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Cow Manure for Improving Carbon-to-Nitrogen Ratio of Tailings, Growth, and Biomass of Water Kale (*Ipomoea reptans* Poir.)

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Abstract

Gold mine tailings are characterized by low organic matter, poor nutrient availability, extreme pH conditions, and unfavorable texture, all of which restrict plant growth. This study evaluated the effects of cow manure (CM) on soil chemical properties, carbon-to-nitrogen (C/N) ratio, and the growth and biomass of water kale (*Ipomoea reptans* Poir.) cultivated in tailings-based media. A greenhouse experiment was arranged in a randomized block design consisting of five treatments: 0% (control), 15%, 30%, 45%, and 60% CM (v/v), each replicated six times. Observations included soil pH, organic carbon, total nitrogen, C/N ratio, plant growth parameters, biomass, and microbial populations at 35 days after planting. The application of CM significantly increased organic C, total N, and the C/N ratio, with the highest values observed at 60% CM. However, higher CM doses reduced substrate pH to slightly acidic levels. The 15% CM treatment produced the greatest improvements in plant growth and biomass, increasing plant height by 21%, stem thickness by 29%, leaf number by 8%, and fresh and dry biomass by 73% and 120%, respectively, compared with the control. Increasing CM above 15% enhanced the C/N ratio but did not further improve plant performance, likely due to reduced nitrogen availability associated with higher organic matter and slower mineralization. CM applications did not significantly affect bacterial or fungal populations in the rhizosphere. Overall, the findings indicate that low-dose CM (15%) is optimal for improving the C/N ratio, supporting nutrient availability, and enhancing water kale growth in tailings. This study demonstrates the potential of organic amendments for rehabilitating gold mine tailings and highlights their role in restoring soil functionality and supporting sustainable revegetation efforts.

INTRODUCTION

The heavy metals, including cadmium, lead, arsenic, mercury, and chromium, can remain in the soil, harming crop health and food safety (Rashid et al., 2023). These contaminants enter agricultural areas from natural and anthropogenic sources (Muhammad et al., 2021; Briffa et al., 2020; Nabila & Budianta, 2025). The contamination of soil with heavy metals should be controlled since it threatens food web and human health.

Gold mining in Indonesia contributes to employment and foreign exchange. Companies with mining business permits conduct the majority of gold mining activities in Indonesia; however, the role of artisanal and small-scale gold mining (ASGM) remains significant. A critical issue with ASGM is tailings management; typically, a lack of systems for storing and processing tailings, which are the residual waste from gold ore extraction.

The gold content in the ore is minimal. Based on data from the Directorate General of Mineral and Coal (Ditjen

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Minerba, 2012), the gold content at the Pongkor Gold Mine in West Java averages 17.14 g t^{-1}. In other places, such as Halmahera, the gold content is only 1 mg kg^{-1} (Yanny et al., 2023), indicating that the tailings volume is far larger than the gold obtained. Another issue is that tailings storage is not well planned, resulting in storage sometimes occurring on productive agricultural land, thereby degrading the land's quality.

The general characteristics of gold mine tailings include a lack of organic matter and macronutrients for plants; however, they contain high levels of micronutrients and heavy metals (Syofiani & Oktabriana, 2018; Hindersah et al., 2018a). Low levels of organic carbon (C) and total nitrogen (N) (Hindersah et al., 2018b), along with extremely high or low acidity (pH) of tailings (Wangi et al., 2023; Dewi et al., 2023), limit the growth of agricultural plants. Both parameters play an important role in enhancing plant production and restoring the ecosystem function of mining land.

The physical properties of tailings limiting plant growth include high solid fractions of sand, clay, or silt (Yuarsah et al., 2017; Arranz-González and Cala-Rivero, 2017), which result in suboptimal porosity and permeability for water availability and root growth. Soil porosity is directly proportional to the content of soil organic matter and the abundance of roots and biota (Robinson et al., 2022). Empirical models explain that soil permeability depends on the percentage of fractions present in the soil (Suharyatun et al., 2023). Enhancing soil porosity and permeability with organic soil conditioners is always recommended to improve plant growth while simultaneously increasing the levels of C and N and the C/N ratio of the soil. These soil properties influence plant growth and the presence and activity of soil bacteria and fungi.

