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Effect of Various Manure Doses on the Choy Sum Grown in Mercury-Contaminated Tailing Substrate

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ABSTRACT

Gold-mine tailings limit plant growth and yield due to physicochemical properties and the high content of Mercury (Hg). The greenhouse experiment evaluated the effect of cow manure (CM) on Carbon, Nitrogen, and Hg content, microbial population as well as plant growth, yield, and Hg content of choy sum (*Brassica chinensis* L.) grown in Hg-contaminated tailing. The experiment used a Randomized Block Design to test various compositions of tailing and CM. The results showed that CM increased organic-C up to 442% and C/N ratio by 82% and decreased Hg in soil and shoot by 48% and 76% respectively, but did not affect bacterial and fungal count in substrate. Meanwhile, 45% of CM in tailings resulted in a greater plant yield of 11.68 g, which is 146.4% higher than that of the control. However, the shoot of treated plants contained Hg higher than the maximum limit of mercury contamination in vegetables. The technical implication of this experiment is that the CM amendment improves tailing quality and increases plant growth, but the leaves of Choy sum are unsafe for consumption.

1. INTRODUCTION

Mineral mining plays a significant role in the economic revenue and development of many countries. Its most positive impacts are providing community livelihoods and foreign exchange for the countries. Indonesia has potential gold reserves of 3.6 billion tons, around 6.9% of the world's total gold reserves. In 2020, 36.50 t of gold was sold in the domestic market and 44.92 t in the foreign market [1].

Gold mining leads to extreme land-use changes, severe destruction of land and water bodies, and loss of vegetation and other biodiversity. In many artisanal and small-scale gold mining (ASGM), the toxic metal mercury (Hg) is used to extract the gold from the mineral ore. Indeed, gold mining wastes, including tailing and wastewater, are contaminated with Hg [2]. The gold production of ASGM in Indonesia is predicted to be 53.8 t a^{-1} to 120 t a^{-1}, with an estimated Hg consumption of 1,727.5 t a^{-1} and Hg emissions of 345.5 t a^{-1} [3, 4]. Therefore, Indonesian policy focuses on accelerating the legalization of ASGM, performing good mining practices, frequently monitoring and evaluating the ASGM performance, and providing access to capital, marketing, and assistance in processing and gold refining facilities to reduce the diverse effect of Hg [3].

In general, tailings of ASGM are not stored and handled under the regulations for post-mining management. Disposing the Hg-contaminated tailing in productive agricultural areas without treatment harms the soil. The Hg content in the soils affected by tailing usually exceeds the threshold limit value for Hg in the soil [5]. High Hg levels in the soil are toxic to plants and the environment; they may accumulate in the edible part of plants [6, 7]. Mercury exposure leads to a decrease in chlorophyll content and photosynthesis, and it reduces transpiration rate and water uptake by plants [8]. Plants generally uptake Hg by the roots, which results in the accumulation of Hg in the roots and leaves [9, 10].

The Hg availability for plant uptake in soil depends on acidity (pH), soil texture, and organic matter (OM). Tailings, in general, are extremely acidic or alkaline. Low pH causes metal desorption and increases its bioavailability [11]. Sandy soil with high infiltration rate reduces the Hg level in the root area [12]. The macronutrients nitrogen (N), phosphorus (P), potassium (K), and organic carbon (C) of tailing are low, but micronutrients and toxic metal content are immense [13]. The tailing usually contains high sand or clay that prevents root growth. The application of OM is suggested to overcome those constraints.

The organic amendment retains metals, provides plant nutrients, and improves water-holding capacity [14, 15]. The OM reduces the bulk density and increases macropores [16-18]. The surface of organic matter has negative charges due to the presence of carboxyl (-COOH) and phenolic (-OH) groups [19] that attract and hold positive charges of metal ions. Negative charges of humic acid in OM can bind heavy metals such as Hg in the form of Hg^{2+} ions in the planting medium [20].

