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Effects of humic acid extracted from organic waste compost on maize seedlings under cadmium stress

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ABSTRACT

Cadmium (Cd) contamination poses a major global threat to agricultural productivity and food safety by entering the human food chain. Our study investigates the potential of humic acid (HA), a naturally occurring substance in compost with potent metal-binding capabilities, to mitigate the impact of Cd stress on maize. Maize seeds were exposed to 2 mg L^{-1} Cd^{2+}, with HA applied at varying concentrations (0 to 0.5 g L^{-1} HA-Cd) to assess its effects on germination and early seedling growth. Cd stress significantly impaired germination rates, indices, and root development. However, the application of 0.05 g L^{-1} HA-Cd improved the germination rate by 56%, while 0.05 and 0.1 g L^{-1} HA-Cd treatments enhanced root length by 17% and 34%, respectively. Conversely, the highest dose (0.5 g L^{-1}) negatively affected growth. HA treatments also increased chlorophyll content and enhanced the uptake of essential nutrients like calcium, magnesium, and iron, while reducing sodium and sulfur levels in shoot tissues. These physiological improvements were closely linked to the growth-promoting effects observed at optimal HA concentrations. The findings from this study not only underline the role of humic acid as a promising ameliorative agent against cadmium toxicity but also establish the optimal concentrations of HA that maximize growth and physiological benefits in maize. This research could pave the way for developing cost-effective, environmentally sustainable strategies to enhance crop resilience against Cd stress, thereby safeguarding food security in contaminated soils.

Keywords: cadmium stress, early maize development, humic acid, seed germination, toxicity.

INTRODUCTION

Cadmium (Cd) contamination is a global agricultural concern, posing significant threats to the food chain and human well-being (Xiao et al., 2017; Zhao et al., 2022a). Its high mobility facilitates its transmission from soil to crops and ultimately to humans, even at low soil concentrations, resulting in reduced plant performance and grain yields (Shah et al., 2023). Accumulation of Cd in higher trophic organisms within the food chain occurs following its absorption by plants (Yang et al., 2021; Li et al., 2021a). Human health is still at risk even though Cd contamination of agricultural soil usually falls within the low to moderate range (Ma et al., 2021a).

Maize (*Zea mays* L.) holds significant global agricultural importance, ranking as the third most essential crop on a worldwide scale (FAO, 2012). In Morocco, maize stands as the third most cultivated crop, following wheat and barley. However, the notable increase in maize imports can be attributed to the insufficient domestic production. This production deficit poses a challenge to the continuous and sustainable growth of the poultry sector, where maize plays an essential role in formulating poultry feeds (A.E.C., 2018). Cadmium induces stress in plants, affecting crucial physiological processes, including enzymatic activity, membrane permeability, and peroxidative metabolism (Pizzeghello et al., 2013). It disrupts fundamental metabolic functions such as germination, growth, and cellular respiration, acting as a potent inhibitor of photosynthesis and interfering with protein synthesis. Cd exposure results in visible changes in plant morphology, including inhibited root and shoot growth, leaf chlorosis, root tip necrosis, reduced water content, and biomass loss. Cd can infiltrate plant roots, disrupting nutrient balance and exacerbating malnutrition issues in the global population (Ma et al., 2021a). Cd has the ability to trigger the generation of reactive oxygen species by disrupting specific metabolic pathways or deactivating enzymes within the antioxidant system, leading to an increase in their levels (Sanita di Toppi and Gabbrielli, 1999). Compost, a commonly found organic waste product, contains inherent humic substances (Aylaj et al., 2023), particularly humic acid (HA), which is renowned for its robust metal-binding capabilities, extensively researched for its interactions with various heavy metals, including Cd (Olk et al., 2019 ; Huang et al., 2019 ; Rashid et al., 2020; An et al., 2022).

Humic acid, the main component of humic substances, possesses numerous functional groups exhibiting diverse properties (Nardi et al., 2016 ; Wang et al., 2021). Previous research has established that HA application enhances plant tolerance to both metals and drought, helps maintain ion balance, and contributes to the scavenging of ROS (Canellas et al., 2015; Asadi Aghbolaghi et al., 2022). Furthermore, due to its strong metal affinity, HA can form complexes with heavy metals, there by altering their bioavailability (Yip et al., 2010). In plant metal stressed, HA regulates toxic metal mobilization and transformation, activates antioxidant enzyme systems, balances hormone levels, encourages the accumulation of stress-responsive compounds like proline, promotes enhanced mineral uptake, and stimulates cellular division and elongation in leaves (Pizzeghello et al., 2013; Asadi Aghbolaghi et al., 2022). According to Huang et al., (2021), the beneficial effects of HA were believed to be associated with changes in DNA methylation levels, particularly in terms of the specific type of demethylation, which subsequently influenced the expression of relevant genes. HA application has been demonstrated to effectively alleviate lead stress in tea trees (Duan et al., 2020) and improve resilience to drought and phosphorus deficiency stress in *Zea mays* L. (Kaya et al., 2020). Furthermore, it promotes the heat stress tolerance of Arabidopsis (*Arabidopsis thaliana* L.) by activating the transcription of heat-shock proteins (Cha et al., 2020).

In addition to its positive effects, the impact of HA on plants varies depending on factors such as concentration range and plant species. For instance, in maize shoots, Asli and Neumann (2010) observed reduced growth due to HA. While most HA varieties used in previous studies are commercially sourced, relying on unsustainable resources and contributing to environmental pollution (Yang et al., 2021). Furthermore, the cost-effective and straightforward extraction of HA from compost makes it an attractive option for agricultural applications, offering a sustainable approach to enhance crop resilience in Cd-contaminated environments.

Our study aims to investigate the effects of HA derived from compost, produced by composting a mixture of immature horse manure and organic municipal solid waste at specific ratios, on maize (*Zea mays* L.) seed germination and seedling growth under varying concentrations of HA and Cd^{2+}. Nevertheless, it remains uncertain whether varying levels of humic acid can mitigate the toxicity of cadmium in plants. This study aimed to emphasize the role of humic acid in attenuating cadmium stress in maize plants. We assessed various morphological parameters, including seed germination percentage, shoot and root length, chlorophyll content, and the accumulation of Cd and mineral elements in the shoots. Our goal was to understand how maize responds to Cd^{2+} when applied alone and in combination with HA, with a focus on the impact of Cd-HA interactions on element uptake in maize seedlings' shoots. Additionally, we aimed to determine the most effective dosage of humic acid in promoting root development, improving nutrient utilization efficiency and enhancing tolerance to cadmium stress.

MATERIALS AND METHODS

Culture conditions and treatments

In the current study, maize plants (*Zea mays* L., NK Dracma variety) were used for experimentation at the Faculty of Sciences in El Jadida, Morocco. The selection of maize as the experimental crop was based on its significant relevance, being a commonly cultivated plant worldwide (Pál et al., 2006; Alharby et al., 2021).

Maize seeds were sterilized with 70% ethanol and then rinsed with distilled water three times. Subsequently, ten maize seeds were placed in Petri dishes with three layers of wetted filter paper at the bottom to prevent water loss and then soaked in either humic acid (HA) or water. Germination took place in complete darkness at 28 °C and 70% ambient humidity. Each Petri dish containing maize seeds was soaked in one of seven concentrations of HA (dissolved in distilled water) (0 gL^{-1}, 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, 0.1 gL^{-1}, 0.5 gL^{-1}). Petri dishes soaked with clean water served as the control (Ck). Additionally, the seeds were moistened with a 10 mL solution of Cd^{2+} ions (single-element, matrix: 2% HNO_3) at two Cd levels (Ck (no added Cd) and HA-Cd (2 mgL^{-1}) to induce Cd stress. When the radicle protruded 1–2 mm from the seed capsule, the number of germinated seeds per Petri dish was determined daily, and root length was measured after 7 days. Plant growth and biochemical tests were conducted at 15 days of exposure. The experimentation was organized with eight treatments, applied to seedlings in a completely randomized design comprising two Cd levels, seven HA treatments, and three replicates per treatment, resulting in a total of 24 pots.

Preparation of humic acid (HA) and composting process

The composting process of organic municipal solid wast commenced with mechanical separation to select the suitable fraction for composting. To achieve the desired carbon-to-nitrogen (C/N) ratio, a blend of immature horse manure and sugarcane bagasse was introduced. The research primarily focused on extracting HA from compost produced via aerobic fermentation in a laboratory-scale reactor. The extraction and fractionation methodology hinged on the unique solubility attributes of humic acids in basic media and fulvic acids in acidic or basic environments. This procedure closely followed the approach outlined by Nardi et al., (1994), as described in earlier research (Aylaj et al., 2023).

The recovered HA from the supernatant was in the form of humic acid salts. The fractionation process for the humic extracts entailed adjusting the medium's acidity, following the methodology proposed by Baglieri et al., (2014).

To characterize the HA fractions, six stock solutions of HA were prepared, each with final concentrations ranging from 5 mgL^{-1} to 500 mgL^{-1}, derived from the compost. CHNOS elemental analysis and UV-Vis spectroscopy were employed for characterization. Accurate solid-state elemental composition analysis was conducted using Thermo Finnigan EA 1110 CHNS equipment, which involved high-temperature combustion. Various physico-chemical parameters, including pH, electrical conductivity (C.E.), humidity, organic carbon (O.C.), nitrogen (N), and organic matter (O.M.), were measured according to AFNOR standards.

For specific absorbance measurements, 12.5 mg of HA was dissolved in one liter of a low-normality bicarbonate solution. This procedure adhered to established protocols by Chen et al., (1977), Swift (1996) and Owen (1996). The UV-Vis absorbance of HA was quantified using a UV-2450 spectrophotometer by SHIMADZU.

