

Influence of Irrigation Water Sources on Growth Performance, Nutritional Composition, and Heavy Metal Accumulation of *Amaranthus hybridus* L.

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Abstract

Water scarcity and rapid urbanization have increased the use of alternative water sources for vegetable production. The growth, nutritional components, and heavy metal deposition of *Amaranthus hybridus* grown in Daki-Biu District, Abuja, Nigeria, were assessed in this study in relation to several irrigation water sources. A pot experiment was conducted using a completely randomized design with four treatments: tap water (control, T1), well water (T2), stream water (T3), and wastewater (T4), with each treatment replicated three times. Irrigation water quality, growth parameters (plant height, number of leaves, leaf area and fresh weight), proximate composition, and heavy metal concentrations were determined using standard laboratory procedures. Data were subjected to analysis of variance (ANOVA) at a 5% significance level. T4 had the highest electrical conductivity ($1268 \mu\text{S}/\text{cm}^{-1}$) and potassium level, while Cadmium and lead exceeded WHO safety limits. The results showed significant differences ($p < 0.01$) among treatments. Plants irrigated with T4 recorded the greatest plant height (58.2 cm), fresh weight (51.0g plant^{-1}), and number of leaves (24.3); however, leaf area was high in plants irrigated with T1 (49.3 cm^2). Crude protein content was highest in T2 (6.09%) and T4 (5.84%), while carbohydrate content was higher under T1 treatment. Heavy metal analysis showed high concentrations of cadmium, lead, and chromium across all treatments, with the highest in T4, exceeding recommended safety limits for vegetable consumption. The findings showed that T4 treatment can enhance the growth, yield, and nutritional quality of *Amaranthus hybridus* but poses significant food safety risks due to heavy metal accumulation.

Keywords: *Amaranthus hybridus*; irrigation water; growth performance; nutritional composition; heavy metal accumulation.

INTRODUCTION

Amaranthus hybridus L., commonly known as African spinach or green amaranth, is an important C4 leafy vegetable which belongs to the Amaranthaceae family. The crop is widely cultivated in tropical and subtropical regions because of its rapid growth, high nutritional value, and adaptability to diverse environmental conditions [32]. It is an important source of dietary protein, vitamins (A, C, K, and B-complex), minerals such as calcium and iron, dietary fibre, and bioactive phytochemicals, making it valuable for improving household nutrition and food security, particularly in developing

countries [35] [32] [26] [3].

The availability of water is an important factor that influences the growth and yield of vegetable crops, including *Amaranthus hybridus*. The increasing demand for vegetables, coupled with rapid urbanization and climate variability, has resulted in declining freshwater availability, thereby increasing the need for alternative irrigation water sources. Urban expansion and population growth have further reduced the availability of freshwater for agricultural production [11] [37].

However, in urban and peri-urban areas where freshwater resources are scarce, farmers are increasingly depending on groundwater, surface water, and untreated wastewater to irrigate vegetable crops [9] [13]. Irrigation has therefore become essential for supplementing rainfall, especially in regions characterized by irregular precipitation patterns or frequent water scarcity [9].

Water availability influences plant growth, yield and nutritional quality of the crop. Both the quantity and quality of irrigation water play important roles in determining plant growth, nutrient uptake, and productivity [13]. Different sources of irrigation water, such as tap water, river water, stream water, and wastewater, vary in physicochemical characteristics and nutrient composition, thereby affecting crop performance and soil properties. For example, Wastewater irrigation can have both beneficial and negative effects on crops, particularly vegetables [4]. It has been reported in previous research that wastewater contains minerals such as nitrogen, phosphorus, and Organic matter, which can improve the physical properties and nutrient content of the soil [19] [37]. However, wastewater may also contain contaminants and potentially toxic elements such as cadmium (Cd), Copper (Cu), Manganese (Mn) and Lead (Pb), which can negatively affect soil quality, crop health, yield and the nutritional content of the vegetable [4] [30] [31]. Studies have also shown that irrigation regimes significantly impact growth parameters (leaf area, plant height) [32].

In Nigeria, irrigation farming is widely practiced supporting year-round vegetable production. However, the quality of irrigation water is not always considered when selecting water sources, particularly in urban and peri-urban farming systems. As a result, farmers may rely on rivers, streams, wells or untreated wastewater that can be contaminated with domestic, industrial, or agricultural pollutants, which can adversely affect soil quality, crop productivity, and the safety of vegetables intended for human consumption [11] [16]. Therefore, this study evaluated the comparative effects of tap water, well water, stream water, and wastewater irrigation on the growth, nutritional quality, and heavy metal accumulation in the edible tissues of *Amaranthus hybridus*. Although numerous studies have investigated the effects of wastewater irrigation on vegetable crops, few have compared multiple irrigation water sources and their combined effects on the growth, nutritional quality, and heavy metal accumulation of *Amaranthus hybridus*. The results of this study can help farmers identify suitable water sources that promote better crop performance and ensure that the vegetables produced are safe and healthy for consumers.

Materials and Methods

2.1 Study Area

The study was conducted in Daki Biu District, Jabi, Abuja, Federal Capital Territory (FCT), Nigeria. The area is characterized by a tropical wet-and-dry climate, with a mean annual temperature of approximately 28°C to 30°C.