In addition to improving soil porosity and permeability, organic matter increases soil organic C and N levels (Li et al., 2022; Jiang et al., 2024). Compared with chemical fertilizers, the application of organic fertilizers increases more organic C levels but the application of organic matter accompanied by chemical fertilizers increases organic C and total N levels and changes the C/N ratio. This ratio affects soil functions such as decomposition of plant residues and the N cycle. Abbasi et al. (2015) explained that N mineralization occurs when organic matter with a C/N ratio of 12.7-26.4 is added to the soil, and spontaneous immobilization occurs when organic matter with a C/N ratio of 36.4-121.5 is added. A C/N ratio of 24 is ideal for soil microbes to stimulate the release and provision of N and P nutrients to plants (Schultheis et al., 2020).

Organic matter provides energy and carbon for heterotrophic soil microbes that play a vital role in the soil nutrient cycle. The application of vermicompost increased the abundance of Proteobacteria and Actinomycetes (Chen et al., 2022), while compost raised the total population of bacteria and fungi, along with their abundance compared to chicken manure and green manure (Alori et al., 2023).

Research on improving the characteristics of gold mine tailings by adding organic materials has not been widely conducted in Indonesia. Therefore, this study was carried out to evaluate changes in soil acidity, organic C and total N levels, the C/N ratio, and the growth and

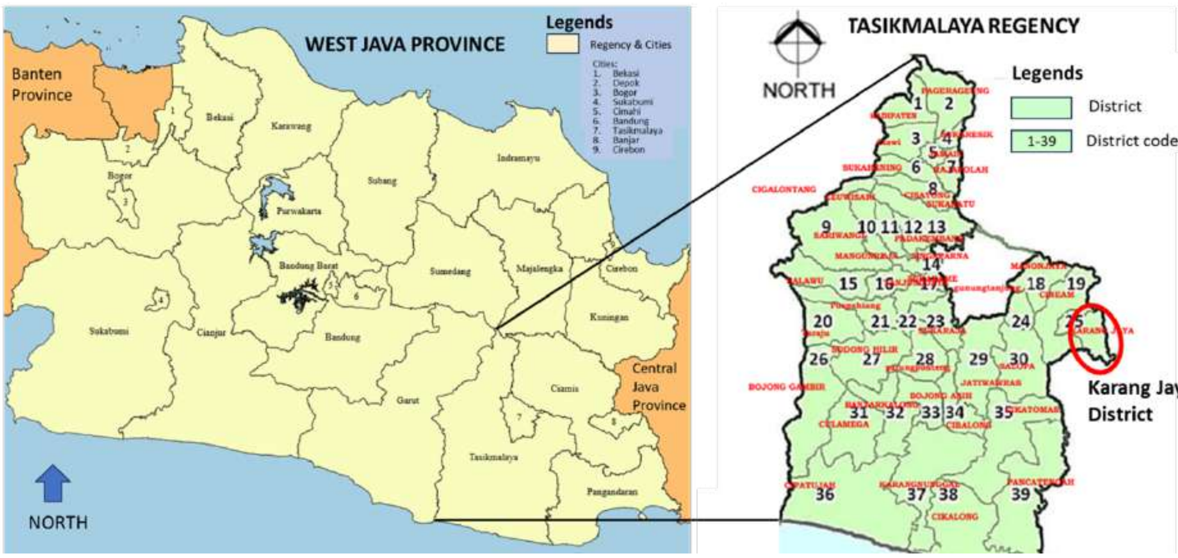

Figure 1. Location of community gold mines in Karanglayung Village, Karangjaya District, Tasikmalaya
Source: Map of West Java from commons.wikimedia.org; Map of Tasikmalaya Regency was from https://www.scribd.com/document/494852997/PETA-KAB-TASIK

