2017). Therefore, it is possible to conclude that Acacia wood-derived biochar at an application rate of 5% can be used to immobilize As and Mn at a higher percentage than that obtained with cow manure. A higher application rate of cow manure may also cause an increase in As and Mn uptake and accumulation by plants.

The plant DWs from the growth studies were related to the level of accumulated As after 120 d of cultivation (Figure 6). The results revealed that *P. purpureum* from the control treatment had the highest accumulation of As in both the aboveground and underground parts, while plants grown in BC5% had the lowest As accumulation, followed by higher levels in BC3% and BC1% in a biochar dose-dependent manner (Figure 6a).

Regarding Mn accumulation after 120 d of cultivation, *P. purpureum* grown in the control group also had the highest Mn accumulation level in both the aboveground and underground parts, while plants from BC5% had the lowest Mn levels in the aboveground and underground parts, followed by slightly higher levels in BC3% (Figure 6b). For the plants grown in the manure-supplemented mine tailings, both As and Mn accumulation was greater than that in the biochar-supplemented tailings but decreased with increasing levels of manure for As (Figure 6c). However, for Mn accumulation, the lowest level was found in CM1% for the aboveground parts and CM3% for the underground parts (Figure 6d).

Overall, the addition of biochar at 5% (w/w) to the mine tailings was able to reduce the accumulation of As and Mn in *P. purpureum* to a greater level than the addition of cow manure at similar levels, even though the latter also had the ability to reduce the accumulated As and Mn concentrations in the plants. However, the addition of manure increased the plant biomass significantly, so in terms of plant DW, As and Mn

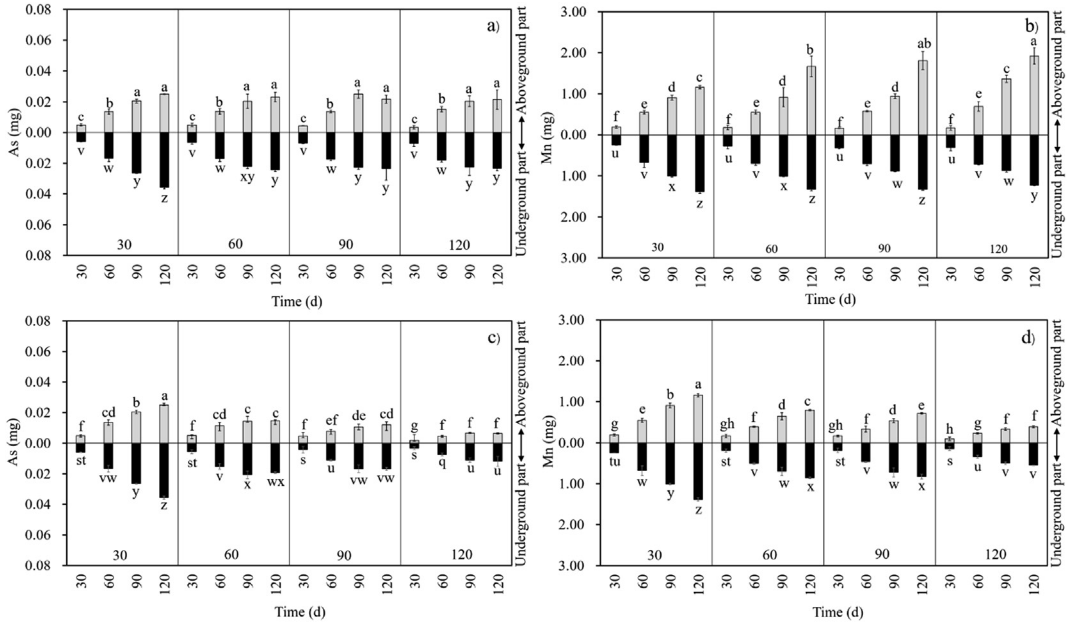

Figure 6. Effects of the addition of (a, b) manure or (c, d) biochar on (a, c) As and (b, d) Mn uptake and accumulation in *P. purpureum* (mg g^{-1} plant DW) over 30, 60, 90, and 120 d of cultivation. Data are shown as the mean ±1 SD, derived from 3 replications. Means with a different letter indicate significant differences in values ($p < 0.05$; DMRT).

accumulation from the mine tailings was higher with manure than with biochar supplementation. Thus, when considering suitable application ratios to reduce the uptake, accumulation, and immobilization of As and Mn using *P. purpureum*, the suitable rate recommended for these mine tailings is the addition of 5% (w/w) biochar with a harvesting period of 120 d. A suitable ratio of manure would be 5% (w/w) for As immobilization, but 3% or 5% (w/w) would be appropriate for immobilization of Mn in mine tailings with harvesting periods of 90 or 60 d, respectively. However, the addition of manure or biochar for phytostabilization will depend on the desired utilization. If the main benefit is to be biomass, then manure should be used. However, if the main benefit is to be plants with a low As and Mn accumulation level, then 5% (w/w) biochar would be better.