Methods of analysis

Parameters plant growth

The fresh and dry masses of both plant shoots and roots were determined by individually weighing each component. Each treatment and replication involved harvesting three maize plants per pot, which were then rinsed with distilled water. Subsequently, the plants were separated into shoots, leaves, and roots for further analysis of growth parameters. The weight of both shoots and roots was recorded to determine the average fresh mass of the materials. Additionally, the lengths of the shoots and roots were measured. Shoot length was quantified from the base to the apex of the plant samples, while root length was measured from the shoot's base to the root apex. Dry matter content was determined after subjecting the biomass to drying in an oven at a temperature of 105 °C. This comprehensive approach provided a detailed understanding of the growth and development of the maize plants under various treatments. The calculation of leaf area for individual leaves and each plant was conducted by employing a caliper to measure both the length and width of the leaves. This process followed the method outlined by Picard (1990).

Chlorophyll content estimation

For determining the chlorophyll a (Chl a) and chlorophyll b (Chl b) we followed the methodology outlined by Maclachlam and Zalik (1963). Fresh maize leaves, approximately weighing 0.1 g, were utilized. These leaves were homogenized using acetone and acid-washed sand, with subsequent spinning for five minutes at 3000 rpm and a temperature of 30 °C. The resulting mixture was blended with dilute acetone and then subjected to measurement at optical densities (OD) of 663 nm and 645 nm using a spectrophotometer. Subsequently, the concentrations of Chl a and Chl b were computed using the provided equations.

$$Chl\ a\ (\mu g\ g\ fw^{-1}) = 12.7 \times OD\ (663\ nm) - [2.69 \times OD\ (645\ nm) \times V/(1000 \times W)] \quad (1)$$

$$Chl\ b\ (\mu g\ g\ fw^{-1}) = 22.9 \times OD\ (645\ nm) - [4.68 \times OD\ (663\ nm) \times V/(1000 \times W)] \quad (2)$$

where: fw is Fresh Material, V is the extraction volume, OD is the optical density, and W is the weight of fresh material.

Mineral accumulation in aerial parts

The analysis of mineral accumulation (Ca, Mg, Na, and S) in shoots was conducted as follows: Plant materials (0.2 g per sample) were initially dried in an oven at 65 °C for a period of 48 to 72 hours. Subsequently, the dried shoot samples were immersed in a mixture of H_2SO_4 and HNO_3 with a volumetric ratio of 1:5 (v/v) for 24 hours. This was followed by combining them with a solution of HNO_3 and $HClO_4$ in a volumetric ratio of 5:1 (v/v). Each sample was then subjected to the analysis of element concentrations, including calcium (Ca), magnesium (Mg), sodium (Na), and sulfur (S), using an ICP-OES type spectrometer.

Determination of cadmium and iron in aerial parts

The procedure for analyzing Fe and Cd in plants adhered to the method outlined by Kalra (1998). For this analysis, 1 gram of dry biomass was collected and crushed, after which it underwent incineration at 500 °C for 4 hours, followed by a cooling period. The resulting ashes were then moistened using a small amount of deionized water and dissolved through the addition of 2 mL of concentrated HCl. The final volume of the solution was adjusted to 50 mL using deionized water. The measurement of metal content was carried out utilizing an spectrometer (Optima 8000 ICP-OES). All preparations are made using double-distilled water. For the determination of cadmium, five concentration standards (0, 0.1, 0.25, 0.5, and 1 µg/ml) were prepared by dilution using single-element standards (Cd^{2+}, matrix: 2% HNO_3) with an initial concentration of 100 µg/ml. In parallel, eight standards with concentrations ranging from 0; 2.5; 5; 7.5; 10; 15; 25 and 50 µg/ml were prepared for the determination of macroelements. These standards were obtained through the dilution of multielement standards (1000 µg/ml). A Yttrium solution with a concentration of 2.5 µg/ml is incorporated as an internal standard in the analysis process.

STATISTICAL ANALYSIS

The means of the experimental values and their corresponding standard deviations were calculated. Aerial and root growth parameters underwent statistical analysis via standard analysis of variance (ANOVA), and the means were compared using the least significant difference test (LSD) at the 5% significance level. This analysis was conducted using SPSS 20.0 software.

Two tolerance indices were utilized, to assess the plant tolerance to cadmium (Cd) toxicity. These indices were calculated by considering the quantity of each parameter (Px) of the Cd-stressed treatment (HA-Cd) in comparison to the control (Ck), and of the Cd-stressed treatment (HA-Cd) in comparison to the control Cd (Ckcd : 0 gL^{-1} HA-Cd), following the method described by Wilkins in 1978:

$$Tolerance\ index\ relative\ to\ Ck = (HA\text{-}Cd)Px/(Ck)Px$$

$$Tolerance\ index\ relative\ to\ Ckcd = (HA\text{-}Cd)Px/(Ckcd)Px$$

RESULTS

Table 1 presents the key chemical characteristics of the compost utilized in the current study for humic acid extraction. This compost stands out for its elevated levels of organic matter. The compost exhibits a slightly alkaline pH, while the electrical conductivity indicates a moderately high value. Additionally, the compost is

Table 1. Physical and chemical characteristics of compost used for extracting humic acid in the maize plant experiment

Theses	pH	CE	TOC	TKN	C/N	NH_4^+	NO_3^-	MgO	CaO	K_2O	P_2O_5	Fe	Zn
Measure unit		ds cm^{-1}	% DM										
[a]Compost	[b]7.88±0.04	3.50± 0.05	28.7± 0.40	2.9± 0.03	9.77± 0.07	0.2± 0.01	0.2± 0.01	4.83± 0.02	8.2± 0.34	10.0± 0.03	5.95± 0.49	1.0± 0.09	0.7± 0.09

Note: [a] mixtures of SMSW and IHM at a ratio 9:1 weight weight^{-1} (ww^{-1}); [b] means ± standard deviation of three replicates, DM: dry matter.

characterized by a substantial proportion of mineral fractions.

The UV-Visible analysis of humic acids is summarized in Table 2, providing specific absorbances, the Welt ratio, and ΔlogK values for HA classification. The Welt ratio (E4/E6) is calculated by dividing the absorbance at 465 nm by the absorbance at 665 nm. Notably, the E4/E6 ratio of the HA used in this study is less than 5, indicating an advanced degree of humification in the compost employed for humic acid extraction (El Herradi et al., 2014). This underscores the prevalence of humic acid over fulvic acid in the compost.

Humic acids can be classified based on their ΔlogK values, representing the difference in the logarithms of the absorbances at 400 nm and 600 nm (Kumada, 1967 ; Cunha et al., 2009). According to the data in Table 2, we can confirm that the HA used belongs to class B in terms of the degree of humification (Kumada, 1988).

Assessment of seed germination

To gain an initial understanding of the methods employed to mitigate Cd toxicity, we assessed seed germination under various humic acid treatments when exposed to Cd. Maize seeds subjected to Cd stress without HA treatments showed a significant reduction ($p < 0.05$) in germination rate (Figure 1) compared to the control. In the control groups (no Cd and HA added), the germination rate increased significantly by 32% compared to the Cd stress only (Table 3b). When HA was added to the medium, the germination rate (GR) increased and reached a level equivalent to the GR of seeds in the control group without Cd stress or HA treatments (Table 3a). However, when compared to the Cd treatment alone, the GR was significantly stimulated, reaching a remarkable 56% increase with 0.05 gL^{-1} HA-Cd (Table 3b).

Cd tolerance indices, expressed as stress index of RRL (relative root length), RGR (relative germination rate), RGI (relative germination index), at 7 d.a.s.

We noted that, the length of the root (LR), germination index (GI), and GR increased with an increasing level of humic acid treatments for seeds exposed to Cd stress (Figure 1). This effect was more evident for seeds treated with 0.1 gL^{-1} HA-Cd in terms of RL (9.84 cm) and with 0.05 gL^{-1} for both GI (123%) and GR (77%). The Cd-tolerant seedlings exhibited higher values at the 0.05 gL^{-1} and 0.1 gL^{-1} HA-Cd levels compared to the other HA levels (Figure 1). No significant difference in seedling (RL, GI, and GR) was observed in seeds exposed to 2 mgL^{-1} of Cd and 0.01 gL^{-1} HA-Cd treatments compared to the control condition. However, a decreasing trend was clearly visible for 0 gL^{-1} and 0.5 gL^{-1} of HA-Cd. The lowest values were observed in the 0.5 gL^{-1} HA-Cd treatment for RL and the 0 gL^{-1} HA-Cd treatment for both GI and GR (Figure 1).

The effect of 2 mgL^{-1} of Cd^{2+} on root length was less distinct compared to the control. When HA concentrations of 0.005 gL^{-1} to 0.025 gL^{-1} were added, the root length slightly increased by 0 % to 15% compared to the control (Table 3a). However, when compared with the control, there was no significant difference ($p < 0.05$) among the treatments with 0 gL^{-1} to 0.025 gL^{-1} HA-Cd.

