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Humates mitigate Cd uptake in the absence of NaCl salinity, but combined application of humates and NaCl enhances Cd mobility & phyto-accumulation

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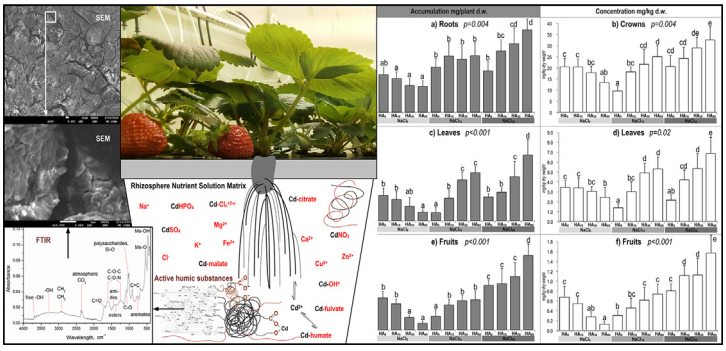
HIGHLIGHTS

- Humic acid (HA) and NaCl reduce a proportion of the most toxic Cd^{2+} form by multifold.
- NaCl increases a proportion of Cl-Cd ionic forms, increasing Cd uptake by strawberry.
- In the absence of NaCl, HA increases a proportion of HA-Cd forms, reducing Cd uptake.
- Combined application of HA and NaCl enhances Cd mobility and uptake by strawberry.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium is among the critical pollutants easily taken up from contaminated media by plants, which can be exploited in the phytoremediation of Cd-contaminated resources, but is also an obstacle in producing food with low Cd content. Crucial variables governing Cd biogeochemistry are complex humates (HA) and chlorides, but the underlying interactions are poorly understood. The aim was to determine the impacts of HA (0–60 mg/L) and NaCl (0–30 mM) on Cd biochemistry in contaminated (2.0 μM Cd) rhizosphere solution and Cd accumulation in various tissues of strawberry (*Fragaria x ananassa*). The results show that salinity (vs. non-saline $NaCl_0$ control) suppressed vegetative and yield parameters, but increased dry matter and Na, Cl and Cd concentration/accumulation in most of the analysed tissues. The HA application in the $NaCl_0$ treatment decreased tissue Cd content; however, at the highest application rates of NaCl and HA, there were increases in the tissue Cd concentration (by 70 %, 100 % and 120 % in crowns, leaves and fruits, respectively) and accumulation (by 110 %, 126 % and 148 % in roots, fruits and leaves, respectively) in comparison to the control ($NaCl_0HA_0$). Tissue Cd concentration/accumulation decreased in the order: roots>crowns>leaves>fruits; the same accumulation pattern was noted for Na and Cl, suggesting that Cd-Cl complexes may represent a major form of Cd taken up. Chemical speciation calculations revealed that the proportions of various Cd forms varied multi-fold across the treatments; in the control (without NaCl and HA), Cd^{2+} dominated (86 %), followed by

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$CdHPO_4$ (6.5 %), $CdSO_4$ (6.2 %) and $CdNO_3^+$. In other treatments the proportion of Cd^{2+} decreased with a corresponding increase of Cd-Cl (from 0.02 % in control to 57 % in Cd + $NaCl_{30}$ treatment) and Cd-HA (from 0 % in control to 44 % in Cd + HA_{60} treatment), which was associated with higher Cd phytoaccumulation. The results represent a theoretical basis for phytoremediation studies and for producing low-Cd food in relatively complex matrices (contaminated soils, reused effluents); in the absence of salinity, amelioration with humates has a great potential to mitigate Cd contamination.

1. Introduction

Environmental resources required for quality food production are becoming restricted due to increased salinity (Murtaza et al., 2019) and metal pollution (Rashid et al., 2020). Both environmental constrains have become exacerbated in the recent decades. Under global climate change, the hotter and drier climate in many areas, accompanied by unsustainable water management (e.g. use of low-quality water resources for irrigation) in (semi)arid agroecosystems, has increased soil salinization (Ondrasek and Rengel, 2021). It is expected that salt-affected areas in agroecosystems will increase over time as land use intensity (El-Kady and Borham, 2020) and water scarcity increase (Ondrasek, 2014). In addition to water scarcity, unsustainable waste management (discharge of municipal and/or industrial wastewaters and effluents) may pollute the environment with different salts (e.g. nitrates, phosphates) as well as toxic metals. Thus, irrigated croplands in (semi)arid areas are most at risk of salinization and metal contamination (Azadi and Raiesi, 2021; Zahedifar and Moosavi, 2020). Among toxic metals, cadmium (Cd) can easily enter the human diet through the soil-plant system, with various chronic and carcinogenic implications in humans (Shi et al., 2021). Due to its high mobility and toxicity, Cd is among the most hazardous substances of concern for human and environmental health.

Excessive salinity can impact negatively on soil physicochemical properties (Romić et al., 2020), diminish soil microbial activity and diversity (Azadi and Raiesi, 2021), compromise water and nutrient uptake by plants, and reduce crop growth and yield quality (Saidimoradi et al., 2019). In addition, recently conducted studies report that salinity may significantly alter Cd biogeochemistry (Azadi and Raiesi, 2021; Lopez-Chuken et al., 2021). Such detrimental outcomes are still not understood fully, but can be related to enhanced Cd solubility and mobility via formation of uncharged or less charged salt-Cd complexes (Azadi and Raiesi, 2021) and/or competition among Cd^{2+} and other cations for adsorption onto the soil organo-mineral matrix (Zahedifar and Moosavi, 2020). From the standpoint of food production, a detrimental impact of combined salinity and Cd contamination might result in significant yield reductions as well as increased Cd accumulation in the edible tissues.

