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Research Article

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Investigation of Nitrate Content of Sage (*Salvia fruticosa* Mill) and Oregano (*Origanum onites*) Plants

Abstract

In this study, the nitrate contents of sage (*Salvia fruticosa* Mill) and oregano (*Origanum onites*) plants and their soils growing naturally between Kekova and Kaş were investigated. The spectrometric method was used in nitrate analysis on leaf samples of plants collected from research areas. NO_3-N contents were determined as mg kg^{-1} dry weight of dry matter. The leaves chlorophyll concentrations were measured with the Minolta SPAD 502 plus chlorophyll meter. DR 2800 (Hach-Lange, USA) Spectrophotometer was used for nitrate analysis in dry and moist soil samples. Nitrate contents of plant leaves were from 140.12 to 363.64 mg kg^{-1} values in sage leaf samples; It was determined that oregano leaf samples varied from 106.06 to 415.58 mg kg^{-1}. Nitrate contents of soils ranged from 2.66 to 24.66 µg g^{-1} in soils where sage is grown; it ranged from 6.24 to 19.82 µg g^{-1} in the soils oregano is grown.

INTRODUCTION

The growth and development process of plants depends on photosynthesis. Nitrogen is an essential component of chlorophyll and enzymes and proteins involved in photosynthesis. During the plant's photosynthesis, chlorophyll absorbs sunlight and converts it into chemical energy. There is a strong relationship between photosynthesis and nitrogen in the plant. Plants take up nitrogen from nitrate and ammonium ions (Marschner, 1995). Soil texture, structure, pH, temperature, and environmental factors have an essential effect on nitrate and ammonium uptake by plants. In well-ventilated soils with a pH of 6 to 8, the nitrification rate increases, and the plants take more nitrate. It was found that NO_3^- and NH_4^+ uptake decreased at low temperatures. Most plants prefer nitrate-nitrogen (NO_3^--N) to ammonium nitrogen (NH_4^+-N**)** (Kacar and Katkat, 2019). Most of the nitrogen taken up by plants is in the nitrate-nitrogen form. Plants tolerate high nitrate-nitrogen concentrations and accumulate more nitrate-nitrogen in their tissues than ammonium (Havlin et al., 1999). Plant species, age, organs, the nitrogen content of the environment, light intensity, temperature, climate, water, soil properties, and harvest time play an essential role in plants' nitrate content (Zhou et al., 2000; Oruç and Ceylan, 2001; Zhang et al., 2020).

Excessive levels of nitrate in foods negatively affect human health (Erkekoğlu and Baydar, 2010; Kayıkcıoglu ve Okur, 2020a; Kayıkcıoglu ve Okur, 2020b). The limit values of nitrate contents in plants vary according to the plant type. According to the Turkish Food Codex, the maximum limit is determined as 2000-4500 mg NO_3^- /kg for lettuce and salad group vegetables whose leaves contain high amounts of nitrate (Anonim, 2008). Özcan and Akbulut (2007) examined the macro and micronutrient contents of 31 types of medicinal and aromatic plants. In reported that their studies, including some species belonging to the Lamiaceae family, the plants' nitrate content ranged from 12.15 to 238.85 mg/kg in dry weight. Fresh and dry leaves of *Origanum onites* and *Salvia fruticosa*, which are members of the *Lamiaceae* family, are consumed as tea. Dry herbage of *O. Onites* used as a seasoning in salads, soups, meat, chicken, vegetable dishes, pickled sauces, and sausages; It has also been used in beverages cheeses. (Bozdemir, 2019). Since these plants have antioxidant and antimicrobial properties, they are used for food storage and extend their shelf life.

In this study, the nitrate contents of sage (*S. fruticosa*) and oregano (*O. onites*) plants and their soils growing naturally between Kekova and Kaş were investigated. The changes in nitrate-nitrogen (NO_3^--N) and chlorophyll concentrations of two different plant species in the same family were compared.

MATERIAL and METHODS

In the research, plant and soil samples were taken from areas where sage (*Salvia fruticosa* Mill.) and oregano (*Origanum onites*) plants naturally grow between Kekova/Demre and Kaş. In the research, the plants' pre-flowering period was taken into account. Ten plant and ten soil samples were taken from the area research species growing. A total of 20 plants and 20 soil samples were examined. The map of the study area is given in Figure 1.

