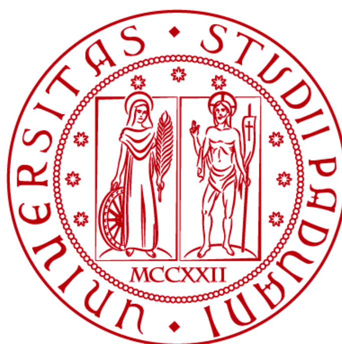
UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI INGEGNERIA CIVILE, EDILE E AMBIENTALE

Department of Civil, Environmental and Architectural Engineering

Corso di Laurea Magistrale in Environmental Engineering



TESI DI LAUREA

TESTING THE PLANT UPTAKE OF CHEMICALS IN FERTILIZERS

Laureando:

UMAR FAROOQ

[1215994]

Relatore

Prof. ALBERTO PIVATO

Correlatore:

Dott. Ing. GIOVANNI BEGGIO

ANNO ACCADEMICO 2021-2022

ACADEMIC YEAR 2021/2022

This page is left blank intentionally.

Acknowledgments

To begin, I would want to show my gratitude to Prof. Pivato Alberto, who has encouraged me in class and identified me as a possible candidate to supervise this research.

My heartfelt appreciation goes to my mentor, Dr. Ing. Giovanni Beggio, who has welcomed me without hesitation and has supported me during my research period as well as my time at Padova University. I would want to express my gratitude to him for all of his valuable guidelines, which have been an enormous help in my research. Apart from my advisers, I would like to express my gratitude to the personnel and colleagues at the SESA laboratory, particularly Director Tiziano Bonato and laboratory colleague Andrea, for their invaluable assistance in carrying out this work at the laboratory and provided all the necessary apparatus for the experiment. Additionally, I would want to express my gratitude to my family and friends who have aided me in one way or another or simply by providing moral support.

A Special thanks go out to Daniele, my husband, for his unlimited love and support, without which this would not have been possible. Finally, I want to express my heartfelt gratitude to everyone who, while I may not have named them by name, has stood by my side morally or in any other way.

Contents

Acknowledgments ii
Contents iii
Figures iv
Tables v
Part-I Theoretical Background 1
1 General Introduction 2
1.1 What is uptake? 2
1.2 Uptake of chemicals by Plants 4
1.3 Why is plant uptake crucial for fertilizers? 4
1.4 How to assess plant uptake and bioaccumulation 5
1.5 Fertilizers 23
1.5.1 Fertilizer Regulations concerning Digestate and compost 23
1.5.2 Criteria for Product function category 1 and 3 26
1.5.3 Process Requirements for CMC 3 and CMC 5 28
Part -II Scientific Article 30
Abstract 31
Riassunto 33
2 Introduction 34
3 Material and Methods 38
3.1 Experimental Design 38
3.1.1 Treatment and Replicates 38
3.1.2 Nutrient Solutions 39
3.1.3 Control Soil and Test soil 39
3.1.4 Plant specie 40
3.2 RHIZOtest Bioassay 41
3.2.1 Preculture Period 41

3.2.2 Test Culture Period 43
3.2.3 Harvests of plants 44
3.3 Plant and soil analysis 45
3.3.1 Concentration and fluxes in plants 45
3.3.2 Statistical analysis 47
4 Results and Discussion 49
4.1 Plant Biomass 49
4.2 Trace Element Concentrations 51
4.3 Net Uptake Flux 53
4.4 Bio-concentration Factor and Translocation Factor 54
4.5 Conclusion 55
Part-III Data 56
Appendices I
A Experimental Design I
B Concentration Values Before Exposure IV
C Concentration Values After Exposure V
D Flux, Bio-accumulation and Translocation Factor VII
E Statistical Analysis. VIII
Bibliography X

Figures

Figure 1.1 - Component material category (CMC); CMC3 and CMC5 24
Figure 1.2 -.PFC1 as fertilizer and PFC3 as a soil improver 25
Figure 3.1 - RHIZOtest short procedure 41
Figure 3.2 - Pot assembly for preculture phase. 42
Figure 3.3 - Assembly Setup for Test culture phase 43
Figure 4.1 - Mean dry weight (g) of roots and shoots of RHIZOtest plants after exposure 49
Figure 4.2 - (Trace) elements extractible concentration (mg kg^{-1} d.w.) of soil matrix 52

