



Effects of N, P and K deficiencies on nutrient accumulation and quality parameters in hydroponic lettuce (*Lactuca sativa* L.) cultivation

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ABSTRACT

This study evaluated the chronological impacts of nitrogen (-N), phosphorus (-P), and potassium (-K) deficiencies on lettuce (*Lactuca sativa* L.) grown hydroponically. Using a randomized complete block design with three replicates, morphological, physiological, physicochemical, and tissue elemental dynamics were assessed across early (Week 1) and chronic (Week 2) stress phases. Macronutrient starvation rapidly and distinctly constrained shoot growth and vegetative development ($P < 0.0001$), while fully nourished controls optimized plant height (30.63 cm) and shoot fresh mass (15.36 g). Individual omissions triggered immediate root-zone adaptations: transient root elongation under -K (15.17 cm) and root thickening under -P. Leaf canopy temperature was a sensitive physiological indicator; -K consistently elevated canopy temperatures (20.03 °C to 19.16 °C) due to disrupted stomatal cooling. Root-zone solutions showed distinct acidification under -N (pH 6.39, Week 1) and alkalization under -K (pH 7.22). ICP spectrometry revealed strong microelement crosstalk during chronic stress: controls maintained optimal molybdenum accumulation (10.75 ppb), whereas -N, -P, or -K triggered complete shutdown of Mo transport, collapsing to 0.09 ppb under -K. This regulatory collapse indicates molybdate transporter efficiency (MOT1) is tightly bound to primary nutrient availability and root energy status. Monitoring non-destructive physiological vectors like early leaf temperature changes, combined with subtle microelement signatures such as Mo dynamics, offers a precise diagnostic framework for early detection of macronutrient starvation before irreversible visual stunting manifests.

Key words: Macronutrient deficiency, lettuce, molybdenum, canopy temperature, hydroponics, early diagnosis.

INTRODUCTION

Lettuce (*Lactuca sativa* L.), a prominent member of the Asteraceae family, is a cool-season leafy vegetable widely cultivated for its broad green leaves. The native range of is generally considered to be the Mediterranean basin and the Near East. More specifically, it is often described as originating from the eastern Mediterranean region or Western Asia. Genetic studies suggest the earliest cultivation began in the Caucasus region roughly 6,000 years ago, followed by domestication in ancient Egypt. From there, it spread throughout Europe, Asia, and North Africa (Majid *et al.*, 8; Wei *et al.*, 14). In Turkey, lettuce cultivation is traditionally carried out in temperate regions during the autumn, winter, or early spring seasons (Chowdhury *et al.*, 2). The crop can be successfully grown in both open fields and protected environments, provided that the climatic conditions are suitable. Globally, lettuce production has reached a substantial commercial scale. As of 2024, global lettuce production amounted to approximately 28.2 million tonnes across 1.28 million hectares, with China leading the global market by accounting for 57% (14.9 million tonnes) of the total

output, followed by the United States and India (FAO, 3). Turkey ranks 7th globally with a production volume of 602,848 tonnes, trailing slightly behind Spain and Italy (FAO 3). The most widely cultivated lettuce varieties in Turkey are curly lettuce (263,829 tonnes), head lettuce (225,542 tonnes), and iceberg lettuce (113,477 tonnes), respectively.

In protected cultivation, soilless farming (hydroponics) is becoming increasingly prevalent worldwide to meet commercial demands and overcome soil-related limitations. Having initiated in the 1930s with greenhouse tomato production, soilless cultivation has evolved into a standard method for many crops, particularly short-season leafy greens. Since the 2000s, this technology has expanded rapidly in the Mediterranean basin, leading to the establishment of modern facilities and the conversion of traditional soil-based greenhouses into hydroponic systems (Bihari *et al.*, 1). In Turkey, soilless agriculture began in Antalya and has expanded to provinces such as İzmir, Adana, Mersin, Aydın, and Denizli. Commercial production in these hydroponic systems is dominated by leafy greens during the winter, alongside high-value crops like tomatoes, peppers, cucumbers, and various cut flowers (Talaz and Nas, 12). Plant nutrition in hydroponics relies

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entirely on the precise delivery of aqueous solutions containing essential elements directly to the root zone, where parameters such as pH and electrical conductivity (EC) must be strictly maintained within optimal ranges (Bihari *et al.*, 1).

Unlike soil, which possesses a natural buffering capacity to tolerate minor irrigation and fertilization errors, hydroponic substrates and solution cultures are highly unforgiving. Even a minor dosage calculation error or technical malfunction can cause prompt, irreversible damage to the entire crop within the system (Voogt and Bar-Yosef, 13). In commercial hydroponic lettuce production, improper or imbalanced nutrient management leads to immediate malnutrition symptoms, causing severe yield and quality losses. Nutrient imbalances or excessive fertilization frequently distort the solution pH and EC levels, which disrupts optimal nutrient uptake, triggers visible chlorosis, necrosis, and morphological deformities, and ultimately reduces the market value of the produce. The primary macronutrients-Nitrogen (N), Phosphorus (P), and Potassium (K)-are foundational to vital physiological mechanisms, including chlorophyll synthesis, energy transfer (ATP), osmotic regulation, and enzyme activation. Consequently, deprivation of any of these macro-elements induces rapid, distinct physiological and morphological modifications in the plant.