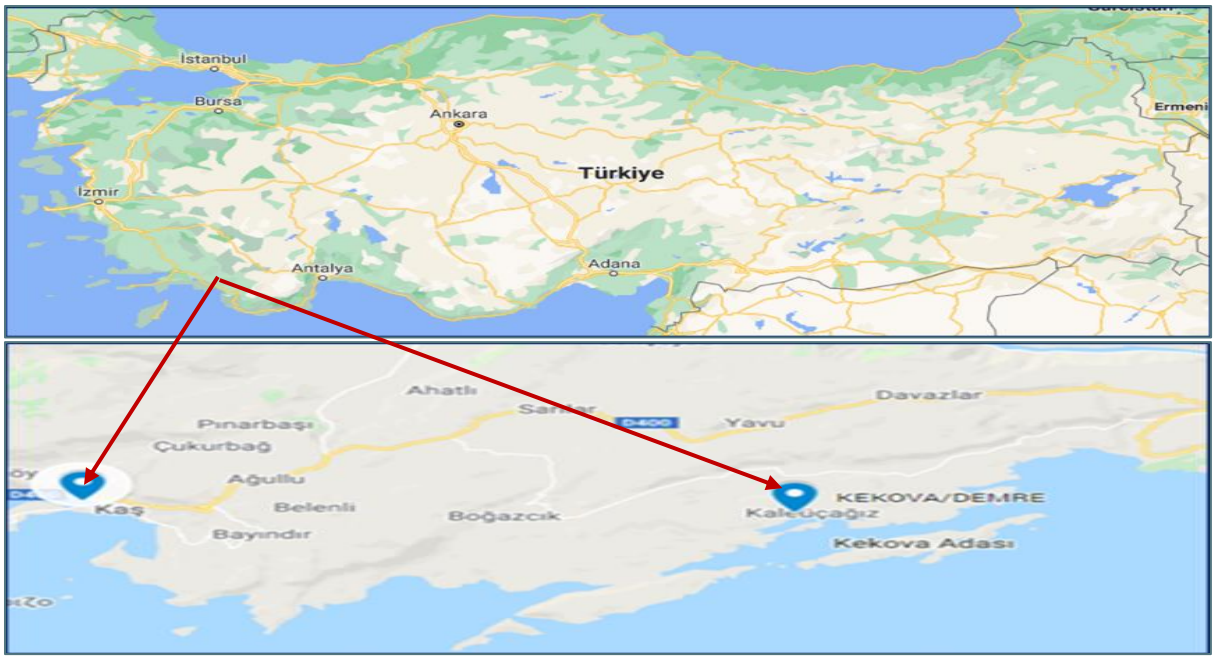

Figure 1. Location of the study area

Plant Analysis

Chlorophyll concentration: In the research area, color intensity values were determined by randomly ten leaf samples from a plant with Minolta SPAD 502 plus chlorophyll measuring device.

NO_3^--N: Plant samples were placed in plastic bags and, as soon as possible, brought to the laboratory, and then the samples were washed with distilled water. They were dried in drying cabinets set at 65ºC. The dried plants' leaves were made ready for analysis by grinding them in the grinding mill. Nitrate contents in leaf samples were determined colorimetrically with salicylic acid application in a spectrophotometer set at 410 nm wavelength. Analysis results have been calculated as mg/kg dry matter (Kacar and İnal, 2008). Each plant sample has been analyzed in duplicate.

Soil Analysis

Soil samples taken from 0-30 cm soil depths were dried in room conditions in the laboratory, and dried soil samples were sieved through a 2 mm sieve (Chapman et al., 1961).

NO_3^--N: The nitrate content in the soil was made based on cadmium metal's reduction of nitrate in soil samples to nitrite. All measurements were determined in the DR 2800 (Hach-Lange, USA) Spectrophotometer using HACH brand standard test kits (Nitra Ver 5 Nitrate Reactive Powder Pad). NO3-N results in soil were evaluated according to Marx and Stevens (1999).

Statistical Analysis

The T-test was performed using the licensed SPSS Statistics Base v23 version to analyze the study's values. The statistical significance level was set at $p < 0.01$.

RESULTS and DISCUSSION

When the NO_3^--N concentrations of leaf samples for both species was seen to range from 106.12 to 415.58 mg kg^{-1}. The highest NO_3^--N ratio was obtained from *S. fruticosa* leaves with 287.25 mg kg^{-1}, while the lowest NO_3^--N ratio was obtained *from O. onites* leaves with 204.83 mg kg^{-1}. It was observed that the change in NO_3^--N content in the leaves of the plants was statistically significant at the 5% level (Table 1). In another study conducted with *O. onites* plants collected from different locations, it was reported that the NO_3^--N contents of the plants varied between 4.33 and 534.63 mg

kg^{-1} (Kocabaş, 2014). Özcan and Akbulut (2007) reported the NO_3^--N content of plants as 174.26 mg kg^{-1} in *Ocimum minimum*, 30.60 mg kg^{-1} in *Salvia aucheri*, 46.27 mg kg^{-1} in *Salvia fruticosa* L. and 137.79 mg kg^{-1} in *Satureja hortensis*.