The biogeochemistry of Cd and the risk of its transfer to human diet strongly depend on many pedovariables, such as the total concentration and speciation of Cd, antagonistic elements and various ligands, and the pH and chemical interactions in the rhizosphere matrix (Ondrasek et al., 2022a). Thus, crop production in salt-affected and Cd-polluted agroecosystems is highly challenging and needs to be managed with respect to the specific environmental conditions.

Recently, increasing interest has been shown in different soil organic amendments to ameliorate Cd-polluted and salt-affected soils (Abbas et al., 2018; Azadi and Raiesi, 2021). Naturally occurring, complexed and recalcitrant organics, such as humics, were shown to be crucial for crop yield and quality (Bacilio et al., 2016; Haghighi and Silva, 2013; Ondrasek et al., 2018), nutrient uptake (Yildirim et al., 2021) as well as Cd biogeochemistry and dynamics in the soil-plant continuum under saline conditions. For instance, it was documented that the addition of humics reduced Cd uptake and toxicity in lettuce grown in contaminated nutrient solution (Haghighi et al., 2010) and in garden cress grown in contaminated soil (Yildirim et al., 2021). By contrast, combined application of humic acid (HA) and NaCl salinity synergistically enhanced Cd mobility in sandy soil and Cd accumulation in strawberry, heightening a health risk from Cd in human diet (Ondrasek et al., 2022a). Due to high physicochemical complexity and heterogeneity of humates (Piccolo, 2001), as well as the strong influence of environmental parameters (e.g. pH, temperature, concentration) that vary on a small scale, the relevant interactions of humates with Cd under saline conditions are yet to be elucidated and explained fully.

This study was aimed at characterizing i) complexation reactions and Cd biogeochemistry under low salinity (0–30 mM NaCl) and low HA concentration (0–60 mg/L) in Cd-contaminated (2.0 μM) rhizosphere solution with a strawberry as a salt-sensitive non-metalophyte, and ii) impacts on Cd phytoextraction, tissue distribution, and vegetative growth and yield. The tested treatments corresponded to the conditions encountered in irrigated agroecosystems with low-quality saline and/or Cd-contaminated wastewaters (e.g. Rashid et al., 2020), and could provide a theoretical basis for water management or phytoremediation in such cropping systems.

2. Material and methods

2.1. Environmental conditions and treatments applied

One uniformly selected Frigo A+ strawberry (*Fragaria x ananassa*, Duch. cv. Albion) transplant (Toolangi Strawberry CO-OP, Toolangi, Victoria, Australia) was placed in a hole cut in tight-fitting lid of PVC pot (5 L) filled with half-strength continuously aerated nutrient solution (Table 1). From day 21 after transplanting (DAT) to the end of trial (70 DAT), strawberry plants were exposed to the full-strength nutrient solution. On 30 DAT, the full-factorial combination of Cd contamination [2.0 μM, as cadmium nitrate tetrahydrate, Ajax Chemicals Ltd., Sydney, Australia], salinity (0, 10 and 30 mM NaCl, Ajax Chemicals Ltd.) and humic acid (0, 10, 30 and 60 mg HA/L, as sodium humate, Sigma-Aldrich, Saint Louis, MI, USA) was applied (Table 1). The HA was dissolved in 0.1 M NaOH; the pH of solution was adjusted to 6.0 by 0.1 M HNO_3 followed by filtering through a 0.45 μm membrane filter. Background concentrations of Cd (<20 μg/L) and Na (120 ± 9 mg/L) were recorded in the tested HA stock solution.

The ultrapure deionized water (18 mΩ cm^{-1}) obtained from a Milli-Q system (Millipore Corp, Milford, CT, USA) was used for the preparation of all stock and nutrient solutions. Nutrient solutions with applied treatments were changed every 3 days and were topped up with Milli-Q water in between solution changes to compensate for transpiration losses. All nutrient solutions were aerated continuously. The pH was maintained at 6.0 ± 0.1 by

Table 1
Composition of full-strength basal nutrient solution (Ondrasek et al., 2022a) and treatments applied.

Component	Basal nutrient solution	Treatments applied
pH	6.00	1. Cd (2.0 μM)
KNO_3	2.5 mM	2. Cd + HA_{10} (10 mg/L HA)
KH_2PO_4	0.5 mM	3. Cd + HA_{30} (30 mg/L HA)
$Ca(NO_3)_2$	2.5 mM	4. Cd + HA_{60} (60 mg/L HA)
$MgSO_4$	1.0 mM	5. Cd + $NaCl_{10}$ (10 mM NaCl)
$FeSO_4$	50 μM	6. Cd + $NaCl_{10}$ + HA_{10}
H_3BO_3	5.0 μM	7. Cd + $NaCl_{10}$ + HA_{30}
$MnCl_2$	3.0 μM	8. Cd + $NaCl_{10}$ + HA_{60}
$ZnSO_4$	0.60 μM	9. Cd + $NaCl_{30}$ (30 mM NaCl)
$CuSO_4$	0.50 μM	10. Cd + $NaCl_{30}$ + HA_{10}
$NiSO_4$	0.10 μM	11. Cd + $NaCl_{30}$ + HA_{30}
Na_2MoO_4	0.02 μM	12. Cd + $NaCl_{30}$ + HA_{60}
[a]MES	1.0 mM	

[a] 2-(N-morpholino)ethanesulfonic acid (pH buffer).

adding 0.1 M KOH or HNO_3 as necessary every 12 h. Plants were grown in a controlled-environment room at the University of Western Australia (31°59′S, 115°49′E; Perth, WA) with 12/12 night/day duration, temperature 17/22 °C, humidity 85/65 %, and 350 μmol m^{-2} s^{-1} photosynthetically active radiation.