Although the general roles of macro-elements are well-documented, a comprehensive understanding of how specific, individual N, P, and K deficiencies alter the chronological progression of vegetative growth, biochemical parameters, and leaf temperature dynamics in hydroponic lettuce remains essential for precision greenhouse management. Therefore, the primary objective of this study was to evaluate the distinct effects of nitrogen, phosphorus, and potassium deficiencies on the morphological growth, leaf color profiles, chlorophyll content (SPAD), leaf temperature, and macro-micro element accumulation profiles of lettuce (*Lactuca sativa* L.) grown in a hydroponic environment. Furthermore, this study aimed to establish reliable visual, physiological, and nutrient-interaction indicators that can be utilized for the early detection and diagnosis of specific macronutrient deficiencies before irreversible commercial damage occurs.

MATERIALS AND METHODS

The experiment was conducted during November–December 2025 in the research greenhouse of the Department of Horticulture, Faculty of Agriculture, Dicle University. The plant material consisted of uniform lettuce seedlings professionally sourced from a commercial nursery in Antalya, Turkey. Throughout the experimental period, the greenhouse

microclimate was strictly controlled, maintaining a diurnal temperature regime of 16–19 °C during the day and 13–15 °C at night. The trial was established according to a randomized complete block design (RCBD) featuring four distinct nutrient treatments, with three independent replicates per treatment and 20 plants embedded within each replicate. A non-circulating hydroponic system was used for the study. The experimental treatments comprised a complete nutrient solution prepared according to the standard Hoagland formulation (control), along with nitrogen-deficient (–N), phosphorus-deficient (–P), and potassium-deficient (–K) nutrient solutions. The plants were cultivated in a closed-loop Nutrient Film Technique (NFT) hydroponic system under controlled greenhouse conditions. The control group received a modified full-strength Hoagland and Arnon (4) nutrient solution formulation, which contained the following macronutrient and micronutrient concentrations: 2.5 mM $\text{Ca}(\text{NO}_3)_2$, 2.5 mM KNO_3 , 1.0 mM KH_2PO_4 , 1.0 mM MgSO_4 , along with a comprehensive micronutrient formulation consisting of 4.5 μM MnCl_2 , 23.1 μM H_3BO_3 , 0.38 μM ZnSO_4 , 0.16 μM CuSO_4 , 0.05 μM H_2MoO_4 , and 45.0 μM Fe-EDTA. Individual macronutrient deficiencies were induced by systematically omitting the respective target element from the nutrient solution while maintaining comparable ionic strength, osmolality, and counter-ion balance.

For nitrogen deficiency (–N), $\text{Ca}(\text{NO}_3)_2$ and KNO_3 were completely omitted and replaced with equivalent concentrations of CaCl_2 and KCl, respectively, thereby maintaining calcium and potassium supply without introducing nitrogen. For phosphorus deficiency (–P), KH_2PO_4 was omitted and replaced with an equivalent concentration of KCl to maintain potassium supply while eliminating phosphate. For potassium deficiency (–K), KNO_3 and KH_2PO_4 were replaced with equivalent concentrations of NaNO_3 and NaH_2PO_4 , respectively, ensuring consistent nitrate and phosphate supply while completely excluding potassium from the nutrient solution. The electrical conductivity (EC) of the circulating hydroponic media was strictly maintained within the operational range of 1.8 - 2.0 mS/cm. The nutrient solution temperature inside the main experimental reservoirs was constantly monitored and stabilized between 22 – 25 °C via automated aquarium heaters and chillers. To ensure optimum nutrient availability, the pH of the circulating solutions was checked daily and adjusted to 5.8 - 6.2. To eliminate any external nutrient contamination in the deficiency treatments, pH adjustments were performed using 0.1 M NaOH or 0.1 M HCl for the potassium- and nitrogen-deficient groups where appropriate, while the control treatments were adjusted using 0.1 M KOH or 0.1 M HNO_3 .

