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Assessment of the Relationship Between Humic Acid Contents and Trace Elements of Some Agricultural Soils in Diyarbakır Region by Multivariate Statistical Methods

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ABSTRACT
There are important relationships between humic acid (HA) and the bioavailability, reactions and mobility of trace elements in the soil. For this reason, soils are tried to be improved chemically, biologically and physically with HA applications. In this study, the relationship of humic acid contents of 118 agricultural soil samples from Diyarbakır region with some trace elements (Al, As, Ba, Be, Cd, Fe, Mn, Pb, Sb, Sn, Se, V and P) was evaluated by multivariate statistical analysis. After the soil samples were solubilized by the microwave wet digestion method, the element contents were determined with the ICP OES (Inductively Coupled Plasma Optical Emission Spectrometer) device. SRM NIST 2586 was used as SRM (Standard Reference Material) for the accuracy of the method. Recovery values were found between 91.6% and 105.9% as a result of the analysis. Humic acid was extracted from soils by the International Society for Humic Substances (IHSS) method and determined using a shaker and centrifuge device. For the accuracy of the method, it was tested with Humic Acid Sodium Salt (HA-Na). Pearson correlation and partial correlation analysis were applied to the obtained data set. In addition, multivariate statistical analyses such as multiple regression HCA (Hierarchical Cluster Analysis) and PCA (Principal Component Analysis) were applied. Multiple regression analysis was performed according to the Step-wise method. Manganese and P ($p< 0.01$) were significant when HA was taken as the dependent variable. According to the Pearson correlation coefficient, the correlation between HA and As ($r = -0.282^{**}$) in soil was negative and significant, while Fe ($r = 0.185^{*}$), Mn ($r = 0.273^{**}$)), Sn ($r = 0.242^{*}$), Se ($r = 0.325^{**}$) and P ($r = 0.315^{**}$) were determined as positive and significant. In clustering and PCA analysis, HA, P Mn and Fe were found to be in the same group. The analyses have shown that HA has a positive effect on the plant nutrients in the soil.

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Diyarbakır Yöresindeki Bazı Tarım Topraklarının Hümik Asit İçerikleri ile Eser Elementler Arasındaki İlişkinin Çok Değişkenli İstatistiksel Yöntemlerle Değerlendirilmesi

ÖZET
Hümik asit (HA) ile topraktaki iz elementlerin biyoyarayışlılığı, reaksiyonları ve hareketliliği arasında önemli ilişkiler bulunmaktadır. Bu nedenle topraklar, HA uygulamalarıyla kimyasal, biyolojik ve fiziksel yönden iyileştirilmeye çalışılmaktadır. Bu çalışmada, Diyarbakır yöresinden 118 adet tarımsal toprak örneklerinin hümik asit içeriklerinin bazı iz elementlerle (Al, As, Ba, Be, Cd, Fe, Mn, Pb, Sb, Sn, Se, V ve P) olan ilişkisi çok değişkenli istatistiksel analizlerle değerlendirilmiştir. Toprak örnekleri mikrodalga yaş yakma yöntemiyle çözünürleştirildikten sonra element içerikleri ICP OES (Inductively Coupled Plasma Optical

Toprak Bilimi

Araştırma Makalesi

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Anahtar Kelimeler
İz element
Hümik asit

Emission Spectrometer) cihazı ile belirlenmiştir. Yöntemin doğruluğu için SRM (Standard Reference Material) olarak SRM NIST 2586 kullanılmıştır. Yapılan analiz sonucunda geri kazanım değerleri %91.6 ile %105.9 arasında bulunmuştur. Hümik asit topraklardan International Society for Humic Substances (IHSS) yöntemiyle ekstrakte edilmiş, çalkalayıcı ve santifrüj cihazı kullanılarak belirlenmiştir. Yöntemin doğruluğu için Hümik Asit Sodyum Tuzu (HA-Na) ile test edilmiştir. Elde edilen veri setine Pearson korelasyonu ve kısmi korelasyon analizi uygulanmıştır. Ayrıca çoklu regresyon, HCA (Hiyerarşik Küme Analizi) ve PCA (Principal Component Analysis) gibi çok değişkenli istatistiksel analizler uygulanmıştır. Çoklu regresyon analizi Step-wise yöntemine göre yapılmıştır. Manganese ve P ($p< 0.01$), HA bağımlı değişken olarak alındığında önemli bulunmuştur. Pearson korelasyon katsayısına göre toprakta HA ile As (r = -0.282**) arasındaki ilişki negatif ve anlamlı iken, Fe (r = 0.185*), Mn (r =0.273**)), Sn (r = 0.242*), Se (r = 0.325**) ve P (r = 0.315**) gibi diğer elementlerle pozitif ve anlamlı olarak belirlenmiştir. Kümeleme ve PCA analizinde HA, P Mn ve Fe'nin aynı grupta olduğu saptanmıştır. Yapılan analizler HA'in toprağın bitki besin elementleri lehinde pozitif etki yaptığını göstermiştir.