2.2 Source of Materials

Seeds of *Amaranthus hybridus* were obtained from a reputable local agricultural supplier in Abuja. Topsoil was collected from a nearby fallow land in Daki Biu and sieved to remove stones, debris and plant residues before being used to fill the 4L plastic bowl that was used as the experimental pots.

2.3 Experimental Design and Treatment

The experiment was arranged in a Randomized Complete Block Design (RCBD) comprising four irrigation water treatments with three replicates, giving a total of twelve (12) experimental units (pots). The treatments are Tap water (Control T₁), Well Water (T₂), Stream Water (T₃) and Wastewater (T₄).

2.4 Physicochemical and Heavy Metal Analysis of Irrigation Water

The samples of each irrigation water source (tap, well, stream and wastewater) were collected in clean labelled bottles before the commencement of the experiment and analyzed for selected physicochemical properties and heavy metal concentrations using standard analytical procedures in the laboratory. The pH of each water sample was measured using a calibrated digital pH meter. The electrical conductivity (EC) and total dissolved solids (TDS) were determined using a calibrated digital conductivity & TDS meter.

Selected heavy metals, namely lead (Pb) and cadmium (Cd), were determined in the water samples using a Thermo Scientific iCE 3000 Series Atomic Absorption Spectrophotometer (AAS), operated in flame mode with an air-acetylene flame

Total coliform count was also determined using standard microbiological procedures and expressed as colony-forming units per 100 mL (CFU/100 mL).

2.5 Nursery and Transplanting

Seeds of *Amaranthus hybridus* were grown in a nursery and irrigated regularly until seedlings emerged. Two weeks after sowing, healthy, uniform seedlings were transplanted into plastic pots containing sandy loam soil collected from the study area. Ten seedlings were transplanted into each pot. Each treatment was irrigated with its designated water source throughout the eight-week experimental period. An equal amount of water was applied to all pots twice daily to maintain uniform soil moisture.

2.5 Data Collection

2.5.1 Morphological Growth Parameters

The growth and yield parameters were measured weekly throughout the experimental period of 8 weeks. The following plant parameters were recorded,

Plant Height (cm): It was measured from the base of the plant to the tip of the highest leaf using a metre rule.

Number of Leaves: The number of leaves was counted on each plant.

Leaf area: leaf area (cm^2) was determined by measuring the length and width of the leaf and was calculated using $0.5 (\text{Length} \times \text{width})$ [29]

Fresh weight (g): The fresh weight of the whole plant was measured after harvest using an electronic scale.

2.5.2 Nutritional Analysis: The harvested leaves were air-dried, milled into a fine powder, and subjected to proximate analysis following the Association of Official Analytical Chemists (AOAC) standard methods to determine crude protein, crude fibre, ash content, carbohydrate and moisture content.

2.5.3 Mineral and Heavy Metal Analysis: At harvest, leaf samples were air-dried, homogenized, and ground into a fine powder. A 2 g portion of each sample was weighed into a 250 cm^3 glass conical flask and digested with 20 cm^3 of nitric acid (HNO_3). The flask was covered with a watch glass and heated on a hot plate at 110°C for 3 h. After evaporation to near dryness, the digest was diluted with 20 cm^3 of 2% (v/v) HNO_3 , filtered through Whatman No. 42 filter paper, and transferred into a 50 cm^3 volumetric flask. The volume was made up to 50 cm^3 with deionized water. The filtrates were analyzed for mineral elements (Ca, Mg, K, Na, Fe) and selected heavy metals (Pb, Cd, Cr) using a Thermo Scientific iCE 3000 Series Atomic Absorption Spectrophotometer (AAS; AA02134104, software version v1.30). The concentrations of the analyzed elements were expressed as mg/kg dry weight. Appropriate calibration standards and reagent blanks were used during analysis, and replicate measurements were performed to ensure analytical reliability.

The concentration is calculated in mg/kg

$$= \frac{(\text{Conc. of sample} - \text{conc. of blank}) \times \text{volume in cm}^3}{\text{Weight of sample} \times 1000} \times 1000$$

2.6 Statistical Analysis

The data collected were subjected to Analysis of Variance (ANOVA) using SPSS at 5% ($p < 0.01$) level of probability.

Result and Discussion

3.1 Physicochemical Properties of Irrigation Water

The physicochemical characteristics of the four irrigation-water sources are presented in Table 1 and 2. The water sources exhibited clear differences in ionic composition, oxygen status, nutrient concentrations, selected potentially toxic elements, and microbial quality. These differences provide an important basis for interpreting the subsequent variation in the growth, nutritional composition, and elemental accumulation of *Amaranthus hybridus*.

The pH values were relatively uniform across the treatments, ranging from 7.13 in well water (T2) to 7.35 in tap water (T1), and the one-way ANOVA confirmed that the differences were not statistically significant, $F(3, 8) = 1.021$, $p = .433$. Thus, within the conditions of the experiment, pH was unlikely to be a major source of treatment differentiation.

The near-neutral pH of the irrigation waters is generally favourable for plant production because nutrient availability and root-zone chemical processes can be adversely affected at strongly acidic or alkaline pH values [22].