Root collar diameter was recorded at the baseline zone of the shoot using a digital vernier caliper (mm). Plant height (cm) and primary root length (cm) were determined by harvesting the designated plants, thoroughly washing the root systems with distilled water to remove external solution residues, separating the root and shoot components, and measuring them with a standardized laboratory ruler. Plant (shoot) fresh weight (g) and root fresh weight (g) were determined immediately after harvest by weighing the separated plant tissues on a high-precision digital analytical balance. For dry matter determination, four representative plants from each replicate were sampled at each measurement period, oven-dried at 70 °C for 48 hours until reaching a constant weight, and their respective shoot and root dry weights (g) were recorded. Canopy leaf temperature (°C) was measured from the 3rd and 4th fully expanded leaves from the top of the plant using a calibrated handheld infrared thermometer. Relative chlorophyll content was monitored non-destructively in SPAD units using a Minolta SPAD-502 chlorophyll meter. Foliar color space parameters (L^* , a^* , b^*) were systematically recorded using a portable PCE-TCR 200 colorimeter to track specific chlorosis and pigment degradation profiles induced by nutrient starvation. The pH and soluble solids content (SSC, %) of the plant leaf extracts were evaluated at each sampling period to determine internal physiological shifts, with SSC measured via a digital pocket refractometer. At the end of each designated week, harvested leaf tissues were thoroughly dried and pulverized. Macroelement (K, Ca, Mg, Na, P) concentrations (%) and microelement (Fe, Mn, Zn, Cu, Mo) concentrations (ppb) were determined via Inductively Coupled Plasma (ICP) spectrometry at the Central Technology and Research Center Laboratory of Şırnak University. Total foliar nitrogen content (%) was analyzed quantitatively using the standard Kjeldahl digestion method at the same centralized facility. All experimental data obtained from the weekly measurements were subjected to a one-way analysis of variance (ANOVA) using the JMP (Version 7.0) statistical software package. The assumptions of normality and homogeneity of variance were verified prior to analysis. Treatment means exhibiting statistically significant differences were evaluated and separated using the Least Significant Difference (LSD) multiple comparison test at a confidence threshold of $P < 0.05$.

RESULTS AND DISCUSSION

Statistical analyses revealed significant differences between groups in the first week regarding root collar diameter ($P < 0.0001$), whereas in the

second week, the structural variation stabilized as adaptive processes balanced development ($P = 0.0214$). The weekly changes in root collar diameter at the end of the study are presented in Figure 1. Whilst a marked increase was observed in the control group in the second week (4.13 mm), the highest value under nitrogen deficiency was initially obtained in the first week, with a steady increase occurring in the second week (4.44 mm). This situation highlights that nitrogen is a critical nutrient for sustainable growth, but its restriction can initially trigger resource reallocation toward the root system to facilitate nutrient mining. Indeed, Kacar and Katkat (6) note that nitrogen deficiency does not strictly limit root-zone structural development in the short term, but substantially reduces overall growth velocity in the long term. Under potassium deficiency, the root collar diameter remained above the control level in the first week (5.05 mm), reaching 5.63 mm in the second week, indicating that potassium limitation does not restrict initial root thickening. Marschner (9) reports that while plants experience severe problems with osmotic regulation and carbohydrate transport under potassium deficiency, transient adaptive increases in localized root diameters are frequently observed in short-term experimental designs as a survival mechanism. Under phosphorus deficiency, a distinct adaptive increase was monitored as values progressed from 4.17 mm to 5.40 mm. Zhang *et al.* (15) report that highly targeted modifications in root morphology, particularly root thickening, cortical cell expansion, and increased root surface area, are universal adaptive responses under localized phosphorus starvation. These findings indicate that while primary macronutrient starvation limits vertical growth, compensatory mechanisms are immediately activated in the plant's root-zone structural diameter to optimize localized resource mining.

Macronutrient deficiencies led to a marked reduction in overall plant height, as structurally

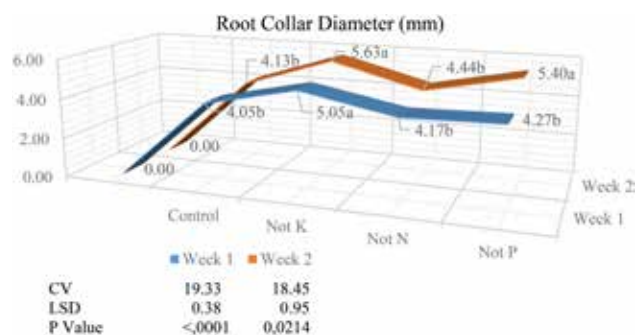


Fig. 1. Weekly changes in root collar diameter of lettuce plants under different nutrient deficiencies.

illustrated in Figure 2. The highest plant height (30.63 cm) was achieved by the fully nourished control group in the second week. In stark contrast, shoot elongation remained strictly limited and clustered at significantly lower levels (within the 25-26 cm range) under all individual K, N, and P omissions. Significant differences were observed between the treatments in the first week ($P = 0.0021$), and this statistical divergence became even more pronounced during the chronic phase in the second week ($P < 0.0001$). This temporal gap indicates that nutrient deficiencies assert an increasingly heavy toll on canopy framework development over time. Under nitrogen deficiency, plant height was highly restricted in the first week (20.04 cm) and showed only a limited, minor increment in the second week (25.18 cm), validating the primary structural role of nitrogen in vegetative development. Kacar and Katkat (6) state that nitrogen deficiency directly limits plant height by restricting photosynthetic capacity, protein assembly, and overall cell division. Marschner (9) also emphasizes that nitrogen supply determines the rate of cell division and structural cell elongation in vegetative meristems, explaining the severe stunting observed under its absence.