Korelasyon
Regresyon
ICP OES

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INTRODUCTION

Soil and water are the most important natural resources for living things and all kinds of life forms and essential food fiber and sheltering production necessary for the continuity of human life (Nebel, 1990; Çobanoğlu, 2001). It is very important to know the nature of the soil, and the interactions of the components in the soil with each other. Understanding the relationship between organic and inorganic substances that make up the main body of the soil is necessary for economically feasible, environmentally friendly and sustainable utilization of soil. Humic acids, an economical form of humus, which is the main component of soil organic matter, can support traditional fertilisation methods with significant influences towards enhancing chemical, physical and biological properties of soil, which have resulted in increasing consumption in the intensive agricultural systems. The addition of humic acids to the soil can stimulate the growth of plants beyond the sustaining mineral nutrients. These advantages of humic acids let it common usage all over the world, due to their readiy accessibility and relatively low cost, in poor organic matter-containing soils (Conte et al., 2005; Tarhan, 2011; Prado et al., 2016; Yang & Hodson, 2019).

Iron, aluminium and manganese oxides; organic matter; phosphates, carbonates and sulphides are important components of soil. The deficiency of certain elements especially those of cationic micro elements are commonly observed in the organic matter scarcity in the soils and plant responds to this stress differently. Some heavy metals mainly essential and beneficial plant nutrients play an important role in the nutrition of plants, animals, and humans, but their excessive concentrations can be toxic. The nutritional functions of humic acids appear especially in the absorption of plant nutrients in nutrient-deficient growth environments. Humic acids adsorb some pesticides applied to the soil, especially herbicides and buffer their toxicity to untargeted organisms and, prevent them from mixing into the ground waters. In addition, it has functions in limiting the mobility and availability of some toxic heavy metals as well as harmful radioactive metals to translocate to the plants (Bozkurt, 2005; Eren, 2020).

Potential toxic metals (As, Ba, Be, Se, V, Sn, Sb, Cd, Pb and Al) are the leading factors that cause pollution as a result of agricultural activities and create greater danger over time. These metals, which cause significant pollution in the soil, not only negatively affect productivity in vegetative production, but also threaten human and animal health by entering the food chain (Dağhan, 2011; Eren & Mert, 2017; Eren, 2019).

As the significance considered, the relationships of humic substances and trace elements in agricultural soils have little attention in the current scientific (Donisa at al., 2003). The aim of this study is to evaluate the relationship of some trace elements with humic acid with some multivariate statistical methods.

MATERIAL and METHOD

Study Area and Samples

Diyarbakır City is located in North Mesopotamia and in the South-eastern Anatolia Region of Turkey. The total area of Diyarbakır is 15.355 km^2, of which about 2000 km^2 is classified as urban. It has the largest urban settlement in Tigris Basin. The continental climate of the area is called as a subtropical plateau climate.

A total of 118 soil samples were collected from agricultural soils according to the sampling criteria in different areas and seasons (May, June and July) shown on the map (Fig 1). The samples were stored at ambient temperature condition in sealed plastic bag to preserve the original quality of the soil. The soil samples were dried in an oven at 80 °C for 12 h. and samples were crushed using a rotary mill at 18 000 rpm and then were packaged in the glass bottles.

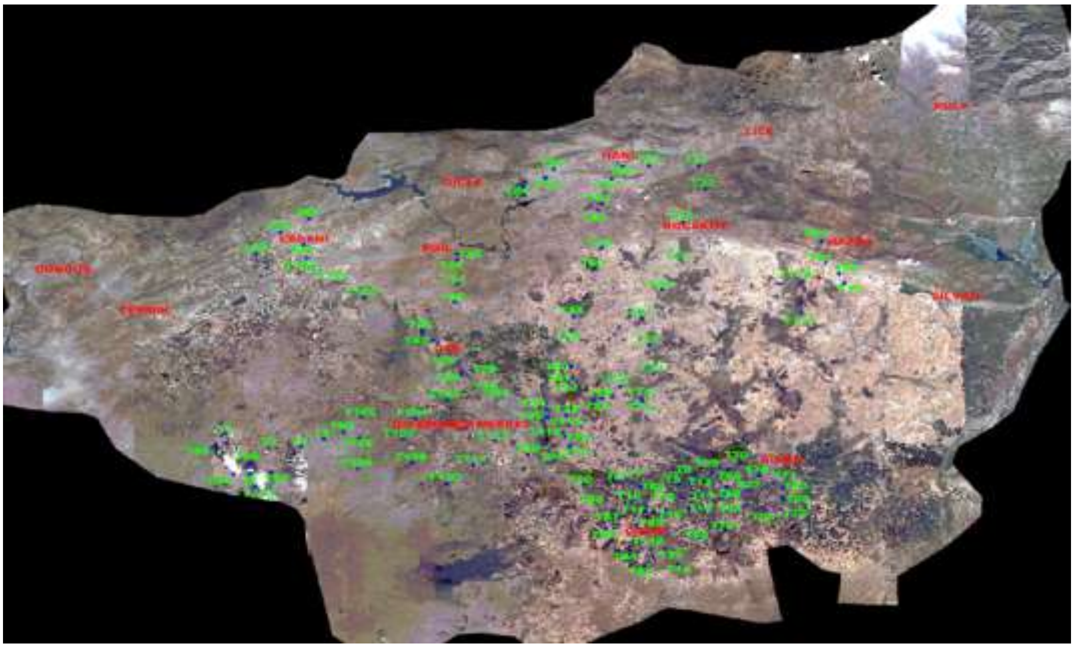

Figure 1. Map of Soil Samples Collected (37.064°, 39.068° latitude, 38.858°, 41.685° longitude)
Şekil 1. *Toplanan Toprak Örneklerinin Haritası (37.064°, 39.068° enlem, 38.858°, 41.685° boylam)*

