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Establishment of correlation between physicochemical properties of soil, micronutrients and citrus greening disease in sweet orange orchards of Marathwada region

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Abstract
The present investigation entitled "Establishment of Correlation Between Physicochemical Properties of soil Micronutrients and Citrus Greening in Sweet Orange of Marathwada Region" was carried out during 2024-2025.The soil and plant samples were collected from 60 different sweet orange orchards in which 30 sweet orange orchards out of which showing citrus greening symptoms in various villages of paithan tehsil in Chh. Sambhajinagar district.
The results revealed that, soil pH is positively correlated with disease incidence (r = 0.894**) and disease severity (r = 0.932**) whereas soil Fe is negatively correlated with disease incidence (r =-0.568**) and disease severity (r =-0.525**) and soil Zn is negatively correlated with disease incidence (r =-0.815**) and disease severity (r =-0.834**). Similar trend follows in tissue analysis also where Iron is negatively correlated with disease incidence (r =-0.441**) and disease severity (r =-0.402**), Cu is negatively correlated with disease incidence (r =-0.408**) and disease severity (r =-0.385**), Mn is negatively correlated with disease incidence (r = 0.494**) and disease severity (r = 0.466**) and Zn is negatively correlated with disease incidence (r =-0.392**) and disease severity (r =-0.363**).These finding suggest that an imbalance and insufficiency of essential macronutrients may be responsible for development of citrus greening incidence and severity in sweet orange orchards.

Keywords: Survey, citrus greening, sweet orange, correlation, healthy vs. unhealthy trees, micronutrients

Introduction

Sweet orange (*Citrus sinensis* (L.)) belongs to the family Rutaceae, specifically the subfamily Aurantioideae, which comprises 33 recognized genera and 203 species. Among all citrus fruits cultivated worldwide, sweet orange accounts for approximately 71% of total citrus production, contributing nearly 50% of global citrus output. According to the Food and Agriculture Organization (FAO), sweet orange is cultivated on 3.8 million hectares, yielding approximately 75.5 million tons annually (FAOSTAT, 2020). The crop holds substantial commercial value in tropical and subtropical regions, with Brazil, India and China being the leading producers at 16.7, 9.8 and 7.6 million tons, respectively, followed by the United States with 4.8 million tons.

India is the second-largest producer of sweet orange and ranks it as the third most cultivated fruit in the country, following mango and banana. Major sweet orange-producing states in India include Maharashtra, Andhra Pradesh, Karnataka, Punjab and Rajasthan, with an annual production of 3.9 million metric tons (MMT). India contributes around 8% of the world's total fruit production. The Marathwada region is especially notable for its extensive sweet orange cultivation and is often referred to as the "mother home" of sweet oranges (Kausadikar, 2005) [11].

Despite its economic significance, sweet orange cultivation is highly susceptible to a range of fungal, bacterial and viral diseases, which cause substantial yield losses. Among these, citrus greening disease, also known as Huanglongbing (HLB), is considered the most destructive. First identified in China in the late 19th century, it is represented with characteristic yellow shoot symptoms and has since been reported under various names across citrus-growing regions-for example, citrus dieback in India, mottle leaf disease in the Philippines and yellow branch or greening in South Africa. Today, it is widely recognized under the name citrus greening. The disease is caused by the gram-negative bacterium *Candidatus liberibacter* spp., a member of the Rhizobiaceae family (Garnier and Bové, 1983) [6].

Initially, citrus greening was thought to be a virus-borne disease, as it was transmissible through grafting-a property associated with viruses at the time. However, in 1967, researchers hypothesized that mycoplasma-like organisms (MLOs) might be the causal agents. These wall-less microorganisms can infiltrate the phloem and disrupt nutrient transport, producing symptoms similar to those of citrus greening (Laflèche and Bové, 1970) [13].

