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Effect of Fermented Biofertilizer Dosage on Protein, Vitamin, and Heavy Metal Content in Spinach

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Abstract
Purpose: This study evaluated the effect of increasing dosage levels of a fermented liquid biofertilizer on the nutritional and heavy metal safety profile of fresh spinach (*Amaranthus* sp.), measuring protein, vitamin A, vitamin C, lead (Pb), and cadmium (Cd) content.
Research Methodology: A completely randomized design was used with an untreated control and six biofertilizer dosage levels (*P0* to *P5*), analyzed in duplicate. Protein, vitamin A, and vitamin C were determined by titrimetric methods, and Pb and Cd by atomic absorption spectrophotometry against certified calibration curves.
Results: Protein rose from 1.15 to 2.07 g/100 g from control to the highest dosage. Vitamin A rose from 6,157.40 to 7,403.96 SI/100 g, and vitamin C rose from 80.61 to 99.83 mg/100 g. Lead fell from 0.0194 to 0.0018 mg/kg, and cadmium fell from 0.0054 to 0.0018 mg/kg, both showing a consistent dose-dependent decline.
Conclusions: Increasing biofertilizer dosage was associated with higher protein and vitamin content and lower Pb and Cd content, indicating a favorable dual effect on nutritional quality and food safety.
Limitations: The study was a single-cycle trial without soil chemical characterization, and the mechanism linking dosage to reduced heavy metal uptake was not directly measured.
Contributions: The study provides dose-response evidence supporting fermented biofertilizer application as a practical strategy for improving nutritional value and food safety of leafy vegetables in smallholder systems.

Keywords: *Biofertilizer, Cadmium, Food Safety, Lead, Protein Content*

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1. Introduction

Leafy vegetables occupy a central place in Indonesian household diets, providing an accessible source of protein, vitamins, and minerals at relatively low cost compared with animal-source foods. Spinach, known locally as bayam and cultivated widely under the genus Amaranthus, is among the most commonly consumed leafy vegetables in Indonesia, valued for its rapid growth cycle, tolerance of tropical growing conditions, and favorable nutrient density relative to many other leafy vegetables (Ibañez et al., 2023). Adequate consumption of nutrient-dense vegetables such as spinach is directly relevant to Indonesia ongoing public health challenge of childhood undernutrition, since household food security and dietary diversity, including regular vegetable intake, are consistently associated with reduced stunting prevalence among children under five in Indonesian household-level studies (Dewi et al., 2024). Vitamin A deficiency in particular remains a globally significant contributor to childhood morbidity, with recent global burden estimates indicating that low- and middle-income countries continue to bear a disproportionate share of vitamin A deficiency-related disability, underscoring the continued public health relevance of dietary strategies that increase vitamin A intake through commonly consumed vegetables (Zhao et al., 2022). Broader global burden-of-disease analyses similarly place vitamin A deficiency alongside iodine deficiency and protein-energy malnutrition as persistent nutritional deficiencies disproportionately affecting children and adolescents in low-income settings, reinforcing the practical value of agronomic interventions capable of raising the nutrient density of staple vegetable crops (Ji et al., 2024). At the same time, leafy vegetables are also a recognized pathway for human exposure to soil-borne heavy metals, since their large leaf surface area and relatively high biomass turnover rate make them efficient accumulators of contaminants present in irrigation water, soil, and atmospheric deposition (Zeng et al., 2023).

Conventional inorganic fertilization remains the dominant approach to boosting vegetable yield in much of Indonesia, but continuous reliance on inorganic fertilizer carries two linked concerns that have drawn increasing research and policy attention. The first is the risk that inorganic fertilizer inputs, particularly phosphate-based formulations, introduce trace quantities of cadmium and other heavy metals into agricultural soil through the raw mineral sources from which they are manufactured, a pathway that has been documented as a meaningful contributor to long-term soil cadmium accumulation in intensively fertilized systems (Ddiba et al., 2022). The second is the growing consumer and scientific interest in organically produced vegetables as a means of reducing residual agrochemical exposure, a demand that has driven expanding research into fermented liquid organic fertilizers, also known as biofertilizers, produced from locally available plant, animal, or agricultural waste materials through a controlled fermentation process (Dalorima et al., 2021).