The treatments were organised in completely randomized design in three replicates. The pots were randomized daily and the treatments lasted for 40 days.

2.2. Biomass sampling and preparation for chemical analyses

The fruits were harvested successively, from the appearance of first marketable fruits (40 DAT) until the end of study (70 DAT), washed with Milli-Q water, dried with paper towel and then frozen at −20 °C as a composite sample. To determine dry fruit yield and mineral composition, fruit samples were defrosted, homogenised, and 100 g was taken as an average subsample before analysis. On 70 DAT, leaves were separated by scissors, rinsed with Milli-Q water and blotted dry. The belowground tissues were rinsed with Milli-Q water, soaked in 5 mM $CaCl_2$ for 20 min, again rinsed with Milli-Q, separated into roots and crown, and weighed fresh and after drying (at 75 °C for 48 h).

Plant samples were homogenised in a stainless-steel grinder (Krups, Bochum, Germany) prior to chemical analyses. The powdered plant sample (~200 mg) was transferred to a 50 mL Erlenmeyer flask and first digested in 5 mL of concentrated HNO_3 at 100 °C for 45 min. The digested mixture was cooled, supplemented with 0.5 mL of concentrated $HClO_4$ and heated at 150 °C for 40 min. The mixture was cooled again and then heated to 180 °C for 10 min to allow dehydration of Si. Cooled digested solutions were transferred to 10 mL vials containing 5 μg of yttrium (an internal standard), and used for instrumental analysis of Cd and Na.

Chlorides (Cl) were extracted from plant samples using the hot water method (Ondrasek et al., 2020). Briefly, ~200 mg of strawberry tissue was put in a vial containing 20 mL of Milli-Q water and placed on a hot plate to bring the suspension to a near boil for 0.5 h. The vials were then shaken for 0.5 h, cooled to room temperature and filtered through a 0.45-μm membrane syringe filters for total Cl detection.

2.3. Chemical analyses and quality control

Total Cd and Na concentrations in biomass digests were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin-Elmer Optima 5300 DV, Waltham, MA, USA). Total Cl in water extracts was measured by colorimetry using an automated continuous flow autoanalyser (Skalar San + +, Skalar Analytical, Breda, Netherlands). Element accumulation was determined based on total concentration and dry weight of specific strawberry tissue. In all measurements, validation of analytical methods was based on quality control such as external calibration, determination of limits of detection and quantitation, use of three reagent blanks containing high-purity water, and standard reference plant material. Limit of detection – LOD (defined as the lowest concentration of a particular element in a sample that can be consistently detected with probability of 95 %; Eichbaum et al., 2014) was 20 μg/L for Cd, 100 μg/L for Na and 500 μg/L for Cl. Measured concentrations in the WEPAL (Wageningen Evaluation Programs for Analytical Laboratories) reference plant material IPE (International Plant-analytical Exchange) 130 were (mean ± SE): 440 ± 75 μg Cd/kg; 595 ± 69 mg Na/kg and 780 ± 140 mg Cl/kg. For samples with a particular elemental concentration below LOD, a half-LOD value was included in statistical analysis. In all reference plant material samples and internal standards, the measured concentrations were within declared values (less than ±5 %).

2.4. Chemical speciation modelling and data processing

Given the strong capacity of chloride and humates to complex Cd, and because different Cd chemical species exhibit differential biogeochemistry and have diverse plant availability, we performed chemical speciation modelling to help explain some relevant mechanisms controlling Cd biochemistry under tested conditions. The calculations of Cd chemical speciation in the nutrient solutions under the tested treatments were performed in the chemical equilibrium software Visual MINTEQ ver. 3.1 (Gustafsson et al., 2018) using the pH value and HA, NaCl and other element concentrations as listed in Table 1. Complexation of Cd with dissolved HA was assessed using the Non-Ideal Competitive Adsorption (NICA)-Donnan model (Kinniburgh et al., 1999; Ondrasek et al., 2018). Activity coefficients were calculated by the Davies equation (appropriate for the ionic strengths of <0.3–1 M as tested in this study). The ambient temperature and CO_2 partial pressure were fixed at 22 °C and 386 Pa, respectively (which corresponded to the tested conditions). All other variables were left as defaults.

The data on vegetative parameters and mineral composition of strawberry tissues were subjected to 2-way ANOVA with NaCl and HA as the main factors and their NaClxHA interaction. Homogeneity of variance was assessed by Levene's test at the 5 % level of significance. The significance of differences among the means was examined using the Tukey's HSD test at $p \leq 0.05$. Statistical analyses were performed in the software package SAS ver. 9.3 (SAS Institute Inc. Cary, NC, USA).

3. Results and discussion

3.1. Vegetative growth and yield under applied treatments

The ANOVA results for the data on vegetative growth and fresh fruit yield parameters of strawberry cv. Albion are presented in Table S1. There was only a significant negative impact of NaCl salinity on all measured parameters, whereas an application of HA as well as the NaClxHA interaction did not influence significantly any of the tested vegetative and yield parameters (Table S1). Compared with the non-saline ($NaCl_0$) control, 30 mM NaCl in the nutrient solution ($NaCl_{30}$) decreased total fresh fruit yield by 34 %, total fruit number by 23 %, number of runners (secondary stems) by 32 %, the length of the longest runner by 45 %, and the leaf area by 48 % (Table 2).