Cow manure is often used by farmers as an organic fertilizer or soil conditioner for improving soil organic-C profile due to the immense content of cellulose [21]. Decomposition of manure provides macronutrient N, P, and K and hence increases soil fertility [22]. Nitrogen is an essential nutrient during the vegetative stage of plants since N is an integral part of chlorophyll [23]. Cow manure has been reported to sustain the viability of microorganisms in the soil, including heterotrophic beneficial microbes that promote plant growth [24].

Compost amendment in mining areas reduces the soil pH, bulk density, and available Hg but increases water-stable aggregate, organic-C, total N and P, and plant growth [25, 26]. However, some types of organic matter increase Hg methylation in mine sites [27]. The influence of OM on the microbial population of tailing is rarely reported, but OM improves heterotrophic microbial count in soil [28, 29].

Plants belonging to Brassicaceae can be used as test plants in the tailing remediation study since they are hyperaccumulators without showing phytotoxic symptoms and are tolerant to heavy metals [30, 31]. Phytoremediation of toxic HMs by Brassica species is a promising technique. However, many species of Brassicaceae are edible and grown as important vegetable in Asia. The ASGM community in the research area is dominated by farmers whose livelihood depends on their land. Vegetable cultivation in such extreme areas requires special precautions regarding the Hg content in leaves. Species of Brassica genera are reported to accumulate Hg and other heavy metals in the edible part [31, 32]. The objective of this study was to investigate the effect of cow manure application to Hg-contaminated tailing on the content of organic C, total N, and Hg as well as the growth and yield of choy sum (*Brassica sinensis* L.), compare the development of the plant in tailing and mineral soil; and determine the bacterial and fungal viability in tailing with various dose of manure.

2. MATERIALS AND METHODS

The pot experiment was conducted in the greenhouse of the Faculty of Agriculture, Universitas Padjadjaran, at an altitude of 726 m above sea level. The area is in the tropics; the average temperature and humidity during the experiment were 14.3℃-27.8℃ and 77.6%-96.3%, respectively.

2.1 Source of tailing, soil and manure

Mercury-contaminated tailings were collected from the ASGM area in the Cigudeg District of Bogor Regency, Indonesia (Figure 1); gold extraction was done by amalgamation. The Hg content at different locations was 223-1,200 mg kg^{-1}, above the threshold limit level of Hg in agricultural soil. The Waste Management Unit of Universitas Padjadjaran provided cow manure (CM), while the Inceptisols soil order was collected from field soil in the Jatinangor Campus of Universitas Padjadjaran.

Chemical properties determination of tailings and CM before the experiment was done according to the Association of Analytical Chemistry proximate analysis [33]. Meanwhile, the tailing physical properties were characterized according to the International Soil Reference and Information Centre [34]. East West Seed Indonesia Hold. Co. produced the seeds of Choy sum cv Shinta.

Figure 1. Location of sample collection in Cibugel, Bogor Regency, Indonesia

The tailings were taken from the disposal pond of ASGM by purposive method at a 0-40 cm depth. The soil was collected from a 30 m^2 field by gridline intersection method at a 5 m distance between sampling points; three composite topsoil samples were collected from 0–20 cm depth.

2.2 Experimental design

Two pot experiments were conducted to grow choy sum in two kinds of substrate: tailing and field soil. Each experiment was set up in a completely randomized block design with five replications to test the different compositions of cow manure in either tailings or soil (Table 1). All treatments were replicated five times for each experimental unit.

Table 1. The composition of tailing- and soil-based substrates mixed with different amounts of cow manure

Treatment Codes	Tailings or Soil (g)	Cow Manure (g)	Percentage of CM in Substrate
p_0	1,000	0	0
p_1	888	112	11.2
p_2	745	225	22.5
p_3	663	337	33.7
p_4	550	450	45.0

The tailings were cleared from debris, grounded, and sieved by a metal sieve with 2 mm openings, while the soil was used directly for the experiment without being grounded or crushed. A total of 1 kg substrates composed of tailing and CM was poured into black polyethylene bags, and the water content was kept at field capacity for a week before transplanting the 14-day-old choy sum seedlings.

