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# Micromineral Content of Swiss Chard (Beta vulgaris L. var. cicla) Leaves Grown on Zeolite-Amended Sandy Soil

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**Abstract.** Swiss chard (*Beta vulgaris* L. var. *cicla*), a popular leafy vegetable grown mainly for its ease of production and nutritional content, is normally used as a good source of microminerals iron (Fe) and zinc (Zn). Improving plant uptake of Fe and Zn could assist in reducing micromineral deficiencies in humans, which are a global problem. A greenhouse pot experiment was conducted at the Agricultural Research Council, Stellenbosch to assess the response in micromineral and soil trace elements uptake in Swiss chard grown under zeolite and sandy soil. The experiment consisted of four treatments, with six replicates arranged in a randomized complete block design. Zeolite was applied at 0%; 10%; 20% and 30% in combination with sandy soil. Swiss chard was harvested for micromineral analysis 143 days after transplanting. Soil samples were also collected at the end of each growing season. Swiss chard leaves were analysed for Fe, Zn, Mn, and Cu content with soil samples also analysed for the same elements. The study found that zeolite did not improve Swiss chard uptake of Fe, Zn and Mn. Soil Fe also had an inverse relationship to zeolite application while the residual effect of zeolite showed the same trend, but only in the second season. This study indicated that zeolite cannot be used to improve micromineral uptake by Swiss chard but can be used to rectify heavy metal-infested soils.

**Keywords:** micromineral, sandy soil, soil amendment, Swiss chard, zeolite

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## 1. Introduction

Vegetables contribute to food security in the world, including in Southern Africa, where even wild leafy vegetables are important [1]. Vegetables are consumed to supply minerals, vitamins, and dietary fibre [2]. Swiss chard (*Beta vulgaris* L. *var. cicla*) is a leafy vegetable that is popular in South Africa for its nutritional content and general ease of production [3]; [4]. The vegetable offers a healthy and affordable alternative in combating micronutrient deficiencies such as iron

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(Fe), zinc (Zn), vitamin A and vitamin C, and has substantially substituted wild leafy vegetables over time [5]. Iron and Zn are among the major problematic microminerals in human health.

Micronutrient deficiency is a global phenomenon that is linked to food insecurity [4], [6]. The deficiencies have been combated with the biofortification of crops, produce fortification and increasing dietary plant varieties [7] - [8]. Soil amendments have also been used to try and improve some of the nutritional contents of vegetables [2]; [9]. Plant uptake of mineral nutrients is linked to nutrient bioavailability which is highly dependent on soil conditions, environmental factors, plant species and the type of nutrient [10]. According to [11] soil properties and soil processes influence the kinetics of sorption reactions, metal concentration in solution and the form of soluble and insoluble chemical species. While environmental factors such as humidity and water play a role in assisting nutrients to solubilize. Some plant species can increase mineral bioavailability by releasing a variety of root exudates which changes the rhizosphere pH leading to better mineral uptake [12]

Soil amendments are incorporated into the soil to improve the physical (soil porosity) and chemical (soil pH) status of the soil. Zeolites have been used to improve soil chemical status, and soil moisture retention ability and to improve crop yields [2]; [13] - [14]. Zeolites are microporous, aluminosilicate minerals of alkaline nature with a high cation exchange capacity (CEC) [15]. Due to their high CEC and adsorption capacity, zeolites have also been used to ameliorate soils contaminated with heavy metals and to improve water quality [16] - [18] Since zeolite is gaining attention as a soil conditioner to enhance soil moisture retention and crop yields, yield quality needs to be monitored to ensure that the harvest is nutritionally adequate. Micromineral deficiencies and excesses are detrimental to crop quality and human health. Zeolite has been shown to improve soil physicochemical and biological characteristics of soils thereby improving features like soil pH and nutrient availability [19] - [20]. Research on the effect of zeolite on the micromineral contents of crops is limited. This study evaluated the uptake of micro minerals in Swiss chard grown in zeolite-amended sandy soil.

