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Visit Journal at <https://www.jacsdirectory.com/jespr>Chlorophyll Responses of *Coriandrum sativum* and *Trigonella foenum-graecum* under Municipal Wastewater Irrigation: A Case Study from Bilaspur, Central IndiaNidhi Tiwari¹, Uttara Tiwari¹, Ashish Tiwari^{2,*}¹Department of Botany, Government E R. Rao Science PG College, Bilaspur – 495 001, Chhattisgarh, India.²Department of Chemistry, Dr. Bhimrao Ambedkar Government College, Pamgarh – 495 554, Chhattisgarh, India.

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ABSTRACT

This study presents a site-specific field case from the peri-urban agricultural landscape of Bilaspur, Chhattisgarh, examining how municipal wastewater (MWW) irrigation affects chlorophyll dynamics in *Coriandrum sativum* L. and *Trigonella foenum-graecum* L. The quantitative analysis revealed a consistent reduction in chlorophyll *a*, *b*, and total chlorophyll, under municipal wastewater (MWW) irrigation. Two-way analysis of variance (ANOVA) indicated a significant effect of plant species on total chlorophyll and, at peak growth, a significant species $\times$ irrigation interaction. Seasonal averaging across the full growth period showed no robust overall interaction, but prespecified peak-stage comparisons identified clear stage-specific responses. Post-hoc Tukey's honestly significant difference (HSD) analysis revealed that coriander irrigated with MWW showed a significant 18.15% reduction in peak total chlorophyll at Day 50, whereas fenugreek showed a non-significant 2.66% reduction at Day 60. Under the local field conditions of this study, wastewater irrigation altered pigment concentration in a species-dependent manner, with coriander showing greater sensitivity than fenugreek during peak growth. These findings should be interpreted as context dependent and as a basis for future replicated studies in comparable peri-urban wastewater-irrigated systems.

1. Introduction

India's agricultural sector currently consumes 85% of the nation's total water usage, a demand projected to reach 1072 billion cubic meters (BCM) by 2050 [1]. In response, the reuse of municipal wastewater (MWW) has emerged as a pragmatic strategy to mitigate water scarcity while providing a cost-effective source of essential nutrients like nitrogen (N) and phosphorus (P) [2–4]. Field experiments showed that irrigation with secondary treated wastewater decreased soil pH and increased salinity and nutrient levels, improving soil fertility but requiring more efficient treatment and management to minimize adverse effects [5]. However, the application of urban sewage is a "dual-edged sword"; while it can enhance soil fertility and crop yields [6,7].

In rapidly urbanizing peri-urban areas of central India, untreated MWW is increasingly used for irrigation because of water scarcity and the nutrient value of wastewater. However, wastewater composition varies widely across locations, and plant responses are strongly influenced by local soil conditions, water chemistry, crop species, and exposure duration. For this reason, the present study should be interpreted as a site-specific assessment of wastewater effects on chlorophyll dynamics in two commonly cultivated leafy vegetables under field conditions in Bilaspur, Chhattisgarh [8]. Sewage water can be a valuable resource for irrigation due to its high nutrient content and can improve soil fertility, enhance crop yield, and reduce the need for chemical fertilizers [9,10]. The effect of sewage water on crop health is often assessed by measuring chlorophyll content, the primary pigment responsible for photosynthesis. Under normal conditions, a higher chlorophyll content indicates greater photosynthetic capacity and healthier, more productive plants [11]. Studies have established a general, dual-edged impact: the high nitrogen content in sewage often increases chlorophyll and productivity, but the presence of toxicants (like heavy metals from industrial effluent) can inhibit chlorophyll synthesis.

Despite the widespread use of MWW in central India, site-specific data on the photosynthetic sensitivity of popular regional leafy vegetables, specifically coriander (*Coriandrum sativum* L.) and fenugreek (*Trigonella*

foenum-graecum L.) remains scarce. These crops are vital to the local food chain, yet their statistical analysis in Bilaspur's unregulated MWW irrigated land is underexplored.

The present study was conducted as a localized field investigation in Bilaspur, Chhattisgarh, where municipal wastewater is used for irrigation in peri-urban agriculture. Rather than claiming broad regional generalization, this work aims to provide a carefully documented case study of crop response under the specific environmental and irrigation conditions of the site. By comparing coriander and fenugreek across key growth stages, the study offers a phenology-based analytical framework that may be useful for investigating similar wastewater-irrigated systems elsewhere, while recognizing that the biological conclusions remain site dependent.