Figure 4.3 - Net uptake flux (ng m^{-2} s^{-1}) for trace elements in the RHIZOtest plants....................53

Tables

Table 1.1 - Reporting pertinent research findings from the scientific literature............................. 6
Table 1.2 - Limit values of contaminants (metals, pathogens) in organic fertilizer 26
Table 1.3 - Criteria requirements for the nutrients for organic fertilizer..27
Table 1.4 - Criteria requirements for soil improvers..27
Table 1.5 - Process criteria for compost and digestate 28
Table 1.6 - Environmental and safety criteria for a component material category 29
Table 3.1 - Nutrient solution employed at various steps of the bioassay.phases 39
Table 4.1 - Concentrations of TEs in Tomato plant shoots and roots...51
Table 4.2 - Bioconcentration(shoots, roots) and Translocation factor for trace elements.........54

Part-I Theoretical Background

This part provides background information on absorption/uptake in plants, why it is critical for fertilizers, and how we may assess and analyze it. Additionally, a concise description of the new fertilizing product Regulation (EU) (2019)/1009, with a focus on organic fertilizers such as digestate other than fresh crop digestate and compost, is provided. Besides, this section also highlights the procedure criteria and requirements for the product function category PFC 1, as well as the component material categories CMC 3 and CMC5.

1 General Introduction

The quality of life has been endangered due to countless technological advances, industrialization and human activities. As industrialization and urbanization accelerate, anthropogenic waste detritus such as industrial wastes, mining wastes, biosolids, manure application, pesticides, fertilizers, toxic chemicals, and wastewater rise, eventually penetrating the soil ecosystem (Rehman *et al.*, 2021). Furthermore, the use of various chemicals for a variety of purposes, including plant protection, has increased. Most chemicals and their derivatives, on the other hand, end up in soil and water, where they interact with the surrounding environment and living species, eventually disrupting the natural equilibrium of the elements in different compartments, including soil.

Emerging contaminants, also known as Contaminants of Emerging Concern (CEC), can include a wide range of chemicals, such as agricultural products, engineered nanomaterials, pharmaceuticals, personal care or household cleaning products and lawn care products, to name a few. These contaminants produced by wastes find their way into rivers, lakes or soil environments, potentially bioaccumulating up the food chain, putting animals and humans in danger if they consume contaminated food (Pullagurala *et al.*, 2018).

1.1 What is uptake?

Uptake refers to the passage of a contaminant into an organism (e.g., plants), which may occur via numerous pathways and involve one or more parts of the organism. The process begins with contact with cell surfaces and tissues (Newman, 2014).

Translocation refers to the transport of chemicals from the point of uptake to other plant components. This ability of the chemical of translocating can be expressed as **Translocation Factor** (TF) (Liu *et al.*, 2021). According to

Bioaccumulation refers to the net accumulation of a pollutant in an organism from multiple environmental sources, including water, air, and solid phases. Food, soil, sediment, or fine particles suspended in air or water are examples of solid phases (Newman, 2014). In another definition

Bioaccumulation Factor (BAF) is defined mathematically as the ratio of the chemical concentration in the organism to that in the surrounding medium (e.g., soil, sediment) (Arnot & Gobas, 2006; Newman, 2014).

$$BAF = \frac{\text{the concentration of chemical equilibrium in an organism (wet weight)}}{\text{mean concentration of a chemical in the reference source (soil or sediment)}}$$

Bioconcentration Factor (BCF) is the net accumulation of a pollutant from water alone. Under controlled conditions, it is measured in laboratory tests (Arnot & Gobas, 2006; Newman, 2014). Bioconcentration Factor (BCF)can evaluate the content of contaminants in organisms, while the translocation factor can measure the concentration of contaminants transferred from one component to another (e.g., from roots to shoots). According to

1.2 Uptake of chemicals by Plants

Plants are found at the bottom of various food chains and are the primary producers of food. In addition, plants are used as a source of food by humans. Thus, they supply vital nutrients for a well-balanced diet, but they can be toxic if they absorb and accumulate harmful pollutants in the soil. These harmful contaminants can build up in their roots, shoots, or both. Since soil is one of the primary sinks for waste chemicals, these contaminants may deliberately or accidentally enter the food chain via different paths, e.g., using pesticides in crop farming, or may transfer into plants from sewage sludge and manure amended soils used as fertilizer in agricultural activity.

