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Research Article

Evaluation by Multivariate Statistical Methods of Relationship with Physicochemical Properties of Humic Acid and Some Soil

***Mehmet DÜZGÜN**
Orcid No:0000-0003-4269-5544
****Uğur BİLGE**
Orcid No: 0000-0001-8909-723X
*****M. Zahir DÜZ**
Orcid No: 0000-0002-3642-4206

*****GAP International Agricultural Research and Training Center, Diyarbakir, Turkey (Corresponding author)

**GAP International Agricultural Research and Training Center, Diyarbakir, Turkey

***Dicle University, Faculty of Science, Department of Chemistry,

mehmet.duzgun@tarimorman.gov.tr

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Abstract

Humic acid is a very important component for soil. In particular, It is an important factor for regulation of plant nutrients. In this study, HA (humic acid) contents of soil samples taken from 12 different locations were determined by appropriate method. After extracting the humic acid from the soil samples, the remaining soil elements concentrations were determined by ICP-OES method. In addition, the element concentrations in the humic acid fraction were determined by the same method. In soil samples were determined organic matter (OM), pH, EC, clay, silt and sand. The relationships between all variables were evaluated by multivariate statistical analysis such as correlation, simple linear regression, PCA (Principal Component Analysis). Spearman rho coefficient was taken into consideration in the correlation analysis. Simple linear regression analysis showed significant regression model between HA and OM, pH, HAfMn (HAf: humic acid fraction), HAfSe, HAfP, TfCd (Tf:soil fraction), TfMn, TfPb, TfSe. In the PCA analysis, 4 factors were found to explain 88.23% of the total change. P1:OM, TPb, TSe, HA P2:TAs, TSb, TBa, TMn, TFe P3: TCd, TP, pH, TSn, Silt, TAl P4: Kil, sand, TBe, EC. In the correlation analysis (N: 12), HA was significant positive with TfSe ($r= 0.615$, $P <0.05$), and significant negative with HAfBe ($r= -0.786$, $P <0.01$) and significant positive with HAfMn ($r= 0.918$, $P <0.01$) and significant negative with HAfSn ($r= -0.700$, $P <0.05$) and significant positive with HAfSe ($r= 0.704$, $P <0.05$) and significant positive with HAfP ($r= 0.700$, $P <0.05$) correlation was found.

Keywords
Humic acid, regression, correlation, ICP-OES

INTRODUCTION

Soil and water are the most important vital sources of people like other living things. Because they form the basis of food production for their lives (Nebel, 1990, Çobanoğlu 2001). The structure of soil, the interactions of components in the soil and their relationship with each other is very important. The determination of the relationships between organic and inorganic substances forming in the soil structure is necessary for improvement and development of healthy products in the soil. The most important factor in the normal growth of plants is the presence of nutrient elements in the soil that they can use when they need it. Soil is the most important component of organic matter. The most important active biochemical component of the soil is humic acids. Advances in agricultural practices play an active role in the rapid depletion of humus which facilitates the uptake of these fertilizers while increasing use of chemical fertilizers. Humic acids can be replace the traditional fertilization methods to support soil structure. The addition of humic acids to the soil can be stimulating the growth of plants beyond the effect of the mineral nutrients. Humic acids are effectively used all over the world due to their benefits in soils containing low organic matter. In the recent years, the benefits of organic matter in the soil and especially humic acids have been scientifically proven. Humic acids help plant growth by providing biological activities and mineral material needs of soil. Humic acids are prevent mixing to the ground water from soil by absorbing the toxic effects of some harmful heavy metals, pesticides and herbicides taken by plants. Potential toxic metals (As, Ba, Be, Se, V, Sn, Sb, Cd, Pb and Al,) are causes of pollution leading by agricultural and industrial activities. These toxic metals, which are causes significant pollution in soil, have negative effects on the productivity of the plant production and they also threaten human and animal health by entering the food chain. The importance of the food products grown in the soil is important in terms of their mineral content and the elements that are applicable for such foods are both nutritional and toxicological. Humic substances in the soil to remove unwanted cations and heavy metal pollutants as well as beneficial elements to plant nutrition. Phosphates and natural minerals in soil are well documented for their ability to dissolve and complex with humic substances. The elements released from rock minerals are facilitates the bioavailability with microbiological activity of the plant in the presence of humic substances. The aim of this study is to determine the concentration of the elements remaining in the 12 soil samples after the extraction of humic acid and the concentration of the elements passing to humic acid and to make some statistical evaluations. The soil samples were studied heavy metals such as Al, As, Ba, Be, Cd, Fe, Mn, Pb, Sb, Sn, Se, V, P concentrations using by ICP-OES technique. This study to provide valuable information on the mineral characteristics and humic substences of soil samples in around Diyarbakır were evaluated with multivariate statistical methods.