Citrus greening (HLB) plays a major role in global citrus decline by drastically reducing both fruit yield and quality. Affected trees exhibit symptoms such as stunted growth, leaf mottling and yellowing, lopsided fruits, premature fruit drop and twig dieback. They also become more susceptible to environmental stressors, including temperature fluctuations and drought. Although the disease was initially overlooked, it's devastating impact became undeniable after it spread to major citrus-growing regions, notably São Paulo, Brazil (2004) and Florida, USA (2005)-both key global citrus hubs (Bové, 2006; Gottwald, 2012) [8].

The growing prevalence and destructive effects of citrus greening underline the urgent need for effective disease management strategies. One critical factor that influences both disease severity and tree health is nutritional imbalance, which weakens plant defense mechanisms and increases susceptibility to infection. Recent research has emphasized that nutritional imbalance can act as a secondary factor aggravating the severity of citrus greening disease. Balanced nutrition, particularly with micronutrients and selected micronutrients, enhances tree tolerance under HLB stress. For instance, Ghimire *et al*. (2023) [7] demonstrated that HLB-affected citrus trees perform better when supplied with complete and balanced nutrition and rootstocks with higher nutrient-uptake efficiency maintained productivity for longer durations. Likewise, Dong *et al*. (2024) [4] reported that boron nutrient imbalance alters leaf cell-wall structure and modifies Asian citrus psyllid feeding behavior, thereby facilitating disease spread in orchards. These findings underline the importance of nutrient balance in influencing both the expression and epidemiology of citrus greening, justifying the present study on establishing correlations between nutrient disorders and disease severity in sweet orange orchards of Marathwada. Therefore, understanding the correlation between nutrient disorders and citrus greening disease is essential for developing sustainable and science-based solutions to mitigate its impact and enhance citrus productivity.

Materials and Methods

The present investigation, titled "Establishment of Correlation Between Physicochemical Properties of soil, Micronutrients and Citrus Greening Disease in Sweet Orange Orchards of Marathwada Region" was conducted during 2024-2025. Representative soil and leaf samples were randomly collected from sixty sweet orange orchards (thirty healthy and thirty infected) located in Chh. Sambhajinagar district of Marathwada region during year 2024-2025. The data collected on various parameters were statistically analysed and presented in form of tables and graphs discussed under the following sub headings:

Survey of citrus greening disease of sweet orange orchards: A survey was conducted on infected and healthy sweet orange orchards in Marathwada region. Sixty orchards were selected with thirty were healthy and thirty infected samples, to detect of correlations with primary, secondary macronutrient disorders so soil and plant analysis was carried out. Geographically, Marathwada region of Maharashtra state is located between 17° 37' to 20° 39' North latitude 74°35' to 78° 22' East longitude, covering total geographical area are 6.4 million ha. Marathwada region falls under semi-arid and tropical climate zones of Maharashtra state. It experiences three main seasons: monsoon, winter and summer. The annual average rainfall is approximately 930 mm. Soils of Marathwada region range from deep black to shallow, light textured types. The majority are medium to deep black soils and categorized as Vertisols and Inceptisols.

Experimental details: Sweet orange selected as fruit crop and Number of orchards: 60 (30 Healthy and 30 citrus greening infected). Assessment of Disease Scale, Incidence and Severity: An extensive survey of sweet orange orchards was conducted in the month of November and December 2024 in Marathwada regions to record the status of citrus greening of sweet orange. The observation of disease incidence and severity in orchards were recorded during the survey of citrus greening of sweet orange. The study was conducted in key sweet orange-producing towns of Chh. Samhajinagar district from Marathwada region, Maharashtra during November-December 2024. Twelve villages were selected from tehsil of Paithan as folows: Paithan (12 orchards), Dadegaon (11), Vadwali (5), Karajkheda (2), Akhatwada (3), Harshi (2), Lohgaon (9), Brahmangavhan (7), Mavasgvhan (6), Sangatpuri(1), Gandhegaon (1), Dawarwadi (1).