## 2. Methods

### 2.1. Research Site and Treatment

A greenhouse pot experiment was carried out at the Agricultural Research Council Infruitec-Nietvoorbij, Stellenbosch, South Africa. The study was conducted over two seasons the first season being late autumn to late spring 2018 and the second season being early autumn to early spring 2019. Six weeks old Swiss chard (Beta vulgaris var. cicla) cv. Ford Hook Giant seedlings were transplanted into zeolite-amended sandy soils. The seedlings were 7 cm tall at the 5 to 7-leaf growth stage. Treatments were 0% zeolite + 100% sand (control), 10% zeolite + 90% sand, 20% zeolite + 80% sand, and 30% zeolite + 70% sand (i.e., weight-to-weight ratios of 0:10, 1:9, 2:8, and 3:7 for zeolite to sandy soil).

There were four treatments with six replicates which were arranged in a randomised complete block design. Each pot contained a mixture of soil and zeolite weighing 12 kg. Basal fertiliser was applied as urea (46% N): 1.17g/pot, single super phosphate (20% P): 3g/pot and potassium chloride (50% K): 1.44g/pot. Urea at 0.33g/pot was side-dressed at 4 and 8 weeks after transplanting. Soil moisture was maintained between 50-70 % pot capacity throughout the study, which is between the suitable 40-80% recommended soil moisture [21]. Weeds were controlled manually while insect pests were controlled using Makhro Cyper® (active ingredient: cypermethrin, 200 g L-1) and Mercaptothion® (active ingredient organophosphate 500 g·L-1) in the first and second seasons respectively.

### 2.2. Harvesting and Sample Processing

For each season, harvesting was done 59 days after transplanting (DAT) followed by 4 subsequent harvests at 21-day intervals. For micromineral analysis, Swiss chard harvested on the last harvest, 143 DAT was considered for both growing seasons. Only matured leaves of length≥15 cm were harvested, the plants were not allowed to flower as Swiss chard is generally consumed before flowering. After harvesting, leaves were oven-dried in a forced air oven at 70°C till constant weight. Dry leaf samples were milled using the Polymix PX-MFC, 90D miller manufactured by Kinemanetica AG, Switzerland. Milled samples were placed in an airtight container and stored in a refrigerator (5°C) till further processing.

### 2.3. Plant Tissue Analysis

Swiss chard samples were dry-ashed in a muffle furnace at 500°C for 6 hours to measure micromineral contents: iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn). Hydrochloric acid extraction method was then used to evaluate the micromineral compositions [22].

### 2.4. Soil Chemical Analysis

Before the application of treatments (baseline) and after harvest (for both seasons), a soil chemical analysis was performed using the Non-affiliated Soil Analysis Work Committee's standard protocols (1990). Soil microminerals (Fe, Cu, Zn, and Mn) were analysed using the 0.02 M EDTA (ethylene diamine tetra acetic acid) titration method while soil pH was analysed using the 1.00 M potassium chloride (KCl) method [22]. Chemical analysis was conducted using only analytical-grade reagents.

### 2.5. Statistical Analysis

Data were analysed using Statistical Analysis System (SAS) software (version 9.4, SAS Institute Inc., Cary, NC, USA, 2000) for Analysis of Variance (ANOVA). Seasonal homogeneity of variance was tested with Levene's test, after which the results of both seasons were merged and studied in a single overall ANOVA. The Shapiro-Wilk test was carried out to test for deviation from normality and insignificant interactions. Fisher's least significant difference was calculated at the 5% level to compare treatment means. For all tests, a probability level of 5% was considered significant

## 3. Results and Discussion

### 3.1. Baseline Soil Characteristics and Zeolite Properties

The baseline soil pH (5.4) was less than the recommended soil pH for optimal Swiss chard growth. The soil microminerals Fe, Zn, Mn, and Cu were at levels 362.6, 6.2, 24.2, and 0.4 mg/kg respectively. The zeolite used in this study had a granular white-to-grey appearance, it could absorb about 400% water on its sinter plate. Its mineralogy was composed of clinoptilolite (>90%) and quarts (<5%), the chemical properties of the used zeolite are shown in Table 1.