Given that plants are an essential component of both animal and human diets, assessing the uptake and accumulation of potentially dangerous organic pollutants in plants is critical for risk assessment. In addition, although pesticides have been used on plants for a long time in agricultural production, other chemicals have just recently gained attention. As a result, plant accumulation is essential for monitoring contaminants spread through a food chain.

1.3 Why is plant uptake crucial for fertilizers?

Plant uptake is essential in assessing how the chemical pollutants present in various fertilizers can be transferred to the food chain. Different organic or inorganic pollutants found in fertilizers, such as salts, Persistent Organic Pollutants (POPs), CEC, and HM, as well as their breakdown products, are taken up by plants, limiting their translocation, runoff, and volatilization. The process of plant uptake is important for bioremediation of contaminated sites and for residues present in food crops that are potentially bioaccumulating up the food chain, putting animals and humans in danger if they consume contaminated food.

Understanding how these pollutants are taken up and translocated by plants (primarily in food crops) is also crucial for building robust models to estimate their accumulation in agricultural products and possible human exposure (Liu *et al.*, 2021). Particular investigation demonstrates that phytotechnology may be uniquely designed for effective exposure avoidance in many applications where plants may be deployed as sensors to identify environmental contamination and possible hazards. Moreover, contaminant transport and fate in various media, such as groundwater, sediment and air, play an essential role in understanding bioavailability and bio-accessibility (Henry *et al.*, 2013).

1.4 How to assess plant uptake and bioaccumulation

Agricultural soils (or all soils) act as a repository for a variety of organic and inorganic contaminants, depending on their source. Soil contamination occurs as a result of both intentional uses of fertilizers containing various agrochemicals and incidental contamination from industrial waste release, irrigation with wastewater or grey water, amendments containing heavy metals and other pollutants-laden sludge (soil conditioning to simulate plant growth), or as a result of atmospheric fallout. Concerns about these pollutants interacting with plants pose two issues: first, are these pollutants absorbed by plants? Second, if they are absorbed, do they stay inside the tissues of the plants? The movement of elements inside the plants is described substantially by two processes, in particular, uptake which is root acquisition of soil components and transport which is the translocation of roots to above-ground tissues. While the magnitude of contaminant translocated by plants is generally species-neutral, not all elements taken up by the roots reach the plant's upper tissues (Su & Liang, 2011).

Predictions regarding the bioaccumulation of these contaminants in fertilizers have prompted the scientific community to investigate their potential impacts on soil-grown plants as well as hydroponic plants. Although plants absorb elements and other nutrients from the soil solution, the subject of root absorption and translocation of these chemical contaminants remains unresolved. Numerous research published since 2009 has documented the absorption and transfer of contaminants from agricultural fertilizer sources. This section discusses current research on the processes of uptake and translocation of various chemical pollutants. *Table 1.1* provides the literature review on the related topic.

Table 1.1 - Reporting pertinent research findings from the scientific literature.(Missing or not reported information is indicated by n/a)