Table 1. Chemical properties of tailings from ASGM in Karanaglayung Village before the experiment

Properties	Value	Status
Acidity	8.08	Alkaline
Organic C (%)	1.57	Low
Total N (%)	0.16	Low
C/N ratio	9.81	Low
Potential P_2O_5 HCl 25% (mg 100 g^{-1})	42.49	High
Available P_2O_5 Bray (mg kg^{-1})	9.45	Average
Potential K_2O HCl 25% (mg 100 g^{-1})	12.28	Low
Exchangeable cations		
Kex (cmol kg^{-1})	0.18	Low
Naex (cmol kg^{-1})	0.21	Low
Caex (cmol kg^{-1})	15.03	High
Mgex (cmol kg^{-1})	6.82	High
Cation exchange capacity (cmol kg^{-1})	3.51	Very low

biomass of water kale (*Ipomoea reptans* Poir.) planted in gold mine tailings with various doses of CM added.

MATERIALS AND METHODS

The pot experiment was conducted in the greenhouse of the Faculty of Agriculture, Padjadjaran University (Unpad) in Jatinangor, Sumedang, located at an altitude of 725 m above sea level from November 2022 to February 2023. Tailings were sourced from a community gold mine in Karangpaningal, Karanglayung Village, Karang Jaya District, Tasikmalaya Regency, with coordinates of 7˚26'40.574608'' S and 108˚21'23.417662'' E (Figure 1). The gold mining site is situated at an altitude of 430 m above sea level and is close to agricultural land, as approximately 38% of the village population is engaged in farming.

The total Hg content of the tailings deposited for about three months was 330 mg/kg. The tailings had a silty clay texture, containing 2% sand, 57% silt, and 41% clay, with a slightly alkaline pH (8.08). The chemical properties of the tailings (Table 1) indicate their low fertility for plant growth.

Cow Manure (CM) was obtained from the Unpad's Compost Processing Installation. The manure contained organic C 41.09%, total N of 2.06%, C/N ratio of 19.95, potential P of 0.56%, and potential K of 0.29%, with acidity and water content of 6.05 and 19.03%, respectively.

Experimental Design

The pot experiment carried out in the greenhouse was arranged in a randomized block design to evaluate four levels of CM (15, 30, 45, and 60%; v/v) mixed with tailing. The control treatment was tailings without CM. Each treatment was replicated six times, with three seeds sown in adjacent holes spaced 5 cm apart.

Experimental Setup

The tailings were air-dried, ground, and sieved through a 0.2 mm sieve. Tailings were mixed evenly with CM according to the CM treatments. From each mixture, 1 kg was taken and placed into a black polybag. Seeds of water kale cv Bangkok LP-1 from PT. East West Seed Indonesia were sown in three planting holes that were 2-cm deep and spaced 5 x 5 cm apart. The planting holes were covered with the same medium and watered with 50 mL of groundwater to moisten the medium around the seeds.

The 15-day-old plants were treated with 2.25 g/pot of NPK compound fertilizer (16-16-16), equivalent to 150 kg ha^{-1}. The fertilizer was placed in three holes, 2 cm deep, spaced 2.5 cm from the stem. The high concentration of clay and silt in the planting media prevented the plants from being watered to field capacity due to the muddying effect. Therefore, the volume of water was gradually increased according to the plant's growth stage. In the final week of the experiment, the plants were watered 100 mL per day. The plants were kept in a greenhouse without pesticides until they were 35 days old.

Observation and Statistical Analysis

The height of the plants, stem thickness, number of leaves, fresh and dry weight of plants, soil pH, organic carbon content, total nitrogen, and microbial population were measured 35 days after planting (DAP). To determine dry weight, fresh plant biomass was heated at 70 °C for two days until a constant weight was achieved. At the end of the experiment, the plants were separated from the planting medium, mixed uniformly, and 50 g of soil samples were collected for soil pH, organic C, and total N analysis. The plant roots were shaken to remove soil, and the soil attached to the root surface was collected and stored at 4 °C for microbiological analysis.