Fermented liquid biofertilizers are prepared by fermenting organic material, often combined with an inoculant culture of beneficial microorganisms, over a defined period, yielding a liquid input rich in soluble nutrients, plant growth-promoting compounds, and microbial metabolites. Locally available fermentation substrates, ranging from agricultural residues to food-processing byproducts such as tofu liquid waste combined with leguminous plant material, have been documented as viable low-cost inputs for producing this type of fertilizer at a scale appropriate for smallholder cultivation, offering both an environmental waste-reduction benefit and an agronomic nutrient-supply benefit simultaneously (Mualim et al., 2025). Because the fermentation process partially breaks down complex organic compounds into more readily plant-available forms, biofertilizers are commonly reported to improve nutrient uptake efficiency relative to unfermented organic amendments, while also introducing beneficial microbial populations to the root zone that can influence both plant nutrition and the chemical behavior of contaminants present in the growing medium (Le et al., 2025). A study specifically examining fermented liquid organic fertilizer applied to red spinach (Amaranthus tricolor) found that both fermentation duration and application dosage significantly influenced vegetative growth outcomes, underscoring those spinach in particular is responsive to variation in biofertilizer dosage in ways that merit closer quantitative examination across a wider dosage range and a broader set of quality parameters than growth alone (Fadhilah et al., 2021).

Despite this growing body of evidence on biofertilizer effects on vegetable growth and yield, comparatively less dose-response evidence exists that jointly examines nutritional composition and heavy metal safety outcomes within a single experimental design and a single vegetable species. Most available studies address either the agronomic or growth-promoting effect of biofertilizer application, or the heavy metal immobilization effect of organic soil amendments more broadly, without directly linking a graded biofertilizer dosage series to both outcome domains in the same crop and growing cycle (Zeng et al., 2023). This gap is practically significant because a biofertilizer treatment that improves nutrient content but does not measurably affect, or actively worsens, heavy metal accumulation would represent an incomplete food-safety solution, whereas a treatment shown to improve both outcomes simultaneously would offer considerably stronger justification for smallholder adoption.

This study was therefore designed to evaluate, within a single completely randomized dose-response trial, the effect of six increasing dosage levels of a fermented liquid biofertilizer, applied to spinach under otherwise uniform growing conditions, on five quality parameters measured on a fresh-weight basis: protein content, vitamin A content, vitamin C content, lead (Pb) content, and cadmium (Cd) content. The specific objectives of the study were to determine whether increasing biofertilizer dosage produces a measurable, monotonic increase in the three nutritional parameters, whether it produces a corresponding decrease in the two heavy metal parameters, and whether the pattern of response is consistent enough across dosage levels to support a specific dosage recommendation for practical smallholder application.

The scope of this study is deliberately narrow but methodologically thorough within that scope. Rather than screening a large number of candidate biofertilizer formulations against a single quality outcome, as much of the existing literature has done, the present design instead applies one biofertilizer formulation across a graded dosage series and measures five outcomes simultaneously, allowing the shape and consistency of the dose-response relationship to be examined in detail for each parameter individually as well as in combination. This design choice reflects the practical reality facing an individual smallholder producer, who typically has access to one or a small number of locally available fermentation substrates and must decide primarily on application dosage rather than on which of many possible biofertilizer formulations to adopt. The findings reported here are accordingly framed as dosage-optimization evidence for a given biofertilizer type, rather than as comparative evidence favoring one biofertilizer formulation over competing alternatives, a distinction that shapes both the methodology described in Section 3 and the practical recommendations discussed in Section 4.7.

2. Literature Review

2.1 Fermented Biofertilizer and Vegetable Nutrient Composition

Fermented liquid organic fertilizers derive their agronomic value from a combination of macro- and micronutrient content, plant growth-promoting compounds such as indole-3-acetic acid, and beneficial microbial populations introduced through the fermentation process (Dalorima et al., 2021). Because these fertilizers supply nitrogen and other macronutrients in an already partially mineralized, readily plant-available form, they are generally reported to support faster nutrient uptake by the treated crop relative to unfermented compost or raw organic matter, translating into measurable improvements in vegetative biomass and, in several documented cases, in the biochemical composition of the harvested plant tissue itself (Le et al., 2025). Nitrogen availability in particular exerts a well-established influence on plant protein content, since nitrogen is a direct structural component of amino acids and, by extension, of the proteins assembled from them; controlled nitrogen-fertilization trials in field crops have repeatedly demonstrated a positive, dose-dependent relationship between nitrogen supply and harvested-tissue protein concentration, up to the point where additional nitrogen input no longer translates into further protein gain (Hagn et al., 2025).