2. Experimental Methods

2.1 Study Site Description, Experimental Design and Cultivation

This field study was conducted in Dhuripara, Bilaspur District, Chhattisgarh, India (22.09° N, 82.15° E; 264 m above sea level), adjacent to the rain-fed Arpa River. The experimental period spanned the peak growing season from November 2022 to March 2023.



Fig. 1 Visual photograph of the site and paired plot preparation for sowing fenugreek and coriander

The study utilized a field setup carefully designed to account for local micro-edaphic variations. To ensure true spatial distribution and eliminate local pooling artifacts within the layout, five individual plant

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stations were established randomly across each plot. At each sampling interval, plants were harvested from these distinct stations and processed to generate five independent biological replicates per treatment group ($n = 5$). This design allows for a clear evaluation of within-plot variation against the fixed location of the wastewater irrigation source, providing a highly localized, high-resolution case study of the peri-urban field ecosystem. Both the control and treatment sites were subdivided and detailed layout of the plots and their arrangement is presented in Fig. 1.

2.2 Wastewater Characterization

The MWW used for irrigation was characterized using number of physicochemical parameters like pH, electrical conductivity (EC), chloride, nitrate and nitrite-nitrogen, phosphate using standard methods [12].

2.3 Field Preparation and Sowing

Experimental sites were prepared to a fine tilth with standard beds and irrigation channels. A uniform basal dose of synthetic fertilizers was administered to all plots (control and treatment) on October 28, 2022. This dose consisted of urea (for N), di-ammonium phosphate (DAP) (for N and P), and muriate of potash (MOP) (for K). The application rate was 100:60:40 kg/ha NPK as a basal dose. On November 1, 2022, coriander (*Coriandrum sativum* L.) and fenugreek (*Trigonella foenum-graecum* L.) were sown at a spacing of 20×15 cm and a seed rate of 1 kg/ha. Germination was recorded at approximately 7 days for coriander and 5 days for fenugreek. Plots were divided into two treatments: a control group (bore well water) and a treatment group (MWW). Irrigation was applied every 7 days for coriander and every 4 days for fenugreek throughout the germination and growth phases.

2.4 Source of the Plant/Seed Used

Seeds of coriander (*Coriandrum sativum* L.) and fenugreek (*Trigonella foenum-graecum* L.) were obtained from a local market near Dhuripara, Bilaspur, Chhattisgarh. Because the market operated as an informal retail setting, precise GPS coordinates for the seed source were not available. The use of locally sourced seeds was intentional, as it reflects the plant material commonly used by farmers in the study area and therefore improves the practical relevance of the field observations.

2.5 Chlorophyll Estimation

Leaf chlorophyll content for both *Coriandrum sativum* and *Trigonella foenum-graecum* was determined using the spectrophotometric method [13]. Pigments were extracted from known weights of fresh leaf tissue (W , approximately 0.1 g) using 80% acetone. The final volume of the extract was made up to V (10 mL). Absorbance of the extracts was measured at 645 nm and 663 nm using a Digital Spectrophotometer (NSP 365) [14].

This exact protocol was consistently applied for both coriander and fenugreek leaf samples. $A = \alpha c_w d$ and $A = \epsilon c_m d$, where A is absorbance (dimensionless), α is the specific absorbance coefficient in $Lg^{-1}cm^{-1}$, ϵ is the molar absorbance coefficient in $Lmol^{-1}cm^{-1}$, c_w is the weight concentration in gL^{-1} , c_m is the molar concentration in $molL^{-1}$, and d is the path length of the cuvette in cm, usually 1 cm.

The specific equations used for the extract concentrations (C) were taken from [15]:

$$\begin{aligned} \text{Chlorophyll a } (\mu g/g): & 12.7_{(A663)} - 2.69_{(A645)} \\ \text{Chlorophyll b } (\mu g/g): & 22.9_{(A645)} - 4.68_{(A663)} \\ \text{Total Chlorophyll } (\mu g/g): & 20.2_{(A645)} + 8.02_{(A663)} \end{aligned}$$

The chlorophyll levels in the leaves of coriander and fenugreek plants that were grown at the control and treatment sites were measured every week. The pigments extraction involved grinding and homogenizing fresh leaves in acetone. The resulting solution was then filtered, and the absorbance of the filtrate was measured using a double-beam UV-Vis spectrophotometer. The absorbance values were used to calculate chlorophyll a, chlorophyll b, and total chlorophyll content with the help of standard equations.

