

Relationship Between Mineral Contents and Canopy Reflectance in Washington Navel Orange Trees (*Citrus sinensis* L. Osbeck)

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The aim of this study is to determine the relationship between mineral contents of Washington Navel (*Citrus sinensis* L. Osbeck) orange trees of different ages and canopy reflectance (RF) of electromagnetic spectrum (EMS) in visible near-infrared region (VNIR). With this aim, nutrients analysis was conducted on orange trees at the ages of 15, 20, 25 and 30 years, which have similar soil properties and similar management techniques. Some spectroradiometric measurements were also carried out. According to the results, the research indicated some important statistical relationships between the RF and the contents of N, P, Mg, Fe, Cu and Zn and also between the normalized difference vegetation index (NDVI) values and the contents of those nutrients in orange trees of different ages. In the study, the most significant relationship appeared in the 25 year old trees. Results show the following relationships in the 25 year old trees; negative between red band and N at $p < 0.001$ level, positive between red band and P at $p < 0.05$ level, negative between Mg and blue and red bands at $p < 0.05$ and $p < 0.001$ level, positive between Cu and near-infrared bands at $p < 0.05$ level. In this study, it is determined that there is a positive relationship at $p < 0.01$ level between the NDVI and N content and positive relationships at $p < 0.05$ level between NDVI and P, Fe, Cu, Zn contents of 25 year old trees. According to these results, the outcome is that it is more appropriate to prefer 25 year old trees during the studies of remote sensing to measure the contents of nutrients in Washington Navel trees of different ages in the Mediterranean climatic zone.

Key Words: Washington navel, Nutrient contents, Canopy reflectance, Normalized difference vegetation index.

INTRODUCTION

Citrus cultivation in Turkey varies in different ecological regions. *Citrus* plants are commonly grown in the Mediterranean and Aegean regions.

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Mediterranean region produces 70-80 % of Turkey's supply of *citrus*. Antalya, one of the major cultivation areas of *Citrus*, makes up the 25 % of the regional production. The separation of the *citrus* plants in the area is as follows: orange with 78 %, lemon with 14 %, tangerine with 7 % and bitter orange and grapefruit with 2 %. Among the orange types grown in the region, Washington Navel (*Citrus sinensis* L. Osbeck) is the most common one with 75 %.

Nutrient elements are the most limiting factors in production of *citrus* in Mediterranean Region. Studies conducted on citrus plant in the Mediterranean region revealed macro and micro nutrient deficiencies. In a study conducted in citrus orchards of Western Mediterranean region, Yalçın *et al.*¹ found out that there was lack of N with 41 %, P with 38 %, K with 37 %, Mg with 29 %, Zn with 91 % and Mn with 84 %. Similar studies throughout the area revealed that there are serious nutritional problems. This results in loss of yield and product quality^{2,3}. However, current methods for estimating the amount of nutrient elements available to growing crops include soil sampling or in-season plant sampling, both of which can be costly and labor intensive. The use of remote sensing techniques to estimate nutrient status could decrease the amount of labour needed for sampling and could reduce the cost associated with sampling and analysis⁴.

Remote sensing is simply obtaining information about an object, area or phenomenon by analyzing data acquired by a device that is not in contact with the object, area or phenomenon⁵. Recently, researchers have evaluated remote sensing techniques for estimating the nutrient status of growing crops by determining the appropriate wavelength or combination of wavelengths to characterize crop nutrient deficiency. Blackmer *et al.*⁶ reported that reflected radiation near 550 and 710 nm was better for detecting N deficiencies compared with reflectance at other wavelengths. Masoni *et al.*⁷ found that Fe, S, Mg and Mn deficiencies decreased absorption and increased reflectance and transmittance in corn, wheat, barley and sunflower leaves. They also noted that mineral deficiencies affected leaf concentration of other elements in addition to the deficient element, with nutrient concentration varying according to species and deficiency level.

Destructive tissue testing is a common way to assess crop nutrient status. Non-destructive methods have been developed to monitor crop nutrient status. Blackmer and Schepers⁸ found that the chlorophyll-meter was a useful method of monitoring corn N status, compared with measuring leaf N concentration, which requires destructive sampling. While the chlorophyll meter is a good indicator of in-season N status, the technique requires time and labour for data collection. The use of remote sensing could help for extensive field sampling while still providing a good detection of deficiencies.

