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Full Length Article

Estimating Zn Content of Pepper under Different Fertilizer Applications Using a Handheld Spectrometer

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Abstract

In this study, the zinc content of pepper plants was determined when different doses of zinc sulphate ($ZnSO_4.7H_2O$) or ethylene diamine tetraacetic acid (Zn-EDTA) were applied using the hyperspectral method. This study was performed in greenhouse and two different fertilizers ($ZnSO_4.7H_2O$ and Zn-EDTA) were applied at five doses to the plants. There were fifty pots that had five recurrences, and each pot contained three pepper plants. Three leaf samples from each pot were taken at five different growing stages for analysis. An ASD Field Spec Handheld spectroradiometer and a plant probe were used to obtain data from the leaves. The zinc (Zn) concentrations were determined by stepwise multiple linear regression analysis of first degree derivation of spectral reflectance peaks with maximum R^2; possible wavelengths for zinc (Zn) measurements were determined. Reflectance peaks, its first order derivatives and the equation of prediction models for zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) and ethylene diamine tetraacetic acid (Zn-EDTA) were compared. To reveal the relationships between Zn concentration of the leaf samples fertilized with either $ZnSO_4.7H_2O$ or Zn-EDTA, the correlation analysis of the vegetation index was performed. The relationships between plant vegetation indices and zinc concentration were visualized by diagrams. The reflectance peaks at 640 nm wavelength were significant for both fertilizers. The highest accuracies were obtained at 400–500 nm for $ZnSO_4$ treatments, whereas the 900–1000 nm wavelengths were better for Zn-EDTA treatments. In conclusion, different spectral wavelengths should be used for the determination of zinc concentration from hyperspectral reflectance peaks, but the vegetative index of the plants cannot be determined by the hyperspectral reflectance method. © 2015 Friends Science Publishers

Keywords: Pepper; Zinc nutrients; Spectroradiometer

Introduction

Spectroradiometric measurement techniques are based entirely on land survey, are slightly more economical compared with traditional laboratory methods, and have the advantage of acquiring data in a short time. Spectroradiometric measurements have been used since the 1970s to determine the chemical composition of plants faster and more economically than traditional laboratory methods (Norris *et al*., 1976; Marten *et al.,* 1989; Wessman, 1994). Determining the deficiency of plant nutrients by using this technique may allow for the development of a method applicable for field use and for more widespread usage compared with traditional laboratory methods.

The factors affecting plant reflectance are nutrient deficiency, water stress, plant diseases, soil salinity, and the angle of incidence of the sun's rays. The behavior of plant pigments is affected by physiological status of the plant, and therefore the measurement and interpretation of the pigments at varying spatial and temporal scales is crucial indicator of a range of properties and processes in vegetation (Nichol and Grace, 2010). The fact that spectral characteristics are associated with leaf chlorophyll and mineral content suggests that nutrient deficiencies can be estimated using spectral methods (Basayigit *et al*., 2008). Başayiğit and Albayrak (2007) analyzed the effect of fertilizer application on spectral reflectance on vetches with hairy fruit among forage crops and estimated N, P and K content with spectroradiometric methods by applying nitrogen fertilizer at different concentrations. The regression coefficients between the estimated and measured reflectance values were in the range of 0.88–0.94 and considered a range to estimate the reflectance values without damaging to the plant under field conditions (Başayiğit and Albayrak, 2007).

Nutrient deficiency in plants can be determined with non-analytical methods, particularly using hyperspectral sensing of the plant. Barley leaves displayed different spectral responses to N, P and K nutrient deficiencies. The contents of plant nutrients can be estimated by using reflectance values gathered from leaves, especially at wavelengths of 450–1000 nm (Christensen *et al.,* 2004). Chlorosis and other symptoms occur on leaves due to high salinity, nutrient deficiencies or disease, and pests causing the reflectance characteristics to change. When white beet reflections were compared, diseased plants showed less

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reflectance compared with healthy plants, especially in the near infrared region (Laudien *et al.,* 2003). Menesatti *et al.* (2010) studied orange leaves' nutritional status utilizing a Vis-NIR portable spectrophotometer and compared the results with standard chemical analyses. They found that model R^2 (observed vs. predicted) was 0.966, while test R^2 (observed vs. predicted) was 0.694 for the nutrient Fe. Spectral reflectance and functional model processing could be useful diagnostic tools for predicting nutrient deficiencies in leaves. Furthermore, these methods could be useful in defining cost-effective and environmentally friendly methods such as vine (Ordóñez *et al.,* 2013).

Slonecker *et al.* (2009) raised ferns on the soil treated with different levels of arsenic (sodium arsenate); N, P, K and trace elements were applied to the plants weekly. The plants were measured with an ASD full-range (400–2500 nm) spectroradiometer. The laboratory analyses were compared to the reflectance values gathered from the measurements and R^2 values were found in the range 0.59–0.75.

In a study to elucidate whether determining zinc deficiency in the apple trees with spectroscopic methods was possible (Basayigit and Dedeoglu, 2012), sample leaves were taken from three different regions and evaluated with a spectroradiometer. Spectral reflectance measurements and the levels of Zn were compared by the stepwise multiple linear regression analysis method, and, for the regions that were considered high, the R^2 values (0.92–0.95) were gathered using six bands (Basayigit and Dedeoglu, 2012).