3.5. Efficiency of manure and biochar in the phytostabilization of As and Mn with P. purpureum

The percentage reduction in As and Mn uptake and accumulation compared with the control treatment revealed that adding biochar at 5% (w/w) reduced the As concentrations in the underground and aboveground parts of *P. purpureum* to the greatest extent, by 63.9% and 78.6%, respectively, followed by adding biochar at 3% (w/w), which reduced the As concentration in the underground parts by 55.4%, while manure at 5% (w/w) reduced the As concentration in the aboveground parts by 69.6% (Figure 7a). For Mn, the addition of biochar at 5% (w/w) reduced the Mn concentrations in the underground and aboveground parts to the highest extent by 69.3% and 72.5%, respectively, followed by biochar at 3% (w/w), which reduced Mn accumulation in the underground and aboveground parts by 63.9% and 57.6%, respectively (Figure 7b). The addition of biochar at 5% (w/w) gave the lowest plant accumulation of As and Mn and was able to immobilize As and Mn in the mine tailings. Accordingly, cow-derived biochar was reported to be efficient in reducing the bioavailability of Cd to *Brassica juncea* to a greater extent than regular manure, with a reduction level of 34.3–69.9% and a reduced accumulation in *B. juncea* of 51.2% and 67.4% at biochar ratios of 6% and 3%, respectively (Kiran et al., 2017). Thus, the application of biochar is an alternative to reduce heavy metals in contaminated soil and enhance nutritional and culinary safety.

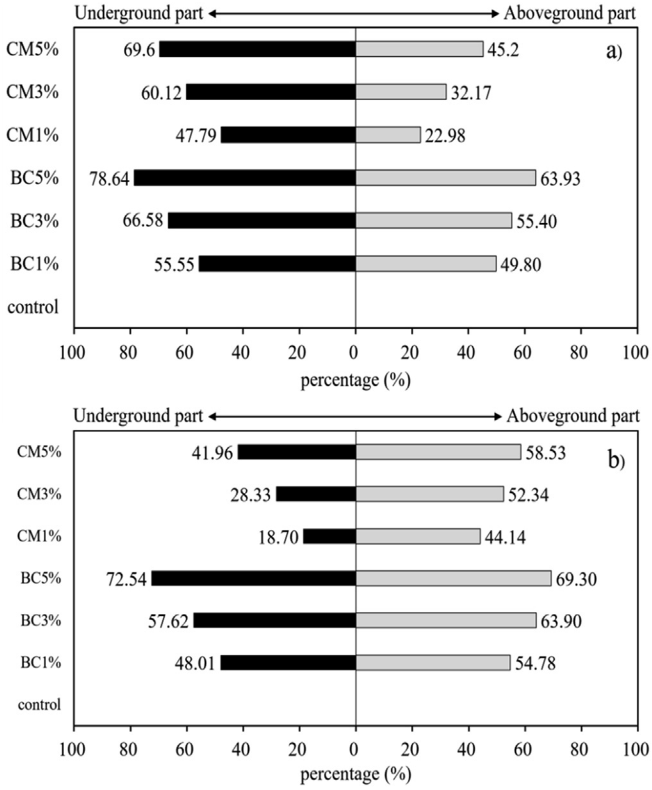

Figure 7. Percentages of (a) As and (b) Mn uptake and accumulation in the above and belowground parts of *P. purpureum*. Data are shown as the mean ±1 SD, derived from 3 replications.

4. Conclusion

The results demonstrated that cow manure and acacia wood-derived biochar have the potential to reduce As and Mn uptake and accumulation in *P. purpureum*, enhance plant growth, and improve mine tailing properties. The suitable ratio to reduce As and Mn uptake and accumulation using *P. purpureum* was the application of 5% (w/w) biochar. However, phytostabilization has limitations due to heavy metals and other pollutants in the ground at high concentrations, which means that the efficiency of treating and restoring ground polluted by heavy metals could be low as well. Thus, phytoextraction should be a primary treatment, followed by phytostabilization to reduce heavy metal accumulation in plants so that the plants can then also be used for other purposes. Furthermore, this method can reduce the potential risk of heavy metal distribution into food chains and the surrounding environment; furthermore, this method can be used to solve the problem of heavy metal-contaminated soil and mine tailings and remediate those areas so that they can be used again. However, there must be a suitable selection of plants, such as fuel crops, for the treatment and restoration of polluted lands for maximum benefits and efficiency.

Declarations

Author contribution statement

Pantawat Sampanpanish: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Anothai Kowitwiwat: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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