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# NATURAL CLINOPTILOLITE MITIGATES HEAVY METAL ACCUMULATION AND IMPROVES NUTRIENT BALANCE IN ALFALFA UNDER WASTEWATER IRRIGATION

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## ABSTRACT

The safe reuse of wastewater in forage production is constrained by the risk of heavy metal accumulation and nutrient imbalance in plant tissues and growth media. Although natural zeolite, mainly composed of clinoptilolite, has been reported to mitigate such risks, the optimal soil–zeolite mixing ratio for alfalfa performance under contrasting irrigation water qualities remains poorly defined. Therefore, this study aimed to evaluate the potential role of natural zeolite in enhancing alfalfa growth and mineral composition while reducing heavy metal uptake under irrigation with treated and raw wastewater. A greenhouse experiment was conducted during the summer of 2024 at Jordan University of Science and Technology (JUST). The study followed a randomized complete block design with 15 treatments, including five soil–zeolite mixtures and three irrigation types: distilled water (DW), treated wastewater (TWW), and raw wastewater (WW). The results showed that alfalfa growth, mineral composition, and heavy metal accumulation were significantly influenced by the interaction between growth media and irrigation type ($P<0.05$). The 25% soil: 75% zeolite (25% S: 75% Z) treatment under WW irrigation recorded the highest plant fresh and dry biomass and the highest nitrogen and phosphorus contents, probably due to improved nutrient retention and root-zone buffering associated with clinoptilolite's high cation exchange capacity. Magnesium and sodium contents increased with increasing zeolite proportion, with the highest values recorded in plants grown in pure zeolite (100% Z) under WW irrigation. Nickel (Ni) and lead (Pb) were detected only under WW irrigation and were absent under DW and TWW. Increasing zeolite proportion reduced Ni and Pb concentrations in both plant tissues and growth media, with greater reductions at higher zeolite proportions. Overall, the 25% S: 75% Z mixture provided the most favorable agronomic balance, as it enhanced alfalfa biomass production and nutrient status while substantially reducing heavy metal accumulation compared with pure soil. These findings emphasize the importance of natural zeolite as a sustainable amendment for safer wastewater reuse in agriculture, forage production, and environmental protection.

***Keywords***: Natural clinoptilolite, Heavy metal immobilization, Wastewater irrigation, Soil amendment, Sustainable agriculture.

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## 1. INTRODUCTION

Jordan is considered one of the most water-scarce countries in the world due to its arid climate, limited freshwater resources, rapid population growth, and the increasing impacts of climate change. In recent decades, declining precipitation and recurrent droughts have substantially reduced the availability of renewable freshwater resources, placing considerable pressure on agricultural production and environmental sustainability (Odeh, 2019; Mohammad et al., 2024). Water scarcity has emerged as one of the greatest global challenges, threatening agricultural productivity, food security, and sustainable development, particularly in arid and semi-arid regions where irrigation represents the largest consumer of freshwater. The combined effects of climate change, urbanization, and increasing water demand are expected to further intensify competition for limited water resources, underscoring the urgent need for sustainable water management and the adoption of alternative irrigation sources (Prisa, 2023; Christou et al., 2024). At the same time, ensuring future food security requires integrating sustainable agricultural practices with innovative crop

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improvement strategies to enhance resource-use efficiency and crop resilience under water-limited conditions. Recent advances in agronomic management, climate-resilient breeding, and modern biotechnological approaches have highlighted their potential to improve agricultural productivity while reducing pressure on natural resources (Ahmad et al., 2026; Mary et al., 2026; Murtaza et al., 2026). Furthermore, sustainable agricultural development has been recognized as a cornerstone for strengthening food security and building resilient farming systems that can adapt to increasingly challenging environmental conditions (Alsanhani, 2026). In this context, the efficient utilization of non-conventional water resources and environmentally friendly soil amendments has become an important strategy for sustaining crop production in water-deficient regions.

In response to this growing water scarcity, several countries in the Middle East, including Jordan, have increasingly adopted the reuse of raw and treated wastewater as a strategic component of agricultural water management and sustainability frameworks (Othman et al., 2021). The reuse of treated wastewater has evolved from a conventional disposal strategy into an important component of the circular economy, in which wastewater is regarded as a valuable source of water, nutrients, and energy rather than a waste product. Properly treated wastewater can provide a reliable irrigation supply while simultaneously recovering nutrients such as nitrogen, phosphorus and potassium, thereby reducing dependence on mineral fertilizers and improving resource-use efficiency (Boularbah et al., 2024; Christou et al., 2024).

Using wastewater for irrigation reduces reliance on limited freshwater resources and decreases the energy and financial costs associated with groundwater extraction (Al-Suhaibani et al., 2021; Othman et al., 2021). In addition, wastewater serves as a supplementary source of nutrients that can enhance soil fertility, improve crop productivity, and contribute to more sustainable agricultural production when appropriate treatment, water quality monitoring, and management practices are implemented (Al-Suhaibani et al., 2021; Sohail et al., 2021; Christou et al., 2024).

However, wastewater can contain elevated levels of pollutants, such as heavy metals. Therefore, the long-term use of wastewater for irrigation raises concerns regarding the accumulation of heavy metals, including lead (Pb), nickel (Ni) and cadmium (Cd). These elements are persistent in the environment, are not readily degraded, and may accumulate in soils and subsequently enter the food chain (Khan et al., 2015; Othman et al., 2021). Heavy metal accumulation poses potential risks to the environment and to human and animal health, highlighting the need for cost-effective and environmentally safe strategies to reduce heavy metal bioavailability in soils and their uptake by plants (Jakkula & Wani 2018; Martins et al., 2023). Repeated irrigation with wastewater may also adversely affect soil quality by altering soil physicochemical properties and disrupting microbial communities responsible for nutrient cycling and organic matter decomposition, thereby reducing long-term soil fertility (Khan et al., 2015; Boularbah et al., 2024). In forage production systems, the uptake of heavy metals by crops such as alfalfa is of particular concern because contaminated biomass may transfer toxic elements to livestock and subsequently enter the human food chain through animal-derived products. Consequently, considerable research has focused on developing environmentally friendly soil amendments capable of immobilizing heavy metals while maintaining crop productivity and supporting the safe reuse of wastewater in agriculture (Martins et al., 2023; Christou et al., 2024).