In plants, in visible near-infrared (VNIR) area of EMS, some changes at spectral reflectance's occur in relation with the age and the growth period. Researches indicate that these changes vary according to plant types and these variables come from the biochemical concentrations, activity of internal-external plant structures and the accumulation of biomass⁹⁻¹¹. Sari *et al.*¹² reported that chlorophyll contents of leaves of orange trees at different age, growth and development stage varied and this variation was statistically significant at blue (420-520 nm) and red (630-690 nm) bands.

In this study, the objective is to determine the relationships between RF in the area of visible near-infrared of EMS and the mineral contents of Washington Navel (*Citrus sinensis* L. Osbeck) orange trees at different ages, which have been commonly cultivated in Mediterranean region.

EXPERIMENTAL

This research was conducted on Aksu River plain near Antalya city (in Mediterranean region) of Turkey (30°53' E, 36°52' N). Four experimental plots of Washington navel (*Citrus sinensis* L. Osbeck) trees were selected from the orchard of the Agricultural Research Institute on the plain. Each plot consisted of randomly selected trees. The ages of the orange trees grown in each plot were 15, 20, 25 and 35 year old. Orange trees growing in each plot had similar soil types (typic xerofluvent), according to the Soil Survey Staff¹³ and agricultural management. All spectral measurements and nutrient analysis were carried out on these trees.

Canopy reflectance (RF) measurements: Canopy reflectance (RF) measurement at 4 different wavelengths (three visible and one near-infrared) was made for 10 trees grown in each plot. Canopy reflectance measurements were assessed on each tree from each orchard under cloudless conditions between 09.30 and 10.30 local standard time. Sari *et al.*¹⁴ reported that, in remote sensing studies on Washington navel (*Citrus sinensis* L. Osbeck) grown in Mediterranean region, the most appropriate time is the fruit setting period. In this study RF measurements were taken during the fruit setting periods of the trees.

Canopy reflectance measurements in the range of 450-900 nm were made using a portable spectroradiometer (Model 100 AX Exotech Inc., USA) which was set to four spectral wavelength bands. Wavelength of the bands termed as CH1 (blue band), CH2 (green band), CH3 (red band) and CH4 (near- infrared band, NIR) were 420-520, 520-600, 630-690 and 760-900 nm, respectively. Calibrations of the sunlight and RF measurements for each orange tree at each orchard were made using the standard BaSO₄ panels according to Jackson *et al.*¹⁵ and Zorca-Tejada *et al.*¹⁶. The spectroradiometer had a 15-degree field-of-view (FOV) and was placed 2 m above (*ca.* 0.20-0.25 m² canopy view) each of randomly selected individual

ten canopies from each plot^{17,18}. All RF measurements were acquired in clear and open air condition.

The normalized difference vegetation index was also evaluated to determine the relationship between age-dependent nutrient contents and canopy reflectance of Washington Navel (*Citrus sinensis* L. Osbeck) trees at fruit setting stages. The NDVI was proposed to evaluate crop stress by many researches such as Penuelas *et al.*¹⁹ and Korobov and Railyan²⁰. The NDVI is calculated from the reflected solar radiation in the near-infrared (CH4) and red (CH3) wavelength bands via the formula: $NDVI = (CH4 - CH3) / (CH4 + CH3)$ ²¹.

Chemical and statistical analysis: Soil samples were taken from each orange orchard and then analyzed for some physical and chemical properties after they had been air-dried and passed through 2 mm sieve. Some physical and chemical properties of soil samples were given in Table-1. For the soil samples, pH was determined in a 1:2.5 ratio soil: water suspension. Total carbonates were determined according to the calcimeter method of Nelson²². Electrical conductivity was determined according to Rhoades²³. Soil texture was determined by hydrometer method²⁴ and organic matter by the Walkley-Black²⁵. Extractable P content was extracted by NaHCO₃²⁶ and determined by a molybdate colorimetric method²⁷; extractable K, Ca and Mg were extracted with NH₄-OAc and determined by atomic absorption spectrophotometer (AAS)²⁶. Soil Cu, Fe, Mn and Zn were extracted with diethylene tetraamine pentaacetic acid (DTPA)²⁸ and then determined by AAS.

