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RESEARCH PAPER

# Effects of humic acid and mineral Zn fertilizer on Zn uptake and maize yield in soils formed on limestone and marl parent materials

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## Abstract

In this study, the effects of zinc (Zn) and humic acid (HA) applications on soils (12 Aridisol soil samples) formed on limestone and marl parent materials, which are very common in Türkiye, were tested by incubation, adsorption, and greenhouse experiments. Adsorption experiments were carried out using the batch sorption technique, and the Langmuir and Freundlich isotherm parameters were calculated. In the incubation, soils were incubated with control, HA, Zn, and HA+Zn, and the DTPA-Zn was tested in five different periods (1, 7, 15, 30, and 90th days). Maize was grown for eight weeks in pots in the greenhouse. The maximum Zn adsorption for all soils ranged between 3333 and 6250 mg kg$^{-1}$ in marl soils and 1042 and 5263 mg kg$^{-1}$ in limestone soils, which decreased to ranges between 2631 and 5555 mg kg$^{-1}$ in marl soils and 1052 and 5000 mg kg$^{-1}$ in limestone soils with HA application, respectively. The desorption rate (%) of adsorbed Zn increased as the initial Zn concentration increased in all soil series formed on marl (smectite clay mineral) and limestone (kaolinite clay mineral) parent materials. The effects of humic acid and Zn application on the fresh and dry weights of maize were found to be statistically significant at the 5% level for marl and limestone parent material. Humic acid application increased the fresh weight of maize compared with that of the control. The highest available Zn was determined in Başkuyu series (0.79 mg kg$^{-1}$) formed on marl parent material, while the lowest available Zn was obtained in Saraççeşme series (0.60 mg kg$^{-1}$) formed on limestone parent material. In the greenhouse, HA increased the fresh weight of maize in soils formed on both parent materials by 0.4 and 19.6%, respectively, compared to the control. Zn fertilization with HA further increased the fresh and dry yields, with 12 mg kg$^{-1}$ performing better. Smax (maximum Zn adsorption of the soil) parameters of soils were negatively correlated with EC, $P_{av}$, $K_{av}$, organic matter, silt, clay, total N, and cation exchange capacity (CEC) of the samples. Although HA alone increases the availability of Zn in plants, HA + Zn applications should be applied together to obtain higher yields.

## Introduction

The formation and composition of soils involve the combined effects of parent material, topography, vegetation, time, and climate (Weil and Brady, 2017; Yavitt, 2000). At a relatively large scale, climate and parent material are the most effective soil-forming factors to gain a specific character in soils. However, hilly topography causes a significant difference in soil properties even on similar types of parent material over a very short distance because of its control over water, heat, and vegetation. The other key soil-forming factor, parent material, regulates the speed of physical, chemical, and biological weathering processes in a specific environment (Delgado and Gomez, 2016). The parent material is also a primary source of nutrients necessary for plant growth and a habitat for soil micro-organisms in natural soils (Trettin et al., 1999). The mineralogical composition of the parent material can influence the movement of fine soil particles and plant nutrients along the soil profile through water, weathering, growing vegetation types, and soil-dwelling organisms (Jacobs, 1998).

Marl is a rock containing clay-sized (<0.002 mm) clastic material and carbonate (calcite). Therefore, it is defined as a transitional rock material between clastic and chemical sedimentary rocks (Šestanović, 2001). Smectite, which predominates in the marl parent material classified as aridisol, is a phyllosilicate mineral characterized by a 2:1 (Si: Al) layer structure (Brown, 1980; Bailey et al., 2015). Smectite clays have a variable net negative charge stabilized by the outward sorption of metal cations (Na, Ca, Mg, and H) on the interlamellar surfaces. The smectite structure has numerous unique properties, including chemical and exchangeable ion structures and small crystal sizes. Furthermore, the structure of smectite is responsible for its highly active surface area, high cation exchange capacity, rare hydration properties, and ability to strongly modify the movement behavior of solutions (Mortland, 1970; Sawhney and Singh, 1997). Furthermore, $Al^{3+}$-saturated clays have strong water polarity associated with $Al^{3+}$, which leads to better adsorption by forming strong H-bonds with insecticides (Sawhney and Singh, 1997). Johnston et al., (2001) and Sheng et al., (2002) reported that some hydrated metal cations, such as $Ca^{2+}$ and $Mg^{2+}$, surrounded by water molecules reduce ion-dipole bonds, preventing direct contact between exchangeable cations and polar functional groups. Furthermore, nonpolar organic compounds can interact with the siloxane surfaces of smectite (Laird and Fleming, 1999). Saturated clays with weakly hydrated cations, such as $NH^{4+}$ and $K^+$, enhanced the adsorption of organic compounds by increasing the size of the adsorptive spheres between exchangeable cations. Marl parent materials have the most erodible surfaces in arid environments, including arid regions (Cerda 2002; Martinez-Mena et al., 2002).

Limestone is a sedimentary rock that accounts for approximately 10% of the sedimentary rocks found on Earth's surface. It is formed by the deposition of shells and shell fragments, or by the direct crystallization of calcium carbonate from water (Blatt and Tracy, 1996). The remaining components may include other carbonate minerals such as dolomite magnesite and aragonite (Jackson, 1997). Kaolinite, which predominates in the limestone parent material, is a weathering product of feldspars. Because kaolinite is electrically stable, its ability to adsorb ions is lower than that of other clay minerals. Kaolinite is a mineral commonly found in soil. It tends to be particularly abundant in weathered soils, such as Ultisols and Oxisols. Very few isomorphous substituents are present in the tetrahedral and/or octahedral layers of kaolinites. Most kaolinites were close to the ideal formula for $Al_2Si_2O_5(OH)_4$. The 1:1 layer had little or no permanent charge owing to the low amount of substitution. The cation exchange capacities and surface areas are typically low. Soils with a high kaolinite ratio are generally less fertile than soils dominated by 2:1 clay minerals (Milliken, 2003).

Illite is a closely related group of nonexpansive clay minerals. It is a secondary mineral precipitate and an example of phyllosilicates or layered aluminosilicates. Its structure is a 2:1 sandwich of silica tetrahedron (T)–alumina octahedron (O)–T layers (USGS, 2019). The formation of illite is generally favored under alkaline conditions and high Al and K concentrations. The cation exchange capacity (CEC) of illite is smaller than that of smectite, but higher than that of kaolinite, typically around 20-40 cmol $kg^{-1}$ (Weil and Brady, 2017).

Different parent materials influence the morphology and mineralogical and physicochemical properties of soils under the same conditions, such as the biosphere, topography, and climate, especially in arid and semi-arid regions. Therefore, differences in soil properties are primarily related to the parent material (Washer and Collins, 1988). Zinc (Zn) content in soil ranges from 10 to 300 mg $kg^{-1}$, depending on the chemistry of the parent material and soil texture, organic matter, and pH (Mihaljevic, 1999), with an estimated global average of 64 mg $kg^{-1}$ (Kabata-Pendias, 2011). Zn is the second most abundant transition metal in living organisms. Zn is critical for generative growth (seed formation) rather than vegetative growth. Zn may be important for pollen grain development, possibly by improving the phytohormone status of plants and protein synthesis (Brown et al., 1993).

Zinc deficiency in soils has been reported worldwide, especially in calcareous soils in arid and semiarid regions. Zinc deficiency in soils and crop plants occurs in almost all countries, especially in cereal-growing areas. Türkiye is among the countries with the most severely Zn-deficient soils. Since Zn deficiency is a major micronutrient deficiency in humans in Türkiye, increases in cereal Zn concentrations by Zn fertilization have significant impacts on human health. In Türkiye, Zn deficiency is a widespread problem in crops (Çakmak et al. 1999) and humans (Çavdar et al., 1983). Zinc concentrations in Turkish soils are some of the lowest ever recorded (Sillanpää, 1990). According to the results obtained from 1,511 soil samples using the Soil-DTPA test (Lindsay and Norvell, 1978), 49.8% of the cultivated soils in Türkiye were classified as Zn-deficient (Eyupogluet al., 1994). The prevalence of Zn deficiency in Turkish soils increased with increasing pH and decreasing organic matter levels in soils collected from different regions. Soils in different regions of Türkiye are generally deficient in Zn, which is reflected in the poor growth of plants under greenhouse conditions (Kacar, 1998). Zinc deficiency is common in plants growing in highly weathered acidic and calcareous soils. In the latter case, Zn deficiency was often associated with Fe deficiency (lime chlorosis). The low availability of Zn in calcareous soils with high pH is due to the adsorption of Zn to clay or $CaCO_3$ rather than the formation of poorly soluble $Zn(OH)_2$ or $ZnCO_3$ (Trehan and Sekhon, 1977). In addition, Zn uptake and translocation to the shoot are inhibited by high bicarbonate and $HCO_3$ concentrations (Dogar and van Hai, 1980).