2.3 Parameters and statistical analysis

The plant height, leaf number, crown diameter, and fresh weight of plants grown in tailing and soil were measured five weeks after planting. At the end of the experiment, the dry weight ratio of shoot to root (S/R) was calculated after heating the biomasses at 70℃ for two days until constant weights were obtained. The soil organic-C and total-N content were determined according to Walkley and Black and the Kjeldahl method, respectively; the C to N ratio (C/N) was then calculated.

The soil acidity was determined using the potentiometric method using a pH meter. All soil analyses were referred to the standard method of the AOAC [33]. The total Hg content of soil and shoot was analyzed using an atomic absorption spectrophotometer after samples were destructed with mixed acids. The total bacteria and fungi population was counted five weeks after planting using the serial dilution plate method on Nutrient agar (NA) and potato dextrose agar (PDA) plates, respectively. All data was subjected to analysis of variance followed by the Duncan Multiple Range Test (DMRT) at $p<0.05$ using the SPSS.

3. RESULTS AND DISCUSSION

3.1 Tailing and manure properties

Table 2 shows the properties of tailing before the experiment. The tailing had low organic-C and total-N, and the C/N ratio was only 5.15. Low organic-C indicates low organic matter content, which are important indicators of soil health [34]. Insufficient concentration of organic-C poses a serious problem for soil fertility, including low availability of nutrients, limited microbial population and biodiversity, and poor soil physical properties that limit root growth as well as nutrients and water uptake.

Table 2. Tailing nutrient profile and solid particle composition before the experiment

Parameter	Unit	Value
pH H_2O	-	8.92
Organic C	(%)	1.03
Total N	(%)	0.18
ratio	-	5.15
Potential P_2O_5	(mg 100 g^{-1})	45.64
Available P_2O_5	(mg kg^{-1})	9.23
Potential K_2O	(mg 100 g^{-1})	15.16
Exchangeable Cations		
K^+	(cmol kg^{-1})	0.23
Na^+	(cmol kg^{-1})	0.54
Ca^{2+}	(cmol kg^{-1})	19.18
Mg^{2+}	(cmol kg^{-1})	1.19
CEC*	(cmol kg^{-1})	21.15
Base saturation	(%)	99.9
Al-dd	(cmol kg^{-1})	0.1
H-dd	(cmol kg^{-1})	0.053
Total Hg	mg kg^{-1}	310
Al saturation	(%)	0.46
Solid Particles		
Sand	(%)	11.87
Silt	(%)	55.56
Clay	(%)	32.57

A low N content indicates the inability of tailing to provide macro-essential N, which is prominent for growth, especially during the vegetative stage. A low C/N ratio inhibits nitrate (NO_3^-) reduction and nitrite (NO_2^-) oxidation and contributes to the leaching of NO_3^- and NO_2^-, mainly during the rainy season [35]. The total phosphorus (P) content in the tailing was medium, but their availability was high. The available P in tailing was high, but N and potassium (K) content was low, resulting in an imbalanced composition of the major essential nutrients. Therefore, inorganic fertilizer and organic matter are needed to grow choy sum in the tailing.

The cation exchange capacity (CEC) of tailing is medium, generally determined by OM and clay content [36]. The CEC in tailing is possibly influenced by high clay content (32.57%) rather than OM content (1.03%). This tailing has the medium ability to hold cations nutrients in the negative charge of tailing particles and prevent them from leaching. The tailing was high, possibly due to the increased pH [31, 37]; therefore, the tailing provided base cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) for plants.

Before the experiment, the pH was 8.9; alkalinity caused the phosphates (PO_4^-) to bind to cations, mainly Ca^{2+} and became unavailable to the plant [38]. However, a high pH induces metal immobilization and limits their uptake [11]. The alkalinity of tailing is supposed to be caused by the high exchangeability of Ca, Na, and Mg. Higher pH restricts the fungal metabolisms, which is optimal in an acid environment. Beneficial soil bacteria, which fulfill their function in the nutrient cycle, usually grow in slightly acid to neutral environments.

The tailing texture was clay loam with a high content of silt (55.56%) and clay (32.57%), a barrier for root growth. The particle size of silt was higher than clay; both solid fractions prevent the downward movement of water. Based on the data in Table 1, the tailing cannot support plant growth, so the application of organic matter is necessary to improve its physical, chemical, and biological properties.