MATERIALS and METHODS

The taken 12 samples of soils 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 105 °C for 12 h. The samples were pulverized with a rotary mill at 18 000 rev min^{-1} and then were packaged in the glass bottles. The points where the samples were taken are shown in Figure 1.

Reagents and Digestion Procedure

The reagents and acids used in this study were of analytical grade and used without further purification. Distilled water was further purified with a Milli-Q ® system (Millipore Corporation, USA) and used throughout the experiments. Nitric acid (HNO_3, 65%) and Hydrogen peroxide (H_2O_2, 30%) were used for the digestion of analytical grade (E. Merck, Darmstadt, Germany). A Milestone Start D microwave digestion system was used for digestion of the soil samples. Microwave (MW) digestion was studied as follows: 0.3 g of soil samples and SRM 2586 NIST Gaithersburg, MD 20899 SC reference material were weighed and transferred into pressure-resistant PTFE vessels. The acid mixture, 3ml HNO_3 + 9ml HCL + 1ml H_2O_2 + 1ml HF was added to each sample and hold on until gas exhausted. The program of the MW digestion is listed in Table I. After the digestion procedure, the residue was filtered and diluted to 50 ml Milli-Q™ water (Millipore Corporation, USA).

Table 1 Temperature program of the microwave digestion system for soil samples

Step	**T (min)**	**T (^{0}C)**	**Power (W)**
1	15	150	1200
2	20	150	1200

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Instrumentation

A model ICAP 6300 Duo Thermo Scientific (ICP-20113107, England) ICP-OES, radial for high concentrations and axial for low concentrations was studied for the determination of Al, Ba, Ca, Mg, P, K, Na, As, Sb, Se, Sn, Be, Fe, Mn and Cd in diluted solutions of the samples. The most sensitive lines without spectral interferences in the sample matrix were used for the analysis. All of the method from sampling to analysis were also applied to blanks in order to evaluate any metal contamination during the analytical procedure.

Table 2. Instrumental operating conditions using ICAP 6300 duo thermo scientific ICP-OES

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.	16 L/min.
Auxiliary flow	0.5 L/min.	0.5 L/min.
Nebulizer flow	0.6 L/min.	0.3 L/min.
Plasma viewing	Radial,Axial	Axial
Source equilibrium delay	20 sec.	20 sec.

Physicochemical Properties of Soil and Isolation of Humic Acid

The texture of the soil according to the Bouyoucos hydrometer method, pH and electrical conductivity (EC) in the saturation sludge, and cation exchange capacity (CEC) by sodium acetate method were determined. Organic matter and Organic carbon(OC) in soil samples were determined by Walcley-Black wet burning method and the results were converted to organic matter values by multiplying with 1.72 organic matter conversion coefficient (table 5). The humic acid (HA) coverage of soil (S) samples was studied using extraction techniques reported by the International Society for Humic Substances (IHSS). Approximately 50 g of soil sample was added to the flasks and 100 ml of 0.5 N NaOH was added and shaken 20 hours, at 25 ^{o}C and 200 rpm in a shaker. After shaking, the extract separated by decantation was centrifuged in 5 min. at 6000 rpm and the suspended solids were separated from the solution phase the obtained red solution was divided into two parts. A part of red solution was titrated with 6M HCl to precipitate humic acids (pH 1-2) and the precipitate was separated by centrifugation and then the humic acids were dried and weighed. Since there is no Standard Reference Material for humic acid, the analysis was made by purchasing the item from Sigma-Aldrich (Humic acid sodium salt lot: STBF9164V, Germany) to evaluate the accuracy of our method. The results we found were close to the content of the material. The label value is 50-60% and and we find 59%. The amount of humic acid in the soil sample was determined by the calculations. The other part of the red solution was determined by ICP-OES to detect trace elements in humic matter. The results are shown in the table 5.

Statistical Analysis

SPSS 21.0 were evaluated using multivariate statistical methods by using statistical package programs.Spearman rho correlation, which is a nonparametric correlation, was taken as the basis for the non-normal and small observations. Regression and PCA (Prencipal Component Analysis) was applied to results. Differences between applications were considered significant if p value was less than 0.05 ($p < 0.05$). Smaller values than LOD were evaluated by taking half of the LOD.