The organic matter (OM) content controls the magnitude of Zn sorption in soil (Montalvo et al., 2016). Organic matter is composed of different fractions such as humic acid (HA) and fulvic acid. Therefore, the surface of organic colloids is dominated by negative charges at low soil pH (Stevenson, 1994). As the OM and soil pH increased, the amount of organic ligands and their charge density increased. It also increases Zn adsorption because of the increased stability of the organic complexes with HA as the pH increases (Boguta and Sokołowska, 2016). Humic acid, a pool of humic substances, tends to form complexes with zinc. However, the stability and solubility of the formed complexes depend on the Zn-HA stoichiometry, plant species, proportion of HA added to the soil, growth medium characteristics, and soil type (Olaetxea et al., 2018). Therefore, depending on the combination of these factors and soil management practices, crop Zn uptake can be improved or reduced (de Morais et al., 2021). Humic substances improve nutrient uptake and plant growth by adding HA and Zn to the soil (Justi, 2019). The effects of humic substances on plant tissues include stimulation of root proliferation, enhancement of $H^+$-ATPase activity in the cell membrane, and changes in the magnitude of nutrient uptake, assimilation, and utilization efficiency (Aguiar et al., 2013; Nardi et al., 2021). HA in soil can improve Zn nutrition (Boguta and Sokołowska, 2016) and plant growth by forming a Zn complex (De Morais et al., 2021). Organometallic complexes increase Zn content in the soil solution and Zn diffusion from the solution to the cell root surface. This is related to the complexation of Zn by organic ligands and the improvement in Zn uptake by plants (Justi et al., 2019). Dissolved Zn in the soil liquid phase is readily available to plants as the Zn concentration in solution contains a small fraction of the total Zn content of the soil (Montalvo et al., 2016). Therefore, HA is expected to contain organic radicals capable of complexing Zn into soluble forms. The use of HA effectively increased the Zn content in the solution, especially at a high soil pH (7.2) (Justi et al., 2019). HA can also buffer soil acidity. It maintains the pH within the optimum range required to provide crops with abundant nutrients, especially Zn, whose availability decreases as pH increases (Montalvo et al., 2016).

The buffering effect of HA on soil acidity is more frequent. When pH is in the alkaline range, less dissolved Zn is present in the soil, while low insoluble forms ($ZnOH^+$ and $ZnOH_2$) are formed in the soil (Alloway, 2009; Montalvo et al., 2016). HA buffers soil acidity and prevents oscillations in soil reaction (Aguiar et al., 2013; Rosa et al., 2018), and consequently promotes the formation of soluble $Zn^{2+}$ free hydrated forms and soluble Zn-organic complexes (Montalvo et al., 2016). HA also depend on the structure-organic functional groups-function properties of HA (Nardi et al., 2021). It is linked to the type and concentration of oxygen, nitrogen and sulphur-containing functional groups in the HA structure (Aguiar et al., 2013; Zanin et al., 2019; Nardi et al., 2021; de Morais et al., 2021).

## Materials and Methods

### Materials

#### The taxnomy of the experimental soils

The characteristics of the experimental soils formed on marl (6 soils) and limestone parent materials belonging to the Aridisol order in the U.S. Soil Taxonomy are given in Table 1. Marl soils are classified as Xeric Hablocambic, Xeric Pedrocalcid and Typic Calciorthid and limestone soils are Vertic Calciorthid, Typic Paleorthid and Typic Calciorthid. The name of marl soils are Atınova, Hacıfakıl, Çatalca, Başkuyu, Kuyubaşı and Seyrantepe soil series and Limestone's soils are Saraççeşme, Tine, Karataş-1, Tuvem-1, Tuvem-1, Tuvem-2 and Karataş-2 soil series. The soil series is characterized by arid and semiarid climate.

#### Some physical and chemical properties of soils

Composite surface soil samples (0-20 cm depth) were brought to laboratory in plastic bags. Air-dried samples were then passed through 2 mm sieve to performe the following analysis: particle size distribution (sand, silt and clay contents) by a hydrometer method (Bouyoucus, 1951), pH and electrical conductivity (EC) in saturation paste (Richard, 1954) calcium carbonate equivalent (CCE) by a manametic method using the Scheibler calcimeter (Soil Survey Staff, 2014) organic matter by modified Walkley-Black method (Sparks, 1996), cation exchange capacity (CEC) by molar Na-Acetate saturation (Richard 1954), total nitrogen (N) by concentional Kjeldahl method (Bremner 1965), DTPA-extractable cationic microelements (Fe, Cu, Mn, Zn) (Lindsay and Norwel, 1978) including certain heavy metals (Ni, Cr, Pb and Cd) by ICP-OES (Perkin-Elmer, DV, 2100).

The physical and chemical properties of the experimental soils were presented in Table 2. The limestone soils had finer texture than the marl soils.

Electrical conductivity values ranged similarly in both parent materials. Soil organic matter contents were higher with limestone soils (1.61 - 2.69%) than the marl soils' (0.89 - 1.69%). The CCE contents of marl

**Table 1.** The soil taxonomy classification of the experimental soils

| Soils | Soil Family | Soil Series | Parent material | Order |
|---|---|---|---|---|
| S1 | Xeric Hablocambic | Atınova | Marl | Aridisol |
| S2 | Xeric Hablocambic | Hacıfakıl | | |
| S3 | Xeric Hablocalcid | Çatalca | | |
| S4 | Xeric Hablocalcid | Başkuyu | | |
| S5 | Xeric Pedrocalcid | Kuyubaşı | | |
| S6 | Typit Calciorthid | Seyrantepe | | |
| S7 | Vertic Calciorthid | Saraççeşme | Limestone | |
| S8 | Typit Paleorthid | Tine | | |
| S9 | Typit Calciorthid | Karataş-1 | | |
| S10 | Vertic Calciorthid | Tüvem-1 | | |
| S11 | Vertic Calciorthid | Tüvem-2 | | |
| S12 | Typit Calciorthid | Karataş-2 | | |

soils (27.41 - 43.40%) were higher than the limestone soils' (5.28 - 24.61%). The total nutrient element concentrations of soils were given in Table 3. The element concentrations were highly affected from the parent materials depending on the overal effects of soil forming factors and agricultural practices.

**Properties of humic acid (HA)**

Some chemical properties of HA used in the experiment are given in Table 4. Organic matter content

**Table 2.** Some chemical and physical properties of the experimental soils

| Soil | Sand % | Silt % | Clay % | Texture Class | OM % | CEC cmol kg$^{-1}$ | pH | CCE % | EC dS m$^{-1}$ | $P_{av}$ kg ha$^{-1}$ | $K_{av}$ kg ha$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 43.0 | 24.0 | 33.0 | CL | 1.28 | 24.2 | 7.54 | 27.4 | 0.782 | 7.84 | 134 |
| S2 | 43.0 | 26.7 | 30.3 | CL | 0.89 | 23.9 | 7.50 | 35.6 | 0.716 | 4.66 | 96 |
| S3 | 41.0 | 34.2 | 24.9 | L | 0.79 | 19.3 | 7.69 | 35.0 | 0.619 | 11.9 | 146 |
| S4 | 39.0 | 33.5 | 27.6 | CL | 1.69 | 23.6 | 7.56 | 35.1 | 0.721 | 0.93 | 106 |
| S5 | 42.2 | 31.5 | 26.3 | L | 1.50 | 20.9 | 7.49 | 43.4 | 0.656 | 4.27 | 158 |
| S6 | 45.0 | 31.5 | 23.6 | L | 1.44 | 19.3 | 7.72 | 39.5 | 0.499 | 5.36 | 102 |
| S7 | 26.2 | 38.2 | 35.6 | CL | 1.97 | 25.6 | 7.51 | 38.7 | 0.717 | 5.74 | 158 |
| S8 | 29.0 | 35.5 | 35.6 | CL | 1.61 | 28.4 | 7.61 | 9.01 | 0.358 | 10.5 | 134 |
| S9 | 23.0 | 38.0 | 39.0 | CL | 2.69 | 29.2 | 7.24 | 7.47 | 0.855 | 24.6 | 201 |
| S10 | 42.2 | 26.2 | 31.6 | CL | 2.20 | 25.7 | 7.76 | 16.3 | 0.506 | 10.3 | 154 |
| S11 | 26.6 | 37.8 | 35.6 | CL | 2.10 | 24.9 | 7.53 | 20.1 | 0.691 | 5.59 | 179 |
| S12 | 40.2 | 32.2 | 27.6 | CL | 2.27 | 24.8 | 7.49 | 5.28 | 0.594 | 4.35 | 206 |

