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Interactions of humic acid with development and uptake by seedlings of Vicia faba *L., (cv. Eresen 87) grown in conditions of aluminium toxicity have been investigated in pot experiments in a controlled greenhouse environment. The objective of the study was to investigate whether humic acid moderates aluminum toxicity. The seedlings were treated with solutions of aluminum chloride (AlCl₃) prepared in Hoagland control nutrient solution (HO) and 50 and100 μM and humic acid + Hoagland (HA) solutions, respectively. The 50 and100 μM HA solutions increased root fresh (RFW) and dry (RDW) weights, where the RFW differed significantly from controls (HO) after statistical evaluation by NCSS (NCSS, Kaysville, Utah) with two-sample T-test range at the 5% level. The results of the current experiment suggested that humic acid had suppressed or counteracted the toxic effect of aluminum (Al³⁺) on both main and lateral root growth. Humic acid seems to block the effect of Al³⁺ on nutrient uptake, as tested by atomic absorption spectrophotometry (AAS) and flame photometery (FP). Al³⁺ content in the roots was significantly decreased by 219% in 50-μM HA and by 49% in 100-μM HA treatments respectively. Potassium (K⁺), sodium (Na⁺), and iron (Fe³⁺) were recorded as the other elements taken up in the greatest amounts among the tested nutrients, in addition to Al³⁺. Humic acid increased the contents of Na⁺, K⁺, manganese (Mn²⁺), and zinc (Zn²⁺) significantly in both concentrations of HA treatment compared to controls. The Fe³⁺ content in the roots decreased, in both treatments of HA, application by 252% and 32% respectively. The reduction in the former was significant.*

Keywords   Aluminum, humic acid, nutrients, roots, *Vicia faba* L.

Introduction

Humic acid as a commercial product contains 44–58% carbon (C), 42–46% oxygen (O), 6–8% hydrogen (H), and 0.5–4% nitrogen (N) as well as many mineral elements (Larcher 2003; Lee and Bartlette 1976). It increases the availability of nutrient elements by promoting the conversion of mineral elements into forms available to plants. According to Chen and Aviad (1990), humic substances usually contain large quantities of trace minerals, derived from decayed plant material, and have hormone-like effects on plant growth and

metabolism. Humic acid includes auxin-like effects (O'Donnell 1973) and cytokinin-like responses that increased in creeping bentgrass (Zhang and Ervin 2004).

Certain components in humic acid that are used in various metabolic processes in the cells can be absorbed and transported directly into the plant vascular system. The greatest uptake of nutrients from the soil can enter into the plant root through the cell membrane (Yılmaz 2007; Tipping 2002; Kulikova, Stepanova, and Koroleva 2005).

Aluminum (Al) is one of the most abundant metal ions in the earth's crust but is not regarded as an essential plant nutrient (Rout, Samantaray, and Das 2001). In soils, Al is found as mineral forms, including hydrous oxides, aluminosilicates, sulfates, and phosphates. In acidic soils Al toxicity symptoms appear as a major growth-limiting factor for crop production, causing reduction in growth of the main axis and of lateral root formation (Foy 1988). The restriction of root growth may eventually result in nutrient stress and/or water stress, as a result of Al toxicity (Haynes and Mokolobate 2001).

There are many research reports that describe how the relationship between root growth and humic acid affects nutrient uptake in various growth conditions. For instance, humic acid caused the increase in length and dry weight of maize plant roots and also enhanced nitrogen (N), phosphorus (P), potassium (K^+), calcium (Ca^{2+}), copper (Cu^{2+}), manganese (Mn^{2+}), zinc (Zn^{2+}), and iron (Fe^{3+}) uptake (Eyheraguibel, Silvestre, and Morard 2008); root length in *Helianthus annuus* L. (Kolsarıcı et al. 2005); and root dry weight in marigold, pepper, strawberries and tomato (Arancon et al. 2004). Humic substances have a variety of effects: increased root dry weight and enhanced micronutrients such as Zn^{2+}, Fe^{3+}, Mn^{2+}, and Cu^{2+} (Sharif, Khattak, and Sarir 2002); increased root dry weight of tomato and cucumber (Atiyeh et al. 2002); stimulated root development and enhanced amounts of N, K^+, Cu^{2+}, and Mn^{2+} in ryegrass (Bidegain et al 2000); and increased root fresh and dry weights in tomato and eggplant (Dursun, Güvenç, and Turan 1999). Adani et al. (1998) reported that humic acid affected fresh and dry weights of tomato roots and resulted in conspicious increase of Fe^{3+} content, depending on the humic resource. The two concentrations (20 and 50 mg L^{-1}) of humic acid, resourced from fertilizer, caused Fe^{3+} to increase to 113% and 123% of control values, whereas leonardite-resourced humic substance increased Fe^{3+} content by 135% and 161% in tomato roots. David, Nelson, and Sanders (1994) reported that more K^+ and Ca^{2+} accumulation occurred in tomato roots grown under greenhouse conditions.