Furthermore, treatment with 0.1 gL^{-1} HA-Cd had a significant positive effect (p-value: 0.011) on root length compared to the control, enhancing root length by 34%, while treatments with 0.5 gL^{-1} HA-Cd inhibited root length and germination

Table 2. Specific absorbances and the welt ratio (E4/E6) of the humic acids studied

Parameter	280	Abs (nm) 465	665	E_4/E_6	Δlog *K*
AH_3	0.174	21.87 10^{-3}	6.97 10^{-3}	3.14	0.66

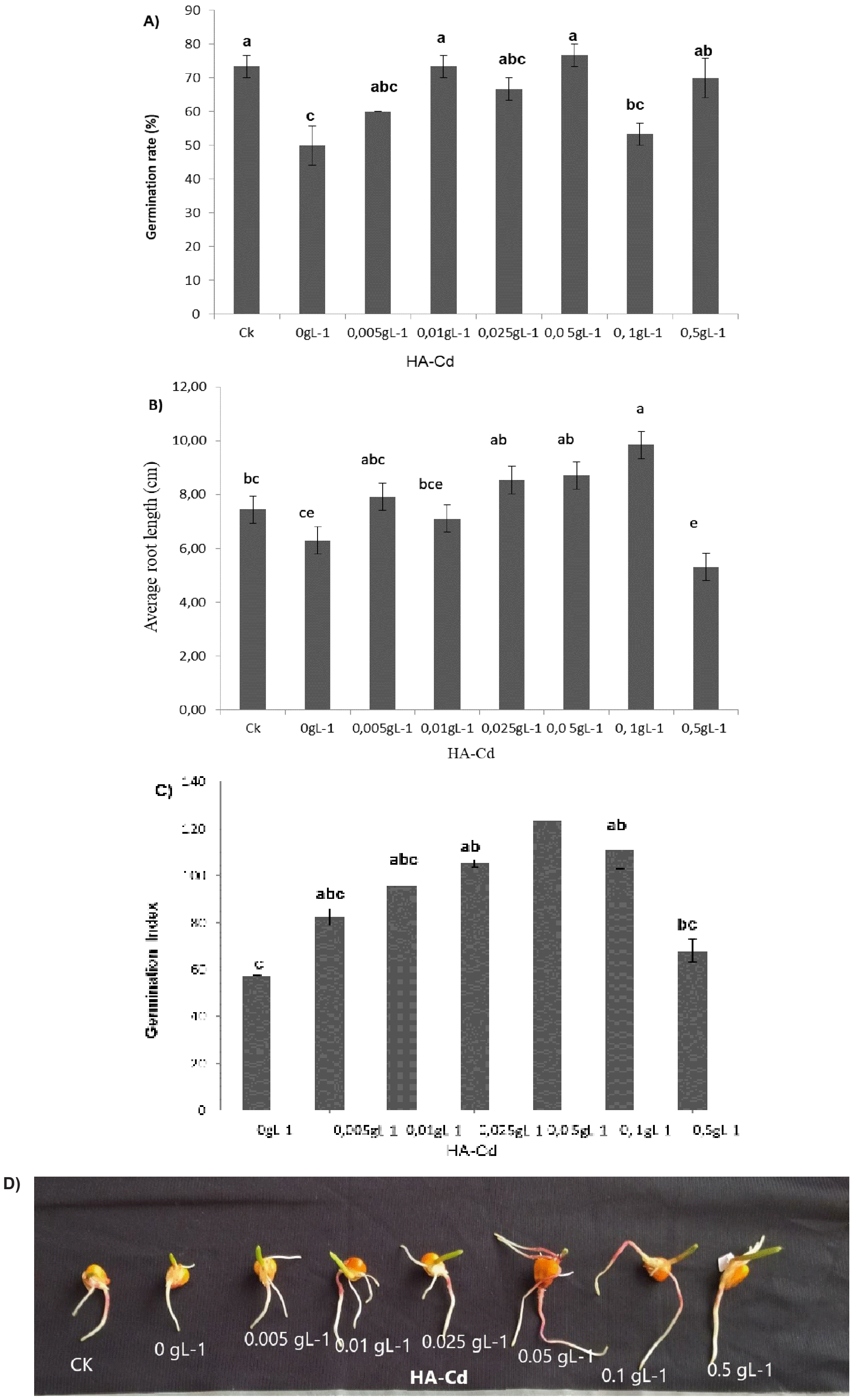

Figure 1. Effect of various humic acid concentrations on the root length (A), germination index (B), germination rate (C), and on the morphological symptoms (D) of maize grown under control and Cd (2 mgL^{-1}) stress at 7 days after sowing

Significance among different treatments at $p \leq 0.05$ levels are denoted by different alphabets and the bars reflect the triplicates means with standard error (±) based on Tukey's multiple range test. HA – humic acid extracted from compost produced by mixtures of separated municipal solid wastes (SMSW) and mixtures of immature horse manure (IHM) at a ratio of 9:1 weight : weight; HA–humic acid extracted at seven concentrations (0 gL^{-1}, 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, 0.1 gL^{-1} and 0.5 gL^{-1}); (CK) a control group with seedlings growing in no-Cd and no HA solution; (Ckcd: 0 gL^{-1} HA-Cd) a Cd exposure group with seedlings growing in Cd and no HA (0 gL^{-1}) solution and (HA-Cd) a group with seedlings growing in Cd stress and various HA concentration.

Table 3. Effect humic acid on physiological parameters of maize grown under Cd^{2+} (2 mgL^{-1}) stress

(a) Parameters	HA- Cd^{c}						
	0 gL^{-1}	0.005 gL^{-1}	0.01 gL^{-1}	0.025 gL^{-1}	0.05 gL^{-1}	0.1 gL^{-1}	0.5 gL^{-1}
RRL	[a]0.85±0.02[b]	1.06±0.02	0.95±0.016	1.15±0.06	1.175±0.05	1.34±0.142	0.71±0.013
RGR	0.68±0.041	0.82±0.03	1±0	0.911±0.034	1.05±0.04	0.73±0.01b	0.95±0.037
GI	0.57±0.04	0.82±0.06	0.95±0.02	1.05±0.07	1.23±0.08	1.11±0.05b	0.68±0.04
(b) Parameters	HA- Cd^{c}						
		0.005 gL^{-1}	0.01 gL^{-1}	0.025 gL^{-1}	0.05 gL^{-1}	0.1 gL^{-1}	0.5 gL^{-1}
RRL-Cd		[d]1.25±0.02[b]	1.12±0.04	1.36±0.04	1.38±0.02	1.57±0.13	0.84±0.03
RGR-Cd		1.23±0.1	1.49±0.1	1.36±0.07	1.56±0.09	1.08±0.06	1.41±0.03
GI-Cd		1.44±0.06	1.16±0.05	1.1±0.06	1.17±0.01	0.78±0.02	0.72±0.08

Note: [a] numbers represent a value calculated as a ratio of a respective parameter obtained under HA-Cd treatment and Ck; [a] bars reflect the triplicates means with standard error (±); [b] HA-Cd humic acid extracted from compost produced by mixtures of separated municipal solid wastes (SMSW) and mixtures of immature horse manure (IHM) at a ratio of 9:1 weight:weight; HA – humic acid extracted at seven concentrations (0 gL^{-1}, 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, 0.1 $gL^{-1}$and 0.5 gL^{-1}); (CK) a control group with seedlings growing in no-Cd and no HA solution; (Ck) a Cd exposure group with seedlings growing in Cd and no HA treatment (Ckcd: 0 gL^{-1} HA-Cd) solution and (HA-Cd) a group with seedlings growing in Cd stress and various HA concentration; [d] value calculated as a ratio of a respective parameter obtained under HA and Cd treatment and without HA treatment (Ckcd : 0 gL^{-1}HA-Cd).

index by 29% and 32% compared to the control, respectively (Table 3a).

Comparatively, Cd stress without HA treatments in the tested range slightly reduced root length by 15%, while the germination index and germination rate were significantly affected ($p < 0.05$), decreasing by 43% and 32%, respectively, in comparison to the control (Table 3a). The results also revealed that treatments with HA-Cd significantly enhanced root length, germination index and germination rate. Notably, germination index reached significant levels at 0.05 gL^{-1}, exhibiting a 23% increase, and root length reached significant levels at 0.1 gL^{-1} HA-Cd treatments, showing a 34% increase compared to the plants in the control group (Table 3a).

Consequently, drawing from germination-related data, it becomes evident that HA treatments exert a direct and positive influence on seed stress tolerance. Moreover, a conspicuous pattern emerges, indicating that as the HA-Cd concentration escalates within the 0–0.1 gL^{-1} spectrum, there is a notable augmentation in root length, germination rate, and germination index. However, beyond this concentration range, were observe a subsequent decline, as visually represented in Figure 1. Statistical analysis of the results concerning seed root length revealed significant differences ($p < 0.001$) among the tested humic acid levels (Table 4a). The application of the Tukey test enabled the classification of data into four groups for LR: 0.1 gL^{-1}, 0.05 gL^{-1}, 0.025 gL^{-1}, and 0.005 gL^{-1} > 0.05 gL^{-1}, 0.025 gL^{-1}, 0.005 gL^{-1}, Ck, and 0.01 gL^{-1} > 0.005 gL^{-1}, Ck, 0.01 gL^{-1}, 0 gL^{-1} > 0.01 gL^{-1}, 0 gL^{-1} and 0.5 gL^{-1}.

Moreover, significant differences in both germination indice and germination rate were observed ($p < 0.01$) (Table 4a). The Tukey test classified the data into three groups for each of these two parameters.

For IG: 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, and Ck > 0.1 gL^{-1}, 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, Ck, and 0.5 gL^{-1} > 0.005 gL^{-1}, 0.01 gL^{-1}, 0.5 gL^{-1}, Ck and 0 gL^{-1}.

For TG: 0.05 gL^{-1}, 0.01 gL^{-1}, Ck, 0.5 gL^{-1}, 0.025 gL^{-1}, 0.005 gL^{-1} > 0.5 gL^{-1}, 0.025 gL^{-1}, 0.005 gL^{-1}, 0.1 gL^{-1} > 0.025 gL^{-1}, 0.005 gL^{-1}, 0.1 gL^{-1}, and 0 gL^{-1}.

Table 4. Analysis of variance of the effect of the humic acid traetments in Cd^{2+} (2 mgL^{-1}) stress on the parameters of germination of maize plant

Parameter	Average root length (cm)	Germination index (%)	Germination rate (%)
F-value	13.262***	5.853**	6.429*
p-value	0.000	0.002	0.01

The Cd^{2+} concentration of 2 mgL^{-1} chosen in the present experiment showed that it is the lower and not lethal concentration. Indeed, it didn't exhibit an unambiguous impact on maize seedlings compared to the control group (treatment with no Cd and no HA). The findings of this study indicate that the addition of HA helps reduce the toxicity of cadmium stress. Based on the above conclusions, concentrations of 0.05 and 0.1 gL^{-1} HA-Cd appear to be suitable for effectively mitigating the damage caused by the cadmium environment on maize seed germination, in contrast to the Cd-only treatment, while higher concentration treatments led to a decline.

