# MICROBIAL FERTILIZER FROM CO-COMPOSTED SLUDGE USING MISHIMAX-50 SYSTEM

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**Abstract -** Sewage sludge from municipal and dairy wastewater treatment in Vietnam poses environmental challenges due to landfill overload and groundwater contamination. This study investigated co-composted sludge-derived microbial fertilizer (MK50) as a sustainable agricultural alternative. Sludge was co-composted with wood chips using mixing and thermal stabilization, producing MK50 with heavy metal levels below Vietnam's safety thresholds. Microbial analysis revealed high densities of phosphate-solubilizing (1.45 × 10^6 CFU/g) and cellulose-degrading (2.16 × 10^6 CFU/g) bacteria, surpassing commercial biofertilizers. Field trials on water spinach and purple amaranth showed MK50 yields (3.33 kg/m² and 5.00 kg/m², respectively) comparable to or better than commercial fertilizers, with improved morphometric parameters ($p < 0.05$). However, MK50's low nitrogen content (2.08%) led to less vibrant foliage. These results suggest MK50's potential for sustainable agriculture by valorizing sludge through microbial activity, though further research is needed to optimize nutrient profiles and long-term efficacy.

**Key words –** Sludge; co-composting; microbial fertilizer; insoluble phosphate bacteria; cellulose decomposer.

## 1. Introduction

Sewage sludge, a byproduct of municipal and industrial wastewater treatment, including dairy processing, presents significant management challenges in Vietnam due to increasing production and limited landfill capacity [1]. Current reliance on landfilling is unsustainable, as overloaded sites and high-moisture leachate pose risks of groundwater contamination [2]. Activated sludge from aerobic treatment harbors diverse microbial communities, making it a valuable resource for producing microbial fertilizers [3]. Co-composting, the controlled aerobic decomposition of organic matter mixed with bulking agents like wood chips or straw, stabilizes sludge, reduces pathogens, and reduce the availability of heavy metals yielding nutrient-rich biofertilizers [4, 5]. This process leverages the metabolic capabilities of sludge-derived microorganisms to transform complex compounds into plant-available nutrients [6].

Microbial activity in soil stimulates plant growth and enhances crop productivity by making essential nutrients more accessible to plants. Soil microorganisms, particularly phosphate-solubilizing bacteria, convert insoluble phosphates into bioavailable forms, enhancing plant phosphorus uptake and promoting growth [7]. Cellulose-degrading microbes break down complex organic matter into simpler compounds, improving soil structure and nutrient availability for crops [8]. The synergistic action of soil microorganisms, including phosphate-solubilizing and cellulose-degrading bacteria supports sustainable agriculture by improving soil fertility and plant vigor.

Microbial communities in sewage sludge exhibit robust metabolic capabilities, rendering them suitable for producing biofertilizers that promote sustainable crop production. Analysis of 19 distinct sludge samples revealed consistent microbial diversity across sources, with dominant phyla including Proteobacteria, Bacteroidetes, and Firmicutes, in descending order of abundance [9]. Proteobacteria demonstrate extensive metabolic versatility, contributing to critical environmental processes such as carbon, nitrogen, sulfur, and phosphorus cycling. Bacteroidetes, often proteolytic, facilitate protein degradation into volatile phenolic acids and ammonia, with their prevalence linked to total solid content under anaerobic conditions. Firmicutes exhibit adaptability in degrading diverse environmental substrates. Research by Zhou et al. demonstrated that sludge-derived fertilizers enhance soil microbial diversity and structure, thereby improving crop performance indicators [10]. Furthermore, compost from sewage sludge ameliorates saline-alkaline soils, increasing soil carbon and nitrogen content [11]. Despite these benefits, the microbial activity and agronomic efficacy of sludge-derived fertilizers, particularly for crops like water spinach (*Ipomoea aquatica*) and purple amaranth (*Amaranthus cruentus*), warrant further investigation.