Leaf samples of orange trees were collected as described by Chapman²⁹ in the fruit setting period that was the most suitable growth and development stage for passive remote sensing techniques as suggested by Sari *et al.*¹⁴. Leaf samples were collected on the day when spectral reflectance measurements were made. For the nutrient elements analysis, a total of 120 leaf samples (10 trees from each plot, 3 replicate and 4 different age) were collected. Leaf samples were washed by distilled water and dried in a forced air oven at 65 °C to a constant weight. Leaf samples were ground separately in a stainless mill to pass through a 20 mesh screen and kept in clean polyethylene bags for analysis. Dried leaf samples of 0.5 g each were digested with 10 mL HNO₃/HClO₄ (4:1) acid mixture on a hot plate. The samples were then heated until a clear solution was obtained. The same procedure was repeated several times. The samples were filtered and diluted to 100 mL with distilled water. Concentrations of K, Ca, Mg, Fe, Zn, Mn and Cu in the digestates were determined by using AAS³⁰. Phosphorus was measured by spectrophotometer³¹ and N was determined by a modified Kjeldahl procedure³⁰.

Correlation coefficients between mineral contents and reflectance data of orange trees at different ages were calculated using a computer program Minitab Win Version 13.

TABLE-1
SOME PHYSICAL AND CHEMICAL PROPERTIES OF THE SOIL SAMPLES TAKEN FROM EACH ORANGE ORCHARD

Orchard no.	Depth (cm)	pH	CaCO ₃ (%)	EC (dS m ⁻¹)	Org. mat. (%)	% Clay			Silt	Text.	N (%)	P (mg kg ⁻¹)				Mg 100 ⁻¹			
						Sand	Clay	Silt				K	Ca	Mg	Fe	Mn	Zn	Cu	
1	0-30	7.50	20.63	1.72	2.60	12.44	37.92	49.64	SiCL	0.14	13.93	0.42	46.95	4.56	11.24	19.06	0.28	6.37	
	30-60	7.83	28.05	1.70	2.00	12.44	41.92	45.64	SiC	0.08	6.32	0.24	41.67	5.34	8.88	12.54	0.12	2.22	
2	0-30	7.80	24.00	1.68	1.80	10.44	45.92	43.64	SiC	0.09	17.91	0.35	48.22	5.41	8.88	14.58	0.50	3.78	
	30-60	7.88	26.10	1.62	1.40	14.44	43.92	41.64	SiC	0.07	11.49	0.29	56.74	6.50	7.90	11.76	0.22	2.11	
3	0-30	7.67	18.63	1.62	2.40	30.44	35.92	33.64	CL	0.16	28.35	0.35	54.49	3.08	11.21	16.32	0.10	2.38	
	30-60	7.60	18.75	1.58	1.60	28.44	33.92	37.64	CL	0.07	6.16	0.16	47.17	4.82	11.76	15.36	0.94	2.89	
4	0-30	7.56	23.63	1.72	2.50	20.44	35.92	43.64	CL	0.14	11.47	0.49	34.30	3.22	12.96	20.46	0.54	2.34	
	30-60	7.60	27.45	1.62	1.30	20.44	33.92	45.64	CL	0.07	6.12	0.28	39.47	4.22	11.18	16.54	0.18	2.29	

1: 15 year-old orchard; 2: 20 year-old orchard; 3: 25 year-old orchard; 4: 35 year-old orchard

TABLE-2
MINIMUM, MAXIMUM AND MEAN VALUES OF NUTRIENT ELEMENTS, CANOPY REFLECTANCE AT THE VISIBLE NEAR-INFRARED REGIONS (CH1, CH2, CH3 CH4 BANDS) AND NDVI VALUES