The current study applied Zn-EDTA or $ZnSO_4$ fertilizers at different dosages to pepper plant samples, determined the composition of plant nutrients, and compared the spectral signs of these two Zn fertilizers.

Materials and Methods

Experimental Design

This study was conducted in the Agricultural Research and Application Centre of Suleyman Demirel University, Isparta-Turkey. The experiment was designed in an environmentally controlled greenhouse. The dimensions of greenhouse were width of 10.0 m, length of 25.0 m and height 3.5 m. The "Amazon F1"pepper cultivar (*Capsicum annum L.*) was used as the experimental material. Fifty randomized pots were used with soil obtained from the surface horizon of Serenli series. The Serenli series is located at the coordinates 293740, 4193875 (UTM 36 N) and formed on colluvial fan. This soil was classified as Typic Haploxeroll and Mollic Fluvisol according to the Soil Taxonomy and FAO/UNESCO (Akgül *et al.,* 2001). During the growing season, mono ammonium phosphate ($NH_4H_2PO_4$) and ammonium nitrate (NH_4NO_3) were applied as fertilizer, and Previcur Energy SL 840 and Mostar 20SP were applied as insecticides. $ZnSO_4$ or Zn-EDTA was applied at 0, 5, 10, 20 or 40 mg kg^{-1} dosages for supplying spectral differences.

Sampling and measurement were performed in controlled greenhouse conditions from April to July. The first sampling process was performed on May 28, 2012; the other sampling processes were performed on 5 dates: June 13, June 25, July 5 and July 16. For 5 periods, 150 total spectral measurements (e.g., 30 for each period) were performed, 3 for each plant to which $ZnSO_4$ or Zn-EDTA was applied at 0, 5, 10, 20 or 40 mg kg^{-1} dosages at each period. During the selection, attention was paid to ensure that the leaves suffered no biological or mechanical effect from any hazardous substance. Measurements and samplings were performed with mature and middle leaves of young shoots. In addition, care was taken that the upper part of the leaves had no pollutants such as dust or pesticide residues.

Hyperspectral Data Measurements and Processing

For spectral measurements, a portable ASD Field Spec Handheld spectroradiometer was used. Using the first replications of the plants (to which one of two different Zn fertilizers were applied for each period at 5 different dosages), leaf samples were taken from 10 pots in total. Thirty measurements were taken in a period, three from each pot. Spectral reflectance with 5 replications was measured with a plant probe that has wavelengths of 325–1075 nm on a computer. For calibration of the device, spectralon made of gypsum block, which is a white reference, was used once for each 10 measurements. Spectral reflections were measured from the leaves by placing the leaf blade (lamina) between the windpipes in a way that it was exposed to the light. ASD RS3 and View Spec Pro software were used to collect and analyze the data in the computer environment. Leaves, on which measurements were conducted, brought to the laboratory and kept in plastic bags in the refrigerated cabinets for analyses.

Statistical Analysis

The levels of Zn gathered from laboratory analysis and spectral data obtained from the plants addressed in this study were evaluated with the Minitab 15 statistical software package via the stepwise multiple linear regression analysis method, which is a multiple comparison test. Using different wavelength combinations obtained by reducing the variables (wavelengths), prediction models that had the highest R^2 values were searched at most 3 bands and by the decreasing the number of bands.

The data acquired in the study were analyzed by ANOVA with factorial arrangement in terms of characteristics emphasized. In the study, there were 5 levels of dosage factor as 0, 5, 10, 20 and 40 mg kg^{-1} and 5 levels of time factor as 1, 2, 3, 4 and 5 stages. To designate the differences between the dosage means, Tukey's test was used.

The Zn nutrient limit values were classified as deficient, adequate and over; the Zn values in these classes were 18–19 mg kg^{-1}, 20–200 mg kg^{-1} and higher than 200 mg kg^{-1}, respectively.

Results

The nutritional status of the experimental plants was evaluated according to Zn nutrient limits. Thirty six percent of the pepper plants that were fertilized with $ZnSO_4$ indicated Zn deficiency, while the remaining 64% had sufficient Zn levels. ANOVA showed that the dosage vs. time interaction effect on Zn characteristics was statistically significant; the differences between the dosage means were variable and not stable for each period.

The differences among the applications resulted in a statistically significant change in plant zinc content for all periods. No difference was observed between the 0, 5 and 10 mg kg^{-1} dosages on May 28, while the 20 and 40 mg kg^{-1} Zn application showed differences compared with May 28 measurements. On June 13, the 40 mg kg^{-1} Zn application was different from 0, 5, 10 and 20 mg kg^{-1} Zn applications. For June 25, there was no difference between the 0, 5, 10, 20 and 40 mg kg^{-1} Zn applications. On July 5, 10 mg kg^{-1} Zn applications differed from other applications. On May 28, 0, 5, 10 and 20 mg kg^{-1} Zn applications were different from the 0, 5 and 20 mg kg^{-1} Zn applications. On May 28 and June 13, the 40 mg kg^{-1} Zn applications were different compared with the 20 mg kg^{-1} Zn applications. It was observed that Zn applications were effective, especially on July 16, and July 5 (Table 1).