We hypothesize that co-composted sludge fertilizer (MK50) promotes crop growth primarily through microbial-mediated transformation of soil nutrients. This study aimed to: (1) assess MK50's safety via heavy metal analysis, (2) characterize the phosphate-solubilizing and cellulose-degrading activities of its microbial communities post-co-composting, and (3) evaluate its impact on crop growth and yield compared to commercial fertilizers.

## 2. Materials and methods

### *2.1. Materials and Sludge collection*

Sewage sludge was collected at a municipal wastewater treatment plant and a dairy processing facility in [Danang, Vietnam]. Sludge samples were collected from various facilities, including wastewater treatment sludge from the Vinamilk dairy plant (VNM) as well as municipal wastewater treatment plants. The samples from the municipal plants were collected and processed in three distinct batches, labeled D5, D6, and D7.

Chemicals were purchased from Xilong Company in China or will be specified. In all tests, including laboratory and field tests, Song Gianh HC-15 microbial organic fertilizer was used as the control group.

### *2.2. Co-Composting*

The sludge was mixed with cedar wood chips at a 4:1 ratio (w/w) to enhance aeration and reduce moisture content. A total of 900 kg of sludge was processed, with incremental additions, around 50 kg of sludge. The mixture was co-composted in a Mishimax-50 composting system (Japan) for 3 months. The system was equipped with automated turning with the speed of 4 rpm to ensure adequate aeration and continuous hot air injection to maintain temperatures of 50–55°C (not over 70°C), facilitating pathogen elimination. The resulting microbial fertilizer (MK50) was air-dried, sieved through a 2 mm mesh, and stored at 4°C for microbial analysis. For field application, the biofertilizer was packaged in sealed bags and stored at ambient temperature until use in experimental crop cultivation.

### *2.3. Sludge Composition Analysis*

To assess the safety of MK50, concentrations of heavy metals (Pb, Cd, Cr, Cu, Zn) in the initial sludge and composted MK50 were analyzed by Quatest 2. Results were compared against Vietnam's hazardous waste thresholds (QCVN 01-189:2019/BNNPTNT) [12].

Prior to and following co-composting, the sewage sludge and resulting fertilizer were analyzed for moisture content, ash content, and total phosphorus (expressed as $P_2O_5$). Moisture content was determined by oven-drying 100 g samples at 105°C for 24 hours until constant weight was achieved [13]. Ash content was measured by incinerating 5 g dried samples at 550°C for 4 hours in a muffle furnace, with the residue weighed to calculate the ash percentage [14]. Total phosphorus ($P_2O_5$) was quantified using the ammonium molybdate spectrophotometric method after acid digestion of 1 g samples, following standard protocols [15]. The nitrogen content of pre and post co-composted sludge were determined using the Kjeldahl method [16]. All analyses were performed in triplicate to ensure accuracy, and results were expressed on a dry weight basis.

### *2.4. Microbial Activity Analysis*

The density of aerobic microorganisms in co-composted sludge (MK50) was determined using the serial dilution method, followed by spread plating on Plate Count Agar (HiMedia, India) and incubation at 30°C for 48 hours [17].

To assess phosphate-solubilizing and cellulose-degrading activities, Pikovskaya's medium containing tricalcium phosphate and Carboxymethylcellulose (CMC) agar were used, respectively [18, 19]. For microbial enumeration, 10 g of co-composted sludge was homogenized in 90 mL of sterile saline, serially diluted, and spread onto the respective agar media. Phosphate-solubilizing bacteria were identified by clear halos around colonies after 7 days of incubation at 30°C, while cellulose-degrading bacteria were detected by clear zones post-staining with Congo red after 1 day of incubation. Microbial densities was expressed as colony-forming units per gram (CFU/g).