Zeolites are crystalline aluminosilicate minerals formed from volcanic ash and saline water through hydrothermal processes. Natural clinoptilolite, one of the most abundant and agriculturally important zeolite minerals, has been widely used in agriculture because of its non-toxic nature, high cation exchange capacity (CEC), and unique porous structure (Cataldo et al., 2021; Kordala & Wyszkowski, 2024). The substitution of $Si^{4+}$ by $Al^{3+}$ within the crystal lattice creates a permanent negative charge, giving natural clinoptilolite a high CEC, large specific surface area, and strong adsorption properties. These characteristics also enhance soil water-holding capacity and nutrient retention, resulting in improved soil fertility (Cataldo et al., 2021; Amirahmadi et al., 2022; Kordala & Wyszkowski, 2024). Heavy metals such as $Pb^{2+}$ and $Ni^{2+}$ are immobilized through ion-exchange and surface adsorption mechanisms, thereby reducing their mobility, bioavailability, and subsequent uptake by plants (Moeen et al., 2020; Martins et al., 2023). In addition, clinoptilolite has a high affinity for ammonium ($NH_4^+$) and other nutrient cations, allowing it to retain nutrients within the root zone, reduce nitrogen losses through leaching, improve nutrient use efficiency and gradually release nutrients according to plant demand (Palanivell et al., 2021; Wang et al., 2025). Furthermore, its high water-holding capacity improves soil moisture retention and facilitates nutrient uptake, thereby supporting plant growth under water-limited conditions (Amirahmadi et al., 2022). Consequently, natural clinoptilolite amendments have been shown to limit the movement of pollutants to deeper soil layers through heavy metal adsorption, while reducing plant heavy metal content and enhancing nutrient status and biomass production (Ghassemi Sahebi et al., 2020; Moeen et al., 2020).

Alfalfa (*Medicago sativa* L.) is one of the most economically important legume crops worldwide, due to its high yield, nitrogen-fixing ability and nutritional value. It serves as a primary livestock feed because of its high protein, fiber, and mineral content and is well-suited for sustainable forage production due to its perennial growth habit and rapid regrowth potential (Latif et al., 2023; Bakhtiar et al., 2025). Although previous studies have demonstrated that natural zeolite, particularly clinoptilolite, can improve soil fertility and reduce heavy metal uptake in several crop species, limited information is available regarding the combined effects of different soil–zeolite mixing ratios under contrasting irrigation water qualities, particularly in alfalfa irrigated with raw and treated wastewater. Furthermore, few studies have simultaneously evaluated plant growth, nutrient composition, heavy metal accumulation, and changes in the growth media within a single experimental framework. Therefore, this study aimed to evaluate the role of natural zeolite in enhancing alfalfa growth and nutrient status while reducing heavy metal uptake under raw and treated wastewater irrigation.

## 2. MATERIALS AND METHODS

### 2.1. Experimental Site and Design

The experiment was conducted at the Agricultural Research Center, Faculty of Agriculture, Jordan University of Science and Technology (JUST), Irbid Governorate, Jordan (32.4950°N, 35.9912°E) (Fig. 1). The study period extended from July 21 to October 20, 2024. During the experimental period, the mean daytime temperature ranged from 26 to 35°C, the nighttime temperature ranged from 18 to 23°C, and the relative humidity varied between 50 and 55%. Plants were grown under natural daylight conditions without supplemental lighting.

The study followed a factorial randomized complete block design (RCBD) with 15 treatment combinations comprising five soil–zeolite mixing ratios and three irrigation water sources, with three replicates per treatment.

