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ARTICLE

Effect of Humic acid and levels of Zinc and Boron on Chemical Behavior of Zinc in Calcareous Soil

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ABSTRACT

There are global concerns about the significant increase in the concentrations of heavy elements due to the increase in agricultural, urban and industrial activities, which poses a threat to the natural environment and humans. This study focused on studying the addition of humic acid and different levels of zinc on the presence of zinc in the soil in its different forms and its readiness for the plant to find the best concentration and with the presence or absence of humic acid. This study was carried out on the sunflower crop, as zinc was added at levels of (0, 15 and 30) kg h^{-1} and humic acid at two concentrations, (0 and 50) kg h^{-1}. Zinc was extracted successively and total and available zinc was measured. The results indicated: Concentration of total and available Zinc associated with carbonate minerals, with oxides, and with organic matter in soil increased with increasing levels of Zinc added to soil, reaching 8.638, 1.049, 1.971, 1.658 and 1.625 mg kg^{-1} soil, respectively. The effect of added humic acid was significant in reducing the concentrations of total Zinc, Zinc bound to carbonate minerals, bound to oxides and residues. The maximum concentrations in case of not adding acid reached 9.313, 1.8957, 1.424, and 4.300 mg kg^{-1} soil, respectively, while the effect was positively in increasing available concentration associated with organic matter, as concentration in case of adding humic acid reached 0.851 and 1.4584 mg kg^{-1} soil, respectively. In the case of adding humic acid, this study is considered good for the results it showed in increasing the available zinc with the presence of humic acid, which is reflected in the plant growth and maintaining this element within the recommended concentrations. We determined Zn speciation in the soil system and evaluated the effects of humic acid application on the basic soil properties and Zn bioavailability.

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1. Introduction

Zinc is one of the most bioavailable and transportable heavy metals and therefore its presence in high concentrations in soil causes plant toxicity and reduces yield and quality [1]. [2] showed that zinc is important as it acts as a cofactor for many enzymes including anhydrase, carbonic acid, phosphatase, dolase and carbopeptide. Zinc also affects photosynthesis. By reducing the activity of hydrocarbonic anhydrases and chlorophyll formation, as the integrity of the cell membrane, its divisions, expansion and protein synthesis depend on optimal zinc nutrition [3]. It was also found that high concentrations of zinc become toxic to the plant, so its concentrations must be maintained within acceptable limits, as the increase in zinc also causes a disturbance in the balance of other elements and may replace other essential elements within the cell, such as Fe. Therefore, the presence of zinc in high concentrations disrupts many plant physiological processes [2]. It must be noted that the deficiency of zinc in the soil is responsible for the decrease in the yield and nutritional value of grain crops. Many studies have indicated a deficiency of zinc in some agricultural soils, as some studies have recommended supplying up to 40 $mg.Kg^{-1}$ as a dietary requirement [4,5]. A study conducted by [6] to show the different forms of zinc present in the soil and the factors that affect the availability of zinc to the plant to reveal the relationship between soil properties and zinc availability and to show the possibility of genetically improving plant varieties to tolerate zinc toxicity. Humic acid is one of the basic components of humus. The molecular structure of humic acid includes aromatic and aliphatic groups. Humic acid also contains active groups such as carboxyl, phenol, ketone, and amine. The aromatic structure is the reason for the biological activity of these compounds due to the large number of functional groups found in each ring. Humic substances are produced as a result of the chemical and biological decomposition of plant and animal remains. After humic acid, it is the best biostimulant as it has a direct and indirect effect on the physiological processes of the plant [7–10]. Adding humic acid not only improves the physical and chemical properties of the soil but also provides better root growth, increased ability to exchange cations, and provision of nutrients [11–13]. In a study conducted to improve the level of zinc concentration in the presence of humic acid in the wheat yield, it was determined that adding zinc at a level of 10 $kg\ ha^{-1}$, in addition to humic acid, achieves an increased yield and records the highest plant height, spike length, and grain filling [14]. In a study conducted by [15] to show the effect of adding humic acids on zinc concentration when growing broccoli in calcareous soil, a good effect was found for humic acid along with zinc element on the yield and plant characteristics. [16,17] found, in a field experiment conducted on sunflower plants, that fertilization with 50 $kg\ ha^{-1}$ of humic acid has a significant effect in increasing dry weight, total and biological yield, and yield per plant. [18] found that adding organic matter that works as soil conditioners, such as sludge and biochar, increases the concentration of zinc in the soil, which causes some problems such as disrupting many physiological processes of the plant. Therefore, in this study, humic acid was added with zinc to increase its availability and reduce its toxicity. Sunflower crop (Helianthus annuus. L) is considered one of the broad strategic oil crops. Spread due to its economic importance and multiple uses, and it is one of the four most important oil crops in the world [19,20]. It is grown in many arid and semi-arid areas and can grow in a wide range of different soils. It is one of drought-resistant crops and has a variety of uses, including oil extraction for oily varieties. It comes in second place after Soybeans because its seeds contain a high percentage of oil, reaching 50%. Therefore, it is an important source for the food oil industry [21]. The research aims to know the effect of humic acid and levels of Zinc and Boron on the chemical behaviour of Zinc in calcareous soil.