Aluminum toxicity is common in acidic soil, induced by acid rains, as a result of industrial pollution and excessive use of fertilizers in agriculture (Wild 1988). On a global basis, 50% of planting areas are accepted as acidic (Von Uexküll and Mutert 1995), which causes yield reductions and toxic effect on plant growth (Matsumoto 2000). Although Al is a nonessential element for plant growth, at 7% it is the third most common element found in soils, nearly everywhere in the earth. Aluminum toxicity increases with increasing acidity of soil (under pH 5) and is then found as the aluminium hexaaqua complex ion $[Al(H_2O)_6^{+3}]$, which is the most toxic form (Matsumoto 2000). It can make a strong compound with water molecules. The most common symptom of Al toxicity in plants is the restriction of root development of main and lateral roots, which appear as shorter, thicker, and brownish colored (Foy 1988; Vardar, Arıcan, and Gözükırmızı 2006).

According to Foy, Chaney, and White (1978), Huang, Grunes, and Kochian (1992), Al interfered with the uptake, transport, and use of Ca^{2+}, Mg^{2+}, and K^+. The toxic effect resembles phosphate deficiency (Kocaçalışkan 2005; Haynes 1982). Nichol et al. (1993) reported that Al decreased Ca^{2+} uptake 69%, NH_4^+ by 40%, and K^+ by 13% in the Al-sensitive plant barley (*Hordeum vulgare* L.). In cacao (*Theobroma cacao* L.) K^+, Ca^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} (Baligar and Fegaria 2005) and in peach P^{3-}, Ca^{2+},

Mg^{2+}, Fe^{3+}, and molybdenum (Mo) were decreased (Graham 2001; Liu and Luan 2001). Aluminum also caused inhibition of K^+ uptake by guard cells of broad bean. In roots of a variety of silver birch (*Betula pendula* Roth) Al decreased K^+, Mg^{2+}, and Fe^{3+} uptake but did not affect P^{3-} concentration; however, lower Al levels increased the amounts of Ca^{2+} in the roots (Kidd and Proctor 2000). According to Ryan and Kochian (1993), 50 µM Al caused Ca^{2+} reduction in an Al-sensitive variety of wheat, whereas Al-tolerant wheat was not affected. Significant reduction of root growth was observed in squash roots after the roots were treated with 50 µM Al (Ahn et al. 2001).

Broad bean is an important nutritious vegetable all over the world and contains 20–36% protein for human and animal consumption. While in Turkey 47,000 tons of dry broad bean has been produced, total production is up to 4 438 510 tons in the world (Anonymous 2005).

There are many reports of Al toxicity affecting nutrient uptake by various plants; however, none of these has focused on the subject of interactions between humic acid and Al. Because there was no report of humic acid affecting Al toxicity in broad bean, this study is aimed as a contribution to this subject. The objectives of this experiment were to assess whether humic acid derived from leonardite affects broad bean grown in Al-treated pots. The study is also based on an earlier investigation of uptake of Na^+, K^+, Ca^{2+} and Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} and their interactions in Hoagland nutrient solution supplemented with humic acid on roots of *Vicia faba* L.

Materials and Methods

Cultivation in Growth Room Condition

The experiments were conducted with broad bean cultivar Eresen 87 in the growth room conditions of the Department of Botany, Marmara University, during the period from November 2007 to 2009. The seeds were obtained from the Aegean Agricultural Research Institute, Izmir. The ingredients of a commercial product of humic acid, derived from leonardite and called Black Gold, was kindly provided by Hektaş Tic. T.A.Ş. Gebze, Kocaeli.

The cultivar seeds, after imbibition in distilled water, were germinated in separate Petri dishes prepared by placing a disk of filter paper in the base of each, arranged for randomized treatment as control (HO) and experimental (HA) groups for 2 weeks. The Hoagland and humic acid treatments were applied to the seeds as full-strength Hoagland–Arnon (1950) solutions (HO) and humic acid (HA) (10 ml L^{-1}) added to Hoagland solutions, respectively. The solutions used in the experiment were formulated as shown in Tables 1 and 2.