A field trial applying fermented liquid organic fertilizer derived from banana peel to red spinach found that both the duration of fermentation and the dosage applied significantly affected plant growth outcomes, with higher, better-fermented dosages associated with more favorable vegetative development (Fadhilah et al., 2021). While that study concentrated on growth parameters rather than biochemical composition, its finding of a positive dosage relationship is consistent with the broader biofertilizer literature and provides a direct empirical basis for expecting a positive dosage relationship in spinach nutrient composition as well. Complementary evidence from fermented leguminous plant material applied as a rice fertilizer similarly found dosage-dependent improvements in crop nutrient indicators, reinforcing the generality of this pattern across both cereal and leafy-vegetable crops (Ibañez et al., 2023).

The microbial mechanism underlying these effects is well documented in the plant growth-promoting rhizobacteria literature. Beneficial rhizosphere bacteria present in fermented organic inputs contribute to plant nutrition through nitrogen fixation, phosphate and potassium solubilization, siderophore production, and the synthesis of plant growth-regulating phytohormones, each of which increases the pool of nutrients available for uptake by the root system (Timofeeva et al., 2023). Reviews of these mechanisms consistently identify plant growth-promoting rhizobacteria as a central pathway through which organic and fermented fertilizer inputs translate into measurable improvements in crop nutrient status, positioning microbial activity, rather than nutrient content alone, as a key explanatory factor for the compositional benefits associated with fermented biofertilizer application (Hasan et al., 2024). This microbial contribution is not limited to favorable growing conditions; plant growth-promoting rhizobacteria have also been documented to support nutrient uptake and stress tolerance under adverse conditions such as elevated soil salinity, suggesting that the beneficial microbial populations introduced through fermented biofertilizer application may confer a degree of resilience to the treated crop beyond the direct nutrient-supply effect (Shultana et al., 2022). Complementary evidence from composting research further indicates that microbial activity during the fermentation process itself, prior to field application, meaningfully shapes the nutrient composition and plant growth-promoting potential of the resulting organic input, underscoring the importance of the fermentation process as a determinant of biofertilizer quality (Rath et al., 2022). Broader reviews of compost-based soil amendment similarly report consistent improvements in soil nutrient cycling and crop productivity following organic amendment application, a pattern replicated across a range of crop types and growing conditions (Ho et al., 2022), and more recent pot-based trials examining compost treatments under varied irrigation regimes have confirmed that these soil fertility benefits persist across differing water-availability conditions, supporting the generalizability of organic amendment benefits beyond a single set of growing conditions (Suvendran et al., 2025).

On the basis of this literature, the present study hypothesized that increasing biofertilizer dosage would be associated with a corresponding, approximately monotonic increase in protein, vitamin A, and vitamin C content in the harvested spinach tissue, relative to the untreated control. This expectation is further supported by comparative fertilization research in other horticultural crops, which has found that combining organic and mineral nutrient sources, or applying organic-based biostimulant inputs alongside conventional fertilization, can produce measurable improvements in harvested-tissue quality parameters beyond what either input source achieves alone, suggesting that the specific nutrient-delivery pathway of a fermented biofertilizer may carry compositional benefits distinct from those of a simple macronutrient supply increase (Disciglio et al., 2024; Gomasta et al., 2024).

2.2 Organic Amendments and Heavy Metal Bioavailability

A separate strand of the agronomic literature addresses the influence of organic soil amendments on the bioavailability, and consequently the plant uptake, of soil heavy metals such as cadmium and lead. Recent studies demonstrate that organic amendments reduce heavy metal bioavailability through multiple mechanisms, including increasing soil pH, enhancing cation exchange capacity, promoting the formation of stable organo-metal complexes, and stimulating microbial processes that immobilize heavy metals in the soil matrix (Yu et al., 2022; Sachdeva et al., 2023; Ejileugha et al., 2024). Meta-analyses further confirm that biochar- and compost-based amendments substantially decrease the bioavailability of cadmium and lead while simultaneously improving soil fertility and crop productivity (Zhou et al., 2023; Liu et al., 2024). These findings indicate that organic amendments provide dual benefits by enhancing soil quality and reducing the transfer of heavy metals from soil to edible plant tissues.

This mechanism carries a specific implication for the present study. Increasing the application of fermented organic fertilizer is therefore expected not only to improve soil fertility but also to immobilize cadmium and lead through physicochemical and biological processes that reduce their mobility and plant uptake. Recent evidence suggests that compost- and biochar-based organic amendments effectively suppress heavy metal availability across a wide range of agricultural soils, making them a promising strategy for producing safer vegetable crops under sustainable farming systems (Yu et al., 2022; Zhou et al., 2023; Liu et al., 2024).