In this study, field experiments were conducted to investigate the effects of humic acid on soil Zn bioavailability and uptake. The main objectives of this study were to 1) determine Zn speciation in the soil system; 2) evaluate the effects of humic acid application on the basic soil properties and Zn bioavailability; and 3) clarify its potential role in Zn accumulation, translocation, and their competitive interactions in S. androgynous. The current study can provide a novel strategy for developing an effective fertilization application method to reduce Zn accumulation and available Zn bioavailability for plants.

2. Materials and methods

In the spring of 2022, a field experiment was conducted at one of the research fields connected to the University of Baghdad's College of Agricultural Engineering Sciences to cultivate sunflowers of the Shamos variety (Helianthus annuus L.). The soil type of the field was determined to be clay. All field operations, including plowing, leveling, and smoothing the soil, were done to prepare the soil for cultivation. Lines were also created. Before planting, field soil samples ranging in thickness from 0 to 30 cm were collected from several fields and thoroughly combined to create a composite sample that accurately represented the field. The dirt was then dried, ground with a wooden hammer, and sieved with a sieve with a hole diameter of 2 mm, and all the required physical and chemical analyses were conducted on it, as shown in **Table 1** according to methods mentioned in [22, 23]. Macronutrient fertilizers were added at a rate of 120 kg N ha^{-1} , 60 kg P ha^{-1} and 100 kg K ha^{-1} according to fertilizer recommendation for crop [24]. Seeds of sunflower plants of Shamos variety were planted, irrigation process was carried out using a drip irrigation system using gravimetric method to measure moisture content of soil for determining time and depth of irrigation. Irrigation was done after draining 50% of available water. Digital meters were used to ensure that water was distributed evenly to experimental units . Field experiment is a study effect of three factors using a randomized complete block design (RCBD) with three replications: first factor is adding solid humic acid (powder) to soil when planting at two levels, which is without addition (control) and 50 kg H^{-1}, symbolized as H_0 and H_1 respectively. Second is adding Zinc in form of hydrated Zinc sulphate ($ZnSO_4.7H_2O$) (23% Zinc) at three levels: without addition (control), 15 kg Zn ha^{-1} , and 30 kg Zn ha^{-1}, symbolized as Zn_0, Zn_1, and Zn_2, respectively. Third factor is adding Boron to soil when planting in form of solid boric acid, which contains 17% Boron, at three levels: without addition (control), 6 kg B^{-1}, and 12 kg B^{-1}, which are symbolized as B_0, B_1, and B_2, respectively.

Thus, number of experimental units is:

2 (humic acid) × 3 (Boron) × 3 (Zinc) × 3 (replicates) = 54 experimental units.

Available Zinc was extracted from soil using DTPA solution (Diethylene Triamine Penta Acetic acid) (0.005 M), Calcium chloride (0.01 M), and TEA (0.01 M) in a ratio of 2:1 (soil: extraction solution) according to [25] described in [26], total concentration of Zinc was estimated after digesting soil using a mixture of two concentrated acids (H_2SO_4 $HCLO_4$) according to method presented in [27]. Successive Zinc extraction was carried out according to method of [28] by taking 2 grams of ground dry soil (2 mm) and placed in a centrifuge tube (50 ml). The water dissolved Zinc was extracted by adding deionized water, while exchangeable Zinc was extracted by adding an ammonium acetate solution (NH_4OAc) (N=1.0) with a reaction degree of pH = 7 from soil portion. Remaining from previous step, Zinc bound to carbonate (Carbonate - bound) was estimated by adding ammonium acetate solution NH_4OAc (N=1.0) with a reaction

In the soil, the quantitative zinc element was measured after incorporating soil samples with a carrier (HF_4, SO_2H_4, HClO) according to the method proposed. The available zinc was extracted using a 0.005 molar DTPA extractor, 0.01 molar 2CaCl and 0.1 molar TEA extractor at pH 0.3 and at a ratio of 0:4 soil: carrier extractor.

Degree of pH = 5 to remaining soil portion, Zinc bound to oxides (Oxide - bound) was extracted by adding hydroxylamine hydrochloride NH_2OH.(0.04 M) (HCl) prepared by dissolving it in 25% of glacial acetic acid (v / v) at pH= 3. Zinc bound to organic matter (organically - bound) was also extracted by adding 15% of a 30% solution of hydrogen peroxide (H_2O_2) at a pH = 2 to remaining part , samples were placed in a water bath at a temperature of 80C° for 5.5 hours with intermittent shaking. After cooling, 5 ml of a solution of 3.2 M ammonium acetate (NH_4OAc) in 20% (v / v) of Nitric acid was added (3N), as for remaining Zinc (residual), it was estimated by subtracting the sum of extraction forms of dissolved and exchanged Zinc bound to carbonates, oxides, and organic matter from total concentration according to method of [29]. Samples were shaken at a speed of 200 rpm in all above stages and the filtrate was separated using a centrifuge at 2500 rpm for 30 minutes. After completing the successive extraction process for each part of Zinc form,. Zinc was determined using an atomic absorption spectrometer (AAS).

3. Results and discussion

3.1 Effect of humic acid , Zinc and Boron levels on Zinc concentration in soil after harvest

Table 1. Some chemical and physical characteristics of study soil before planting.

