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Evaluation of Humic Acid Content and Zinc Levels in Soil, Olive Fruit and Leaf Samples in Edremit Bay

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Abstract

Edremit Bay is located on west coast of Turkey and region's economy mainly relies on olive products. In this study, Zn(II) content of soil, olive fruit and olive leaf samples obtained from Burhaniye and Küçükkuyu in Edremit Bay were analyzed. Additionally, humic acid contents of soil samples were determined and compared with a commercial fertilizer additive. Determination of Zn(II) in mineralized samples were achieved using flame atomic absorption spectrometer. The humic acids were fractionated and identified using Fourier transform infrared (FT-IR) spectrometer. The obtained results confirmed the relationship between humic acid in soils and zinc availability of plants.

Keywords: Humic acid, zinc, plant nutrition, bioavailability, olive

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INTRODUCTION

Zinc is a trace element found in soil, plants and animals and essential for a healthy growth. This element plays important role in protein and enzyme functions. Insufficient amount of zinc affects the metabolic processes and reproduction in plants^[1-2]. Zinc deficiency is widespread in soils depending to low pH and/or organic matter. In zinc deficiency in plants chlorosis and crinkled/downward curl visualization on leaves can be seen^[3, 4]. Also, zinc deficiency adversely affects physical growth, gastrointestinal and immune system in humans.

The total amount of zinc in soil is distributed fractions such as water soluble, exchangeable, non-exchangeable (adsorbed on insoluble metal oxides) and organically bound (covalently bounded to humic substances)^[5]. It is well known that, availability of trace elements highly depends on the soil properties. The parameters pH, texture and organic matter content may significantly affect the availability of minerals in plants^[6]. Humic

substances (HS) are a general category of naturally occurring organic matter and formed by decomposition of plant remains, animals and microorganisms in soils and sediments^[7]. HS are generally characterized as yellow to black in color, heterogeneous and high molecular weight molecules. Humic (HA) and fulvic acids are the major components of HS. Fulvic acids are completely soluble in aqueous medium, whereas humic acid is insoluble under acidic conditions ($\text{pH} \leq 2$) and can be easily fractionated^[8, 9].

HA and fulvic acid contain carboxylic and phenolic groups and can easily form anionic complexes with metals and covalently bonded species with organic molecules. Therefore, they play important role in the solubility, transport and accumulation of trace metals in soil^[10, 11]. Furthermore, these substances can also ameliorate the deleterious effect of toxicants such as heavy metals and organic pollutants. There are many reports associated to sorption capacities and complexation properties of HAs with

various metal ions such as copper, zinc, iron and cadmium^[12, 13].

The aim of this study is to estimate relations between humic acid content of soil and zinc content of soil, olive leaf and fruit samples. Characterization of HA fractions were achieved by FT-IR and zinc content of samples were determined by FAAS.

MATERIAL AND METHODS

Instrumentation

A Unicam 929A flame atomic absorption spectrometer (FAAS) with deuterium lamp background corrector was utilized for determination of Zn(II) in air-acetylene flame. The hollow cathode lamp for zinc was manufactured by Unicam Analytical System. Working conditions of FAAS is given in Table 1.

Table 1: Experimental Conditions for FAAS.

Element	Wavelength (nm)	Lamp Current (mA)	Burner Height (mm)	Bandwidth (mm)	Fuel Gas Flow Rate (L/min)
Zn	213.9	5	15	0.5	0.58

Mineralization of oil fruit and leaf samples were achieved using Cem Mars 5 Microwave digestion system. Humic acid fractions of soils and commercial fertilizer additive samples were compared with Perkin Elmer BX 1600 FT-IR spectrometer. Thermo Orion 5 Star model pH meter, Sartorius TE214S electronic balance, Heidolph MR 3001 K model magnetic stirrer and Eppendorf Research micro pipettes were used for the present work.

Reagents

All chemicals used were analytical-reagent grade. All solutions were prepared with water that obtained by reverse osmosis system. Glassware and vessels were kept in 10% (v/v) nitric acid for 24 h, rinsed with deionized water and dried in a dust free environment. The 1000 mg/L stock standard solution was prepared using Merck Titrisol 109953 zinc standard (1000 mg zinc; ZnCl₂ in 0.06% HCl). Working standard solutions were prepared by diluting the stock solution. Concentrated HNO₃ (Merck) was used for mineralization of samples. NaOH and HCl (Merck) were used for extraction and precipitation of HAs, respectively. Soil, olive fruit and leaf samples were collected from two locations called Küçükuyu and Burhaniye from Edremit Bay, Turkey. Fertilizer additive sample was obtained

commercially from Balıkesir and used for comparison of HAs.