Table 1
Liquid humic acid ingredients

Ingredient	Volume (% w/w)
Total organic substances	9.5
Total humic acid + fulvic acid	18.4
Water-soluble K_2O	4.0
pH	10–12

Table 2
Experimental groups and solutions

Treatment	Composition of solutions
HO50 and HO100 (control)	50 and 100 μM AlCl$_3$ added to 1-L Hoagland solutions
HA50 and HA100	50 and 100 μM AlCl$_3$ added to 1 L Hoagland solution containing 10 ml liquid humic acid

The seeds were transferred into single pots containing 280 g sterilized Gardol compost media. The pH was stabilized at 4.5 by adding sulfuric acid (H$_2$SO$_4$) and checked once in every 2 days. The pots were set up in a completely randomized block (Mead and Curnow 1983) at 23 $\pm$ 2 °C (Cordovilla et al. 1999), 55% $\pm$ 5 humidity, and exposed to 4000–4200 lux light intensity for 14-/10-h day and night periods, respectively.

The seedlings were treated with Hoagland solutions containing Al and humic acid + Hoagland (HO 50, 100 μM and HA 50, 100 μM) respectively (Table 2). During 2 months, the control and experimental sets were were given, at 3-day intervals, 30 ml Hoagland (HO) and Hoagland + 10 ml L^{-1} humic acid (HA) solutions, respectively. At the harvesting time after 2 months of growth, the roots were cut from each plant, fresh and dry weights of each sample were recorded, and the samples were prepared the methods of Roberts et al. (1993), Beadle (1993), and Mackey and Neal (1993) for determination of the nutrient contents of the roots.

Nutrient Analyses

For the nutrient analyses, samples were prepared by the wet ashing method described by Kaçar (1972). The dried samples were crushed using mortar and pestle. The powder from each sample was transferred to an Erlenmeyer flask, to which were added 6 ml nitric acid + perchloric acid solutions. The samples were kept for 30 min in a water bath at 40 °C for digestion and the solution removed by heating at 150–180 °C until reduced to 1-ml extracts. This residue was dissolved with distilled water and made up to 100 ml in a standard flask. The samples for determination of Na$^+$, K$^+$, Ca^{2+} and Cu^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, and Al^{3+} were analyzed using flame photometers (FP) (Jenway, Dunmow, UK), flame atomic absorption spectrophotometers (FAAS), and Varian Liberty Series II atomic emission spectrophotometers (ICP-AES) respectively (Varian Medical Systems, Palo Alto, Calif., USA). FAAS was used in the determinations of Cu^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, and Al^{3+}. Air-acetylene (C$_2$H$_2$) was used in the determinations of Cu^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} and nitrous oxide (N$_2$O)-C$_2$H$_2$, was used in the determination of Al^{3+}.

Statistical Analysis

The data obtained from the experiments, in which six replicate plants for each treatment were set up in a randomized manner, were subjected to NCSS (2004) for two-sample T-test range at 5% to determine significance of differences between means. Means are indicated with standard error ($\pm$SE).

Results and Discussion

Effects of Humic Acids on Root Growth under Aluminum Treatment

The root fresh weight (RFW) significantly increased in HA50 and HA100 treatments as 38% and 24% respectively compared to control groups at the level of $\alpha = 0.05$. At the same level, humic acid increased RDW in both treatments of 50 and 100 HA as 0.82% and 13%, respectively, but without any significant differences. In both cases humic acid clearly increased root biomass and seemed to decrease the toxic effect of Al^{3+} on growth of bean roots. Decrease in root biomass has been reported in various plants. The available reports state that Al toxicity causes inhibition of root development in silver birch (*Betula pendula* Roth) (Kidd and Proctor 2000), inhibits growth in root length and decreases RDW in soy bean (*Glycine max* (L.) Merr.) (Shamsi et al. 2007), decreases RDW in sorghum by increasing Al concentration (Ohki 1987), and decreases RDW in peach [*Prunus persica* (L.) Batsch] (Chibiliti and Byrne 1990). In the present research we observed that broad bean root growth is also affected by Al^{3+} toxicity without humic acid and showed damage in root tips as well as inhibition of lateral root formation (Tables 3 and 4). This positive effect of humic acid was also observed in a maize root system, which was inhibited by Al (Harper et al. 1995). When organic acids (citrate, oxalate, tartarate, malate, and lactate) were tested with humic acid, they also decreased Al toxicity in soy bean [*Glycine max* (L.) Merr.], increased RDW, and enhanced root length. It was concluded that as little as $1 \, g \, L^{-1}$ humic acid was as effective as $1000 \, \mu M$ citrate on those parameters (Ginting, Johnson, and Wilkens 1998). The present results agree with these studies in providing evidence of a positive effect of humic acid on root weight and length, in the presence of Al^{3+} (Tables 3 and 4). Humic acid seems to chelate Al^{3+}, making it inactive, and blocking Al in the rhizosphere.