The total dissolved solids (TDS) and electrical conductivity (EC) differed among water sources. Across treatments, TDS increased from 35.28 mg L^{-1} in tap water to 674.10 mg L^{-1} in wastewater, while EC increased from $68.15 \mu\text{S cm}^{-1}$ in tap water to $1268 \mu\text{S cm}^{-1}$ in wastewater. Both TDS, $F(3, 8) = 2615.904$, $p < .001$, and EC, $F(3, 8) = 1232.455$, $p < .001$ showed highly significant treatment effects. The elevated EC observed in the wastewater indicates a higher concentration of dissolved ions and suggests a greater potential for salinity pressure [21]. Prolonged exposure to elevated salinity can reduce plant water uptake through osmotic effects and may contribute to progressive accumulation of salt in the soil [1] [21]. Recent reviews have similarly reported that salts and nutrients present in reclaimed wastewater can affect soil crop systems, with the magnitude of the effect depending on water quality and management practices [21].

Dissolved oxygen (DO) also varied among the treatments, ranging from 0.90 mg L^{-1} in wastewater to 2.90 mg L^{-1} in well water, with a highly significant treatment effect, $F(3, 8) = 453.251$, $p < .001$. DO is an important water quality parameter, particularly in wastewater treatment system [20]. The very large F-statistic nevertheless indicates that the irrigation sources were clearly differentiated with respect to this water-quality attribute.

Nitrate concentration ranged from 0.69 mg L^{-1} in tap water to 2.62 mg L^{-1} in wastewater, while phosphate remained low between 0.01 mg L^{-1} and 0.02 mg L^{-1} . Nitrate differed significantly among the water sources, $F(3, 8) = 2087.717$, $p < .001$, whereas phosphate showed no significant difference, $F(3, 8) = 1.700$, $p = .244$. Potassium showed one of the strongest treatment effects, increasing from 2.68 mg L^{-1} in tap water to 44.78 mg L^{-1} in wastewater, $F(3, 8) = 2732.535$, $p < .001$. The relatively high potassium concentrations in wastewater and well water may have contributed to the enhanced vegetative performance observed later in the experiment because potassium is central to stomatal regulation, osmotic balance, enzyme activation, and general plant metabolism [22].

Table 1 also shows the elevated concentration of heavy metals, particularly lead (Pb) and cadmium (Cd). Lead concentrations ranged from 0.05 mg L^{-1} in tap water to 0.20 mg L^{-1} in well water, and the treatment effect was highly significant, $F(3, 8) = 564.000$, $p < .001$. The highest Pb observed in well water exceeded the WHO permissible limit (0.05 mg L^{-1}). Cadmium concentrations were comparatively similar across the water source (0.10 – 0.12 mg L^{-1}) and exceeded the recommended limit of 0.003 mg L^{-1} in all treatments and did not differ significantly among treatments, $F(3, 8) = 2.000$, $p = .193$. The absence of a significant treatment effect for Cd should therefore not be interpreted as an indication of safety; rather, it suggests that Cd concentrations were consistently elevated across the irrigation water sources.

Heavy metals such as Cd and Pb are environmental contaminants that are potentially toxic, non-biodegradable, and can accumulate in soils and plant tissues, posing long-term health risks to consumers [5]. Similar findings have been reported in recent studies, where irrigation with contaminated water sources resulted in elevated heavy metal concentrations in agricultural soils and crops [17][34].

Microbiological quality also varied substantially. Total coliform counts ranged from 1 CFU per 100 mL in tap water to 228 CFU per 100 mL in stream water, with a highly significant treatment effect, $F(3,8) = 3183.689$, $p < .001$.

Although the reported counts were below the reference value used in the original table, their presence demonstrates differential microbial quality among the irrigation sources. This is relevant for leafy vegetables because irrigation water can contribute to microbial contamination of produce, particularly where water is of poor sanitary quality. Taken together, the water-quality results indicate a trade-off: wastewater and well water supplied comparatively greater concentrations of some nutrients, particularly potassium, but also showed higher ionic loads and, in the case of wastewater, elevated Pb and Cd concerns. The findings therefore support the need to evaluate irrigation-water quality not solely in terms of crop growth, but also in relation to edible-product safety.

Table 1: Physicochemical properties of the four sources of Irrigation Water

S/N	Parameters	T1-(Tap)	T2-(Well)	T3-(Stream)	T4-(Waste water)	WHO Limits
1	Ph	7.35	7.13	7.16	7.34	6.5-8.5
2	TDS (mg/l)	35.28	513.1	155.0	674.1	1000
3	E.C (µS/cm)	68.15	1004	286.8	1268	1000
4	Dissolved Oxygen	1.7	2.9	2.7	0.9	5
5	Nitrate (mg/l)	0.69	2.62	0.72	2.62	<5
6	Phosphate (mg/l)	0.02	0.02	0.01	0.01	0.5
7	Potassium (mg/l)	2.68	38.48	11.67	44.78	2
8	Lead (mg/l)	0.05	0.20	0.06	0.10	0.05
9	Cadmium (mg/l)	0.11	0.10	0.12	0.11	0.003
10	Total Coliform (per 100ml)	1	52	228	45	<1000

Table 2: Analysis of variance of physicochemical and microbiological water-quality parameters across the experimental groups

