



Uptake of cyclic C_6O_4 in maize and tomato: Results from a greenhouse study

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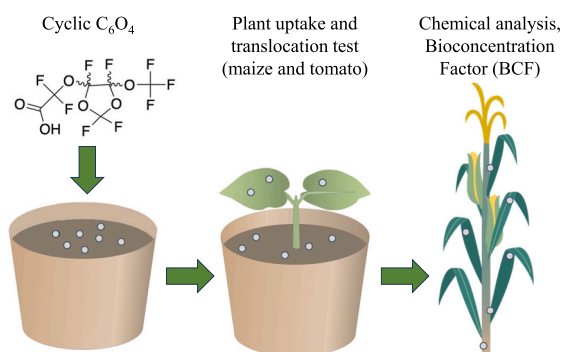
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HIGHLIGHTS

- Uptake from soil and translocation of cC_6O_4 are studied on terrestrial plants (maize and tomato).
- The uptake (as BCF) is low but the behavior of cC_6O_4 is substantially different in the two plants.
- For maize the maximum concentration is in roots while for tomato is in leaves.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyclic C_6O_4 (cC_6O_4 , CAS number 1190931-27-1) is a perfluoralkyl ether used as a polymerization aid in the synthesis of fluoropolymers and produced since 2011 as substitute of PFOA. This work reports the first data on bioaccumulation of cC_6O_4 on terrestrial plants (maize and tomato). In general, the observed accumulation and translocation of cC_6O_4 in plants is low or negligible. For maize a bioconcentration factor ($BCF_{dw/dw}$) of about 39 was observed in the root compartment and much lower ($BCF_{dw/dw} = 12$) in the aboveground tissues. In tomato the observed BCFs are substantially lower, with a maximum of 2.5 in leaves. The differences observed between the uptake and distribution of cC_6O_4 in maize and tomato plants are probably due to differences in plant physiology (but also in the experimental design of the tests). Maize plants grown at different concentrations in this study did not show relevant differences in term of biomass and growth, while tomato plants exposed to cC_6O_4 were subject to a delay in the ripening of the fruits (and relative biomass). The overall results are discussed in comparison with literature data available for legacy PFASs but the comparison is difficult due to differences in the experimental design. It is relevant to note that the concentrations tested in this study are significantly higher than expected environmental concentrations.

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1. Introduction

Per- and polyfluorinated alkyl substances (PFASs) are chemicals containing one or more fully fluorinated methyl or methylene carbon atom with no H/Cl/Br/I atom attached to it (OECD, 2021) as distinctive structural feature.

The general term of PFAS defines a very complex class of chemicals including thousands of compounds almost completely xenobiotic (i.e., not present in the environment before their artificial production), characterized by extremely variable structure and properties (physical, chemical, and biological). However, a property common to PFASs is the persistence due to the stability of the C—F bond (Butt et al., 2014).

PFASs have been extensively used in several industrial and consumer products, and many of these chemicals are detected in the global environment and biota. Over the last two decades, many national and international agreements have been developed to control and limit PFASs production and emission. To specifically address concerns related to PFASs toxicity, in recent years alternative products have been developed. Among these, cyclic C_6O_4 (cC_6O_4) was registered and patented by Solvay in 2011, as substitute of PFOA (perfluorooctanoic acid).

A review on the available knowledge on environmental and ecotoxicological characteristics of cC_6O_4 have been already published (Bizzotto et al., 2023). The available information on the aquatic environment includes short and long term ecotoxicity data on bacteria, algae, crustaceans, and fish, as well as some data on bioaccumulation. Moreover, a long term mesocosm experiment was also performed (Rico et al., 2024). With regards to terrestrial organisms, a recent study investigated the toxicity and bioaccumulation potential of cC_6O_4 in earthworm (Bizzotto et al., 2024), while information on the uptake and translocation of cC_6O_4 in terrestrial plants is investigated in the present study.

With regard to other PFASs, several studies have investigated their potential for accumulation and translocation inside terrestrial plants via the vascular system; most of the available studies focus on the uptake and translocation of perfluoroalkyl acids (PFAAs) from soil to plants. The accumulation of PFASs in plants is influenced by several factors, including their physicochemical properties, plant physiological characteristics and soil properties (Ghisi et al., 2019; Lesmeister et al., 2021; Li et al., 2022; Xu et al., 2022; Lv et al., 2023; Adu et al., 2023; Scearce et al., 2023; Lasee et al., 2021). Root uptake from soil and water is considered the primary pathway for PFASs to enter plants. PFASs can translocate from soil solutions to vascular tissues of plant roots through apoplastic, symplastic, or transmembrane pathways, and then accumulate in the cell walls, root cell organelles and intercellular spaces of the plant roots cortex; from roots, PFASs are then transferred to the aerial parts (shoots, leaves and fruits) via transpiration.

It is important to understand the potential for plant uptake and accumulation of PFASs, since these processes can significantly affect the fate and transport of these compounds in the environment (Adu et al., 2023); additionally, the PFASs accumulation in edible plants can also play a significant role for the human exposure. Moreover, there is the need to better understand the plants uptake of the new PFASs alternatives, characterized by different structures compared with legacy PFOS and PFOA.