S1: Atınova soil series, S2: Hacıfakıl soil series, S3: Çatalca soil series, S4: Başkuyu soil series, S5: Kuyubaşı soil series, S6: Seyrantepe soil series, S7: Saraççeşme soil series, S8: Tine soil series, S9: Karataş-1 soil series, S10: Tüvem-1 soil series, S11: Tüvem-2 soil series, S12: Karataş-2 soil series, OM: Organic matter, CEC: Cation exchange capacity, pH: Soil reaction, CCE: Calcium carbonate equivalent, EC: Electrical conductivity, $P_{av}$: Available $P_2O_5$, $K_{av}$: Available $K_2O$, C: Clay, L: Loam

**Table 3.** Total concentration of some plant nutrients in the experimental soils

| Soil Series | g kg$^{-1}$ | | | | | mg kg$^{-1}$ | |
|---|---|---|---|---|---|---|---|
| | P | Mn | N | K | Fe | Cu | Zn |
| S1 | 0.42 | 0.51 | 0.64 | 18.07 | 16.85 | 18.4 | 32.2 |
| S2 | 0.43 | 0.95 | 0.45 | 15.04 | 17.94 | 30.7 | 83.1 |
| S3 | 0.62 | 0.76 | 0.40 | 17.96 | 10.64 | 20.6 | 52.7 |
| S4 | 0.69 | 0.97 | 0.85 | 11.75 | 12.93 | 35.1 | 96.1 |
| S5 | 0.72 | 0.82 | 0.75 | 15.73 | 22.34 | 29.1 | 52.6 |
| S6 | 0.52 | 0.9 | 0.72 | 1.211 | 18.06 | 29.8 | 97.0 |
| S7 | 0.51 | 0.96 | 0.99 | 12.38 | 14.04 | 34.3 | 88.7 |
| S8 | 0.55 | 0.79 | 0.81 | 10.36 | 12.57 | 22.8 | 66.1 |
| S9 | 0.11 | 0.56 | 1.35 | 9.05 | 10.20 | 12.0 | 28.0 |
| S10 | 0.17 | 0.91 | 1.10 | 14.72 | 10.20 | 26.4 | 78.7 |
| S11 | 0.52 | 0.82 | 1.05 | 11.43 | 19.67 | 35.4 | 58.9 |
| S12 | 0.24 | 0.80 | 1.14 | 8.42 | 10.21 | 29.3 | 68.0 |

S1: Atınova soil series, S2: Hacıfakıl soil series, S3: Çatalca soil series, S4: Başkuyu soil series, S5: Kuyubaşı soil series, S6: Seyrantepe soil series, S7: Saraççeşme soil series, S8: Tine soil series, S9: Karataş-1 soil series, S10: Tüvem-1 soil series, S11: Tüvem-2 soil series, S12: Karataş-2 soil series

was 43.3%, total humic acid value was 20.8% and electrical conductivity was 0.27 dS m$^{-1}$. The HA was highly acidic with a pH of 3.3 and its heavy metal contents were at trace amounts below the detection limits of ICP-OES.

**Table 4.** Chemical properties of the humic acid

| Parameters | Results |
|---|---|
| Organic matter (%) | 43.3 |
| Σ Humic acid (%) | 20.8 |
| Σ Nitrogen (%) | 0.17 |
| pH | 3.3 |
| Electrical conductivity (dS m$^{-1}$) | 0.27 |
| Σ Phosphorus (%) | 1.71 |
| Σ Potassium (mg kg$^{-1}$) | 395 |
| Σ Calcium (mg kg$^{-1}$) | 83.5 |
| Σ Magnesium (mg kg$^{-1}$) | 2125 |
| Σ Iron (mg kg$^{-1}$) | 3.12 |
| Σ Zinc (mg kg$^{-1}$) | 1.87 |
| Σ Manganese (mg kg$^{-1}$) | <0.02 |
| Σ Nickel (mg kg$^{-1}$) | <0.02 |
| Σ Chromium (mg kg$^{-1}$) | <0.02 |
| Σ Lead (mg kg$^{-1}$) | <0.02 |
| Σ Cadmium (mg kg$^{-1}$) | <0.02 |

## Method

### Adsorption and desorption experiments

Triplicate scoops of 2 g of the soils were equilibrated with 25 mL of Zn solutions containing of 0, 6.5 - 32.5 - 65 - 162.5 - 325 - 487.5 - 650 mg L$^{-1}$ prepared in 0.01 M $Ca(NO_3)_2$ background solution to keep ionic strength constant. The suspentions were shaken for 24 h at 25°C on an end-over-end shaker. Then the solution phase were separated by sequential centrifugation at 3000 rpm for 10 min and filtering through Whatman 2 filter paper. Immediately after adsorption, the desorption batches were performed by equilibrating the Zn-loaded-soils with 20 mL of 0.01 $Ca(NO_3)_2$ solutions for 24 h at 25°C. Then, the Zn concentration of the supernatants obtained from the adsorption ad desorption batches were determined by ICP-OES. The amounts of adsorbed and desorbed Zn was calculated from the difference in the equilibrium and initial Zn concentrations (Alumaa, 2001). The sorption data were then tested for the comformation of linear Langmuir and Freundlich isotherms as given below:

$$\frac{Ce}{S} = \frac{1}{k\ Smax} + \frac{1}{Smax}$$

Where S amount of Zn adsorbed (mg kg$^{-1}$), Ce Zn concentration of the equilibrium solution (mg L$^{-1}$), Smax maximum Zn adsorption of the soil (mg kg$^{-1}$), k bonding energy coefficient (mg mL$^{-1}$).

The exponential forms to the Freundlich model is:

$$S= K_F Ce^{1/n}$$

Where $K_F$ Freundlich constant and n constant.

In order to reduce the deviation from the Langmuir model, the total amounts of the Zn was included into the S. This improved the determination coefficient ($R^2 \geq 0.948$) of the regression line without ommiting any sorption data point at the lower-end.

**Clay mineralogy**

In order to carry out clay analyses in soils, carbonates, oxide minerals and organic matter were sequentially removed by the following methods: carbonates by 1 N acetate buffer (pH. 4.5-5), organic matter by 30% $H_2O_2$ on a hot plate, sesquioxides by citrate-bicarbonate-dithionite on a hot plate at 80°C.

The X-ray diffractograms of the soil samples were obtained to determine clay types (Whittig and Allardice, 1986). The semi-quantitative percentages of the minerals were calculated by the ratio of the individual peaks to total peak area. The results were presented in Table 5. The content of smectite clay minerals varied between 37.18% and 29.75%, kaolinite clay minerals between 59.08% and 43.87% and illite clay minerals between 18.95-11.17%. The dominance of the clays in the soils were smectite 42.86 %, kaolinite 35.71% and illite 21.43%. The smectite clays were dominant in the limestone soils whereas kaoline was dominant in the marl soils. Illite however were present at equal amounts in soils developed on both parent materials.