For quantitative activity assessment, 10 g of sludge was incubated in liquid Pikovskaya's or CMC medium containing tricalcium phosphate or CMC, respectively. After 7 days (phosphate) or 1 day (cellulose), cultures were centrifuged at 4000 rpm for 10 minutes. One milliliter of supernatant was reacted with ammonium molybdate or dinitrosalicylic acid (DNS) reagents to quantify soluble phosphate or reducing sugars, respectively [20, 21]. Soluble phosphate was measured spectrophotometrically at 820 nm, calculated using the standard curve $y = 0.2469x + 0.2515$ ($R^2 = 0.9669$). Reducing sugars from CMC hydrolysis were quantified at 540 nm, based on the standard curve $y = 2.9367x - 0.0058$ ($R^2 = 0.99924$).

### *2.5. Field Trials*

Field experiments were conducted in Tuy Loan, Vietnam from August–October, 2024 on water spinach (*Ipomoea aquatica*) and purple amaranth (*Amaranthus cruentus*). Two treatments were applied: (1) MK50 (5 t/ha +100 kg/ha top-dressing) and (2) commercial fertilizer (5 t/ha organic base + 100 kg/ha NPK top-dressing). Each plot measured 3 m². Crops were grown under standard irrigation and management practices.

Growth parameters (stem length, leaf length, leaf width) were measured on 33 randomly selected plants per plot at harvest (28-30 days post-planting) using a digital caliper. Yield was determined as total biomass per unit area (kg/m²).

### *2.6. Statistical Analysis*

Growth parameter data from field trials were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc test to compare treatments ($p < 0.05$). The yield of each crop treated with fertilizers was summarized as means per $m^2$. All analyses were performed using R (version 4.2.1) [18].

## 3. Results and Discussion

### *3.1. Evaluation of Sludge Composition*

The sludge was analyzed for hazardous heavy metal content before treatment, with the results presented in Table 1. Nutrient compositions relevant to agricultural soil, both before and after treatment, are shown in Table 2.

The results indicate that heavy metal concentrations in the input sludge were significant lower than the hazardous waste thresholds specified in the National Technical Regulation (QCVN 01-189:2019/BNNPTNT). The results indicate that the sludge complies with the standards for both toxic and safe substances, making it suitable for disposal in the treatment system and for agricultural use. Additionally, previous studies have demonstrated that using composted sludge can effectively reduce toxic substances and harmful microorganisms [4-6].

Additionally, the sludge contained significant amounts of organic carbon, nitrogen, and available phosphorus. These nutrient components remained largely unchanged during treatment, as demonstrated by the minimal differences in values before and after processing (Table 2). The treatment process significantly reduced the sludge's moisture content from over 90% to below 40%. This reduction not only mitigated odor but also improved the sensory characteristics of the sludge. Lower moisture content enhances the efficiency of storage and utilization of the treated product, confirming its safety for agricultural applications. However, nutrient analysis indicates that the sludge is not a particularly rich source of nutrients for crops due to its relatively low nutrient content. Following research will focus on analyzing the biological activity of microbial communities in the treated sludge to evaluate its potential as a microbial fertilizer, providing beneficial microorganisms for agricultural soils.

***Tables 1.*** *The hazardous heavy metal content before treatment*

| No. | Parameter | Results | Unit | QCVN 01-189:2019/BNNPTNT, hazardous waste thresholds |
|---|---|---|---|---|
| 1 | As | 5.122 | mg/kg | ≤10 |
| 4 | Cd | 2.135 | mg/kg | ≤5 |
| 5 | Pb | 119.5 | mg/kg | ≤200 |
| 10 | Hg | <0.3 | mg/kg | ≤2 |
| 13 | Total oil | 128.6 | mg/kg | 1,000 |
| 14 | Phenol | ND (MDL=0.3) | mg/kg | 20,000 |
| 15 | Benzene | ND (MDL=0.5) | mg/kg | 10 |
| 16 | Chlorobenzene | ND (MDL=0.5) | mg/kg | 1,400 |
| 17 | Toluene | ND (MDL=0.5) | mg/kg | 20,000 |
| 18 | Naphthalene | ND (MDL=1.0) | mg/kg | 1,000 |