Such negative effects of salinity on plant growth (Table 2) are common in glycophytes, such as soybean (Sytar et al., 2017), faba bean (Benidire et al., 2020), rice (Ul Haq et al., 2014), strawberry (Ferreira et al., 2019; Saidimoradi et al., 2019) and many others (Ondrasek and Rengel, 2021). These salinity effects can be explained by: i) the severe structural disorganization of nucleus, chloroplast and mitochondria associated with Na^+ and/or Cl^- accumulation (Abbas et al., 2018), nutrient (e.g. K^+, Mg^{2+}, Ca^{2+}) imbalances (Saidimoradi et al., 2019), reduction in photosynthesis, and alterations in numerous other physiological functions (Ondrasek and Rengel, 2021). However, some other negative impacts under excessive salinity, such as leaf chlorosis or leaf-edge necrosis, leaf senescence and even plant mortality, were not observed in the tested strawberry cv. Albion (data not shown).

Table 2
Impact of salinity on fruit yield and vegetative growth parameters of strawberry cv. Albion 40 days after salinity treatment commencement. Means (standard errors in parenthesis; $n = 12$) with a different letter in a column are significantly different according to Tukey's HSD test ($p < 0.05$). The 2-way ANOVA showed the NaClxHA interaction to be non-significant. Hence, the data were averaged across the HA rates.

Salinity treatment	Fruit yield, g/plant	Fruit number/plant	Runner number/plant	The longest runner, cm	Leaf area, cm^2
$NaCl_0$	105(9.1)a	7.5(0.4)a	3.1(0.3)a	36.4(2.9)a	182(9.1)a
$NaCl_{10}$	98(8.2)a	6.9(0.4)a	2.9(0.3)a	30.1(3.2)b	155(7.5)b
$NaCl_{30}$	69(5.1)b	5.8(0.3)b	2.1(0.2)b	20.1(2.6)c	94(6.0)c

Even though strawberry is one of the most salt-sensitive glycophyte crops (e.g. yield decreased by 33 % for every 1 mS/cm increase in salinity beyond the salinity threshold of ~1 mS/cm) (Ferreira et al., 2019), numerous studies have confirmed significant differences among strawberry genotypes under salt exposure. For instance, some recent studies also reported that cv. Albion (as a relatively new cultivar released in 2004) is one of the most salt-tolerant strawberry varieties regarding growth and fruit yield, commercial fruit size, plant survival, total soluble sugars in fruits, and Na^+ exclusion from leaves under salinity stress (Ferreira et al., 2019; Sun et al., 2015), and thus should be exploited as a valuable germplasm in breeding approaches for creating genotypes with increased salt tolerance.

3.2. Dry matter and elemental concentration and content in strawberry tissues

The summary of ANOVA for the data on dry matter content, and concentration and accumulation of Na, Cl and Cd in tested strawberry tissues is presented in Table S2. The NaClxHA interaction was non-significant, and the HA rates did not significantly alter dry matter amount in any of the analysed strawberry organs. Similar results regarding the HA effects on plant biomass have been recorded in nutrient solution studies with barley (Cabrera et al., 1988), ryegrass (Kalis et al., 2006) and radish (Ondrasek et al., 2018), and in soil experiments with tobacco (Evangelou et al., 2004; Yu et al., 2017).

Application of NaCl significantly increased dry matter content of all strawberry parts, as well as Na, Cl and Cd concentration and accumulation in most of the analysed tissues (Table S2). Increased dry matter content under NaCl treatment has been reported in strawberry cultivars, including Albion (Sánchez-González et al., 2017), as a consequence of osmotic stress (reduced water content) increasing relative concentrations of sugars, organic acid anions and other compounds (Awang et al., 1993).

As expected, NaCl salinity significantly enhanced both concentration and content of Na and Cl in all analysed tissues. Compared with the non-saline control, concentrations of Na and Cl increased in roots by 5.6- and 6-fold, in crowns by 13- and 14-fold, in fruits by 19- and 24-fold and in leaves by 5.2- and 4.8-fold, respectively (Table 3). Similar magnitudes of increase were also recorded in tissue contents of Na and Cl (Table 3). The Na and Cl concentrations in tested tissues followed the order roots>crowns>fruits>leaves, whereas the content of both elements was in the order crowns>roots>leaves>fruits (Table 3). Similar results recently have been reported for cv. Albion and other commercial strawberry cultivars in the field (Ferreira et al., 2019) and greenhouse conditions (Ondrasek et al., 2022a; Saidimoradi et al., 2019; Sun et al., 2015).

Table 3
Impact of salinity on dry mater (DM) and concentration and content of Na and Cl in tested tissues of strawberry cv. Albion 40 days after treatment commencement. Means (standard errors in parenthesis; $n = 12$) with a different letter in a row are significantly different according to Tukey's HSD test ($p < 0.05$). The 2-way ANOVA showed the NaClxHA interaction to be non-significant. Hence, the data were averaged across the HA rates.