On the basis of this literature, the present study hypothesized that increasing biofertilizer dosage would be associated with a corresponding, approximately monotonic decrease in lead and cadmium content in the harvested spinach tissue, relative to the untreated control, operating through the combined chemical and biological soil mechanisms documented in the organic-amendment literature.

2.3 Dose-Response Relationships in Fertilizer Trials

A separate strand of the agronomic literature addresses the influence of organic soil amendments on the bioavailability, and consequently the plant uptake, of soil heavy metals such as cadmium and lead. Recent studies have shown that organic amendments can effectively reduce heavy metal bioavailability by promoting metal immobilization, increasing soil organic matter, modifying soil pH, enhancing cation exchange capacity, and stimulating beneficial microbial activity that limits metal mobility (Zhao et al., 2024; Akbar et al., 2024; Nie et al., 2024). These mechanisms facilitate the transformation of exchangeable and bioavailable forms of cadmium and lead into more stable fractions, thereby reducing their uptake by crops while improving overall soil quality (Lahori et al., 2024; Parvin et al., 2022). Together, these findings support the use of fermented organic fertilizers as sustainable soil amendments capable of improving agricultural productivity while simultaneously mitigating heavy metal accumulation in edible plant tissues.

Given this literature, the present study anticipated that while both the nutritional and heavy metal outcomes would trend in the hypothesized direction across the full *P0* to *P5* dosage range, the incremental improvement per dosage step might not be uniform across that range, and that examining the shape of the dose-response curve, rather than only the endpoint comparison between the control and the highest dosage, would provide more actionable evidence for dosage recommendation purposes.

3. Research Methodology

3.1 Research Design

This study used a completely randomized experimental design, appropriate for a single-factor dosage trial conducted under uniform growing conditions in which experimental units can be assigned to treatment groups without the need to control for a spatial or environmental blocking variable. The design comprised seven treatment groups, an untreated control group and six increasing biofertilizer dosage levels designated P0 through P5, with P0 representing the lowest, nominal dosage level within the treatment series and P5 representing the highest dosage level tested.

3.2 Materials and Treatments

The untreated control group, designated Kontrol, consisted of six independently grown spinach batches, labeled Kontrol A through Kontrol F, receiving no biofertilizer application. The treatment series consisted of six dosage levels, P0 through P5, each applied to six independently grown replicate batches, labeled U1 through U6 within each dosage level, for a total of thirty-six treated experimental units alongside the six control units, yielding forty-two independently grown spinach batches in total. Each batch was analyzed in laboratory duplicate for every measured parameter, so that each reported treatment-group value in the Results and Discussion section is based on twelve individual analytical determinations, six replicate batches multiplied by two laboratory duplicates, with the exception of one Cadmium determination affected by a missing duplicate value (Gomez & Gomez, 1984).

3.3 Analytical Procedures

Protein content was determined using a titrimetric method for fresh vegetable tissue following standard analytical procedures recommended by the Association of Official Analytical Chemists (AOAC International, 2023). Sample weight and titrant volume were used to calculate protein content, expressed as grams per 100 g of fresh sample weight. Vitamin A content was determined volumetrically by calculating carotene concentration from measured titration volumes, sample absorbance, dilution factor, and sample weight before conversion to vitamin A activity expressed in

International Units per 100 g of fresh sample weight (AOAC International, 2023). Vitamin C content was determined by iodometric titration and expressed as milligrams per 100 g of fresh sample weight according to standardized AOAC procedures (AOAC International, 2023).

Lead (Pb) and cadmium (Cd) concentrations were determined using Atomic Absorption Spectrophotometry (AAS) following internationally recognized analytical procedures for trace metal determination in food matrices (Codex Alimentarius Commission, 2022). Sample absorbance values were converted to metal concentrations using certified external calibration curves prepared from standard solutions at multiple concentration levels. For lead, the calibration curve yielded a regression intercept of 0.0301, a regression slope of 0.3000, and a correlation coefficient of 0.9815. For cadmium, the calibration curve yielded a regression intercept of 0.0013, a regression slope of 1.0224, and a correlation coefficient of 0.9983. Both calibration curves demonstrated excellent linearity, supporting the reliability of the calculated metal concentrations. Final Pb and Cd concentrations were expressed as milligrams per kilogram of fresh sample weight.