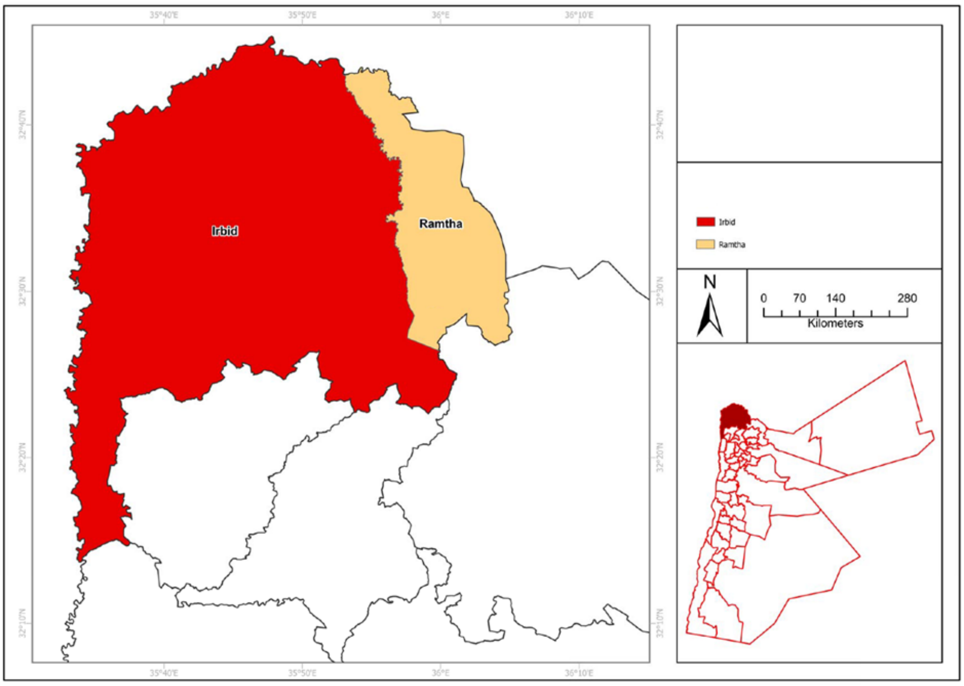


**Fig. 1**: Geographic location of the experimental site at the Agricultural Research Center, Faculty of Agriculture, Jordan University of Science and Technology (JUST), Irbid Governorate, northern Jordan. The map shows the location of the study area within Jordan and the administrative boundaries of the region.

### 2.2. Experimental Treatments

The growth media treatments consisted of pure soil (100% S), pure zeolite (100% Z), and three mixtures of soil and natural clinoptilolite at varying ratios: 75% soil with 25% zeolite (75% S: 25% Z), 50% soil with 50% zeolite (50% S: 50% Z), and 25% soil with 75% zeolite (25% S: 75% Z). Irrigation water treatments consisted of distilled water (DW), treated wastewater (TWW) and raw wastewater (WW).

The five mixing ratios were selected to represent a gradient from untreated soil to pure clinoptilolite, enabling the evaluation of dose-dependent effects on plant growth, nutrient availability and heavy metal immobilization under different irrigation water qualities. This design also facilitated the identification of the most effective soil–clinoptilolite ratio for improving alfalfa performance while reducing heavy metal uptake.

The experimental soil was characterized before planting, and its initial physical and chemical properties are presented in Table 1. Natural clinoptilolite used in this study was commercially obtained from a local supplier in Jordan. According to the manufacturer's specifications, the material contained approximately 85% clinoptilolite, had a particle size of 0.5 to 1.5 mm, and exhibited a cation exchange capacity (CEC) of approximately 180 cmol (+) $kg^{-1}$. The chemical composition of natural clinoptilolite and the irrigation water sources is presented in Table 2. The chemical composition of the irrigation water sources was determined before the experiment, and water quality was assumed to remain constant throughout the experimental period; therefore, no periodic chemical analyses were performed.

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**Table 1:** The physical and chemical properties of the soil used in this study (*n* = 3)

| Parameter | Value |
|---|---|
| Soil texture | Clay loam |
| pH | 7.80 |
| EC (dS $m^{-1}$) | 1.24 |
| N (%) | 0.072 |
| P (mg $kg^{-1}$) | 1.42 |
| K (mg $kg^{-1}$) | 54.95 |
| Ca (mg $kg^{-1}$) | 1512.76 |
| Mg (mg $kg^{-1}$) | 228.95 |
| Na (mg $kg^{-1}$) | 419.65 |
| C:N ratio | 27.63 |
| Organic matter (%) | 3.42 |

EC = electrical conductivity; N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Na = sodium; C: N ratio = carbon-to-nitrogen ratio; dS $m^{-1}$ = decisiemens per meter; mg $kg^{-1}$ = milligram per kilogram.

**Table 2:** Chemical composition of natural clinoptilolite, treated wastewater (TWW) and raw wastewater (WW) used in the experiment

| Chemical component | Natural clinoptilolite (ppm) | TWW (ppm) | WW (ppm) |
|---|---|---|---|
| N | 0.008 | 29.00 | 44.69 |
| P | 179.00 | 2.72 | 2.52 |
| K | 1,953 | 20.13 | 18.12 |
| Na | 11,252 | 175.39 | 222.20 |
| Ca | 3,220 | 35.70 | 16.86 |
| Mg | 397.00 | 12.38 | 0.61 |

N = nitrogen; P = phosphorus; K = potassium; Na = sodium; Ca = calcium; Mg = magnesium; ppm = parts per million.

### 2.3. Planting and Irrigation Management

A total of 45 plastic pots (20×20 cm) were filled with the prepared growth media. No sterilization procedures were applied to the growth media prior to planting. Eight alfalfa seeds (*Medicago sativa* L.) were sown directly into each pot under greenhouse conditions. After seedling emergence, plants were thinned at the two- to three-leaf stage, and four healthy, uniform seedlings were maintained per pot throughout the experiment. Each pot received 1.23 g of a slow-release NPK fertilizer (18:18:5) at the start of the experiment.

During the growing period, each treatment received its assigned irrigation water source. Pots were irrigated three times per week during hot periods and twice per week during periods of moderate temperature. Field capacity was determined gravimetrically as the difference between the dry weight of the growth medium and its water-saturated weight after allowing excess water to drain for 24 h. The amount of irrigation water applied was adjusted throughout the experiment to maintain the moisture content of the growth media close to field capacity, following the method described by Almaz et al. (2023). Pots were monitored regularly, and weeds were removed manually whenever necessary.

### 2.4. Data Collection and Measurements

Plant height was measured from the base to the tip of the tallest shoot. Fresh weight was recorded immediately after harvest, and dry weight was determined after oven-drying plant samples at 70°C for 48 hours, followed by weighing using a precision analytical balance (±0.001 g).

The mineral composition of alfalfa shoots, including nitrogen (N), potassium (K), magnesium (Mg) and sodium (Na), was determined using the Kjeldahl method (AOAC, 1995). The K: Na ratio was calculated as the quotient of potassium and sodium concentrations. Phosphorus concentration was determined by dry ashing followed by colorimetric analysis at 410 nm (Chapman & Pratt, 1962).