Effects of Humic Acids on Nutrient Content of the Roots of Broad Bean Seedlings

The concentrations of different nutrients, namely, Al, K, Cu, Na, Ca, Fe, Zn, and Mn, in the roots of broad bean plants were determined after growth in both Hoagland (HO) and Hoagland + humic acid (HA) solutions, each one added at concentrations of 50 and $100\mu M$ Al chloride hexahydrate ($AlCl_3 \cdot 6H_2O$). The results obtained at the harvesting stage, after 2 months of growth, were evaluated as $mg \, g^{-1}$ of total dry weight. The nutrient concentrations among the controls and experimental treatments are presented in Table 4.

Table 3
Growth parameters of roots of *Vicia faba*, grown under aluminum stress, at harvesting time

	Experimental group			
Growth parameter	HO50	HA50	HO100	HA100
Root fresh weight (g)	3.30 ± 0.43	$4.57 \pm 0.08^*$	4.60 ± 0.49	$5.72 \pm 0.20^{**}$
Root dry weight (g)	0.29 ± 0.14	0.53 ± 0.08	0.58 ± 0.05	0.66 ± 0.04

*Significantly different from HO50.
**Significantly different from HO100.

Table 4

Nutrient composition of broad bean (*Vicia faba* L.) cv. ERESEN 87 roots

	Nutrient (mg/g) in dry weight							
Treatment	Na	K	Ca	Al	Cu	Fe	Mn	Zn
HO 50	2.136 ± 0.28	12.55 ± 1.73	1.33 ± 0.29	7.86 ± 1.01	0.02 ± 0.00	4.64 ± 1.12	0.28 ± 0.04	0.06 ± 0.01
HA 50	12.296* ± 0.79	33.41* ± 1.15	1.24 ± 0.04	2.47* ± 0.36	0.11* ± 0.08	1.32* ± 0.41	0.54* ± 0.02	0.21* ± 0.05
HO 100	2.436 ± 0.33	6.28 ± 0.91	1.22 ± 0.29	7.53 ± 2.64	0.02 ± 0.00	5.10 ± 2.36	0.23 ± 0.04	0.07 ± 0.00
HA 100	15.405** ± 0.86	37.60** ± 3.49	2.49** ± 0.33	5.07 ± 1.62	0.02 ± 0.00	3.88 ± 1.38	0.91** ± 0.08	0.16** ± 0.01

*Significantly different from HO 50.
**Significantly different from HO 100.

Aluminum

As one the common constituents of the earth and plant, Al^{3+} content varies greatly in plants and its physiological function is still unclear (Kabata-Pendias and Pendias 2001), although there is some evidence that low levels have a beneficial effect on plant growth (Clark 1977; Foy, Chaney, and White 1978).

It is reported that Al^{3+} injury occurs in many plants grown in acidic soil and it is accepted as a limiting factor in crop production (Reid 1976; Foy, Chaney, and White 1978; Foy 1983; Kochian 1995; Watanabe and Osaki 2002). The symptoms appear first in the roots as inhibition of cell division as well as direct damage of the plasma membrane (Matsumoto 2000), so plants have shorter, thicker brownish roots and less development of lateral roots (Foy 1988; Vardar, Arıcan, and Gözükırmızı 2006).

Convenient Al^{3+} uptake conditions were set up experimentally by providing Al solution with Hoagland nutrient solution (HO) as well as the remedial effect of humic acid. The concentration of Al showed that there were significant differences between the treatments HO50 and HA50 in broad bean roots (Table 4). While a significant decrease appears in HA 50 as 219%, the decrease in HA100 appeared as 49% without any significance at the same level of $\alpha = 0.05$.

Al^{3+} mostly can cause distortion in root formation without humic acid in the media but some plants might vary in Al uptake, depending on their sensitivity or tolerance to Al^{3+} toxicity. The Al^{3+} content increased in roots of soy bean (*Glycine max* (L.) Merr.) (Shamsi et al. 2007), *Abies firma* Siebold and Zucc (Yoshida, Takenaka, and Tezuka 2005), sorghum (Ohki 1987), and potato (*Solanum tuberosum* L.; Lee 1971). The increase of Al^{3+} content in the roots is greater (average 7.8 times) than in the aerial parts of broad bean (Büyükkeskin 2008).

Few reports have been found of removing Al^{3+} toxicity in the growth media for various plants. Suthipradit, Edwards, and Asher (1990) tested fulvic acid, malic acid, and oxalic acid for removing the toxic effects of Al^{3+} on soybean [*Glycine max* (L.) Merr.], cowpea (*Vigna unguiculata* L.), and mung bean (*Vigna radiata* L.) on root length. They reported that fulvic acid is able to remove toxic effects of Al better than malic and oxalic acids, which failed to remove Al toxicity. These results may be mostly related with the functional groups of humic acid, which contains greater amounts of oxygen and can bind strongly with monomeric Al^{3+} and thus inhibit Al^{3+} uptake by the roots.