Variable	15 Year old			20 Year old			25 Year old			35 Year old		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
N (%)	2.20	2.80	2.48	2.10	2.70	2.40	2.10	2.70	2.42	1.90	2.30	2.14
P (mg kg ⁻¹)	0.11	0.17	0.15	0.10	0.18	0.12	0.08	0.14	0.12	0.10	0.15	0.12
K (%)	0.68	0.94	0.79	0.73	1.00	0.87	0.59	0.97	0.73	0.89	1.23	1.03
Ca (%)	4.55	4.97	4.75	3.70	4.35	4.05	4.10	4.85	4.56	4.25	5.22	4.74
Mg (%)	0.35	0.49	0.41	0.37	0.49	0.44	0.35	0.54	0.46	0.34	0.44	0.38
Fe (mg kg ⁻¹)	82.00	129.00	103.90	71.00	117.00	89.30	46.00	104.00	88.30	76.00	172.00	117.50
Cu (mg kg ⁻¹)	22.00	31.50	26.14	15.50	19.20	17.38	11.50	25.20	19.30	46.60	92.20	67.29
Zn (mg kg ⁻¹)	16.30	21.90	19.58	8.10	16.20	11.79	6.80	10.80	9.51	13.20	21.30	15.13
Mn (mg kg ⁻¹)	22.70	29.80	24.96	14.10	21.30	18.25	16.50	22.50	19.84	17.00	31.20	20.88
Ref CH1	30.24	45.36	37.37	22.68	44.28	34.78	20.52	43.20	31.97	12.96	27.00	20.30
Ref CH2	42.26	73.38	59.82	56.35	79.25	69.32	48.72	70.44	61.93	37.57	73.96	50.19
Ref CH3	24.96	41.92	33.72	26.85	36.27	33.06	25.43	40.04	31.56	17.90	30.14	23.50
Ref CH4	383.40	573.82	487.64	436.22	661.58	571.69	331.85	614.29	500.04	345.91	536.76	435.80
NDVI	0.80	0.90	0.87	0.85	0.92	0.89	0.78	0.92	0.88	0.85	0.93	0.90

RESULTS AND DISCUSSION

The minimum, maximum and mean values of nutrient elements (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu), the canopy reflectance values at the wavelength bands (CH1, CH2, CH3 and CH4) and NDVI were presented in Table-2.

As presented in Table-2, although similar cultivation techniques have been applied, the mineral contents and canopy reflectance data of trees at different ages show some changes during the fruit setting period due to the plant physiology. This situation shows that Washington Navel trees use the energy coming from electromagnetic spectrum actively. Ericson³² indicated that orange trees go through some physiological activities during the growth period that is called 'fruit setting period'. Similarly, in a study conducted on orange and lemon blocks of bitter orange origin at the ages of 15-25, Edwards and Wheaton³³ reported that the trees used the maximum spectral energy in June-Fruit Setting period. Also, the trees at different ages used and direct the energy of EMS in the area of VNIR (CH1, CH2, CH3 and CH4 wavelength bands) actively during the same period depending on leaf chlorophyll content and age-related physiological activities.

Understanding the spectral features of Washington Navel (*Citrus sinensis* L. Osbeck) orchard is important to monitor the seasonal canopy properties and biochemical changes in plantations. Similar to spectral reflectance of typical living plants^{34,35}, the whole different age orange trees in the present study showed lower reflectance values at CH1 and CH3 wavelength bands and significantly higher reflectance values at the CH2 and CH4 wavelength bands in the fruit setting stage (Fig. 1).

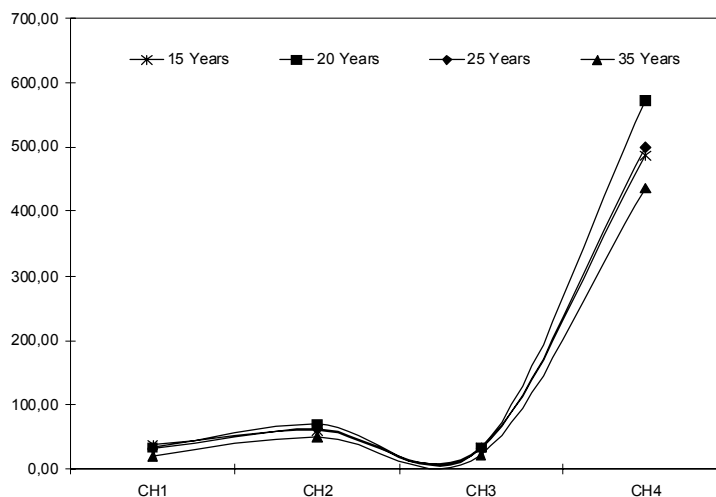


Fig. 1. Canopy reflectance graphics of Washington Navel orange trees

As presented in Fig. 1, Washington Navel (*Citrus sinensis* L. Osbeck) trees at different ages absorbed the energy through the chlorophyll pigments at the CH1 and CH3 wavelength bands, which are the chlorophyll absorption area of EMS. At the near infrared (CH4) wavelength band; however, they reflected considerable amounts of energy due to the structural features and stress conditions. Rees³⁴ reported that there are significant relationships among chlorophyll content, water content and health of plant in the area of visible and especially near infrared wavelength of EMS and various physiological features of the plant can be measured in that way. In a study on wollypod vetch plant, Basayigit and Albayrak³⁶ reported that serious relationships exist between VNIR area of EMS and doses of fertilizers with different amounts of N, P and K.