Reagents and Digestion Procedure

Analytical grade (E. Merck, Darmstadt, Germany) nitric acid (HNO_3, 65%), HCl (37%), HF (40%) and hydrogen peroxide (H_2O_2, 30%) were used for the digestion of soil samples by means of a Milestone Start D microwave digestion system (MW). The procedures were as follows: 0.3 g of soil samples or reference material (SRM 2586 NIST Gaithersburg, MD 20899 SC), the acid mixture (3 mL HNO_3 + 9 mL HCl + 1 mL H_2O_2+ 1 mL HF) were placed in a pressure-resistant PTFE vessels, and was added to each sample and hold on until gas exhausted. The protocol of the MW digestion is given in Table 1. After the digestion procedure, the digests were filtered through Whatman 42 filter paper and diluted to 50 mL with deionized water.

The instrumental operating protocols are given in Table 2 and The Detection and Quantification Limits of Elements, Analytical Wavelengths and Accuracy Assessment of Analysis CRM SOİL-A and SRM 2586 by ICP OES showed in Table 3.

Table 1. Microwave digestion procedure for soil samples
Çizelge 1. *Toprak numuneleri için mikrodalga yakma prosedürü*

Step	***T (min)***	***T (°C)***	***Power (W)***
1	15	150	1200
2	20	150	1200

Accuracy and Precision of Analytical Method

The ICP OES (Thermo ICAP 6300) were calibrated with a multi-element Standard solutions (High-purity, ICV-4, 1408726, Charleston). As, Se and Sb elements were analyzed in hydride system and working standard solutions (10, 20, 40, 60 and 100 µg L^{-1}) were prepared by adding of ascorbic acid and 1.0 % KI and then they were diluted with 10 % HCl as the samples. The quantification (LOQ) and detection limits (LOD) for each metal were calculated as follows: 10 independent analyses of a blank solution spiked with the metal at a level of lower concentration of the analytical curve were performed. The quantification (LOQ) and detection limits (LOD) were calculated from the standard deviation (α) of all measurements determinations (LOD = 3 x (α) and LOQ = 10 x (α). The certified standard reference materials (SRM 2586 NIST Gaithersburg, MD 20899 SC) and (CRM-SOIL-A, Lot:1309920 Charleston) were used to assess the accuracy and precision of the method.

Table 2. Instrumental Operating Conditions Using ICP-OES
Çizelge 2. *ICP OES İçin Cihaz Çalışma Koşulları*

Parameters	*Working conditions*	*Hydride System conditions*
Power	1150 W	1350 W
Rotation speed of pump for Flush	100 rpm	50 rpm
Rotation speed of pump for analysis	50 rpm	30 rpm
Pump rest time	5 sec.	5 sec.
Purge gas	Argon	Argon
Plasma gas	Argon	Argon
Plasma flow	12 L min^{-1}	16 L min^{-1}
Auxiliary flow	0.5 L min^{-1}	0.5 L min^{-1}
Nebulizer flow	0.6 L min^{-1}	0.3 L min^{-1}
Plasma viewing	Radial, Axial	Axial
Source equilibrium delay	20 sec.	20 sec.

Table 3. The Detection and Quantification Limits of Elements, Analytical Wavelengths and Accuracy Assessment of Analysis CRM SOİL-A and SRM 2586 by ICP OES
Çizelge 3. *Elementlerin Tayin ve Dedeksiyon sınırları, Dalgaboyları ve CRM SOİL-A ile SRM 2586 Referans Maddelerinin ICP OES ile Analizlerinin Sonuçları*

			CRM-SOİL-A			*SRM - 2586*		
Chemical Elements (λ nm)	*LOD ($\mu g\ g^{-1}$)*	*LOQ ($\mu g\ g^{-1}$)*	*Found values ($\mu g\ g^{-1}$)*	*Certified values ($\mu g\ g^{-1}$)*	*Recovery (%)*	*Found values ($\mu g\ g^{-1}$)*	*Certified values ($\mu g\ g^{-1}$)*	*Recovery (%)*
Al (396.152)	0.0716	0.2389	495±5.500	500±3.000	99	63526±540	66520±760	95
As (189.042)	0.0016	0.0050	0.19±0.002	0.20±0.010	95	8.2±2.200	8.7±1.50	94
Ba (493.409)	0.0015	0.0051	4.85±0.060	5.00±0.050	97	402±24.0	413±18.0	97
Be (234.861)	0.0005	0.0017	-	-	-	1.32	1.4	94
Cd (214.438)	0.0005	0.0018	0.0029±0.00025	0.003	97	2.60±0.620	2.71±0.54	96
Fe (259.940)	0.0190	0.0634	203.6±2.500	200±1.00	102	50315±962	51610±890	97
Mn (257.610)	0.0017	0.0057	0.098±0.002	0.100±0.001	98	1024±32.0	1000±18.0	102
Pb (261.418)	0.0227	0.0759	0.38±0.030	0.40±0.020	95	412±13.0	432±17.0	95
Sb (206.833)	0.0067	0.0224	0.031±0.003	0.030±0.001	103	-	-	-
Sn (189.918)	0.0037	0.0123	-	-	-	-	-	-
Se (196.090)	0.0030	0.0100	0.0095±0.0008	0.010±0.001	95	0.55±0.090	0.6	92
V (292.402)	0.0077	0.0259	0.98±0.050	0.100±0.005	98	152±12.0	160	95
P (177.495)	0.1682	0.5606	10.6±0.080	10.0±0.100	106	1029±96.0	1001±77.0	103