Total N in the planting medium was determined using the Kjeldahl method, while the concentration of organic C was determined using the Walkley and Black method (AOAC, 2019). The C/N ratio was calculated as the ratio of organic carbon to total nitrogen. The calculation of the population of soil bacteria and fungi was carried out using the serial dilution plate method on nutrient agar and potato dextrose media, respectively (Ben-David and Davidson, 2014).

Analysis of variance (F test, $p < 0.05$) was conducted for all observed variables. Duncan's Multiple Range Test at $p < 0.05$ was applied if the experimental treatment significantly affected the measured parameters. The average data for each parameter are presented in a table or histogram, accompanied by the standard deviation.

RESULTS AND DISCUSSION

Substrate Properties

The acidity of the tailings before being mixed with CM was 8.08. Adding 60% of CM to the tailings significantly lowered the pH of the substrate to a slightly acidic level, below 6 (Table 2). The ability of CM to decrease pH is clear in substrates with higher CM amounts. Soil acidity decreases as organic matter decomposes; in conditions of low substrate porosity, oxygen levels drop, and heterotrophic microbes obtain energy from fermenting organic matter, producing organic acids (Bailey-Serres et al., 2012). Planting kale requires a pH around 6.00 - 6.5 (Nitasari and Wahidah, 2020), so a pH below six could restrict plant growth.

The increase in organic C, total N, and C/N ratio in the substrate containing CM was proportional with the increase in CM dose (Table 2). The content of organic C and total N before the experiment was 1.57% and 0.16%, respectively. The substrate without CM contained slightly more organic C and relatively the same total N as the tailings before the experiment (Table 1). However, applying CM of 15% and 60% increased organic C by 72.45% and 281.82%, respectively, compared to the control. The increase in both parameters caused a rise in C/N ratio the substrate compared to the control. In this experiment, the source of total N was NPK fertilizer and CM. However, each treatment received the same NPK dose, so different CM doses caused the difference in total N. Before the experiment, CM contained 2.06% total N.

The increase in organic C and total N levels in this experiment aligns with the rise in soil organic matter levels, which is reported up to 2.36% compared to the application of N fertilizer, and 55.38% when organic matter is combined with N fertilizer (Jiang et al., 2024). Soil organic C and total N increased by 4.92% and 8.33%, respectively, with the addition of organic fertilizer (Li et al., 2022). Carbon is originally taken from the atmosphere by plants through photosynthesis, while nitrogen is mainly absorbed by roots from the soil and stored in plant biomass. Soil organic matter releases organic carbon and total nitrogen through enzymatic decomposition by soil microbes (Bai et al., 2023). Therefore, soil with organic matter amendments contains higher levels of organic C and total N.

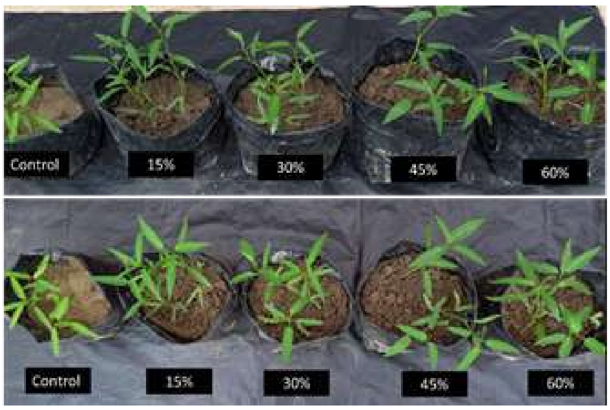

Figure 2. Water kale grown in the tailing-based substrate with various doses of cow manure

Table 2. Effects of various doses of cow manure on organic C, total N, and C/N of tailing-based substrate

Cow Manure (%)	pH	Organic C (%)	Total N(%)	C/N
Control	6.63 ± 0.11 b	1.96 ± 0.17 a	0.15 ± 0.08 a	13.27 ± 0.95 a
15	6.45 ± 0.10 b	3.38 ± 0.15 b	0.22 ± 0.03 b	14.97 ± 1.27 ab
30	6.37 ± 0.16 b	5.20 ±0.59 c	0.32 ± 0.02 c	16.06 ± 2.45 ab
45	6.33 ± 0.07 b	6.24 ±0.68 d	0.34 ±0.02 c	18.10 ± 2.38 bc
60	5.98 ± 0.41 a	7.47 ±1.51 e	0.39 ±0.06 d	19.07 ± 3.78 c

Remark: Values in columns followed by the similar numbers show no significant difference according to Duncan's multiple range test at $p < 0.05$.