Zn contents were sufficient in all plants treated with Zn-EDTA and the contents of Zn-EDTA vs. time interaction was significant in these plants. This indicates that differences between the Zn applications means were not stable for each period (Table 2).

At the same time, there was no difference between 10 and 20 mg kg^{-1} Zn applications. Within June 13measurements, there were no variances between 5, 10 and 20 mg kg^{-1} applications, but the 0 and 40 mg kg^{-1} applications were different from the 5, 10 and 40 mg kg^{-1} applications. There was also no difference between 0, 5, 10 and 20 mg kg^{-1} Zn applications from the June 25 measurements. The 40 mg kg^{-1} Zn application, however, was different from 0, 5, 10 and 20 mg kg^{-1} Zn applications. The 0, 5, 20, and 40 mg kg^{-1} applications were the same, while 10 mg kg^{-1} Zn application was different from 0, 5, 20, and 40 mg kg^{-1} applications on July 5. There was no difference between 5 and 40 mg kg^{-1} Zn applications on July 16. The 0 mg kg^{-1} Zn application was different from the other applications (Table 2). According to the evaluation, variation for plant Zn content was identified with the 0 mg kg^{-1} Zn applications at May 28, June 13, July 5 and July 16 measurements. The pepper plant did not require levels of Zn content higher than found in the stock soil from earlier growing seasons. Therefore, high concentrate of Zn applications were more effective than low contents applications in the most recent growing season.

The differences in $ZnSO_4$ applications on July 16 were partially effective. When Zn-EDTA was applied to plants, the differences in Zn applications were effective on May 28 and June 13.

The differences between the Zn applications per period in the test plants with $ZnSO_4$ affected the spectral reflections, and the dosage effects made the reflectance graphs more obvious. This difference was distinct only on July 16 in the visible region and on June 25 in the infrared region. These data suggest that the difference between the dosages becomes clearer as the plant grows and it affects the reflectance in the infrared region; however, there is no difference in the visible region between the different dosage applications on May 5 of plant growth (Fig. 1).

Derivative spectral curves of plants to which $ZnSO_4$ was applied showed refractions between 500 and 770 nm on

Table 1: The influence of $ZnSO_4$ applications on the reflectance values of pepper

Dosage(mg kg-^{1})	May 5	June 13	June 25	July 5	July 16	Mean
0	38.20±2.01a	33.19±1.66a	29.68±2.27a	29.08±5.14a	22.33±1.41a	31.08±1.77
5	34.92±1.17a	33.89±6.64a	17.175±0.0439a	20.10±0.367ab	23.34±0.712a	24.57±2.14
10	36.98±0.0722a	36.11±1.09 a	17.69±6.13a	8.00±1.123b	15.51±5.87ab	22.40±3.67
20	5.16±1.62b	32.5±19.5 a	21.40±0.924a	25.84±0.0600a	5.62±1.77b	18.00±3.78
40	7,53±2.20b	11.80±1.58 b	16.22±7.38a	21.50±7.75ab	30.88±1.44a	17.59±2.87
Mean	25.25±4.32	28.34±3.89	20.43±2.13	20.90±2.49	19.63±2.73	

Table 2: Influence of ZnEDTAapplications on the reflectance values of pepper

Dosage(mg kg-^{1})	May 5	June 13	June 25	July 5	July 16	Mean
0	39.26±1.66d	33.03±3.16b	27.66±1.17b	34.85±0.673 ab	35.46±0.375c	34.05±0.20
5	53.62±1.56bc	49.65±0.216 a	33.82±1.83ab	42.18±4.86a	59.97±1.46ab	47.85±2.61
10	68.94±1.33a	48.69±8.08a	27.31±0.773b	27.44±0.145 b	61.13±1.45a	46.70±4.77
20	48.00±4.74cd	33.62±0.908 b	25.32±1.33b	37.00±1.34ab	49.63±4.59b	38.72±2.70
40	63.17±1.47ab	47.83±0.16a	39.61±1.83a	40.13±1.78a	55.00±1.9ab	49.15±2.49
Mean	54.60±2.98	42.56±2.53	30.75±1.51	36.32±1.64	52.24±2.64	

*Means followed by the same letters are not significantly different ($p<0.05$)