**Table 5.** Semi-quantitative clay contents (%) of the experimental soils

| Soil | Dominance | Smectite | Illite | Kaolinite |
|---|---|---|---|---|
| S1 | Smectite > Kaolinite > Illite | 41.6 | 21.9 | 36.5 |
| **S2** | Smectite > Kaolinite > Illite | 45.4 | 11.5 | 43.2 |
| **S3** | Smectite > Kaolinite > Illite | 58.2 | 4.2 | 37.6 |
| **S4** | Smectite > Kaolinite > Illite | 55.2 | 8.2 | 36.6 |
| **S5** | Kaolinite > Smectite > Illite | 31.6 | 12.4 | 55.9 |
| **S6** | Kaolinite > Illite > Smectite | 16.2 | 32.7 | 51.0 |
| **S7** | Kaolinite > Illite > Smectite | 5.33 | 44.1 | 50.6 |
| **S8** | Kaolinite > Illite > Smectite | 20.4 | 20.9 | 58.6 |
| **S9** | Kaolinite > Smectite > Illite | 31.8 | 3.53 | 64.7 |
| **S10** | Smectite > Kaolinite> Illite | 50.6 | 16.4 | 32.9 |
| **S11** | Kaolinite > Smectite > Illite | 29.7 | 11.2 | 59.1 |
| **S12** | Kaolinite > Smectite > Illite | 37.2 | 18.9 | 43.9 |

S1: Atınova soil series, S2: Hacıfakıl soil series, S3: Çatalca soil series, S4: Başkuyu soil series, S5: Kuyubaşı soil series, S6: Seyrantepe soil series, S7: Saraççeşme soil series, S8: Tine soil series, S9: Karataş-1 soil series, S10: Tüvem-1n soil series, S11: Tüvem-2 soil series, S12: Karataş-2 soil series

**Incubation experiments**

According to the differences in adsorption isotherms (lime, composition, etc.), four soils (S2 and S4 soil series on marl parent material and S7 and S8 soil series on limestone parent material) were selected to represent both marl and limestone parent materials. Theses soils were used to test time-dependent effects of HA acid treatments on the DTPA extractability of Zn over 90 days. The experiment consisted of HA, Zn chemical fertiliser ($ZnSO_4$), humic acid+Zn chemical fertiliser, and the control (without HA and Zn) treatments in triplicates. The experiment was set up in completly-randomised design. The soil were incubated at constant moisture content in the field capacity and 25±2°C. Soil samples were taken on the 1, 7, 15, 30 and 90th days of incubation and analysed for DTPA-Zn (Lindsay and Norwel, 1978).

**Greenhouse experiments**

A representative soil samples were selected from each parent material by taking into account the results of adsorption/desorption and incubation experiments. The greenhouse experiment was conducted in triplicates with two soils (Başkuyu soil series (S4) on marl parent material and Tine soil series (S8) on limestone parent material), two doses of humic acid (0% and 2%) and 5 levels of $ZnSO_4$ (0 - 4 - 8 - 12 - 16 mg Zn $kg^{-1}$). Soil samples were passed through a 5-mesh sieve and 2 kg soil was filled into plastic pots on dry weight basis. Plant nutrient solutions were prepared and sprayed on the soil and mixed thoroughly to maintain homogeneity. Maize seeds (*Zea mays* var. *indentata*) were sown after soaking 24 hours before sowing. The plant were irrigated in field capacity. At the end of the vegetation period of 8 weeks, they were harvested just above the soil and their heights and fresh weights were determined and then washed with distilled water and dried at 65°C to a constant weight and dry weights were determined. Plant samples were then ground and homogenized to a particle size of ≤0.5 mm and prepared for analysis.

### Plant analysis

Scoops of 0.5 g plant samples were digested in a microwave oven in $HNO_3/H_2O_2$ mixture (8/2, v/v). Then the plant nutrient concentration of the digests were analysed for plant nurients by means of ICP-OES. The total nitrogen was determined by the conventional Kjeldahl method.

### Statistical analysis

Data were analyzed using SPSS 19.0 statistical software. The differences between the treatment means were tested using the Duncan's multiple range test. The underlying coherence between the investigated parameters were revealed by Pearson's correlation (SPSS 19.0, SPSS Inc. 2011). The incubation data were subjected to repeated measure analysis procedures to simultanously test the between (treatment effects) and within effects (the time-effect) and the mean separation was performed by Bonferroni test at p≤0.05.

## Results

### Zn adsorption isotherms

Adsorption isotherms of 12 soil samples without and with humic acid application are given in Figure 1. Zn adsorption data were characterized at acceptable

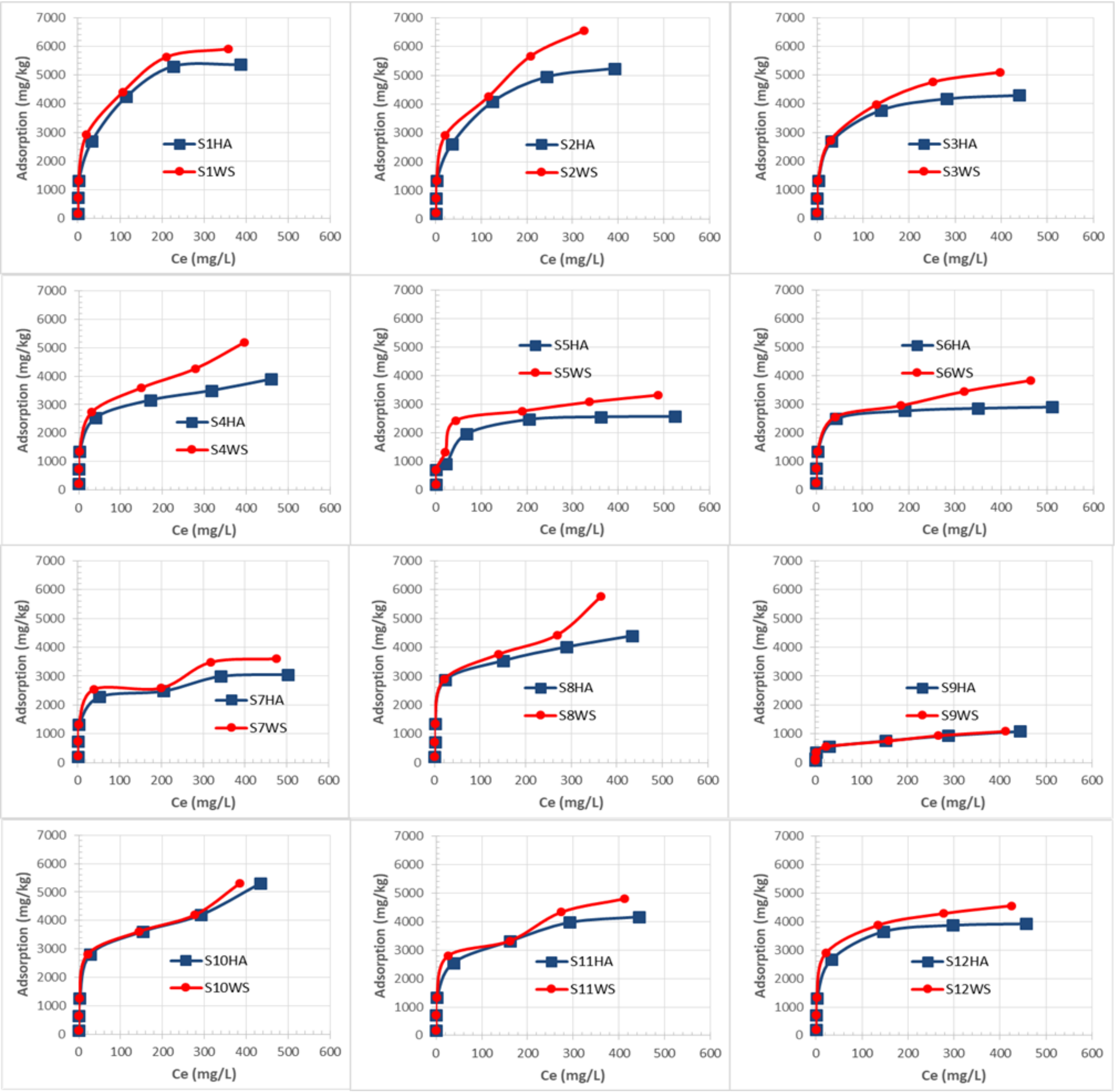

**Figure 1.** The adsorption isotherms of the experimental soils with and without humic acid treatment. Soils numbered 1-6 are marl soils and 7-12 are limestone soils

levels with both isotherms. The adsorption maximum values of Langmuir isotherm (b) without and with humic acid application ranged between 1042-6250 mg $kg^{-1}$ and 1052-5555 mg $kg^{-1}$, respectively. The maximum adsorption values were significantly reduced by the HA treatment. This was apparent at higher Zn loadings (Figure 1). The binding energy (k) varied between 0.061-0.137 and 0.056-0.267 in soils without and with humic acid application, respectively. In the Freundlich isotherms, the adsorption power (n) varied between 0.171-0.231 and 0.225-0.384 in soils without and with humic acid treatment, respectively. $K_f$ values varied between 326-1564 and 550-2019 in soils without and with humic acid application, respectively. The regression coefficients for the Langmuir isotherm without and with humic acid application were $R^2$=0.958-0.997 and $R^2$=0.990-0.999, while the regression coefficients for the Freundlich isotherm were $R^2$=0.472-968 and $R^2$=0.653-0.968, respectively.