Variable	Source	Sum of Squares	df	Mean Square	F	p-value
pH	Between Groups	.122	3	.041	1.021	.433
	Within Groups	.317	8	.040	—	—
	Total	.439	11	—	—	—
TDS	Between Groups	807748.562	3	269249.521	2615.904	< .001
	Within Groups	823.423	8	102.928	—	—
	Total	808571.986	11	—	—	—
Conductivity	Between Groups	2932566.261	3	977522.087	1232.455	< .001
	Within Groups	6345.202	8	793.150	—	—
	Total	2938911.463	11	—	—	—
Dissolved oxygen	Between Groups	7.773	3	2.591	453.251	< .001
	Within Groups	.046	8	.006	—	—
	Total	7.819	11	—	—	—
Nitrate	Between Groups	7.203	3	2.401	2087.717	< .001
	Within Groups	.009	8	.001	—	—
	Total	7.212	11	—	—	—
Phosphate	Between Groups	.000	3	.000	1.700	.244
	Within Groups	.001	8	.000	—	—
	Total	.001	11	—	—	—
Potassium	Between Groups	3742.206	3	1247.402	2732.535	< .001
	Within Groups	3.652	8	.457	—	—
	Total	3745.858	11	—	—	—
Lead	Between Groups	.042	3	.014	564.000	< .001
	Within Groups	.000	8	.000	—	—
	Total	.043	11	—	—	—
Cadmium	Between Groups	.001	3	.000	2.000	.193
	Within Groups	.001	8	.000	—	—
	Total	.001	11	—	—	—
Total coliform	Between Groups	90435.000	3	30145.000	3183.689	< .001
	Within Groups	75.749	8	9.469	—	—
	Total	90510.749	11	—	—	—

Note: ANOVA = analysis of variance; TDS = total dissolved solids. F statistics are reported with numerator and denominator degrees of freedom of 3 and 8, respectively. Values originally reported as .000 are presented as $p < .001$ in accordance with standard journal reporting practice. Statistical significance was assessed at $\alpha = .05$.

3.2 Growth Performance

3.2.1 Plant Height and Number of Leaves

Plant height of *Amaranthus hybridus* over the eight-week growth period is presented in Figure 1 (mean $\pm$ standard deviation). Plants irrigated with wastewater (T4) consistently had the greatest plant height throughout the experiment, followed by well water (T2), stream water (T3), and tap water (T1), which had the lowest height. The enhanced plant height observed under wastewater irrigation can be attributed to high nutrient content of the wastewater, especially nitrogen and potassium, as indicated earlier in Table 1. Nitrogen plays a critical role in promoting vegetative growth by enhancing cell division and elongation, while potassium regulates water balance and enzyme activity, contributing to overall plant vigor [2] [22]. Potassium also contributes positively to plant growth [15]. The combined availability of these nutrients in wastewater likely contributed to the superior plant height recorded in this treatment. These findings align with previous studies, which reported that wastewater irrigation significantly enhances plant height and overall growth due to its nutrient-rich composition [30]. Similarly, enhanced stem elongation and vegetative growth have been observed in crops irrigated with nutrient-enriched water sources [34].

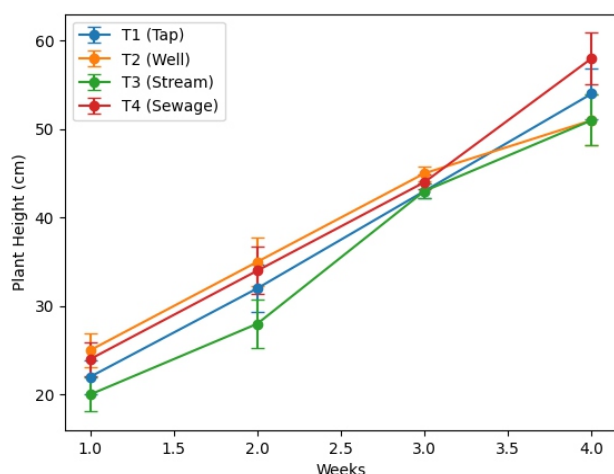


Figure 1: Effect of different irrigation water (Tap water T1, Well water T2, Stream water T3 and Wastewater T4) on the plant height of *Amaranthus hybridus* over an eight-week cultivation period (mean $\pm$ standard deviation)

Figure 2 below presents the effect of different irrigation water sources on the number of leaves of *Amaranthus hybridus* over an eight-week growth period (mean $\pm$ standard deviation). Leaf number increased progressively across all treatments with time, indicating active vegetative growth. Plants irrigated with wastewater (T4) consistently recorded the highest number of leaves throughout the growth period, followed by well water (T2), stream water (T3), and tap water (T1), which exhibited the lowest number of leaves. The observed differences among treatments indicate that irrigation water quality influenced leaf production. The enhanced leaf production observed under wastewater irrigation is likely attributable to the higher nutrient content of the wastewater, particularly nitrogen and potassium, as indicated by the physicochemical characteristics of the irrigation water (Table 1).