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Liu *et al.*, 2021)	Wheat (Triticum aestivum L.)	n/a	■ Imidacloprid ■ Dimethoate ■ Fosthiazate ■ Pirimicarb ■ Atrazine ■ Chlorantraniliprole ■ Ethoprophos ■ Triadimefon ■ Tebuconazole ■ Flusilazole ■ Difenoconazole	■ **Typology:** Hydroponic ■ **Scale:** Growth chamber ■ **Nr. of controls:** 2 (1 plant-free control -spiked solution only- and pesticide-free control - only plant on Hoagland solution-). ■ **Nr. of tested dosages:** 1 (100 ng/L) ■ **Nr. of replicates per dosage:** 3 ■ **Nr. of individuals per replicate:** 60 seedlings ■ **Size of reactor/container replicate:** 6 L (30cmX24cmX10cm) ■ **Nr. of plants in one analyzed sample:** 6 ■ **Direct Measures:** Six plants were taken out of the solution as one sample and three replicates were performed at time intervals of 2, 6, 12, 24, 48, 72, 96, 120 and 144 h	Seeds were sterilized with a solution of 5% sodium hypochlorite solution for 10 min and then rinsed with deionized water. After imbibing in deionized water for 16 h, the seeds were germinated in polyvinyl chloride (PVC) seedling tray for 4 days	PVC box containing 6 L of Hoagland solution spiked with 100 ng/L of each tested chemical; Two control treatments were prepared including a wheat-free control (spiked so- lution only) to monitor the loss of pesticides and a pesticide-free control (wheat only). To avoid potential pesticide photolysis and minimize algal growth, the boxes were wrapped with aluminum foil and the gap between the lid and the wheat seedlings was filled with a sponge.	■ **Duration:** 14 days ■ **Procedure:** Transfer the seedlings to a PVC box with 6 L of half-strength Hoagland solution. The pH of the hydroponic solution was 6.5. ■ **Condition:** The container was put in a temperature-controlled growth chamber at 25/20 °C (day/night) with 60% humidity. A 16:8 h daily light cycle was used with 250 mmol/ m^2 s fluorescent light.	■ **Duration:** 2 to 144 h ■ **Procedure:** 60 seedlings (root length of 15 ± 1 cm; shoot height of 20 ± 1 cm) were transferred into each replicate. ■ **Condition:** Same as those in Pre-growth	One plant sample (6 individuals) was taken out of the hydroponic solution from each replicate at time intervals of 2, 6, 12, 24, 48, 72, 96, 120 and 144 h. A shoot and a root sample were derived from each plant sample. The hydroponic solutions (test replicates and controls) were also sampled at the same time interval. All the samples were stored at -20°C before analysis.	Pesticide concentrations in sampled shoots, roots and hydroponic solutions. Pesticides extraction by a modified QuECHERS method and quantification by an LC-MS/MS system. Water, lipids and carbohydrates contents of root samples.	■ Uptake kinetics of pesticides in plant $\mathbf{C_{tissue\,(t)} = C_{tissue,eq}(1-e^{-kt})}$ $C_{tissue\,(t)}$ = concentration in sample at time t $C_{tissue,eq}$ = equilibrium concentration k = uptake rate constant (per hour). ■ RCF and TF $\mathbf{RCF = C_{root}/C_{water};\ TF = C_{shoot}/C_{root}}$ Where Croot, Cshoot, and Cwater are the concentrations of each pesticide in the root, shoot and solution samples. ■ Quasi-equilibrium factor ($\alpha_{pt.}$) to explore the relationships between the levels of pesticides in wheat plants and external water as a function of time: $\boldsymbol{\alpha_{pt} = (C_{pt}/C_w)/(f_{pw} + f_{ch} * K_{ch} + f_{lip} * K_{lip0}}$ f_{pw}, f_{ch}, and f_{lip} =%wt of water, carbohydrates and lipids in the root on the basis of fresh weight; K_{ch} and K_{lip} = the carbohydrate-water partition coefficient and the lipid-water partition coefficient of each compound.