In this study, we conducted greenhouse experiments to evaluate cC_6O_4 uptake in two agricultural crops: the plant uptake and translocation tests were carried out according to USEPA (2012), evaluating two different species (maize, *Zea mays* L., and tomato, *Solanum lycopersicum* L.) exposed to cC_6O_4 spiked soils. The exposure was aimed to verify the potential uptake and translocation of cC_6O_4 in different tissues of the tested macrophytes, observing in the meanwhile also any macroscopic morphological alteration. To the best of our knowledge, this is the first study evaluating the bioconcentration behavior of cC_6O_4 in terrestrial plants grown under controlled laboratory conditions.

2. Materials and methods

2.1. Chemicals and soil spiking

The cC_6O_4 (CAS 1190931-27-1; molecular formula: $C_6H_4 F_9NO_6$; complete chemical name: acetic acid, 2,2-difluoro-2-[[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy]-, ammonium salt) was gently provided by Solvay Specialty Polymers Italy S.p.A. (batch number: 07040 PS). Solubility of cC_6O_4 in water is >667 g/L and the critical micellar concentration (CMC) is 36–56 g/L; when in aqueous solution, it completely dissociates in the anionic form. Other properties of environmental relevance are the very low vapour pressure (7.5E-5 Pa at 25 °C), low n-Octanol/Water partition coefficient (log K_{ow} = 1.3) and low Organic Carbon adsorption coefficient (log K_{oc} = 1.04) (Bizzotto et al., 2023).

The experimental activity used ultra-pure water as solvent. The originally sent cC_6O_4 solution from Solvay (aqueous solution of cyclic C_6O_4 ammonium salt, cC_6O_4 concentration in water 39.1 % weight/weight, density of the solution 1.18 g/mL) was used as a stock solution for the spiking activity. The stock solution was kept in the original holder and managed and stored according to the provided safety data sheet. For the uptake and translocation test, the cC_6O_4 was added to the soil via solubilization in deionized water that was used to wet the soil samples to reach from 40 % to 60 % of the total water holding capacity. Spiked samples were mixed thoroughly with a stainless-steel shovel to reach the target concentrations. To verify and validate the tested concentrations, chemical analyses of cC_6O_4 were performed on pooled samples of the tested soils, collected at the beginning of the test. Specifically, the soil samples collected at the beginning of the maize were frozen, stored and analyzed after the end of the exposure period; a residual concentration of cC_6O_4 was detected in the unspiked soil used as control for the maize test, probably due to a cross-contamination event occurred during the soil preparation and spiking. Additional control measures were implemented for the tomato test; soil samples of the tomato test were analyzed before the test began, in order to verify the absence of cC_6O_4 in the unspiked soil and to confirm exposure concentrations. Test results are reported below referring to soil measured concentrations.

2.2. Plant uptake and translocation test

The plant uptake and translocation tests were carried out according to USEPA, 2012, evaluating bioaccumulation of cC_6O_4 in maize (*Z. mays*) and tomato (*S. lycopersicum*) exposed to spiked soils; these species were selected since representative of valuable agricultural crops and characterized by different physiological features. Additionally, both the species were used in previous studies for the evaluation of PFAS distribution in plant compartments.

The tests were performed in different periods; the test with maize was conducted in 2022 (spring) while the exposure of tomato was run in 2023 (early summer); the experimental settings of the 2023 test was refined and optimized on the basis of experience gained in 2022. Specifically, in 2023 special care was paid to the spiking activities, implementing additional control measures to confirm exposure concentrations before starting the test and to avoid cross-contamination events; additionally, the procedure for tissue sampling and the samples number were optimized (analyzing composite samples). The overall experimental conditions are summarized in Table 1, while the properties of the soils used for the tests are reported in Table 2. In both tests, all organisms (including the control) were from the same source and were grown in identical test chambers with the same amount of substrate (from the same source); additionally, the control (unspiked soil) and the spiked soils were cultivated in the same greenhouse.

For both tested species, the exposure occurred at the University of Naples Federico II greenhouse facility with natural photoperiod and temperature. Seedlings were obtained in polystyrene trays (i.e., non-

Table 1

Experimental conditions of the plant uptake and translocation test.

	Maize (<i>Z. mays</i>)	Tomato (<i>S. lycopersicum</i>)
Test protocol	USEPA, 2012	
Test substance application method	Root exposure (cC ₆ O ₄ spiked in soils)	
Soil	Natural soil collected from the wild from a site far from anthropic activities	Commercial soil bought from a garden center
Replicates	For each exposure scenario and the control, 13 pots (4 L each), one seedling per pot; only 3 replicates (3 plants) were used for chemical analysis (for each tissue, analyses were run on 3 samples collected from 3 different plants).	For each exposure scenario and the control, 10 pots (4 L each), one seedling per pot: all the replicates were used to prepare composite samples for chemical analyses (for each tissue, 1 composite sample prepared from the ten replicates).
Exposure period	28 days (28th April – 26th May 2022)	52 days (6th June - 28th July 2023)
Test treatment levels (cC ₆ O ₄ , measured concentration)	Unspiked soil (namely, the control): 0.0101 mg/kg d. w.	Unspiked soil (control): < limit of detection (LOD)
Analyzed tissues	Spiked soils: 0.0192–30.8 mg/kg d.w. Root and above ground tissue (stem+ leaves)	Spiked soils: 0.59–30.6 mg/kg d.w. Root, stem, leaves, fruits
Greenhouse conditions	Temperature did not exceed 25 ± 3 °C during the day, while the temperature during the night felt in the 20 ± 6 °C interval; relative humidity was within 55 % and 85 % during the light period	Temperature did not exceed 30 ± 3 °C during the day, while the temperature during the night felt in the 25 ± 2 °C interval; relative humidity was within 60 % and 85 % during the light period
Light quality	Natural sunlight and photoperiod	
Watering	Bottom watering as needed avoiding overflow events, using tap water (no nutrient added)	