The acidity and water content of CM were 6.05 and 19.03%, respectively. The CM comprises 41.09 organic C, 2.06% total N, 0.56% total P_2O_5, and 0.29% total K_2O. The C/N ratio of cow manure was 19.94; the microbes actively degraded the organic matter without immobilizing the N.

3.2 Plant growth and yield

Plant growth in tailing was delayed compared to that in field soil (Table 3). The average plant height, leaf number, and crown diameter of choy sum grown in tailing were 16.12 cm, 5.44 cm, and 4.62 cm, respectively, while in soil, they were 26.70 cm, 6.6 cm, and 5.72 cm. Manure application in tailing and soil enhanced the growth traits, while the leaf number of plants grown in tailing did not change significantly (Table 3).

The significant effect of manure on the plant growth traits (Table 3) led to an increase in the dry weight of shoots and roots (Table 4). Both dry matters were gradually enhanced by increasing the manure dose. For plants treated with 11.2% of manure, the dry weight of shoots increased to 111%, while 45% of manure (p_4) increased shoots and roots dry weight up to 433% and 200% compared to the control, respectively (Table 4). The shoot-to-root ratio (S/R) changed significantly after each manure dose application, suggesting that manure induced shoot growth and then photosynthesis of choy sum leaves. The improvement in biomass is related to the adequate supply of nutrients and water during plant development due to substrate improvement.

The yield of choy sum in tailing with any dose of CM was remarkably increased (Figure 2). The fresh weight of plants with a higher dose of CM was five times higher than the control, while the yield increment of plants with 11.2% manure was twofold compared to the control.

Table 3. Morphological traits of plants grown in tailing and soil with various doses of manure at five weeks after transplanting

Treatments	Plant Height (cm)		Leaves Number		Crown Diameter (mm)	
	Tailing	Soil	Tailing	Soil	Tailing	Soil
p_0	13.96 ± 0.53a	23.90 ± 0.95a	4.60 ± 0.34	5.80 ± 0.44a	2.98 ± 0.36a	4.48 ± 0.34a
p_1	18.88 ± 0.50b	25.32 ± 2.43b	5.00 ± 0.45	6.40 ± 0.48ab	4.02 ± 0.70b	4.96 ± 0.84a
p_2	21.98 ± 0.65d	27.42 ± 1.19d	5.80 ± 0.69	6.80 ± 0.52cd	4.86 ± 0.53b	5.92 ± 0.38b
p_3	21.20 ± 0.32c	26.14 ± 2.35c	5.40 ± 1.02	6.60 ± 0.50b	4.96 ± 0.42b	6.18 ± 0.56b
p_4	22.92 ± 0.64e	30.74 ± 1.53e	6.40 ± 0.56	7.40 ± 0.56d	6.30 ± 1.07c	7.06 ± 0.59c

Values in the column followed by the same letters are not significantly different based on the DMRT with p <0.05. p_0: Tailing or soil; p_1: 112 g manure + 888 g tailing/soil; p_2: 225 g manure + 745 g tailing/soil; p_3: 337 g manure + 663 g tailing/soil; p_4:450 g manure + 550 g tailing/soil.

Table 4. Dry biomass of five-week-old choy sum grown in tailing treated with manure

Treatments	Shoot Dry Weight (g)	Roots Dry Weight (g)	S/R
p_0	0.18 ± 0.07a	0.028 ± 0.017bc	9.3 ± 6.3d
p_1	0.38 ± 0.13ab	0.019 ± 0.004c	17.5 ± 4.3a
p_2	0.61 ± 0.23bc	0.046 ± 0.007a	14.2 ± 5.0c
p_3	0.70 ± 0.08c	0.037 ± 0.011b	19.9 ± 4.8a
p_4	0.96 ± 0.14d	0.059 ± 0.017a	17.4 ± 5.0b

Values in the column followed by the same letters are not significantly different based on the DMRT with p <0.05. p_0: tailing without manure; p_1: 112 g manure + 888 g tailing; p_2: 225 g manure + 745 g tailing; p_3: 337 g manure + 663 g tailing; p_4: 450 g manure + 550 g tailing.