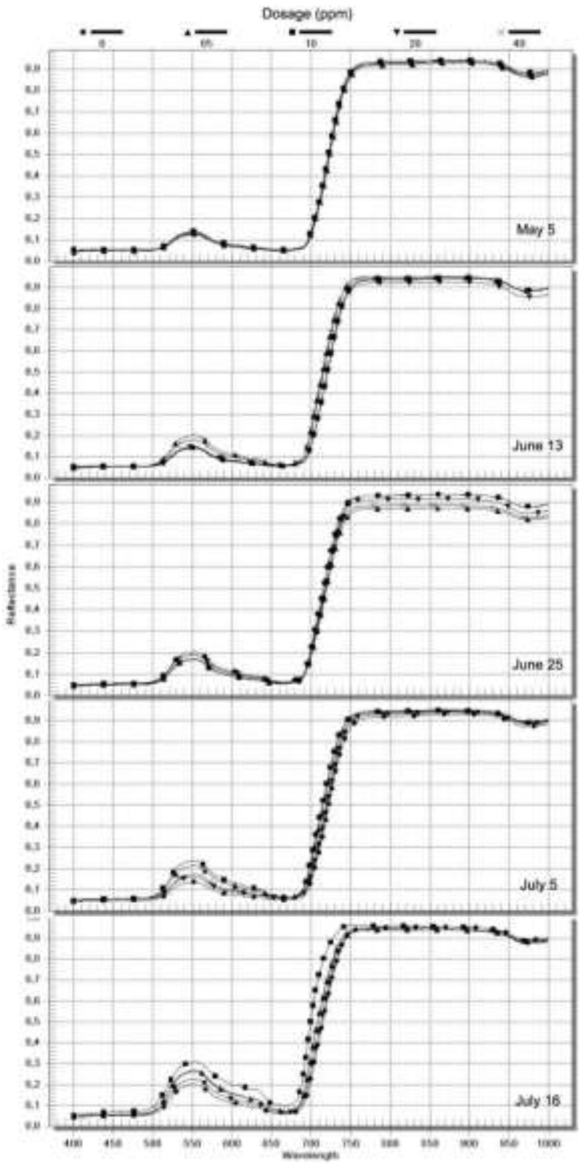

Fig. 1: The spectral curves of $ZnSO_4$ applications in five different periods

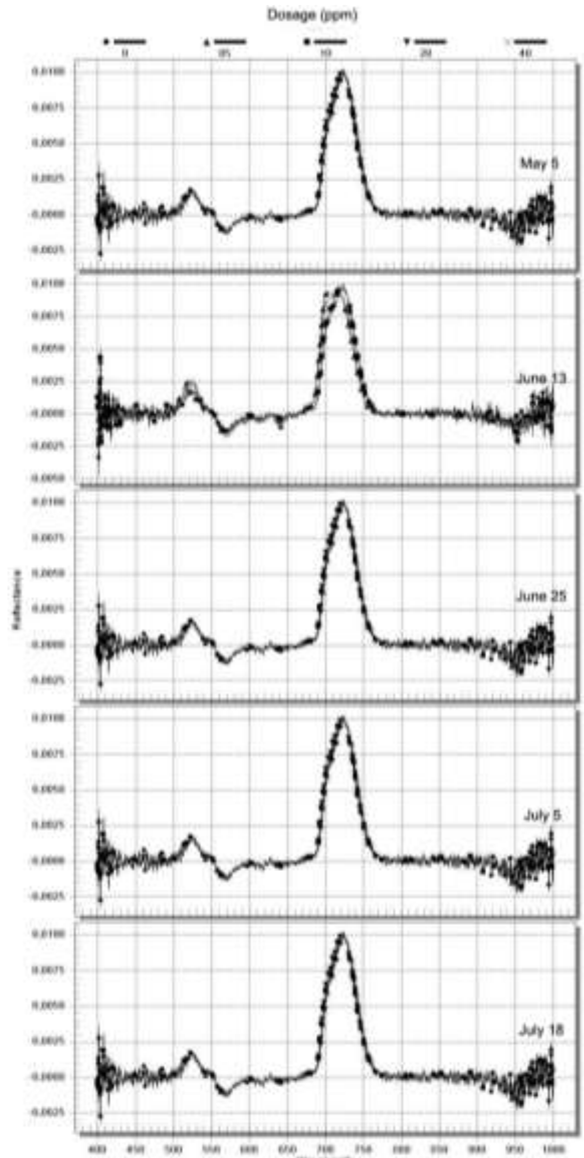

Fig. 2: The derivative spectral curves of $ZnSO_4$ applications in five different periods

May 28, June 13, June 25, July 5 and July 16 (Fig. 2). There were 40 refractions at 640 nm on spectral curves and 25 refractions at each 500, 525, 550, 570, 600, and 770 nm; 10 refractions each at 540 and 620 nm; and 5 refractions each at the 610, 623, 630, 650, 660, 680, and 690 nm wavelengths. Each of the 700 and 720 nm wavelengths showed 7 refractions. For the 715 nm wavelength, only the first refraction was observed.

In addition, when the number of distinct refractions in the spectral curve was compared, the numbers of peaks were 40 for May 5, 50 for June 13, 62 for June 25, 47 for July 5, and 41 for July 16; the highest number of peaks was observed on June 25.

When the reflectance characteristic for the Zn-EDTA application was analyzed, the reflections in the visible region (400–700 nm) on May 5showed an increase for the 0, 10, 40, 20 and 5 mg kg^{-1} Zn applications. There was no significant difference in the near-infrared region. The difference depending on the periods of test plants, treated with Zn-EDTA, was also observed in reflectance curves and; this was most apparent on June 25. Increasing the Zn application in both the visible and infrared regions on June 25resulted in an increase of reflectance (Fig. 3).