Adjective	Units	Value
Soil reaction (pH) 1:1	—	7.65
Electrical conductivity (EC) 1:1	dS.m^{-1}	2.82
Cation exchange capacity (CEC)	Cmole + kg^{-1}	24.53
Organic matter	gm kg^{-1}	6.1
Carbonate minerals		225.8
Available Nitrogen	mg kg^{-1} soil	21.14
Available Phosphorus		5.45
Available Potassium		68.25
Available Zinc		0.88
Total Zinc		13.75
Bulk density	cm^{-3} cm^{-3}	1.37
Available water	Mg cm^{-3}	0.14
Sand	gm kg^{-1}	425
Silt		275
Clay		300
Texture		Clay Loam

1—Total Zinc

Figure 1 shows significant differences increasing in total Zinc concentration at post-harvest stage, Zn_2 treatment excelled with 8.638 mg kg^{-1} soil compared to Zn_0 treatment, which gave 8.5987 mg kg^{-1} soil. This is attributed to levels of Zinc added to soil. As for effect of humic acid on total Zinc concentration, treatment H_0 as it gave 9.313 mg kg^{-1} soil, while treatment H_1 gave 7.923 mg kg^{-1} soil. This may be attributed to role of humic acid in chelating Zinc. It is present in soil and when organic matter decomposes, Zinc becomes available for absorption by plant. Humic acid also effects in improving chemical properties of soil and increasing exchange capacity of cations, which reduce remaining Zinc to compensate for Zinc absorbed by plant and maintain the balance of Zinc forms in soil. In contrast to treatment in which humic acid was not added, this is consistent with findings of[30] who showed that organic matter retain Zinc and increase its availability for plants. AS for effect of added Boron on total Zinc concentration in soil, treatment B_0 achieved 9.3928 mg kg^{-1} soil, while 7.849 mg kg^{-1} soil was recorded for treatment B_2. This may be attributed to added Boron leading to an increase in plant growth and set vegetative in flowering stage, thus increasing root secretions, reducing soil pH, increasing the availability and absorption of Zinc in soil by plant, which led to preparing the deficiency of total Zinc to fill deficiency, this is consistent with what [31] stated in that reason for decrease in total Zinc concentration in soil is due to depletion of available Zinc and creation of suitable conditions for process of decomposition of total Zinc sources, such as low soil reactivity as a result of activity of physiological processes of roots and microorganisms, which results in weathering of minerals containing Zinc.

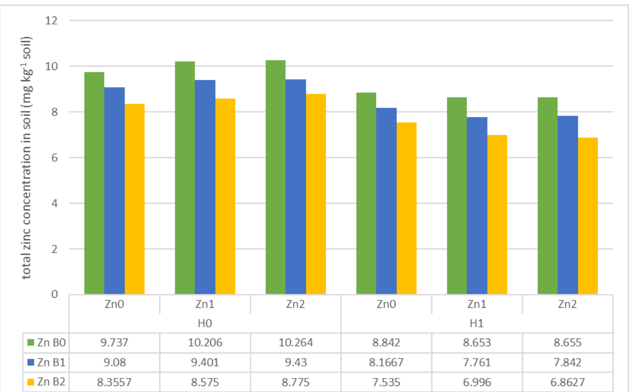


Figure 1. Impact of humic acid, boron, and zinc concentrations on the post-harvest total zinc concentration in soil (mg kg^{-1} soil).

The results of the binary interaction between humic acid and zinc showed that there were significant differences in the total zinc, and the HOZn2 treatment was effective. The highest quantity was found in soil at 9.4897 mg kg^{-1}, while the H1Zn2 treatment yielded 7.7866 mg kg^{-1}. Regarding humic acid and boron, there were significant differences in the overall zinc concentration in the soil; the H0B0 treatment had the greatest concentration, 10.0690 mg kg⁻¹ was in H_1B_2 treatment, which gave 7.1312 mg kg^{-1}.Boron and Zinc, the treatment achieved Zn_2B_0 had The maximum concentration of total Zinc, reaching 9.459 mg kg^{-1} soil, while minimal concentration when treated with Zn_1B_2, which reached 7.785 mg kg^{-1} soil. Adding humic acid affected concentration Zinc in soil after100 days of planting, as we find that the concentration of available Zinc in all treatments is less than its content in flowering stage, and this may be due to increase in biological growth and nutritional needs (maturity stage) during this period and occurrence of absorption of nutrients from soil to carrying out plant's vital activities, which leads to release of a portion of Zinc. Results of triple interaction of humic acid , levels of Zinc and Boron showed significant differences between treatments, $H_0Zn_2B_0$ treatment achieved The maximum concentration of 10.2640 mg kg^{-1} soil, while The minimal concentration in $H_1Zn_2B_2$ treatment reached 6.8627 mg kg-1soil, adding organic matter led to availability of micronutrients and Zinc was transformed from non-available forms such as total Zinc during weathering and biogeochemical processes when any deficiency occurs to form of available Zinc to meet plant's need [32].

2—Zinc dissolved in soil

According to **Figure 2**, every research element had a noteworthy impact on raising the dissolved zinc concentration in the soil solution following harvest. Compared to Zn0 treatment, which produced a minimum concentration of 0.1363 mg kg^{-1}, Zn2 treatment was substantially superior and produced a maximum concentration of 0.299 mg kg^{-1}. Humic acid addition also significantly increased the amount of dissolved zinc in the soil; treatment H1 had the highest concentration of 0.263 mg kg^{-1}, while treatment H0 had 0.1720 mg kg^{-1} soil. Regarding the impact of additional boron, treatment B2 produced soil with a maximum content of 0.267 mg kg^{-1}.while B0's therapy provided The lowest concentration of 0.173 mg kg^{-1} in the soil might be explained by the addition of zinc at varying concentrations, which raised the amount of soluble zinc in the soil. The addition of humic acid along with organic acid root exudates lowers soil pH, which influences the rise in soluble zinc concentration in the soil. According to [33–35] added boron also plays a part in increasing vegetative and root growth, which in turn increases root secretions and their crucial role in reducing soil interaction and increasing concentration of soluble zinc.