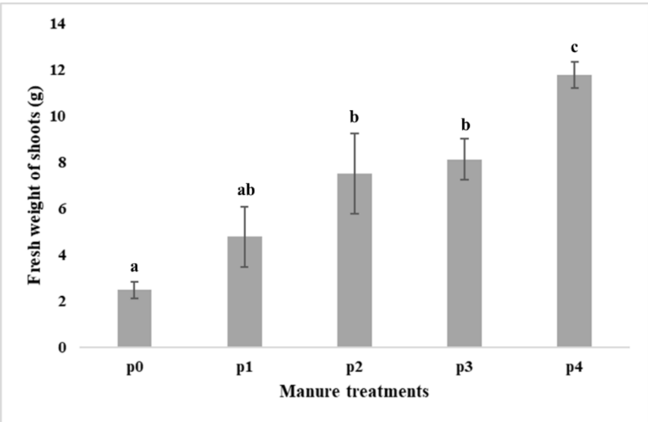

Figure 2. Shoot fresh weight of 5-week-old choy sum grown in tailing treated with manure

Letters indicate a significant difference (p < 0.05) between treatments based on the DMRT with p <0.05. p_1 = 112 g manure + 888 g tailing; p_2 = 225 g manure + 745 g tailing; p_3 = 337 g manure + 663 g tailing; p_4 = 450 g manure + 550 g tailing.

3.3 Substrate properties

Higher content of CM in tailing-based substrates significantly increased the organic-C, total N, and C/N ratio of substrates five weeks after transplanting (Table 5). Higher doses of CM resulted in higher organic-C and total-N compared to the lower CM doses. However, 33.7% and 45% CM decreased the C/N ratio significantly compared to the substrate with 22.5% CM (Table 5). Soil organic carbon is a quantifiable part of soil organic matter that can be calculated by multiply the organic-C content by 1.72 (Table 5). The C and N are essential to improve the nutrient status of tailing for plant growth. The C/N ratio determines the availability of N in soil for root uptake. The organic-C content of the substrate was very low without CM (< 2.0), but a low dose of CM amendment increased the OC to a lower level (2.0 - 3.5), while medium- and high-dose of CM increased the OC content to a high value (6.0 - 8.0).

The C/N ratio of all substrates was less than 20, which mobilized the N for plant uptake. In this research, the C/N ratio did not exceed 25, which caused N immobilization when the soil could not provide enough nitrogen for the plants. The low C/N ratio (9.3) of the control plants demonstrated N mineralization, while the C/N ratio of 19.9 in treated soil resulted in a balance of N mineralization and immobilization [38, 39].

The use of 33.7% and 45% CM decreased Hg of substrates (Table 6), while the total Hg of substrates with a lower dose of CM was similar to that of the control. A decrease in Hg of substrate and plant shoots is caused by Hg dilution by CM. Organic matters are reported to induce Hg methylation [27], lowering total Hg in substrates.

The shoot dry matter from six replications of each treatment was mixed due to the low weight of the samples to analyze; two subsamples were taken for Hg analysis. The Hg content in the shoot decreased after CM application; some treatments reduced the Hg of shoots to less than 1 mg kg^{-1} (Table 6). The pH of substrates was neutral, which increased mercury hydroxide adsorption by soil colloids and decreased mercury bioavailability [11, 40]. The negative charges of OM hold positive charges of metal ions to reduce their bioavailability [20]. Despite the neutral pH of substrates (Table 6), Hg content in the edible part of choy sum, including leaves and stems, was > 50 μg kg^{-1}; the threshold limit of mercury in edible parts of plants [41].