The severity of citrus greening disease was recorded using the disease scale developed by Pustika *et al*. (2008). As shown in table 1 by Pustika *et al*. (2008) [17].

Table 1: Disease scale for recording citrus greening disease severity

Scale	Description
0	Leaves on branch asymptomatic
1	<50% leaves on branch showing interveinal chlorosis
2	>50% leaves on branch showing interveinal chlorosis
3	<50% leaves on branch showing vein corking
4	>50% leaves on branch showing vein corking
5	<50% leaves on branch showing interveinal chlorosis and vein corking
6	>50% leaves on branch showing interveinal chlorosis and vein corking
7	Branch showing die back, <50% leaves abscised
8	Branch showing die back, >50% leaves abscised
9	All leaves on branch abscised, branch desiccated

By Pustika *et al.* (2008) [17]

The percent incidence was calculated by recording observations on plant units infected such leaves. The percent disease severity was calculated by recording observations of the area or a volume of a plant tissue disease.

Disease prevalence and percent disease severity index (DSI) were calculated using following formulas. To evaluate percent incidence 20 plants from each of the four corners and 20 plants from center (focus) were arbitrarily selected totaling in 100 plants per field. percent incidence was done by using the following formula; given by Masyahit *et al*. (2013) [14].

$$\%\text{Incidence} = \frac{\text{number of infected plants}}{\text{Total in number of observed plants}} \text{x } 100$$

To evaluate percent DSI was calculated by using a formula adopted from Lakshmi *et al*. (2011).

$$\%\text{DSI} = \frac{\text{Sum of all disease ratings } \Sigma \text{ n x v}}{\text{Number of leaves examined x maximum disease grade}} \text{x100}$$

Where, ′n′ and ′v′ represent the number of samples in each numerical rating and grade in each numerical rating respectively. Representative soil samples were collected from selected within the canopy, at 0.5 m away from tree trunk and at a depth of 30 cm, following standard procedures procedure during December 2024 for estimation of different nutrients in order to know the soil nutrient status of the orchard soils. Collected soil samples were brought to the laboratory and dried under shade by spreading on white glared sheets and covered with white coloured paper for avoiding contamination with the extraneous material. After drying a part of each sample meant for micronutrient analysis was ground in wooden mortar and pestle and preserved in polythene bag with proper labelling. The precautions outlined by Jackson (1973) [9] were followed in order to avoid contamination. Similarly, other part of each sample was ground with porcelain mortar pestle passed through 0.5 mm sieve and stored in polythene bags with proper labelling for subsequent (analysis) estimation of physicochemical characteristics.

Method of soil analysis: Water holding capacity, bulk density, pH, Electrical Conductivity, Organic Carbon, Calcium Carbonate and Micronutrients like Fe, Cu, Mn and Zn are these details of procedure adopted and references are given below in following table:

Table 2: Soil Nutrient parameters and their methods of analysis

Sr. No.	Particulars/Nutrient	Methods	References
1	WHC	Keen-boxes	(keen and Rackowski, 1921)
2	Bulk Density	Clod coating	(Black and hartage,1986)
3	pH	1:2:5 (Soil:Water)	Jackson (1973)
4	EC	1:2:5 (Soil:Water)	Jackson (1973)
5	Organic Carbon	Wet Digestion	Jackson (1973)
6	$CaCO_3$	Rapid titration	Piper (1966)
7	DTPA Fe, Cu, Mn, Zn	AAS Machine	Lindsay&Norvell (1978)

Plant analysis: The leaf sampling was done from the twigs of current season growth at index 4th leaf from die apex in April 2024-25 as per study with sample size at 50 leaves per samples (Bhargava and Dhandar, 1987) [1]. The collected leaf samples were brought to the laboratory. The samples were air dried on perfectly clean surface at room temperature for 2-3 days in dust free atmosphere free from any kind of contaminants. Samples were placed in hot air oven at 60 °C for 48 hrs and grinded in an electric stainless-steel mill using 0.5 mm sieve. Then the samples were placed in oven to dry for few hours more till constant weight and stored in plastic jars for analysis.: Fine powdered plant sample (0.5 gram) was taken in 100 ml conical flask, 4 ml of concentrated nitric acid was added to it and kept for overnight. On next day, 9 ml of diacid mixture (HNO_3 and $HClO_4$, 9:4) was added and digested on hot plate as described by Piper (1966) [16].