Isolation of Humic Acid From Soil

The humic acid (HA) coverage of soil (T) samples were studied using extraction techniques reported by the International Society for Humic Substances (IHSS) (Schnitzer & Khan 1972; Stevenson, 1982). 1 g of Humic acid sodium salt was weighed into the centrifuge tube and 50 ml of 0.5 N NaOH was added. 200 rpm for 20 hours at 25 °C in a shaker and agitated. After the agitation was completed, the extract separated by decantation was centrifuged at 6000 rpm for 30 minutes and all the suspended solid part was separated from the solution phase. This process was continued until the solution turned into a light tea color. All the extracts were then combined and the alkaline solution completely separated from the solid phase was acidified with 6 M HCl to a pH between 1.0 and 2.0 (pH meter, Inolab 720). Humic acid precipitated because its solubility was very low between pH 1.0 and 2.0. After the solution was kept in the refrigerator for 24 hours, the humic acids became visible and dark colored humic acids were obtained. Humic acid was dried in an oven at 65 °C for 24 hours and its amount was calculated according to the formula below.

The same procedures were applied to 118 soil samples, the amount of soil was taken as 50 g.

% Humic Acid = (m/n).100

m: sample weighed after oven (g)

n: ODW of the sample taken (Oven dry weight value, g)

Statistical Analysis

The data were subjected to multivariate statistical analysis procedures by using SPSS 21.0 statistical package programs. In order to reveal the coherence between the measured parameters and differentiate the origin of metals Pearson correlation, multiple regression, cluster analysis (CA) and principal component analysis (PCA) were performed after normalizing the data set (Lu et al. 2012; Zhang, 2006). The Kaiser-Meyer-Olkin (KMO) coefficient above 0.60 showed that our data set was suitable for PCA. The Barlett Test of Sphericity significance value was found to be 0.01. PCA results were interpreted according to hypothetical chemical element sources (Peris et al., 2008; Yuan et al., 2013). Hierarchical CA was performed according to Ward method (Cai et al., 2012; Chen et al., 2012; Franco-Uria et al., 2009; Mico et al., 2006; Xia et al., 2011). The results are summarized in a dendrogram. As the K-S (Kolmogorov Smirnov) normality test revealed that the data-set can be considered normally distributed ($p>0.05$) unless otherwise the related data set were transformed by log transformation. Differences between applications were considered significant if $p<0.05$ and when the analysis results were below the LOD, they were accepted as the half of the LOD.

RESULTS and DISCUSSION

In this study, a total of 118 soil samples collected from agricultural lands in different regions of Diyarbakır determined the relationships between humic acid (HA) and trace elements (Al, Fe, Ba, P, Sn, Cd, Pb, V, Sb, As, Mn, Be, Se). Statistical analysis such as Pearson correlation multiple regression analysis, HCA (Hierarchical clustering analysis, Fig. 5-6) and PCA (Principle component analysis, Fig. 3) were applied to results and The amount of humic acid obtained from soil samples showed in Table 4.

Pearson correlation was applied to results and the relationship in between HA and elements evaluated by coefficient showed a correlation of elements and humic acid with each other. Pearson correlation coefficient indicated that As showed a negative correlation with HA, while Fe, Mn, Sn and Se showed a positive correlation with humic acid and all variables stuations were showed in Table 6.

Partial correlation coefficients showed variables situations when HA stabilized and all coefficients were showed in Table 7.

The descriptive statistics for the total trace element concentration of experimental soils showed in Table 5.

The results of HA were not showed normal distribution therefore log transformation applied to results and results were showed normalized distribution (Fig. 2).

Principal component Analysis (PCA) was applied to data-sets before this KMO (Kaiser-Mayer-Olkin) coefficient and Barlett globality test were determined. These coefficients showed data suitable for component analysis. KMO coefficient of 0.624 was found and Barlett globality test was significant at $p\leq 0.01$. When scree plot Eigenvalue bigger than 1 value, it were found as 5 components, P1: Mn, Fe, Pb, Cd, Sb, P2: HA and P, P3: V, Al, As, P4: Be, Se, P5: Ba and Se (Fig.3)

Table 4. The amount of humic acid obtained from soil samples
Çizelge 4. *Toprak örneklerinden elde edilen hümik asit miktarı*