Table 2

Properties of the natural and commercial soil used for the uptake and translocation assays. Data reported in the table refer to unspiked soils. Only soil texture is referred to the raw unsieved soil, while all other analyses are referred to the soil fraction resulting after the raw soil sieving process (4 mm, stainless steel sieves).

Property	Natural soil used for maize test	Commercial soil used for tomato test	Method
pH	7.45 ± 0.05	7.80 ± 0.03	ISO 10390 (2021)
Grain size	Gravel 0.4 %, Sand 99.5 %, Silt 0.8 %, Clay 0.02 %	Gravel 0.2 %, Sand 83.5 %, Silt 16.28 %, Clay 0.02 %	ISO 11277 (2020)
Water content	55.57 ± 0.14 %	61.00 ± 0.01 %	ISO 11465 (1993)
Water Holding Capacity	89.37 ± 1.14 %	159.00 ± 6.5 %	ISO 11268-1, Annex C (2012)
Cationic Exchange Capacity (CSC)	48.3 ± 4.8 cmol/kg	57.3 ± 5.8 cmol/kg	ISO 11260 (2018)
Organic Carbon	3.6 ± 0.5 %	7.6 ± 0.6 %	ISO 10694 (1995)

toxic, and devoid of nutrients capable of promoting the growth of fungi, bacteria, and other microorganisms). Seedlings were transferred to new test containers in polypropylene with drainage holes having equal size and volume. Pots were filled with spiked or control soils. Irrigation was periodically administered preventing any soil leaching phenomena. Nutrients were not supplied during exposure; no pesticides were administered.

Both plants were tested at two different concentrations of cC₆O₄. The highest concentrations measured in the environment (topsoil sampled very close to the industrial production site of cC₆O₄) are in the order of a few µg/kg d.w. (Valsecchi, 2022). To reduce analytical problems, possible at very low concentrations, the lowest concentration tested is higher but relatively close to realistic environmental levels. The highest concentration tested in both tests is around 30 mg/kg d.w. The reported concentrations are analytically measured. Therefore, they are slightly different in the two experiments.

For maize, the duration of the exposure period was equal to the length of time required to achieve sufficient biomass for analysis (28 days), while for the tomato test the exposure was prolonged until fruits were mature (52 days) at least in the negative controls.

At the harvesting time, each part of the related macrophyte was freshly subdivided and separately stored (starting from the control up to the highest exposure concentration) for chemical analysis.

In the case of *Z. mays*, two sub-samples were produced per replicate: i) root; ii) stem+leaves (i.e., plants were not sufficiently developed to easily separate stem and leaves). For *S. lycopersicum*, the tissues were sampled when the fruits of the control were ripe (and almost ripe in the cC₆O₄ spiked soils) and four sub-samples were produced per replicate: i) root, ii) stem, iii) leaves, and iv) fruits. Preliminary testing highlighted, for tomatoes, the relevance of special care to efficiently remove residual earth trapped from the root; therefore, roots of the tomato plants were cleaned by soaking in water and then gently dried with paper towel.

2.3. Analysis of cC₆O₄ in soil and plant tissues

Chemical analysis of cC₆O₄ in soil and plant tissues were performed at the laboratory Merieux NutriSciences Italia (Resana, Italy).

The determination of cC₆O₄ in soil and in biological matrices was conducted applying methods developed and validated internally by Merieux NutriSciences (further details in Supplementary Material); both the methods are based on LC-MS/MS detection. For soil, the method is based on ASTM D7968: 2017. Soil samples were dried in oven at 105 °C and sieved at 2 mm; extraction was then performed using methanol and water under alkaline condition; chemical analysis was run in triplicate. For biological samples, the method is based on FDA Foods Program Compendium of Analytical Laboratory Methods (Method number C-010.01). Briefly, biological samples are shaken for 30 s with water and 15 % sodium chloride solution; then, acetonitrile is added, and the samples are shaken vigorously for 8 min. The extract was then centrifuged; the organic layer was transferred in another tube and dried with magnesium sulfate. After another centrifugation, the extract was purified with charcoal, concentrated to dryness, then dissolved with a solution containing internal reference material and analyzed by LC-MS/MS. Limit of detection (LOD) for cC₆O₄ in soil and biological tissues were respectively 0.053 µg/kg d.w. and 0.31 µg/kg w.w. All concentrations of cC₆O₄ reported in this study refer to the anionic form.