Growth media pH and electrical conductivity (EC) were measured using a 1:4 suspension (25 g medium in 100 mL deionized water), as described by Motsara & Roy (2008).

The concentrations of nickel (Ni) and lead (Pb) were determined in plant tissues and growth media. For plant tissue analysis, shoot samples were first oven-dried and ground to a fine powder. Approximately 0.5 g of the homogenized material were subjected to digestion using a mixture of concentrated nitric acid ($HNO_3$) and hydrogen peroxide ($H_2O_2$) in a closed-vessel microwave digestion system. The resulting digests were then analyzed for Ni and Pb concentrations using inductively coupled plasma mass spectrometry (ICP-MS) as described by AOAC (2023).

To assess heavy metal content in the growth media, air-dried soil and zeolite samples were sieved to pass through a 2 mm mesh. Subsamples weighing 0.50 g were digested with concentrated $HNO_3$ and $H_2O_2$ under controlled conditions, following which the filtrates were analyzed by ICP-MS for Ni and Pb quantification as described by Motsara and Roy (2008). The Ni and Pb were selected for analysis because preliminary characterization of the treated and raw wastewater identified these as the heavy metals of concern in the irrigation water sources used in this study. Accordingly, the effectiveness of natural clinoptilolite in reducing their uptake by alfalfa was evaluated.

### 2.5. Statistical Analysis

Data collected from this research were statistically analyzed by the General Linear Model (GLM) procedure using the statistical software package SAS version 9.4 (2013). Before conducting the GLM analysis, the assumptions of normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Mean comparisons were performed using the Least Significant Difference (LSD) test at the 5% probability level ($P<0.05$).

## 3. RESULTS AND DISCUSSION

### 3.1. Growth Characteristics

Results, presented in Fig. 2A, showed that alfalfa plant height was significantly influenced by both the growth

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media and the irrigation water source (P<0.05). Compared with the corresponding 100% soil control, mixed soil–zeolite media generally improved plant height under TWW and WW irrigation, whereas pure zeolite did not show a comparable response. Under TWW irrigation, the mixed media increased plant height by 18.8 to 23.2% relative to plants grown in 100% soil, with the highest response observed in the 50% S: 50% Z treatment. Under WW irrigation, the increase was more pronounced, ranging from 30.0 to 34.6% in the mixed media, with the highest value recorded in 75% S: 25% Z, followed by 25% S: 75% Z. The improvement in plant height under mixed soil-zeolite media may indicate more favorable root-zone conditions compared with pure soil or pure zeolite. However, because plant height is an indirect growth indicator, the possible roles of improved nutrient availability and reduced heavy metal uptake should be interpreted together with the biomass, nutrient composition, and heavy metal data. In contrast, 100% Z produced comparatively lower plant height, particularly under TWW irrigation, indicating that excessive zeolite proportion alone may not provide sufficient nutrient buffering or balanced physical conditions for optimal alfalfa growth. These findings are consistent with those of Amirahmadi et al. (2022) and Jawad et al. (2023), who attributed growth reduction at excessive zeolite proportions to increased water loss and absence of organic matter.

Fresh and dry biomass also differed significantly among treatment combinations (Fig. 2B and 2C). Relative to the corresponding 100% soil control, the 25% S: 75% Z medium increased fresh and dry weight by 15.4 and 34.1% under TWW irrigation and by 33.2 and 19.3% under WW irrigation, respectively. In contrast, under WW irrigation, the 75% S: 25% Z and 50% S: 50% Z treatments reduced both fresh and dry biomass relative to 100% soil. These results indicate that the biomass response depended on both zeolite proportion and irrigation water quality, with 25% S: 75% Z being the most effective medium for biomass production under WW irrigation. The higher nutrient content of wastewater, particularly nitrogen, may also have contributed to increased vegetative growth.

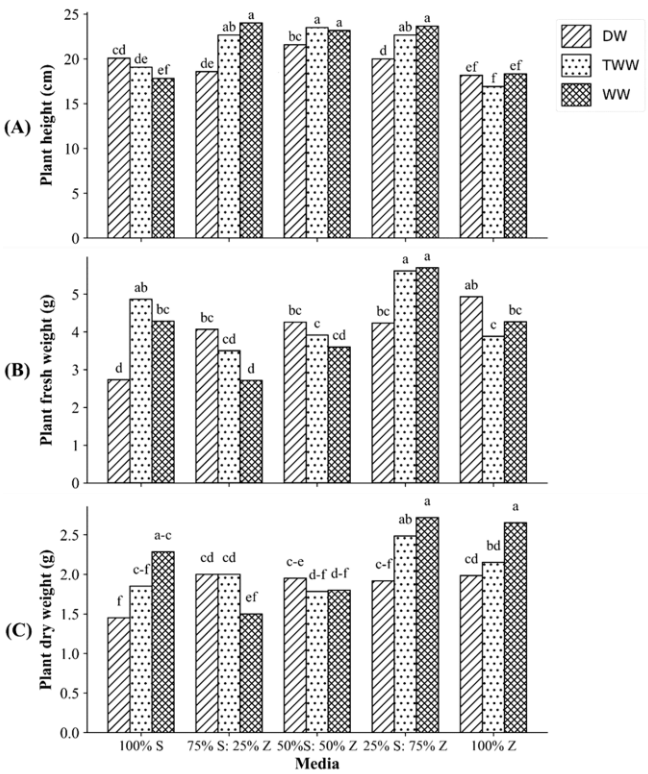


**Fig. 2:** Effect of different growing media and water irrigation treatments on alfalfa plant height (A), fresh weight (B), and dry weight (C). Bars represent mean ± SE (n = 3). Different letters indicate significant differences at P<0.05. (LSD: plant height =1.52, plant fresh weight =0.93 and plant dry weight =0.48).