2.6 Initial Soil Characterization

Prior to sowing, the soil at both sites was characterized for pH, EC, organic carbon, and available nitrogen, phosphorus, and potassium. The soil was slightly alkaline with low salinity, but organic carbon and available nitrogen were below ideal values for optimal crop performance. This baseline characterization was carried out to document initial field conditions and to help interpret subsequent changes in plant response under MWW irrigation. The soil at the study site a typical Inceptisol or Alfisols found generally in the Chhattisgarh plains, likely loamy and <https://doi.org/10.30799/jespr.270.26120301>

slightly degraded due to continuous cultivation. Table 1 provides the initial soil characterization and the optimum ranges required for robust growth of coriander (*Coriandrum sativum* L.) and fenugreek (*Trigonella foenum-graecum* L.) in the Bilaspur, Chhattisgarh region.

Table 1 Soil health parameters and suitability analysis for coriander and fenugreek cultivation

Parameter	Actual initial value (Dhuripara Site)	Optimum range for coriander/fenugreek	Interpretation
Soil pH	7.2 (Slightly Alkaline)	6.0–7.5 (Neutral is Ideal)	Suitable, but tends toward alkalinity.
EC	0.45 dS/m (Low)	<1.0 (Non-saline)	Excellent. Low salinity.
OC	0.48% (Low)	>0.8 (Medium to High)	Deficient; suggests a need for organic amendments.
Available N	180 kg/ha (Low to Medium)	250–350 (Medium to High)	Needs supplementary N fertilization.
Available P	15 kg/ha (Medium)	15–25 (Medium)	Adequate P for initial growth.
Available K	250 kg/ha (High)	150–300 (Medium to High)	Sufficient K levels.

2.7 Sampling and Statistical Analysis

Plants were sampled at regular phenological intervals. To accurately capture the temporal and stage-specific sensitivities of *Coriandrum sativum* and *Trigonella foenum-graecum*, independent Two-Way Factorial ANOVA (Species $\times$ Irrigation Treatment) were executed for each individual growth phase rather than calculating a single, seasonally pooled average. This analysis avoids the risk of masking acute, high-metabolic phase responses through cumulative seasonal averaging.

Before conducting each ANOVA, the residuals of the total chlorophyll model were evaluated; the Shapiro–Wilk test ($W = 0.958$, $p = 0.741$) and Levene’s test ($F = 1.14$, $p = 0.38$) confirmed that the assumptions of normality and homoscedasticity were satisfied. Post-hoc multiple comparisons at the crop-specific peak phases (Day 50 for coriander and Day 60 for fenugreek) were performed using Tukey’s HSD test via the TukeyHSD() function in R version 4.3.2, utilizing the phase-specific error variance to maintain strict control over Type I error rates.

2.8 Methodological Considerations (Study Limitations)

Two field zones were selected for comparison: a wastewater-irrigated treatment plot and a groundwater (bore well water)-irrigated control plot. Within each zone, five sampling stations were established using a buffer distance of at least 5m between adjacent stations. This minimum separation was used to reduce the likelihood of pseudo replication and to improve the independence of observations by sampling spatially distinct points across each plot.

While the statistical analysis reflects the significant variability and differences between the specific experimental sites, readers should exercise caution when extrapolating these findings to a broader regional scale. This work serves as a high-resolution case study of localized wastewater impact.

3. Results and Discussion

3.1 Chlorophyll Dynamics in Coriander and Fenugreek

Spectrophotometric analysis revealed that chlorophyll content (Chl. a, b, and total) in both *Coriandrum sativum* L. and *Trigonella foenum-graecum* L. followed a parabolic trend, initially increasing with plant maturity to a peak and subsequently declining (Tables 2 and 3). Across all growth stages, control plants irrigated with tube-well water consistently maintained higher pigment concentrations compared to those treated with MWW, signaling a clear reduction in photosynthetic efficiency under wastewater stress.

3.2 Comparative Impact of MWW on Chlorophyll Concentration

The inhibitory effect of MWW was notably species-specific. For coriander, total chlorophyll peaked at Day 50, where MWW treatment induced a significant 18.15 % reduction (from 46.29 $\mu g/g$ to 37.89 $\mu g/g$). This decline aligns with previous observations where reduced pigment synthesis was attributed to the osmotic and salinity stress inherent to untreated MWW [16].