The statistical responses regarding primary root length across the sequential measurement periods are presented in Figure 3. In the early stress phase (Week 1), the longest root extension was monitored in plants subjected to potassium deficiency (13.13 cm), whilst the shortest baseline was recorded in the control group (7.56 cm). This rapid early elongation represents a classic morphological escape response to low-K availability, where plants accelerate primary root mining to seek deeper mobile ions. By the second week, the differences between groups narrowed and lost statistical significance ($P = 0.0734$), demonstrating that long-term root adaptation mechanisms eventually balance out vertical developmental rates. Nevertheless, plants

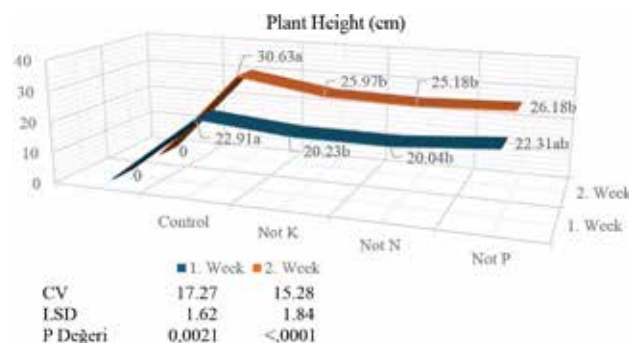


Fig. 2. Weekly changes in plant height at the end of the study.

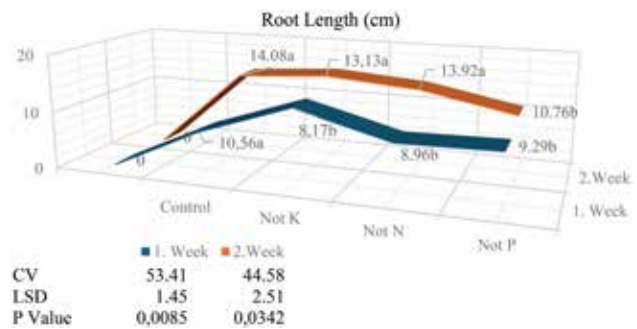


Fig. 3. Weekly changes in root length of the plants obtained at the end of the study.

under phosphorus starvation maintained a steady, progressive increase in root length (9.29 cm - 10.76 cm), and the K-deficient group continuously tracked higher values than all other groups (13.13 cm - 15.17 cm). As supported by Zhang *et al.* (15), under phosphate starvation, shoot-directed carbon is actively redirected to stimulate root systems, leading to modified root architecture to increase the probability of capturing sparse inorganic anions.

The temporal variations in total shoot biomass are illustrated in Figure 4, which documents the weekly changes in the fresh weight of the plants. In the complete control group, the total plant fresh weight reached its peak in the second week (15.36 g). Conversely, individual macronutrient starvation imposed clear boundaries on overall biomass incorporation, with the differences remaining statistically limited due to wide within-group deviations ($P > 0.05$). Despite the lack of strict statistical significance, the biological trends were clear: nitrogen deficiency (12.59 g) and potassium deficiency (13.41 g) led to the most severe limitations in fresh weight accumulation compared to the control baseline, while phosphorus-deficient plants maintained a slightly higher intermediate value of 14.32 g in the final week. Kacar and Katkat (6) note that nitrogen starvation

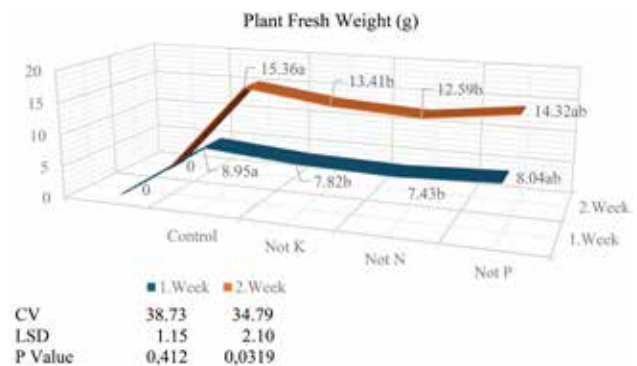


Fig. 4. Weekly changes in shoot fresh weight of lettuce plants under different nutrient deficiencies.

severely curtails total photosynthetic leaf surface area, thereby restricting fresh mass accumulation. Marschner (9) further expands that both nitrogen and potassium are heavily involved in cellular turgor maintenance and expansion, meaning their joint deficiency limits the physical capacity of tissues to hold water and build fresh volume.

An analysis of the root fresh weight data, detailed in Figure 5, highlights that the control group achieved the highest root mass in both the first (1.13 g) and second weeks (1.31 g). All induced nutrient deficiencies restricted the expansion of root fresh biomass. In particular, phosphorus deficiency exerted the most severe restriction, resulting in the absolute lowest value of 1.02 g by the end of the second week. Parallel restrictions were monitored under N deficiency (1.07 g in Week 1 to 1.16 g in Week 2) and K deficiency (1.17 g in Week 1 to 1.24 g in Week 2). Statistical analysis confirmed that these variations were highly significant in both the early ($P = 0.0324$) and chronic ($P = 0.0256$) measurement windows. This strict inhibition under low phosphorus confirms that while roots may attempt adaptive elongation (as seen in Fig. 3), the complete lack of inorganic phosphate fundamentally limits tissue density and cellular water retention, severely lowering total root fresh mass accumulation.