The improved biomass response in the 25% S: 75% Z treatment may be explained by the combined effects of natural clinoptilolite on the root zone. Its high cation exchange capacity can improve nutrient retention and availability, while its porous structure may enhance water-holding capacity. In addition, clinoptilolite can reduce the mobility of potentially toxic metal ions through adsorption and ion-exchange reactions, thereby limiting heavy metal stress under wastewater irrigation (Moeen et al., 2020; Mondal et al., 2021). Ghassemi Sahebi et al. (2020) reported that clinoptilolite application improved sorghum yield and yield components under treated wastewater and saline-water irrigation, indicating that zeolite can improve crop performance by modifying root-zone water and nutrient conditions under marginal irrigation water sources.

### 3.2. Plant Chemical Content

As shown in Table 3, the nitrogen (N) content in alfalfa shoot was significantly affected by both the growing media and irrigation water source. Compared with the corresponding 100% soil control, mixed soil-zeolite media slightly increased nitrogen content, particularly under WW irrigation. Under WW, the 50% S : 50% Z and 25% S : 75% Z treatments increased shoot nitrogen by 3.5 and 3.8%, respectively, relative to 100% soil. In contrast, 100% Z reduced shoot nitrogen by 36.2, 15.2 and 15.3% under DW, TWW, and WW irrigation, respectively, compared with the corresponding 100% S treatments. This indicates that zeolite was more effective when combined with soil than when used alone. The slight improvement in nitrogen content under mixed media, especially under WW irrigation, may be associated with the ability of clinoptilolite to retain ammonium and reduce nitrogen losses from the root zone, thereby improving nitrogen availability for plant uptake. However, the lower nitrogen content under 100% Z may reflect the limited capacity of pure clinoptilolite to supply nitrogen when used without soil, because the material contained very low intrinsic nitrogen and did not contribute organic matter to the growth medium. This suggests that clinoptilolite can retain ammonium and improve nitrogen-use efficiency through its high cation exchange capacity, but its effectiveness depends on the presence of an external nitrogen source or a soil matrix that supports nutrient buffering. Palanivell et al. (2021) reported that clinoptilolite zeolite improved nitrogen adsorption–desorption behavior and soil buffering capacity, while Wang et al. (2025) found that zeolite increased soil cation exchange capacity, reduced nitrogen gaseous losses, and improved nitrate retention under urea fertilization.

**Table 3:** The effect of growing media and irrigation water source on alfalfa nitrogen (N), phosphorus (P) and potassium (K) content

| Media | N (%) | | | P (mg/g) | | | K (mg/g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | DW | TWW | WW | DW | TWW | WW | DW | TWW | WW |
| 100% S | 3.62ab | 3.61ab | 3.72ab | 1.93de | 2.02cde | 2.34abc | 34.14b | 36.88a | 35.45ab |
| 75% S : 25% Z | 3.54b | 3.63ab | 3.76ab | 2.66a | 2.27bcd | 2.15cd | 31.97cd | 36.82a | 33.51bc |
| 50% S : 50% Z | 3.62ab | 3.73ab | 3.85a | 2.12cd | 2.18cd | 2.19cd | 34.30b | 31.35d | 34.55b |
| 25% S : 75% Z | 3.73ab | 3.70ab | 3.86a | 2.35abc | 2.12cd | 2.59ab | 33.55bc | 29.11e | 28.80e |
| 100% Z | 2.31d | 3.06c | 3.15c | 1.65ef | 1.34f | 1.92de | 17.42h | 20.87g | 23.20f |
| LSD | 0.28 | | | 0.38 | | | 2.16 | | |

Means followed by the same letters are not significantly different at P<0.05.

Compared with the corresponding 100% soil, mixed soil-zeolite media increased phosphorus content under DW and TWW irrigation, whereas the response under WW irrigation was more treatment-dependent. Under DW irrigation, soil-zeolite mixtures increased phosphorus content by 9.8 to 37.8%, with the highest response observed in the 75% S: 25% Z treatment. Under TWW irrigation, mixed media increased phosphorus content by 4.9 to 12.4% relative to 100% S, while 100% Z reduced phosphorus content by 33.7%. Under WW irrigation, the 25% S: 75% Z treatment increased phosphorus content by 10.7% relative to 100% soil, whereas 75% S: 25% Z, 50% S: 50% Z, and 100% Z showed lower values than the soil control (Table 3). These results indicate that phosphorus uptake was influenced by both zeolite proportion and irrigation water quality, with moderate to high soil-zeolite mixtures supporting phosphorus accumulation more effectively than pure zeolite. Paliaga et al. (2025) found that enriched zeolite amendments combined with treated wastewater irrigation improved soil fertility by increasing total nitrogen and available phosphorus, enhanced plant nitrogen uptake, and stimulated favorable soil bacterial groups in a tomato pot experiment, supporting the role of zeolite-based amendments in improving root-zone nutrient conditions under wastewater irrigation. However, the lower phosphorus content in 100% zeolite may reflect the limited ability of clinoptilolite alone to supply phosphorus, particularly in the absence of soil-derived phosphorus pools. Palanivell et al. (2021) indicated that zeolite has limited capacity to adsorb or supply phosphorus due to electrostatic repulsion of phosphate anions, and that low zeolite rates are sufficient to maintain plant phosphorus availability when soil-derived phosphorus pools are present, whereas higher zeolite proportions provide no additional benefit for phosphorus uptake.