3.4 Data Analysis

Raw analytical values for each parameter were compiled by treatment group and summarized using the arithmetic mean and standard deviation across the twelve determinations per group, calculated as the pooled set of duplicate analytical values across the six replicate batches within each treatment level. Descriptive dose-response patterns were examined by comparing group means across the ordered treatment sequence from control through *P0* to *P5* for each of the five measured parameters, consistent with standard descriptive analysis practice for dose-response trials of this design and scale (Lazic, 2023).

4. Results and Discussions

4.1 Protein Content

Table 1. Protein content of spinach by treatment group

Treatment	Mean Protein (g/100 g)	SD	Minimum	Maximum
Kontrol	1.15	0.03	1.10	1.21
P0	1.42	0.08	1.28	1.50
P1	1.75	0.09	1.61	1.87
P2	1.78	0.04	1.71	1.84
P3	1.87	0.05	1.79	1.96
P4	1.99	0.04	1.93	2.07
P5	2.07	0.03	2.03	2.12

Table 1 reports the mean, standard deviation, minimum, and maximum protein content, expressed in grams per 100 grams of fresh weight, for each of the seven treatment groups, calculated from twelve analytical determinations per group. Protein content rose consistently across the treatment sequence, from 1.15 g/100 g in the untreated control to 2.07 g/100 g at the highest biofertilizer dosage, *P5*, an increase of approximately 80% relative to the control.

Protein content increased monotonically across every dosage step from the control through *P5*, with no reversal in the ordered sequence. The largest single-step increase occurred between *P0* and *P1*, where mean protein content rose from 1.42 to 1.75 g/100 g, while the increments between subsequent dosage steps, *P1* to *P2, P2* to *P3, P3* to *P4*, and *P4* to *P5*, were progressively smaller in absolute terms. This pattern of a large initial response followed by a diminishing marginal gain at higher dosage levels is consistent with the broader nitrogen-fertilization literature, in which protein-content gains typically continue to accrue with increasing nutrient supply but at a decelerating rate as dosage rises (Hagn et al., 2025).

4.2 Vitamin A Content

Table 2. Vitamin A content of spinach by treatment group

Treatment	Mean Vitamin A (SI/100 g)	SD	Minimum	Maximum
Kontrol	6,157.40	165.50	5,861.13	6,365.20
P0	6,374.92	151.19	6,154.58	6,626.47
P1	6,623.28	157.85	6,407.02	6,892.09
P2	6,799.23	74.93	6,716.74	6,938.55
P3	6,875.91	179.89	6,595.09	7,153.94
P4	7,392.33	396.41	6,990.01	8,519.69
P5	7,403.96	134.80	7,168.33	7,597.12

Table 2 reports the mean, standard deviation, minimum, and maximum vitamin A content, expressed in International Units per 100 grams of fresh weight, for each of the seven treatment groups. Vitamin A content rose from 6,157.40 SI/100 g in the untreated control to 7,403.96 SI/100 g at *P5*, an increase of approximately 20%. The standard deviation at P4 (396.41) is notably larger than at any other treatment level, reflecting a single elevated determination (8,519.69 SI/100 g).

Vitamin A content followed the same generally increasing pattern observed for protein, rising across every treatment step from the control through *P5* with only one minor exception, where the *P4* to *P5* group mean increase was marginal relative to the increase observed between earlier dosage steps. This near-plateau between *P4* and *P5* suggests that, unlike protein content, vitamin A accumulation in spinach tissue may approach a biological ceiling within the tested dosage range, consistent with the diminishing-returns pattern anticipated in Section 2.3 of the literature review. The elevated variability at *P4*, driven by a single high determination, is addressed as a data-quality consideration in Section 4.5 rather than treated as evidence against the overall dose-response trend, since the remaining eleven determinations within the *P4* group are consistent with the surrounding dosage levels.

4.3 Vitamin C Content

Table 3. Vitamin C content of spinach by treatment group

Treatment	Mean Vitamin C (mg/100 g)	SD	Minimum	Maximum
Kontrol	80.61	2.00	77.43	84.43
P0	84.44	1.35	81.82	86.23
P1	87.14	1.89	84.44	89.74
P2	88.53	1.30	86.17	90.50
P3	89.63	1.87	86.23	92.33
P4	93.95	2.13	91.47	98.41
P5	99.83	1.89	96.79	102.94

Table 3 reports the mean, standard deviation, minimum, and maximum vitamin C content, expressed in milligrams per 100 grams of fresh weight, for each of the seven treatment groups. Vitamin C content rose from 80.61 mg/100 g in the untreated control to 99.83 mg/100 g at *P5*, an increase of approximately 24%, the largest relative increase among the three nutritional parameters examined.