To assess the influence of both HA and Cd stress on maize plants, our experiment was extended to cover 15 days of maize seedling growth. Various parameters, including leaf area, root and shoot length, and maize shoot fresh biomass, were measured. While there were no significant differences ($p > 0.05$) observed in leaf area, shoot fresh weight, and shoot length among the HA-Cd treatments (Table 5a). A net decrease of 20%, 24%, 23%, and 10% (compared to the control) was observed in root length at 15 days after sowing (d.a.s) for treatments with 0 gL^{-1} HA-Cd, 0.005 gL^{-1} HA-Cd, 0.01gL^{-1} HA-Cd, and 0.5 gL^{-1} HA-Cd, respectively. Interestingly, an increase of approximately 11% and 28% in root length (compared to the control) was also noted for treatments with HA-Cd concentrations of 0.05 gL^{-1} and 0.1 gL^{-1}, respectively (Table 6a). The statistical analysis of the results for root length at 15 days indicated significant differences (p-value: 0.05) among the tested HA levels (Table 5a).

In this study, when compared to seedlings exposed to Cd without the addition of HA (0 gL^{-1} HA-Cd), the inclusion of HA demonstrated a significant reduction in the toxicity of cadmium stress on root length at 15 d.a.s (Figure 2). Root length increased by 13%, 33%, 39%, and 60% with the introduction of HA-Cd at concentrations of 0.5 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, and 0.1 gL^{-1}, respectively (Table 6b).

The LSD tests revealed a significant reduction in fresh shoot weight (FSW) at the 0.5 gL^{-1} HA-Cd level compared to various other treatments, ranging from 6% to 10%. This decrease was significantly lower than the FSW observed in the CK treatment (p-value: 0.018), 0 gL^{-1} HA-Cd (p-value: 0.023), 0.01 gL^{-1} HA-Cd (p-value: 0.049), 0.05 gL^{-1} HA-Cd (p-value: 0.039), and even 0.025 gL^{-1} HA-Cd (p-value: 0.026).

In our experimental context, LA exhibited an increase ranging from 0.03% to 20% in all treatments compared to the control (Table 6a), but this difference was not statistically significant ($p < 0.05$).

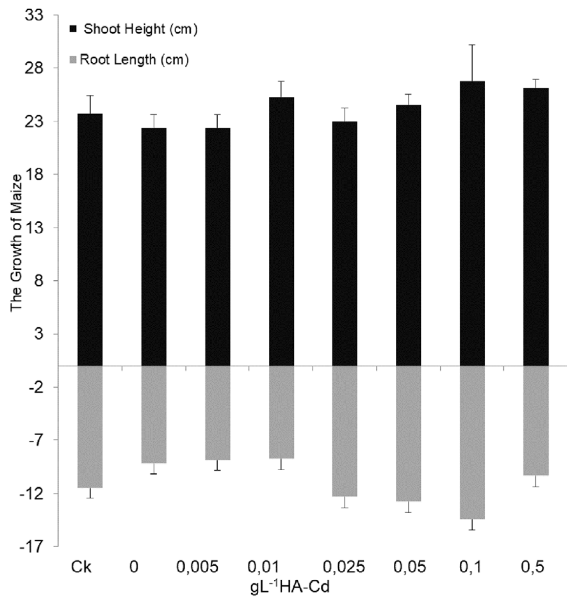

Figure 2. Effect of various humic acid concentrations on the shoot height and root length at 15 days after sowing of maize grown under control and Cd (2 mgL^{-1}) stress

Table 5. Analysis of variance of the effect of the humic acid traetments in Cd^{2+} (2 mgL^{-1}) stress on the parameters of maize seedling, chlorophyll content and mineral shoot content of plant maize at 15 d.a.s.

(a)	Root length	Shoot height	Leaf area	Fresh shoot weight	Chl a	Chl b
F-value	2.658 NS	1.020	0.821 NS	1.533 NS	10.505	282.721
p-value	0.05	0.454	0.584	0.226	0.000 ***	0.000 ***
b)	Cd	Ca	Mg	Na	S	Fe
F-value	8.212***	71.8***	86.756***	652.265***	88.678***	18.277***
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Note: *** significant différences at p<0.001, ** significant différences at $p < 0.01$, * significant differences at $p < 0.05$ and NS: not significant. LSD at 5%. Numbers represent values of F at 5%.

Table 6. Effect humic acid on physiological parameters of maize grown under Cd^{2+} (2 mgL^{-1}) stress. Cd tolerance indices, expressed as stress index of RRL (relative root length), RSH (relative shoot height), RLA (relative leaf area), RFSW (relative fresh shoot weight) RCHLa (relative content of chlorophyll a), RCHLb (relative content of chlorophyll b) at 15 d.a.s.

a) Cd tolerance of parameters	HA- Cd[c]						
	0 gL^{-1}	0.005 gL^{-1}	0.01 gL^{-1}	0.025 gL^{-1}	0.05 gL^{-1}	0.1 gL^{-1}	0.5 gL^{-1}
RRL	[a]0.8±0.01[b]	0.76±0.05	0.766±0.01	1.064±0.09	1.113±0.04	1.28±0.2	0.905±0.02
RSH	0.95± 0.01	0.94±0.01	1.07±0.01	0.9±0.02	1.04±0.07	1.15±0.17	1.11±0.03
RLA	1.14±0.07	1.03±0.03	1.01±0.05	1.11±0.05	1.2±0.04	1.2±0.16	1.12±0.14
RFSW	1±0.01	0.97±0.03	0.98±0.01	0.99±0.01	0.98±0.01	1.001±0.005	0.91±0.01
RChla	0.70±0.001	0.63±0.001	0.78±0.001	0.55±0.10	0.56±0.001	0.95±0.05	0.88±0.002
RChlb	0.74±0.002	0.5±0.001	0.78±0.004	0.68±0.002	0.57±0.004	0.96±0.005	0.88±0.0004
b) Cd tolerance of parameters	HA- Cd[c]						
		0.005 gL^{-1}	0.01 gL^{-1}	0.025 gL^{-1}	0.05 gL^{-1}	0.1 gL^{-1}	0.5 gL^{-1}
RRL-Cd		[d]0.96±0.07[b]	0.96±0.02	1.33±0.12	1.39±0.04	1.6±0.25	1.13±0.01
RSH		1.00±0.01	1.13±0.01	1.02±0.02	1.10±0.06	1.21±0.16	1.17±0.03
RLA		0.91±0.04	0.88±0.02	0.98±0.02	1.05±0.05	1.04±0.07	0.97±0.08
RFSW		0.98±0.04	0.99±0.00	1.00±0.00	0.99±0.01	1.01±0.00	0.91±0.02
RChla		0.91±0.15	1.13±0.00	0.80±0.00	0.80±0.08	1.36±0.00	1.27±0.00
RChlb		0.67±0.00	1.05±-	0.92±0.00	0.77±0.00	1.29±0.01	1.19±0.00

Note: numbers represent a value calculated as a ratio of a respective parameter obtained under HA-Cd treatment and Ck. [a] bars reflect the triplicates means with standard error (±). [b] HA-Cd humic acid extracted from compost produced by mixtures of separated municipal solid wastes (SMSW) and mixtures of immature horse manure (IHM) at a ratio of 9:1 weight:weight; HA – humic acid extracted at seven concentrations (0 gL^{-1}, 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, 0.1 $gL^{-1}$and 0. 5 gL^{-1}); (CK) a control group with seedlings growing in no-Cd and no HA solution; (Ck) a Cd exposure group with seedlings growing in Cd and no HA treatment (Ckcd: 0 gL^{-1} HA-Cd) solution and (HA-Cd) a group with seedlings growing in Cd stress and various HA concentration. [d] value calculated as a ratio of a respective parameter obtained under HA and Cd treatment and without HA treatment (Ckcd : 0 gL^{-1}HA-Cd).

Chlorophyll content

The phytotoxic effects of cadmium led to a reduction in chlorophyll a and chlorophyll b content in maize seedlings. A significant reduction of 30% and 26% was recorded in Chl a and Chl b in the Cd-treated maize plants, respectively, compared to the controls without Cd supplementation (Table 6a). The application of humic acid enhanced chlorophyll content (both a and b) in leaves compared to Cd treatment alone (Figure 3). Therefore, no statistically significant difference ($p < 0.05$) was recorded in Chl a for treatments with 0.01 gL^{-1} HA-Cd, 0.1 gL^{-1} HA-Cd, and 0.5 gL^{-1} HA-Cd, in comparison to the control without HA and Cd supplementation. However, the HA supply at levels of 0.005 gL^{-1}, 0.025 gL^{-1}, and 0.05 gL^{-1} showed no statistically significant difference ($p < 0.05$) in Chl a content compared to plants grown under Cd alone without HA supplementation. No statistically significant difference ($p < 0.05$) was recorded in Chl b for treatments with 0.1 gL^{-1} HA-Cd compared to the control. The lowest observed chlorophyll a and b content was noted at HA concentrations of 0.025 gL^{-1} and 0.05 gL^{-1} (Figure 3). Enhanced values of Chl a and Chl b content were observed with 0.1 gL^{-1} HA-Cd and 0.5 gL^{-1} HA-Cd treatments. These treatments increased Chl a by 36% and 27% and Chl b by 29% and 19%, respectively, compared to seedlings exposed solely to Cd (0 gL^{-1} HA-Cd) (Table 6b).

Estimation of Cd, Ca, Mg, Na, S and Fe content

Cd and HA-Cd exposure had a significant effect ($p \leq 0.001$) on the accumulation of Cd, Ca, Mg, Na, S and Fe in maize shoots at 15 d.a.s (Table 5b). As shown in Table 5, Cd stress increase significantly ($p < 0.001$) Cd levels in treated maize plants in comparison with control, with a notable increase observed in all treated plants, ranging from 18 to 32-fold (Table 7a).