The linear relationships between RF and mineral contents of orange trees in the four different age groups in the fruit setting stage were investigated. The results at this analysis were presented in Table-3.

As seen in Table-3, between the RF of Washington Navel (*Citrus sinensis* L. Osbeck) trees at different ages in the VNIR area of EMS, NDVI values and mineral contents (N, P, Mg, Fe, Cu, Zn and Mn) significant relationships were found. However, no statistical relationship could be recorded between K and Ca contents of the trees and the any wavelength area of EMS.

N and Fe are the crucial elements needed in the emergence of chlorophyll pigments. The most important function of P is to form the pyrophosphate bonds, which make the energy transfer possible. With this active function, phosphorus plays a significant role in the photosynthesis³⁷. There exists a linear relationship between any increase in N and Fe contents and chlorophyll content³⁸. Some researchers such as Gilbert *et al.*³⁹, Volterrani *et al.*⁴⁰ and Daughtry and Wathall⁴¹ claimed that CH1 and CH3 wavelength bands of EMS is the chlorophyll absorption area and that spectral reflectance declines. This situation, as reported by the researchers, explains the negative relationships between N content and spectral reflectance in CH1 and CH3 wavelength bands ($p < 0.01$, $p < 0.001$) at 20 year old trees and in CH3 wavelength band ($p < 0.001$, $p < 0.001$ and $p < 0.05$, respectively) at 15, 25 and 35 year old trees. Similarly, negative relationships have been found between Fe content and RF in CH3 wavelength band at $p < 0.05$ level at 15 and 35 year old trees. Between P content and RF in CH1 and CH3 wavelength bands at 15-20-35-year-old trees, no statistical relationship was observed. However, between P content and RF in CH3 band of EMS at 25-year-old trees a negative relationship at $p < 0.05$ levels were detected (Table-3).

In relation with the age and the health of the plant, chlorophyll content of the leaves may increase. This increase is an indirect sign of intensive photosynthesis activity and health of the plant. As a result, a healthy plant