Table 5. Effect of various doses of manure on organic Carbon and total Nitrogen of tailing-based substrate

Treatments	Organic C (%)	Organic Matter (%)	Total N (%)	C/N Ratio
p_0	1.45 ± 0.15a	2.52 ± 0.59a	0.19 ± 0.02a	7.69 ± 0.96a
p_1	2.11 ± 0.34a	3.67 ± 1.30a	0.26 ± 0.04b	8.33 ± 0.64a
p_2	5.94 ± 0.21b	10.34 ± 0.79b	0.32 ± 0.01c	18.88 ± 0.99c
p_3	7.48 ± 0.37c	13.02 ± 1.41c	0.45 ± 0.01d	16.74 ± 0.66c
p_4	7.87 ± 0.19c	13.69 ± 0.72c	0.56 ± 0.02e	14.00 ± 0.43b

Values in a column followed by the same letters are not significantly different based on the DMRT with p <0.05. p_0: Tailing; p_1: 112 g manure + 888 g tailing; p_2: 225 g manure + 745 g tailing; p_3: 337 g manure + 663 g tailing; p_4:450 g manure + 550 g tailing.

Table 6. Effect of manure on Hg content in the substrate and choy sum shoot, and pH of substrates

Treatments	Hg in Soil ($mg\ kg^{-1}$)	Hg in Shoot ($mg\ kg^{-1}$)	pH Substrates
p_0	148.45 ± 8.99b	4.89 ± 0.04	7.08 ± 0.02d
p_1	141.12 ± 8.67b	2.41± 0.14	6.71 ± 0.05c
p_2	172.55 ± 17.59b	1.64 ± 0.07	6.69 ± 0.06c
p_3	91.59 ± 4.80a	0.98 ± 0.11	6.46 ± 0.05b
p_4	77.00 ± 4.19a	1.15 ± 0.11	6.13 ± 0.03a

Values in a column followed by the same letters are not significantly different based on the DMRT with $p < 0.05$. p_0: Tailing; p_1: 112 g manure + 888 g tailing; p_2: 225 g manure + 745 g tailing; p_3: 337 g manure + 663 g tailing; p_4:450 g manure + 550 g tailing.

3.4 Population of bacteria and fungi

The dose of CM did not affect the population of bacteria and fungi in the substrate (Figure 3). The log value of total bacterial and fungal count was 10 and 4, respectively, typically found in the rhizosphere, where nutrients are continuously available for their proliferation. The total count of both microbes did not distinguish their function and taxonomic group due to using general media in microbial enumeration. Nonetheless, the study verified that Hg-resistance microbes are present in tailing-based substrates. The microbes can be developed as bioremediation agents of Hg-contaminated sites.

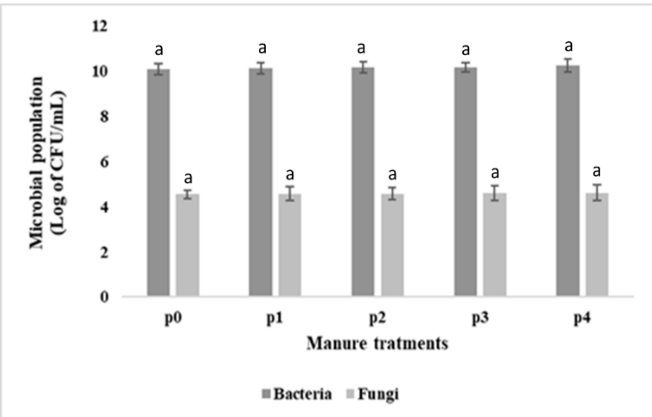

Figure 3. Effect of cow manure on the population of total bacteria and fungi in the choy sum's growth media at five weeks after transplanting

The application of CM increased plant height and crown diameter, although the difference in the leaf number of control and treated plants is not significant (Table 4). Organic substances, including agricultural waste, are sources of soil organic material that play an essential role in improving the physical properties of soil media. In this study, the tailings texture is clay loam with a predominance of sand fraction (Table 1). Adding CM with an organic-C content of 41% reduces the proportion of sand and clay fractions in the substrate and improves soil structure [16]. Despite the short experiment, OM might not yet play a role in improving soil aggregation in the substrate, but a looser substrate will facilitate root growth and, subsequently, shoot growth.