Methods of plant analysis: The plant samples were used for estimation of Iron, Copper, Manganese and Zinc. These details of procedure adopted and references are given below in following table;

Table 3: Plant Nutrient parameters and their methods of analysis

Nutrient	Method	Reference
Fe, Cu, Mn, Zn	Atomic Absorption Spectrophotometer	Piper (1966) [16]

Statistical analysis

Coefficient of correlation (r) between nutrients disorders on the one hand & soil chemical properties and leaf nutrient concentration on other at healthy and infected plant respectively. ("Statistical Methods for Agricultural Workers" by Panse and Sukhatme 1985) [15].

Results

An extensive survey was conducted during November and December 2024 in the Chh. Sambhajinagar district of the Marathwada region to assess the status of citrus greening in sweet orange orchards. Disease incidence across surveyed orchards ranged from 35.87% to 54.41% with an average incidence of 43.98%. The highest incidence was recorded in Paithan village (54.41%) while the lowest was observed in Karajkheda village (35.87%). These findings are consistent with the observations reported by Jagtap (2011) [10], who also documented significant variation in citrus greening prevalence across orchards within the same region.

The Soil physicochemical properties (WHC, BD, pH, EC, OC, $CaCO_3$) varied between healthy and diseased orchards indicating that orchards should be well managed in terms of soil physical and chemical properties in unhealthy orchards. The soil micronutrient levels like Cu and Mn were found to sufficiency range across both healthy and diseased orchards indicating that orchards well managed in terms of Cu and Mn.In case of micronutrient like Fe and Zn found to be within sufficiency range across healthy orchards but in unhealthy orchards had found deficiency of Fe and Zn indicating that orchards should be well managed in terms of soil fertility. However, Similar trend is seen in plant analysis also sufficiency range of micronutrient in healthy trees and deficiency found in unhealthy orchards despite clear differences emerged in leaf nutrient content between healthy and diseased trees. Leaves of diseased trees showed lower levels of of Iron, Copper, Manganese and Zinc when compared to healthy trees.

The correlation analysis confirmed these observations.That the soil pH is positively correlated with disease incidence (r = 0.894**) and disease severity (r = 0.932**) whereas soil Fe is negatively correlated with disease incidence (r =-0.568**) and disease severity (r =-0.525**) and soil Zn is negatively correlated with disease incidence (r =-0.815**) and disease severity (r =-0.834**). Similar trend follows in tissue analysis also where Iron is negatively correlated with disease incidence (r =-0.441**) and disease severity (r =-0.402**), Cu is negatively correlated with disease incidence (r =-0.408**) and disease severity (r =-0.385**), Mn is negatively correlated with disease incidence (r = 0.494**) and disease severity (r = 0.466**) and Zn is negatively correlated with disease incidence (r =-0.392**) and disease severity (r =-0.363**)

Similar findings were reported by Dong *et al*. (2021) [4], who demonstrated that nutrient imbalance in soil, particularly Iron and Zinc and nutrient imbalance in plant, particularly of Iron, Copper, Manganese and Zinc shows citrus greening severity in citrus orchards of China. our results demonstrate similar trends in Marathwada orchards. Likewise, Pustika *et al*. (2008) [17] noted that deficiency of essential micronutrients predisposes citrus plants to stronger HLB symptom expression. These results strengthen the view that nutrient imbalance significantly influences disease progression On the other hand, Gottwald *et al*. (2012) [8] observed that nutrient management alone had inconsequential effects on HLB control in Florida citrus orchards. The present findings partly contrast with their study, suggesting that while nutrient management may not cure the disease it can certainly modulate symptom severity and plant tolerance in affected regions.