Parameter	Tissue	Treatment		
		$NaCl_0$	$NaCl_{10}$	$NaCl_{30}$
DMg/plant	Leaves	3.43(0.2)a	5.50(0.4)b	6.05(0.4)b
	Fruits	1.10(0.01)a	1.05(0.01)b	1.10(0.01)a
	Crowns	6.30(0.3)a	6.77(0.5)a	6.55(0.5)a
	Roots	2.50(0.1)a	3.00(0.2)a	3.70(0.2)b
Elemental concentration in tissues (g/kg)				
Na	Leaves	0.04(0.0)a	0.10(0.0)b	0.21(0.0)c
	Fruits	0.03(0.0)a	0.17b(0.0)b	0.58(0.0)c
	Crowns	0.29 (0.0)a	2.28(0.01)b	3.90(0.01)c
	Roots	0.79(0.0)a	2.50(0.1)b	4.45(0.4)c
Cl	Leaves	0.09(0.0)a	0.21(0.0)b	0.44(0.0)c
	Fruits	0.05(0.0)a	0.28(0.0)b	1.2(0.01)c
	Crowns	0.55(0.01)a	5.0(0.3)b	7.7(0.5)c
	Roots	1.5(0.01)a	5.7(0.6)b	9.1(1.0)c
Elemental content in tissues (mg/plant)				
Na	Leaves	0.14(0.0)a	0.70(0.0)b	1.10(0.01)c
	Fruits	0.10(0.0)a	0.45(0.0)b	0.95(0.01)c
	Crowns	1.84(0.01)a	14.8(1.2)b	25.3(3.2)c
	Roots	2.03(0.01)a	7.50(0.4)b	15.9(2.1)c
Cl	Leaves	0.3(0.0)a	1.2(0.0)b	2.0(0.0)c
	Fruits	0.2(0.0)a	0.9(0.0)b	1.8(0.0)c
	Crowns	4.0(0.01)a	30.2(4.1)b	55(6.2)c
	Roots	4.5(0.2)a	14.2(1.4)b	37(4.4)c

Albion has been confirmed (Ferreira et al., 2019) as a strawberry cultivar with the highest survival rate (94 %), with no significant disturbance in total plant dry weight under salinity treatment (~25 mM NaCl), and with limited leaf Na^+ (but not Cl^-) accumulation in every tissue. Some of key features underlying salinity tolerance of cv. Albion are likely related to uptake rate, transport, accumulation and (re)distribution of Na^+ and Cl^- ions among plant tissues.

Both Cl^- and Na^+ have a relatively low concentration threshold above which they induce Na/Cl toxicity, impair nutrient uptake (e.g. K^+, NO_3^-) and cause numerous other physiological imbalances associated with salt stress (Ondrasek et al., 2022b). One of plant adaptive responses to excessive salinity is salt exclusion from shoots (i.e. root accumulation), thus maintaining low concentration of Na^+/Cl^- in leaves and other shoot tissues. Importantly, it was shown that the addition of HA to soil (Ali et al., 2020; Kaya et al., 2018) or nutrient solution (Jarošová et al., 2016; Saidimoradi et al., 2019) can mitigate salt stress in crops. For instance, application of HA to nutrient solution lowered concentration of Na^+ but increased that of K^+ in leaf and root tissues of two strawberry cultivars regardless of the presence or absence of salinity stress (Saidimoradi et al., 2019). However, under conditions tested in the present study, HA application did not limit accumulation of Na^+ and Cl^- in shoots and fruits (Table 3; Table S2).

3.3. Cadmium accumulation and distribution in the strawberry tissues

The interaction of salinity and HA (NaClxHA) influenced tissue Cd concentration (in crowns, leaves and fruits) and accumulation (in roots, leaves and fruits) (Table S2). In non-saline treatments ($NaCl_0$), addition of HA to rhizosphere solution mostly reduced tissue Cd accumulation (Fig. 1a, c, e) and concentration (Fig. 1b, d, f,). However, under increased NaCl and HA rates, Cd concentration was increased by 70 % in crowns (Fig. 1b), 1-fold in leaves (Fig. 1d) and 1.2-fold in fruits (Fig. 1f), whereas Cd content was elevated by 1.1-fold in roots (Fig. 1a), 1.48-fold in leaves (Fig. 1c) and 1.26-fold in fruits (Fig. 1e) in comparison to the control ($NaCl_0HA_0$). In general, tissue Cd concentration and accumulation increased in this order: fruits<leaves<crowns<roots, following the same accumulation/concentration pattern as in case of Na and Cl (Table 2). In general, such results could compromise food quality, i.e. suggesting that Cd-Cl complexes may represent a major form of Cd taken up; hence, there may be an increased risk of Cd exposure from food grown in substrates with elevated Cl concentration (discussed later).

Similar observations of increased Cd uptake with the application of HA and NaCl (individually or in combination) have been reported in various plant species (Park et al., 2013), including strawberry (Ondrasek et al., 2022a). For instance, in radish grown in Cd-contaminated nutrient solution, HA addition intensified transfer of Cd to shoots and, importantly, to the edible hypocotyl (Ondrasek et al., 2018). Similarly, in Cd-contaminated nutrient solution, Kalis et al. (2006) found that the addition of HA (30 mg/L) increased root uptake and content of Cd in perennial ryegrass. With soil amelioration by HA (2 % *w*/w), Evangelou et al. (2004) reported a 10-fold increase in Cd accumulation in tobacco shoots. Recently, Gao et al. (2022) recorded a 2.2-fold HA-mediated increase in Cd accumulation in *Sedum alfredii*. Tobacco grown in two Cd-contaminated soils showed a 14–46 % increase in Cd content in leaves after humics application (Yang et al., 2013). In strawberry cv. Albion grown in Cd-contaminated quartz-based media, significantly enhanced Cd concentration (up to 135-fold in fruits) was recorded in the combined HA and NaCl treatment (Ondrasek et al., 2022a). In contrast to these reports on exacerbated Cd uptake and accumulation in various tissues by HA application, the effects of HA on alleviating the Cd toxicity and reducing Cd uptake were demonstrated in hybrid *Pennisetum* (Song et al., 2020), garden cress (Yildirim et al., 2021), rice (Zhang et al., 2020), pakchoi (Li et al., 2021) and wheat (Zhou et al., 2020).