For both the $ZnSO_4$ and Zn-EDTA applications, the most significant reflectance was observed in infrared region on June 25. In the measurements of $ZnSO_4$ application samples on June 25, increasing the Zn application in

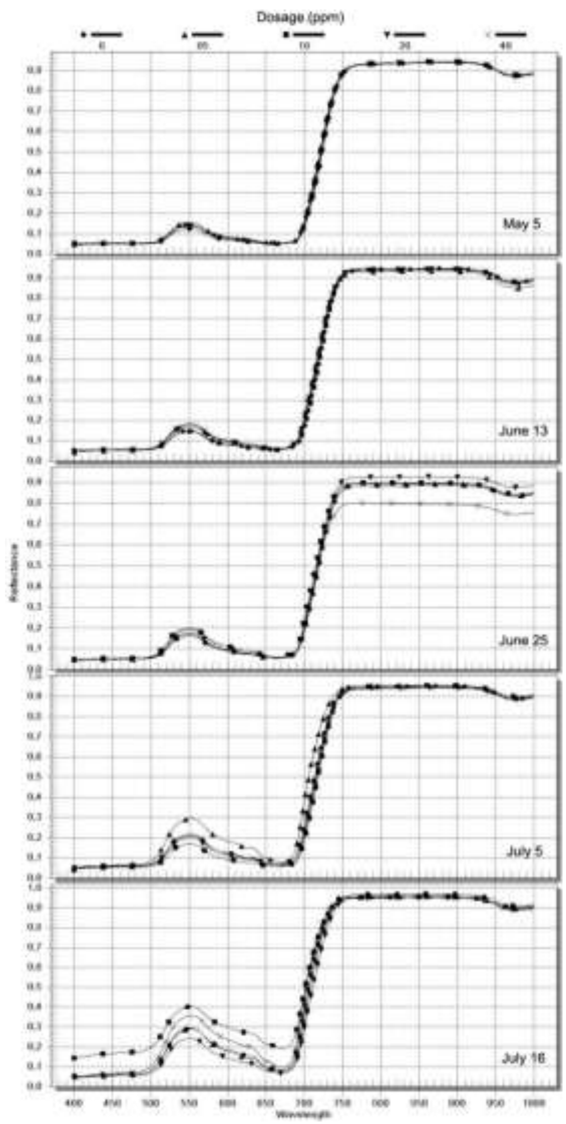

Fig. 3: The spectral curves of ZnEDTA applications in five different periods

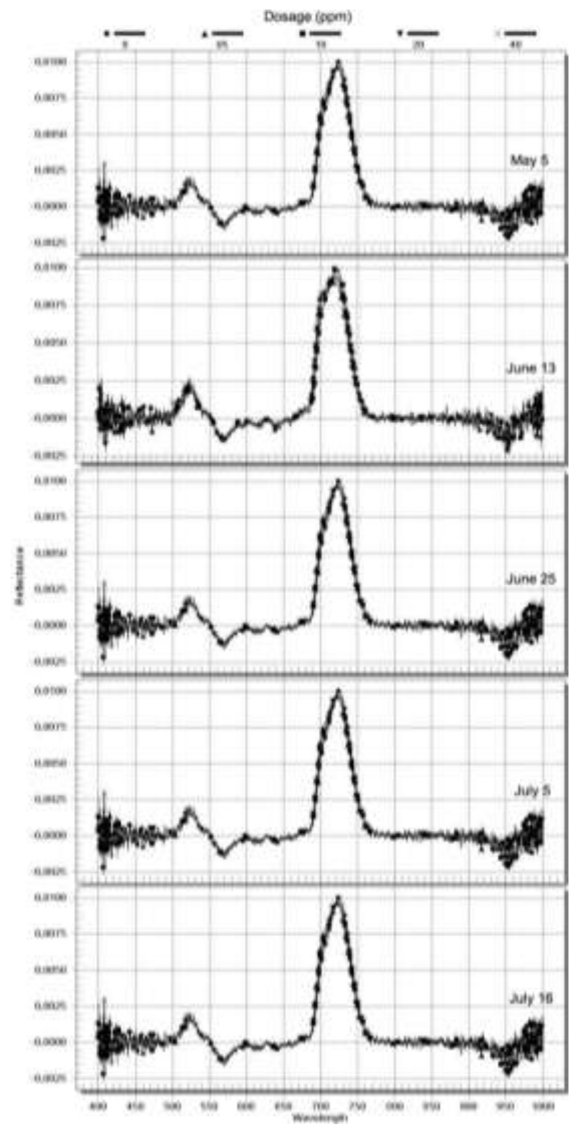

Fig. 4: The derivative spectral curves of ZnEDTA applications in five different periods

infrared region decreased the reflectance. While increasing the Zn application both in infrared and visible region increased the reflectance in Zn-EDTA application samples on June 25. At the same time, for both fertilizer applications, the Zn application of 10 mg kg^{-1} generally showed a difference compared with other applications. The measurements of the experimental plants from June 25 produced the highest reflectance values. The reflectance measurements taken on June 25 were exposed to both fertilizer applications showed the same results in the near-infrared region, but not in the visible region.

The wavelengths which showed the most refraction was determined by taking first derivatives of reflectance curves and by this method wavelength that can be widely used were located (Fig. 4).