Figure 2. Impact of humic acid, boron and zinc levels, and soluble zinc concentration in soil following harvest (mg kg^{-1} soil).

Results of interaction between humic acid and Zinc indicated in concentrations of dissolved Zinc in soil, if the H_1Zn_2 treatment achieved the The maximum concentration, amounting to 0.3743 mg kg^{-1} soil. In contrast, H_0Zn_0 treatment gave lowest, amounting to 0.1153 mg kg^{-1} soil, this may be attributed to important properties of Humic acid in its ability to form dissolved complexes with nutrient ions, and these results are consistent with what was found by [36], who showed that increase in availability and solubility of microelements in soil is due to levels of humic acids added to soil. As for interaction between levels of humic acid and Boron, there was a significant effect in increasing concentration of dissolved Zinc, H_1B_2 treatment, which 0.3267 mg kg^{-1} soil, was superior, while lowest was in comparison H_0B_0 treatment, which gave 0.1347 mg kg^{-1} soil, and this difference can be it can be attributed to effective role of humic acid and Boron added to soil in maintaining concentration of dissolved Zinc in soil, These findings concur with those of[37], who showed that humic acids are among most important types of organic materials that directly affect soil fertility, as they can increase avalibility and dissolution of micronutrients such as Zinc. Interaction between Boron and Zinc, Zn_2B_2 treatment achieved the highest of dissolved Zinc, reaching 0.3740 mg kg^{-1} soil, while the lowest was in Zn_0B_0 treatment, 0.1050 mg kg^{-1}, addition of Boron and Zinc has an important role in increasing vegetative and root growth and thus increasing root secretions that reduce soil pH, which led to an increase in concentration of dissolved Zinc, this is consistent with what was found by [26], as they showed that adding fertilizers containing Zinc and Boron significantly affected their concentration in soil. Results also showed triple interaction between Humic acid, Zinc and Boron levels, with significant differences between treatments, $H_1Zn_2B_2$ treatment excelled with The maximum concentration amounting to 0.483 mg kg⁻¹ soil, while The minimal concentration was in comparison treatment ($H_0Zn_0B_0$) 0.0850 mg kg^{-1} soil, This is because raising the levels of zinc and boron in the soil solution increased the concentration of dissolved zinc, and this increase was directly correlated with the amount added. Boron also played a role in lowering the pH, which increased the concentration of dissolved zinc in the soil solution because of root secretions caused by the plant's increased vegetative and root growth as well as the addition of humic acid, which effectively lowers pH. These findings are in line with those of[38–40].

3—Exchangeable Zinc in soil

Significant differences were observed in the rising concentration of exchangeable zinc in the soil solution, as demonstrated in **Figure 3**. The Zn0 treatment resulted in the lowest concentration of 0.2885 mg/kg of soil, while the Zn2 0.749 mg/kg. The addition of humic acid led to an increase in zinc concentration, with the H1 treatment yielding a maximum concentration of 0.588 mg/kg of soil, significantly higher than the H0 comparison treatment, which recorded a minimum concentration of 0.449 mg/kg. These findings are consistent with[41] study, which suggested that humic acid interacts with organic acid root secretions to lower pH, thereby enhancing the soil's exchangeable zinc availability. The addition of boron also significantly increased the concentration of exchangeable zinc in the soil. The B2 treatment was particularly effective, achieving a minimum zinc concentration of 0.476 mg/kg in soil, compared to the 0.562 mg/kg recorded for the comparator treatment, B0. This is in line with the findings of[42], who reported that adding boron to soil promotes root growth and exudation, reducing soil reactivity and enhancing the availability and absorption of micronutrients.

Furthermore, the interaction between humic acid and zinc resulted in a marked increase in exchangeable zinc concentrations post-harvest, with the H1Zn2 treatment being particularly successful. The maximum zinc concentration recorded was 0.827 mg/kg, whereas the H0Zn0 comparative treatment yielded the lowest concentration of 0.228 mg/kg[43], who demonstrated that increasing humic acid content in soil improves the availability of micronutrients. The correlation between humic acid and boron was most favorable in the H1B2 treatment (0.6510 mg/kg of soil) and least concentrated in the H0B0 treatment (0.425 mg/kg). This aligns with the description provided by[37] regarding the role of humic acids in enhancing soil fertility and nutrient concentrations.

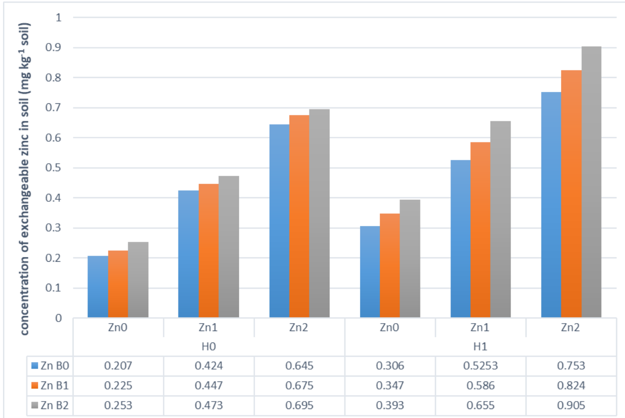


Figure 3. Impact of humic acid, zinc, and boron concentrations on the concentration of exchangeable zinc in soil following harvest (mg kg⁻¹ soil).