Samples	*HA (%)*	*Samples*	*HA (%)*	*Samples*	*HA (%)*	*Samples*	*HA (%)*	*Samples*	*HA (%)*
T1	0.360	**T25**	0.102	**T49**	0.390	**T73**	0.171	**T97**	0.092
T2	1.020	**T26**	0.142	**T50**	0.142	**T74**	0.152	**T98**	0.040
T3	0.133	**T27**	0.096	**T51**	0.440	**T75**	0.095	**T99**	0.085
T4	0.112	**T28**	0.170	**T52**	0.106	**T76**	0.113	**T100**	0.076
T5	2.340	**T29**	0.455	**T53**	0.134	**T77**	0.106	**T101**	0.070
T6	0.133	**T30**	0.146	**T54**	0.200	**T78**	0.130	**T102**	0.087
T7	0.390	**T31**	0.126	**T55**	0.230	**T79**	0.103	**T103**	0.230
T8	0.146	**T32**	0.211	**T56**	0.080	**T80**	0.133	**T104**	0.158
T9	0.076	**T33**	0.170	**T57**	0.076	**T81**	0.120	**T105**	0.177
T10	0.115	**T34**	0.130	**T58**	0.122	**T82**	0.132	**T106**	0.172
T11	0.230	**T35**	0.144	**T59**	0.151	**T83**	0.105	**T107**	0.166
T12	0.112	**T36**	0.090	**T60**	0.441	**T84**	0.156	**T108**	0.082
T13	0.121	**T37**	0.070	**T61**	0.092	**T85**	0.142	**T109**	0.360
T14	0.155	**T38**	0.141	**T62**	0.181	**T86**	0.130	**T110**	0.933
T15	0.074	**T39**	0.221	**T63**	0.133	**T87**	0.095	**T111**	0.110
T16	0.142	**T40**	0.154	**T64**	0.132	**T88**	0.097	**T112**	0.126
T17	0.190	**T41**	0.126	**T65**	0.232	**T89**	0.113	**T113**	0.172
T18	0.112	**T42**	0.110	**T66**	0.174	**T90**	0.220	**T114**	0.131
T19	0.121	**T43**	0.087	**T67**	0.190	**T91**	0.116	**T115**	1.230
T20	0.100	**T44**	0.121	**T68**	0.240	**T92**	0.076	**T116**	0.910
T21	0.133	**T45**	0.235	**T69**	0.122	**T93**	0.152	**T117**	0.210
T22	0.164	**T46**	0.090	**T70**	0.151	**T94**	1.920	**T118**	0.111
T23	0.100	**T47**	0.086	**T71**	0.177	**T95**	0.104		
T24	0.122	**T48**	0.132	**T72**	0.160	**T96**	1.080		

*:significant ($P<0.05$), **:significant ($P<0.01$)

Table 5. Descriptive statistics for total trace element concentration of experimental soils
Çizelge 5. *Toprakların toplam iz elementi içeriğine ait tanımlayıcı analizler merkezi eğilim ve dağılım ölçüleri*

Elements	*N*	*Mean*	*Median*	*Standard deviationnn*	*standard error*	*Distorti*	*Stickiness*	*Min.*	*Max.*	*CV (%)*
Al	118	45967.9	44811.5	16117.9	1483.7	-0.401	0.129	1522	81696	35.06
As	118	6.77	7.29	4.42	0.406	-0.083	-0.839	0.0008	16.5	65.24
Ba	117	209.9	203	75.03	6.93	1.023	3.072	63	555	35.74
Be	116	0.746	0.74	0.371	0.034	1.734	8.641	0.0003	2.88	49.74
Cd	118	0.968	0.945	0.487	0.044	-0.161	-0.311	0.00025	2	50.27
Fe	118	46587.7	45236	14651.3	1348.7	0.470	0.327	13367	86647	31.44
Mn	118	1249.6	1126.5	529.4	27.5	1.352	1.474	419	3006	42.36
Pb	118	40.5	40.25	11.3	1.03	0.511	0.778	15	74.8	27.85
Sb	118	4.43	4.37	1.81	0.16	0.767	0.680	1.54	9.92	40.79
Sn	118	0.495	0.435	0.248	0.02	1.484	3.029	0.08	1.55	50.10
Se	115	0.856	0.83	0.464	0.04	1.011	2.741	0.093	2.8	54.20
V	89	134.1	133	51.8	5.49	-0.041	-0.226	5.83	252	38.62
P	118	860.2	787	304.6	14.44	1.779	4.578	200	2154	35.40
HA (%)	118	0.221	0.133	0.316	0.029	4.643	24.471	0.040	2.340	143.63

Tablo 6. Humik asit ve toplam iz element konsantrasyonları arasındaki Pearson korelasyon katsayısı
Çizelge 6. *Pearson correlation coefficients between humic acid and total trace element concentrations*

	Al	*As*	*Ba*	*Be*	*Cd*	*Fe*	*Mn*	*Pb*	*Sb*	*Sn*	*Se*	*V*	*P*	*HA*
Al														
As	-0.178													
Ba	0.398**	-0.411**												
Be	-0.035	-0.060	-0.043											
Cd	0.020	-0.030	0.005	-0.027										
Fe	0.623**	-0.405**	0.428**	0.054	0.032									
Mn	0.374**	-0.595**	0.471**	0.021	0.104	0.734**								
Pb	0.105	0.008	0.129	0.057	0.338**	0.245**	0.225*							
Sb	0.181*	-0.311**	0.082	0.231*	0.352**	0.441**	0.515**	0.252**						
Sn	0.112	-0.279**	0.193*	-0.220*	-0.175	0.250**	0.208*	0.023	0.066					
Se	0.135	-0.066	0.025	0.179	-0.007	0.447**	0.368**	0.140	0.299**	0.224*				
V	-0.451**	-0.399**	0.100	0.029	0.043	-0.088	0.248*	-0.237*	-0.005	0.299**	0.103			
P	-0.081	0.143	-0.071	-0.113	0.101	-0.035	-0.042	-0.125	-0.046	0.161	0.138	0.111		
HA	-0.004	-0.282**	0.002	-0.098	0.082	0.185*	0.273**	0.011	0.087	0.242**	0.325**	0.040	0.315	