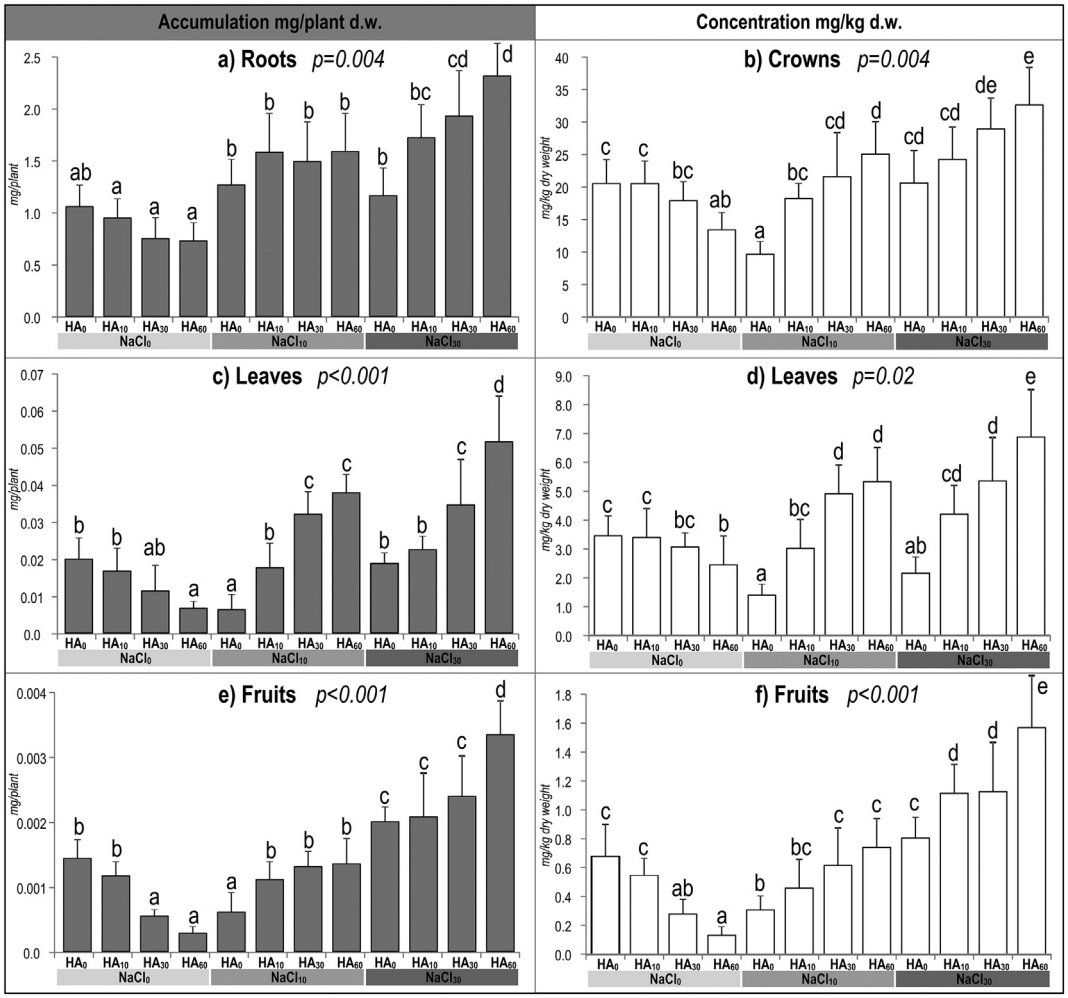

Fig. 1. Mean content and concentration of Cd in tissues of strawberry cv. Albion 40 days after exposure to Cd (2.0 μM), humic acid (0–60 mg/L) and NaCl (0–30 mM) treatments. Means with standard error; $n = 3$. Bars with a different letter are significantly different according to Tukey's HSD test ($p < 0.05$).

The inconsistent or even contradictory results on the effects of HA and NaCl salinity on Cd speciation and phytoavailability are likely due to complex structure of HA and markedly different experimental conditions in different studies (e.g. pH, salinity, soil or nutrient solution matrix). For instance, both inhibitory and stimulatory effects of HA on Cd accumulation were observed with tobacco on four different soils, whereby HA addition suppressed Cd phytoaccumulation on acid and paddy soils, but a significant stimulatory effect on tobacco leaf Cd content was found on alkaline and calcareous soils (Yang et al., 2013). In general, the effects of HA on decreasing availability of Cd and other metals were reported mostly in acid conditions, whereas the stimulating effects were observed in alkaline conditions (Yang et al., 2013). Thus, an environmental pH reaction is one of the master variables that strongly influence Cd biochemistry and Cd transfer to test plant (Fig. 1).

The significant HAxNaCl interaction (Table S2) influenced Cd concentration and content in most of the analysed tissues (Fig. 1). Such results can be explained by slightly acidic pH (6.0) conditions favouring Cd-HA and Cd-Cl complexation (Table 1), which would have influenced Cd phytoavailability. Both Cd-HA and Cd-Cl complexes are recognised as relatively stable, which would impact Cd physicochemical retention, solubility and transfer from soil and/or solution matrices to plants (Ondrasek et al., 2022a; Park et al., 2013; Yang et al., 2013).

3.4. Cadmium chemical speciation modelling in the tested treatments

The summarised results of the modelled Cd chemical speciation in the tested nutrient solutions using the NICA-Donnan approach and the Visual MINTEQ interface are presented in Table 4. The relative proportions of the six chemical forms of Cd varied multi-fold in different treatments, with Cd^{2+} ion dominant in the dissolved phase (with no precipitation apparent; data not shown). In the control (without NaCl salinity and HA), 86 % of Cd was present as Cd^{2+}. However, in all other tested treatments, the proportion of Cd^{2+} decreased. For instance, the addition of HA (up to 60 mg/L) to the rhizosphere solution decreased the proportion of Cd^{2+} to 48 %, mostly because of an increase in Cd-HA complexes (up to 44 %). By contrast, NaCl (up to 30 mM) decreased the proportion of Cd^{2+} (to 39 %), mostly due to the formation of Cd-Cl complexes (an increase to 57 %) (Table 3). The combined application of NaCl and HA depleted Cd^{2+} even further, i.e. to 37 % in Cd + $NaCl_{10}$ + HA_{60} and to 27 % in Cd + $NaCl_{30}$ + HA_{60} (Table 4).