In terms of the interaction between boron and zinc levels, the Zn2B2 treatment achieved the highest concentration of exchangeable zinc at 0.800 mg/kg of soil, while the Zn0B0 treatment had the lowest concentration at 0.2565 mg/kg. These differences can be attributed to the factors previously discussed. The H1Zn2B2 treatment reached the highest concentration of 0.905 mg/kg of soil, whereas the H0Zn0B0 treatment had the lowest, at 0.207 mg/kg of soil. The triple interaction between the three research components revealed notable variations across the treatments. Humic acid's ability to lower pH and improve the chemical and fertility properties, combined with boron's effects on root growth and zinc's role in enhancing micronutrient availability and absorption, explain these results[44]. In addition to promoting plant growth and root development, the combination of zinc and boron also increases the zinc content in the soil.

4—Available Zinc in soil

Figure 4 shows that there concentration of available Zinc in soil, Zn_2 treatment excelled and gave The maximum concentration of 1.049 mg kg^{-1} soil compared to Zn_0 treatment, which gave the The minimal concentration of 0.4248 mg kg^{-1} soil, this is due to fact that adding Zinc at different levels has important role in increasing concentration of available Zinc in soil, This aligns with the assertions made by[21, 33]. who showed that adding fertilizers containing Zinc leads to an increase in concentration of Zinc in soil with increasing added levels. H_1 had The maximum concentration of 0.851 mg kg^{-1} soil compared to treatment H_0, which gave The minimal concentration of 0.6213 mg kg^{-1} soil. This is attributed to role of humic acid and root exudates in reducing pH, increasing plant growth, and increasing availability of Zinc in soil. It is permissible to improve soil's chemical, physical and fertility properties[45]. As for effect of added Boron on available Zinc concentrations, B_2 treatment gave The maximum concentration of 0.829 mg kg^{-1} soil, while B_0 treatment (comparison treatment) gave The minimal concentration of 0.649 mg kg^{-1} soil, important role in reducing pH and increasing concentration of soluble Zinc, this is consistent with what was shown by[46], who showed that absorption of Boron by plant leads to development and growth of root, which leads to an increase in area surface growth of roots and formation of new root hairs that produce amino and organic acids , secrete them outward and reduce soil pH, which leads to an increase in availability of micronutrients. Results of the binary interaction between humic acid and Zinc concentrations of viable Zinc in soil after harvest. The H1Zn2 treatment achieved the maximum concentration of 1.201 mg kg^{-1} soil, while the comparison treatment H0Zn0 gave the minimal concentration of 0.343 mg kg^{-1} soil.

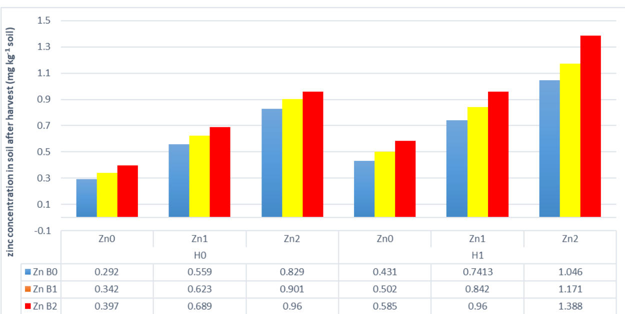


Figure 4. Impact of humic acid, boron and zinc levels, and accessible zinc concentration in soil after harvest (mg kg^{-1} soil).

This may be due to to important properties of humic acid in chelating micronutrients in soil and increasing their availability, these results are consistent with what was found by[34] who indicated that humic acid improve properties of soil by acting as a chelating agent for micronutrients in soil and increasing their availability and absorption. The effect of interference between levels of humic acid and Boron significantly increased concentration of available Zinc, as H_1B_2 treatment, which amounted to 0.9777 mg kg^{-1} of soil, excelled, while The minimal concentration was in H_0B_0 treatment, which gave 0.560 mg kg^{-1}. effective role of humic acid and Boron added to soil in increasing plant growth and root set, an increase in root secretions, which increase the concentration of dissolved and then exchanged Zinc. The findings of[47] are in line with these outcomes. who showed that humic acid is effective source carbon has a role in microbial activity, in addition to increasing availability of some nutrients through its chelation of some minor elements and formation of complex, soluble chelated compounds, which increases chances of their absorption by plants. Likewise, role of humic acids and their positive effect in increasing soil fertility and thus increasing concentrations of some nutrients. Boron also has A major role in increasing vegetative and root growth, which helps in increasing the secretion of organic acids, which contribute to reducing soil pH and increasing concentrations of available micronutrients.

As for the bilateral interaction between Boron and Zinc, Zn_2B_2 treatment achieved The maximum concentration of available Zinc, reaching 1.174 mg kg^{-1} soil, while The minimal concentration was in Zn_0B_0 treatment, which amounted to 0.361 mg kg^{-1} soil. This may be attributed to the same reasons mentioned above that showed the effect of adding different levels of Zinc and Boron to increase the concentration of available Zinc. Results of triple interaction between three study factors, which are humic acid, Zinc and Boron levels, showed that there were significant differences between treatments, $H_1Zn_2B_2$ treatment was characterised by The maximum concentration, which reached 1.388 mg kg^{-1} soil, while The minimal concentration was in comparison treatment $H_0Zn_0B_0$, which amounted to 0.292 mg kg^{-1} soil. This is consistent with what[48] mentioned, who explained role of organic acids in improving soil chemical properties.