Aliquots of the different vegetal tissues were wet weighted and dry weighted (after at least 24 h at 105 °C until constant dry weight) to calculate the water content.

2.4. Data analysis

To allow comparison across soils and crops, Bioconcentration Factors (BCF) were calculated dividing plant tissue concentrations (reported on wet weight) by soil concentrations expressed on dry weight (Eq. (1)):

$$BCF_{w.w./d.w.} \text{ (kg d.w./kg w.w.)} = C_{\text{tissue},w.w.} / C_{\text{soil},d.w.} \quad (1)$$

where $C_{\text{tissue},w.w.}$ (mg/kg w.w.) is the concentration of cC₆O₄ in the plant tissues (root, stem, leaves and fruit for tomato, root and above-ground tissues for maize), $C_{\text{soil},d.w.}$ (mg/kg d.w.) is the mean values of soil concentrations measured at the beginning of the test. Since chemical analysis on maize tissue were performed on 3 samples from different

replicates of the same exposure scenarios, for maize BCF are expressed as mean value with standard deviation.

To evaluate the plant uptake of cC_6O_4 in comparison with literature data available for other PFASs, BCF ($BCF_{dw/dw}$) were also estimated on the basis of tissue concentrations expressed on dry weight (estimated applying the following equation: $C_{tissue_{dw}} = C_{tissue_{ww}} / (1 - \text{water content fraction})$). Additionally, to evaluate the potential influence of soil properties, organic carbon normalized BCFs were also calculated ($BCF_{OC} = BCF_{dw/dw} \times f_{OC}$, where f_{OC} is the fraction of soil organic carbon).

As reported by Blaine et al. (2013), the BCF calculation relies on several assumptions, such as the absence of chemical transformation in the plant (or during the plant extraction process) and negligible atmospheric exchange, presuming therefore that root uptake from soil is the dominant uptake pathway; these assumptions were considered reasonable since cC_6O_4 is very stable and ionized at environmental pH values, and therefore generally nonvolatile (similarly to PFAAs, Blaine et al., 2013, 2014).

Finally, for comparative purposes Translocation factors (TF) were calculated as the ratio of aboveground tissues to root concentration ($TF_{leaves/root}$, $TF_{stem/root}$); for tomato, the ratio of fruit concentrations to leaves concentrations ($TF_{fruit/leaves}$) was also calculated.

3. Results and discussion

3.1. Uptake and translocation of cC_6O_4 in maize and tomato

In the maize test, a residual concentration of cC_6O_4 was detected in the soil used for the control (0.010 mg/kg d.w.), probably due to a cross-contamination event occurred during the soil spiking procedure; therefore, for maize the control was considered as a tested concentration. Specifically, due to similarity of the two lowest tested concentrations and of resulting BCF values (reported in full details in Table 1-SM), an average BCF was estimated for maize considering the overall results from the exposure scenarios of 0.0101 (namely, the control) and 0.0192 mg/kg d.w., while the exposure scenario of 30.8 mg/kg d.w. was considered separately (Tables 3 and 4). In the tomato test, cC_6O_4 was not detected in the control soil, neither in any tissues of plants grown in the control soil (Table 3); since the control plants grew closed to the other exposure scenarios, the finding of no detectable cC_6O_4 in the control plants suggests a negligible volatilization from soil as well as aerial uptake of cC_6O_4 in the plants (as expected based on its physico-chemical properties). The overall BCFs are reported in Table 4; the $BCF_{dw/dw}$ were estimated considering the average values of water content measured for the different plant tissues (maize: root 89 %, aboveground tissues 87 %; tomato: root 44 %, stem 34 %, leaves 30 %, analyzed fruit 42 %).

For maize, the BCFs observed in the two exposure scenarios are different, with lower values in plants grown with 30.8 mg/kg d.w. of

Table 4

Bioconcentration Factors ($BCF_{ww/dw}$ calculated using measured concentrations, and $BCF_{dw/dw}$ estimated on the basis of water content in tissues) determined for cC_6O_4 in different tissues of maize and tomato. The BCF determined for maize in the exposure scenario 0.015 mg/kg d.w. represents the mean value of BCFs determined for soil with measured concentration 0.0101 and 0.0192 mg/kg d.w.

Plant	Tissue	Exposure scenarios – cC ₆ O ₄ in soil			
		0.015 mg/ kg	0.59 mg/ kg	30 mg/kg	Mean
BCF _{ww/dw} = C _{tissue_{ww}} /C _{soil_{dw}}					
Maize	Roots	7.71 (± 2.70)		1.17 (± 0.55)	4.44
	Above ground tissues	2.98 (± 1.49)		0.33 (± 0.16)	1.66
Tomato	Roots		0.04	0.01	0.03
	Stem		0.04	0.01	0.02
	Leaves		1.92	1.6	1.76
	Fruit		0.02	0.02	0.02
BCF _{dw/dw} = (C _{tissue_{ww}} /(1 - water content fraction))/C _{soil_{dw}}					
Maize	Roots	68.23 ± 23.86		10.33 ± 4.84	39.28
	Above ground tissues	22.60 ± 11.26		2.52 ± 1.24	12.56
Tomato	Roots		0.08	0.02	0.05
	Stem		0.06	0.01	0.04
	Leaves		2.74	2.29	2.51
	Fruit		0.04	0.03	0.04
BCF _{OC} = BCF _{dw/dw} x f _{OC}					
Maize	Roots	2.46 ± 0.86		0.37 ± 0.17	1.41
	Above ground tissues	0.81 ± 0.41		0.09 ± 0.04	0.45
Tomato	Roots		0.006	0.001	0.004
	Stem		0.004	0.001	0.003
	Leaves		0.208	0.174	0.191
	Fruit		0.003	0.002	0.003

cC_6O_4 in soil. Additionally, roots show BCFs higher than the maize aboveground tissue.