The CM contained a high total N of 2.06%, essential for leafy vegetable choy sum with leaves as edible parts [42]. Nitrogen promotes the cell division process and then vegetative growth; besides, N is the main constituent of protein. Nitrogen is a major nutrient in the chlorophyll and plays a vital role in the photosynthesis process. Sufficient N will ensure plant metabolism and facilitate plant organ growth, such as leaves, stems, and roots. The CM amendment can increase the total N in tailings. The CM enhances the total N content in the planting media. The total N in soil consisted of available $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$, as well as inorganic N such as NH_3, which are indirectly involved in the N cycle to provide available N for plant uptake.

Cow manure provides the macronutrients N, P, and K for plants, although their amounts are not as high as chemical fertilizer. Improving aeration and substrate porosity after CM amendment facilitates water and nutrient uptake. Manure also provides other macro- and micronutrients that play a prominent role in plant metabolism; cow manure is known to release Fe and Mn for photosynthesis and Mo for N metabolism [43]. Carbohydrates produced during photosynthesis are transported to all parts of the plant and become the primary energy source in plant metabolism, including cell division. Yield increments of food crops grown in mineral and peat soil have also been reported due to manure amendment [44, 45].

The organic C relates to the OM content in the substrate and is a good indicator of the abundance of microbes involved in the OM decomposition and nutrient cycle. After the experiment, the manure-treated tailing had at least 2% organic C. Applying a higher dose of CM increased organic C up to 7.87%, equal to 13.69% of OM. As long as the added CM contained a C/N ratio of less than 20, the decomposition process of organic matter would not cause N immobilization. In this study, no plants showed symptoms of chlorosis. The C/N ratio also determines the OM decomposition by heterotroph microbes because they require organic-C as an energy source and N as the main element of protein synthesis.

Providing OM over a long period decreases Hg availability since Hg in the planting medium is bound by negative charges of hydroxyl and carboxylate in the surface of OM particles [19]. Nevertheless, the Hg analysis in this research did not differentiate Hg species, so the Hg in the substrate (Table 5) was organic, inorganic, or gaseous. The decrease in Hg levels in the growing media compared to the control might be caused by dilution and methylation [27], which is released into the atmosphere.

The bacteria and fungi survive in the rhizosphere of choy sum grown in tailing-based substrates for five weeks. The microbial viability is correlated with the root exudation, which provides organic C, nutrients, and water for microbial survival. Unfortunately, the bacterial and fungal populations did not increase following the OM amendment. The experiment only counts the viable cells of bacteria and fungi, which cannot describe their function for soil fertility or soil bioremediation. This study found the presence of Hg microbes under extreme conditions, which is tailing contaminated with 310 $mg\ kg^{-1}$ of Hg. Nonetheless, the insignificant effect of CM on both microbial populations might be due to the short duration of the experiment and the non-differentiate media of NA and PDA.

The pot experiment was conducted only for short-term observation of plant growth and substrate quality in a controlled environment. The experimental method might not be directly utilized for field conditions. However, this study provides two valuable information for farmers: 1. The possibility of reducing Hg content in Hg-contaminated gold-mine tailing by mixing with CM; and 2. Accumulation of Hg in the edible part of Choy sum causes the vegetable to remain unsafe to be consumed. The local government should disseminate this finding to farmers who intend to grow vegetables in tailing deposit areas.

A study of the long-term impacts of CM on Hg dynamics in soil and their accumulation in edible parts of vegetables is needed. This experiment is for leafy vegetables and other vegetables, including roots, tubers, bulbs, fruits, and flowers.

4. CONCLUSIONS

The application of various doses of CM improved choy sum's growth and yield in mercury-contaminated tailing. However, the growth of choy sum in the tailing-based substrate was lower than that in field soil with a similar dose of manure. The organic-C, total N, and C/N ratio of substrate increased significantly following manure amendment. Any manure dose decreased the Hg of the substrate and its accumulation in plant shoots significantly but did not affect the bacterial and fungal population in the rhizosphere of choy sum. The highest content of manure (45%) in substrate resulted in the highest biomass and yield of choy sum and the lowest Hg level in substate and shoot.

This experiment suggested using manure for choy sum. However, the results of this research cannot yet be applied to farmers' fields because the Hg levels in choy sum leaves were still higher than the minimum threshold limit of Hg.

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