Preparation of Soil, Fruit and Leaf Samples

Soil samples were collected according to soil sampling protocol (TS 3909) of Turkish Standards Institution (TSE) from two locations (Küçükuyu and Burhaniye). Sampling was achieved at a depth of 0–30 cm from various points for each location. The collected samples were mixed and 1.0 kg of composite soil samples were transported to laboratory in polyethylene bags. The samples were air dried at room temperature, ground in a porcelain mortar and passed through a 2 mm sieve prior to the experiments.

Olive fruit and leaf samples were hand-picked from two locations at the same time and from the same orchards as soil samples. Undamaged leaves and fruits were collected from different sites of trees, separately packaged in labeled polyethylene bags and transferred to the laboratory. The leaf samples were washed with tap water and thoroughly rinsed with pure water. The samples were dried in an oven at 105°C prior to pulverization. The fine powder was put into polyethylene containers and preserved in a desiccator until digestion.

The fruit samples were washed with tap water to remove any adsorbed soil particulates and rinsed with pure water. The surface moisture was removed using filter paper. Then, fruits were pitted with a manual stainless steel pitting machine and the flesh was homogenized with a blender. The homogenized samples were stored in polyethylene containers at -18°C until analysis.

Mineralization of Soil, Fruit and Leaf Samples

Mineralization of soil sample was achieved under reflux with HNO_3 . A 0.1 g sample was weighed accurately on the analytical balance and placed in a 250 mL flask. To minimize sample splash and facilitate digestion, sample was slurried with 0.5 mL pure water. Then, 20.0 mL of aqua regia and heated under reflux for 10 h. Finally, the resultant digest was filtered with a Sartorius filter disc (125 mm diameter, blue dot, grade 391) and diluted up to 100 mL in volumetric flask^[14].

Microwave assisted digestion procedure was carried out for mineralization of olive fruit and leaf samples. Accordingly, about 0.3 g samples were weighed directly in PTFE vessels and 5.0 mL of concentrated HNO_3 was added. Then, the vessels were closed immediately. The operational conditions and the heating program used were carried out according to these conditions: a ramp time of 25 min to reach 150°C and a hold time of 25 min at 150°C . After the vessels had cooled, the solutions were transferred to a volumetric flask and diluted up to 50.0 mL^[15].

The digestion procedures were applied for each sample in triplicate and the blank

solutions were prepared in same way. Zn(II) determination were achieved using FAAS in mineralized samples.

Fractionation of HA

HA was extracted by the dilute alkali method^[16]. A 10 g of soil sample was mixed with 0.1 M NaOH solution (1:10) and incubated at room temperature for 24 h. Then, the mixture was centrifuged for 30 min at 4600 rpm. The supernatant was decanted and then filtered to remove any soil particles.

The pH of the alkaline solution was adjusted to 2 with 6 M HCl for fractionation of humic acid. The mixture was left overnight at $+4^{\circ}\text{C}$ and then filtered to obtain brownish humic acid. Finally, the purified HA was dried at room temperature, ground and stored at room temperature. All HA contents are expressed as "g/kg" by mass on dry soil basis.

RESULTS AND DISCUSSION

Characterization of Humic Acids

Küçükkuyu and Burhaniye soil samples were extracted according given procedure and HA contents were determined. Accordingly, HA contents were 12.3 g/kg for Burhaniye, 3.9 g/kg for Küçükkuyu and 115.0 g/kg for fertilizer additive. The obtained data was compared with literature and it is seen that, the amount of HAs are compatible with soil samples^[17, 18]. The FT-IR spectra of HAs that isolated from Burhaniye, Küçükkuyu and fertilizer additive samples were recorded as KBr pellets and the results given in Figure 1.