The dynamic patterns regarding total dry matter accumulation are presented in Figure 6. The fully nourished control plants recorded the highest plant dry weight (1.18 g) during the second week. Chronic deprivation of individual macronutrients restricted dry matter accumulation, with K deficiency (1.00 g), N deficiency (0.94 g), and P deficiency (1.06 g) consistently exhibiting depressed values compared to the control. In the early phase (Week 1), dry matter values were uniformly low, with all deficiency treatments suppressed within a tight baseline range of 0.56 - 0.73 g. Soundy *et al.* (11) observed similar structural drops in lettuce models, concluding that dry shoot mass fractions increase linearly with adequate

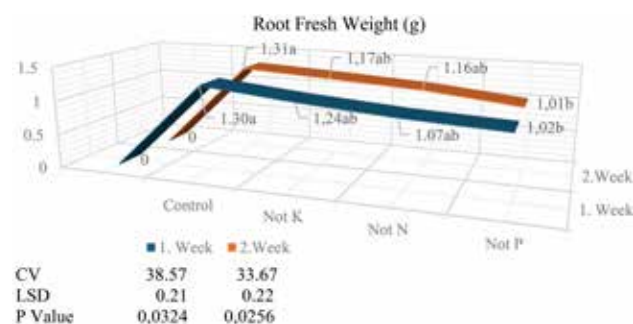


Fig. 5. Weekly changes in root fresh weight of lettuce plants under different nutrient deficiencies.

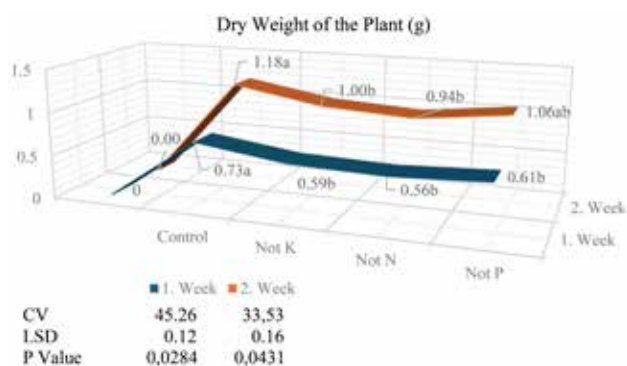


Fig. 6. Weekly changes in plant dry weight of lettuce plants under different nutrient deficiencies.

nitrogen availability, as nitrogen availability directly governs the sink-source relationships that drive dry matter deposition.

An examination of the root dry weight allocation, visualized in Figure 7, shows that the complete control treatments maximized root dry matter in both the first week (0.15 g) and the second week (0.47 g). Single-element starvation significantly limited structural root dry matter accumulation ($P < 0.05$). Specifically, N and P deficiencies remained heavily restricted, remaining within a very low range of 0.12 - 0.13 g during the second week, representing a major drop compared to the control. Potassium deficiency also restricted dry root allocation, resulting in a low level of 0.15 g. Statistical analysis revealed that these differences were highly significant in both the first week ($P < 0.0001$) and the second week ($P = 0.0095$). According to Kacar and Katkat (6), phosphorus is an absolute prerequisite for structural energy metabolism and ATP synthesis; its deficiency directly stalls the metabolic energy pathways required for root dry matter accumulation, causing the severe structural constraints observed here.

The weekly temperature fluctuations monitored across the plant leaf canopy are detailed in Figure 8. In the complete control group, leaf temperature

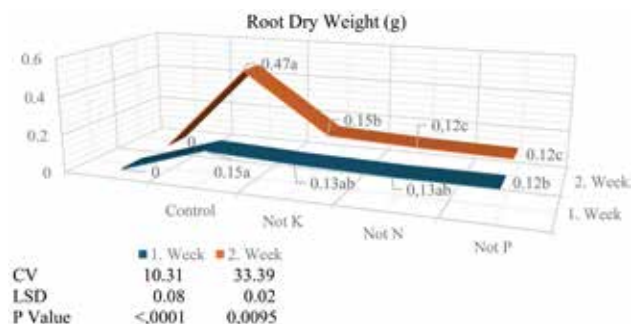


Fig. 7. Weekly changes in root dry weight of lettuce plants under different nutrient deficiencies.

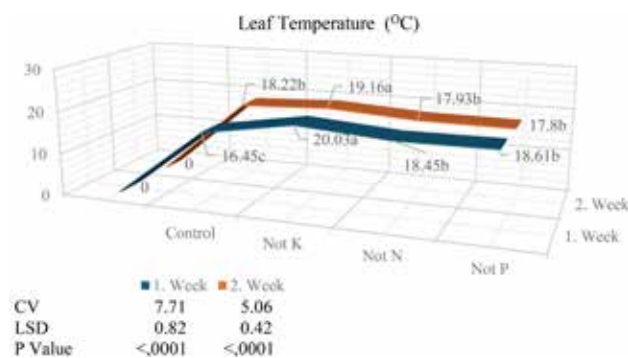


Fig. 8. Weekly changes in leaf canopy temperature of lettuce plants under different nutrient deficiencies.

remained stable and moderate (16.45 °C 18.22 °C), demonstrating well-regulated transpirational cooling under optimal nutritional status. Conversely, the highest leaf canopy temperatures were consistently measured under potassium deficiency (20.03 °C in Week 1 and 19.16 °C in Week 2). This distinct heating effect is directly linked to the essential physiological function of K⁺ ions in governing the osmotic turgor of guard cells. As emphasized by Marschner (9), potassium acts as the primary osmoticum regulating stomatal opening; its absence triggers uncoordinated stomatal closure, suppressing transpirational airflow and raising leaf surface temperatures. Under nitrogen deficiency, leaf temperature was similarly elevated above the control baseline (18.45 °C - 17.93 °C). This phenomenon is supported by Liu *et al.* (7), who noted that nitrogen limitations drastically reduce total photosynthetic sink demand and stomatal conductance, causing a net decrease in functional transpirational cooling.