Table 3. Effect of various doses of cow manure on the height of water kale grown on the tailing-based substrate at day 35

Cow Manure (%)	Plant height (cm)		Stem thickness (mm)		Leaf number	
Control	18.00 ± 1.14	a	1.58 ± 0.05	a	10.39 ± 0.44	a
15	21.83 ± 0.44	c	2.04 ± 0.06	c	11.22 ± 0.17	b
30	19.66 ± 0.90	b	1.91 ± 0.05	bc	10.72 ± 0.33	a
45	19.46 ± 0.49	b	1.88 ± 0.13	b	10.83 ± 0.41	b
60	18.92 ± 0.74	ab	1.82 ± 0.03	ab	10.67 ± 0.37	ab

Remark: Values in columns followed by the same number show no significant difference according to Duncan's multiple range test at $p < 0.05$.

Table 4. Effect of various doses of cow manure on the height of water kale grown in Inceptisols at day 35

Cow Manure (%)	Plant height		Stem thickness		Leaf number	
Control	31.53 ± 0.47	ab	3.94 ± 0.37	a	13.31 ± 0.47	ab
15	38.20 ± 0.65	c	4.93 ± 0.49	c	13.82 ± 0.65	c
30	33.60 ± 0.56	b	4.57 ± 0.43	bc	13.51 ± 0.56	b
45	39.03 ± 0.49	a	4.21 ± 0.07	b	12.60 ± 0.49	a
60	36.47 ± 0.73	ab	4.25 ± 0.24	b	12.84 ± 0.73	ab

Remark: Values in columns followed by the similar numbers show no significant difference according to Duncan's multiple range test at $p < 0.05$

Plant Growth and Biomass

Despite the slow development of water kale, CM increased plant height, stem thickness, and leaf number (Figure 2 and Table 3). The effect of 15% CM on growth parameters was better than the other treatments, and it indicates that using chemical fertilizer alone is not sufficient; therefore, it is necessary to add organic fertilizer. Plants treated with CM 15% are tallest at 35 DAP, measuring 21.83 cm, an increase of 21.27% compared to the control treatment. The average height, stem thickness, and leaf number of plants grown in tailing-based substrates were 19.75 cm, 1,86 cm, and approximately 10 leaves (Table 3), compared to 36 cm, 4.38 cm, and 13 leaves of plants grown in Inceptisols (Table 4). The significantly reduced plant height in the tailings-based substrate compared to that in mineral soil with organic matter treatment was also reported (Hasan and Pakaya, 2020).

The water kale was utilized as a test plant because it responds well to the addition of organic matter and tolerates suboptimal soil conditions (Muntashilah et al., 2015; Zahanis et al., 2018). Their ability to withstand degraded soil conditions was clearly demonstrated in this research; the plants survived in tailings-based substrates despite the limited growth.

Application of 15, 30, 45, and 60% CM showed a better effect on stem thickness and leaf number and was significantly different from the control (Table 3). At 35 DAP, the treatment of 15% CM mixed with chemical fertilizer showed a tendency to produce thicker stems than the control. The maximum stem thickness was 2.04 mm, which is a 29.12% increase compared to the control. This treatment also increased the leaf number more than other treatments and was significantly different from the control. The highest leaf number was 11.22, representing a 7.99% increase over the control (Table 3). Cow manure provides macro-and micronutrients for plant growth and leaf development, as shown in aromatic pepper and shallot (Baiyeri et al., 2016; Mulyati et al, 2022). Stem thickness and leaf number are important growth characteristics to support maximum production (Gallegos-Cedillo et al., 2021).