Nitrogen is essential for vegetative growth, as it promotes cell division and leaf initiation, while potassium also enhances plant growth [15] [22]. The abundance of these nutrients in wastewater likely accelerated meristematic activity, thereby increasing leaf formation. The present findings agree with previous reports that nutrient-rich irrigation water significantly improves vegetative growth, including leaf production [30] [34]. However, it is important to note that increased leaf production may also increase the plant's capacity to absorb contaminants. Although wastewater irrigation enhanced vegetative growth, the elevated concentrations of lead (Pb) and cadmium (Cd) detected in the irrigation water (Table 1) raise concerns regarding the potential accumulation of these toxic metals in the edible tissues of *Amaranthus hybridus*. Leafy vegetables, due to their large surface area and high transpiration rates, are particularly prone to accumulating heavy metals and contaminants when irrigated with polluted water [17]. Therefore, while wastewater enhances leaf production, it may simultaneously elevate food safety risks.

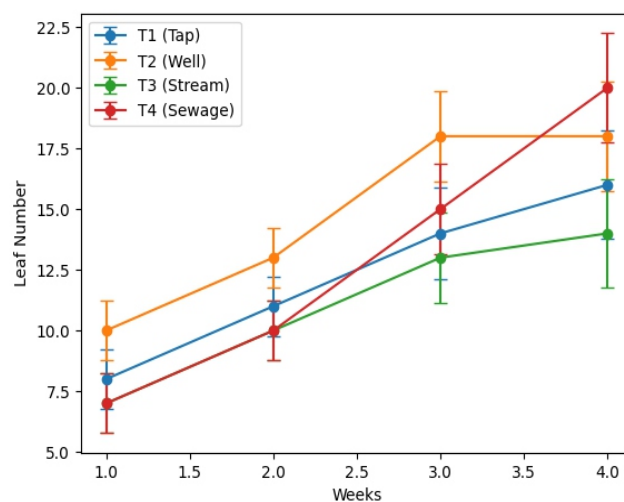


Figure 2: Effect of different irrigation water sources (Tap water T1, Well water T2, Stream T3 and Wastewater T4) on the leaf number of *Amaranthus hybridus* over an eight-week cultivation period (mean $\pm$ standard deviation)

One-way analysis of variance of irrigation-water effects on plant height

In Table 3, the one-way ANOVA revealed that treatment did not produce a statistically significant effect on plant height at week 2, $F(3, 8) = 2.532$, $p = 0.131$. This indicates that the observed differences in plant height among treatments at this early stage were not sufficiently large to establish a treatment-related effect. However, statistically significant treatment effects were observed at weeks 4, 6 and 8. At week 4, the treatment effect was highly significant, $F(3, 8) = 84.502$, $p < 0.001$. This effect became more pronounced at week 6, $F(3, 8) = 46.421$, $p < 0.001$, and was particularly strong at week 8, $F(3, 8) = 387.885$, $p < 0.001$.

Table 3: Analysis of Variance of the Effect of Treatment on Plant height at Weeks 2, 4, 6 and 8

Week	Source	Sum of Squares	df	Mean Square	F	P
2	Between Groups	27.877	3	9.292	2.532	.131
	Within Groups	29.360	8	3.670		
	Total	57.237	11			
4	Between Groups	86.403	3	28.801	84.502	< .001
	Within Groups	2.727	8	.341		
	Total	89.130	11			
6	Between Groups	7.554	3	2.518	46.421	< .001
	Within Groups	.434	8	.054		
	Total	7.988	11			
8	Between Groups	111.391	3	37.130	387.885	< .001
	Within Groups	.766	8	.096		
	Total	112.157	11			

Note: ANOVA = analysis of variance; df= degrees of freedom; F= F-statistic; p= significance probability. Values reported as $p < .001$ where the statistical software output displayed .000.

One-way analysis of variance of irrigation-water effects on leaf number

In Table 4, treatment had a statistically significant effect on leaf number throughout the assessment period. At week 2, the effect was significant, $F(3, 8) = 8.190$, $p = 0.008$, while significance was also maintained at week 4, $F(3, 8) = 6.519$, $p = 0.015$. The magnitude of the treatment effect increased considerably at week 6, $F(3, 8) = 48.333$, $p < 0.001$, and remained highly significant at week 8, $F(3, 8) = 71.111$, $p < 0.001$. These results demonstrate that treatment differences in leaf production were statistically discernible from the early assessment stage and became more pronounced during subsequent growth stages.

Table 4: Analysis of Variance of the Effect of Treatment on Leaf number at Weeks 2, 4, 6 and 8

Week	Source	Sum of Squares	df	Mean Square	F	P
2	Between Groups	14.333	3	4.778	8.190	.008
	Within Groups	4.667	8	.583		
	Total	19.000	11			
4	Between Groups	14.667	3	4.889	6.519	.015
	Within Groups	6.000	8	.750		
	Total	20.667	11			
6	Between Groups	48.333	3	16.111	48.333	< .001
	Within Groups	2.667	8	.333		
	Total	51.000	11			
8	Between Groups	106.667	3	35.556	71.111	< .001
	Within Groups	4.000	8	.500		
	Total	110.667	11			

Note: ANOVA = analysis of variance; df= degrees of freedom; F= F-statistic; p= significance probability. Values reported as $p < .001$ where the statistical software output displayed .000.

3.2.2 Leaf Area and Fresh Weight

Figure 3 illustrates the effect of different irrigation water sources on the leaf area of *Amaranthus hybridus* over an eight-week growth period (mean $\pm$ standard deviation). Across the experimental period, plants irrigated with tap water (T1) consistently exhibited the largest leaf area, followed by stream water (T3), wastewater (T4), and well water (T2), which recorded the smallest leaf area.