The dynamic shifts in the root zone solution pH are presented in Figure 9. The control solution maintained a balanced state, hovering around 6.51 in the first week and 6.75 in the second week. In the nitrogen-deficient treatment, a distinct drop toward a more acidic state (6.39) was monitored during

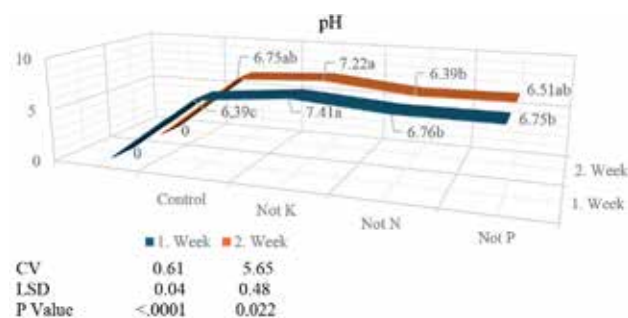


Fig. 9. Weekly changes in hydroponic solution pH under different nutrient deficiencies.

the early stress phase (Week 1). Kacar and Katkat (6) clarify that when plants are starved of mobile nitrate (NO₃⁻) anions, net cation uptake continues to outpace anion influx. To maintain internal cellular electrical neutrality, the roots actively extrude protons (H⁺) into the surrounding medium, leading to the observed drop in environmental pH. In contrast, potassium deficiency induced a notable alkalization of the surrounding hydroponic medium, elevating the pH values to 7.41 and 7.22 across the respective measurement periods ($P < 0.05$). This reflects a reverse ion balancing mechanism where the plant suppresses proton release or increases bicarbonate/hydroxyl extrusion due to a complete lack of external K⁺ cation influx.

When evaluating the soluble solids content (SSC, %), clear chronological differences were documented (Figure 10). The fully nourished control group accumulated the highest final SSC level (5.86%) in the second week. While nitrogen deficiency did not severely restrict carbohydrate accumulation in the short term (5.79%), potassium (4.69%) and phosphorus (4.57%) deprivations led to a highly significant reduction in foliar SSC values ($P < 0.0001$). This pattern confirms that internal sugar synthesis and subsequent vascular phloem loading are heavily dependent on K-regulated enzyme activation and P-mediated phosphorylation mechanisms. As confirmed by Marschner (9), potassium is crucial for long-distance carbohydrate translocations out of source leaves, meaning its deficiency traps carbohydrates or breaks down soluble solid accumulations across developing vegetative tissues.

An analysis of the SPAD chlorophyll indices, illustrated in Figure 11, reveals that the control plants recorded the highest chlorophyll concentration (21.98) by the second week. Chlorophyll levels were severely depressed under nitrogen deficiency (14.76 in Week 1; 14.66 in Week 2), providing a

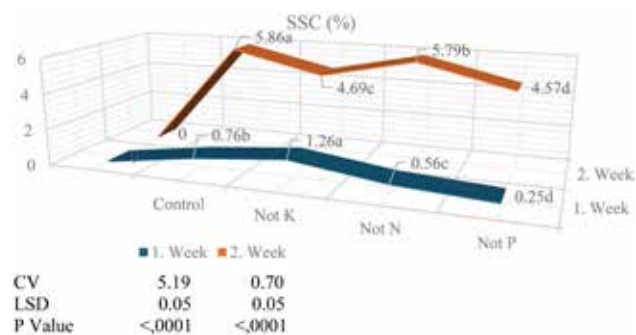


Fig. 10. Weekly changes in soluble solids content (SSC) of lettuce plants under different nutrient deficiencies.

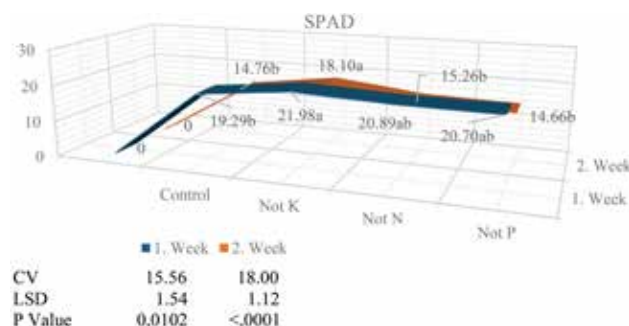


Fig. 11. Weekly changes in SPAD chlorophyll values of lettuce plants under different nutrient deficiencies.

clear diagnostic confirmation that N availability is the primary bottleneck for structural chlorophyll synthesis. The higher rate of nitrogen application in hydroponic lettuce models yields a strong, linear positive correlation with leaf SPAD values, as nitrogen constitutes a central core component of the chlorophyll porphyrin ring structure. Potassium deficiency (18.10 - 19.29) and phosphorus deficiency (15.26 - 20.29) also exhibited lower SPAD values compared to the control group, though their limiting effects were characterized as moderate relative to the severe constraints imposed by nitrogen starvation.