Table 1. NO_3^--N (mg kg^{-1}) and chlorophyll values (SPAD 502) of plant samples

Example No	NO_3^--N in plant mg kg^{-1}		Chlorophyll concentration SPAD value	
	S. fruticosa	***O. Onites***	***S. fruticosa***	***O. Onites***
1	393.64	138.38	47.12	29.31
2	384.98	132.86	42.68	27.04
3	287.73	233.94	36.40	34.73
4	255.41	208.62	27.48	30.89
5	290.04	106.12	31.20	21.79
6	353.33	248.62	41.81	35.15
7	314.07	111.81	38.88	22.48
8	271.08	205.63	36.10	32.45
9	140.12	246.75	25.80	32.84
10	182.12	415.58	30.48	42.87
Minimum	140.12	106.12	25.8	21.79
Maximum	393.64	415.58	47.12	42.87
Mean± SD	287.25 ± 81.61 a	204.83 ± 92.47b	35.80 ± 6.99	30.96 ± 6.27
Significance	p<0.05		ns	
NO_3^--N in plant* Chlorophyll concentration Correlation coefficient (r):0.93 p<0.01				

SD: Standard deviation, ns: Not significant

Chlorophyll concentrations (SPAD value) of *S. fruticosa* and *O. onites* leaves ranged from 21.79 to 47.12. The highest chlorophyll concentrations with an average of 35.80 were found in *S. fruticosa*. However, the difference between Chlorophyll concentrations of plant leaves was not statistically significant. Chlorophyll concentration in *O. Onites* leaves ranged from 30.96 to 35.80. In another study conducted on *O. onites*, chlorophyll measurement values (Minolta SPAD 502 plus) in leaves were reported to range from 27.93 and 46.05 (Kocabaş, 2014). When the correlation analyzes between the NO_3^--N contents and chlorophyll concentrations of *S. fruticosa* and *O. onites* plants were examined, a positive relationship ($p<0.01$) was found between the NO_3^--N content and chlorophyll concentrations (Table 1). In many studies, it has been stated that there is a positive relationship between the chlorophyll concentrations measured with the SPAD chlorophyll meter and the total nitrogen and NO_3^--N contents of the plants (Wood et al., 1992; Liu et al., 2006).

Table 2. NO_3^--N concentrations of soil samples (µg g^{-1})

Example No	**NO_3^--N in soil (µg g^{-1})**	
	S. fruticosa	***O. Onites***
1	7.34	11.87
2	8.18	13.09
3	9.70	19.82
4	6.06	9.90
5	17.28	16.48
6	2.66	10.69
7	12.00	9.95
8	7.98	6.24
9	24.66	19.52
10	20.04	11.38
Minimum	2.66	6.24
Maximum	24.66	19.82
Mean ± SD	11.59 ± 6.92	12.89 ± 4.41
Significance	ns	

SD: Standard deviation, ns: Not significant

When evaluated in general, it was seen that the nitrate content of the soil samples taken from the study areas ranged from 2.66 to 24.66 µg g^{-1}. The average NO_3^--N content of soils where *O. onites* was growing is higher than the average NO_3^--N content of soils where *S. fruticosa* was growing. However, this difference in NO_3^--N content of soils is not statistically significant (Table 2).

Table 3. Evaluation of NO_3^--N concentrations in soil

NO_3^--N (µg/g)	**Evaluation**	***S. fruticosa***	***O. Onites***	***In general total***
<10	low	60%	30%	45%
10-20	medium	30%	70%	50%
20-30	high	10%	-	5%
>30	excessive	-	-	

If a general evaluation has been made about the NO_3^--N content of the soil samples, it was found that 50% of the samples were at a medium level, 45% at a low level, and 5% at a high level (Table 3). It has been stated by the EPA (US Environmental Protection Agency) that the nitrate content in soil solution can generally be between 10-45 µg g^{-1} NO_3^--N. Nitrate nitrogen (NO_3^--N) has been considered a potential pollutant by the Environmental Protection Agency (EPA). Because NO_3^--N, which is present in excess in the soil, can pass into rivers by flow and groundwater through seepage and become an environmental hazard. As a result, it was determined that the nitrate-nitrogen (NO_3^--N) contents of the leaves of the plants showed a statistically significant difference between the species. There is a strong positive correlation between nitrate-nitrogen contents of plant leaves and chlorophyll concentrations. As is known, nitrogen is a crucial chlorophyll component that plays a role in photosynthesis. There was no statistically significant change in the amount of nitrate-nitrogen found in soil samples taken from soils where plant species were grown. A significant difference was found between the nitrate content of plants; however, to be able to say that the sage plant takes more nitrate from the soil than the oregano plant, it is thought

that studies on nitrogen applications are needed in the cultivation of these plants.

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