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Dal Ferro *et al.*, 2021)	■ Lettuce (Lactuca sativa L., var. redial) ■ Spinach (Spinacia oleracea L., var. hunter F1)	Alternative fertilizers: Municipal wastewater treatment plant (WWTP) effluents	Perfluoroalkyl acids Perfluoroalkyl carboxylic acids (PFCAs): ■ PFBA, ■ PFPeA, ■ PFHxA, ■ PFHpA, ■ PFOA, ■ PFNA, ■ PFDA, ■ PFUnA, ■ PFDoA, ■ PFTrA, ■ PFTeA Perfluoroalkyl sulfonic acids (PFSAs): ■ PFBS, ■ PFHxS, ■ PFOS	■ **Typology:** Hydrophonic ■ **Scale:** Greenhouse ■ **Nr. of controls:** 1 (Solution without PFFAS) ■ **Nr. of tested dosages:** 3 (1st with PFAAs-spiked drinking water solution of 500 ng L-1-worst case scenario; 2nd and 3rd from two WWTPs with the presence of PFAAs concentration of 100 and 600 ng/L) ■ **Nr. of replicates per dosage:** 12 as growing PVC pipes (6 for each specie i.e., 6 for lettuce and 6 for spinach) ■ **Nr. of individuals per replicate:** 10 ■ **Size of reactor/container replicate** 4 modules; each module consisted of 12 growing PVC pipes (2m long, 1 m above ground level; Pipes diameter = 10cm, water depth = 5 cm) and contained 120 pots each. ■ **Nr. of plants in one analyzed sample:** 60	n/a	Every solution was stored in a 350 L plastic tank. Solutions were enriched with the fertilizers required by crops according to regional guidelines for soilless cultivation to keep macronutrients and micronutrients homogeneous among tested treatments. The flow rate of the solutions was constant at 0.6 L min-1.t	■ **Duration:** 3 weeks ■ **Procedure:** ■ **Condition:** No information on the procedure provided.	■ **Duration:** 45 days for lettuce; 55 days for Spinach ■ **Procedure:** Plants were pre-grown in soil, then transferred to hydroponics and replanted into mesh pots with expanded clay Leca. The test solutions flowed through PVC pipes from an accumulation tank. Each tested water solution was recirculated from an accumulation tank back to the tank through a 12 V water pump. With the spiked nutrient solution and those from the WWTPs, the plastic tanks were refilled twice for a total of 100 L. ■ **Condition:** The greenhouse temperature was 12°C (minmax = 5–30°C) and relative humidity was 70% (minmax = 40–90%) during the experiment.	After harvesting, plants were split into shoots and roots. The spinach rosette was sampled without separating the leaves of the lettuce. Samples were cleaned with deionized water, dried briefly, weighed separately to assess fresh biomass, deposited in polypropylene containers, and heated to 65°C in a UF260 type oven with forced air circulation. Later, the dry biomass was weighed individually.	Twelve shoots and roots per treatment were randomly selected for chemical analysis. Eleven perfluoroalkyl carboxylic acids and three perfluoroalkyl sulfonic acids were determined weekly in the hydroponic solution, sampled before entering the PVC pipe, as well as quantified at the end of the growing cycle in crop roots and shoots. The selection of compounds was due to their frequent presence in groundwater and surface waters by local authorities.	Quantification Bioconcentration Factor ■ Roots concentration factor **RCF =** $\mathbf{C(PFAA)_{roots}/C(PFAA)_{solution}}$ ■ Shoots concentration factor (**LCF**) **LCF =** $\mathbf{C(PFAA)_{shoots}/C(PFAA)_{solution}}$ ■ Root-shoot translocation factor (**TF**) **TF =** $\mathbf{C(PFFA)_{shoots}/C(PFFA)_{roots}}$ Crop PFAAs concentrations were expressed on a dry weight basis. No loss of PFAAs degradation or volatilization was considered

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Akenga *et al.*, 2021)	Lettuce (Lactuca sativa)	n/a	Antivirals and Antiretrovirals (ARVDs): ■ Lamivudine (LVD) ■ Nevirapine (NVP) ■ Efavirenz (EFV) ■ Oseltamivir (OSV) ■ Phosphate.	■ **Typology:** Hydroponic ■ **Scale:** Greenhouse ■ **Nr. of controls:** 1 ■ **Nr. of tested dosages:** 3 (in total 4 including control with unspiked nutrient solution) ■ **Nr. of replicates per dosage**: 6 per exposure (24 in total) ■ **Nr. of individuals per replicate:** n/a ■ **Size of reactor/container replicate:** Glass container filled with aluminum foil ■ **Nr. of plants in one analyzed sample:** n/a	n/a	■ **Standards and stock solutions:** Stock solutions and standards were prepared and stored following SANTE/11813/2017 (2018) guidelines of the European Commission. All stock solutions were prepared in a 50:50 (v/v) MeOH:HPLC water mixture. MeOH was used to make NVP and EFV stock solutions. The four ARVD combinations were diluted from 0.1 to 100 g L^{-1} in water.	■ **Duration:** 10 days ■ **Procedure:** Analora seedlings (10 days old) were purchased from Defland Nurseries in the UK. Preparing the plants for the exposure test required seven days of soaking in a diluted water-fertilizer solution (hydroponic).	■ **Duration:** 21 days ■ **Procedure:** Each seedling received 400 mL of the four ARVD mix standard nutrient solution (water and commercial fertilizer) (Flora Gro, NPK 3:1:6 at a concentration of 0.5 mL L-1). The nutritional solution only reached the roots since the sample containers (glass) were lined with aluminum foil and sealed. 10 minutes of aeration per hour. Day 7 and 14 exposure solution replacement ■ **Condition:** Relative humidity = 70/90% ± 5% (day/night) Fluorescent Light intensity 350± 50 mol/m2/sec, photoperiod 16:8.	After the examination, the plant samples were promptly washed with HPW and dried thoroughly. The roots and leaves of lettuce were weighed individually. The samples were then freeze-dried before extraction and analysis.	Potential physiological effects on the plant were assessed by comparing the biomass of the control (root and leaves) with the biomass ARVD exposed samples. Plant uptake and translocation test were conducted according to OCSPPC 850.4800.	The bioconcentration factor (BCF), root concentration factor (RCF), leaf concentration factor (LCF), and translocation factor (TF) were used to characterize ARVD uptake in this report. The organic analyte's movement from the root to above-ground tissues is quantified by TF. **BCF = C_{plant} / $C_{exposure\ solution}$** **RCF = C_{root} / $C_{exposure\ solution}$** **LCF = C_{leaf} / $C_{exposure\ solution}$** **TF = C_{leaf} / C_{root}** where C_{leaf}, C_{root}, C_{plant}, and $C_{exposure\ solution}$ is the API concentration in the leaf, root, plant and nutrient solution, respectively.