Table 7. The Partial correlation relationship in between elements and stabilized humic acid in soil samples
Çizelge 7. *Toprak örneklerinde elementler ve stabilize hümik asit arasındaki kısmi korelasyon ilişkisi*

	Al	*As*	*Ba*	*Be*	*Cd*	*Fe*	*Mn*	*Pb*	*Sb*	*Sn*	*Se*	*V*	*P*
Al													
As	0.020												
Ba	0.268*	-0.324**											
Be	-0.010	-0.160	0.005										
Cd	0.423**	-0.045	0.135	-0.246*									
Fe	0.636**	-0.221*	0.310**	0.223*	0.555**								
Mn	0.283**	-0.479**	0.361**	0.204	0.424**	0.663**							
Pb	0.265*	-0.131	0.249*	-0.075	0.465**	0.509**	0.507**						
Sb	0.188	-0.141	-0.037	0.289**	0.244*	0.521**	0.579**	0.300**					
Sn	-0.002	-0.141	0.120	-0.270*	0.169	0.070	0.109	0.118	-0.015				
Se	0.094	0.068	-0.127	0.257*	-0.098	0.323**	0.216	0.150	0.276*	0.293**			
V	-0.567**	-0.310**	0.005	0.145	-0.124	-0.273*	0.119	-0.206	-0.097	0.225*	0.006		
P	-0.138	0.379**	-0.173	-0.054	-0.055	-0.318**	-0.338**	-0.303**	-0.157	-0.014	-0.051	-0.084	

When we performed multiple regression analysis with Stepwise Method, it was possible to develop two models with P and Mn at significance level $p<0.01$ when the dependent variable was HA. In the regression model, $R^2 = 0.407$, $F = 23.69$ were found. The model equation is shown below; HA= -0.294+0.000201*Mn+0.000282*P. The R^2, determination coefficient, F is Fisher coefficient, N is the sample number. As the HA independent variable, The HA can explain a significant portion of variation (R^2) in the dependent variable, as shown in Fig 4 for As, Mn, P, and Se in the regression models.

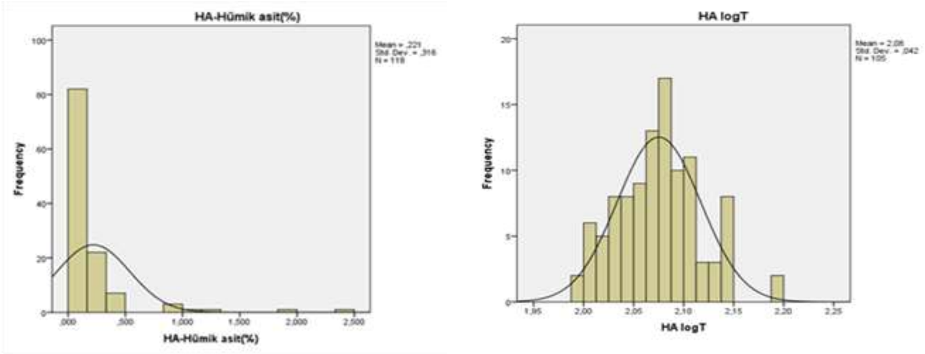

Figure 2. Histograms with normal distribution curve for the original and log-transformed data set
Şekil 2. *Orijinal ve log dönüşümü ile normalleştirilmiş veri setinin histogram ve normal dağılım eğrisi*

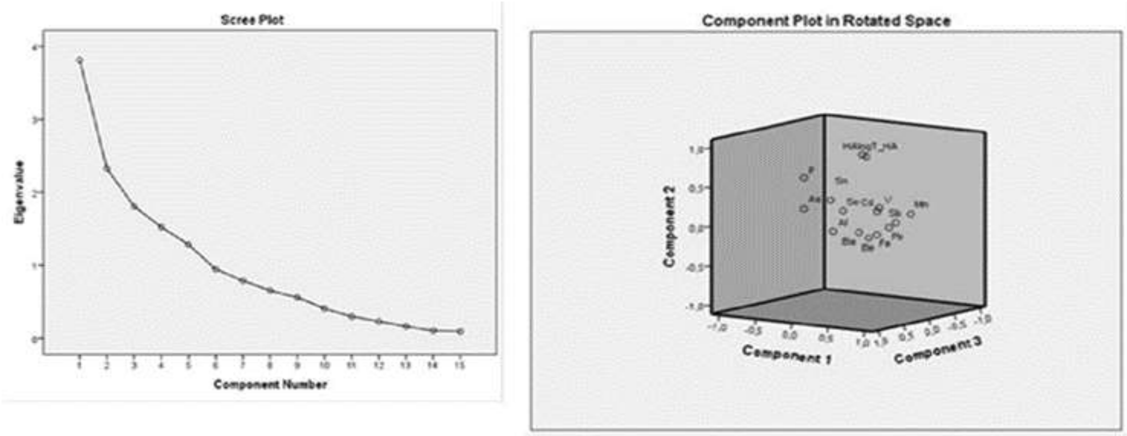

Figure 3. Scree plot and component graphs
Şekil 3. *Scree plot ve bileşen grafikleri*