Derivative spectral curves of the Zn-EDTA-treated plants showed identified refractions between 500–770 nm during crop growing season. There were 25 spectral refractions at 500, 525, 550, 570, 600 and 640 nm on curves; 20 refractions each at 620, 630 and 770 nm; and 16 refractions at the 720 nm wavelength. For the 680 nm wavelength, 15 refractions were observed, 6 refractions at the 700 nm wavelength; and 5 refractions at each of the 650, 660, 670, 725, and 750 nm wavelengths (Fig.4).

In addition, when the number of distinct refractions in graphs was compared, the number of peaks was 50 for May 5, 50 for June 13, 55 for June 25, 47 for July 5, and 65 for July 16; the number of peaks in July 16 was the highest. The reflectance and zinc values of $ZnSO_4$ samples were evaluated with multiple linear regression analysis. The accuracy coefficients (R^2) were between 0.29 and 1 in the prediction models formed from the result of multiple linear

Table 3: The Zn prediction models of spectral curve for $ZnSO_4$ applications in five different periods

Periods	Wavelength No.	Model	Equation	R^2
May 5	1	Zn=327.8+(-5672*459nm)	y = 0.650x + 8.685	0.650
	2	Zn=326+(-14182*459nm)+(8674*492nm)	y = 0.994x + 0.125	0.994
	3	Zn=323.9+(-14575*459nm)+(8613*492nm)+(551*406nm)	y = 1x + 0.018	1
June 13	1	Zn=166.91+(-2036*682nm)	y = 0.536x + 12.23	0.536
	2	Zn=-27.56+(-3650*682nm)+(5166,9*500nm)	y = 0.998x + 0.076	0.998
	3	Zn=-29.87+(-2471*682nm)+(5638,6*500nm)+(-1698*677nm)	y = 0.999x + 0.023	1
June 25	1	Zn=-101+(2579.1*404nm)	y = 0.774x + 4.425	0.774
	2	Zn=-103.4+(5040.5*404nm)+(-2339.2*410nm)	y = 0.991x + 0.189	0.991
	3	Zn =-121.7+(4688.9*404nm)+ (-2275.8*410nm)+(39.19*738nm)	y = 1x – 0.028	1
July 5	1	Zn=50.33+(-204.61*526nm)	y = 0.658x + 7.414	0.658
	2	Zn=-559.06+(-360.55*526nm)+(713.41*970nm)	y = 0.999x + 0.007	0.999
	3	Zn=-508.31+(-432.59*526nm)+(594.98*970nm)+(79.93*737nm)	y =1x + 0.001	1
July 16	1	Zn=78.5+(-1231.1*410nm)	y=0.286x+13.32	0.286
	2	Zn=105+(-6600.1*410nm)+(4651.49*413nm)	y=0.993x+0.126	0.993
	3	Zn=90.84+(-6064.6*410nm)+(5044.27*413nm)+(-502.48*491nm)	y=1x + 0.003	1

Table 4: The Zn prediction models of derivative spectral curve for $ZnSO_4$ applications in five different periods

Periods	Wavelength No.	Model	Equation	R^2
May 5	1	Zn=141.3+(-2041*500nm)	y=0.5108x + 14.383	0.5108
June 13	1	Zn=-307.7+(356*770nm)	y=0.2723x + 18.824	0.2723
June 25	1	Zn=-36.37+(818*500nm)	y=0.3647x + 9.7287	0.3648
July 5	2	Zn=1586.12+(4377*500nm)+(-1983*770nm)	y=0.6667x + 5.5448	0.6663
July 16	1	Zn=692.4+(-713*770nm)	y=0.3274x + 12.416	0.3275

Table 5: The Zn prediction models of spectral curve for ZnEDTA applications in five different periods

Periods	Wavelength No.	Model	Equation	R^2
May 5	1	Zn=-5053+(5433.4*902nm)	y = 0.9699x+1.217	0.9699
	2	Zn =-5022+(5477*902nm)+(-1135.4*686nm)	y=0.9999x+0.2746	0.9999
	3	Zn=-4814+(5248.2*902nm)+(-1462.4*686nm)+(512.5*483nm)	y=1x + 0.2805	1
June 13	1	Zn=285.63+(-4736.5*413nm)	y=0.9428x+2,4731	0.9428
	2	Zn=87.39+(-5115.9*413nm)+(248.1*991nm)	y=0.9998x-0,0167	0.9998
	3	Zn=89.52+(-5144.4*413nm)+(239.7*991nm)+(130.6*411nm)	y=1x-0.0115	1
June 25	1	Zn=122.7+(-110*994nm)	y=0.7318x + 8.4587	0.7325
	2	Zn=103.1+(-1658*994nm)+(1574*986nm)	y=0.9958x + 0.3794	0.9975
	3	Zn=108.6+(-1695*994nm)+(1410*986nm)+(193*997nm)	y=1.001x-0.1589	1
July 5	1	Zn=818.1+(-876.6*986nm)	y=0.8138x+6.5205	0.8138
	2	Zn =722.4+(-777.3*986nm2)+(33*548nm)	y=0.9039x+3.4712	0.9013
	3	Zn=540.1+(-574.7*986nm)+(3187*548nm)+(-3119*555nm)	y=1.002-0.0172	1
July 16	1	Zn=1114+(-1114.1*901nm)	y=0.5329x+23.94	0.5329
	2	Zn=2034+(-13686.9*901nm)+(11605.6*893nm)	y=0.9746x+0.9976	0.9746
	3	Zn=2013+(-13285.3*901nm)+(10775.8*893nm)+(468,1*952nm)	y=1x-0.0649	1