Overall, the study establishes that higher soil pH levels, in conjunction with deficiencies of soil Iron and Zinc and deficiencies of plant of Iron, Copper, Manganese and Zinc are strongly associated with citrus greening incidence and severity. This indicates that nutrient imbalance acts as a secondary factor aggravating the disease and maintaining optimum nutrient balance could play an important role in integrated management strategies for citrus greening.

Table 4: Citrus greening disease incidence and severity in sweet orange orchards

Village & Orchards No.	Taluka	DI (%)	DSI (%)
Paithan-1	Paithan	49.51	47.28
Paithan-2	Paithan	47.99	46.04
Paithan-3	Paithan	42.88	41.24
Paithan-4	Paithan	37.88	34.95
Paithan-5	Paithan	41.23	38.61
Paithan-6	Paithan	51.44	48.70
Paithan-7	Paithan	38.12	36.97
Paithan-8	Paithan	50.88	47.96
Paithan-9	Paithan	36.22	34.93
Paithan-10	Paithan	43.45	41.29
Paithan-11	Paithan	48.23	47.27
Paithan-12	Paithan	44.55	41.55
Mean		44.36	42.23
Range		36.22-51.44	34.93-48.7
Dadegaon-1	Paithan	36.32	34.93
Dadegaon-2	Paithan	45.23	42.07
Dadegaon-3	Paithan	38.66	37.17
Dadegaon-4	Paithan	40.21	37.97
Dadegaon-5	Paithan	41.33	40.04
Dadegaon-6	Paithan	44.83	42.03
Dadegaon-7	Paithan	52.55	50.04
Dadegaon-8	Paithan	42.33	40.95
Mean		42.68	40.65
Range		36.32-52.55	34.93-50.04
Vadwali-1	Paithan	39.45	37.28
Vadwali-2	Paithan	50.32	47.31
Vadwali-3	Paithan	41.44	40.04
Vadwali-4	Paithan	38.01	35.39
Mean		42.30	40.0
Range		38.01-50.32	35.39-50.04
Karajkheda	Paithan	43.12	41.28
Karajkheda	Paithan	47.32	45.51
Mean		45.22	43.39
Range		43.12-47.32	41.28-45.51
Akhatwada-1	Paithan	35.87	34.57
Akhatwada-2	Paithan	41.88	40.50
Mean		38.87	37.53
Range		35.87-41.88	34.57-40.5
Harshi-1	Paithan	54.41	51.04
Harshi-2	Paithan	51.55	49.27
Mean		50.15	49.87
Range		49.27-51.04	48.99-50.76

Table 5: Comparison between soil nutrient content of healthy and unhealthy orchards

Nutrient	Healthy		Unhealthy	
	Range	Mean	Range	Mean
Fe	4.1-6.39	5.32	2.12-7.07	3.84
Cu	1.01-1.8	1.26	0.96-2.20	1.37
Mn	4.44-6.71	5.73	4.56-6.80	5.85
Zn	1.1-1.97	1.52	0.22-1.22	0.72

Table 6: Comparison between leaf nutrient content of healthy and unhealthy trees

Nutrient	Healthy		Unhealthy		Optimum
	Range	Mean	Range	Mean	Range
Fe	102.05-180.70	151.47	71.50-96.23	84.09	99.46-182.23
Cu	4.37-7.13	5.79	3.01-3.28	2.85	3.41-7.42
Mn	19.89-40.30	32.92	10.73-26.07	18.44	26.88-40.64
Zn	14.21-22.10	17.42	10.05-13.98	12.21	14.3-22.31

Optimum range given by Kausadikar (2005)

Table 7: Correlation of soil parameters with disease incidence and disease severity Index

Parameters	Disease incidence	Disease severity index
Soil pH	(r = 0.894**)	(r = 0.932**)
DTPA Iron	(r = - 0.568**)	(r = -0.525**)
DTPA Zinc	(r = -0.815**)	(r = -0.834**)