A significant increase in Cd concentration was observed in the shoots following the application

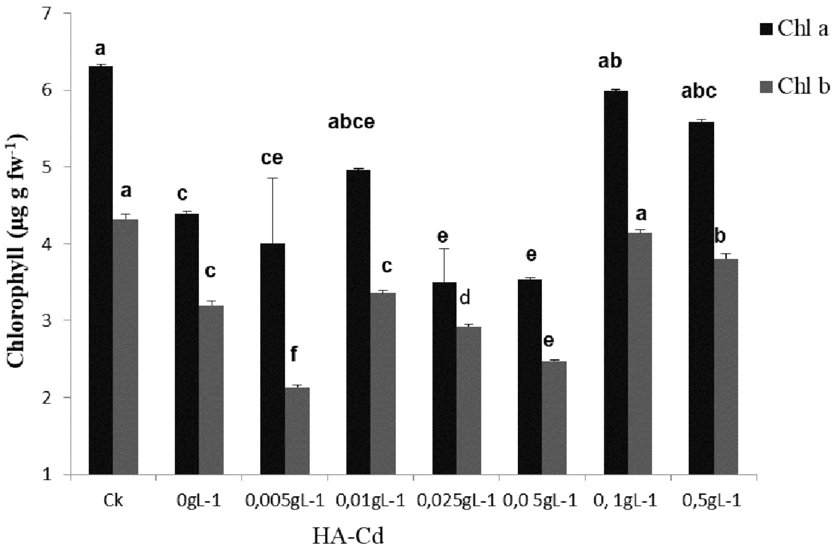

Figure 3. Effect humic acid on chlorophyll a content (Chla), and chlorophyll b content (Chlb) at 15 d.a.s Significance among different treatments at $p \leq 0.05$ levels are denoted by different alphabets and the bars reflect the triplicates means with standard error (±) based on Tukey's multiple range test. HA – humic acid extracted from compost produced by mixtures of separated municipal solid wastes (SMSW) and mixtures of IHM at a ratio of 9:1 weight : weight; HA–humic acid extracted at seven concentrations (0 gL^{-1}, 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, 0.1 gL^{-1} and 0.5 gL^{-1}); (CK) a control group with seedlings growing in no-Cd and no HA solution; (Ckcd: 0 gL^{-1} HA-Cd) a Cd exposure group with seedlings growing in Cd and no HA (0 gL^{-1}) solution and (HA-Cd) a group with seedlings growing in Cd stress and various HA concentration.

Table 7. Effect humic acid on mineral element of maize grown under Cd^{2+} (2 mgL^{-1}) stress. Cd tolerance indices, expressed as stress index of relative shoot content of element mineral

a) Cd tolerance of parameters	HA- Cd[c]						
	0 gL^{-1}	0.005 gL^{-1}	0.01 gL^{-1}	0.025 gL^{-1}	0.05 gL^{-1}	0.1 gL^{-1}	0.5 gL^{-1}
Cd	[a]18.40±0.3[b]	19.74±0.82	18.13±0.20	21.38±0.51	28.14±1.00	31.61±1.31	19.11±0.26
Ca	0.95±0.00	1.02±0.01	0.98±0.01	1.10±0.00	1.06±0.00	1.18±0.02	1.44±0.00
Mg	0.89±0.00	0.85±0.01	0.92±0.00	1.21±0.00	1.00±0.00	1.23±0.02	1.04±0.00
Na	0.56±0.00	0.75±0.01	0.72±0.00	1.20±0.00	0.83±0.00	0.79±0.01	1.48±0.00
S	0.84±0.01	0.70±0.01	0.97±0.00	1.24±0.01	0.93±0.00	0.87±0.01	1.08±0.01
Fe	0.57±0.05	0.34±0.00	0.87±0.06	1.48±0.03	1.03±0.07	0.63±0.00	0.76±0.08
b) Cd tolerance of parameters	HA- Cd[c]						
		0.005 gL^{-1}	0.01 gL^{-1}	0.025 gL^{-1}	0.05 gL^{-1}	0.1 gL^{-1}	0.5 gL^{-1}
Cd		[d]1.07±0.03[a]	0.99±0.01	1.16±0.03	1.53±0.07	1.72±0.04	1.04±0.03
Ca		1.06±0.02	1.02±0.00	1.14±0.01	1.10±0.01	1.22±0.02	1.50±0.00
Mg		0.95±0.02	1.03±0.00	1.36±0.01	1.12±0.01	1.38±0.02	1.16±0.00
Na		1.34±0.02	1.28±0.01	2.14±0.01	1.49±0.00	1.41±0.02	2.65±0.01
S		0.84±0.00	1.15±0.02	1.48±0.03	1.10±0.02	1.03±0.01	1.28±0.01
Fe		0.60±0.05	1.52±0.02	2.65±0.24	1.80±0.05	1.12±0.09	1.34±0.13

Note: numbers represent a value calculated as a ratio of a respective parameter obtained under HA-Cd treatment and Ck. [a] bars reflect the triplicates means with standard error (±). [b] HA-Cd humic acid extracted from compost produced by mixtures of separated municipal solid wastes (SMSW) and mixtures of immature horse manure (IHM) at a ratio of 9:1 weight : weight; HA – humic acid extracted at seven concentrations (0 gL^{-1}, 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, 0.1 gL^{-1}and 0.5 gL^{-1}); (CK) a control group with seedlings growing in no-Cd and no HA solution; (Ckcd: 0 gL^{-1} HA-Cd) a Cd exposure group with seedlings growing in Cd and no HA treatment solution and (HA-Cd) a group with seedlings growing in Cd stress and various HA concentration. [d] value calculated as a ratio of a respective parameter obtained under HA and Cd treatment and without HA treatment (Ckcd : 0 gL^{-1}HA-Cd).

of HA-Cd at concentrations of 0.05 gL^{-1} and 0.1 gL^{-1}, with the most pronounced increase (8.34 mg kg^{-1} dw) noted at the 0.1 gL^{-1} HA dose (Figure 4). In contrast, the lowest concentrations were recorded in both the control group (Ck) and treatments with HA-Cd at 0 gL^{-1}, 0.01 gL^{-1}, and 0.5 gL^{-1}, ranging from 4.72 to 5.11 mg kg^{-1} dw. These values displayed no statistically significant differences ($p < 0.05$) among them.

Specifically, Cd^{2+} concentrations in shoots treated with HA-Cd at levels of 0.05 gL^{-1} and 0.1 gL^{-1} were significantly increased by 28-fold and 32-fold, respectively, compared to the control plants (Table 7a). Additionally, the shoot Cd^{2+} contents were higher at 0.05 gL^{-1} and 0.1 gL^{-1} of HA-Cd by 53% and 71%, respectively, in comparison to the Cd-only treatment (Table 7b).

In the case of calcium accumulation in maize shoots, higher levels of HA supply (ranging from 0.025 gL^{-1} to 0.5 gL^{-1} of HA-Cd) resulted in calcium concentrations ranging from 4.46 mg/g to 6.08 mg/g dw (Figure 5). These concentrations were notably higher than those observed in both the control (Ck) and treatments with 0 gL^{-1} to 0.01 gL^{-1} of HA-Cd. The lowest calcium concentration was observed in treatments with cadmium application alone (0 gL^{-1} of HA-Cd) (Figure 5). However, as the HA level increased, calcium absorption in maize shoots increased as well, with an enhancement ranging from 10% to 50% compared to the treatment with Cd only (Table 7b). The highest enhancement was recorded at a concentration of 0.5 gL^{-1} HA-Cd (Figure 5).

No significant effect ($p < 0.05$) on calcium content was observed in maize seedlings treated with Cd at levels of 0 gL^{-1} to 0.01 gL^{-1} of HA-Cd and in untreated control plants. However, for the treatments with 0.1 gL^{-1} and 0.5 gL^{-1} of HA-Cd, a significant difference was observed (p-value: 0.00). Additionally, under cadmium-induced stress in maize, an increased calcium absorption was observed starting from the application of 0.025 gL^{-1} of HA. Compared to the control, calcium absorption notably increased by 18% and 44% in plants treated with HA-Cd at concentrations of 0.1 gL^{-1} and 0.5 gL^{-1}, respectively.

The addition of cadmium to the medium of maize seedlings inhibited the absorption of magnesium. However, HA supply enhanced this absorption, but it was effective starting from a concentration of 0.025 gL^{-1} (Figure 5). In terms of magnesium accumulation in maize shoots, HA supply at levels of 0.025 gL^{-1} and 0.1 gL^{-1} resulted in significantly higher magnesium concentrations, approximately 4.37 mg/gdw and 4.41 mg/gdw, respectively (Figure 5). Magnesium absorption notably increased by an average of 22% compared to the control and 37% compared to the

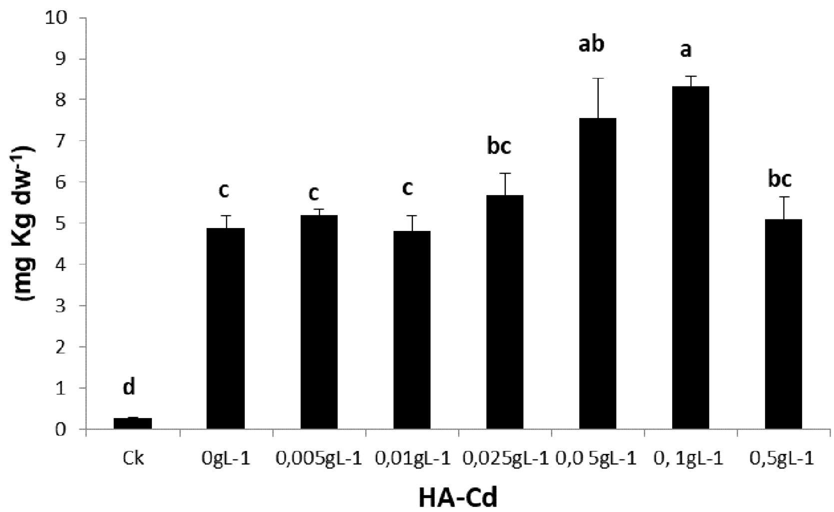

Figure 4. Effect humic acid on Cd shoot content of maize grown under Cd^{2+} (2 mgL^{-1}) stress
Significance among different treatments at $p \leq 0.05$ levels are denoted by different alphabets and the bars reflect the triplicates means with standard error (±) based on Tukey's multiple range test. HA – humic acid extracted from compost produced by mixtures of SMSW and mixtures of IHM at a ratio of 9:1 weight : weight; HA–humic acid extracted at seven concentrations (0 gL^{-1}, 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, 0.1 gL^{-1} and 0.5 gL^{-1}); (CK) a control group with seedlings growing in no-Cd and no HA solution; (Ckcd: 0 gL^{-1} HA-Cd) a Cd exposure group with seedlings growing in Cd and no HA (0 gL^{-1}) solution and (HA-Cd) a group with seedlings growing in Cd stress and various HA concentration.