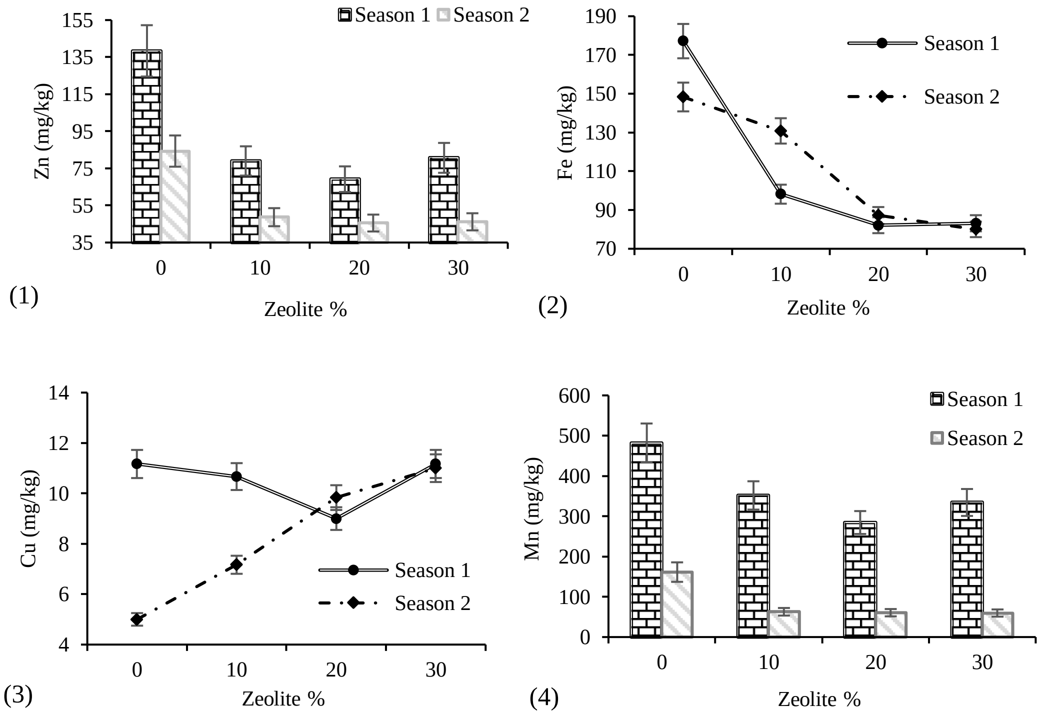
**Table 1**. Chemical Content of the Zeolite Used

| Zeolite Chemical Properties | Value |
|---|---|
| pH (30 g in 60 mL water) | 8 |
| Cation exchange capacity (mg/kg) | 16 |
| SiO2 (%) | 64,3 |
| Al2O2 (%) | 12,7 |
| TiO2 (%) | 0,1 |
| MgO (%) | 1,3 |
| Na2O (%) | 2,3 |
| Fe2O2 (%) | 1,3 |
| CaO (%) | 1,2 |
| K2O (%) | 1,7 |

### 3.2. Swiss Chard Micro Mineral Content Responses to Zeolite Application

The micromineral contents of Swiss chard from this study were found to be at higher levels in the non-amended treatment, with the exception of Cu in the second season of the study. The micromineral concentrations of Swiss chard (Fig 1) and those of the soil (Fig 2) were generally higher in season 1, with exception of Mn. The superior mineral element concentrations in season 1 are largely attributed to the availability of nutrients in higher concentrations in the soil, as well as from applied fertiliser. However, it seems zeolite might be absorbing minerals, making them unavailable to the plant, a trend that seems to be true for Zn and Fe [23].

The FAO/WHO maximum permissible values for Fe, Cu, Zn, and Mn in vegetables are 425.5, 73.3, 99.3, and 500 mg/kg respectively [24]. The Fe and Cu contents in Swiss chard leaves were substantially lower than the permissible levels, with values ranging from 80 to 177.17 mg/kg and 5 to 11.17 mg/kg, respectively. Manganese obtained on the non-amended treatment was within proximity of the permissible level during the first growing season (482 mg/kg), however, all the other treatments were considerably less. Zinc levels were higher than the permissible value in Swiss chard grown on the non-amended control in season 1 (138.4 mg/kg), with all the other obtained Zn values falling within the permissible levels.




Note: Season 1 = year 1 of experiment (2018), Season 2 = year 2 of experiment (2019)

**Figure 1**. Responses of Swiss Chard Micromineral Composition to Zeolite Application. Overlapping Bars Show No Significance at $p<0.05$

[7] found Swiss chard leaves with equivalents of 442.10 mg/kg (dw) Zn and concluded that consuming 100 g (fw) of the Swiss chard would give about 18% of the Zn recommended daily allowance (RDA). None of the Swiss chard in this study came close to that. The consumption of 100 g (fw) from the Swiss chard grown in the non-amended soil would give about 7% of the RDA while the consumption of the same amounts from the zeolite amended treatments would give between 3 and 5.5% of the RDA. Although Swiss chard consumption would have to be almost double to acquire a similar amount of Fe, the yield benefits associated with it can compensate [25].