Based on the modelling results (Table 4), Cd mobility and phytoavailability would follow a decreasing order: Cd^{2+} > Cd-Cl ≥ $CdSO_4$ ≥ $CdNO_3$ ≥ $CdHPO_4$ > Cd-HA (see also Kabata-Pendias, 2004). However, considering the calculated proportions of various Cd chemical forms (Table 3), the most relevant Cd forms in this study likely were Cd^{2+}, Cd-Cl, Cd-HA, $CdSO_4$ and $CdHPO_4$. Accordingly, an increase in the proportion of Cd-Cl (e.g. from 0.02 % in control to 57 % in Cd + $NaCl_{30}$ treatment) and Cd-HA (e.g. from 0 % in control to 44 % in Cd + HA_{60} treatment) was accompanied by significantly higher Cd root uptake and its accumulation in the tested strawberry tissues (Fig. 1). Simultaneously, the proportions of other inorganic Cd pools decreased under the same conditions, e.g. Cd-NO_3 from 6.2 to 1.1 %, Cd-SO_4 from 1.3 to 0.3 % and $CdHPO_4$ from 6.5 to 1.4 % (Table 4). These findings are important

Table 4
Cadmium chemical speciation modelling (Visual MINTEQ ver. 3.1) in the nutrient solution in which strawberry cv. Albion was grown under various treatments.

Treatments applied	Relative distribution (%) of chemical species					
	Cd^{2+}	Cd-Cl complexes	Cd-HA complexes	$CdSO_4$	$CdNO_3^+$	$CdHPO_4$
1. Cd (2.0 μM)	86	0.03	0.0	6.2	1.3	6.5
2. Cd + HA_{10} (10 mg/L HA)	78	0.02	9.4	5.6	1.1	5.8
3. Cd + HA_{30} (30 mg/L HA)	64	0.02	26	4.6	0.90	4.8
4. Cd + HA_{60} (60 mg/L HA)	48	0.02	44	3.5	0.70	3.6
5. Cd + $NaCl_{10}$ (10 mM NaCl)	60	32	0.0	3.4	0.80	3.8
6. Cd + $NaCl_{10}$ + HA_{10}	55	30	7.8	3.2	0.75	3.5
7. Cd + $NaCl_{10}$ + HA_{30}	47	25	22	2.7	0.60	3.0
8. Cd + $NaCl_{10}$ + HA_{60}	37	20	38	2.2	0.50	2.4
9. Cd + $NaCl_{30}$ (30 mM NaCl)	39	57	0.0	1.7	0.40	2.0
10. Cd + $NaCl_{30}$ + HA_{10}	37	53	6.1	1.6	0.40	1.9
11. Cd + $NaCl_{30}$ + HA_{30}	32	47	12	1.4	0.40	1.6
12. Cd + $NaCl_{30}$ + HA_{60}	27	39	32	1.1	0.30	1.40

because Cd-Cl forms could stimulate Cd phytoavailability and its enhanced rhizosphere-plant transfer as reported in sunflower, kenaf and sorghum (Hattori et al., 2006) as well as tobacco (Lopez-Chuken et al., 2021).

The modelled results indicated that even at relatively low HA concentration (up to 60 mg/L) and salinity (up to 30 mM), the relative distribution of Cd forms can vary markedly under stable, slightly acidic pH (6.0) conditions conducive to complexation of Cd with Cl^- and humates. Based on in-depth surface Scanning Electron Microscopy (SEM) examination (Ondrasek et al., 2022a), the HA applied in the present study contain highly complex organics that differ in shape and size, with globular-like structure and heterogeneous micro-to-nano porosity, which promotes formation of macromolecules and agglomerates and result in a supramolecular structure (Giovanela et al., 2010; Piccolo, 2001) (Fig. 2). Albeit still not fully physico-chemically characterized, HA are recognised as a key variable in metal speciation due to their high potential to chelate metals (Park et al., 2013; Yang et al., 2013), forming less mobile and less phytoavailable metalo-complexes compared with a free cationic metal form (e.g. Fig. 2).

The Fourier-transform infrared (FTIR) spectrum of the HA used in the present study revealed the abundance of various polar functional groups, such as free —OH, hydrogen-bound —OH, —CH_2— and —CH_3 groups from aliphatic hydrocarbons, C—O from esters and amides, as well as other radicals from alcohols or polysaccharides (Ondrasek et al., 2022a) (Fig. 2). Most of these functional HA-derived radicals have a strong affinity for free Cd^{2+} (i.e. the most mobile and toxic form), impacting its availability and uptake by strawberry roots and accumulation in various tissues (Figs. 1 and 2); these findings were consistent with accelerated Cd soil-plant transfer and enhanced Cd (hyper)accumulation by *Sedum alfredii* after HA addition (Gao et al., 2022). Similarly, the enhanced potential availability and accumulation of Cd (as well as Cu, Pb and Ni) after HA application were recorded in sunflower, field mustard (*Brassica campestris*)