***Table 2.*** *Nutrient compositions of sludge relevant to agricultural soil, both before and after treatment*

| No. | Parameter | Unit | Before treatment | After treatment |
|---|---|---|---|---|
| 1 | Humidity | % | 90.42 | 37.05 |
| 2 | Ash | % | 19.93 | 20.16 |
| 3 | Total organic carbon | g/100g dried matter | 26.73 | 26.44 |
| 4 | Total nitrogen | g/100g dried matter | 2.64 | 2.08 |
| 5 | $P_2O_5$ | g/100g dried matter | 2.29 | 2.85 |

### *3.2. Evaluation of Density and Activity of Beneficial Microbial Communities in Treated Sludge*

This study examined the density and activity of beneficial microbial communities in treated sludge, specifically focusing on their capabilities for phosphate solubilization and cellulose degradation. The total density of aerobic microbes in treated sludge was significantly lower-by a factor of 3 to 6.7-compared to a commercial organic-microbial fertilizer control (SG). However, the density of beneficial microbes that can solubilize insoluble phosphates and degrade cellulose in treated sludge was significantly higher than in the control group (Figure 1). According to Vietnamese standards, microbial fertilizers must contain at least 10^6 CFU/g of each beneficial microbial type based on specific product claims when cultured on designated media. The densities of phosphate-solubilizing and cellulose-degrading microbes in treated sludge met this standard, although variability was observed based on the sludge source. For instance, VNM sludge exhibited a phosphate-solubilizing microbial density of 1.45 × 10^6 CFU/g (with a range of 1.39–1.52 × 10^6 CFU/g) and a cellulose-degrading microbial density of 2.16 × 10^6 CFU/g (with a range of 2.08–2.53 × 10^6 CFU/g). In contrast, the density of cellulose-degrading microbes in municipal wastewater sludge varied significantly across different batches. Batch D5 had a low density of less than 0.6 × 10^6 CFU/g, while batches D6 and D7 demonstrated considerably higher densities, ranging from 1.76 to 2.83 × 10^6 CFU/g, respectively. Overall, the proportion of beneficial microbes relative to total aerobic microbes in treated sludge-based microbial fertilizers was significantly higher than in the commercial control (Figure 1). This suggests that smaller quantities of sludge-derived fertilizers could achieve comparable efficacy to commercial products.

No clear zones of phosphate solubilization were observed around colonies of co-composted municipal wastewater sludge on media containing insoluble phosphates across all three batches (D5, D6, D7). This absence may reflect limitations in the solid-phase culturing method. Soluble product formation was assessed in liquid media supplemented with insoluble phosphates and cellulose substrates to more accurately evaluate microbial activity. Following a 7-day incubation period, solubilized phosphate was quantified via a colorimetric assay using molybdate reagent, with concentrations determined against a pre-established phosphate standard curve. Similarly, cellulose degradation activity was measured by quantifying reducing sugars via a colorimetric 3,5-dinitrosalicylic acid (DNS) assay, referenced to a pre-established standard curve (Figure 2).

Phosphate solubilization activity correlated closely with bacterial density, as evidenced by significantly higher phosphate ion concentrations in the culture media of VNM samples after 7 days of incubation. Although no phosphate solubilization zones were observed on solid media for municipal wastewater sludge samples (D5, D6, D7), increased phosphate ion levels in liquid media confirmed the presence of insoluble phosphate solubilization activity in these samples. Quantitative analysis provided greater precision in assessing activity: bacterial density in the commercial organic-microbial fertilizer (SG) was approximately 1.8 times lower than in the VNM sludge sample, yet its phosphate solubilization activity was fivefold lower, corresponding to significantly smaller solubilization zones around colonies compared to VNM. In contrast, cellulose degradation activity, measured as CMCase activity, showed minimal variation across samples, with the D7 sample exhibiting the highest activity, surpassing VNM by only 0.1 U/mL. During co-composting, sludge microbial communities were processed with wood chip substrates, which likely enhanced the CMCase activity of indigenous microbes.

Collectively, these results demonstrate that the beneficial microbial communities in co-composted sludge exhibit robust activity, highlighting their potential as a viable source of microbial fertilizers or for other applications requiring diverse and active microbial populations.