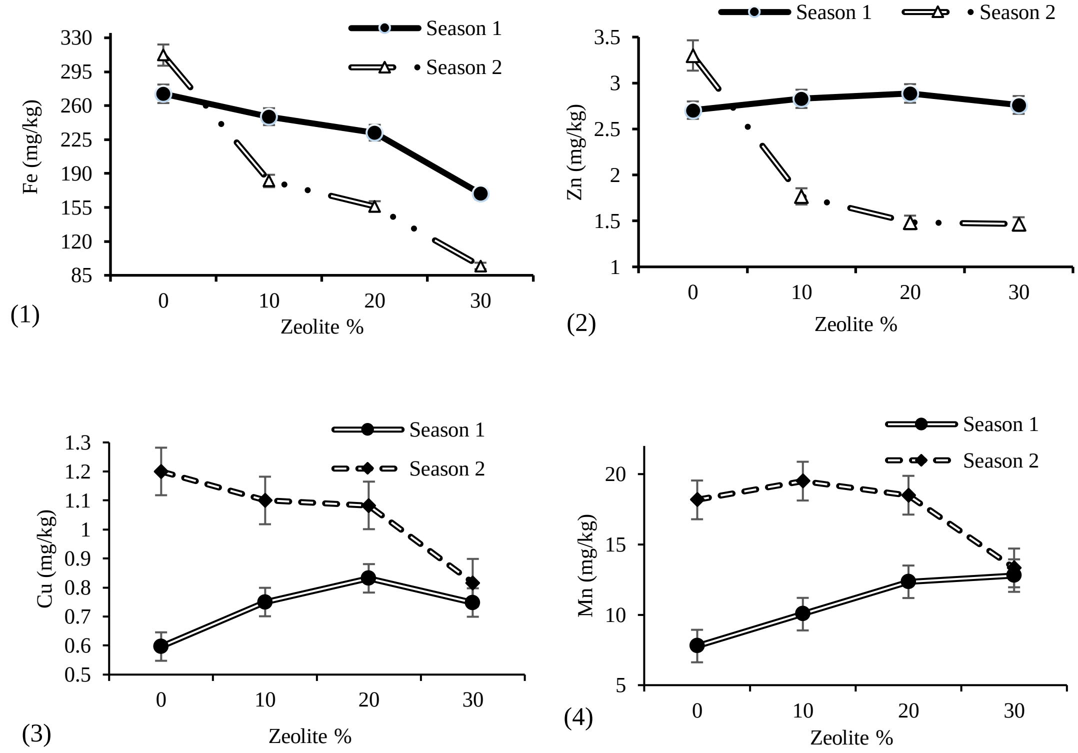
The accumulation of micro minerals in Swiss chard leave observed from the data represented in this study contrasted with the findings of [25], who found no differences in Cu, Fe, Mn, and Zn in the leaf concentrations of Castanea sativa Mill among treatments with 0 and 20% zeolite application (soil: zeolite v/v). Significant increases in Mn and Cu in soybean grown on soil amended with zeolite were found by [26]. The increasing trend from their study is only constant with the trend observed on Cu obtained in season 2. This discrepancy can be attributed to the higher CaO and lower MgO contents of the zeolite that was used in their studies. Calcium increases the rate of Mn uptake by plants, whereas Mg has the opposite effect [27]. [28] utilised zeolite with similar chemical properties (% content) and found increased contents of Zn, Mn and Cu on leaves of common bean. The increase in Zn and Mn was likened to Zn and Mn fertilisation and zeolite application. Zinc uptake by plants depends on the content and form of Zn, the genetic characterises of plants and soil chemical characteristics [29]. This is due to some plant species releasing root exudates which change the rhizosphere pH (chemical status) leading to better mineral uptake [12]. Additionally, the constant accumulation increments of Mn and Cu in zeolite-amended treatments observed in the two studies [26]; [28] could potentially be due to genetic characteristics associated with the plant family Fabaceae as plant genotypes also govern nutrient accumulation [29]. Beans and soybeans have also been endorsed as high sources of Cu and Mn [30].