Potassium/Sodium

The results of nutrient analyses indicated that, as is usual in plants, the concentration of potassium in broad bean roots was greawter than that of all other nutrients (Table 4). At the level of $\alpha = 0.05$ K^+ content increased significantly in both HA50 and HA100 treatments as 166% and 499% respectively, compared to controls (HO50 and HO100).

The available reports state only that K^+ content changes under Al^{3+} treatments; for example, K^+ content decreased in the roots of silver birch (*Betula pendula* Roth) (Kidd and Proctor 2000), peach [*Prunus persica* (L.) Batsch] (Chibiliti and Byrne 1990), tomato *Lycopersicon esculentum* Mill. (Simon et al. 1994), cacao (*Theobroma cacao* L.) (Baligar and Fageria 2005), and potato (*Solanum tuberosum* L.) under increased Al^{3+} concentration (Lee 1971). According to Kochian, Pineros, and Hoekenga (2005), Al^{3+} caused reduction of cation uptake by inhibiting H-ATPase activity directly in the plasm membrane. Thus, humic acid may have reduced the toxicity of Al^{3+} concentrations in the medium by making complexes with Al^{3+}, possibly allowing K^+ to enter more freely in to the roots.

Sodium ions are not essential for the growth of most land plants; however, sodium can still enter plant cells via several routes. Voltage-independent cation (VIC) channels are considered the major route for Na^+ entry into plant cells (Amtmann and Sanders 1999; Schachtman and Liu 1999; Tyerman and Skerrett 1999; White 1999).

Sodium is the element with the greatest increased ratio among the nutrients tested in the experiment, like K^+. The concentration showed that there were significant differences in both treatments of HA50 and HA100 (Table 4). As with K^+ increase, Na^+ accumulated significantly in HA 50 and HA100 by 476% and 532%, respectively, at the same level of $\alpha = 0.05$ compared to controls. This significant increase of Na^+ and K^+ in broad bean and also morphological changes of roots are accompanied by decreasing Al^{3+} content in the root. Sodium is known to have a negative effect on salt-sensitive plants. The ratio of K^+/Na^+ was 2.65 in HA 50 whereas it was 2.44 in HA 100 applications where Na^+ content increased by Al^{3+} concentration increase.

Calcium

Calcium content showed no significant differences between the HO50 and HA50 treatments, although Ca^{2+} content decreased by 7% in HA50. Nevertheless, it significantly increased by 105% in 100HA compared to 100HO at the level of $\alpha = 0.05$ (Table 4).

The available reports state that Ca^{2+} content changes only under Al^{3+} treatment in roots of different plant species. For instance, the literature states that Ca^{2+} decreases in roots of silver birch (*Betula pendula* Roth; Kidd and Proctor 2000), soybean [*Glycine max* (L.) Merr.; Shamsi et al. 2007], *Abies firma* Siebold and Zucc (Yoshida, Takenaka, and Tezuka 2005), peach [*Prunus persica* (L.) Batsch; Chibiliti and Byrne 1990], tomato (*Lycopersicon esculentum* Mill.; Simon et al. 1994), and *Piptatherum miliaceum* (L.) Coss (mountain rice; Zavas, Symeonidis, and Karataglis 1996). According to Kabata-Pendias and Pendias (2001), excessive Al^{3+} in plants induces Ca^{2+} deficiency or may cause reduction in its transportation. In this experiment, addition of humic acid to the solution decreased. Ca^{2+} content in HA50 but significantly increased its concentration in 100HA. This seems to relate to reports that $Al^{3+,}$ which blocks addition of channel proteins directly at the plasm membrane, may cause reduction of uptake of cations such as K^+ and Ca^{2+} (Pineros and Tester 1997). Humic acid is believed to chealate toxic Al^{3+} in the growth media by making a HA-Al complex, by which it is thought that humic acid may reduce the negative effect of Al^{3+} toxicity on Ca^{2+} uptake.

Copper

Copper is the most abundant element in mafic and intermediate rocks, and its form in soil is of great importance in agronomy, but its process of absorption is still far from clear (Kabata-Pendias and Pendias 2001). According to Stevenson and Fitch (1981) the maximum amount of Cu^{2+} can be bound to humic and fulvic acids and sorped ranges from 48 to 160 mg per g of humic acid. In broad bean root the Cu^{2+} concentrations differed significantly among the treatments in 50HO and 50HA, which increased by 494% in HA. However, Cu^{2+} content in the broad bean roots decreased in 100HA (by 26%) without any significance at the same level of $\alpha = 0.05$ (Table 4).