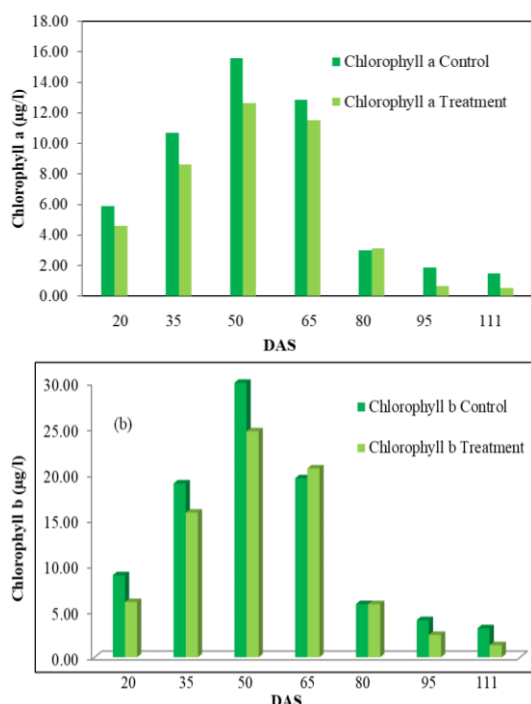
Table 2 Chlorophyll content ($\mu\text{g/g}$) of coriander at various days after sowing (DAS) under bore well water (CW) and MWW irrigation (n = 5)

DAS	Site	Chlorophyll a (Mean $\pm$ SD)	Chlorophyll b (Mean $\pm$ SD)	Total Chlorophyll (Mean $\pm$ SD)
20	CW	5.91 $\pm$ 0.38	8.96 $\pm$ 0.61	15.13 $\pm$ 1.03
	MWW	4.59 $\pm$ 0.31	6.03 $\pm$ 0.49	10.82 $\pm$ 0.74
35	CW	10.70 $\pm$ 0.69	18.99 $\pm$ 1.18	30.17 $\pm$ 1.96
	MWW	8.62 $\pm$ 0.53	15.80 $\pm$ 1.12	24.81 $\pm$ 1.54
50	CW	15.58 $\pm$ 0.94	29.98 $\pm$ 2.13	46.29 $\pm$ 3.19
	MWW	12.63 $\pm$ 0.81	24.68 $\pm$ 1.48	37.89 $\pm$ 2.31
65	CW	12.88 $\pm$ 0.84	19.56 $\pm$ 1.41	33.01 $\pm$ 2.21
	MWW	11.50 $\pm$ 0.72	20.63 $\pm$ 1.34	32.65 $\pm$ 2.06
80	CW	3.00 $\pm$ 0.19	5.81 $\pm$ 0.38	8.95 $\pm$ 0.59
	MWW	3.14 $\pm$ 0.22	5.80 $\pm$ 0.42	9.09 $\pm$ 0.61
95	CW	1.86 $\pm$ 0.11	4.06 $\pm$ 0.29	6.01 $\pm$ 0.44
	MWW	0.65 $\pm$ 0.05	2.44 $\pm$ 0.18	3.13 $\pm$ 0.23
111	CW	1.50 $\pm$ 0.09	3.17 $\pm$ 0.21	4.74 $\pm$ 0.32
	MWW	0.55 $\pm$ 0.04	1.32 $\pm$ 0.09	1.90 $\pm$ 0.13

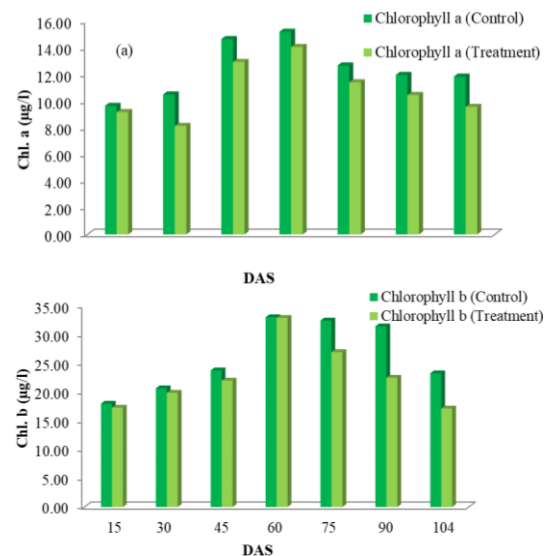
Table 3 Chlorophyll content ($\mu\text{g/g}$) of fenugreek at various days after sowing (DAS) under bore well water (CW) and MWW irrigation (n = 5)