Statistical data of HA and some elements

According to the results of the statistical analysis data, the relationship between humic acid and phosphorus element was better than other elements. A significant portion of soil P is bound to soil organic matter (Stevenson, 1994) and soil organic matter can only accumulate in recalcitrant fractions such as HA, fulvic acids and humin (Usta, 1995). According to the results, minimum and maximum values of P in soil were between 200- 2154 mg kg^{-1}, and the CV values were found as 35.40%. In the factor (PCA) analysis, HA and P were in the same principal component (N:118). This indicates above mentioned co-occurrence and co-accumulation fact of P and soil organic matter. According to Pearson correlation, P was found to show a significant positive correlation with HA (r = 0.315**). In the multiple regression analysis, a significant regression model was observed with P and Mn (P <0.01) when HA was the dependent variable with Step-wise regression procedures. The hierarchical cluster and factor analysis results pointed out similar coherence between P and HA.

The descriptive statistics describing the nature and occurrence of some elements and humic acid were calculated and briefly given below.

Aluminium: The mean, minimum and maximum values of Al were 45967.9, 1522, and 81696 mg kg^{-1}, respectively. The coefficient of variation (CV) was found to be 35.06%. In factor analysis, Al, V, and As were in the same in the same principal component (N: 118), in addition, Al, Ba, Cd, Mn, and Fe were also in close relation in the CA (Cluster analysis). The PCA and correlation analysis were relived a significant correlation coefficients Al-V (0.81), As-Fe (0.85), As-Pb (0.56), Cd-Sn (0.71), Fe-Mn (0.70), Fe-V (0.59) are reported in the literature (Rodriguez et al., 2015). The PCA and CA analysis aggregate Fe and Mn always in the same group due to the accumulation of these elements in the soil formation processes. A significant correlation was found between Fe and humic acid (r = 0.383*) according to Gürel et al. (2015). It can be stated that the soil organic matter and Fe concentrations of soils developing from any parent material is largely dependent on the weathering levels controlled by mainly water and thermal regime of any specific location (Weil and Brady, 2016; Usta, 1995). In general, the more weathering causes the higher organic matter, including HA and sesquioxides (mainly Fe, Al oxi(hyd)oxides in the soil) accumulation.

Regression graphs of some elements showing significant correlation with humic acid showed in Fig.4.

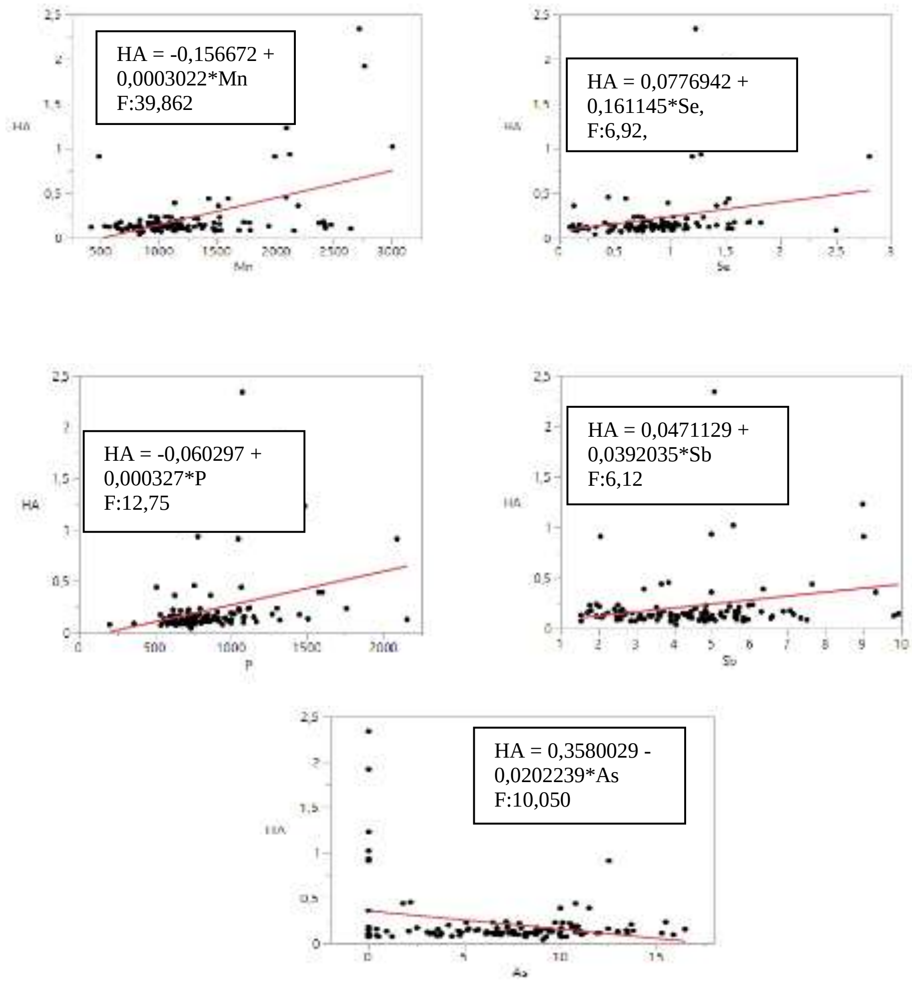

Figure 4. Regression graphs of some elements showing significant correlation with humic acid
Şekil 4. *Hümik asit ile önemli korelasyon gösteren bazı elementlerin regresyon grafikleri*

Cadmium: The mean, minimum and maximum values of Cd were found as 0.968, <0.0005, and 2 mg kg^{-1}, respectively. The CV value was 50.25%. In factor analysis, Cd, Mn, Fe, Pb, and Sb were the same factor and in cluster analysis Cd, Mn and Fe were in the same group (N: 118). This can be explained as Fe and Mn oxides have excessive adsorption capacity for Cd. (Alloway, 1996).