The Cu^{2+} concentrations in the roots have altered only under Al^{3+} treatments in available reports. While Cu^{2+} content increased in roots of soybean [*Glycine max* (L.) Merr.; Shamsi et al. 2007], *Abies firma* Siebold and Zucc (Yoshida, Takenaka, and Tezuka 2005), and tomato (*Lycopersicon esculentum* Mill.; Simon et al. 1994), Cu^{2+} content decreased in

roots of peach (*Prunus persica* (L.) Batsch; Chibiliti and Byrne 1990). The concentration of Cu^{2+} in broad bean root (max. 0.107 mg g^{-1}) supports the findings by Graham (1981) that the rates of Cu^{2+} absorption by higher plant roots are among the lowest of the essential elements, ranging 0.01 to 20 μM of dry weight of roots.

Iron

Iron comprises 5% of the lithosphere and occurs as small particles or is associated with the surfaces of other minerals in the forms of oxides and hydroxides. Nevertheless, Fe^{3+} appears as a chelated form in soils rich in organic matter (Kabata-Pendias and Pendias 2001). It is believed that generally fundamental absorption of this cation is as Fe^{3+} that has been reduced from Fe^{3+} by the ability of roots, and its organic complexes play an important role in plant nutrition at normal soil pH levels (Chaney, Brown, and Tiffin 1972; Kabata-Pendias and Pendias 2001).

In the present experiment, Fe concentrations differed significantly among the treatments in 50HO and 50HA, and decreased by 252% in HA compared to control treatment. Fe^{3+} content in the broad bean roots also decreased in 100HA to 13%, without any significance at the same level of $\alpha = 0.05$ (Table 4).

Despite many reports that Fe^{3+} content changes in various plants under Al^{3+} treatment, none available showed any humic acid effect on Al^{3+} toxicity. The researches reported that Fe^{3+} content decreased in the roots of silver birch (*Betula pendula* Roth; Kidd and Proctor 2000), soybean (*Glycine max* (L.) Merr.; Shamsi et al. 2007), and tomato (*Lycopersicon esculentum* Mill.; Simon et al. 1994). Yoshida, Takenaka, and Tezuka (2005) reported that there is no change of Fe^{3+} content in *Abies firma* Siebold and Zucc roots; and Chibiliti and Byrne (1990) showed the same in peach [*Prunus persica* (L.) Batsch] roots. In contrast, Al^{3+} stress caused Fe^{3+} to increase in the roots of plants (Foy 1992; Foy and Fleming 1982). Humic acid may decrease the Al^{3+} toxicity on Fe^{3+} content because Al^{3+} stress can stimulate callose formation, which can prevent transport between cells in plant tissue through the plasmadesmata (Sivaguru et al. 2000).

Manganese

Mn^{2+} as one of most abundant trace elements is commonly present in rocks, in amounts ranging from 350 to 2000 ppm, and is governed by various environmental factors. It may be in concentrated forms, such as concretions and nodules, as well as simple and complex ions and several oxides of variable composition that are essential in plant nutrition and control the behavior of several other micronutrients (Kabata-Pendias and Pendias 2001).

In this experiment, Mn^{2+} content significantly increased in roots of broad bean seedlings, both in HA50 and HA100 treatments, by 92% and 294%, respectively, compared to controls (HO 50 and HO100) at the level of $\alpha = 0.05$ (Table 4).

There are only a few available reports stating that Al stress causes Mn^{2+} content to decrease in various plant roots. For instance, Yoshida, Takenaka, and Tezuka (2005) in *Abies firma* Siebold and Zucc; Batsch Chibiliti and Byrne (1990) in peach [*Prunus persica* (L.) Batsch]; and Simon et al. (1994) in tomato (*Lycopersicon esculentum* Mill.) roots reported Mn^{2+} reduction when the plants were treated by Al^{3+}. There is no study focused on Mn^{2+} uptake mechanisms in the plant root under Al toxicity and its behavior after HA treatment. The findings obtained in the present study indicated an increase in the mobilization rate and the availability of Mn^{2+} at suitable pH (4.5), to levels that are generally accepted as being toxic (Foy, Scott, and Fisher 1988). The toxic effect of Mn^{2+} was not

detected in broad bean roots, because its absorption appeared as a small proportion. Mn^{2+} is also rapidly taken up and translocated within plants; Mn^{2+} does not bind to insoluble organic ligands in root tissues and xylem fluid (Kabata-Pendias and Pendias 2001).