Swiss chard leaves are considered a rich source of Fe, they have also been used as sources of Mn, Zn and Cu [7]. The leaf accumulation of these minerals tends to differ due to the differences in plant genetics Differences in micromineral accumulation in this study were seen based on zeolite treatment and season of growth. The other explanation could be because only the last harvest was considered, meaning that as the number of harvests increases, there will be a need to supplement microminerals by adding more fertiliser. There are also reports of antagonistic relationships between some minerals during plant assimilation [31]. [25] and [32] found evidence of an antagonistic relationship for common uptake between Fe and Mn. The micromineral concentrations of this study did not provide such evidence. However, it may be considered that Cu and the rest of the microminerals (Fe, Mn and Zn) did have an antagonist relationship, as its accumulation on Swiss chard leaves had a nearly opposite trend compared to other microminerals.

### 3.3. Effect of zeolite on soil trace elements

Essential trace elements such as Zn and Fe have bio-important functions in the metabolism of human beings, animals and plants. Their increased accumulation in the soil can cause detrimental effects on the environment. The normal soil limits of Fe, Zn, Mn and Cu range from 30 to 500, 10 to 300, 300 to 1000 and 10 to 40 mg/kg respectively [24]. From this study, after growing seasons 1 and 2 only Fe compared better to the above-mentioned limits. For the baseline soil used to mix the treatments, both Fe and Zn were comparable to the recommended limits. The low levels of these trace elements in the baseline soil are expected as sandy soils tend to have limited nutrient retention capabilities [33]. Additionally considering that the baseline soil pH was 5.4 the soil Fe and Zn were probably available in their cationic forms [13].

In this study, Fe availability on both the soil and Swiss chard decreased ($p<0.05$) with zeolite application (Fig 1 and 2). However, the soil Fe was generally greater in the first growing season while in Swiss chard accumulation, it was generally more with Swiss chard harvested in the second season. Additionally, soil Zn showed no differences ($p<0.05$) in season 2, while its levels on Swiss chard reduced as zeolite application increased. Soil Cu and Mn on the other hand did not relate to Swiss chard accumulation in both seasons.




Note: Season 1 = year 1 of experiment (2018), Season 2 = year 2 of experiment (2019)

**Figure 2**. Effect of Zeolite on Soil Trace Element Availability: Overlapping Bars Show no Significance at $p<0.05$

The inverse relationship of soil Fe concentrations to soil pH observed in this study is consistent with the findings of [34]. They found that soil pH significantly affected the concentrations of Fe but not that of Mn and Zn. They found superior concentrations of Fe on soils with a pH of 4.5 than those with a pH of 8.0. Zeolite did not affect soil Zn in the first growing season, however, in the second season soil Zn contents contradicted the above statement. [35] suggest that zeolite can retain Zn and slowly release it into the soil solution as soon as 120 hrs after application. The true effect of zeolite absorbing Zn in this study was observed only in the second season which is consistent with the findings reported by [1].

The major characteristics of zeolite that makes it attractive to agriculture are its high CEC and its ability to sorb nutrients and slowly release them [36]. Apart from the general increase in soil pH observed in this study, which is one of the governing factors for soil nutrient availability, (soil pH data reported by [25]), the result is consistent with findings by [37]. The decreasing soil Fe (both seasons), Cu and Zn (Second season) content can be attributed to the blocking mechanism of zeolite due to ion exchange and metal adsorption into the lattice of its tectosilicate [16]. This characteristic of zeolite has been used to sorb heavy metals in wastewater and sewage sludge compost [13]; [38]. Zeolite uptakes heavy metals that are present in their "easily available fraction" forms and exchanges them with Na and K cations from its structure [13].

## 4. Conclusion and Recommendation

The results obtained in this study indicate that zeolite may not necessarily be used to improve or increase micromineral contents in Swiss chard leaves, without supplementing with fertiliser that contains micronutrients. However, zeolite application has an inverse relationship with most of the soil trace elements and can be used to rectify heavy metal-infested soils. Given this benefit and other direct benefits attributed to zeolite, such as improved crop yields, the application of zeolite in combination with other soil amendments to boost micromineral assimilation still needs testing, especially at the field level.

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