Potassium (K) content also varied significantly among treatment combinations. Significant reductions were observed at higher zeolite proportions compared with the corresponding 100% soil control. Under DW irrigation, potassium content decreased by 6.4% in the 75% S: 25% Z treatment and by 49.0% in 100% Z. Under TWW irrigation, the decline was more pronounced, with potassium content decreasing by 15.0, 21.1, and 43.4% in the 50% S: 50% Z, 25% S: 75% Z and 100% Z treatments, respectively. Under WW irrigation, significant reductions were observed in 25% S: 75% Z and 100% Z, where potassium content declined by 18.8 and 34.6%, respectively. These findings indicate that excessive zeolite proportions, particularly pure zeolite, reduced potassium accumulation in alfalfa shoots. This reduction may be related to competitive cation exchange within the clinoptilolite matrix, where potassium availability and uptake can be affected by the relative abundance of other cations such as sodium, calcium, magnesium, and ammonium. Therefore, although zeolite can improve nutrient retention, excessive application may disturb ionic balance and reduce potassium uptake efficiency (Mondal et al., 2021; Kučera et al., 2025).

Results presented in Table 4 showed that sodium (Na) content was significantly influenced by both zeolite proportion and irrigation water quality, with higher zeolite proportions generally increasing shoot sodium content and the strongest response occurring under WW irrigation. Under DW irrigation, all zeolite-containing media significantly increased shoot sodium content, with the greatest increase observed in 100% Z. Under TWW irrigation, significant increases were mainly observed at zeolite proportions of 50% or higher, where sodium content increased by 23.7, 34.8, and 112.9% in the 50% S: 50% Z, 25% S: 75% Z and 100% Z treatments, respectively. Under WW irrigation, the response was more pronounced, with sodium content increasing by 34.1, 84.8, 98.5, and 214.1% in the 75% S: 25% Z, 50% S: 50% Z, 25% S: 75% Z and 100% Z treatments, respectively, relative to 100% soil. These findings indicate that excessive zeolite application, particularly pure zeolite, promoted sodium accumulation in alfalfa shoots. This response may be associated with ion-exchange processes in clinoptilolite, which can release exchangeable sodium into the root zone or modify the balance among competing cations, thus increasing sodium availability for plant uptake. Such sodium accumulation may disturb ionic balance in plant tissues and could adversely affect forage quality under long-term wastewater irrigation (Assaha et al., 2017; Hajlaoui et al., 2023). Similar increases in sodium release and electrical conductivity in zeolite-amended systems have been reported under wastewater and saline conditions (Taheri-Sodejani et al., 2015; Mondal et al., 2021).

**Table 4:** The effect of growing media and irrigation water source on alfalfa sodium (Na) and magnesium (Mg) content

| Media | Na (mg/g) | | | Mg (mg/g) | | |
|---|---|---|---|---|---|---|
| | DW | TWW | WW | DW | TWW | WW |
| 100% S | 1.19h | 2.87ef | 2.70fg | 11.57efg | 11.60efg | 14.61b |
| 75% S : 25% Z | 2.37g | 3.11e | 3.62d | 11.30efg | 10.97fg | 16.44a |
| 50% S : 50% Z | 2.38g | 3.55d | 4.99c | 10.69g | 13.59bc | 11.85ef |
| 25% S : 75% Z | 3.78d | 3.87d | 5.36c | 12.28de | 12.31de | 13.18cd |
| 100% Z | 5.19c | 6.11b | 8.48a | 16.99a | 16.60a | 17.43a |
| LSD | 0.41 | | | 1.04 | | |

Means followed by the same letters are not significantly different at P<0.05.

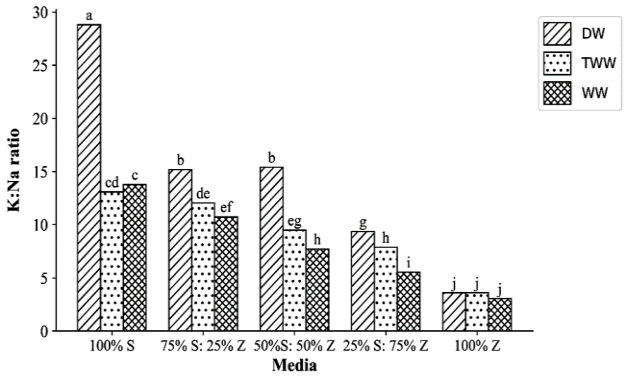


**Fig. 3:** Effect of different growing media and water irrigation treatments on alfalfa plant potassium–sodium ratio. Bars represent mean ± SE (n = 3). Different letters indicate significant differences at P<0.05 (LSD =1.36).

As shown in Fig. 3, increasing zeolite proportion resulted in a decrease in the K: Na ratio in alfalfa shoots. The highest ratio was observed in plants grown in 100% S under DW irrigation, whereas the lowest ratios were recorded in 100% Z across all irrigation water sources. Because a higher K:Na ratio is commonly associated with better ionic balance, enzyme activity, osmotic regulation, and membrane stability under saline or sodium-rich conditions (Assaha et al., 2017; Hajlaoui et al., 2023), the reduced ratio at high zeolite proportions indicates increased sodium accumulation relative to potassium. Compared with 100% Z, mixed soil-zeolite media maintained higher K: Na ratios, suggesting enhanced ionic homeostasis compared with pure zeolite. This response may be related to greater potassium retention and uptake, lower sodium accumulation, and the contribution of magnesium to essential metabolic processes (Assaha et al., 2017; Jēkabsone et al., 2023).