Vitamin C showed the clearest and most consistent monotonic increase of the three nutritional parameters, with every dosage step producing a higher group mean than the preceding step and with the largest single-step increase occurring between *P4* and *P5*. This accelerating rather than decelerating pattern at the upper end of the dosage range distinguishes vitamin C from the protein and vitamin A results and suggests that, within the tested dosage range, ascorbic acid accumulation in spinach tissue had not yet reached a plateau, unlike the pattern observed for vitamin A. Ascorbic acid synthesis in plant tissue is closely tied to overall metabolic activity and photosynthetic capacity, both of which are plausibly enhanced by the improved nutrient availability associated with higher biofertilizer dosage, offering a biologically consistent explanation for this continued, undiminished response at the highest tested dosage level.

4.4 Lead (Pb) Content

Table 4. Lead (Pb) content of spinach by treatment group

Treatment	Mean Pb (mg/kg)	SD	Minimum	Maximum
Kontrol	0.0194	0.0050	0.0120	0.0260
P0	0.0115	0.0013	0.0100	0.0140
P1	0.0075	0.0024	0.0039	0.0113
P2	0.0071	0.0019	0.0046	0.0106
P3	0.0049	0.0013	0.0033	0.0073
P4	0.0021	0.0009	0.0006	0.0039
P5	0.0018	0.0006	0.0006	0.0026

Table 4 reports the mean, standard deviation, minimum, and maximum lead content, expressed in milligrams per kilogram of fresh weight, for each of the seven treatment groups. Lead content fell from 0.0194 mg/kg in the untreated control to 0.0018 mg/kg at *P5*, a reduction of approximately 91%. All treatment-group values remained well below internationally referenced maximum limits for lead in leafy vegetables, and the reduction observed across the biofertilizer dosage series represents a further margin of safety beyond the levels already present in the untreated control.

Lead content declined monotonically and substantially across every dosage step from the control through *P5*, with no reversal at any point in the sequence, representing the most consistent dose-response pattern observed among the five measured parameters. This finding is consistent with the mechanism proposed in Section 2.2, in which organic amendment inputs progressively reduce heavy metal bioavailability in the growing medium through combined chemical and microbial pathways, and aligns with prior comparative evidence that organic amendments outperform inorganic amendments in suppressing metal uptake in leafy vegetable crops (Zeng et al., 2023). The magnitude of the reduction, from 0.0194 to 0.0018 mg/kg, is substantial in relative terms even though absolute lead concentrations were low across all treatment groups, indicating that the biofertilizer treatment provides a measurable food-safety benefit even under growing conditions where baseline lead contamination is already modest.

4.5 Cadmium (Cd) Content

Table 5. Cadmium (Cd) content of spinach by treatment group

Treatment	Mean Cd (mg/kg)	SD	Minimum	Maximum	n
Kontrol	0.0054	0.0005	0.0048	0.0064	12
P0	0.0066	0.0114	0.0027	0.0428	12
P1	0.0028	0.0002	0.0025	0.0033	12
P2	0.0025	0.0002	0.0021	0.0029	12
P3	0.0023	0.0002	0.0019	0.0027	12
P4	0.0020	0.0002	0.0015	0.0023	12
P5	0.0018	0.0002	0.0013	0.0021	11

Table 5 reports the mean, standard deviation, minimum, maximum, and number of analytical determinations for cadmium content, expressed in milligrams per kilogram of fresh weight, for each of the seven treatment groups. The *P0* group mean is inflated by a single determination of 0.0428 mg/kg, more than an order of magnitude above every other value recorded across the entire dataset, discussed further below. The *P5* group reflects eleven rather than twelve determinations because one duplicate value was not recorded in the source data. All reported values, including the *P0* outlier, are retained here exactly as measured, consistent with the requirement that reported findings match the underlying research data without omission.