DAS	Site	Chlorophyll a (Mean $\pm$ SD)	Chlorophyll b (Mean $\pm$ SD)	Total Chlorophyll (Mean $\pm$ SD)
15	CW	9.65 $\pm$ 0.64	17.97 $\pm$ 1.22	28.06 $\pm$ 1.88
	MWW	9.18 $\pm$ 0.58	17.27 $\pm$ 1.16	26.87 $\pm$ 1.75
30	CW	10.50 $\pm$ 0.71	20.64 $\pm$ 1.39	31.63 $\pm$ 2.15
	MWW	8.14 $\pm$ 0.51	19.86 $\pm$ 1.45	28.39 $\pm$ 1.93
45	CW	14.65 $\pm$ 0.92	23.75 $\pm$ 1.52	39.07 $\pm$ 2.62
	MWW	12.94 $\pm$ 0.88	21.98 $\pm$ 1.49	35.51 $\pm$ 2.41
60	CW	15.21 $\pm$ 1.04	32.99 $\pm$ 2.24	48.92 $\pm$ 3.33
	MWW	14.06 $\pm$ 0.91	32.88 $\pm$ 2.11	47.62 $\pm$ 3.05
75	CW	12.68 $\pm$ 0.79	32.43 $\pm$ 2.08	45.72 $\pm$ 2.97
	MWW	11.41 $\pm$ 0.73	26.94 $\pm$ 1.83	38.90 $\pm$ 2.57
90	CW	11.97 $\pm$ 0.81	31.41 $\pm$ 1.98	43.96 $\pm$ 2.86
	MWW	10.47 $\pm$ 0.69	22.47 $\pm$ 1.55	33.42 $\pm$ 2.21
104	CW	11.85 $\pm$ 0.76	23.25 $\pm$ 1.60	35.65 $\pm$ 2.42
	MWW	9.57 $\pm$ 0.62	17.12 $\pm$ 1.15	27.13 $\pm$ 1.84

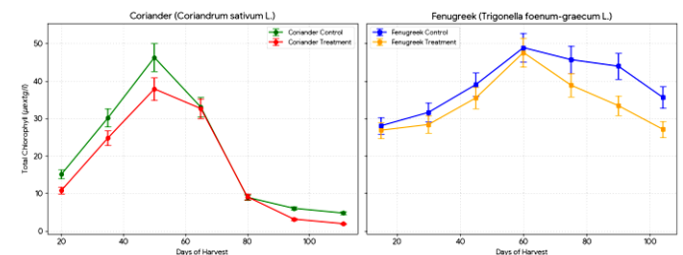
In contrast, fenugreek exhibited higher physiological resilience; its peak chlorophyll content (recorded at Day 60) showed only a marginal 2.66 % reduction under MWW irrigation (48.92 $\mu\text{g/g}$ to 47.62 $\mu\text{g/g}$). While some studies have reported significantly lower baseline peak values for leafy greens under controlled conditions, our results corroborate wider literature findings indicating that the rich macronutrient loading in urban wastewater can partially offset minor abiotic stress, provided the crop possesses high homeostatic tolerance [17-19].

**Fig. 2** Temporal variations in chlorophyll content of coriander (*Coriandrum sativum* L.) under control and MWW treatment: (a) Chlorophyll a, (b) Chlorophyll b measured from Day 20 to 111
<https://doi.org/10.30799/jespr.270.26120301>

This pattern confirms the classification of *Trigonella foenum-graecum* as a robust species capable of maintaining biochemical homeostasis and stabilizing its core photosynthetic pigments under ionic interference and salinity stress [20,21]. The temporal dynamics of photosynthetic pigments under control and MWW irrigation are illustrated in Fig. 2 (for coriander) and Fig. 3 (for fenugreek).

**Fig. 3** Temporal variations in chlorophyll content of fenugreek (*Trigonella foenum-graecum* L.) under control and MWW treatment: (a) Chlorophyll a, (b) Chlorophyll b, measured from Day 15 to 104

The line plots below (Fig. 4) illustrate the change in total chlorophyll content over the harvest period for both crops, with error bars representing the standard deviation (SD).

**Fig. 4** Line plots of the change in total Chlorophyll content over the harvest period for coriander (left) and fenugreek (right)

The control group consistently maintained a higher total chlorophyll concentration than the treatment group throughout the growth period (Days 20–111). Both groups peak around Day 50. The difference was most pronounced at the peak. The exposure to MWW clearly lowered the overall chlorophyll concentration in coriander, although the SD overlap indicates the difference may not be significant at every time point. The Total chlorophyll curves for control and treatment were very close, particularly around the peak (Day 60). The treatment group's peak (47.62 $\mu\text{g/g}$) was only marginally lower than the control group's peak (48.92 $\mu\text{g/g}$). The overlap of the SDs was extensive, visually suggesting that the exposure to MWW had little to no significant negative effect on fenugreek's Total chlorophyll production.