**Zn desorption isotherms**

In general, the amount of Zn desorbed increased with increasing initial Zn concentration and HA treatment (Table 6). In the non-HA treatments in the marl parent material in the Kuyubaşı series Xeric Pedrocalcid soil (S5), 16.9% of the highest amount of Zn adsorbed was desorbed and this rate increased to 25.6% with humic acid application. In general, the apercentages of desorbed Zn remained constant after 325 mg $L^{-1}$ Zn treatment. At the 650 mg $L^{-1}$ initial concentration, 22.2% of the Zn amount was desorbed in the limestone parent material in the Saraççeşme series Vertic Calciorthid soil (S7), and this rate increased to 28.7% with the application of humic acid.

**Incubation period**

During incubation, Zn contents of the soil samples were taken on 1, 7, 15, 30 and 90 days. In the Zn incubation experiment, the difference between soils (formed on marl and limestone parent materials) and treatments (humic acid, chemical fertilizer, humic acid + chemical fertilizer application and the control) was statistically significant at 1% level (Table 7). Soil and treatment interaction was found to be significant at 1% level. The

**Table 6.** Desorption ratio (%) of the adsorbed Zn as a function of the initial Zn concentrations, humic acid treatments and parent material

| | Init. Zn (mg $L^{-1}$) | S1 | | S2 | | S3 | | S4 | | S5 | | S6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Humic acid treatments | | | | | | | | | | | |
| | | (-) | (+) | (-) | (+) | (-) | (+) | (-) | (+) | (-) | (+) | (-) | (+) |
| Marl soils | 6.5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 32.5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 65 | 0.34 | 0.63 | 0.91 | 1.10 | 1.94 | 4.67 | 1.31 | 2.40 | 2.00 | 2.27 | 2.22 | 2.42 |
| | 162.5 | 2.19 | 3.48 | 3.73 | 4.63 | 2.93 | 5.51 | 4.80 | 6.61 | 5.29 | 7.19 | 6.07 | 6.62 |
| | 325 | 3.76 | 6.57 | 6.82 | 7.96 | 6.96 | 10.1 | 7.26 | 10.2 | 9.88 | 12.1 | 10.1 | 11.0 |
| | 487.5 | 3.05 | 10.6 | 7.20 | 9.77 | 7.49 | 12.2 | 9.22 | 14.9 | 14.2 | 18.6 | 16.0 | 17.6 |
| | 650 | 3.18 | 10.6 | 8.49 | 13.5 | 10.3 | 19.4 | 9.54 | 17.2 | 16.9 | 25.6 | 16.1 | 17.6 |
| | Init. Zn (mg $L^{-1}$) | S7 | | S8 | | S9 | | S10 | | S11 | | S12 | |
| | | Humic acid treatments | | | | | | | | | | | |
| | | (-) | (+) | (-) | (+) | (-) | (+) | (-) | (+) | (-) | (+) | (-) | (+) |
| Limestone soils | 6.5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 32.5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 65 | 2.02 | 2.22 | 0.90 | 0.98 | 1.51 | 1.65 | 1.59 | 1.75 | 1.24 | 1.74 | 1.59 | 1.37 |
| | 162.5 | 5.48 | 6.68 | 3.78 | 5.60 | 5.53 | 6.33 | 4.43 | 5.07 | 4.37 | 6.96 | 5.24 | 5.03 |
| | 325 | 12.8 | 14.6 | 7.69 | 11.2 | 7.81 | 8.13 | 7.39 | 9.59 | 5.69 | 11.0 | 8.48 | 10.1 |
| | 487.5 | 20.5 | 26.1 | 11.0 | 14.2 | 15.9 | 18.1 | 11.6 | 15.1 | 10.6 | 15.3 | 13.0 | 15.6 |
| | 650 | 22.2 | 28.7 | 10.2 | 16.5 | 18.0 | 22.6 | 13.3 | 19.2 | 10.6 | 18.9 | 14.7 | 19.4 |

S1: Atınova soil series, S2: Hacıfakıl soil series, S3: Çatalca soil series, S4: Başkuyu soil series, S5: Kuyubaşı soil series, S6: Seyrantepe soil series, S7: Saraççeşme soil series, S8: Tine soil series, S9: Karataş-1 soil series, S10: Tüvem-1 soil series, S11: Tüvem-2 soil series, S12: Karataş-2 soil series

**Table 7.** Time-dependent DTPA-Zn concentrations (mg $kg^{-1}$) of the experimental

| Soils | Treatments | Incubation periods (Days) | | | | | Treat.mean | Soil mean | Grand mean |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 7 | 15 | 30 | 90 | | | |
| S4 | WS | 0.08 | 0.09 | 0.19 | 0.09 | 0.12 | 0.12 b | 0.79 A | 0.68 |
| | WS + Zn | 1.17 | 1.51 | 1.30 | 1.58 | 1.13 | 1.34 a | | |
| | WS + HA | 0.08 | 0.11 | 1.57 | 0.08 | 0.14 | 0.40 b | | |
| | WS + Zn + HA | 1.17 | 1.42 | 1.27 | 1.49 | 1.20 | 1.31 a | | |
| | Incubation mean | 0.63 | 0.78 | 1.08 | 0.81 | 0.65 | | | |
| S2 | WS | 0.08 | 0.15 | 0.14 | 0.08 | 0.12 | 0.12 b | 0.70 B | |
| | WS + Zn | 1.23 | 1.52 | 1.33 | 1.20 | 0.28 | 1.11 a | | |
| | WS + HA | 0.07 | 1.13 | 0.13 | 0.07 | 0.12 | 0.30 b | | |
| | WS + Zn + HA | 1.15 | 1.46 | 1.33 | 1.19 | 1.26 | 1.28 a | | |
| | Incubation mean | 0.63 | 1.07 | 0.73 | 0.64 | 0.44 | | | |
| S7 | WS | 0.06 | 0.08 | 0.15 | 0.07 | 0.11 | 0.10 b | 0.60 C | |
| | WS + Zn | 1.05 | 1.55 | 1.19 | 1.19 | 1.16 | 1.23 a | | |
| | WS + HA | 0.06 | 0.10 | 0.13 | 0.06 | 0.10 | 0.09 b | | |
| | WS + Zn + HA | 1.04 | 1.41 | 1.17 | 0.17 | 1.15 | 0.99 a | | |
| | Incubation mean | 0.55 | 0.79 | 0.66 | 0.37 | 0.63 | | | |
| S8 | WS | 0.06 | 0.08 | 0.19 | 0.06 | 0.12 | 0.10 b | 0.62 C | |
| | WS + Zn | 1.06 | 1.31 | 1.13 | 1.11 | 1.10 | 1.14 a | | |
| | WS + HA | 0.05 | 0.08 | 0.12 | 0.06 | 0.11 | 0.08 b | | |
| | WS + Zn + HA | 1.08 | 1.30 | 1.13 | 1.10 | 1.08 | 1.14 a | | |
| | Incubation mean | 0.56 | 0.69 | 0.64 | 0.58 | 0.60 | | | |
| | Grand mean of incubation periods | 0.59 b | 0.83 a | 0.78 a | 0.60 b | 0.58 b | | | |

| | | | |
|---|---|---|---|
| $F_i$ | 12.028 | $P_i$ | 0.000** |
| $F_t$ | 375.859 | $P_t$ | 0.000** |
| $F_{pm}$ | 8.128 | $P_{pm}$ | 0.000** |
| $F_{t \times pm}$ | 2.293 | $P_{t \times pm}$ | 0.018* |

S4: Başkuyu soil series, S2: Hacıfakıl soil series, S7: Saraççeşme soil series, S8: Tine soil series, WS: Whole soil, S7: Saraççeşme soil series, i: Incubation periods, t: treatments, pm: parent material

effects of Zn on soils formed on marl and limestone parent materials due to humic acid application were insignificant. Humic acid applications increased the available Zn content compared to the control in the soil formed on marl parent material. The highest available Zn content was determined in Başkuyu series (0.79 mg $kg^{-1}$) formed on marl parent material, while the lowest DTPA-Zn content was obtained in Saraççeşme series (0.60 mg $kg^{-1}$) formed on limestone parent material. The highest Zn content of the soils (1.34 mg $kg^{-1}$) was increased by humic acid application in Başkuyu series formed on marl parent material, while the lowest available Zn content (0.08 mg $kg^{-1}$) was determined in chemical fertilizer application in Tine series formed on limestone parent material. Humic acid and humic acid + chemical fertilizer applications statistically affected at the same level the available Zn content of the soils. In addition, control and only chemical fertilizer applications had statistically increased the available Zn content of soils.