The superior leaf area observed under tap water irrigation may be attributed to its better physicochemical quality, particularly its lower concentrations of heavy metals and dissolved contaminants (Table 1). Thereby providing a more favourable environment for leaf expansion.

The relatively small leaf area observed under wastewater and well-water irrigation may be associated with elevated concentrations of lead (Pb) and cadmium (Cd), which can interfere with normal physiological processes in plants. Heavy metal stress has been shown to impair cellular metabolism, reduce photosynthetic efficiency, and inhibit cell division and expansion, thereby hindering leaf development [4] [12]. Leaf expansion is highly dependent on optimal cell division and turgor pressure. The excess lead (Pb) accumulation in *Amaranthus hybridus* can impair stomatal function and photosynthetic activity, which can result in reduced leaf development [18]. These findings indicate that while wastewater and well water may enhance certain vegetative growth parameters by increasing nutrient availability, heavy metal contamination can offset these benefits by limiting leaf expansion.

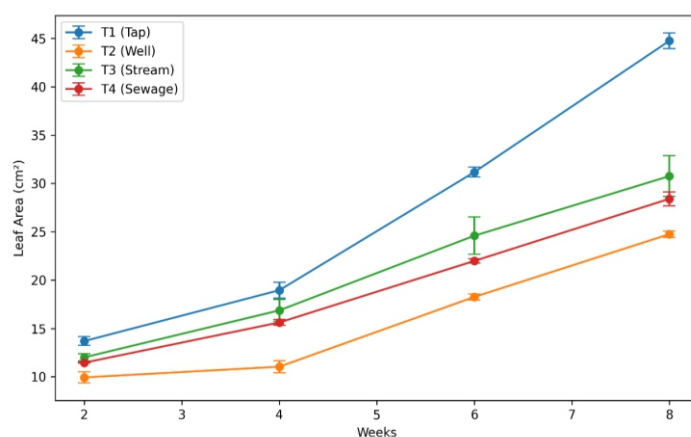


Figure 3: illustrates the effect of different irrigation water (Tap water T1, well water T2, Stream water T3 and Stream water T4) sources on the leaf area of *Amaranthus hybridus* over the eight-week growth period (mean $\pm$ standard deviation).

Figure 4 illustrates the effect of different irrigation water sources on the fresh weight of *A. hybridus* expressed as whole plant weight and edible portion. Wastewater (T4) produced the highest yield, with a whole plant weight of 0.380 kg and an edible portion of 0.140 kg. This was followed by well water (T2), stream water (T3), and tap water (T1), which recorded the lowest yield (0.170 kg whole plant and 0.060 kg edible portion). The greater fresh weight observed under wastewater irrigation is likely attributable to its higher concentrations of essential nutrients, particularly nitrogen and potassium (Table 1), which promote vegetative growth, water uptake, and biomass accumulation. The relatively small error bars across all treatments indicate low variability among the three replicate measurements, suggesting good experimental consistency. The greater biomass recorded under wastewater irrigation agrees with earlier evidence that nutrient-containing wastewater can enhance crop growth and productivity [30].

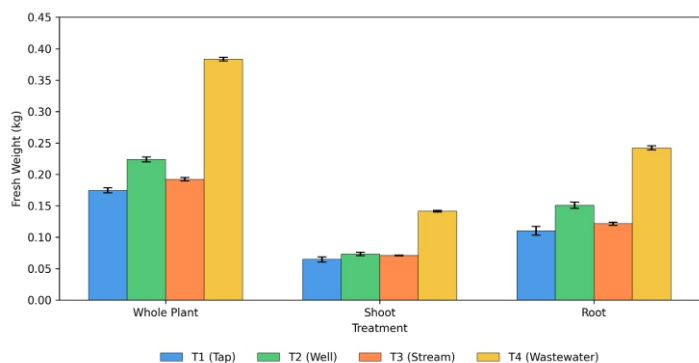


Figure 4: Effect of different irrigation water sources (Tap water T1, Well water T2, Stream water T3 and Wastewater T4) on the harvested fresh weight of *Amaranthus hybridus* (mean $\pm$ standard deviation).

The findings therefore suggest that wastewater can provide supplementary nutrients that stimulate the vegetative growth of *Amaranthus hybridus*. However, given the elevated heavy metal concentrations observed in the wastewater (Table 1), the agronomic benefits of wastewater irrigation should be weighed against the potential risk of heavy metal accumulation in edible tissues and the associated food safety concerns.

Table 5 demonstrated a statistically significant treatment effect on leaf area at all assessment periods. At week 2, a significant difference was observed among treatments, $F(3, 8) = 12.627$, $p = 0.002$. The treatment effect increased substantially at week 4, $F(3, 8) = 92.934$, $p < 0.001$, and was considerably stronger at week 6, $F(3, 8) = 450.049$, $p < 0.001$. The largest F-statistic was recorded at week 8, $F(3, 8) = 547.907$, $p < 0.001$, indicating very strong evidence of differences in leaf area among the treatments at the final assessment.