TABLE-3
CORRELATION COEFFICIENTS BETWEEN NUTRIENT ELEMENTS AND CANOPY REFLECTANCE

Orchd. Ages	Reflect (W/m ²)	N (%)	P (mg kg ⁻¹)	%				(mg kg ⁻¹)			
				K	Ca	Mg		Fe	Cu	Zn	Mn
15	Ref CH1	-0.520ns	-0.479 ns	-0.495 ns	-0.017 ns	-0.496 ns	-0.192 ns	0.033 ns	0.145 ns	-0.012 ns	
	Ref CH2	0.307 ns	0.466 ns	0.430 ns	0.421 ns	0.370 ns	0.147 ns	0.406 ns	0.305 ns	0.446 ns	
	Ref CH3	-0.932***	-0.618 ns	-0.432 ns	-0.131 ns	-0.602 ns	-0.656*	0.368 ns	0.256 ns	-0.269 ns	
	Ref CH4	0.537 ns	0.596 ns	0.328 ns	0.025 ns	0.367 ns	0.406 ns	0.673*	0.497 ns	0.482 ns	
	NDVI	0.833**	0.730*	0.407 ns	0.032 ns	0.536 ns	0.639*	0.637*	0.060 ns	0.450 ns	
20	Ref CH1	-0.777**	-0.491 ns	-0.094 ns	-0.164 ns	-0.528 ns	-0.132 ns	0.307 ns	0.094 ns	-0.182 ns	
	Ref CH2	0.310 ns	0.077 ns	0.129 ns	0.373 ns	0.761*	0.088 ns	0.107 ns	0.319 ns	0.078 ns	
	Ref CH3	-0.896***	-0.531 ns	-0.026 ns	-0.091 ns	-0.491 ns	-0.181 ns	0.424 ns	0.066 ns	-0.375 ns	
	Ref CH4	0.672*	0.208 ns	0.197 ns	0.187 ns	0.593 ns	0.088 ns	0.056 ns	0.297 ns	0.195 ns	
	NDVI	0.792**	0.279 ns	0.203 ns	0.250 ns	0.620 ns	0.016 ns	0.241 ns	0.314 ns	0.293 ns	
25	Ref CH1	-0.465 ns	-0.093 ns	-0.202 ns	-0.042 ns	-0.703*	-0.321 ns	0.166 ns	0.227 ns	-0.457 ns	
	Ref CH2	0.406 ns	0.416 ns	0.203 ns	0.118 ns	0.312 ns	0.129 ns	0.127 ns	0.364 ns	0.308 ns	
	Ref CH3	-0.910***	-0.705*	-0.269 ns	-0.280 ns	-0.873***	-0.522 ns	0.510 ns	0.445 ns	-0.168 ns	
	Ref CH4	0.630 ns	0.501 ns	0.352 ns	0.546 ns	0.193 ns	0.458 ns	0.643*	0.581 ns	0.354 ns	
	NDVI	0.837**	0.746*	0.354 ns	0.570 ns	0.531 ns	0.648*	0.736*	0.676*	0.292 ns	
35	Ref CH1	-0.375 ns	-0.379 ns	-0.586 ns	-0.431 ns	-0.627 ns	-0.233 ns	0.511 ns	0.029 ns	-0.290 ns	
	Ref CH2	0.074 ns	0.011 ns	0.162 ns	0.286 ns	0.413 ns	0.058 ns	0.057 ns	0.532 ns	0.058 ns	
	Ref CH3	-0.682*	-0.610 ns	-0.514 ns	-0.345 ns	-0.595 ns	-0.634*	0.762*	0.400 ns	-0.508 ns	
	Ref CH4	0.219 ns	0.811**	0.249 ns	0.445 ns	0.403 ns	0.424 ns	0.604 ns	0.383 ns	0.744*	
	NDVI	0.550 ns	0.761*	0.249 ns	0.052 ns	0.615 ns	0.680*	0.806**	0.378 ns	0.630 ns	

***Significant at the 0.1 % level; **Significant at the 1 % level; *Significant at the 5 % level; ns = Non significant.

gives high reflectance data in the CH4 wavelength band of the EMS. According to the research findings, among the relationships between spectral reflectance in the CH4 wavelength band of the EMS and N, P and Fe contents, which play a crucial role in the formation of chlorophyll pigments and the energy transfer, no relationship with Fe was detected. Meanwhile, only at 20 year old trees with N content ($p < 0.05$) and at 35 year old trees with P content ($p < 0.05$) significant positive relationships were detected. The fact that there's no relationship for the CH4 wavelength band in the 15-25-year old trees may be due to differences in their physiological structures. However, the present study could not reveal a clear answer.

The most widely used vegetation index applied to optical sensor data has been the normalized difference vegetation index. This index relies heavily on the spectral contrast between CH4 and CH3 wavelength bands of the spectrum. Normalized difference vegetation index (NDVI) is defined as the difference between the reflectances in the CH4 and CH3 regions of the spectrum normalized to the sum of these reflectance's⁴².

The energy reflected by the plant in CH3 and CH4 wavelength bands of EMS directly affects NDVI values. Related to with biomass, water content, thickness, chlorophyll content and leaf pigment concentration, energy absorption of the plant in CH3 wavelength band of EMS and high reflectance of the energy in CH4 wavelength band cause some changes in NDVI values. Because of this reason, there is a positive relationship between NDVI and the elements essential for the formation of chlorophyll. According to the research findings, except for the 35-year-old trees, in the trees at the ages of 15, 20, 25 a positive relationship between N content and NDVI values at $p < 0.01$ level was observed. Between NDVI and P content in the trees at the ages of 15, 25 and 35 years, there is a positive relationship at $p < 0.05$ level. Similarly, between NDVI and Fe content in the trees of 15, 25 and 35 years, a positive relationship at $p < 0.05$ level was determined. These results are in agreement with the literatures^{43,44}.