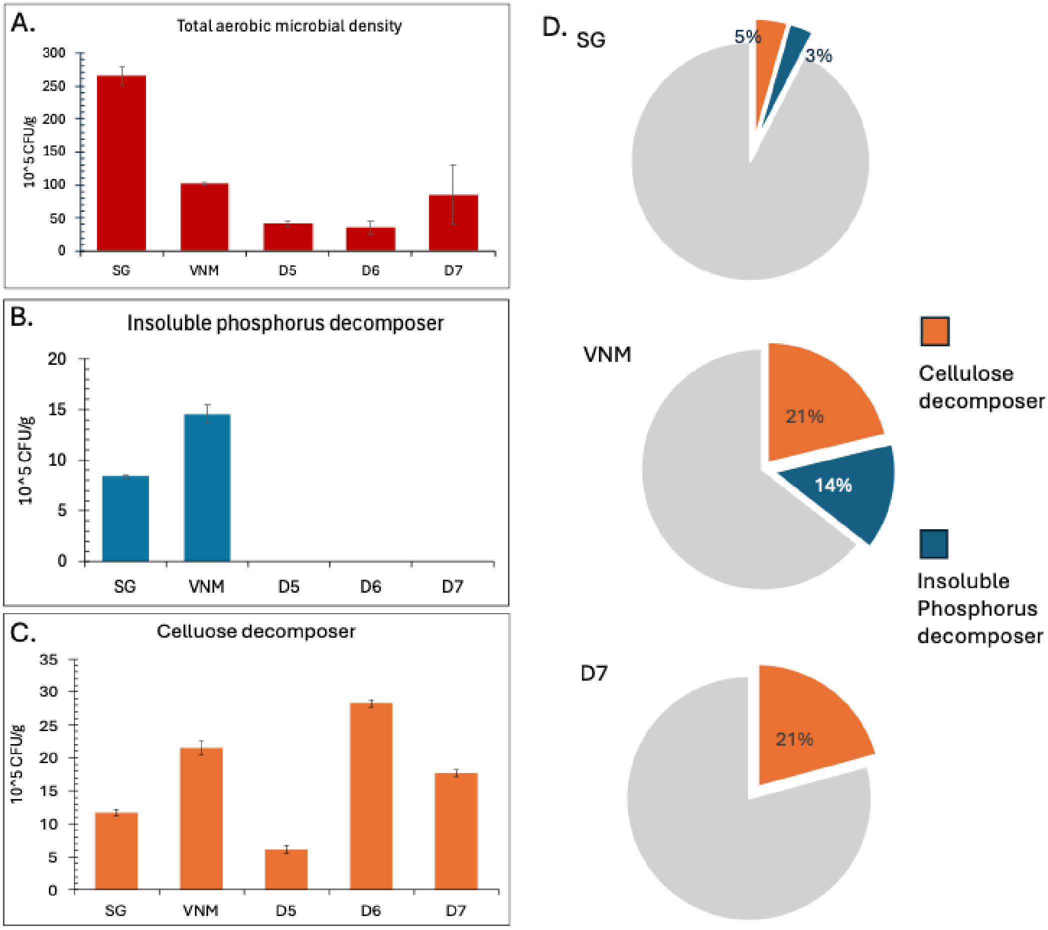


***Figure 1****. The density of total aerobic microorganisms (A.), insoluble P decomposer (B.), and cellulose decomposer (C.) in sludge after treatment. The sludge was collected from different sources including wastewater treatment sludge from the Vinamilk dairy plant (VNM), and municipal wastewater treatment plants (D5, D6, D7). The commercial organic fertilizer (SG) was used as positive control. (D.) Pie charts illustrating the percentage of beneficial microbial density relative to total aerobic microbial density in various sludge samples. Dark blue segments represent the proportion of phosphate-solubilizing microorganisms, and orange segments represent cellulose-degrading microorganisms*