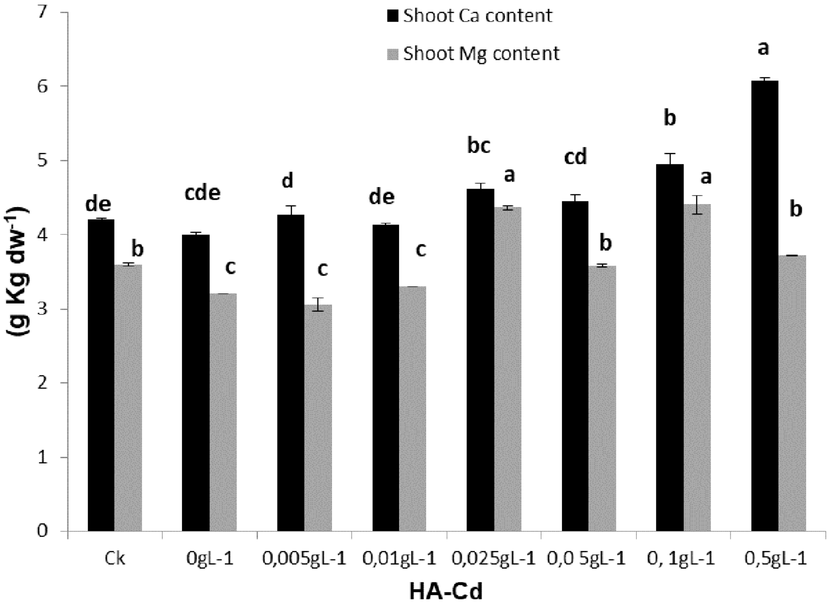

Figure 5. Effect humic acid on Ca and Mg shoot content of maize grown under Cd^{2+} (2 mgL^{-1}) stress
Significance among different treatments at $p \leq 0.05$ levels are denoted by different alphabets and the bars reflect the triplicates means with standard error (±) based on Tukey's multiple range test. HA – humic acid extracted from compost produced by mixtures of separated municipal solid wastes (SMSW) and mixtures of IHM at a ratio of 9:1 weight : weight; HA–humic acid extracted at seven concentrations (0 gL^{-1}, 0.005gL^{-1}, 0.01gL^{-1}, 0.025 gL^{-1}, 0.05gL^{-1}, 0.1gL^{-1} and 0.5gL^{-1}); (CK) a control group with seedlings growing in no-Cd and no HA solution; (Ckcd: 0gL^{-1} HA-Cd) a Cd exposure group with seedlings growing in Cd and no HA (0 gL^{-1}) solution and (HA-Cd) a group with seedlings growing in Cd stress and various HA concentration.

treatment with Cd alone for both 0.025 gL^{-1} and 0.1 gL^{-1} of HA-Cd supply (Table 7b). However, when subjected to HA-Cd treatment at levels of 0.05 gL^{-1} and 0.5 gL^{-1}, it appeared that the magnesium content in maize shoots was restricted, with no significant difference observed in either of these treatments compared to the control. Consequently, Cd alone (0 gL^{-1}), as well as 0.005 gL^{-1} and 0.01 gL^{-1} of HA-Cd, inhibited the magnesium content in maize shoots, leading to a decrease of 11%, 15%, and 8%, respectively, compared to the control, with no significant difference ($p < 0.05$) observed among them (Table 7a). Analyzing the accumulated magnesium content, it is evident that plants treated with 0.025 gL^{-1} and 0.1 gL^{-1} HA-Cd accumulate higher amounts of magnesium in maize shoots under Cd stress than those treated with Cd alone, showing an increase of 36% and 38% (Table 7b).

Cadmium alone, as well as HA-Cd treatments, significantly inhibited the accumulation of sodium content in maize shoots, except for HA-Cd concentrations of 0.025 gL^{-1} and 0.5 gL^{-1}, which increased sodium accumulation by 20% and 48%, respectively, compared to the control (Table 7a). Additionally, Cd alone showed a more significant ($p < 0.001$) decrease in sodium absorption, at 44%, compared to the control (Table 7a). In contrast, treatments of 0.005 gL^{-1}, 0.01 gL^{-1}, 0.05 gL^{-1} and 0.1 gL^{-1} HA-Cd reduced sodium accumulation by varying degrees, ranging from 17% to 29% compared to the control (Table 7a). Furthermore, in comparison to Cd stress alone, the application of HA-Cd significantly increased the proportion of sodium content, with a notable increase observed in all treated plants, ranging from 1.28 to 2.65-fold (Table 7b). No statistically significant difference was observed in plants treated with 0.005 gL^{-1}, 0.01 gL^{-1}, and 0.1 gL^{-1} (p-value: 0.37), as well as in those treated with 0.05 gL^{-1} and 0.1 gL^{-1} (p-value: 0.25).

In the present study, both the HA enrichment pathway and Cd-induced stress revealed distinct response patterns in sodium accumulation in maize shoots across all treatments. Specifically, sodium accumulation in the HA-Cd treatment at 0.005 gL^{-1} was lower than that in the 0.05 gL^{-1} treatment and the control (Ck). Similarly, the 0.1 gL^{-1} treatment exhibited lower sodium accumulation compared to the 0.05 gL^{-1} and 0.01 gL^{-1} treatments, and, finally, the control had lower sodium accumulation compared to the 0.025 gL^{-1} and 0.5

gL^{-1} treatments (Figure 6). Furthermore, in this experiment, the treatment with Cd alone showed the lowest sodium accumulation, while the treatment with 0.5 gL^{-1} HA-Cd demonstrated the highest sodium accumulation (Figure 6).

In our study, we investigated the impact of Cadmium and HA-Cd treatments on the accumulation of sulfur (S) and iron (Fe) in maize shoots. Our findings revealed significant variations in the response patterns across different treatments. Cadmium alone, along with HA-Cd treatments, significantly inhibited the accumulation of sulfur and iron in maize shoots, except at HA-Cd concentrations of 0.025 gL^{-1} (Figure 6). At this level, we observed a remarkable increase in S and Fe accumulation by 24% and 48%, respectively, compared to the control group (Table 7a). Cd alone exhibited a more significant decrease in sulfur and iron absorption, at 16% and 43% respectively, compared to the control group ($p < 0.001$) (Table 7a). For HA-Cd treatments at 0.005 gL^{-1}, 0.01 gL^{-1}, 0.05 gL^{-1}, 0.1 gL^{-1} and 0.5 gL^{-1}, we observed varying degrees of reduction in S and Fe accumulation, ranging from 3% to 30% for S and 0% to 66% for Fe compared to the control (Table 7a).

Comparing treatments to Cd stress alone, the application of HA-Cd significantly increased the proportion of S and Fe content in all treated plants. The increase ranged from 1.03 to 1.48-fold for S and 1.12 to 2.65-fold for Fe (Table 7b).

No statistically significant difference ($p < 0.05$) in sulfur content was observed among plants treated with Cd only, 0.1 gL^{-1}, and 0.05 gL^{-1}, as well as among those treated with 0.05 gL^{-1}, 0.01 gL^{-1}, and the control (Ck). No statistically significant difference was observed in Ck and 0.5 gL^{-1} (p-value: 0.08) for S.

Similarly, for iron content, no statistically significant difference ($p < 0.05$) was observed for treatments at 0.05 gL^{-1}, Cd only, and 10 gL^{-1}. Additionally, there was no statistically significant difference in Fe content among plants treated with 0.1 gL^{-1}, 0.5 gL^{-1}, 0.01 gL^{-1}, Ck, and 0.05 gL^{-1}. No statistically significant difference was observed for Fe in plants treated with Cd only, 0.1 gL^{-1}, 0.5 gL^{-1} and 0.01 gL^{-1}. Furthermore, no statistically significant difference was observed for Fe content among treatments at 0.5 gL^{-1}, 0.01 gL^{-1}, Ck and 0.05 gL^{-1}.

In our study, both the HA enrichment pathway and Cd-induced stress led to distinct response patterns in sulfur and iron accumulation in maize shoots across all treatments.

Sulfur accumulation in the HA-Cd treatment at 0.005 gL^{-1} was lower than that in the Cd-only treatment, 0.1 gL^{-1} and 0.05 gL^{-1} treatments (Table 7b). Similarly, The Cd-only and 0.1 gL^{-1}

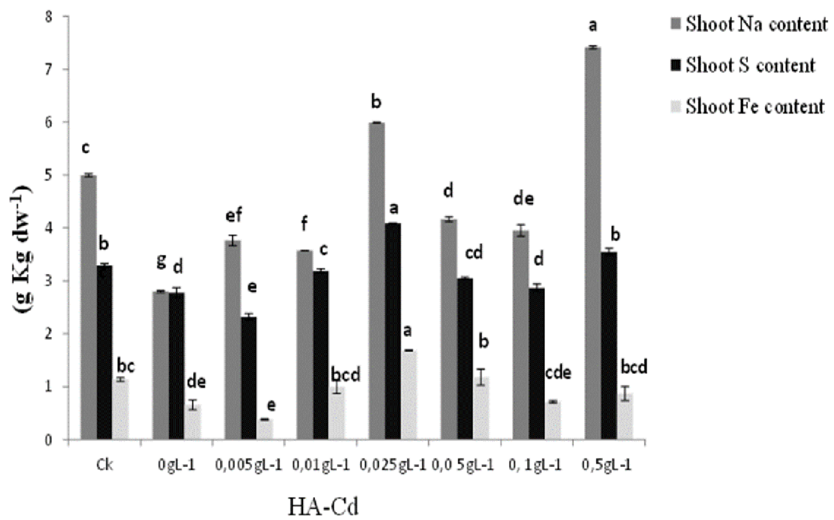

Figure 6. Effect humic acid on elemental mineral shoot content (Na, S and Fe) of maize grown under Cd^{2+} (2 mgL^{-1}) stress

Significance among different treatments at $p \leq 0.05$ levels are denoted by different alphabets and the bars reflect the triplicates means with standard error (±) based on Tukey's multiple range test. HA – humic acid extracted from compost produced by mixtures of SMSW and mixtures of IHM at a ratio of 9:1 weight : weight; HA extracted at seven concentrations (0 gL^{-1}, 0.005 gL^{-1}, 0.01 gL^{-1}, 0.025 gL^{-1}, 0.05 gL^{-1}, 0.1 gL^{-1} and 0.5 gL^{-1}); (CK) a control group with seedlings growing in no-Cd and no HA solution; (Ckcd: 0 gL^{-1} HA-Cd) a Cd exposure group with seedlings growing in Cd and no HA (0 gL^{-1}) solution and (HA-Cd) a group with seedlings growing in Cd stress and various HA concentration.

treatments exhibited lower Fe accumulation compared to the 0.01 gL^{-1} and control treatments, with the 0.05 gL^{-1} and 0.01 gL^{-1} treatments being lower than 0.5 gL^{-1} (Figure 6). The control and 0.5 gL^{-1} treatments had lower Fe accumulation compared to the 0.025 gL^{-1} treatments (Figure 6).