Zinc

It is believed that Zn^{2+} can be found in forms of free and complexed ions in soil solution; however, its absorption has greatly differed among plant species as well as growth media (Kabata-Pendias and Pendias 2001). The available studies reported that Al interferes with uptake and transport of Zn^{2+} as with other essential nutrients such as K^+, Cu^{2+}, Fe^{3+}, and Mn^{2+} (Foy 1984). Although in the soil, Zn^{2+} has been reported to be linear with the nutrient solution and its soluble forms are readily available to plants (Kabata-Pendias and Pendias 2001), Al^{3+} toxicity caused callose formation, which can prevent transportation along the plasmesmata from cell to cell (Sivaguru et al. 2000). The toxicity limits of Zn^{2+} varied with plant species, genotypes, and growth stage, ranging from 100 ppm to 500 ppm as upper toxic levels in dry weight (Davis, Beckett, and Wollan 1978; Macnicol and Beckett 1985), but in the roots the critical concentration of Zn^{2+} is greater, being immobilized in the cell wall or complexed in nondiffusible Zn^{2+} proteins, despite that the roots often contain much more Zn^{2+} than aerial parts of plants.

In the present study, Zn^{2+} concentrations differed significantly among the treatments in 50HO and 50HA, which increased 252% in HA. Although Zn^{2+} decreased nearly 50% less than in HA50, Zn^{2+} concentration significantly increased in 100 HA treatment compared to the control (HO100) (Table 4).

There are a few studies reporting reduction in Zn^{2+} content in the roots of various plants such as *Abies firma* Siebold and Zucc (Yoshida, Takenaka, and Tezuka 2005), tomato (*Lycopersicon esculentum* Mill.; Simon et al. 1994), and cacao (*Theobroma cacao* L.; Baligar and Fageria 2005). According to Lee (1971), the addition of 20 ppm Al to the growth media suppressed Zn absorption in potato roots. These studies state that Al caused a reduction in the uptake of Zn^{2+} and other nutrients that can be related to the blocking effect of Al^{3+} on ion-carrier protein affecting ion mobility (Kochian, Pineros, and Hoekenga 2005). The present study revealed that HA treatment causes significant increase in Zn^{2+}, which may be related to the breaking of the blocking effect of Al^{3+} and may reduce callose formation in the root cells. This is a subject that merits further investigation.

Discussion

This study revealed a significant effect of humic acid on root development and changes in nutrient content in broad bean exposed to Al^{3+} contamination. The result of the current experiment showed that humic acid suppressed the toxic effect of Al^{3+} on the growth of the main and lateral roots. Better growth and development was also evident, in the greater RFW and RDW, together with rather effective regulation of ions in the roots after humic acid treatments (Table 1, Figure 1). The restored effects of humic acid were noted to a greater extent, particularly in the RFW in both HA treatments. Aluminum toxicity mostly affects cell walls by reducing their flexibility and expansion (Kochian, Pineros, and Hoekenga 2005), and therefore there is insufficient root development. Aluminum problems in the uptake of water and nutrients occurred in roots that had been exposed to Al^{3+} (Kocaçalışkan 2005).

The organic acids are beneficial substances if they reduce the binding capacity of the Al^{3+} ions to the root cell wall or cell membrane. Organic acids, which are released
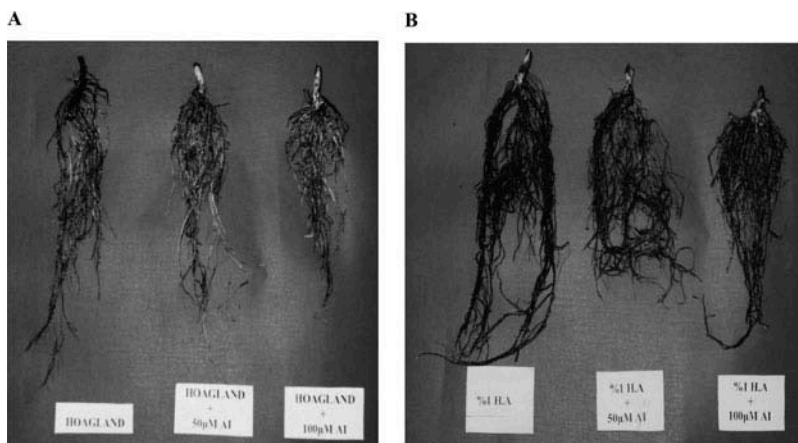
Figure 1. Broad bean roots grown under aluminum stress at harvesting time: (A) control treatment (Hoagland + AlCl$_3$) (HO) and (b) control + humic acid (HA).

from plants, vary depending on plant species and include citrate, oxalate, and malate. The family of broad bean (*Fabaceae*) produces citrate against Al^{3+} toxicity (Yang et al. 2000). Humic and fulvic acids are organic substances that can make a complex monomeric form with Al^{3+} that is nontoxic to most plants (Tan and Binger 1986; Gardner and Al-Hamdani 1997; Suthipradit, Edwards, and Asher 1990; Harper et al. 1995). Humic acid treatments can make a contribution to broad bean root expansion by increasing the mechanisms of water and nutrient uptake, which are very important to the plants.