### Greenhouse Experiment

### Maize performance on the marl soil

The effect of humic acid and Zn applications on the fresh and dry weights of maize was found to be statistically significant at the 5% level (Table 8). Humic acid applications (HA 2%) increased the fresh weight of maize compared to the control (HA 0%). The lowest fresh and dry weights of maize for marl parent material were 58.0 and 24.3 g for the S4, 65.5 and 26.4 g for the 2% HA obtained from the control treatment, whereas the highest fresh and dry weights of maize were 82.2 and 34.9 g for the marl parent material (S4), 86.3 and 35.7 g for the 2% HA obtained from the 2% HA + 12 mg $kg^{-1}$ Zn treatment, respectively.

### Maize performance on the limestone soil

The effect of humic acid and Zn applications on the fresh and dry weights of maize was statistically significant (p≤ 5%) (Table 8). Humic acid applications increased the fresh weight of maize compared to the control. The lowest fresh and dry weights were 65.8 and 24.2 g for the limestone parent material soil (S8), 78.7 and 26.2 g for the 2% HA obtained from the control treatment; whereas the highest fresh and dry weights were 86.0 and 34.6 g for the the limestone parent material soil (S8), 84.8 and 35.0 g for the 2% HA obtained from the 2% HA + 12 mg $kg^{-1}$ Zn treatment, respectively.

## Discussion

In soil, Zn availability is controlled by the intensity of adsorption and precipitation reactions. Zn sorption increases as the soil pH are higher due to the rise of negative charges on soil colloid surfaces and the increased presence of adsorbed and precipitated Zn forms in soil (Montalvo et al., 2016). Langmuir and Freundlich adsorption models were applied in Zn adsorption study, and adsorption parameters were determined from these models (Table 9). Langmuir and Freundlich adsorption isotherms of 12 soil samples without and with humic acid application were determined. In these soils, adsorption data for applications are described at acceptable levels with both isotherms. The adsorption data of all the soils were better described by the linear Langmuir model compared with the Freundlich model. For all soils, the Langmuir isotherm showed significant results both in the control soils without humic acid and in the soils with 2% humic acid. Freundlich isotherms showed significant results only in soils with humic acid. Regarding the physical and physicochemical properties of the soil, texture (clay content), organic matter and pH were the factors that sharply influenced soil Zn availability. Organic matter content controls the magnitude of Zn sorption in soil (Montalvo et al., 2016). Organic matter is composed of different fractions, including humic acid and fulvic acids, which are organic matter pools characterized by low isoelectric points; thus, even at low soil pH levels, negative charges prevail on the surface of organic colloids (Stevenson, 1994). Soil properties can modulate Zn interaction, adsorption and precipitation with soil components, including organic matter pools (Broadley et al., 2012; Alloway, 2009; Montalvo et al., 2016). When associated with organic ligands, Zn availability for crops depends on the chemical stability, solubility and reactivity of the bonds formed between the humic matrix and the metal in the complexes formed (Boguta and Sokołowska, 2016; Rose et al., 2014).

**Table 8.** The effects of humic acid (HA) and Zn treatments on the fresh and dry biomasses of maize grown on the marl (S4) and limestone soil (S8) in the greenhouse experiment

| Zn treatments, mg $kg^{-1}$ | Marl parent material (S4) | | | | Limestone parent material (S8) | | | |
|---|---|---|---|---|---|---|---|---|
| | Fresh biomass, g $pot^{-1}$ HA (0%) | Fresh biomass, g $pot^{-1}$ HA (2%) | Dry biomass, g $pot^{-1}$ HA (0%) | Dry biomass, g $pot^{-1}$ HA (2%) | Fresh biomass, g $pot^{-1}$ HA (0%) | Fresh biomass, g $pot^{-1}$ HA (2%) | Dry biomass, g $pot^{-1}$ HA (0%) | Dry biomass, g $pot^{-1}$ HA (2%) |
| 0 | 58.0 e | 65.5 d | 24.3 d | 26.4 e | 65.8 d | 78.7 b | 24.2 d | 26.2 e |
| 4 | 71.2 d | 74.3 c | 29.2 c | 29.0 d | 82.2 b | 83.5 a | 28.4 c | 28.6 d |
| 8 | 78.3 b | 77.5 b | 32.2 b | 32.1 c | 82.5 b | 84.7 a | 31.7 b | 31.9 c |
| 12 | 82.2 a | 86.3 a | 34.9 a | 35.7 a | 86.0 a | 84.8 a | 34.6 a | 35.0 a |
| 16 | 75.3 c | 79.5 b | 33.0 b | 34.1 b | 75.2 c | 75.5 c | 31.8 b | 33.5 b |

Different letter in the same column indicate a significant difference at p≤0.05

In this study, soils formed on marl and limestone parent material showed differences in the desorption rates of adsorbed Zn at initial Zn concentrations (6.5-32.5-65-162.5-325-487.5-650 mg $L^{-1}$). These differences in soil properties (Table 2 and 3) may have caused differences in the effects of humic acid application on sorption isotherm parameters. As a function of initial concentrations, the soil scavange the Zn by variety of sorption/precipitation reactions that each has different irreversibility and bounding strength. The desorption rate (%) of adsorbed Zn increased as the initial Zn concentration increased in the all soil series formed on marl (smectite clay mineral) and limestone (kaolinite clay mineral) parent material (Table 6). An increase in clay content is a key factor to increase Zn adsorption in soils. However, the density of negative charges in tropical clay minerals and Al and Fe oxides is lower than those in organic colloids (Alloway, 2009; Broadley et al., 2012; Montalvo et al., 2016). Moreover, Zn availability to plants is controlled by soil type, minerals associated with clay and Fe and Al oxides, soil parent material, total Zn content, soil pH, concentrations of organic

**Table 9**. The effects of humic acid treatment on the sorption isotherm parameters

| | | Langmuir isotherm | | | Freundlich isotherm | | |
|---|---|---|---|---|---|---|---|
| Soil | Treatment | $S_{max}$ (mg kg$^{-1}$) | k | $R^2$ | $K_F$ (mg kg$^{-1}$) | 1/n | $R^2$ |
| S1 | HA | 5555 | 0.069 | 0.991 | 674 | 0.384 | 0.898 |
| | WS | 5882 | 0.090 | 0.990 | 1482 | 0.231 | 0.886 |
| S2 | HA | 5263 | 0.066 | 0.990 | 769 | 0.342 | 0.901 |
| | WS | 6250 | 0.065 | 0.969 | 1564 | 0.222 | 0.905 |
| S3 | HA | 4347 | 0.116 | 0.998 | 804 | 0.304 | 0.873 |
| | WS | 5000 | 0.087 | 0.991 | 1383 | 0.213 | 0.896 |
| S4 | HA | 3846 | 0.090 | 0.992 | 883 | 0.256 | 0.890 |
| | WS | 5000 | 0.064 | 0.973 | 1414 | 0.196 | 0.902 |
| S5 | HA | 2631 | 0.060 | 0.995 | 550 | 0.258 | 0.816 |
| | WS | 3333 | 0.061 | 0.993 | 1077 | 0.173 | 0.845 |
| S6 | HA | 2857 | 0.267 | 0.999 | 845 | 0.225 | 0.842 |
| | WS | 3704 | 0.083 | 0.988 | 1259 | 0.174 | 0.886 |
| S7 | HA | 3030 | 0.095 | 0.993 | 790 | 0.233 | 0.853 |
| | WS | 3571 | 0.075 | 0.979 | 1213 | 0.171 | 0.875 |
| S8 | HA | 4348 | 0.106 | 0.993 | 795 | 0.311 | 0.892 |
| | WS | 5263 | 0.072 | 0.959 | 1469 | 0.210 | 0.901 |
| S9 | HA | 1052 | 0.059 | 0.979 | 2019 | 0.264 | 0.935 |
| | WS | 1042 | 0.069 | 0.976 | 326 | 0.183 | 0.947 |
| S10 | HA | 5000 | 0.056 | 0.970 | 766 | 0.326 | 0.880 |
| | WS | 5000 | 0.071 | 0.948 | 1249 | 0.227 | 0.872 |
| S11 | HA | 4167 | 0.084 | 0.976 | 832 | 0.282 | 0.871 |
| | WS | 4762 | 0.071 | 0.976 | 1336 | 0.202 | 0.890 |
| S12 | HA | 4000 | 0.153 | 0.999 | 840 | 0.283 | 0.888 |
| | WS | 4545 | 0.137 | 0.997 | 1386 | 0.203 | 0.895 |