Leaf color space parameters (L^* , a^* , b^*) effectively mirrored these internal physiological disruptions (Table 1). Brightness values (L^*) were consistently optimized in the control plants (49.86 - 47.80), indicating healthy light-scattering across fully expanded leaf tissues. The greenness component (a^*) remained stable and highly negative in the control group. The yellowing coordinates (b^*), however, expanded significantly under chronic phosphorus (97.70) and potassium (95.25) deficiencies relative to the control group (73.45), capturing the onset of severe chlorosis and structural breakdown of the photosynthetic canopy. Under nitrogen deficiency, the more negative a^* values pointed toward a transient intensification of green color in localized leaf segments. This localized

compaction can be associated with restricted cell expansion under severe N stress, which initially concentrates remaining plastids before complete chlorosis takes over.

The direct analytical tracking of macro- and microelements via ICP-spectrometry revealed highly precise diagnostic shifts under specific single-element deprivation regimes (Table 2). Total foliar nitrogen accumulation provided a strict validation of the hydroponic setup ($P < 0.001$, Table 3). Control plants successfully accumulated optimal N densities (4.11% to 4.59%), which aligns perfectly with Soundy *et al.* (11) who defined the sufficient foliar N threshold for lettuce between 4.0 - 5.0%. In contrast, the targeted N-omission group successfully dropped into a severe deficiency state (2.67% to 2.77%), falling well below the critical 3.9% deficiency baseline outlined in historical diagnostics (Jones *et al.*, 5). Concurrently, specific omissions of potassium and phosphorus successfully brought target tissue concentrations down to their baseline depletion limits (0.08 % for K and 0.04 % for P in the second week), proving that the intended deficiency stresses were cleanly achieved. In the early stress phase (Week 1), nitrogen deficiency induced a transient increase in foliar Ca (0.21%) and Mg (0.22%) concentrations compared to the control group, suggesting a compensatory cation uptake synergistic response under nitrate limitation. Conversely, phosphorus starvation significantly restricted early calcium (0.14%) and magnesium (0.15%) accumulation in lettuce leaves (Müftüoğlu *et al.*, 10).

Among the microelement interactions during the chronic stress phase, molybdenum (Mo) accumulation displayed a highly powerful and statistically robust diagnostic response ($P < 0.001$). While the completely nourished control plants achieved a mean density of 10.75 ppb, all single-element deprivation treatments experienced an absolute collapse in Mo accumulation, falling to 0.29 ppb under -N, 0.22 ppb under -P, and a minimum of

Table 1: Weekly changes in L^* , a^* , and b^* values in plant leaves obtained at the end of the study.

Applications	1. Week			2. Week		
	L^*	a^*	b^*	L^*	a^*	b^*
Control	49,86±1,32 a	-30,94±0,84 a	83,61±3,03 a	47,80±2,25 a	-30,62±1,89 a	73,45±6,57 b
K	44,08±1,08 b	-31,85±1,61 a	86,50±3,22 a	44,50±1,18 a	-34,35±1,18 a	95,25±1,20 ab
N	41,84±1,27 b	-31,51±0,71 a	79,22±3,53 a	45,58±2,29 a	-32,00±1,56 a	89,90±5,92 ab
P	45,85±1,17 ab	-33,10±0,87 a	88,62±3,16 a	45,98±1,45 a	-34,29±0,28 a	97,70±4,49 a
CV	17,98476214	-22,51348908	25,82170721	10,42944817	-10,77323643	14,4792559
LSD	3,85	3,12	9,45	5,34	4,18	18,42
P Value	0,0125	0,6481	0,3205	0,7482	0,3088	0,0468

Table 2. Changes in weekly macro- and micro-nutrient content in plant leaves at the end of the study.