Table 5. Effect of various doses of cow manure on the biomass of kale grown on the tailing-based substrate at day 35

Cow Manure (%)	Fresh weight (g)		Dry weight (g)	
0	1.13 ± 0.08	a	0.10 ± 0.008	a
15	1.95 ± 0.19	d	0.22 ± 0.01	c
30	1.69 ± 0.08	c	0.15 ± 0.02	b
45	1.48 ± 0.10	b	0.13 ± 0.006	a
60	1.39 ± 0.12	b	0.13 ± 0.007	a

Remark: Values in columns followed by the similar numbers show no significant difference according to Duncan's multiple range test at $p < 0.05$

Treatment of 15% CM tends to produce higher fresh and dry weights and is statistically different from treatments of 30%, 45%, 60% CM, and the control. Table 5 shows that the 15% CM treatment consistently yielded the highest fresh and dry weights compared to other treatments, and these differences are statistically significant. The highest fresh weight and dry weight were 1.95 g and 0.22 g, respectively, representing increases of 72.57% and 120% compared to the control treatment. A plant's structural development, reflected by its height and stem thickness, plays a role in determining its total biomass (Zhong et al., 2019). While an increased leaf count boosts photosynthetic efficiency, thereby elevating organic compound production essential for growth and biomass accumulation (Bielczynski et al., 2017).

However, 60% of CM significantly reduced the dry weight of intact plants and roots compared to the application of 15% of CM. The increase in C/N ratio to 16-19 in the substrate with 30-60% of CM caused the nitrogen availability in the soil to depend mainly on the enzymatic degradation activity on organic matter. Low N in soil is associated with a low rate of microbial respiration of organic matter (Hadas et al., 2004).

Bacterial and Fungal Population in the Rhizosphere

Low and high doses of CM did not change the total soil microbial population (Figure 3). On a logarithmic scale, the populations of heterotrophic bacteria and fungi in the rhizosphere of kale in all treatments were log 10 and 4, respectively. This value equals 1010 CFU/g for bacteria and 104 CFU/g for fungi.

Acidity, organic C content, and soil C/N ratio are the most critical factors affecting the enzyme activity of soil heterotrophic microbes and the composition of the microbial community across soil types and climate zones (Xu et al., 2020). The application of organic matter increases the gene pool of microbes involved in C degradation from organic matter and N mineralization (Xie et al., 2022). Microbes with heterotrophic metabolism in the soil heavily depend on organic matter.

The C/N ratio did not affect the bacterial and fungal populations in nutrient agar and potato dextrose agar, respectively. These media cannot reflect the diversity and types of metabolism. The certainty of changes in microbial composition can be determined through metagenomic analysis. According to Gawol et al. (2022), the soil C/N ratio influences the bacterial community and determines the diversity of bacterial Phylum, Class, Order, and Family. Conversely, fungi are highly adaptable to changes in the C/N ratio, which ranges from 7 to 126 (Camenzind et al., 2021); in this study, the C/N ratio of the planting medium was 13.27 to 19.0.

Organic carbon and total nitrogen determine soil chemical quality. However, in this experiment, an increase in both parameters in the planting medium still limits the growth of water kale. In this study, substrate texture was a factor that inhibited plant growth more than carbon and nitrogen levels or the C/N ratio. Growth was hindered by the dominance of fine particles in the planting medium; clay particles