Table 5: Analysis of Variance of the Effect of Treatment on Leaf area at Weeks 2, 4, 6 and 8

Week	Source	Sum of Squares	df	Mean Square	F	P
2	Between Groups	16.542	3	5.514	12.627	.002
	Within Groups	3.493	8	.437		
	Total	20.035	11			
4	Between Groups	105.424	3	35.141	92.934	< .001
	Within Groups	3.025	8	.378		
	Total	108.449	11			
6	Between Groups	267.071	3	89.024	450.049	< .001
	Within Groups	1.582	8	.198		
	Total	268.653	11			
8	Between Groups	683.760	3	227.920	547.907	< .001
	Within Groups	3.328	8	.416		
	Total	687.088	11			

Note. ANOVA = analysis of variance; df = degrees of freedom; F = F-statistic; p = significance probability. Values reported as $p < .001$ where the statistical software output displayed .000.

3.3 Proximate Composition

The proximate composition of *Amaranthus hybridus* under the four irrigation regimes is presented in Table 6. Irrigation-water source was associated with significant differences in ash, crude lipid, crude protein, crude fibre, and carbohydrate, whereas moisture content did not differ significantly. These results shows that the source of irrigation water influenced several aspects of the nutritional profile, particularly protein and carbohydrate partitioning.

Moisture content is varied only slightly (87.27–89.60%), with tap water (T₁) recording the highest value. The small variation suggests that the irrigation source had little influence on tissue water content. High moisture content is typical of leafy vegetables and contributes to their freshness, although it may reduce post-harvest shelf life [33].

Ash content was higher in plants irrigated with well water (T₂) and stream water (T₃), indicating greater mineral accumulation. Similarly, crude protein content was highest in the wastewater (6.14%) and well water (6.09%) treatments, reflecting the greater availability of nutrients, particularly nitrogen, in these irrigation sources. Nitrogen plays a central role in amino acid and protein biosynthesis; consequently, nitrogen-rich irrigation water can enhance the protein content of leafy vegetables [22] [34]. Crude fibre content was highest in stream water-irrigated plants, suggesting enhanced structural development and cell wall formation under this treatment. In contrast, carbohydrate content was highest in the tap water treatment (1.89%) and lowest in the wastewater treatment (0.39%). The lower carbohydrate content in wastewater-irrigated plants, together with their higher crude protein content, suggests that increased nitrogen availability promoted carbon partitioning towards protein synthesis at the expense of carbohydrate accumulation, consistent with the carbon–nitrogen balance mechanism [36] [38]. Overall, the findings indicate that irrigation with well water and wastewater improved the nutritional quality of *A. hybridus* by increasing protein and mineral content. However, considering the elevated concentrations of lead and cadmium detected in the wastewater (Table 2), the nutritional benefits associated with wastewater irrigation should be balanced against the potential risk of heavy metal accumulation and its implications for food safety.

Table 6: Proximate composition of *Amaranthus hybridus* irrigated with different water sources

S/NO	Parameters	T1 (Tap)	T2 (Well)	T3 (Stream)	T4 (Waste)
1	Moisture (%)	89.60	87.27	87.38	88.46
2	Ash (%)	2.71	3.24	3.14	2.67
3	Crude Lipid (%)	0.01	0.03	0.05	0.02
4	Crude Protein (%)	3.29	6.09	4.71	6.14
5	Crude Fibre (%)	2.50	2.91	3.21	2.62
6	Carbohydrate (%)	1.89	0.46	1.51	0.39

The ANOVA results for proximate composition are presented in Table 7. Significant treatment effects were observed for ash, $F(3, 8) = 43.356$, $p < .001$; crude lipid, $F(3, 8) = 8.750$, $p = .007$; crude protein, $F(3, 8) = 271.882$, $p < .001$; crude fibre, $F(3, 8) = 35.658$, $p < .001$; and carbohydrate, $F(3, 8) = 1581.651$, $p < .001$. Moisture was the only parameter without a statistically significant treatment effect, $F(3, 8) = 2.710$, $p = .115$. The magnitude of the F-statistics for carbohydrate and protein indicates particularly strong separation among the treatment means relative to within-group variability. Nevertheless, the significant omnibus tests should be followed by post-hoc comparisons before specific pairwise claims are made.

Table 7: Analysis of variance of proximate composition across the experimental groups

Variable	Source	Sum of Squares	df	Mean Square	F	p-value
Moisture	Between Groups	10.689	3	3.563	2.710	.115
	Within Groups	10.518	8	1.315	—	—
	Total	21.207	11	—	—	—
Ash	Between Groups	.767	3	.256	43.356	< .001
	Within Groups	.047	8	.006	—	—
	Total	.815	11	—	—	—
Crude lipid	Between Groups	.003	3	.001	8.750	.007
	Within Groups	.001	8	.000	—	—
	Total	.003	11	—	—	—
Crude protein	Between Groups	14.702	3	4.901	271.882	< .001
	Within Groups	.144	8	.018	—	—
	Total	14.846	11	—	—	—
Crude fibre	Between Groups	.907	3	.302	35.658	< .001
	Within Groups	.068	8	.008	—	—
	Total	.974	11	—	—	—
Carbohydrate	Between Groups	5.101	3	1.700	1581.651	< .001
	Within Groups	.009	8	.001	—	—
	Total	5.109	11	—	—	—

Note: ANOVA = analysis of variance. *F* statistics are reported with numerator and denominator degrees of freedom of 3 and 8, respectively. Values originally reported as .000 are presented as *p* < .001. Statistical significance was evaluated at $\alpha = .05$. Post-hoc testing is necessary to determine the specific pairwise group differences underlying significant omnibus effects.