On the contrary, the leaves of the tomato plants present BCFs higher than all the other tissues (root, stem, and fruit), thus indicating the presence of translocation primarily in the leaves; for tomato, the BCFs determined in the two exposure scenarios are very similar.

Results of this study do not allow a clear comprehension of the differences observed in the uptake of cC_6O_4 in maize and tomato, that could be influenced by several factors, including the plant physiology and the experimental design, characterized by differences in the length of the exposure period and by the use of different soils, whose inherent properties (pH, fertility, organic matter content, and texture) can

Table 3

Measured concentrations of cC_6O_4 in soil and in plant tissues. For tomato, chemical analyses on plants were run on 1 composite samples, while for maize analyses were run on 3 different plants (data are fully reported in SM).

cC_6O_4 in soil (mg/kg d.w.)			cC_6O_4 in MAIZE (mg/kg w.w.)									
			Root					Stem + leaves				
0.0101	±	0.0039	0.087	±	0.039	0.038	±	0.015				
0.0192	±	0.0063	0.131	±	0.018	0.042	±	0.022				
30.8	±	9.7	36.0	±	16.9	10.2	±	5.0				
cC_6O_4 in soil (mg/kg d.w.)			cC_6O_4 in TOMATO (mg/kg w.w.)									
			Root		Stem		Leaves		Fruit			
<LOD			<LOD		<LOD		<LOD		<LOD			
0.59	±	0.18	0.0253	±	0.0087	0.022	±	0.0076	1.13	±	0.39	0.0137 ± 0.0047
30.6	±	9.5	0.271	±	0.093	0.281	±	0.097	49	±	17	0.54 ± 0.19

significantly influence the bioavailability of chemicals (Mei et al., 2021). The soil organic matter (SOM) is a significant sorbent for PFASs thus reducing the root uptake; however, the mechanisms underlying the sorption and bioaccumulation of PFASs are not yet well understood (Mei et al., 2021; Searce et al., 2023). In this regard, considering the physico-chemical properties of cC_6O_4 (characterized by a low Organic Carbon adsorption coefficient, $\log K_{oc} = 1.04$), it is possible that differences in the plant uptake were influenced more by plant physiology rather than by sorption processes due to the different soil properties (e.g. organic matter).

Additionally, it is unclear if the procedure adopted for the sample preparation may have influenced the results. Specifically, the maize after 28 d exposure presented well developed seminal and nodal roots that were easily separated by shaking from the soil thus the root complex was not rinsed; conversely, tomato plants presented a highly developed taproot system trapping the soil, thus the root complex required to be rinsed several times into ultrapure water to be freed from the substrate.

It is also necessary to highlight the specific differences between maize and tomato: the measured water content in the maize was almost the double than that observed in tomato plants, thus influencing the potential uptake of the highly hydrophilic cC_6O_4 . Water uptake in macrophytes can be influenced by various factors, some of which are environmental, such as soil hydraulic conductivity, soil moisture pressure head, and atmospheric demand on the plant system, while others are related to the plant itself, including rooting depth, root density distribution, and transpiration. This comprehensive list alone underscores the complexity of estimating the amount of water drawn from the soil.

In this sense, it is interesting to note that the BCF estimated considering the tissue concentrations expressed on dry weight ($BCF_{dw/dw}$) (Table 4) present very similar values in the leaves of tomato and in the aboveground tissue of maize plants exposed to 30 mg/kg of cC_6O_4 , while it is confirmed the different uptake in the root compartment of the two species. However, regarding root uptake, while for maize a strong negative correlation of the BCF in root with the concentration of pollutant in soil was found; for tomato the accumulation in the root is similar in both exposure scenarios. This result is consistent with Mei et al. (2021) that via a large-scale meta-analysis evidenced how BCF in root tissues presented no clear relationship to soil PFAS concentration thus resulting more a plant-by-plant specific evidence.

Additionally, it is recognized that the plant physiology can significantly influence the uptake and translocation of contaminants (Lv et al., 2023; Searce et al., 2023). For example, C4 plants (i.e., maize) can form a large biological harvest in comparison to C3 plants (i.e., tomato), allowing fast biomass accumulation with high water use efficiency (Leegood and Edwards, 1996). For this reason, C4 plants are regarded in the current literature as the best candidates for application in phytoremediation (Gorelova et al., 2023). In particular, transpiration is determined by factors such as plant type, species and varieties, stomatal conductance of the plant, and leaf area of plants (leaf area index, LAI). Therefore, while it may be hypothesized that maize plants would have a greater uptake due to their higher LAI, it must also be considered that, owing to their higher water use efficiency, they produce more biomass with less water compared to tomatoes.