5—Zinc bound to oxides

Figure 5 shows a significant increase in the concentration of Zinc bound to oxides in the soil after harvest. The Zn2 treatment exhibited the maximum concentration, reaching 1.658 mg kg^{-1} soil, while the Zn0 treatment recorded the minimal concentration at 0.8287 mg kg^{-1} soil, which is attributed to the amount of Zinc added. In terms of the effect of humic acid on Zinc concentration associated with oxides, it was found to be negative. The H0 treatment was significantly superior, yielding the maximum concentration of 1.424 mg kg^{-1} soil, whereas the H1 treatment had a minimal concentration of 1.043 mg kg^{-1} soil. This is due to the role of humic acid in lowering the concentration of Zinc bound to oxides in the soil by reducing soil pH, which increases Zinc availability and decreases its deposition or adsorption by soil components. These findings align with [49], who demonstrated that organic matter in soil alters various physical and chemical reactions, affecting the availability of micronutrients.

The results also indicated a significant effect of added Boron on the concentration of Zinc bound to oxides in the soil. The B0 treatment achieved the maximum concentration at 1.554 mg kg^{-1} soil, while the minimal concentration was observed in the B2 treatment at 0.917 mg kg^{-1} soil. This may be attributed to the impact of Boron on Zinc concentration in the soil.

The interaction between humic acid and Zinc showed a significant increase in the concentration of Zinc associated with oxides in the soil at the post-harvest stage. The H0Zn2 treatment had the The maximum concentration at 1.8431 mg kg^{-1} soil, while the H1Zn0 treatment recorded the lowest at 0.6743 mg kg^{-1} soil. which increased the Zinc bound to oxides and added humic acid, reduced Zinc concentrations bound to other soil components such as oxides. These results are consistent with [50], who noted that the addition of organic matter to soil enhances Zinc availability by promoting its decomposition. Studies have shown a strong correlation between Zinc availability and organic matter, particularly Zinc bound to organic matter.

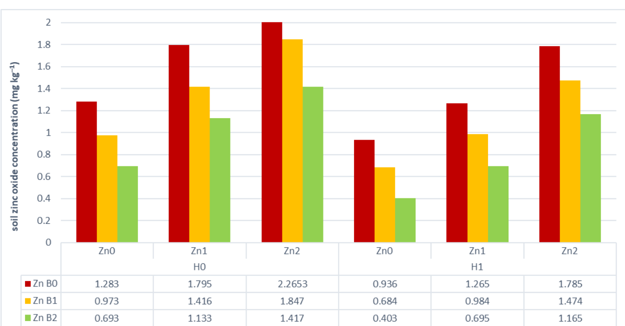


Figure 5. Impacts of humic acid, boron, and zinc levels on post-harvest soil zinc oxide concentration (mg kg^{-1}) for zinc.

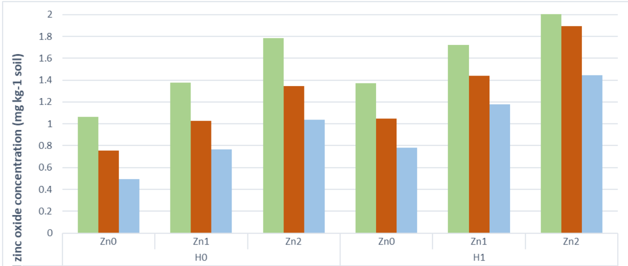
Constitutes about 1.5–2.3% of total Zinc, and organic matter can stabilize Zinc with a strong bond, which causes reduced adsorption by oxides and other soil components. The interaction between humic acid and boron levels revealed a noteworthy variation in the reduction of zinc concentration linked to oxides, as H_0B_0 treatment was superior, 1.7811 mg kg^{-1} of soil, The minimal concentration was in H_1B_2 treatment, which gave 0.754 mg kg^{-1}, this may be due to adding Zinc to soil leads to an increase in its adsorption on surfaces of oxides present in soil, presence of humic acid due to reduce Zinc bound to oxides by retaining Zinc and reducing its association with other soil components. This is inline with what was shown by [49], who showed that dynamics and transformations of Zinc in soil is subject to various factors, and organic matter is one of most important factors and is characterized by having direct and indirect effects on nutrient transformations, as organic matter is a source of organic carbon in soil, which represents approximately 60% of organic matter content that due to retain Zinc, increasing its availability and reducing its adsorption by carbonate and oxide minerals. As for dual interaction between Boron and Zinc, Zn_2B_0 treatment achieved The maximum concentration of Zinc bound to oxides, reaching 2.025 mg kg^{-1} soil, while The minimal concentration was in Zn_0B_2 treatment, which reached 0.754 mg kg^{-1} soil. This may be attributed to role of Boron and humic acid, which lead to reducing concentration of Zinc bound to oxides because added humic acid and Boron due to lower soil pH and root secretions, increase concentration of available Zinc in soil solution, and reduce adsorption of Zinc bound to oxides, these results are consistent with what [51] found, who showed that increasing soil content of organic matter it led to a decrease in Zinc adsorption and thus an increase in its availability in soil solution, this attributed because organic matter form soluble compounds of Zinc in form of chelates that have ability to retain Zinc in a dissolved form in soil solution.