In addition, sulfur shoots accumulation in the HA-Cd treatment at 0.005 gL^{-1} was lower than that in the 0.5 gL^{-1} and 0.01 gL^{-1} treatments. The Cd-only treatment showed lower sulfur accumulation compared to the control treatment, with the 0.1 gL^{-1} treatment being lower than 0.05 gL^{-1}. The 0.5 gL^{-1}, 0.01 gL^{-1}, control, and 0.05 gL^{-1} treatments had lower S accumulation compared to the 0.025 gL^{-1} treatments. Notably, in this experiment, the treatment with 0.005 gL^{-1} HA-Cd showed the lowest S and Fe accumulation, while the treatment with 0.025 gL^{-1} HA-Cd demonstrated the highest sulfur and iron accumulation (Figure 6).

DISCUSSION

The compost used in this study shows favorable characteristics for humic acid extraction, particularly due to its richness in organic matter (Table 1). The slightly alkaline pH and moderately high EC indicate a well-stabilized compost with potential agronomic value. The significant mineral content could influence the extraction yield and the final composition of the humic acids (Kumada, 1988).

Cadmium stress presents a complex environmental challenge that poses a significant threat to plant growth and crop production. HA, a crucial component of humic substances, has been shown to exert a positive impact on plant growth (Aylaj et al., 2023).

In the current study, cadmium stress significantly and adversely affected the GI and GR of maize seeds. Notably, radicle length (RL) remained unaffected by Cd exposure. Interestingly, the behavior of GR in maize seeds differed from that of RL and GI. GR was more sensitive to the toxic effects of cadmium, exhibiting a substantial decrease of 32% when compared to the control. In contrast, RL showed no significant effect under Cd stress treatment alone. This decline in GR due to decreased water uptake by seeds (Kaya et al., 2021).

This result is in line with a study on seed germination under environmental stress conducted by Aylaj and Adani in 2023. In their findings, the authors exposed sugar beet and tomato seeds to varying levels of toxicity using concentrated immature compost extracts. They observed that the toxic components present in the compost extract had a less pronounced impact on the root length of tomatoes compared to the germination rate. Furthermore, tomato seeds exhibited greater radicle length when compared to sugar beet species, suggesting an adaptation of root length in tolerant species under environmental stress.

Similarly, Yang et al., (2023) confirmed that Cd supply had no effect and even increased the root length of maize. Furthermore, the findings published by Vatehova et al., in 2016, focusing on maize hybrids, confirmed that tolerant varieties (Almansa) developed longer roots under Cd stress, even when subjected to the same level of cadmium accumulation in the roots, compared to sensitive varieties (Novania). These results suggest that maize plants may undergo a transition from growth adaptation to a toxic response under moderate cadmium stress. The capacity of seeds to germinate when exposed to Cd can be elucidated through three hypotheses that are not mutually exclusive: differences in the sensitivity of essential hydrolytic enzymes during germination to Cd; the proportionate role of cell division and elongation in organ growth; and the existence of protective mechanisms within seeds to counteract various stress factors (Jaouania et al., 2018; Li et al., 2005).

However, the application of humic acid (HA) derived from compost, which was produced by composting a mixture of separated municipal solid wast and immature horse manure at specific ratios, notably improved the germination of maize seeds. This improvement was evident in terms of increased GR, enhanced GI, and stimulated RL at 7 days after sowing (d.a.s) under Cd stress conditions. This effect was particularly pronounced in maize seedlings treated with 0.05 gL^{-1} and 0.1 gL^{-1} HA-Cd. In the 0.05 gL^{-1} treatment, the germination index exhibited a significant 23% increase, while in the 0.1 gL^{-1} HA-Cd treatment, root length showed a significant 34% increase compared to the control group. Compared to the control, HA improved the germination rate of maize seeds under moderate Cd stress, reaching the value of Ck at concentrations ranging from 0.01 gL^{-1} to 0.05 gL^{-1} HA-Cd, although it was significantly inhibited by Cd toxicity. However, the concentration of 0.005 gL^{-1} HA-Cd was not sufficient to enhance GR, and at concentrations of 0.1 gL^{-1} and 0.5 gL^{-1}, GR values were further inhibited. Moreover, the 0.05 gL^{-1} HA-Cd treatment notably enhanced the germination rate of maize seeds, showing a remarkable 56% increase compared to Cd-stressed seeds without HA. Our result is consistent with a experience on alkali-stressed maize germination under sufficient HA supply (Yang et al., 2023). In their findings, the authors confirmed that the germination rate tended to rise between 0.05 and 0.5 mgL^{-1} as the HA concentration increased and then decrease with further increase.

Our results strongly suggest that optimal concentrations of humic acid successfully relieve the detrimental impacts of cadmium exposure on maize seed germination. HA has the potential to enhance cell membrane permeability, leading to increased nutrient and water absorption, improved root uptake capacity for nutrients and water, and ultimately, enhanced seed germination and root length. The Cd^{2+} concentration of 2 mgL^{-1} chosen in the present experiment showed that it is the lower and not lethal concentration. Indeed, it didn't exhibit an unambiguous impact on maize seedlings compared to the control group.

Both Cd and AH, extracted and supplied to the plants at different doses, did not yield any significant change in biomass in terms of leaf area, shoot fresh weight, and shoot length at 15 d.a.s.

The biphasic response to cadmium in the environment is commonly defined by a beneficial or stimulatory effect at low doses, succeeded by an inhibitory or toxic effect at high doses. Plants cultivated in environments contaminated with Cd or other heavy metals commonly exhibit this pattern (Li et al., 2021; An et al., 2022). Growth media containing trace or low concentrations of heavy metals typically do not exhibit toxicity to plants; instead, they may even promote plant growth (Li et al., 2021; An et al., 2022). For instance, the growth, including the biomass of leaves, and roots, of rice plants was observed to increase when grown in nutrient solutions supplemented with 0.01–0.005 mL^{-1} Cd (Zhang et al., 2007).

We observed a net decrease in root length 20%, 24%, 23%, and 10% (compared to the control) at 15 (d.a.s) for treatments with 0 gL^{-1} HA-Cd, 0.05 gL^{-1} HA-Cd, 0.1 gL^{-1} HA-Cd, and 0.5 gL^{-1} HA-Cd, respectively (Table 6a). Interestingly, an increase of approximately 11% and 28% in root length was also noted for treatments with HA-Cd concentrations of 0.05 gL^{-1} and 0.1 gL^{-1}, respectively.

Root growth also reflects root activity and has a direct impact on plant stress tolerance (Liu et al., 2019b). Aylaj et al., (2023) also observed that in a stressful environment, the application of humic acid at levels of 0.1 gL^{-1} and 1 gL^{-1}, provided by co-compost, enhanced the root length of turnip plants when grown in sandy, nutrient-poor soil. The presence of Cd or other heavy metals in the growth medium has a significant impact on root length compared to the shoot. This is primarily because roots are directly subjected to the stress caused by cadmium (Krantev et al., 2008).

In light of the conclusions drawn above, concentrations of 0.05 and 0.1 mgL^{-1} HA-Cd appear to be suitable for effectively mitigating the adverse impact of the cadmium-laden environment on the germination and early growth of maize seedlings, in contrast to the Cd-only treatment, while higher concentration treatments led to a decline (Figure 1).

A comparable trend was also showed in the Chl a and Chl b content in leaves of maize seedlings. Cd stress significantly inhibited the Chl a and b content of plants after 15 days, as evidenced by a decrease of 30% and 26%, respectively, when compared to the controls without Cd supplementation (Table 6a).

Comparable findings were reported by Shah et al., (2023), indicating that Cd-induced stress in maize has a detrimental impact on chlorophyll synthesis. This decline in chlorophyll content occurs because Cd enters the cell, disrupting the Calvin cycle (Hussain et al., 2013), which results in the degradation of photosynthetic pigments and damage to the photosynthetic capabilities of leaves (Rehman et al., 2019 ; Wang et al., 2021). Consequently, this tends to a decrease in chlorophyll concentration in the leaves (ALKahtani et al., 2020 ; Zhang et al., 2020).