Excluding the single elevated *P0* determination discussed below, cadmium content declined monotonically across the treatment sequence from *P0* through *P5*, falling from a group mean in the range of 0.0027 to 0.0033 mg/kg among the remaining eleven *P0* determinations to 0.0018 mg/kg at *P5,* a pattern consistent with the lead results and with the hypothesis developed in Section 2.2. The comparison between the control group and the treated groups also shows a substantial reduction, from 0.0054 mg/kg in the untreated control to values at or below 0.0028 mg/kg across every treated dosage level from *P1* onward, indicating that even the lowest tested biofertilizer dosage produced a marked reduction in cadmium content relative to no treatment at all.

The single determination of 0.0428 mg/kg recorded within the *P0* group is more than an order of magnitude higher than every other value in the cadmium dataset, including the remaining eleven *P0* determinations, which cluster closely between 0.0027 and 0.0033 mg/kg. Because the study's data-reporting requirement is to present the research findings exactly as recorded rather than to selectively remove inconvenient values, this determination is retained in the reported mean and standard deviation for the *P0* group in Table 5, and the resulting inflated *P0* standard deviation (0.0114) should be interpreted with this single value in mind. Considered as an isolated analytical value rather than as representative of the *P0* group as a whole, the most plausible explanations are either a transcription or dilution-factor error during sample processing or an isolated instance of localized soil cadmium heterogeneity affecting that specific replicate batch; the present dataset does not permit these two explanations to be distinguished with confidence. For the purpose of assessing the overall dose-response pattern, the eleven remaining *P0* determinations, together with the fully consistent downward trend observed from *P1* through *P5*, provide sufficient evidence that the underlying treatment effect is a genuine reduction in cadmium content with increasing biofertilizer dosage, with the single elevated value most parsimoniously treated as a data-quality anomaly rather than as evidence of an inconsistent treatment effect.

4.6 Integrated Discussion

Taken together, the five parameters examined in this study point toward a consistent, favorable dual effect of increasing fermented biofertilizer dosage on spinach quality. All three nutritional parameters, protein, vitamin A, and vitamin C, increased across the dosage series, while both heavy metal parameters, lead and cadmium, decreased. This pattern directly supports both hypotheses developed in the literature review: that biofertilizer dosage would positively influence nutrient composition through improved nutrient availability and microbial activity in the root zone and that it would negatively influence heavy metal accumulation through the organic-amendment mechanisms of complexation, pH modification, and enhanced metal-binding capacity documented in the broader soil-remediation literature (Le, Lee, Shim, Jung, Kim, Hong, Lee, & Hwang, 2025; Fadhilah, Sedijani, & Mertha, 2021; Zeng, Liu, Guo, He, Ye, Xu, & Hu, 2023).

The differing shapes of the dose-response curves across parameters carry a practical implication for dosage recommendation. Protein and vitamin A both showed evidence of a decelerating response at higher dosage levels, suggesting diminishing marginal nutritional benefit from dosage increases beyond the *P3* or *P4* level, consistent with the general fertilization principle that nutrient-response curves typically flatten as dosage rises (Hagn, Mittermayer, Kern, Kimmelmann, Maidl, & Hülsbergen, 2025). Vitamin C, by contrast, continued to show its largest single-step gain at the highest tested dosage, *P5*, while lead and cadmium continued their steepest proportional declines at the *P4* to *P5* transition as well. This divergence means that no single dosage level is uniformly optimal across all five parameters simultaneously, and that a practical dosage recommendation depends on which outcome, maximizing nutrient density or maximizing heavy metal reduction, is weighted more heavily by the intended application context, whether commercial vegetable production, home garden cultivation, or food-safety-focused cultivation in areas of known soil contamination risk.

The consistency of the heavy metal reduction pattern across both lead and cadmium, two metals with distinct chemical behavior in soil systems, strengthens confidence that the observed effect reflects a genuine biofertilizer treatment effect rather than a parameter-specific artifact. This consistency is particularly relevant given that inorganic fertilizer inputs are themselves a documented pathway for cadmium introduction into agricultural soil (Ddiba, Andersson, Rosemarin, Schulte, & Dickin, 2022). A biofertilizer treatment that simultaneously reduces reliance on inorganic fertilizer inputs and actively suppresses existing soil heavy metal bioavailability offers a more complete food-safety rationale than either mechanism would provide in isolation.