Results showed that triple interaction between three study factors, which are humic acid, Zinc and Boron levels, showed significant differences between treatments, as $H_0Zn_2B_0$ treatment was characterized by The maximum concentration, which reached 2.265 mg kg^{-1} soil, while The minimal concentration was in $H_1Zn_0B_2$ treatment, which amounted to 0.403 mg kg^{-1} soil. Adding organic matter to soil increases availability of micronutrients and releases some microelements when they decompose in soil, including Zinc. Organic acids added to soil due to chelate Zinc and preserve it from adsorption to surfaces of iron or manganese oxides or carbonate minerals or loss due to their possession of effective functional groups. Boron also has a role in reducing concentration of Zinc bound to surfaces of oxides by increasing plant growth and increasing root secretions, thus reducing soil pH, increasing Zinc availability, and reducing Zinc adsorption on oxide surfaces.

6—Zinc bound to organic matter

Figure 6 highlights significant differences in the increase of Zinc concentration associated with organic matter after harvest. The Zn2 treatment resulted in the highest concentration at 1.625 mg kg⁻¹ of soil, while the Zn0 treatment had the lowest concentration at 0.919 mg kg⁻¹ of soil. This difference likely stems from the direct increase in Zinc added to the soil. This finding is consistent with the research by [52], who observed that organic matter in soil plays a role in chelating added Zinc. Regarding the impact of humic acid on Zinc concentration bound to organic matter, the H1 treatment showed a significantly higher concentration of 1.4584 mg kg⁻¹ of soil, in contrast to the H0 treatment, which had the lowest concentration at 1.0716 mg kg⁻¹ of soil. This effect is attributed to humic acid's ability to retain Zinc and prevent its loss through its active functional groups. This observation aligns with [53], who emphasised that organic matter improves various soil properties affecting micronutrient availability by chelating and adsorbing micronutrients like Zinc, which are then released into the soil solution in available forms during decomposition. The effect of added Boron on Zinc concentration associated with organic matter revealed that the B0 concentration of 1.594 mg kg⁻¹ of soil, while the B2 treatment had the lowest concentration at 0.949 mg kg⁻¹ of soil. This outcome is due to Boron's role in promoting plant growth and increasing root area, which reduces soil pH and enhances Zinc availability. This finding is supported by [54, 55], who explained that adding Boron increases root length, diameter, and surface area, leading to more root secretions, reduced soil pH, and greater availability of micronutrients.

The interaction between humic acid and Zinc significantly increased Zinc concentrations bound to organic matter in soil post-harvest. The H1Zn2 treatment exhibited the highest concentration at 1.8617 mg kg⁻¹ of soil, while the H0Zn0 treatment had the lowest concentration at 0.771 mg kg⁻¹ of soil. This increase is attributed to the greater amount of Zinc bound to organic matter, with humic acid enhancing this through adsorption and chelation on organic matter surfaces. [56] noted that humic acid plays a crucial role in the adsorption of microelements, carrying a negative charge that binds ions, and influencing element adsorption on metal surfaces based on several factors, including soil interaction, complex nature, adsorbent surface properties, element concentration, and humic substance percentage.



	H0 Zn0	H0 Zn1	H0 Zn2	H1 Zn0	H1 Zn1	H1 Zn2
Zn B0	1.063	1.375	1.784	1.374	1.724	2.245
Zn B1	0.756	1.025	1.345	1.047	1.437	1.896
Zn B2	0.494	0.765	1.037	0.783	1.176	1.444

Figure 6. Impact of humic acid, zinc, and boron levels on post-harvest soil zinc oxide concentration (mg kg⁻¹ soil).

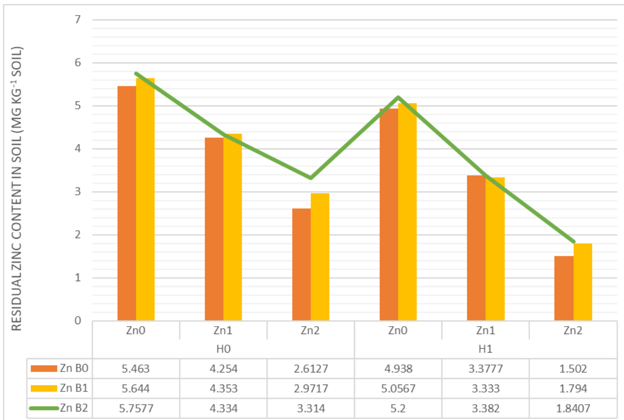
The interaction also significantly reduced Zinc concentrations associated with organic matter in soil, as the H1B0 treatment was superior, reaching 1.7810 mg kg⁻¹ of soil, while the lowest concentration was in the H0B2 treatment at 0.765 mg kg⁻¹ of soil. The binary interaction between Boron and Zinc showed that the Zn2B0 treatment achieved the highest concentration of Zinc bound to organic matter at 2.014 mg kg⁻¹ soil, while the lowest concentration was in the Zn0B2 treatment at 0.638 mg kg⁻¹ soil. The increase in Zinc concentration bound to organic matter is directly proportional to the amount of Zinc added to the soil. Organic matter in soil plays a significant role in the absorption of Zinc, increasing its concentration. Regarding Boron, the results indicated an inverse relationship with Zinc associated with the organic matter due to Boron's role in growth, root expansion, and cell division, which increases root secretions, reduces soil pH and enhances the available concentration of Zinc at the expense of Zinc bound to other soil components. This is consistent with the findings of [57]. The results of the triple interaction of humic acid, Zinc, and Boron levels showed significant differences between treatments. The H1Zn2B0 treatment was characterized by the highest concentration, reaching 2.245 mg kg⁻¹ soil, while the lowest concentration was in the H0Zn0B2 treatment at 0.4940 mg kg⁻¹ soil. Adding organic matter to soil affects the availability of micronutrients, and when decomposed, some micronutrients, including Zinc, are released. Organic acids are added to soil to chelate Zinc and preserve it from loss due to their effective functional groups. This observation is consistent with [58], who demonstrated in their study that there are significant differences in the mechanisms of interactions between humic acids and Zinc ions through complexation reactions, where protons are exchanged. This relationship can be useful in predicting environmental impacts