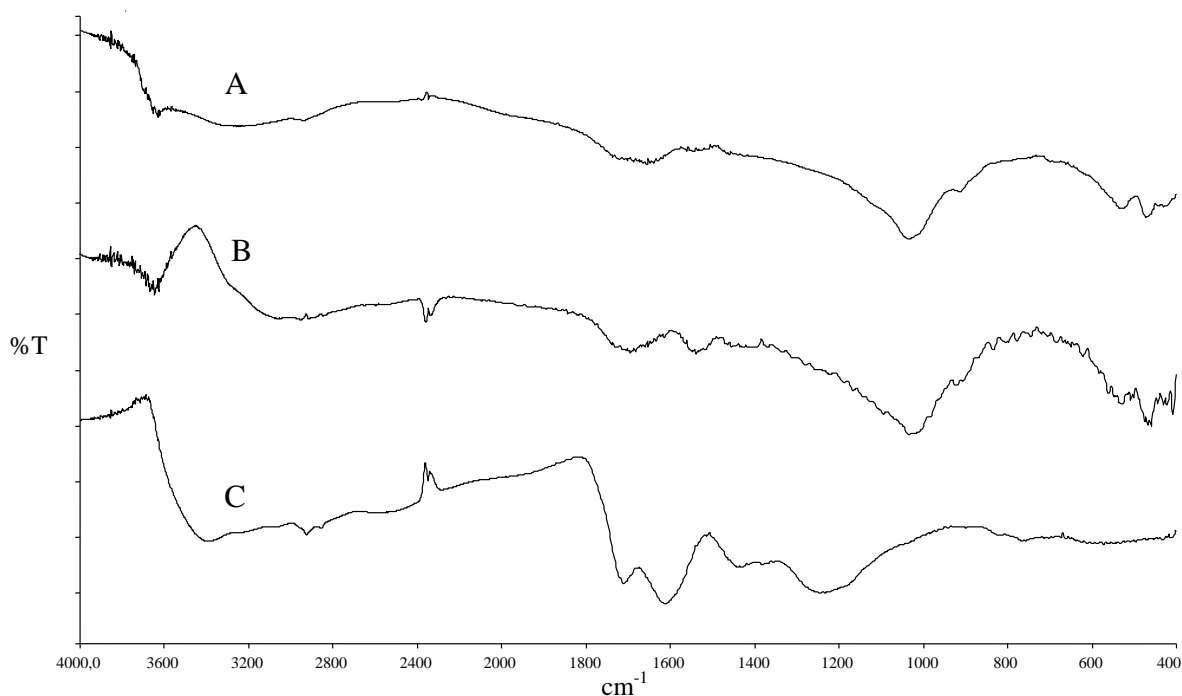


Fig. 1: FT-IR Spectra of HAs Fractionated from Burhaniye Soil Sample (A), Küçükkuuyu Soil Sample (B), Fertilizer Additive Sample (C).

The FT-IR spectra indicate similarities between humic acid fractions of Burhaniye, Küçükkuuyu and commercial fertilizer additive because of similar structures and functional groups. A FT-IR spectrum of humic acid contains typical peaks and reported in numerous studies^[17]. In Figure 1, broad band between 3600–3200 cm^{-1} is due to OH stretching of phenols and/or alcohols. Stretching of aliphatic C-H from 3000–2800 cm^{-1} shows a hydrocarbon skeleton.

Furthermore, carboxylic and ketonic carbonyl stretching (C=O) and stretching vibration of conjugated C=C or H-bonded carbonyl C=O are seen at $\sim 1700 \text{ cm}^{-1}$, at 1630 cm^{-1} , respectively. The strong absorption at 1034 cm^{-1} represents C-O of alcohol and the bands below 1000 cm^{-1} suggest the presence of substituted aromatics.

Zinc Content of Soil, Olive Leaf and Fruit Samples

Zn(II) content of mineralized samples were determined using FAAS. The Zn(II)

levels determined were based on dry weight of soil and leaf samples and wet weight of fruit samples.

The results show that, Zn(II) content of soils were 389.6 and 520.4 mg/kg for Burhaniye and Küçükkuuyu, respectively. A survey of literature reveals that, Zn(II) content of soils varies according to the sampling location such as rural or urban areas. In reported studies total Zn(II) content of soil samples were in the range of 34.6–5929.0 mg/kg^[19–21]. The data showed that total zinc contents of soils are in normal levels for plant growth.

Analysis results for olive fruit and leaf samples of Küçükkuuyu were 18.3 and 41.3 mg/kg for Zn(II), respectively. Similarly, Zn(II) contents were 21.7 mg/kg for fruit and 51.0 mg/kg for leaf in Burhaniye samples. Although, lower concentration of total zinc in Burhaniye soil sample, it is seen that HA plays an important role on the availability and plants growth which absorb more Zn(II), as indicated in literature^[5, 6].

Table 2: Comparison of Zinc Levels in Olive Fruit and Leaf Samples from Different Countries and Regions.

No.	Fruit (mg/kg)	Leaf (mg/kg)	Ref.
1	-	13.3–31.1	[22]
2	6.1–74.6	14.2–128.0	[23]
3	-	~15.0~50.0	[24]
4	6.1–7.7	-	[25]
5	11.16–14.00	12.8–26.80	[26]
6	18.3–21.7	41.3–51.0	This Study

In Table 2, reported results are given for analysis of olive fruit and leaf samples from different countries and regions. It is seen that, accumulation of Zn(II) in fruit and leaf samples is lower than the critical threshold for human^[27]. In any case, direct consumption of olive fruits, even those exhibiting the highest concentrations do not seem to pose any risk on individuals when consumed.

CONCLUSION

It is known that humic substances may significantly reduce water evaporation and increase water holding capacity. HAs are generally used for plant nutrition and to reduce trace metal accumulation in soils. This study offers basic information for total zinc content in soil and olive samples, isolation of humic acid and relationship with zinc content in plant and humic acid amount. However more detailed investigation for a larger area with more sampling is needed to assess the humic acid-zinc relationship. In further studies, it is aimed that determination of fulvic acid amount will be achieved and which humic substance is more affective on Zn(II) availability will be disclosed. Furthermore, fractionation analysis in olive fruit samples can be carried out to obtain bioavailable amount and humic acid-Zn(II) relationship can be demonstrated more clearly.

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