Previous studies indicated that the PFASs enter crop plant root via apoplastic (between and through cell walls), symplastic (through cells via plasmodesmata), or transmembrane pathways and the transport mechanisms of different perfluorinated compounds vary depending on the plant species (Searce et al., 2023; Mei et al., 2021). Searce et al. (2023) report that, in the root cortex, the symplastic and transmembrane pathways are selective against the transport of some PFASs (particularly long-chain compounds), while the apoplastic pathway is not. Beyond the root cortex, another factor regulating the PFASs distribution is the Casparian strip, a lignin suberin-rich layer in the root endodermis, that acts as an apoplast barrier between epidermis and endodermis in root, thus limiting PFAAs from entering vascular tissue through apoplast

pathway (Mei et al., 2021; Lv et al., 2023). As a result, the passage of contaminants into aboveground plant compartments is limited to those compounds that can travel through the selective symplastic and transmembrane pathways, where long-chain compounds are filtered out to a greater extent due to the larger molecular size and greater hydrophobicity (Searce et al., 2023). It is relevant to note that the Casparian strip is normally absent in immature root tips, although currently there is still a lack of information regarding the variability of PFASs concentrations during the root growth and development. Searce et al. (2023) concluded that the total PFASs content of root vegetables may be influenced by the ratio of cortex tissue relative to tissue within the vascular cylinder, and the presence, quantity, and location of secondary growth characteristics (e.g., cambial layers). Moreover, from 2 to 3 cortex layers are present in tomato species (Ron et al., 2013), while from 8 to 15 in maize (Ortiz-Ramírez et al., 2021) probably explaining the lower translocation observed in maize respect to tomato.

Additionally, crop physiological features such as fine root area, percentage of mature roots, and leaf blade area can influence the uptake, due to the greater volumes of transpirational flow (Searce et al., 2023).

To compare the plant uptake of cC_6O_4 with that of legacy PFASs (specifically, perfluoroalkyl carboxylic acids, PFCAs, and perfluoroalkyl sulfonic acids, PFASs), a literature search was conducted selecting studies conducted on the same plant species (maize and tomato) and with similar experimental design (soil exposure; pot experiment in greenhouse; further details on the experimental design are reported in SM). Most of the studies report BCFs values determined considering tissue dry weight concentrations; therefore, the $BCF_{dw/dw}$ was the metric chosen for the comparison. Figs. 1 and 2 report a graphical comparison of the $BCF_{dw/dw}$ determined for cC_6O_4 (mean value considering the different exposure scenarios) and PFASs respectively in maize and tomato under controlled exposure experiments, as reported in scientific literature (maize studies: Stahl et al., 2009; Krippner et al., 2015; Wen et al., 2016 and Navarro et al., 2017; tomato studies: Blaine et al., 2014; Navarro et al., 2017; Lal et al., 2020, Lasee et al., 2021). Studies not performed under controlled lab conditions (Felizeter et al., 2021; Zhang et al., 2018; both studies investigating PFASs uptake in maize) were not used for a direct comparison of BCF due to potential variability in PFASs soil concentrations and, therefore, related uncertainties in BCF values; however, since these studies investigated PFASs distribution in different maize tissues, they were considered valuable to compare the translocation factors (TF) observed for PFASs with the one estimated for cC_6O_4 in this study (Fig. 1).

In the maize experiment, the BCF of cC_6O_4 in the aboveground tissues is higher than that of PFOA (and other long-chain PFASs) and appears similar to BCFs determined for short-chain compounds, as determined under controlled greenhouse studies (Krippner et al., 2015; Stahl et al., 2009; Navarro et al., 2017; Wen et al., 2016). However, the TF of cC_6O_4 in maize is lower than 1, indicating therefore an enhanced accumulation in root and reduced translocation of cC_6O_4 from root to shoot, similarly to what observed for PFOA and PFOS.

In the tomato test, the BCFs of cC_6O_4 estimated for root, stem and fruit are very low (and lower than BCFs determined for the other PFASs, although a certain variability is observed in literature data), and only the BCF determined for the leaves is similar to literature values for PFOA. Considering the TF, a value higher than 1 is observed only for tomato leaves, suggesting therefore translocation of cC_6O_4 in leaves but not in fruits or in the other tissues, similarly to what observed also for most of the other PFASs. It is recognized that the uptake and translocation of PFASs in plants can be influenced by several factors (including also plant features). In the case of cC_6O_4 , it is likely that its high solubility can determine accumulation in the leaves (through the transpirational flow), although this uptake seems less relevant than what observed in PFAAs (as demonstrated by the comparison of the BCF determined for leaves). Previous studies concluded that, in terrestrial invertebrates, cC_6O_4 likely undergoes uptake and elimination rate radically different to what observed for PFAAs (Bizzotto et al., 2024); results from this study appear to confirm that cC_6O_4 behaves differently from the legacy PFASs,