7—Residual Zinc

Figure 7 illustrates the significant variations in residual Zinc concentrations after harvest. The Zn0 treatment showed the highest concentration at 5.343 mg kg^{-1} soil, while the Zn2 treatment recorded the lowest at 2.3392 mg kg^{-1} soil. Regarding the impact of humic acid on residual Zinc, the H0 treatment yielded the highest concentration of 4.3005 mg kg^{-1} soil, in contrast to the H1 treatment, which had the lowest concentration at 3.3805 mg kg^{-1} soil. Organic matter enhances the soil's physical and chemical properties, thereby increasing the availability of micronutrients. Various factors such as soil pH, electrical conductivity, organic matter content, active lime, soil texture, moisture, clay and silt percentages, clay type, and ion concentrations all influence micronutrient availability in soil [59]. The addition of Boron also significantly affected residual Zinc levels, with the B2 treatment showing the highest concentration at 3.9714 mg kg^{-1} soil, compared to the B0 treatment, which had the lowest at 3.6912 mg kg^{-1} soil.

The interaction between humic acid and Zinc revealed significant differences in residual Zinc concentrations post-harvest. The H0Zn0 treatment reached the highest concentration at 5.6216 mg kg^{-1} soil, whereas the H1Zn2 treatment recorded the lowest at 1.7122 mg kg^{-1} soil. This effect can be attributed to humic acid's ability to enhance Zinc availability by lowering soil pH, chelating Zinc, and releasing it from residual forms to balance deficiencies in other Zinc forms, achieving equilibrium among Zinc bound to soil components. This observation aligns with [60], who noted that substantial amounts of residual Zinc in certain treatments result from Zinc's tendency to become unavailable, requiring various factors to render it accessible. Under favourable conditions, this stored Zinc becomes available and helps maintain a balance with other Zinc forms.

Regarding the interaction between humic acid and Boron, significant differences in residual Zinc concentration in the soil were observed. The H0B2 treatment achieved the highest concentration at 4.4686 mg kg^{-1} soil, while the lowest concentration was recorded in the H1B0 treatment, at 3.2726 mg kg^{-1} soil. This effect may be attributed to humic acid, which reduces the remaining Zinc concentration in the soil by altering its chemical and physical properties, facilitating the transformation of Zinc from unavailable forms to accessible forms, thus maintaining a balance of Zinc in the soil. This finding aligns with [61], who noted that adding humic acid to the soil increases the availability of micronutrients and creates a balance among the different forms of elements in the soil.



	H0 Zn0	H0 Zn1	H0 Zn2	H1 Zn0	H1 Zn1	H1 Zn2
Zn B0	5.463	4.254	2.6127	4.938	3.3777	1.502
Zn B1	5.644	4.353	2.9717	5.0567	3.333	1.794
Zn B2	5.7577	4.334	3.314	5.2	3.382	1.8407

Figure 7. Impact of humic acid, zinc, and boron on post-harvest residual zinc content in soil (mg kg^{-1} soil).

The Zn0B2 treatment resulted in the highest residual Zinc concentration (5.478 mg kg^{-1} soil) in the context of the interaction between Boron and Zinc, while the Zn2B0 treatment produced the lowest concentration (2.057 mg kg^{-1} soil). This may be due to the high Boron content, which increases the amount of Zinc remaining in the soil

4. Conclusions

The relationship of Zn with soil factors is understood quite deeply. The present review discussed several important factors playing roles in the regulation of Zn mobility and bioavailability in soil. interaction between humic acid and zinc showed that there were significant differences in the total zinc, and the H0Zn2 treatment was effective. The highest quantity was found in soil at 9.4897 mg kg^{-1}, while the H1Zn2 treatment yielded 7.7866 mg kg^{-1}. Regarding humic acid and boron, there were significant differences in the overall zinc concentration in the soil; the H0B0 treatment had the greatest concentration,10.0690 mg kg^{-1}. In conclusion, this field experiment demonstrated the complex interactions between humic acid, Zinc, and Boron in calcareous soils and their significant impact on the chemical behaviour of Zinc. The addition of humic acid notably reduced the concentrations of total Zinc and Zinc bound to carbonate minerals, oxides, and residues while increasing the available Zinc associated with organic matter. Zinc addition led to higher total and available Zinc concentrations in the soil, highlighting its role in enhancing Zinc availability. The results suggest that the strategic use of humic acid, Zinc, and Boron can optimize Zinc availability in sunflower cultivation, potentially improving crop yield and nutrient content.

Author Contributions

All authors contributed equally to all stages of the study, from conceptualization and study design to data collection, analysis, writing of the manuscript, and final approval of the published version.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data will be available on request from the author.

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