RESULTS and DISCUSSION

In this study, the limit of detection (LOD), the limit of quantification (LOQ) and the certified reference materials were used in order to verify the accuracy of ICP-OES method for each element. The accuracy of the entire proposed method was confirmed by standard reference material analysis. The physicochemical properties of some soil (12 samples, Table 3) with the high HA ratio extracted from soil were determined. The multivariate statistical analysis such as correlation, regression, PCA (Principle component analysis),

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Table 3 . Physicochemical properties of some soil samples and the amount of humic acid. (CEC: Cation Exchange Capacity, OC ; Organic carbon)

Sample No	Organic matter (%)	OC	CEC (meq/100g)	HA (%)	pH	EC	Clay (%)	Plate (%)	Sand (%)	Texture
S116	2.31	1.39	44	0.910	7.50	0.29	30.74	39.14	30.12	Clay- Loam
S115	2.51	1.45	-	1.230	6.60	0.22	45.50	29.50	25.0	Clay
S113	0.70	0.13	31.4	0.172	7.83	0.31	55.24	22.88	21.88	Clay
S37	0.48	0.28	30.7	0.070	8.16	0.16	14.52	16.44	69.40	Sand -Loam
S19	0.77	0.45	-	0.121	8.10	0.23	29.04	18.56	52.40	Sand-Clay-Loam
S29	3.42	1.98	-	0.455	7.95	0.24	47.60	25.36	27.04	Clay
S94	5.54	3.21	35.6	1.920	6.15	0.25	30.45	20.35	49.20	Sand-Clay-Loam
S46	0.76	0.44	-	0.090	7.71	0.30	66.96	17.56	15.48	Clay
S60	4.52	2.62	25.3	0.441	7.87	0.25	43.10	25.66	31.24	Clay
S47	1.30	0.75	33.3	0.086	7.70	0.43	53.04	25.72	21.24	Clay
S5	11.2	6.49	39.3	2.340	6.95	0.43	46.20	29.30	24.50	Clay
S2	3.39	1.92	33.2	1.020	6.12	0.30	27.70	43.27	29.03	Loam

Table 4. The Analysis of elements 12 soil samples fraction obtained from different area of Diyarbakır (mg/kg)

Samples	Al	As	Ba	Be	Cd	Fe	Mn
T116	21432±357	2.63±0.07	33±0.87	0.24±0.01	2±0.01	19437±321	326±4
T115	66159±754	<0.0016	150±1.05	1.11±0.01	1.5±0.09	10086±290	2223±23
T113	58296±795	1.15±0.15	129±2.85	0.74±0.03	2.34±0.07	51795±823	666±11
T96	22030±228	<0.0016	104.1±1.13	<0.0005	<0.0005	80450±713	1413±11.4
T37	58488±2005	<0.0016	152±1.05	0.08±0.008	2.14±0.07	33648±272	667±21
T19	40419±925	2.65±0.07	125±2.25	0.27±0.013	1.85±0.06	37878±807	531±12
T29	52984±927	0.06±0.02	125±0.21	0.32±0.003	2.2±0.08	44827±775	746±13
T94	49251±409	<0.0016	141±2.1	<0.0005	1.4±0.05	88288±1207	5645±74
T46	76618±1891	<0.0016	168±1.8	1.2±0.05	2.32±0.009	66417±1624	1021±24
T60	53100±1558	0.73±0.16	112±2.25	0.36±0.02	2.62±0.02	46666±1264	1079±28
T47	77095±2175	<0.0016	139±2.85	1±0.07	2.23±0.05	61689±1146	1181±31
T5	40969±68	<0.0016	141±1.5	0.23±0.006	1.89±0.04	51442±195	1801±6.7
T2	34063±486	<0.0016	230±1.5	0.14±0.04	2.2±0.02	59356±1032	3134±23

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Table 5 The Analysis of elements 12 soil samples fraction obtained from different area of Diyarbakır (mg/kg)) (continuation)