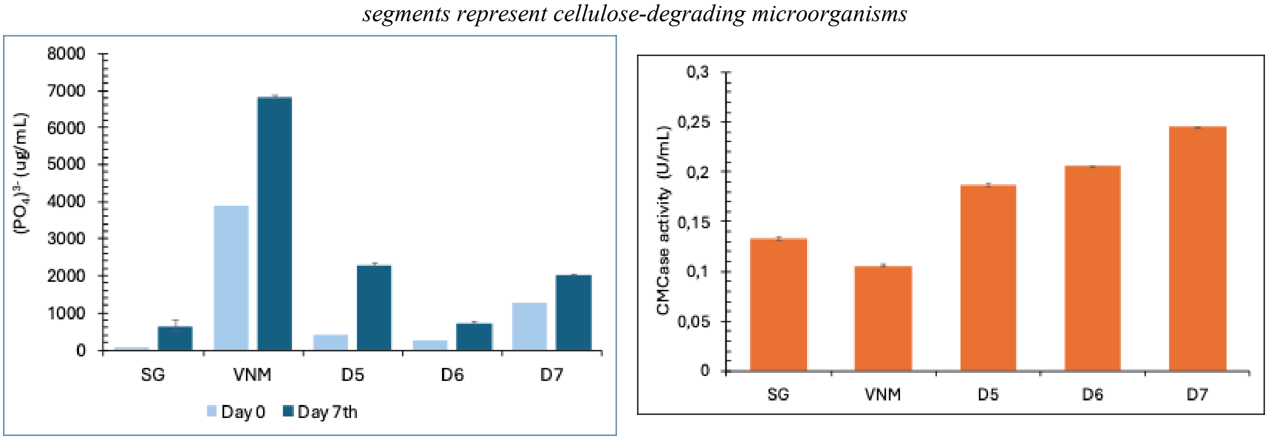


***Figure 2****. Microbial activity of phosphate solubilization (left) and cellulose degradation (right) in co-composted sludge from various sources. Sludge samples were collected from the Vinamilk dairy plant wastewater treatment facility (VNM) and municipal wastewater treatment plants (D5, D6, D7). A commercial organic-microbial fertilizer (SG) served as the positive control. CMCase activity was determined by subtracting the activity of heat-inactivated enzyme (boiled for 10 minutes)*

### *3.3. Effects of Microbial Fertilizer Derived from Co-Composted Sludge on Crop Cultivation*

Treated sludge, processed through co-composting, was utilized as a microbial fertilizer source for experimental cultivation of water spinach (*Ipomoea aquatica*) and purple amaranth (*Amaranthus cruentus*) in the fields of farmers belonging to the Tuy Loan Vegetable Cooperative. At each farm, four parallel plots were established: two for water spinach and two for purple amaranth. For the control plots (Commercial), farmers applied conventional care and fertilization practices. In the experimental plots, conventional fertilizers were entirely replaced with microbial fertilizer derived from co-composted sludge. In large-scale field experiments, the microbial fertilizer, designated as MK50, was exclusively sourced from sludge obtained from an municipal wastewater treatment plant due to the large quantities required for field-scale trials. Laboratory analyses revealed no significant differences in microbial density (>10^6 CFU/g) or degradation activities (CMCase and phosphorus solubilization) among batches or compared to the VNM sample. This consistency justified the use of mixed sludge to simplify processing and ensure sufficient material for field application. The VNM sludge was excluded from field trials due to limited availability and its role as a comparative reference rather than the primary focus of this study.