Magnesium (Mg) content was significantly affected by the treatment combinations and increased markedly in plants grown in 100% Z under all irrigation sources (Table 4). Compared with the corresponding 100% soil control, 100% Z increased magnesium content by approximately 46.8, 43.1 and 19.3% under DW, TWW, and WW irrigation, respectively. Under WW irrigation, 75% S: 25% Z also increased magnesium content by 12.5% relative to 100% soil, whereas 50% S: 50% Z and 25% S: 75% Z showed lower magnesium accumulation. This response suggests that while zeolite can retain magnesium ions, it may also act as a slow-release reservoir under favorable moisture and nutrient conditions, thereby maintaining magnesium availability in the rhizosphere and facilitating plant uptake. Collectively, these findings indicate that zeolite amendment influenced magnesium dynamics by balancing cation retention and bioavailability, with plant uptake responses strongly affected by zeolite proportion and irrigation water quality (Cieśla et al., 2019; Martins et al., 2023). Similar dose-dependent effects of zeolite on plant response and soil physicochemical properties have been reported by Szatanik-Kloc et al. (2021), while Kordala & Wyszkowski (2024) emphasized that clinoptilolite behavior is largely governed by its cation exchange capacity and exchangeable cation composition.

### 3.3. Soil pH and EC Levels

The effects of growth media and irrigation water source on the pH and electrical conductivity of the alfalfa growth media are presented in Table 5. The pH values were not significantly affected by zeolite proportion or irrigation water quality, ranging from 7.79 to 8.27 across treatments and indicating slightly alkaline conditions. Neither the soil–zeolite mixture nor the type of irrigation water resulted in marked changes in media pH. However, a slight increase was observed in 100% Z under treated WW. Similar trends have been reported in previous studies, where zeolite application was associated with increased soil pH due to ion-exchange reactions and enhanced cation retention (Taheri-Sodejani et al., 2015; Moeen et al., 2020).

**Table 5:** The effect of growing media and irrigation treatments on the pH and electrical conductivity (EC) of alfalfa growing media

| Media | pH | | | EC (dS/m) | | |
|---|---|---|---|---|---|---|
| | DW | TWW | WW | DW | TWW | WW |
| 100% S | 7.92 | 7.83 | 8.05 | 1.00e | 1.94a | 1.93a |
| 75% S : 25% Z | 7.93 | 8.13 | 8.01 | 1.00e | 1.33cd | 1.31cd |
| 50% S : 50% Z | 7.84 | 8.05 | 7.98 | 1.16de | 1.24cd | 1.95a |
| 25% S : 75% Z | 7.79 | 7.87 | 8.05 | 1.63b | 1.66b | 2.11a |
| 100% Z | 8.23 | 8.27 | 8.15 | 1.32cd | 1.37c | 1.40c |
| LSD | NS | | | 0.21 | | |

Means followed by the same letters are not significantly different at P<0.05.

On the other hand, electrical conductivity (EC) was significantly affected by both growing medium and irrigation water source. Irrigation with WW and TWW resulted in higher EC values compared to DW, indicating greater accumulation of soluble salts in the root zone; however, the magnitude of this increase varied according to growth medium. The highest EC was observed in the 25% S: 75% Z medium under WW, whereas the lowest EC values were recorded under DW in 100% S and 75% S: 25% Z treatments (Table 5). This response is consistent with Senila et al. (2022), who reported that natural zeolite application can increase pH, EC, and sodium levels. These findings indicate that although zeolite improved several growth and nutrient parameters, high zeolite proportions under wastewater irrigation may increase salt accumulation, suggesting the need for salinity monitoring during long-term application to avoid progressive salinity buildup.

### 3.4. Heavy Metals Concentration

***3.4.1. Heavy Metals Concentration in Plant Shoot:*** Nickel (Ni) and lead (Pb) concentrations in alfalfa shoots were significantly affected by the interaction between growth medium and irrigation water source (Table 6). Both metals concentrations in alfalfa shoots were significantly higher in all growth media irrigated with WW compared with those receiving DW or TWW, under which both metals were undetectable (0.000 ppm). Under WW irrigation, a significant decline in both nickel and lead concentrations was observed with increasing zeolite proportion in alfalfa shoots. Compared to the 100% soil, nickel concentration decreased by 17.3% in 75% S: 25% Z and by 39.8% in 50% S: 50% Z. Greater reductions were observed at higher zeolite proportions, reaching 61.2% in 25% S: 75% Z and 68.6% in 100% Z. Similarly, lead concentration decreased by 13.4, 33.0, 39.1 and 50.3%, respectively, in the same treatments. These results demonstrate that zeolite addition reduced heavy metal accumulation in alfalfa shoots, with the strongest reduction observed at higher zeolite proportions. Comparable results have been reported by Hasanabadi et al. (2015) and Argiri & Tsadilas (2016) on alfalfa, Ibrahim et al. (2014) on lettuce, and Rahmany-Samani et al. (2023) on rice. The reduction in nickel and lead accumulation with increasing zeolite content can be attributed to the high cation exchange capacity and large surface area of zeolite, which enhances metal adsorption and immobilization in the root zone, thereby limiting their bioavailability and translocation to aerial plant parts (Mondal et al., 2021; Wang et al., 2025). In addition to immobilization, the greater biomass production observed in some zeolite-amended treatments, particularly 25% S: 75% Z under WW irrigation, may have contributed partly to a dilution effect, whereby absorbed metals were distributed within a larger amount of plant tissue. Similar dilution effects have been reported in crop biomass, where increased plant growth reduced the concentration of heavy metals in plant tissues despite metal entry into the soil-plant system (Yin et al., 2024).

**Table 6:** The effect of media composition and irrigation water source on the concentrations of nickel (Ni) and lead (Pb) in the alfalfa plant

| Media | Ni (ppm) | | | Pb (ppm) | | |
|---|---|---|---|---|---|---|
| | DW | TWW | WW | DW | TWW | WW |
| 100% S | 0.000e | 0.000e | 0.392a | 0.000e | 0.000e | 0.179a |
| 75% S : 25% Z | 0.000e | 0.000e | 0.324b | 0.000e | 0.000e | 0.155b |
| 50% S : 50% Z | 0.000e | 0.000e | 0.236c | 0.000e | 0.000e | 0.120c |
| 25% S : 75% Z | 0.000e | 0.000e | 0.152d | 0.000e | 0.000e | 0.109c |
| 100% Z | 0.000e | 0.000e | 0.123d | 0.000e | 0.000e | 0.089d |
| LSD | 0.037 | | | 0.012 | | |

Means followed by the same letters are not significantly different at P<0.05.