Humic Acid: The mean, minimum and maximum values of HA were determined as 0.221, 0.040, and 2.340 (%), respectively. According to Şahin (2012), HA reported a HA abundance of 29.3% in Chernozem soil while Gürel et al. (2015) found HA content between %0.35 and %2.09 in the Blacksea region's soils, moreover according to Tokay and Yaşar (2008) in Küçükkuyu and Burhaniye soils HA were found %0.38 and %1.23 respectively. CV value of humic acids in the soils was found as 143.63% and the range and variation of the results were too large, the data were far from normal distribution thus log transformation was applied to normalize the data. In factor and HCA analysis HA and P were the same group (N: 118).

Hierarchical clustering analysis was performed and the summarized version was given in the form of a dendrogram. When all variables are taken into account, the clustered version of the samples and the clustered version of the variables on the basis of the samples are given in Figure 5 and Figure 6.

CONCLUSIONS

Heavy metals were found to be above the permissible threshold values in some soils, but generally, the soils of the region were found to be in safe range for the respective trace elements. Both Pearson correlation analysis and multiple regression, PCA and CA analyses showed that the elements useful to the soil (P, Fe, Mn and Se) were more attached to the soils with

HA, whereas toxic elements such as As and Cd were less bound. Naturally, this is an important condition for the healthy growth of plants. Therefore, it has been concluded that HA can be a good regulator for agricultural soils and can be used for this purpose.

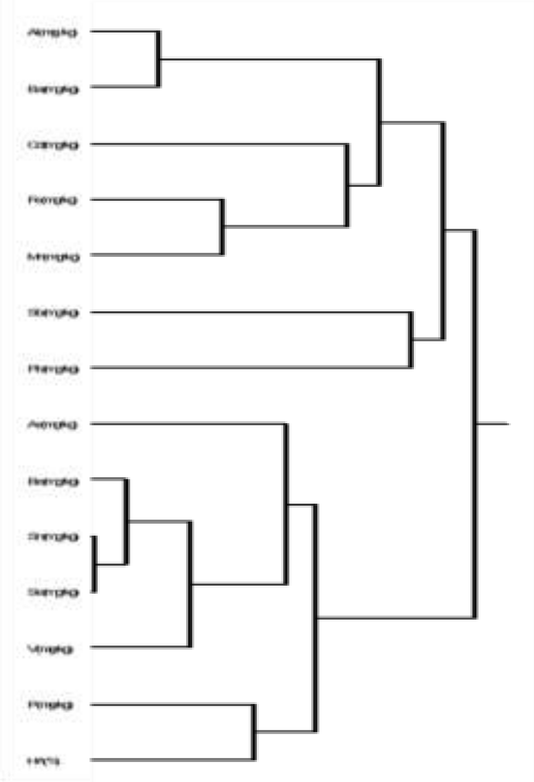

Fig. 5. Dendrogram of clustering analysis of variables
Şekil 5. *Değişkenlerin kümeleme analizinin dendrogramı*

Except for Karacadağ region, all agricultural soils in Diyarbakır province were found to be poor in terms of humic acid (Table4, Fig 1). Thus, it is advisable for producers to improve their soils with HA fertilizers.

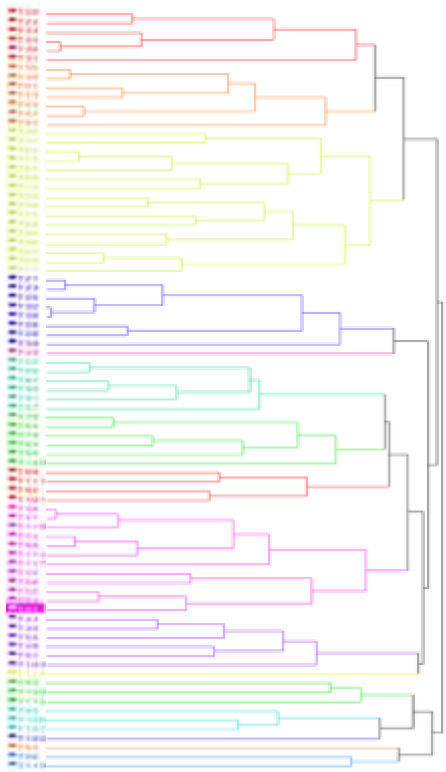

Fig. 6. Dendrogram of clustering analysis of all samples
Şekil 6. *Tüm örneklerin kümeleme analizinin dendrogramı*

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Contribution rate statement summary of researchers

The authors declare that they have contributed equally to the article.

Conflict of interest statement

The authors of the article declare that there is no conflict of interest between them.

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