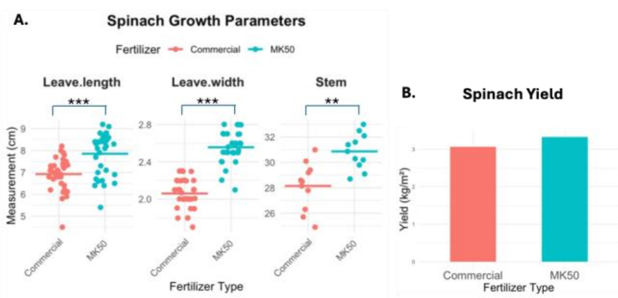


***Figure 3.*** *The development of water spinach cultivating with different fertilizer types in the field (A) Scatter plots showing stem length, leaf length, and leaf width under treated sludge with co-composting (MK50) and Commercial fertilizer treatments, with solid lines indicating mean values. (B) Bar showing yield per plot. **p=0.0025, ***p<0.0001 show the significant different between spinach cultivating by MK50 and commercial fertilizer by t-test*

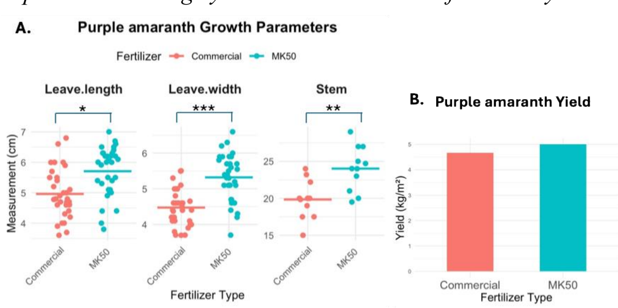


***Figure 4.*** *The development of purple amaranth cultivating with different fertilizer types in the field (A) Scatter plots showing stem length, leaf length, and leaf width under treated sludge with co-composting (MK50) and Commercial fertilizer treatments, with solid lines indicating mean values. (B) Bar showing yield per plot. *p=0.0199, **p=0.0057, ***p=0.0017 show the significant different between purple amaranth cultivating by MK50 and commercial fertilizer by t-test*

In field trials conducted on water spinach (*Ipomoea aquatica*) and purple amaranth (*Amaranthus cruentus*), the application of microbial fertilizer derived from co-composted sludge (MK50) resulted in yields comparable to, and occasionally exceeding, those obtained with commercial fertilizers (comprising organic base fertilizers and chemical top-dressing fertilizers). The yield of water spinach and purple amaranth under MK50 treatment was 3.33 kg/m² and 5.00 kg/m², respectively, compared to 3.07 kg/m² and 4.67 kg/m² for commercial fertilizers. Morphometric parameters, including stem length, leaf length, and leaf width, were significantly higher ($p < 0.05$) in both crops when treated with MK50 compared to the control plots. However, water spinach treated with commercial fertilizers exhibited a darker green coloration, which is more visually appealing to consumers. This difference may be attributed to the higher nitrogen content in the NPK fertilizer applied during the top-dressing phase, which promotes darker and thicker foliage. In contrast, the nitrogen content in the MK50 microbial fertilizer was relatively low (approximately 2.08%), potentially insufficient to support optimal leaf development during the vegetative growth stage. Nevertheless, the microbial fertilizer derived from treated sludge demonstrates significant potential for agricultural applications. Further research is required to optimize its formulation and develop detailed application guidelines for farmers, necessitating long-term and in-depth studies.

## 4. Conclusion

This study confirms the efficacy and safety of microbial fertilizer derived from co-composted sludge (MK50) for sustainable crop production. Initial sludge analysis revealed heavy metal concentrations below hazardous thresholds (QCVN 01-189:2019/BNNPTNT), ensuring its suitability for agricultural use. The treated sludge exhibited robust microbial activity, with phosphate-solubilizing and cellulose-degrading microbial densities significantly exceeding commercial organic-microbial fertilizers. Field trials on water spinach (*Ipomoea aquatica*) and purple amaranth (*Amaranthus cruentus*) demonstrated that MK50 achieved yields comparable to or surpassing commercial fertilizers, alongside significantly improved morphometric parameters ($p < 0.05$). However, lower nitrogen content (2.08%) in MK50 led to less vibrant foliage compared to NPK-treated plants. Further research is needed to optimize MK50's nutrient profile, validate this hypothesis through mechanistic studies, and conduct replicated trials to ensure yield consistency, paving the way for its widespread adoption in sustainable agriculture.

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