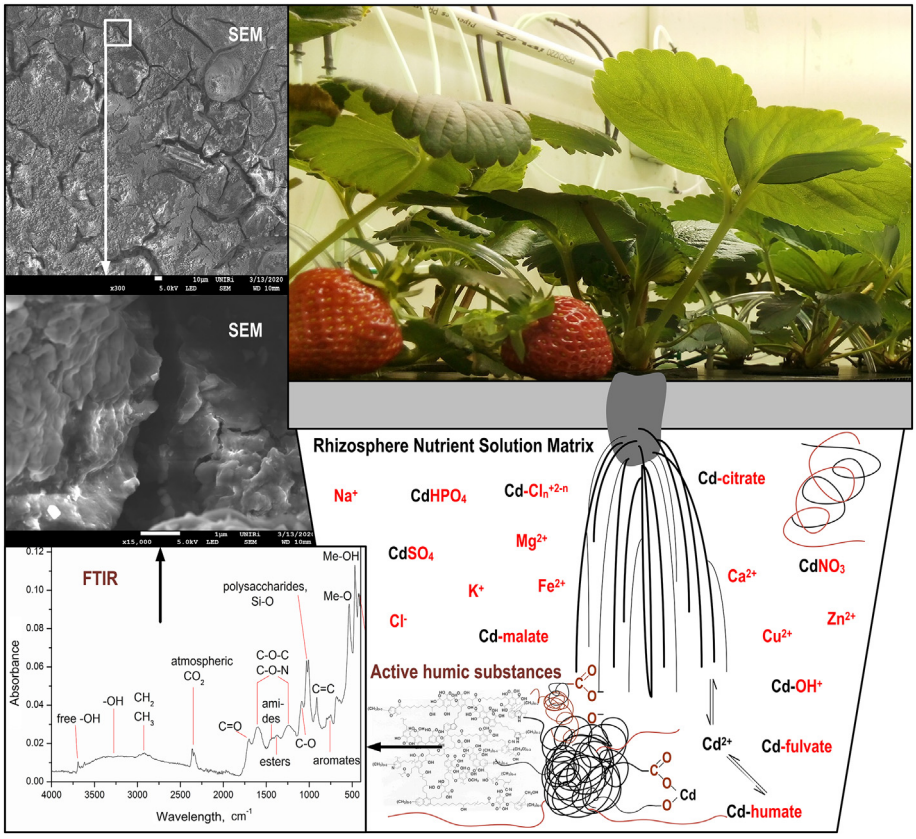

Fig. 2. Possible mechanisms governing Cd biogeochemistry in the strawberry root zone based on (i) the recently reported FTIR and SEM spectral footprints of the humic acids used in the present study (Ondrasek et al., 2022a) and (ii) the calculated Cd chemical speciation (Table 3).

and grass *Festuca arundinacea* (Park et al., 2013). The activation and acceleration of Cd mobility by HA (Fig. 1) are especially relevant given the relatively homogenous conditions in the aqueous medium tested in the present study vs. organo-mineral soil matrix, where additional physicochemical sorption mechanisms of Cd on secondary clay minerals can minimize Cd soil-plant transfer. In addition, although HA are too large to be taken up by root cells, the HA fragments formed by microbial decomposition and/or dissociation in the rhizosphere solution could be absorbed by roots (Evangelou et al., 2004), potentially contributing to enhanced Cd uptake by strawberry (Fig. 1).

The modifications of the root-zone solution by pH and chemical amendments have to be taken into account in explaining interactions between Cd and (in)organic ligands (chlorides, humates) because all these factors play a crucial role in Cd solubility, availability and uptake. It is postulated that at higher pH (>5) as tested here, enhanced deprotonation of humate polyanions would result in a higher negative charge and more extended (i.e. opened) configuration (Baker and Khalili, 2005; see also Fig. 2) because of electrostatic repulsion between the negative charges on HA surface (Baker and Khalili, 2005). Thus, under conditions tested in the present study, the more open humate configuration likely allowed faster diffusion and greater binding of Cd^{2+} from nutrient solution to negatively charged sites on HA (Fig. 2). In addition, a more open HA structure could contribute to effective release of Cd bound on the outer surface of HA supramolecules and its uptake by strawberry roots.

From the practical standpoint, an appropriate adjustment of pH reaction and concentration of humates and chlorides in saline and/or low-quality wastewaters (e.g. Cd-contaminated, HA-enriched) might contribute to: i) their faster and more effective phytoremediation by using some metallophyte and/or halophyte species and ii) sustainable "metal-free" food production, but additional studies are needed.

4. Conclusions

Well-known salt-stress impacts on the glycophyte species strawberry, such as suppressed vegetative growth and yield parameters, were confirmed, and were associated with increased dry matter content in eaves and roots and Na, Cl and Cd accumulation in all strawberry tissues. The study showed that the risk of uptake and accumulation of toxic Cd could be mediated by HA addition only in the absence of salinity, whereas in the treatments with combined HA (up to 60 mg/L) and salinity addition (up to 30 mM NaCl), this impact was opposite (increased Cd phytoaccumulation).

Calculations showed the most toxic free Cd^{2+} form was most abundant (86 %) in treatments without HA and NaCl, whereas in the other treatments the increased proportions of Cd-Cl (up to 57 %) and Cd-HA (up to 44 %) complexes were associated with markedly increased Cd phytoaccumulation. The work on elucidating the effects of the crucial Cd biogeochemistry variables (e.g., pH, presence of competing metallic ions, Cd-binding compounds) in complex media (e.g., metal-contaminated organo-mineral soils, metal-contaminated wastewater) on the combined effects of HA and salinity is warranted.

CRediT authorship contribution statement

GO designed and conducted the experiment. GO, ZR, DR, VT, RS, SR and JH processed data. GO drafted the manuscript. All authors were involved in data interpretation, discussion and final manuscript preparation.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157649.

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