From a sustainability perspective, the observed nickel and lead concentrations in alfalfa shoots remained below commonly reported maximum permissible limits for forage crops (5.0 ppm for nickel and lead) (Kim 2023; Fatima & Bayram, 2024). This indicates that natural clinoptilolite may support safer wastewater reuse by reducing heavy metal transfer to forage biomass. Nevertheless, the use of wastewater irrigation should be accompanied by continuous monitoring of heavy metals, salinity, and forage quality, particularly under long-term field conditions, to ensure protection of soil health, livestock nutrition, and agroecosystem sustainability (Othman et al., 2021; Saleh et al., 2025).

***3.4.2. Heavy Metals Concentration in the Growth Media:*** Nickel and lead concentrations in the growth media were detected only under WW irrigation, whereas both metals were undetectable under DW and TWW irrigation (Table 7). Nickel concentration in the growth media did not statistically differ among treatments. However, under WW irrigation, zeolite-amended media showed lower nickel concentrations than the 100% soil control, with decreases ranging from 26.0% to 62.9% in the 75% S: 25% Z and 25% S: 75% Z treatments, respectively. In contrast, lead concentration in the growth media was significantly reduced by zeolite addition under WW irrigation. Compared with the 100% soil control, lead reductions ranged from 14.8% in 75% S: 25% Z to 49.9% in 100% Z.

**Table 7:** The effect of media and irrigation water treatments on the concentrations of nickel (Ni) and lead (Pb) in the alfalfa growing media

| Media | Ni (ppm) | | | Pb (ppm) | | |
|---|---|---|---|---|---|---|
| | DW | TWW | WW | DW | TWW | WW |
| 100% S | 0.000 | 0.000 | 0.439 | 0.000[e] | 0.000[e] | 0.345[a] |
| 75% S : 25% Z | 0.000 | 0.000 | 0.325 | 0.000[e] | 0.000[e] | 0.294[b] |
| 50% S : 50% Z | 0.000 | 0.000 | 0.263 | 0.000[e] | 0.000[e] | 0.235[c] |
| 25% S : 75% Z | 0.000 | 0.000 | 0.163 | 0.000[e] | 0.000[e] | 0.215[c] |
| 100% Z | 0.000 | 0.000 | 0.313 | 0.000[e] | 0.000[e] | 0.173[d] |
| LSD | NS | | | 0.022 | | |

Means followed by the same letters are not significantly different at $P<0.05$.

The reduction in heavy metal accumulation in plant shoots, together with the changes observed in the growth media, suggests that natural clinoptilolite reduced metal mobility and bioavailability in the root zone. This effect can be explained by the negatively charged aluminosilicate framework, high cation exchange capacity, porous structure, and large surface area of clinoptilolite. These properties allow metal cations to be retained through adsorption and ion-exchange reactions, limiting their movement in the growth medium and reducing their availability for plant uptake (Rodríguez-Iznaga et al., 2022; Kordala & Wyszkowski, 2024). Similar reductions in bioavailable or extractable heavy metals following zeolite application have been reported by Senila et al. (2022), who found that natural zeolite reduced cadmium and lead bioavailability in contaminated soil. Moeen et al. (2020) also reported reductions in extractable cadmium, lead, copper and zinc, accompanied by increased soil pH and cation exchange capacity, while Mondal et al. (2021) described zeolite as an effective amendment for improving soil chemical stability and reducing heavy metal mobility.

From a practical perspective, the 25% S: 75% Z mixture appears to be the most suitable soil–zeolite mixing ratio under raw wastewater irrigation because it provided the best compromise between biomass production, nutrient accumulation, and reduction of nickel and lead uptake. Although higher zeolite proportions further reduced heavy metal accumulation, pure zeolite increased Na accumulation and reduced the K: Na ratio, indicating a potential risk of ionic imbalance under long-term wastewater irrigation. Therefore, farmers using wastewater for forage production should avoid excessive zeolite application and should preferentially use mixed soil–zeolite media, particularly the 25% S: 75% Z ratio, while monitoring salinity, sodium accumulation, and forage quality under field conditions.

## 4. CONCLUSION

The present study demonstrated that natural zeolite, mainly composed of clinoptilolite, can improve alfalfa performance and reduce heavy metal uptake under wastewater irrigation by modifying nutrient availability and metal mobility in the growth media. The findings show that mixed soil–zeolite media were more agronomically effective than either pure soil or pure zeolite. Among the tested treatments, the 25% soil: 75% zeolite mixture provided the most favorable balance between biomass production, nitrogen and phosphorus uptake, and mitigation of nickel and lead accumulation under raw wastewater irrigation. In contrast, pure zeolite was less suitable as a standalone medium because an excessive zeolite proportion promoted sodium accumulation and reduced the K:Na ratio, indicating a potential risk of ionic imbalance.

Overall, these findings support the use of natural zeolite as a sustainable amendment for improving the safety and productivity of wastewater-irrigated alfalfa systems. The results highlight the importance of optimizing soil–zeolite mixing ratios rather than using zeolite alone, particularly under raw wastewater irrigation where heavy metal mitigation must be balanced against potential sodium accumulation. Future field-scale studies are recommended to validate these greenhouse results under long-term conditions, assess economic feasibility, and refine soil–zeolite mixing guidelines for different soil types, crops, and wastewater qualities.

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