Changes in ionic status of roots are crucial, and the results of the current experiment revealed that the concentration of humic acid treatments increased Na$^+$, K$^+$, Ca^{2+}, Fe^{3+}, and Mn^{2+} contents in both 50 and 100 HA compared to the same concentrations of Hoagland solutions (50 HO and 100 HO). Ions K$^+$, Na$^+$, and Fe^{3+} are recorded as representing the greatest amounts of elements accumulated by roots, among the elements studied in this experiment. The Na$^+$ and K$^+$ contents were increased significantly by increasing Al^{3+} concentrations (HA 50 and HA 100), where the increase was by 25% in Na$^+$ and by 13% in K$^+$ in HA 100 compared to HA 50.

Calcium uptake is one of the most important nutrient interactions with Al^{3+}. The Ca^{2+} uptake seemed to be suppressed while the plant was exposed only to Al^{3+}, and it was significantly increased by humic acid treatment. In the presence of Al^{3+} without humic acid, the reduction in Ca^{2+} content agreed with the findings of Huang, Grunes, and Kochian (1992) and Kabata-Pendias and Pendias (2001), who found Ca^{2+} deficiency or decreased Ca^{2+} transport. After humic acid treatment, it was also revealed that the Na$^+$, K$^+$, Ca^{2+} (in 100 HA), Cu^{2+} (in 50 HA), and Mn^{2+} and Zn^{2+} are released after breaking the blocking effects of Al^{3+} on nutrient uptake.

The increasing Mn^{2+} and Zn^{2+} concentrations observed here are noteworthy. Both nutrients increased significantly in the presence of Al^{3+} and HA compared to HO treatments. The results of the present investigation agreed with the findings of Haug and Vitorello (1996), Graham (2001), Kochian, Pineros, and Hoekenga (2005), and Lee (1971), who postulated that Al treatments cause the reduction of Ca^{2+}, K$^+$, Fe^{3+}, and Zn^{2+} by inhibiting the activity of H$^+$-ATPase and that the resulting ion balance changes in various

plants. In contrast to these reports, Fe^{3+} content still decreased under humic acid treatment and also phosphorus, which we have not tested in this experiment.

Aluminum is noted to a lesser extent particularly under humic acid treatment with HO and HA. These results agree with the findings of Stevenson and Vance (1989) and Stevenson (1994), who reported Al^{3+} can make strong compounds with oxygen-carrying molecules. According to them, functional groups of humic acids which contain of high amounts of oxygen, such as COOH, phenolic-OH, enolic-OH, alkalic OH, C=O, and $-NH_2$, can make strong complexes with Al and other metalic ions by water-bridging electrostatic attraction and chelation. In this experiment, no symptoms of severe physical damage could be detected in root formations (Figure 1). This might indicate that the Al ions might bind less to the root membrane or cell wall after chelation by humic acid or other organic substances, such as citrate, released from the roots (Kochian 1995; Ryan, Delhaize, and Randall 1995; Yang et al. 2000).

The increasing Na^+ content is rather interesting, although increase in K^+ uptake is meaningful in terms of K^+/Na^+ relations in the membranes. The presence of the competition between Na^+ and K^+ causes significant negative effects on plant growth, and Na concentration often exceeds K^+ accumulation (Schachtman and Liu 1999). Despite inducing salinity, Na^+ has an important negative effect for salt-sensitive plants such as broad bean. However, because there was a change (decrease in HA treatment but still in favor of Na^+) of the ratios of K^+/Na^+ in the root area at the same concentration (at 50 HO, 50 HA, 100 HO, and 100 HA) in which K and Na ions still might compete for entry into plant root cells. No extremely harmful effect was detected in the roots of broad bean, This is might be related to counteraction by the presence of carboxylic and phenolic –OH groups that humic acid can bind with clay particles and cations so that ions of lower valency than Al^{3+} can be held in the rhizosphere close to the root surface and absorbed easily by the increased CEC (cation exchange capacity) of the soil. This has been evidenced by the significantly increased contents of Na^+, K^+, Mn^{2+}, and Zn^{2+}. However, despite Cu^{2+} and Ca^{2+} appearing to increase significantly in HA 50 and HA 100, respectively, the other effects of the treatment need more investigation.

The present finding that humic acid can be used to compensate for the toxic effect of Al in soils should be further studied in *Vicia faba* and in other crops to gain more evidence.

Acknowledgments

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