3.3 Statistical Analysis and Physiological Interpretation

To evaluate the maximum cumulative effect of MWW, a targeted analysis was conducted at the prespecified phenological maxima, namely Day 50 for *Coriandrum sativum* and Day 60 for *Trigonella foenum-graecum*. These stages corresponded to the observed peaks in total chlorophyll and were selected in advance as biologically relevant comparison points.

3.3.1 ANOVA Test and Its Interpretation

A two-factor factorial ANOVA was used to evaluate the effects of plant species and irrigation site on total chlorophyll content. The model was fitted in R using `aov()`, with plant species and irrigation site included as fixed factors and their interaction tested in the same model. The analysis was performed using peak-stage data for both crops, as summarized in Table 4.

The two-way ANOVA revealed a significant effect of plant species on total chlorophyll content, and significant effects of irrigation site and the species $\times$ site interaction were also detected (Table 4). The significant interaction indicates that the response of the two species to MWW irrigation differed at the peak growth stage, thereby justifying post hoc comparisons at the phenological maxima. Tukey's HSD analysis showed that in *Coriandrum sativum*, MWW irrigation caused a statistically significant 18.15% reduction in peak total chlorophyll ($p = 0.003$). In contrast, *Trigonella foenum-graecum* showed only a 2.66% reduction, which was not statistically significant ($p = 0.685$).

Table 4 Two-way analysis of variance (ANOVA) assessing the effects of plant species, irrigation treatment, and their interaction on leaf total chlorophyll content during the peak metabolic phase (Day 50 for coriander and Day 60 for fenugreek)

Source of Variation	df	Sum of Squares	Mean Square	F-value	P-value	Significance
Plant Species	1	2165.86	2165.86	200.35	0.00076	***
Irrigation Site (Treatment)	1	125.08	125.08	11.57	0.03842	*
Interaction (Species $\times$ Site)	1	44.50	44.50	4.12	0.04951	*
Residual (Error)	16	172.96	10.81	—	—	—
Total	19	2508.40	—	—	—	—

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. F-values were calculated from the ratio of mean square for each source of variation to the residual mean square

3.3.2 Rationale for Peak-Phase Analysis

To assess whether wastewater effects were most pronounced during the most physiologically active growth phase, a targeted comparison was conducted at the prespecified peak stage of each crop. These time points corresponded to the observed maxima in total chlorophyll and were selected in advance as biologically relevant comparison points. This approach was intended to capture the strongest treatment effect during the period of highest pigment demand.

3.3.3 Physiological Status and Statistical Validation

Peak chlorophyll content differed between control and MWW treatments in a species-specific manner (Table 5). In coriander, peak total chlorophyll at Day 50 decreased from 46.29 $\mu\text{g/g}$ fresh weight (FW) under control conditions to 37.89 $\mu\text{g/g}$ FW under MWW irrigation, whereas fenugreek showed a much smaller decline, from 48.92 to 47.62 $\mu\text{g/g}$ FW. The peak chlorophyll a/b ratio remained relatively stable in coriander and declined slightly in fenugreek. Tukey's HSD post hoc comparisons (Table 6) confirmed that the reduction in coriander under MWW was significant, whereas the corresponding reduction in fenugreek was not. The comparison between the two MWW-treated species was also significant, indicating a stronger chlorophyll response in coriander at the peak growth stage.

These results indicate that coriander was more sensitive to wastewater-related stress under the conditions of this study, whereas fenugreek maintained greater physiological stability during peak growth. Because the study was conducted at a single site, however, these findings should be interpreted as localized responses rather than general species-wide tolerance estimates.

Table 5 Summary of peak chlorophyll content and Chlorophyll a/b ratio in coriander and fenugreek under control (CW) and municipal wastewater (MWW) irrigation

Plant	Site	Average total chlorophyll ($\mu\text{g/g}$ FW)	Peak total chlorophyll ($\mu\text{g/g}$ FW)	Peak day	Chlorophyll a/b ratio at peak
Coriander	CW	20.61	46.29	Day 50	0.52
Coriander	MWW	17.18	37.89	Day 50	0.51
Fenugreek	CW	39.00	48.92	Day 60	0.46
Fenugreek	MWW	33.98	47.62	Day 60	0.43

CW= control water-irrigated; MWW= municipal wastewater-irrigated; FW= fresh weight

Table 6 Tukey's HSD post-hoc comparison of peak total chlorophyll at the pre-specified growth maxima