S1: Atınova soil series, S2: Hacıfakıl soil series, S3: Çatalca soil series, S4: Başkuyu soil series, S5: Kuyubaşı soil series, S6: Seyrantepe soil series, S7: Saraççeşme soil series, S8: Tine soil series, S9: Karataş-1 soil series, S10: Tüvem-1 soil series, S11: Tüvem-2 soil series, S12: Karataş-2 soil series, HA: Humic acid, WS: Whole soil, Smax: Maximum Zn adsorption of the soil, k: Bonding energy coefficient, $K_F$: Freundlich K, n: Constant

matter, Ca, calcite, bicarbonate, and phosphate found in the soil, solution or in labile forms prone to be solubilized and to react with zinc with subsequent formation of precipitates and high-stability organometallic complexes (Alloway, 2009; Boguta and Sokołowska, 2016; Broadley et al., 2012; Montalvo et al., 2016). Smectite is one of the largest and most important classes of the phyllosilicate clay-mineral group. They are common in temperate soils and tend to dominate the cationic adsorption chemistry of these soils due to their cation exchange capacity and very high specific surface (Kloprogge and Frost, 1999). Desorption is an important property that determines the mobility of adsorbed elements or metals in contaminated areas. The amount of desorption varies depending on the nature of the adsorption event, i.e. whether the binding is by physical, electrostatic bonds or covalent bonds. Physically adsorbed cations are usually capable of being readily replaced or desorbed by other cations present in the soil solution. On the other hand, ions or molecules bound by covalent bonds are not easily desorbed because they bind to the adsorbent with high energy and usually released back to the solution phase with the complete breakdown of the adsorbent. In soil 9, none of the Zn absorbed at the lowest concentration was desorbed. At the 650 mg L$^{-1}$ initial concentration, 18.0% of the Zn amount was desorbed in the limestone parent material in the Karataş-1 series Typit Calciorthid soil family (S9) in the Aridisol and this rate increased to 22.6% with the application of humic acid. The amount of Zn that could be desorbed or replaced by Ca was calculated from the adsorbed Zn at each initial concentration applied. Accordingly, at the lowest concentrations applied, almost none of the absorbed Zn was desorbed. The amount of absorbed Zn was almost not desorbed at the lowest concentrations applied (6.5 - 32.5 - 65 mg L$^{-1}$). This shows that especially Zn adsorbs with a high energy and is retained with a high energy even at high concentrations. The other point is that the adsorption or precipitation mechanisms of Zn cause the release of 2 H$^+$ ion on a stochiomeric base (Uygur and Rimmer, 2000). This reduces the pH of equilibrium solution below 6.5, in turn, the physisorption processes take place that increase the desorption rate. In addition, humic acid application showed that desorption was higher than the application without humic acid because of chelation reaction between the HA and Zn and possible blockage of specific sorption site on the colloidal surfaceses (Spark et al., 1997). This suggests that Zn may be highly immobilized in these soils, especially at high concentrations. The fact that Zn is not desorbed at low concentrations indicates that Zn deficiency is a key fact in soils with poor organic matter content and high pH under insufficient fertilization practices, to limit plant performance. The release of trace elements over time (desorption rate) is important for plant nutrition. In general, desorption of soils increased Zn mobility due to humic acid application.

Bivariate Pearson correlations coefficients between the soil properties and the sorption parameters were given in Table 10. Smax parameter was negatively correlated with soil EC, $P_{av}$, $K_{av}$, OM, silt, clay, total N and CEC. The properties negatively correlated with Smax indicates that the property function a reduced sorption site, co-input of Zn by previously applied agricultural practices and chelate with it. The parameters such as pH, CCE, sand, total K, P, Fe, Zn, Cu, Mn and CEC positively correlated with has a primary rol over accumulation or fixation of Zn in the soil. Regarding the soil's physical and physicochemical properties, the texture (clay content), organic matter and pH were the factors that sharply influenced soil Zn availability. Soil properties are capable of modulating Zn interaction, adsorption, and precipitation with soil components, including organic matter pools (Alloway, 2009; Broadley et al., 2012; Montalvo et al., 2016). When associated with organic ligands, the Zn availability to crops relies on the chemical stability, solubility, and reactivity of the bonds formed between the humic matrix and the metal in the complexes formed. Generally, Fe and Al oxides have a larger and stronger sorption sites due to a chemisorption process take place on their surface, can coat relatively lesser soil component such as carbonates and sand sized fractions (Uygur and Rimmer, 2000). Smax parameter was significantly and positively correlated with $K_F$ (0.940, $p < 0.01$) and n (0.755, −0.47, $p < 0.01$) values.

The DTPA-Zn measured troughout the entire incubation period of 90-day are given in Table 7. The availability of plant nutrients during the growth period ensures a good performance along with a desirable yield and yield quality.  The release of trace elements retained by sorption sites is required to sustainably meet the trace element needs of the plant (Uygur and Rimmer, 2000). In this study, HA+Zn application increased the DTPA-Zn concentration of the soils compared to only HA application in all soil series formed on marl (smectite clay mineral) and limestone (kaolinite clay mineral) parent material during the incubation period. When Zn was mixed with HA, free sulfates were

**Table 10.** Bivariate Pearson correlations between the soil properties and the sorption parameters

| Parameters | Smax | k | $K_F$ | n |
|---|---|---|---|---|
| k | 0.110 | | | |
| $K_F$ | $0.940^{**}$ | 0.206 | | |
| n | $0.755^{**}$ | 0.154 | 0.514 | |
| EC (dS $m^{-1}$) | -0.301 | -0.136 | -0.413 | -0.114 |
| pH | 0.559 | 0.038 | $0.628^{*}$ | 0.314 |
| CCE (%) | 0.137 | -0.408 | 0.259 | -0.323 |
| $P_{av}$ (kg $ha^{-1}$) | $-0.584^{*}$ | -0.107 | $-0.761^{**}$ | 0.017 |
| $K_{av}$ (kg $ha^{-1}$) | -0.541 | 0.421 | -0.551 | -0.171 |
| OM (%) | $-0.619^{*}$ | 0.138 | $-0.629^{*}$ | -0.300 |
| Sand (%) | 0.526 | 0.213 | 0.530 | 0.339 |
| Silt (%) | $-0.582^{*}$ | -0.101 | -0.439 | $-0.631^{*}$ |
| Clay (%) | -0.252 | -0.252 | -0.397 | 0.080 |
| ΣN (%) | $-0.620^{*}$ | 0.139 | $-0.629^{*}$ | -0.300 |
| ΣK (%) | 0.459 | -0.240 | 0.359 | 0.410 |
| ΣP (%) | 0.277 | -0.338 | 0.449 | -0.267 |
| ΣFe (%) | 0.117 | -0.373 | 0.196 | -0.255 |
| ΣZn (mg $kg^{-1}$) | 0.279 | -0.085 | 0.453 | -0.202 |
| ΣCu (mg $kg^{-1}$) | 0.313 | -0.076 | 0.504 | -0.248 |
| ΣMn (mg $kg^{-1}$) | 0.250 | -0.219 | 0.404 | -0.223 |
| CEC (cmol $kg^{-1}$) | -0.210 | -0.069 | -0.352 | 0.149 |