	Elements	Control	- N	- P	- K	P-Value	LSD	CV (%)
1 st Week	Ca (%)	0.19 ab	0.21 a	0.14 b	0.20 a	0.012	0.03	11.8
	K (%)	0.67 a	0.51 b	0.55 b	0.18 c	<0.001	0.11	12.4
	Mg (%)	0.20 a	0.22 a	0.15 b	0.20 a	0.018	0.04	12.1
	Na (%)	0.20 b	0.25 a	0.21 b	0.20 b	0.009	0.04	13.7
	P (%)	0.11 a	0.13 a	0.03 b	0.12 a	<0.001	0.03	14.5
	Fe (ppb)	3.17 b	13.34 a	5.35 b	2.73 b	0.021	2.43	10.9
	Zn (ppb)	12.68 b	36.76 a	10.88 b	14.79 b	0.015	4.25	12.4
	Cu (ppb)	2.75 b	8.66 a	2.59 b	2.35 b	0.017	2.13	13.2
	Mn (ppb)	7.34 b	29.78 a	8.43 b	11.43 b	0.014	3.34	12.7
	Mo (ppb)	0.31 b	0.99 a	0.27 b	0.35 b	0.008	0.14	15.9
2 nd Week	Ca (%)	0.33 a	0.16 b	0.11 c	0.20 b	0.011	0.05	11.5
	K (%)	0.40 a	0.17 b	0.22 b	0.08 c	<0.001	0.06	13.2
	Mg (%)	0.31 a	0.16 b	0.12 c	0.20 b	0.019	0.05	12.9
	Na (%)	0.34 a	0.17 b	0.21 b	0.19 b	0.010	0.05	14.3
	P (%)	0.33 a	0.13 b	0.04 c	0.12 b	<0.001	0.04	14.7
	Fe (ppb)	1.28 b	7.15 a	5.47 a	0.74 b	0.016	2.02	11.1
	Zn (ppb)	10.62 b	11.27 a	10.09 a	3.47 b	0.022	3.62	12.6
	Cu (ppb)	9.97 a	4.54 b	3.80 b	0.61 c	0.018	2.21	12.9
	Mn (ppb)	7.55 b	11.34 a	10.00 a	1.82 c	0.013	2.61	13.8
	Mo (ppb)	10.75 a	0.29 b	0.22 b	0.09 c	<0.001	0.19	13.7

Note: Different lowercase letters within the same row represent statistically significant differences according to the LSD test ($P < 0.05$).

Table 3. Weekly total nitrogen content in plant leaves at the end of the study.

Treatment	1 st Week (%)	2 nd Week (%)
Control	4.11 a	4.59 a
K	4.03 b	4.28 b
P	3.79 c	3.94 c
N	2.67 d	2.77 d
P-Value	< 0.001	< 0.001
LSD	0.062	0.054
CV (%)	0.90	0.73

Note: Different lowercase letters within the same column represent statistically significant differences according to the LSD test ($P < 0.05$).

0.09 ppb under -K starvation. This dramatic response highlights a crucial physiological crosstalk. The molybdate transport via specific root transporters (MOT1) is a highly active, metabolically driven process that is tightly coupled with functional nitrogen assimilation requirements and overall root system energy status. When any primary macronutrient (N, P, or K) is completely omitted, the root systems suffer severe energy depletion or disrupted enzyme

signals, leading to a complete shutdown of Mo uptake mechanics. This dynamic implies that monitoring precise microelement variations offers a highly sensitive diagnostic tool for detecting macronutrient deficiencies well before visible visual stunting manifests on the crop canopy.

The statistical results regarding the total nitrogen content in plant leaves, which were collected at one-week intervals, are presented in Table 3. The effect of nutrient deficiencies on the total nitrogen (N) content in the leaves of lettuce plants grown in a soilless medium was found to be significant in both the first-week and second-week measurements ($p < 0.001$). In the first-week measurements, the highest total nitrogen content was observed in the control group at 4.11%, followed by the K-deficient group at 4.03% and the P-deficient group at 3.79%. As expected, the lowest nitrogen content was obtained from the N-deficient treatment at 2.67%. In the second week of the experiment, nitrogen accumulation was observed to increase over time in all treatments. The control group again showed the highest value at 4.59%, while the K-free plot and the P-free plot were at statistically different levels with values of 4.28% and 3.94%, respectively. In the N-free group, the nitrogen

content remained at 2.77%, causing the statistical gap between it and the other treatments to widen further.

This study systematically evaluated the chronological effects of individual N, P, and K deficiencies on the morpho-physiological, physicochemical, and elemental characteristics of hydroponic lettuce during early and chronic stress phases. Individual macronutrient omissions induced distinct and progressive responses in plant growth and physiology. All deficiency treatments restricted shoot growth and structural development, with N deficiency producing the greatest reductions in biomass accumulation and chlorophyll content, highlighting the critical role of nitrogen in vegetative growth. Canopy temperature increased particularly under K deficiency, indicating impaired stomatal regulation and altered plant water relations. Root-zone pH also responded differentially, showing acidification under N deficiency and alkalization under K deficiency, reflecting changes in nutrient uptake and ion balance. Elemental analysis revealed substantial interactions between macronutrient deficiencies and micronutrient accumulation. Notably, N, P, and K deficiencies markedly reduced Mo accumulation during chronic stress compared with adequately nourished plants, suggesting strong interactions between macronutrient availability and Mo uptake. Overall, early changes in leaf temperature, chlorophyll status, and elemental composition can provide useful indicators for detecting macronutrient deficiencies before severe visual symptoms and growth impairment become evident in hydroponic lettuce.

AUTHORS' CONTRIBUTION

Conceptualization of research (EA.); Designing of the experiments (AT); Contribution of experimental materials (MA); Execution of field/lab experiments and data collection (VP); Analysis of data and interpretation (EA); Preparation of the manuscript (VP,AS).

DECLARATION

The authors have no conflict of interest.

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