3.4 Heavy Metals & Mineral Content

In this study, the heavy metal composition and mineral content in *Amaranthus hybridus* were determined (Table 8). The irrigation water source significantly influenced the accumulation of heavy metals and mineral elements in *Amaranthus hybridus*. Nickel concentrations differed significantly among treatments, with the highest in samples treated with well water (3.57 mg/kg). Although nickel is an essential micronutrient involved in enzyme activity, excessive accumulation can be toxic to both plants and humans [5]. The elevated level observed in *Amaranthus hybridus* treated with well water may indicate geological contamination or leaching from surrounding soils.

Cadmium concentrations ranged from 0.68 mg/kg in the tap water treatment to 0.83 mg/kg in the wastewater treatment, exceeding the Codex Alimentarius maximum permissible limit of 0.20 mg/kg for leafy vegetables [8]. Cadmium is a non-essential and highly toxic metal that readily accumulates in plant tissues and may cause kidney dysfunction, skeletal disorders, and other adverse health effects following prolonged dietary exposure [17]. The high Cd levels observed across all treatments suggest that contamination may not be limited to irrigation alone but may also involve soil background levels or environmental deposition.

Lead concentrations ranged from 0.22 mg/kg in *Amaranthus hybridus* exposed to the tap water to 1.65 mg/kg in the

wastewater treatment samples, indicating substantial contamination associated with wastewater irrigation. Lead is not required for plant metabolism and interferes with photosynthesis, nutrient uptake, and normal physiological processes while posing serious health risks to consumers [18]. Essential macronutrients, including calcium and magnesium, were abundant in *Amaranthus hybridus* across all treatments, reflecting adequate nutrient availability. These elements are critical for cell wall development, membrane stability, enzyme activation, and photosynthesis [22]. Sodium showed greater variation among treatments, while potassium remained relatively stable, suggesting consistent uptake irrespective of irrigation source. Copper and manganese were present at concentrations that support normal plant metabolism, although excessive accumulation of these micronutrients may become phytotoxic [27].

These results show that irrigation water quality strongly influenced the elemental composition of *Amaranthus hybridus*. Although wastewater and well water enhanced the accumulation of essential mineral nutrients, the elevated concentrations of cadmium and lead represent a potential food safety concern. These findings are consistent with previous reports that leafy vegetables readily accumulate heavy metals due to their high transpiration rates and extensive leaf surface area, thereby increasing the risk of metal transfer into the human food chain [17] [24].

Table 8: Determination of Heavy Metals and Selected Mineral Elements in Vegetables

	Heavy metal (mg/kg)					Mineral Elements in the vegetables					
	Pb	Ni	Fe	Cd	Cr	Ca	Mg	Na	K	Cu	Mn
T1	0.22	1.89	31.03	0.68	40.69	1600.83	4219.45	36.33	787.73	34.22	22.31
T2	0.45	3.57	45.61	0.75	40.59	1895.12	4392.98	108.71	766.00	31.12	25.68
T3	0.61	1.57	35.60	0.71	40.91	2280.92	4345.00	77.43	782.10	33.31	24.66
T4	1.65	1.33	36.09	0.83	41.70	1979.18	4347.77	99.55	762.7	34.58	23.85

CONCLUSION

This study shows that the irrigation water source significantly influenced the growth, yield, nutritional composition, and heavy metal accumulation of *Amaranthus hybridus*. Wastewater and well water irrigation enhanced vegetative growth and improved the nutritional quality of the crop by increasing crude protein and mineral contents, which reflects the contribution of dissolved nutrients to plant development. However, wastewater irrigation also resulted in high concentrations of toxic heavy metals, particularly cadmium (0.83) and lead (1.65), which exceeded recommended food safety limits and may pose health risks to consumers. These findings highlight the trade-off between improved crop productivity and food safety, highlighting that while wastewater represents a nutrient-rich alternative irrigation source, its use without adequate treatment and monitoring is unsuitable for the sustainable production of leafy vegetables. Consequently, effective irrigation water management is essential to maximize crop productivity while safeguarding environmental quality and public health.

RECOMMENDATIONS: Although the findings of this study indicate that wastewater irrigation improves yield and enhances the nutritional quality of *Amaranthus hybridus*, the associated risk of heavy metal contamination poses a serious threat to consumer health. Based on these results, the following recommendations are made,

- i. Farmers should avoid using untreated wastewater for irrigation. In instances where the use is unavoidable, the water should be properly treated to reduce contaminants, particularly heavy metals such as lead and cadmium.
- ii. There should be regular monitoring and testing of irrigation water, soil, and plant tissues to detect and control the accumulation of toxic elements. Relevant regulatory bodies should also establish and enforce permissible limits for contaminants in irrigation water used for vegetable production.
- iii. Farmers should be educated and enlightened on the health risks associated with untreated wastewater and trained in safer irrigation methods and improved crop management practices.

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