The application of HA had a positive impact on chlorophyll content (both a and b) in leaves compared to the treatment with Cd alone. Notably, the highest values of Chl a and Chl b content were observed with 0.1 gL^{-1} HA-Cd and 0.5 gL^{-1} HA-Cd treatments, with the most pronounced increase (6 and 4.14 µg gfw^{-1}, respectivly) noted at the 0.1 gL^{-1} HA-Cd dose**.** These treatments resulted in significant increases of 36% in Chl a and 29% in Chl b for the former, and 27% in Chl a and 19% in Chl b for the latter, in contrast to seedlings that were only exposed to Cd (0 gL^{-1} HA-Cd), as shown in the Table 6b. The lowest observed levels of chlorophyll a and b content were noted at humic acid concentrations of 0.025 gL^{-1} and 0.05 gL^{-1} (Figure 3). As previously reported, similar protective effects against adverse stress have been extensively documented in *Zea mays* (maize) when humic acid is applied, as observed in a study by Yang et al., (2023). HA plays a crucial role in enhancing plant responses to chlorophyll synthesis, involving various elements, including the presence of phytohormones, aromatic compounds, alterations in membrane potentialb and, dissociation of organic acids, as highlighted by Souza et al., (2022). Due to its structural complexity, HA may contain numerous physiologically active substances that can influence chlorophyll production, as suggested by Muscolo et al., (2013).

Our findings revealed higher Cd levels in maize plants stressed with cadmium compared to the control group. Notably, a significant increase in Cd concentration was noted in the shoots after the application of HA-Cd at concentrations of 0.05 gL^{-1} and 0.1 gL^{-1}, with the most substantial increase (8.34 mg Kg^{-1}dw) occurring at the 0.1 gL^{-1} HA dose (Figure 4). Specifically, the shoot Cd^{2+} contents were 53% higher at 0.05 gL^{-1} and 71% higher at 0.1 gL^{-1} of HA-Cd compared to the Cd-only treatment (Table 7b).

Metal accumulation data reveals that *Zea mays*, when cultivated in soil containing cadmium at concentrations between 20 to 25 mg/kg, accumulates over 50% of this cadmium content in its shoot. This observation is in line with research findings conducted by Nawaz et al., (2020) and Shah et al., (2023). Multiple studies have reported that maize plants exhibit a greater capacity for cadmium absorption and translocation to aboveground portions compared to other cereal crops (Khan and Bano, 2016). Furthermore, this data highlights that the highest cadmium concentration is typically found in the stem, followed by the leaf, and lastly, the seed, aligning with observations made by Sharma and Agrawal (2006).

Previous research has also confirmed that higher levels of humic acid lead to increased cadmium accumulation in shoot tissues (Rashid et al., 2022; Huang et al., 2023). The existence of functional groups, particularly carboxylic and hydroxyl groups, in HA serves as crucial binding sites for metals. This structural characteristic facilitates the formation of cadmium organic acid complexes with five or six membered ring structures, there by promoting the mobility of Cd in both the growth medium and within plants, as demonstrated by Fei et al., (2004). These findings align with prior research, which supports the idea that increased nitrogen fertilization (An et al., 2022), higher organic matter content (Ma et al., 2020), or elevated levels of humic acid (Rashid et al., 2022 ; Huang et al., 2023) result in enhanced Cd mobility in both the growth medium and within the plant.

The alleviation of Cd toxicity in shoots and roots due to the supply of HA can be attributed to several factors. These factors include the immobilization of cadmium on the root surface, the presence of Cd within the symplasm of root cells, and its encapsulation by phytochelatins within the vacuoles of these cells (Huang et al., 2019).

In current study, we have obtained results consistent with other research findings, demonstrating the active impact of humic acid on increasing Cd concentration as its dosage increases (Rashid et al., 2022; Huang et al., 2023). It's worth noting that heavy metal availability tends to rise as the pH of the growth medium decreases, as previously observed (Wang et al., 2017b).

The substantial improvement in the uptake and transport of cadmium in roots due to the application of HA can be attributed to various factors. These factors include the transpiration stream in the xylem, the dilution effect as aboveground plant parts grow, and the activation of genes responsible for metal transporters in the roots (Ma et al., 2021b). This genetic activation leads to an increased expression of relevant genes, ultimately enhancing Cd uptake and accumulation, as documented in studies by Lin et al., (2011) and Yang et al., (2020).

Previous research have consistently shown that free Cd^{2+} ions are more detrimental to crops than Cd-organic acid complexes (Wu et al., 2003). Consequently, the formation of metal chelates by organic acids and Cd not only enhances Cd mobility within plants but also mitigates its toxicity to them.

A study has confirmed a significant decrease in the bioavailability of Cd when the tolerant wheat cultivar NARC-2011 was grown in Cd-contaminated soils irrigated with sewage. This decrease in bioavailability was linked with a reduction in the proportion of mobile Cd fractions, specifically the exchangeable and reducible fractions, and an increase in the immobile Cd fractions, which included the oxidizable and residual forms (Rashid et al., 2022). In our study, it was noted that as the humic acid level increased, calcium absorption in maize shoots also increased, showing enhancements ranging from 10% to 50% when compared to treatments with Cd alone (Table 7b). The most significant enhancement was recorded at a concentration of 0.5 gL^{-1} HA-Cd, while the lowest calcium concentration was noted in treatments with cadmium application alone (0 gL^{-1} of HA-Cd) (Figure 5).

One funding conducted by An et al., (2022) confirmed a positive correlation between root cadmium content and root calcium content, suggesting that Cd^{2+} enters root surface guard cells through calcium channels.

The introduction of cadmium into the maize seedling medium resulted in a reduction in magnesium absorption (Figure 5). However, the addition of HA improved magnesium absorption, with significant effects observed starting from a concentration of 0.05 gL^{-1}. Notably, magnesium absorption increased by an average of 22% when compared to the control and 37% when compared to the treatment with cadmium alone, for both the 0.025 gL^{-1} and 0.1 gL^{-1} HA-Cd supply (Table 7a and 7b).

Conversely, cadmium alone exhibited a more significant decrease in sulfur and iron absorption, at 16% and 43%, respectively, compared to the control group ($p < 0.001$) (Table 7a).

When comparing treatments to Cd stress alone, the application of HA-Cd significantly increased the proportion of sulfur and iron content in all treated plants. The increase ranged from 1.03 to 1.48-fold for S and 1.12 to 2.65-fold for Fe (Table 7b).

In situations where transition metals are highly concentrated in the extracellular environment, passive absorption processes with low specificity may transport unwanted ions, such as Cd^{2+}, which could potentially compete with essential cations like Zn^{2+}, Cu^{2+}, Fe^{2+}, or Ca^{2+} (Fox and Guerinot 1998 ; Perfus-Barbeoch et al., 2002 ; Clemens 2006).

Both cadmium alone and the HA-Cd treatments had a significant inhibitory effect on the accumulation of sodium content in maize shoots. However, it is noteworthy that at HA-Cd levels of 0.025 gL^{-1} and 0.5 gL^{-1}, there was an increase in sodium accumulation by 20% and 48%, respectively, compared to the control (Table 7a). This minor portion of the accumulated sodium in shoots is attributable to the sequestration of Na by phytochelatins in the vacuoles of root cells, there by limiting the translocation of sodium from roots to shoots.

To summarize, our appropriate application of humic acid derived from compost, produced by composting a mixture of immature horse manure and separated municipal solid wast at specific ratios, at concentrations of 0.5 gL^{-1} and 0.1gL^{-1} HA-Cd, magnified the dilution effect in maize seedlings. This amplification resulted in improved growth indices (GI and RL), increased chlorophyll, calcium, magnesium, sulfur, iron contents, and a decrease in sodium level.

The impact of HA on root development was assessed by immersing seeds in an HA solution for germination experiments. Importantly, the liquid medium did not contain any additional nutrients, and the growth of seedlings relied solely on the nutrients stored within the seeds. Therefore, the noticed variations in the morphology of seedlings growth of maize plants in response to the application of HA can be ascribed directly to differences in HA concentrations. Our study has revealed that adequate HA application can enhance maize plant tolerance to its surrounding environment.

CONCLUSIONS

Our study demonstrated that cadmium toxicity negatively impacted the germination and seedling growth of maize plants at a concentration of 2 mgL^{-1} Cd^{2+} in a controlled experimental setup. Cd stress significantly reduced chlorophyll a and b content in leaves after 15 days, showing a 30% and 26% decrease, respectively, compared to controls without Cd supplementation. When compared to the control group, stressed maize plants strssed with cadmium showed higher levels of the metal.

The introduction of cadmium into the growth medium hindered magnesium absorption and significantly impeded the accumulation of sodium and sulfur in maize shoots.

We suggest that humic acid can be a promising basis for promoting subsequent growth by preserving germination and seedling development in the presence of cadmium induced stress conditions. Notably, applying HA-Cd at levels of 0.05 gL^{-1} and 0.1 gL^{-1} led to a significant increase in cadmium concentration in shoots, with the most substantial increase (8.34 mg Kg^{-1} dw) occurring at the 0.1 gL^{-1} HA dose. Specifically, shoot Cd^{2+} contents were 53% higher at 0.05 gL^{-1} and 71% higher at 0.1 gL^{-1} of HA-Cd compared to Cd-only treatment.

In comparison to Cd treatment alone, the germination rate was significantly stimulated, showing a remarkable 56% increase with 0.05 gL^{-1} HA-Cd. Treatment with 0.05 gL^{-1} and 0.1 gL^{-1} HA-Cd had a significant positive effect ($p < 0.05$) on root length compared to the control, enhancing root length by 17% and 34%, respectively. Conversely, treatments with 0.5 gL^{-1} HA-Cd inhibited root length and the germination index by 29% and 32%, respectively, compared to the control.

Our findings show that the combined application of HA and cadmium stress actively influences the increase in Cd concentration as its dosage increases. The appropriate application of HA made from compost, produced by composting a mixture of immature horse manure and separated municipal solid wast at specific ratios, at concentrations of 0.05 gL^{-1} and 0.1 gL^{-1} HA-Cd, amplified the dilution effect in maize seedlings. The humic acid dose that promoted growth correlated with increased chlorophyll, calcium, magnesium, and iron contents in shoots, while reducing sodium and sulfur levels. Conversely, the dose that inhibited maize growth exhibited the opposite effect, diminishing chlorophyll, calcium, magnesium, and iron contents, while elevating sodium and sulfur levels. Our findings offer significant recommendations for the utilization of humic acid in agricultural practices when faced with cadmium stress, aiming to enhance plant productivity.

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