Table 8: Correlation of leaf nutrient with disease incidence and disease severity index

Leaf nutrient	Disease incidence	Disease severity index
Fe	(r = - 0.441**)	(r = - 0.402**)
Cu	(r = - 0.408**)	(r = - 0.385**)
Mn	(r = - 0.494**)	(r = - 0.466**)
Zn	(r = - 0.392**)	(r = - 0.363**)

* values are different from 0 with significant levels p=0.05.
**values are different from 0 with significant levels p=0.01.

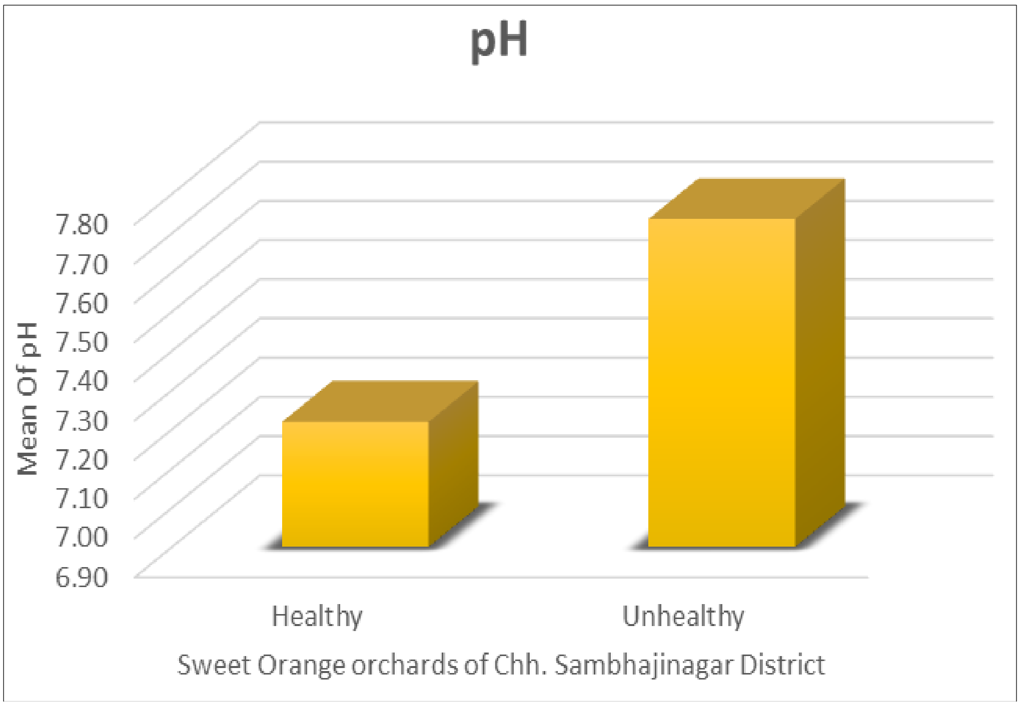

Fig 1: Soil pH comparison between healthy and unhealthy sweet orange orchards

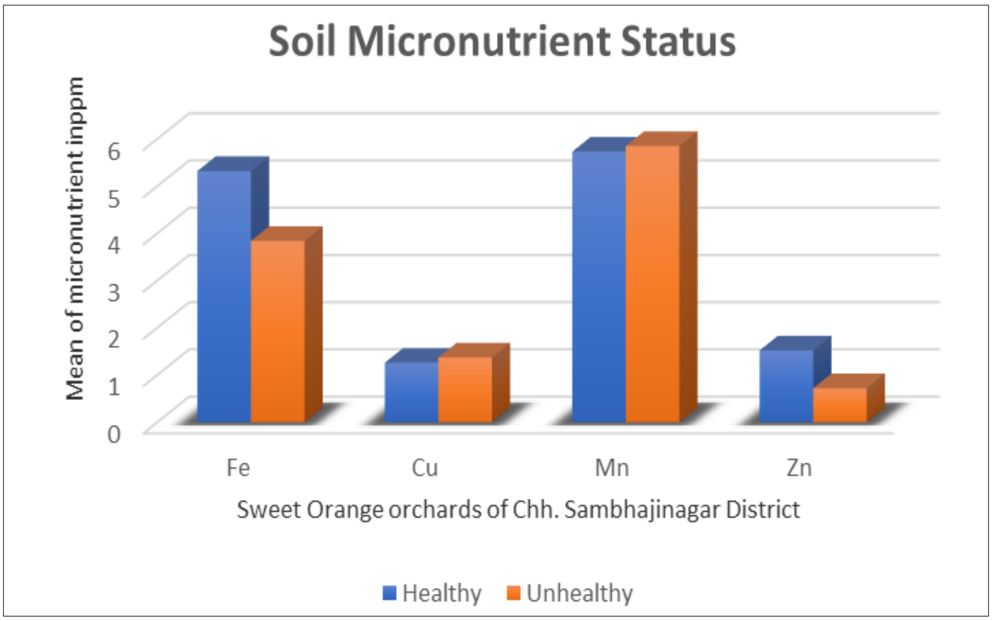

Fig 2: Soil Micronutrients Status between healthy and unhealthy sweet orange orchards