Table 6: The ZnpredictionmodelsofderivativespectralcurveforZnEDTAapplicationsinfivedifferentperiods

Periods	Wavelength No.	Model	Equation	R^2
May 5	1	Zn=114.3+(-145*725nm)	y=0.4482x+ 18.578	0.4490
	2	Zn=210.3+(-686*725nm)+(3218*630nm)	y=0.8581x+4.951	0.8591
	3	Zn=923.8+(-657*725nm)+(3267*630nm)+(-790*770nm)	y=0.9978x + 0.2474	0.9990
June 13	1	Zn=-161.16+(3544*500nm)	y = 0.536x+21.901	0.5361
	2	Zn=-125.477+(7718*500nm)+(-4311*680nm)	y=0.9234x+3.6346	0.9234
	3	Zn=-1.037+(6033*500nm)+(-5833*680nm)+(629*525nm)	y=0.984x + 0.7451	0.9839
June 25	1	Zn=818.1+(-876.6*986nm)	y=0.8138x+6.5205	0.8138
	2	Zn =722.4+(-777.3*986nm2)+(33*548nm)	y=0.9039x+3.4712	0.9013
	3	Zn=540.1+(-574.7*986nm)+(3187*548nm)+(-3119*555nm)	y=1.002-0.0172	1
July 5	Not predicted data			
July 16	1	Zn=-1090+(1195*770nm)	y=0.7011x + 15.151	0.7009

regression analysis performed at May 5, June 13, June 25, July 5, and July 16 (Table 3). The accuracy coefficients (R^2) were 1 in the prediction models formed using the reflectance values gathered from 3 wavelengths in all periods. The accuracy coefficient of the prediction models gathered using a single wavelength from July 16 of reflectance measurements showed the lowest value. The highest accuracy coefficient (R^2=0.774) of prediction models produced by using reflectance values that belong to a single wavelength was acquired in June 25. It should be noted that 60% of the selected wavelengths were found between 400 and 500 nm in the models developed for predicting the Zn content of the $ZnSO_4$-treated plants.

The accuracy coefficients (R^2) of the prediction models formed on May 5, June 13, July 5 and July 16 were between 0.27 and 0.51 and the highest accuracy coefficient was acquired from May 5 using a single wavelength (Table 4).

The reflectance and zinc values of Zn-EDTA samples were evaluated with multiple linear regression analysis. The accuracy coefficients (R^2) were between 0.53 and 1 in the prediction models formed from result of multiple linear regression analysis performed at all periods for the Zn-EDTA-treated test plants (Table 5). The accuracy coefficients (R^2) were 1 in the prediction models produced using the reflectance values gathered from 3 wavelengths in all periods. The highest accuracy coefficient (R^2=0.97) of prediction models gathered from reflectance values from a single wavelength was acquired on May 5. Approximately 60% of selected wavelengths were between 900 and 1000 nm in models developed for predicting the Zn content of experimental plants.

Using identified wavelengths in the derivative spectral curves of Zn-EDTA applications, the Zn equations of prediction model were developed (Table 6). The equation with highest accuracy coefficient (R^2) of 0.99 was produced using 3 wavelengths from May 5, when the prediction models were analyzed. The lowest accuracy coefficient (R^2) of 0.45 was produced using 1 wavelength from May 5.

Discussion

The wavelength 640 nm was the widely used in the derivative figures gathered from reflectance measurements of $ZnSO_4$-treated; the wavelengths between 500 and 600 nm were also significant. In addition, the 770 nm wavelength was significant. The most distinguishing data from the $ZnSO_4$ treatment was acquired on June 25.

Meanwhile, similar results were gathered from Zn-EDTA application. The 640 nm was the most used wavelength, followed by the wavelengths between 500 and 600 nm. The 680 and 720 nm wavelengths were also significantly different from $ZnSO_4$-treated. The most distinguishing data from the Zn-EDTA application was acquired on May 5.

The accuracy coefficient was 1, using the 3 wavelengths in prediction models produced by comparing the spectral measurements and results of laboratory analysis with multiple linear regression analysis for both the $ZnSO_4$ and Zn-EDTA applications. The highest accuracy coefficient was R^2=0.97 in the equations produced by using a single wavelength in the Zn-EDTA application. The highest Zn content values were acquired from the Zn-EDTA-treated pepper plants applied with increasing Zn applications.