Samples	Pb	Sb	Sn	Se	V	P	Ca	Mg	K
T116	18.8±0.34	1.68±0.13	0.16±0.003	0.36	52.5±0.75	2022±24	110545±270	9296±183	2666±40
T115	28±0.84	7.36±0.16	0.22±0.002	1.31	99±0.81	1030±4.8	7661±280	7716±110	3547±7.6
T113	26.7±0.8	3.73±0.18	0.16±0.003	0.27±0.01	76.8±0.97	588±6.4	24740±308	15749±244	5096±34
T96	40.7±0.35	7.07±0.22	0.61±0.04	1.68±0.05	170.2±0.1	530±6.23	4832±16.4	13591±73	8270±99.5
T37	13.9±0.12	3.30±0.16	0.15±0.002	0.23±0.02	129±0.4	495±11	27728±618	14002±552	3089±37
T19	12.9±0.36	3.11±0.37	0.17±0.004	0.41±0.01	116±1.2	530±2.25	47793±258	10807±302	2277±35
T29	18.2±0.88	3.52±0.36	0.20±0.10	0.25±0.002	103±1.1	660±8.2	38675±58	14325±304	4163±47
T94	42.3±0.07	5.10±0.10	0.18±0.001	1.80±0.1	177±1	1145±3.15	7712±52.3	7175±38.6	5074±227
T46	28.9±0.34	4.73±0.24	0.27±0.003	0.76±0.1	ND	529±3.29	10033±225	12408±377	5976±142
T60	24.8±0.88	3.68±0.2	0.17±0.002	0.48±0.13	ND	443±10	13818±478	6621±162	2068±45
T47	28.62±0.82	4.96±0.40	0.27±0.006	0.9±0.06	ND	516±4.7	14945±74	10973±310	5160±107
T5	32.9±0.81	3.44±0.2	0.17±0.005	2.11±0.17	82±0.9	494±6.3	9421±96	9774±38	1723±21
T2	33.1±0.41	4.11±0.09	0.17±0.003	3±0.27	146±0.93	667±6.7	5652±68	9927±178	1435±24

Table 6 The Analysis of elements transferred to humic acid fraction from agricultural soil samples (mg/Kg)

Samples	Al	As	Ba	Be	Cd	Fe	Mn
T116	3436±130	5.87	0.186	<0.0005	<0.0005	331±5.84	0.014
T115	353±34	3.47	0.095	<0.0005	<0.0005	176±7	0.011
T113	212±23	9.4±2	0.091	<0.0005	<0.0005	249±16	0.0073
T37	5784±239	ND	123±0.17	0.62	<0.0005	0.11	0.0051
T96	10748±67	6.55±0.12	10.4±0.8	<0.0005	<0.0005	1286±9.3	35.8±0.18
T29	429±0.04	4.9±0.003	14.8	<0.0005	<0.0005	32.5±0.12	0.0065
T94	1531±10	4.27±0.55	23.6	<0.0005	<0.0005	281±0.25	1.37±0.17
T46	1366±202	16.8±3	0.12	0.28	<0.0005	303±3.2	0.0047
T60	393±12	4.31	0.0068	<0.0005	<0.0005	384±16	0.0098
T47	1369±203	29.3	0.027	0.57	<0.0005	696±13	0.0062
T5	2602±46	3.15±0.49	0.045	<0.0005	0.0025	1597±36	5.2±0.009
T2	683±28	4.12±0.59	0.046	<0.0005	<0.0005	519±3.2	0.9

Table 7 The Analysis of elements transferred to humic acid fraction from agricultural soil samples (mg/Kg)(continuation)

Samples	Pb	Sb	Sn	Se	V	P	Ca	Mg	K
T116	0.012	<0.0067	0.071	<0.0030	73±7.3	1836±0.45	1491±13	116±12	ND
T115	0.0086	<0.0067	0.066	<0.0030	196±1.61	2797±13	663±4	0.020	ND
T113	0.0096	<0.0067	0.085	<0.0030	<0.0077	1279±84	672±0.4	109±11	ND
T37	0.019	<0.0067	0.14	<0.0030	<0.0077	553±13	11008±48	<0.0029	ND
T96	1.32±0.08	<0.0067	<0.0037	<0.0030	<0.0077	2481±16.3	376.5±16	2.45±2.4	ND
T29	0.0052	<0.0067	0.07	0.38	136±2.1	2182±17	14±0.003	<0.0029	ND
T94	0.0098	<0.0067	0.026	0.80	154±1.98	1886±0.92	738±2.2	<0.0029	ND
T46	0.012	<0.0067	0.15	<0.0030	<0.0077	1042±13	2229±76	0.025	ND
T60	0.0074	<0.0067	0.10	0.016	<0.0077	1542±12	600±21	0.076	ND
T47	0.0067	<0.0067	0.24	<0.0030	4.06±0.2	2176±11	279±35	0.031	ND
T5	0.0079	<0.0067	0.11	0.89	202±1.9	2790±2.33	694±7.8	147±3.7	ND
T2	0.0070	<0.0067	0.068	0.59	21.7±0.91	2873±7.16	475±5.3	8.6±0.65	ND

It was shown regression analysis results in Table 7. Humic acid showed significant properties with some other valuables.