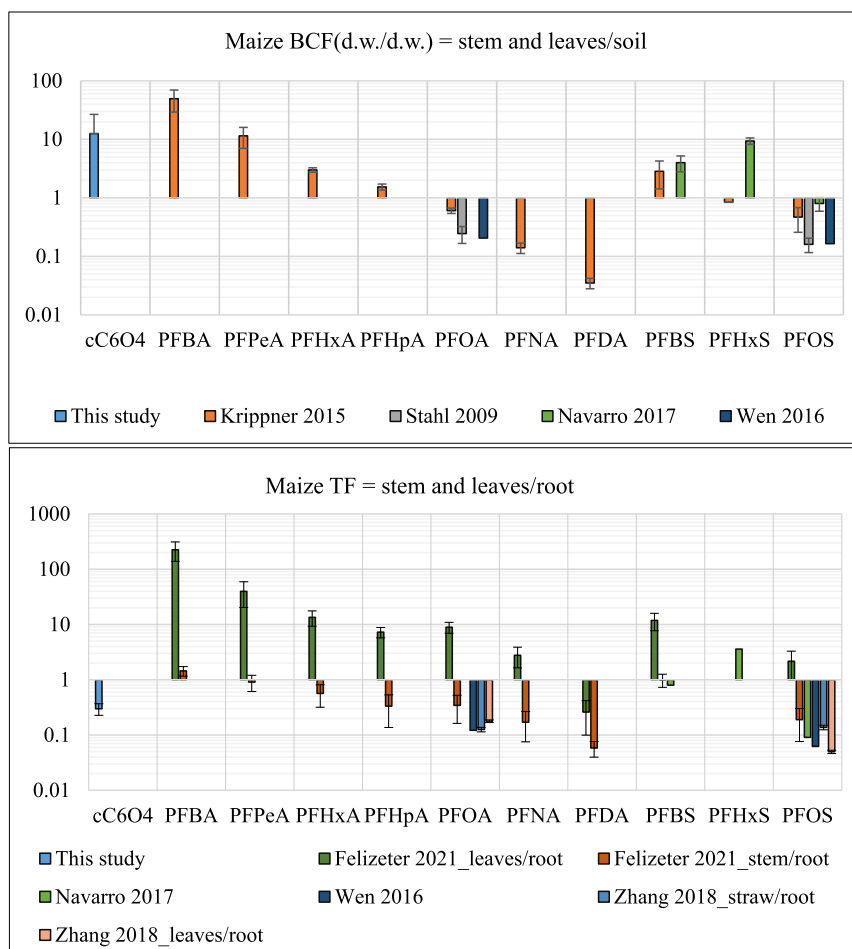


Fig. 1. Comparison of the mean values of BCF and TF determined for cC₆O₄ (this study) with values reported in literature for PFASs in maize (Krippner et al., 2015; Stahl et al., 2009; Navarro et al., 2017; Wen et al., 2016; Felizeter et al., 2021; Zhang et al., 2018). The figure refers to the mean values of BCF and TF calculated for studies evaluating different exposure scenarios (Felizeter et al., 2021, Zhang et al., 2018 and this study).

although the comparison is limited by the high variability in the experimental design and related factors. The differences between cC₆O₄ and PFAAs are also confirmed by the mass distribution (percentage to total plant content) of these chemicals in different plant tissues, as determined on the basis of measured tissue concentrations and the overall produced plant biomass. Specifically, considering the cC₆O₄ mass balance in the maize test, in all the tested exposure scenarios it is observed that the total amount of cC₆O₄ in the plants is equally distributed between root (47–50 %) and the above ground tissue (50–53 %). For maize (pot study), Navarro et al. (2017) reported that PFOS presented a higher accumulation in roots (89 %) while PFBS and PFHxS were preferentially found in leaves (>80 %).

In the tomato test, the cC₆O₄ was mostly stored in leaves (~97–98 % in the tested exposure scenarios); as comparison, Navarro et al. (2017), in their tomato study, observed that PFOS (75 %) and the long-chain PFCAs (C7–C10: PFHpA, PFOA, PFNA, PFDA) preferentially remained in roots (54–96 %) while the short-chain PFCAs (C4–C6: PFBA, PFPeA, PFHxA) tended to be translocated to above-ground tissues (leaf: 31–56 %, and fruit: 32–48 %).

3.2. Influence of cC₆O₄ on the plant growth

The experiments presented in this study were aimed at evaluating the uptake of cC₆O₄ in plants and not its phytotoxicity; however, during the test, observations regarding plant growth (expressed as wet biomass at the end of the test) were recorded and are here discussed as preliminary indications.

Full details about the wet biomass measured for maize and tomato at the end of the exposure period are reported in SM.

With regard to maize, plant exposed at 0.0192 mg/kg of cC₆O₄ for 28 days showed a slight but statistically significant stimulatory effect ($p < 0.05$, Holm-Sidak analysis), with higher growth compared to the other exposure concentrations. No statistically significant differences were observed among the other exposure scenarios (Table 3-SM).

At the end of the test on tomato, a statistically significant difference (about 20 % of biomass reduction, $p < 0.05$, Holm-Sidak analysis) was observed between the control and the pots treated with cC₆O₄, but not between the treatments at different concentrations (Fig. 3). The total number of fruits was similar in all the exposure scenarios, but the number of ripe fruits (and thus, their weight) decreased at increasing concentrations, suggesting a delay in fruit ripening. Additionally, at the harvesting time, a widespread foliar necrosis was observed at 30.6 mg/kg d.w. As further observation, it was noted that soils spiked with 30.6 mg/kg d.w. of cC₆O₄ appeared more moistened than the lower concentrations, suggesting an impaired ability of tomato plants in absorbing water with potential subsequent criticalities in functional development.