Approximately 60% of the selected wavelengths were between 400 and 500 nm in the models developed from the $ZnSO_4$-treated plants and about 60% were between 900 and 1000 nm in the models developed for predicting Zn content of Zn-EDTA-treated plants. The accuracy coefficient (R^2) values were lowest in last periods of model equations produced using a single wavelength at each of the fertilizer applications. Models developed from Zn-EDTA application produced more successful results than the models produced from $ZnSO_4$ application in predicted equations. Similarly, R^2 values were found 0.99, 0.68, 0.94, 0.92 and 0.98 from regression analysis conducted to identify the N, Mg, Fe, Zn and chlorophyll content. In a study with apple trees using spectral methods, the R^2 values were found 0.97, 0.99, 0.71, 0.92, and 0.99 for P, K, Ca, Cu and Mn, respectively (Basayigit *et al.*, 2009). Basayigit and Dedeoglu (2012) identified Zn deficiency in apple trees with spectroscopic methods. In their study, deficiency of zinc was accurately predicted using 6 wavelengths and their R^2 values were 0.92, 0.95 and 0.94 for three locations (Basayigit and Dedeoglu, 2012).

To specify photosynthetic pigments with hyperspectral reflectance techniques, Blackburn (2006) stated that chlorophyll can be identified at 530–600 nm wavelengths. Using spectral methods (Osbourne *et al.*, 2002), N and P deficiency in corn and their antagonistic interactions could be identified by using stepwise multiple linear regression analysis with the values gathered at 350–1000 nm wavelengths.

Conclusion

Significant differences in the near-infrared region (700–1000 nm) were seen on June 25 in both of the fertilizer applied in this study. Consequently, it was concluded that different wavelengths should be used for reflectance curves and derivative transformations to identify Zn content in the pepper plant according to the hyper-spectral reflectance methods.

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References

Akgül, M., L. Başayiğit, Y. Uçar and M. Müjdeci, 2001. *The Soils of Atabey Plain*. Research report. Suleyman Demirel University Agriculture Faculty Press, Isparta, Turkey

Başayiğit, L. and S. Albayrak, 2007. The use of the reflectance measurement to predict the N, P and K content of wollypod vetch under different N, P and K fertilization. *Asian J. Chem.*, 19: 5609–5619

Basayigit, L., S. Albayrak and H. Şenol, 2009. Analysis of VNIR reflectance for prediction of macro and micro nutrient and chlorophyll contents in apple trees (*Maluscommunis). Asian J. Chem.,* 21: 1302–1308

Basayigit, L., S. Albayrak, H. Şenol and H. Akgül, 2008. Estimation possibility of plant nutrition contents using spectroradiometer data. *National Congress of Plant Nutrition and Fertilizer,* pp: 163–174. Konya, Turkey

Basayigit, L. and M. Dedeoglu, 2012. The determination of Zn deficiency on apple orchards using visible near infrared (VNIR) spectroscopy methods. *TABAD Res. J. Agric. Sci.,* 5: 64–67

Blackburn, G.A., 2006. Hyperspectral remote sensing of plant pigments. *J. Exp. Bot.,* 58: 855–867

Christensen, L., B. Bennedsen, R. Jorgensen, and H. Nielsen, 2004. Modeling nitrogen and phosphorous content at early growth stages in spring barley using spectral line scanning. *Biosys. Eng.,* 88: 19–24

Laudien, R., G. Bareth and R. Doluschitz, 2003. *Analysis Hyper Spectral Field Data for Detection of Sugar Beet Disease*, pp: 375–381. European Federation for Information Technology in Agriculture Conference, Deprecen, Hungary

Marten, G.C., J.S. Shenk and F.E. Barton, 1989. *Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality*. USDA ARS, Agricultural Handbook, Washington DC, USA

Menesatti, P., F. Antonucci, F. Pallottino, G. Roccuzzo, M. Allegra, F. Stagno and F. Intrigliolo, 2010. Estimation of plant nutritional status by VIS–NIR spectrophotometric analysis on orange leaves (*Citrus sinensis* (L) Osbeck*cv* Tarocco). *Biosys.Engineer.,* 105: 448–454

Nichol, C.J. and J. Grace, 2010. Determination of leaf pigment content in *Calluna vulgaris* shoots from spectral reflectance. *Int. J. Remote Sens.,* 31: 5409–5422

Norris, K.H., R.F. Barnes, J.E. Moore and J.S. Shenk., 1976. Predicting forage quality by infrared reflectance spectroscopy. *J. Anim. Sci.,* 43: 889–897

Ordóñez, C., J.R., Rodríguez-Pérez, J.J. Moreira and E. Sanz, 2013. Using hyperspectral spectrometry and functional models to characterize vine-leaf composition. *IEEE Trans. Geosci. Remote Sens.,* 51: 2610–2618

Osbourne, S.L., J.S. Schepers, D. Francis and M.R. Schlemmer, 2002. Detection of phosphorus and nitrogen deficiencies in corn using spectral radiance measurements. *Agron. J.,* 94: 1215–1221

Slonecker, T., B. Haack and S. Price, 2009. Spectroscopic analysis of arsenic uptake in pterisferns. *Remote Sens.,* 10: 644–675

Wessman, C.A., 1994. Estimating canopy biochemistry through imaging spectroscopy. *In*: *Imaging Spectroscopy a Tool for Environmental Observation*, pp: 57–69.Hill, J. and J. Me´gier (eds.). ECSC, EEC, Brussels and Luxembourg

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