Another nutrient element, which is expected to have an important statistical relationship with the mineral contents of the orange trees and the reflectance data in VNIR area of EMS, is magnesium. Magnesium is the central atom of chlorophyll and it is directly related to the chlorophyll content of plant³⁸. In the study, no relationship with Mg content in the 15 and 35 year old trees was obtained. On the other hand, in 20 year old trees, between the spectral reflectance in CH2 wavelength band of EMS and Mg content a positive relationship at $p < 0.05$ level was found. Also, in the 25-year-old trees, between spectral reflectance in CH1 and CH3 wavelength bands, which are chlorophyll absorption bands and Mg content, negative relationships at the levels of $p < 0.05$ and $p < 0.001$, respectively, were detected. In plants having high chlorophyll content, considerable increases

in the reflectance of CH2 wavelength band of EMS were reported³⁴. This report is supported by the positive relationship between spectral reflectance in CH2 wavelength band at 20 year old trees and Mg content and the negative one between Mg content and spectral reflectance's in CH1 and CH3 wavelength bands in 25 year old trees. These observations were in agreement with the previous studies conducted on different plant species⁴⁵⁻⁴⁸. However, the fact that no relationship has been found in VNIR area of EMS in the trees at other ages may be due to some processes taking place in plant physiological structures. Therefore, more detailed studies on trees at these ages are needed.

In the present study, a positive statistical relationship between Cu content in the 15 and 25-year old trees and spectral reflectance in CH4 wavelength band at $p < 0.05$ level was determined. Also there is a positive relationship between Cu content in 35 year old trees and spectral reflectance in CH3 wavelength band with the level of $p < 0.05$. When the relationship between NDVI values and Cu content is evaluated, except for 20 year old trees, statistically positive relationships in the trees at the ages of 15 and 25 at $p < 0.05$ level and in 35 year old trees at $p < 0.01$ level were recorded. Cu plays an essential role in the chlorophyll stability and synthesis and also has effects on carbohydrate and protein metabolism in plants³⁷. These statistical relationships between spectral reflectance in CH4 wavelength band of EMS and NDVI value and Cu content of orange trees indicates that Cu deficiency be detected by using the remote sensing techniques and technologies. Positive relationships found between spectral reflectance in CH4 wavelength band of EMS, NDVI and Cu content of Washington Navel trees support these findings⁴².

Zinc is associated with nitrogen metabolism in plants. Zn has also some effects on photosynthesis activity. In case of zinc deficiency, protein synthesis and protein concentration, which is one of the most important parameters for fertility change on a large scale. Therefore, Zn is a highly valuable element in plant growth⁴⁹. According to the data obtained in the study, however, only in 25 year old trees, there is a positive relationship at $p < 0.05$ level between Zn content and NDVI values. No significant relationship was found in the trees at other ages. With respect to the results, Zn is effective on the physiology of mature trees especially above 25 years of age. In the same way, the fact that a relationship was found between Mn, which plays an important role in chloroplast structure and the growth of leaf-cells⁴⁹ and the usage of energy by orange leaves in CH4 wavelength band points out that nutrients such as Zn and Mn are affective on plants above 25 years of age in terms of physiology. This relationship can be used in remote sensing techniques and technologies. The fact that no significant relationship could be found between this nutrients and either NDVI or CH4 wavelength band

may be due to smaller cell sizes and low chloroplast content related to low Zn and Mn requirement at young orange trees. However, more detailed studies should be conducted on this issue.

Results of this study demonstrated that the relationships between nutrient contents and canopy reflectance were certainly affected by the plant ages. In addition, significant relationships were found between RF and the contents of N, P, Mg, Fe, Cu and Zn in orange trees.

In the study, when all the nutrients contents and values of NDVI and VNIR area of EMS are taken into consideration, the strongest relationship of all was found in 25 year old trees. This can be explained by more balanced physiological features of the trees that have reached enough maturity and their ability to respond rapidly to any changes in nutrient status. This opinion is supported by the statement that, in the Mediterranean region, the average optimum periods of Washington Navel (*Citrus sinensis* L. Osbeck) trees are between 25-30 years. Therefore, it is concluded that it is more appropriate to prefer 25 year old trees in studies toward determining nutrient contents of Washington Navel (*Citrus sinensis* L. Osbeck) trees of different ages. To verify these findings, additional studies should be conducted and data obtained from Washington Navel (*Citrus sinensis* L. Osbeck) trees of different ages under increasing doses of nutrient element applications should be evaluated for this purpose.

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