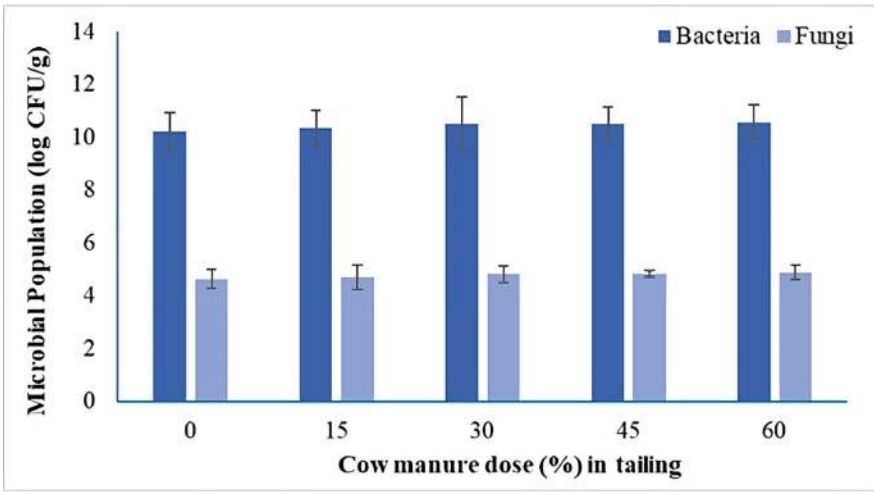

Figure 3. Total bacterial and fungal counts in the rhizosphere of water kale grown in tailing-based substrates with various doses of Cow Manure.

(diameter 0.002 mm) and silt (diameter 0.002-0.05 mm) reduced soil porosity, leading to decreased water availability. The dense texture of tailings increases specific gravity and reduces the capacity of tailings to store water (Veldkamp et al., 2020). This condition diminishes the ability of plant roots to absorb water and nutrients.

A higher CM content in the substrate can lower the pH and enhance the mobilization and absorption of essential and non-essential metals (Sintorini et al., 2021). Sufficient metal absorption can elevate the levels of essential metals, which are beneficial for human health; for example, iron interferes with the growth of primary and lateral roots (Li et al., 2016). Therefore, future research must consider the levels of heavy metals in the shoots since the concentrations of metals and heavy metals in tailings are generally high (Hindersah et al., 2018a; Okewale and Grobler, 2023).

The C/N ratio in soil determines the balance of important elements for plant growth and microbial health (Klopp, 2024). Ideally, the soil C/N ratio is 10 (Schultheis et al., 2020), allowing the soil to function optimally for decomposing plant residues and ensuring the N cycle. In this experiment, the C/N ratio did not exceed 25 when microbes immobilized N into their cells (Robertson and Groffman, 2015), thereby reducing the availability of N for plants. There was no chlorosis in the young leaves of any plant during the experiment, indicating that no N immobilization occurred.

Generally, plant growth parameters increased in tailings with various doses of CM. Increasing the C/N ratio induces organic matter degradation by beneficial soil microbes. Nonetheless, the plate count method for determining fungal and bacterial populations cannot distinguish their functions and pathogenicity. It is suggested that organic matter enhances the function of bacteria or fungi that release secondary metabolites into the soil to stimulate plant growth (Orozco-Mosqueda et al., 2023). Therefore, plant growth with CM is better because it plays a dual role in improving the structure of tailings, providing more nutrients, especially N, and inducing the proliferation of phytohormone-producing bacteria.

CONCLUSIONS

Adding 15-60% of CM into the tailings improved organic carbon, total nitrogen, and carbon-to-nitrogen (C/N) ratio of the planting medium, as well as the growth and biomass of water kale plants grown in pots. Only the application of 60% CM slightly reduced the soil pH to acidic levels. However, all doses of CM did not alter the total bacterial and fungal populations in the rhizosphere. This greenhouse experiment determined that adding 15% CM into a tailing-based substrate resulted in improved C/N ratios and pH for plant growth compared to other CM levels. The 30-60% CM increased the C/N ratio of the planting medium, potentially reducing nitrogen (N) availability for plant uptake. Therefore, plant growth with 15% CM is better than that with higher doses of CM. Further research recommends using CM doses of less than 15%. This experiment confirms that organic matter is crucial for improving soil C/N ratios and promoting plant growth. CM is recommended for rehabilitating gold mine tailings, primarily to support revegetation alongside chemical fertilizers that supply essential nutrients, such as nitrogen (N), phosphorus (P), and potassium (K).

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