As mentioned above, due to the aim of the experiments, these results must be considered as purely indicative and not suitable for deriving ecotoxicological endpoints (e.g., EC50, NOEC). However, a very preliminary comparison can be made with some literature data on PFCAs and PFSAs, selecting studies conducted with comparable experimental design (Table 5-SM).

As a rule, despite the variability among different plants and different chemicals, the toxicity of PFASs on terrestrial plants appears to be



Fig. 2. Comparison of the mean values of BCF and TF determined for cC₆O₄ (this study) with values reported in literature for PFASs in tomato (Blaine et al., 2014; Navarro et al., 2017; Lal et al., 2020; Lasee et al., 2021). The figure refers to the mean values of BCF and TF calculated for studies evaluating different exposure scenarios (Lal et al., 2020; Lasee et al., 2021; this study).

relatively low, with NOEC values orders of magnitude higher than environmentally relevant concentrations (Li et al., 2022).

4. Conclusions

The present work contributes to increase the knowledge of behavior and effects of cC₆O₄ on terrestrial ecosystems.

The results of this study show a relatively low average uptake and translocation of cC₆O₄ in both the tested terrestrial plants. A certain variability was observed between the uptake and distribution of cC₆O₄ in maize and tomato plants, mainly due to the differences in plant

structure and physiology (but also to the experimental design of the tests). For maize, the BCF determined for the root compartment was higher than those observed for the aboveground tissues; for tomato, results suggests that cC₆O₄ is accumulated primarily in leaves (probably due to the transpiration flow) while there is not a preferential accumulation in fruits or in other tissues (stem and roots).

A comparison with data on bioconcentration in plants of legacy PFASs available in the literature is quite difficult due to the high variability in the experimental design and related factors. It seems that cC₆O₄ behaves differently from the others PFASs tested, however, even among those, substantial differences are evident.

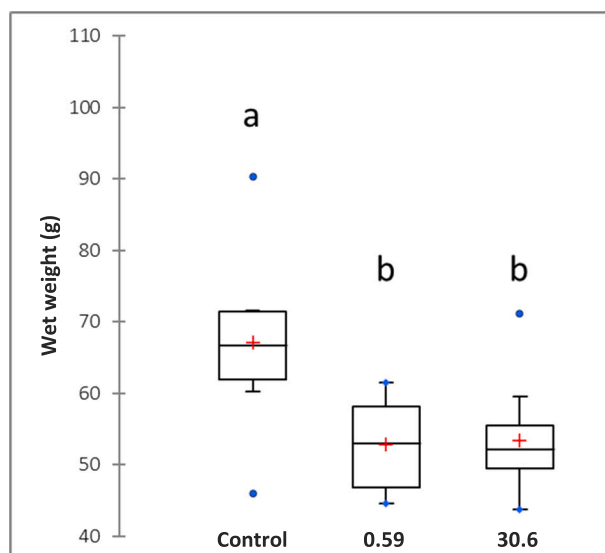


Fig. 3. Total biomass (g) of tomato plants exposed to the control and cC_6O_4 spiked soil. The wet weight trend at 0.59 and 30.6 mg/kg d.w. of cC_6O_4 is compared to the control; equal letters indicate no significant differences between treatments (Holm-Sidak method; $\alpha = 0.05$).

Although the described experiments were aimed to evaluate the uptake and not the toxicity of cC_6O_4 , some observation may also be made on the effects on the tested plants. Maize plants grown in different exposure scenarios did not show relevant differences in term of biomass and growth, while tomato plants exposed to cC_6O_4 were subject to a delay in the ripening of the fruits (and relative biomass). Additionally, a widespread foliar necrosis was observed in plants exposed at ~ 30 mg/kg d.w.

Finally, it is relevant to note that the concentrations tested in this study are significantly higher than expected environmental concentrations. As an example, preliminary investigations conducted very close to the industrial production site of cC_6O_4 showed that maximum level of the compound in soil is of a few $\mu\text{g/kg}$ d.w., (about 1–2 orders of magnitude lower than the lowest concentrations tested in this study) while measured concentrations in grass (herbaceous Gramineae) allowed to estimate a $BCF_{ww/dw}$ of ~ 3 (Valsecchi, 2022), similarly to what observed in this study (BCF determined for the leaves of maize and tomato).

CRedit authorship contribution statement

Elisa C. Bizzotto: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Data curation, Conceptualization. **Giovanni Libralato:** Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation, Formal analysis. **Antonino de Natale:** Writing – original draft. **Petra Scanferla:** Project administration, Funding acquisition. **Marco Vighi:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Antonio Marcomini:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Author and co-authors reports financial support was provided by Solvay Specialty Polymers Italy S.p.A. Marco Vighi reports a relationship with Solvay Specialty Polymers Italy S.p.A. that includes: consulting or advisory.

Data availability

Data will be made available on request.

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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.2024.171613>.

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