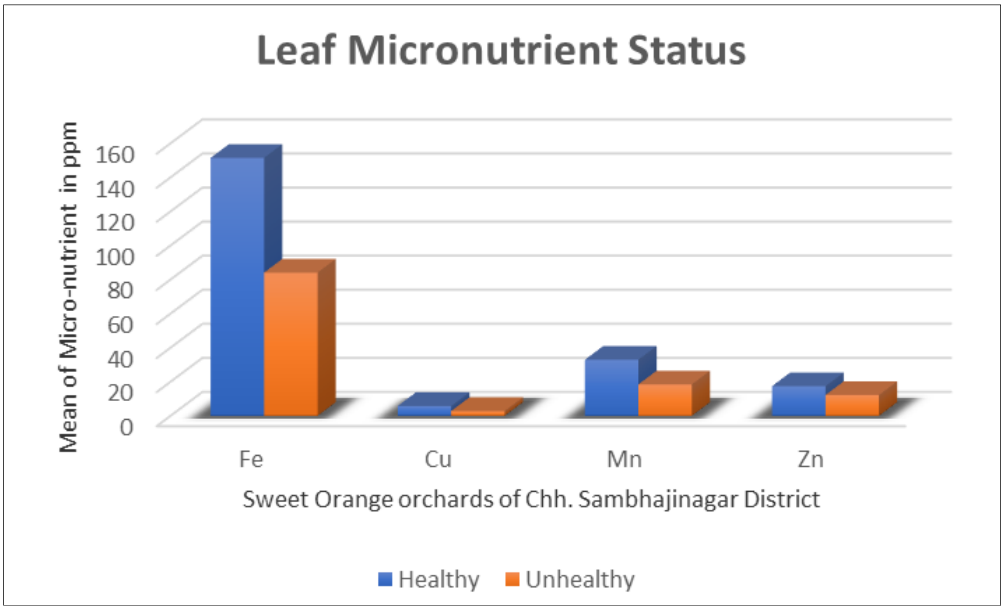

Fig 3: Leaf Micronutrients Status between healthy and unhealthy sweet orange orchards

Conclusion

There was a significant variation in the DTPA micronutrient like Fe and Zn content across soil samples from unhealthy sweet orange orchards in the Chh. Sambhajinagar district. The levels of Copper (Cu), Manganese (Mn) were within the sufficiency range in all orchard soils. However, higher soil pH levels and lower concentrations of of Iron, Copper, Manganese and Zinc in leaf tissues may be responsible for the incidence and severity of citrus greening disease in sweet orange trees.

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