Σ: Total, k: Bonding energy coefficient, $K_F$: Freundlich K, n: Constant, EC: Electrical conductivity, pH: Soil reaction in saturation paste, CCE: Calcium carbonate equivalent, $P_{av}$: Available $P_2O_5$, $K_{av}$: Available $K_2O$, OM: Organic matter, CEC: Cation exchange capacity retained in the complex formed. In addition, bonds related to sulfone groups were formed, which are indicative of the interaction of HA with $ZnSO_4$ and the synthesis of Zn-HA complexes. Sulfur groups present in the HA+$ZnSO_4$ mixture are indicative of Zn-HA complexation. This is probably due to the presence of functional groups containing oxygen, nitrogen and sulfur on the HA surface (Aguiar et al., 2013; Nardi et al., 2021; Zanin et al., 2019). There was no statistical difference between Zn treatment and Zn+HA, treatment in terms of DTPA-Zn concentrations of soils in both soil series (Başkuyu, Hacıfakıl, Saraççeşme and Tine) regardless the parent material during the incubation period. The DTPA-Zn concentration of the soils was higher in the soil series formed in the marl parent material dominated by smectite clay mineral than in the limestone parent material, especially in Başkuyu soil series. The mechanisms and processes of Zn interaction with HA include ionic or uncoordinated forms, undefined complexes, bidentate chelates and bidentate bridging coordination bonds (Boguta and Sokołowska, 2016). The interaction of organic ligands with Zn is beneficial for providing Zn to plants, considering that free Zn ions are prone to react with soil constituents through sorption and precipitation, which are the main processes regulating Zn availability in soil (Montalvo et al., 2016; Olaetxea et al., 2018). Organic matter is also a source of soluble organic compounds that can bind micronutrients through the formation of soluble organo-metal complexes (Wang and Xing, 2005). In this study, S4, which had a higher Zn content than S2, S7 and S8, presented a higher soil Zn-DTPA content in the Başkuyu soil series formed on the marl parent material with smectite clay mineral during the incubation period.

The effects of humic acid (HA) and Zn treatments on the fresh and dry biomasses of maize grown on the marl (S4) and limestone soil (S8) in the greenhouse experiment were given in Table 8. The highest fresh and dry weight of maize was 86.3 and 35.7 g from 2% HA+12 mg $kg^{-1}$ Zn application in the Başkuyu soil series formed on the marl parent material with smectite clay mineral, respectively. Humic acid applications in soil formed on marl (smectite clay mineral) and limestone (kaolinite clay mineral) parent material increased the fresh weight of maize compared to the control. Humic substances are formed by the chemical and biological breakdown of plant and animal residues and provide an important source of organic carbon in soil. The most prominent biostimulant is humic acid, which has direct and indirect effects on the morpho-physiological growth of plants (Peña-Méndez et al., 2005). HA is mainly derived from humic substances and the final decomposed material contains 60% of the organic matter in the soil (Muscolo et al., 2013). On the other hand, application of HA to soil not only improves various soil properties (such as structural stability, biological activity and affecting soil pH), but also promotes better root development, water holding capacity, carbon sequestration, cation exchange capacity and nutrient availability in the rhizosphere (Gümüş and Şeker, 2015). Solution cations have an influence on the rate and amount of HA sorbed onto smectite. The adsorption of HA by mineral surfaces is mostly influenced by solution pH, ionic strength and the type of exchangeable cations. These factors also influence the structure conformation of HA (Essington, 2015). HA and clay complexes contribute to soil structure and water holding capacity and play an important role in the cycling of various nutrients and pollutants by taking part in reductive and oxidative reactions (Sparks, 2003). The presence of $Ca^{2+}$ improves the binding between the mineral surface and HA. $Ca^{2+}$ is more effective than $K^+$ in eliminating the repulsive charges between the smectite surface and weathered humates and fulvates. Furthermore, the $Ca^{2+}$ ions reduce the HA solubility (Li et

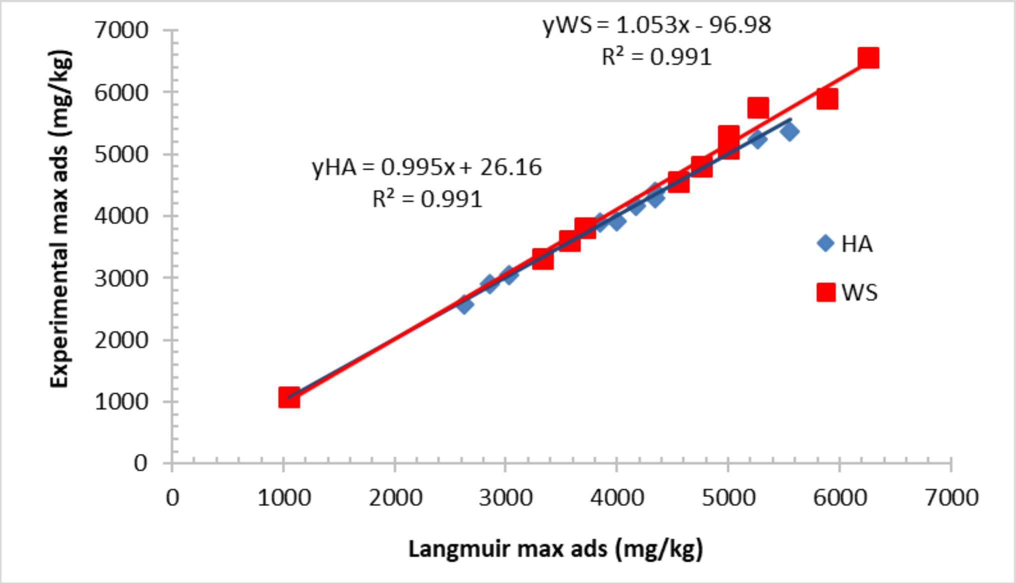

**Figure 2.** Scatter diagram for the experimental max adsorption vs. predicted ones with (HA) and without (WS) humic acid (HA) treatment

al., 2004) by bridging clay and surfaces. This infact further decreases the efficiency of potentially high energy sites for Zn sorption by changing the nature of colloidal surfaces. Therefore, higher calcium carbonate containing Başkuyu soil (S4, 35.1%) had higher DTPA-Zn in overall than the Tine soil series (S8, 9.01%) due to apparent drawback the effect of excessive soil carbonates in the soil. Similarly, the heavy fraction of soil organic corbon such as HA accumulated twice as much SOC at the $CaCO_3^-$bearing site, in Ca prevalent soils than the soils with less Ca (Rowley et al., 2021).

## Conclusion

It was determined that the clay types were different in 12 different soil samples with two different parent materials belonging to the Arisol order. While the dominant clay mineral in the soils formed on marl parent material was smectite, the dominant clay mineral in the soils formed on limestone parent material was kaolinite. Because of adsorption analysis, the parameters of Langmuir and Freundlich models were determined. Langmuir model was found to be more suitable for Zn adsorption than Freundlich model. There was no significant difference between Langmuir and Freundlich isotherm parameters in soils formed on marl and limestone parent material with and without humic acid. The desorption rate (%) of adsorbed Zn increased as the initial Zn concentration increased in the all soil series formed on marl (smectite clay mineral) and limestone (kaolinite clay mineral) parent material. In the incubation study, humic acid applications increased the available Zn content in soils formed on both parent materials compared to the control. Humic acid applications increased the fresh weight of maize compared to the control. The highest available Zn was determined in Başkuyu series (0.79 mg $kg^{-1}$) formed on marl parent material, while the lowest available Zn was obtained in Saraççeşme series (0.60 mg $kg^{-1}$) formed on limestone parent material. In the greenhouse experiment, humic acid applications increased the fresh weight of maize in soils formed on both parent materials by 0.4 and 19.6% compared to to the control. Zn fertilization with HA further increased the fresh and dry yield that 12 mg $kg^{-1}$ performed better. The lowest wet and dry weight values of maize were obtained from the control in the soil formed on marl parent material, while the highest wet and dry weight values were obtained from 2% humic acid + 12 mg $kg^{-1}$ Zn treatment. Smax (maximum Zn adsorption of the soil) parameter of soils were negatively correlated with EC, $P_{av}$, $K_{av}$, organic matter, silt, clay, total N and cation exchange capacity (CEC) of the samples. Although humic acid alone increases the plant availability of Zn, HA + Zn applications should be applied together in order to obtain higher yields. It is necessary to improve the results obtained by conducting studies for soils with different parent material and soil families.

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## Author Contribution

**M.K.:** Conceptualization, investigation, methodology, validation, software, validation, formal analysis, investigation, resources, data curation, writing-original draft preparation, writing-review and editing, visualization, supervision, statistical analysis, project administration. **G.D. and N.G.:** methodology, formal analysis. **S.U., V.U. and M.O.A.:** Methodology, validation, software, validation, formal analysis, investigation, resources, data curation, writing-original draft preparation, writing-review and editing, visualization, supervision, statistical analysis. **Z.D.:** Writing-original draft preparation, writing-review and editing, visualization, statistical analysis, supervision, resources, data curation, all authors reviewed the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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