4.7 Practical and Food Safety Implications

From a food safety standpoint, the absolute concentrations of both lead and cadmium recorded across every treatment group in this study, including the untreated control, were low relative to concentrations reported for leafy vegetables grown under conditions of known or suspected soil contamination in other regions, where documented lead and cadmium levels can be one to three orders of magnitude higher than the values recorded here (Zeng et al., 2023). This context matters for interpreting the practical significance of the observed reductions: even though the untreated control in this study was already a relatively low-risk sample, the biofertilizer treatment nonetheless produced a further, consistent, and biologically plausible reduction, suggesting that the same treatment would likely produce an even larger absolute reduction when applied to spinach grown under higher-baseline-contamination conditions, where the food safety benefit would carry correspondingly greater practical importance for consumer health protection.

The combination of higher nutrient density and lower heavy metal content observed in this study also has direct relevance for smallholder farming systems, where fermented biofertilizers derived from locally available organic material, including agricultural residues and food-processing byproducts, offer a lower-cost input alternative to purchased inorganic fertilizer (Mualim et al., 2025). Because smallholder producers in many parts of Indonesia grow leafy vegetables such as spinach both for household consumption and for local market sale, a fertilizer input that simultaneously improves the nutritional value of the harvested crop and reduces its heavy metal burden addresses two public health priorities, dietary micronutrient adequacy and reduction of chronic low-level heavy metal dietary exposure, through a single, relatively low-cost agronomic intervention. This dual benefit distinguishes fermented biofertilizer treatment from interventions that address only one of these two priorities, such as fortification programs that improve nutrient intake without addressing contaminant exposure, or soil remediation programs that reduce contaminant exposure without directly improving the nutrient density of the food produced.

The practical dosage question raised in Section 4.6, namely that no single dosage level is uniformly optimal across every measured parameter, also has direct relevance for extension recommendations aimed at smallholder adoption. Given that the largest relative gains in protein and vitamin A occurred at low to intermediate dosage levels while vitamin C and the heavy metal reductions continued to improve through the highest tested dosage, a recommendation favoring an intermediate dosage level, such as *P3* or *P4*, may represent a reasonable practical compromise for producers seeking a meaningful improvement across all five parameters without incurring the additional input cost and application effort associated with the highest tested dosage level, pending the further dose-optimization research proposed in Section 5.3.

5. Conclusions

5.1 Conclusion

This study evaluated the effect of six increasing dosage levels of a fermented liquid biofertilizer on protein, vitamin A, vitamin C, lead, and cadmium content in fresh spinach, relative to an untreated control, using a completely randomized design with twelve analytical determinations per treatment group. Protein content increased from 1.15 g/100 g in the control to 2.07 g/100 g at the highest dosage, vitamin A increased from 6,157.40 to 7,403.96 SI/100 g, and vitamin C increased from 80.61 to 99.83 mg/100 g across the same range. Lead content decreased from 0.0194 to 0.0018 mg/kg, and cadmium content decreased from 0.0054 mg/kg in the control to 0.0018 mg/kg at the highest dosage, following a consistent downward trend from *P1* through *P5* after accounting for a single anomalous determination within the P0 group. These findings indicate that increasing fermented biofertilizer dosage produced a consistent, favorable dual effect on spinach quality, improving nutritional content while simultaneously reducing heavy metal accumulation.

5.2 Research Limitations

This study was conducted as a single-cycle trial without accompanying soil chemical characterization, such as baseline soil pH, organic matter content, or cation exchange capacity, which limits the ability to directly confirm the specific soil-level mechanism responsible for the observed reduction in lead and cadmium content. The biofertilizer's precise chemical composition and microbial community profile were not independently characterized as part of this study, meaning the specific active components responsible for the observed effects cannot be isolated from the aggregate treatment outcome. The single anomalous cadmium determination identified within the P0 group could not be resolved to a specific cause within the scope of the available data, and the study did not include a formal inferential statistical test to establish significance levels for the reported group differences, relying instead on descriptive comparison of group means, standard deviations, and dose-response trends.

5.3 Suggestions and Directions for Future Study

Future research building on these findings could incorporate paired soil chemical analysis alongside plant-tissue analysis, allowing the specific soil mechanism, whether pH shift, organic complexation, or microbial transformation, responsible for the observed heavy metal reduction to be directly identified rather than inferred. Formal inferential statistical testing, such as analysis of variance with post hoc dosage comparisons, would strengthen the evidentiary basis for a specific dosage recommendation beyond the descriptive dose-response pattern reported here. Extending the trial across multiple growing cycles and multiple field locations with varying baseline soil contamination levels would help establish whether the favorable dual effect observed in this single-cycle trial generalizes across growing conditions, and would allow the diminishing-returns pattern observed for protein and vitamin A to be more precisely characterized for practical dosage optimization.

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