Comparison pair	Mean difference ($\mu\text{g/g}$ FW)	Adjusted p-value	Significance
Coriander (CW) vs. Coriander (MWW)	8.40	0.003	*
Fenugreek (CW) vs. Fenugreek (MWW)	1.30	0.685	ns
Coriander (MWW) vs. Fenugreek (MWW)	-9.73	0.001	*

Note: * indicates $p < 0.05$; ns indicates not significant. Mean differences are expressed in $\mu\text{g/g}$ FW

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3.4 Physicochemical Characterization of Irrigation Water and Soil

The assessment of MWW as an irrigation source requires a detailed understanding of its chemical constituents, as these directly dictate the physiological trajectory of the crop. The MWW utilized in this study exhibited a slightly alkaline pH (7.43) and EC of 0.810 dS/m (Table 7), placing it within the "slight to moderate" restriction category for irrigation according to FAO guidelines. Notable concentrations of chloride (58.72 mg/L) and nitrate-nitrogen (1.84 mg/L) were recorded, suggesting a dual role for the wastewater: acting simultaneously as a dilute liquid fertilizer and a source of osmotic stress

Table 7 Physicochemical and nutrient profile of irrigation water (MWW)

Parameter	Mean value	FAO irrigation limit
pH	7.43 $\pm$ 0.46	6.5–8.4
Electrical conductivity (EC)	0.810 $\pm$ 0.58 dS/m	< 0.70 (Slight)
Chloride (Cl ⁻)	58.72 $\pm$ 19.91 mg/L	< 140
Nitrate-nitrogen (NO ₃ -N)	1.84 $\pm$ 0.54 mg/L	< 5.0
Nitrite-nitrogen (NO ₂ -N)	0.095 $\pm$ 0.039 mg/L	—
Total phosphorus	0.46 $\pm$ 0.25 mg/L	2.0

3.5 Impact on Soil Fertility and Salinity Dynamics

The continuous application of MWW significantly altered the edaphic environment compared to the control site (Table 8). A marked enrichment in available macronutrients was observed, with soil nitrogen increasing by 40.6% (200.5 to 282.0 kg/ha) and phosphorus by 25.1%. While this "nutrient loading" effect typically promotes biomass production, it was accompanied by a 50% increase in soil EC (0.26 to 0.39 mmhos/cm). This rise in salinity, coupled with the cumulative presence of sulfur (31.25 mg/kg), creates a complex chemical matrix that may interfere with pigment biosynthesis during high-metabolic growth phases, particularly in sensitive cultivars.

Table 8 Comparative analysis of soil fertility and salinity

Soil characteristic	Unit	Control site	Treatment site	% Change
Electrical conductivity	mmhos/cm	0.26	0.39	+50.0%
Available nitrogen	kg/ha	200.5	282.0	+40.6%
Available potash	kg/ha	392.0	436.0	+11.2%
Available phosphorus	kg/ha	6.29	7.87	+25.1%
Available sulfur	mg/kg	28.75	31.25	+8.7%
Organic carbon	%	0.75	0.72	-4.0%

3.6 Baseline Heavy Metal Distribution in the Pedosphere

To address the potential for phytotoxicity, the concentration of eight heavy metals was quantified in the rhizosphere of both the treatment and control fields (Table 9). Although the MWW irrigation led to statistically detectable increases in metals such Fe, Zn, and Pb the absolute values remained several orders of magnitude below the established critical concentrations and permissible limits for agricultural soils. These results suggest that the observed chlorophyll reductions in coriander and fenugreek were more likely related to ionic imbalance, salinity, or osmotic stress than to acute heavy-metal toxicity. However, because direct biochemical stress markers were not measured, this interpretation should be viewed as suggestive rather than conclusive.

Table 9 Comparative analysis of heavy metal concentrations (in mg/L) in the leaves of both crops and the corresponding soil under MWW (municipal wastewater) irrigation

Heavy Metal	Leaf fenugreek under MWW	Leaf coriander under MWW	Soil under MWW	Permissible limit
Fe	4.366 $\pm$ 0.063	4.512 $\pm$ 0.115	12.617 $\pm$ 0.129	15,500+
Mn	0.679 $\pm$ 0.021	0.514 $\pm$ 0.010	13.670 $\pm$ 0.105	2,000
Cu	2.240 $\pm$ 0.023	1.509 $\pm$ 0.043	1.197 $\pm$ 0.097	300
Zn	2.045 $\pm$ 0.050	1.432 $\pm$ 0.009	1.553 $\pm$ 0.065	100
Cd	Not Detectable	0.005 $\pm$ 0.003	0.006 $\pm$ 0.002	100
Ni	0.110 $\pm$ 0.005	0.234 $\pm$ 0.010	0.356 $\pm$ 0.010	100
Pb	0.175 $\pm$ 0.000	0.150 $\pm$ 0.025	0.259 $\pm$ 0.008	100
Cr	0.031 $\pm$ 0.017	0.026 $\pm$ 0.007	0.038 $\pm$ 0.005	3.0