Table 8. Ragression Analysis of between HA and Soil Properties

HA-OM	N=12, P<0.01, Significant	R^2= 0.752 , F:30.242 model ;**OM = 0.537+ 3.432*HA**
HA-PH	N=12, P<0.01, Significant	R^2 =0.594, F=14.649, model; **PH = 7.935+ (-0.743)*HA**
HA-HAf Mn	N=12, P<0.01, Significant	R^2 =0.641, F:16.080, Model **; HAfMn = -0.595 1.612*HA**
HA –HAf Se	N=12, P<0.01, Significant	R^2 =0.671, F:18.369, Model ; **HAfSe = -0.054+ 0.375*HA**
HA-HAf P	N=12, P<0.05, Significant	R^2 =0.405 ,F:6.138, Model ;**HAfP = 1411.8+ 621.2*HA**
HA-Tf Cd	N=12, P<0.05, Significant	R^2 = 0.396, F:6.546, Model ; **TfCd = 2.270+(-0.288)*HA**
HA-Tf Mn	N=12, P<0.05, Significant	R^2 = 0.441, F:7.899 , Model ; **Tf Mn =618.7+ 1309.5*HA**
HA-Tf Pb	N=12, P<0.05, Significant	R^2 = 0.416, F:7.111, Model ; **TfPb = 20.4+ 7.232*HA**
HA –Tf Se	N=12, P<0.05, Significant	R^2 =0.479 , F:9.190, Model; **TfSe = 0.396+ 0.804*HA**

HAf : ** Fraction of humik acid **Sf : Fraction of soil

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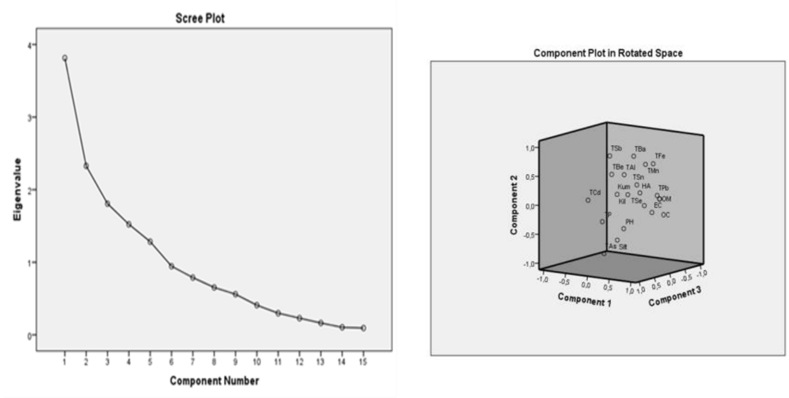

Figure 1. The scree plot curve, basic component graph of total elements and physicochemical properties of soil and humic acid.

Principal Component Analysis (PCA) of the elements and humic acid was performed. Kaiser-Meyer-Olkin (KMO) coefficient above 0.60 showed that our data set is suitable for PCA. Barlett Sphericity Test significance was found to be 0.01. The KMO coefficient was found to be 0.624 and it was significant at P <0.01. In the factor analysis application, 5 basic factors with eigenvalues greater than 1 were determined. 71.70% of the total change is explained by these factors (fig.1). The F1 (Factor): Mn, Fe, Pb, Cd, and Sb, F2: HA and P, F3: V, Al, As, F4: Be, Se, F5: Ba and Sn were determined by using varimax as a rotation method. The F1, OM, OC, TPb, TSe, HA, F2: TAs, TSb, TBa, TMn, TFe, F3: TCd, TP, PH, TSn, Silt, TAl, F4: Clay, Sand, TBe, EC,

CONCLUSIONS

According to literature, some heavy toxic metals considered to be dangerous, were found to be smaller in some soil fractions existing humic acid-rich. According to Table 5, some minerals that are useful for plants were found enriched by humic acid. Therefore, the agriculture lands which are poor in terms of humic acid are important to increase humic acid levels. In addition to, the stubble fires is well known to be harmful for agricultural soils.

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