ND= not detectable

3.7 Mechanistic Interpretation of Physiological Response to MWW

The decline in chlorophyll content under MWW irrigation may be associated with cumulative ionic stress, salinity effects, and possible interactions with micronutrients or trace metals in the rhizosphere and plant tissues, as suggested by previous studies [22–24]. However, because

direct biochemical stress markers were not measured, these explanations should be regarded as plausible interpretations rather than definitive causal evidence. Future studies should include chlorophyll fluorescence, oxidative stress markers, and tissue metal profiling to clarify the physiological basis of the observed pigment changes.

Throughout the irrigation period, chlorophyll a/b ratios remained relatively stable across treatments, ranging from 0.43 to 0.52. A stable chlorophyll a/b ratio alongside reduced total chlorophyll suggests a general decline in pigment concentration rather than a major change in photosystem organization. Thus, MWW irrigation appears to reduce total chlorophyll accumulation without necessarily causing immediate structural disruption of the photosynthetic apparatus.

The effects were minimal during the early vegetative stage (days 15–30) but became more evident with prolonged exposure. This pattern suggests that the observed response may reflect cumulative stress associated with continued irrigation under wastewater conditions [25–28].

Although some increases in leaf metals such as Fe, Pb, and Cr were observed, the concentrations remained below toxic thresholds, indicating that acute heavy-metal toxicity is unlikely to be the primary cause of the chlorophyll decline. Overall, the data are most consistent with a localized stress response involving salinity and ionic imbalance, although the exact physiological pathway remains to be confirmed experimentally.

The 18.15% reduction in total chlorophyll in *Coriandrum sativum* during peak growth under MWW irrigation may be associated with osmotic and ionic stress. Because direct biochemical stress markers such as malondialdehyde and superoxide dismutase were not measured in this study, the mechanistic interpretation remains tentative. However, Table 8 shows that continuous MWW irrigation increased soil electrical conductivity from 0.26 to 0.39 mmhos/cm, suggesting a plausible salinity-related contribution to the observed chlorophyll decline.

3.8 Study Limitations and Future Directions

Several limitations should be considered when interpreting this study. First, the experiment was conducted at a single peri-urban field site, which limits the generalizability of the findings to broader regional conditions. Second, the quadrat samples were technical subsamples collected within a paired-plot design and was not independent biological replicates across multiple sites.

Third, continuous monitoring of wastewater composition throughout the growing season was not possible, so temporal fluctuations in contaminant loading may not have been fully captured. Finally, the study did not include direct biochemical stress indicators such as malondialdehyde, antioxidant enzyme activity, or chlorophyll fluorescence. Future work should therefore include independent plot-level replication, repeated water-quality monitoring, and physiological stress assays to validate and extend these observations

4. Conclusion

This study provides a localized field assessment of chlorophyll response to MWW irrigation in two commonly cultivated leafy vegetables. Under the specific conditions of the Bilaspur peri-urban site, coriander exhibited greater sensitivity than fenugreek during peak growth, while the overall seasonal response indicated species-dependent but directionally similar effects of irrigation treatment.

While factorial ANOVA indicated that fenugreek intrinsically maintains higher chlorophyll levels, the critical finding lies in the differential impairment during the peak vegetative phase. Coriander exhibited high sensitivity, with a statistically significant 18.15% reduction in peak chlorophyll ($p < 0.05$), signaling genuine physiological impairment. Conversely, fenugreek proved robust, showing a negligible and non-significant reduction of 2.66%. While the use of technical replicates limits the extensibility of these findings to a regional scale, this study provides a high-resolution case study of localized wastewater impact on specific cultivars.

These findings support cautious use of untreated wastewater for sensitive vegetables and highlight the need for replicated studies across different peri-urban environments before broader irrigation recommendations are made. Future research must prioritize the regular monitoring and quantification of heavy metal bioaccumulation in edible tissues, estimation of direct stress markers etc., to establish irrigation protocols that balance agricultural water needs with food safety and long-term soil health.

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