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Mousavi *et al.*, 2021)	Valerian (Valeriana officinalis L.)	■ Phosphate (PO4) ■ Methionine (Met)	■ Cadmium (Cd) ■ Zinc (Zn)	■ **Typology:** Hydroponic ■ **Scale:** Greenhouse ■ **Nr. of controls:** ■ **Nr. of tested dosages:** 2 ■ **Nr. of replicates per dosage:** 3 ■ **Nr. of individuals per replicate:** 4 ■ **Size of reactor/container replicate**: 5L ■ **Nr. of plants in one analyzed sample:**	The seeds were surface-sterilized with 1% H_2O_2 for 30 min and then rinsed three times in sterile water. The seeds were soaked in distilled water for 24 h, then germinated in wet filter papers in Petri dishes (within 3 days) at 25 °C, and thereafter transferred to a sand culture moistened with deionized water.	The nutrient solutions were prepared with greenhouse tap water having a pH of 6.9 and an EC of 0.74 dS m- 1.	■ **Duration:** 3 weeks + 2 weeks (in Cd-free nutrient solution) ■ **Procedure:** The seedlings were transplanted into small pots with phosphate solutions (900, 1200, and 1500 μM). The nutritional solutions in the growing containers were aerated and replaced twice a week. These solutions were kept between 5.8 and 6.0 by adding 0.1 M HCL or KOH as required. Then they were cultivated for two weeks in a Cd-free nutrient solution. ■ **Condition:** 12 hours of daylight, 26/30°C Day/night temperature, and 75/85% humidity.	■ **Duration:** 4 days ■ **Procedure:** After development in the cd-free nutrient solution, 72 seedlings were randomly chosen for Cd absorption studies. 4 plants per replication were moved to a 5-L plastic beaker and grown for 4 days in the same nutrient solutions as previously, but with 400 M or no methionine (Met) and 10 M Cd (NO3)2, resulting in a Met: Cd molar ratio of 40:1. To reduce positional non-uniformity, container placements were changed randomly every 24 hours. The containers were pre-sterilized with 5% NaClO to prevent fast Met breakdown in solution. Every two days, the solutions changed. ■ **Condition:** Same as in pre-growth of plants	After 4 days of exposure to the treatment solutions, the seedlings were harvested by cutting with the stainless-steel razor blade at the stem point leveled to the upper surface of plant supporting plates to separate roots from shoots. The shoots were rinsed in tap water and then washed three times with pure water and blotted dry with tissue paper, weighed and oven-dried at 65℃ for 72 h. After determination of dry mass, the plant samples were ground using a stainless-steel mill for elemental analysis.	Dried powder samples were burned in a muffle furnace at 500 ℃ for 6 h and then digested with 2 mL of 20% HCl (6 N) for 5 min at 60 ℃ on a heating block. The extract was cooled, filtered and finally diluted to a volume of 25 mL with distilled deionized water and stored in plastic vials until analyzed for Cd and Zn were by atomic absorption spectroscopy (AAS). Each root system was divided into two parts. One was directly oven dried just like the shoots, while the other was first washed with an EDTA solution to remove apoplastic Cd. The Cd remaining in the EDTA-washed roots was considered to be symplastic Cd, the removed Cd as apoplastic Cd. The amount of the latter was calculated as the difference between Cd in the root samples without and with EDTA-washing.	They investigated the effects of exogenous methionine (Met) and different phosphate (PO_4) concentrations on Cd uptake. The root-to-shoot translocation factor was determined as the ratio between the Cd content of the shoot to that of the EDTA-washed roots. **$TF = C(Cd)_{shoots} / C(Cd)_{root}$** Similarly; **$TF = C(Zn)_{shoots} / C(Zn)_{root}$**

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	Preparation of test replicates	Pre-growth of plants	Plants exposure	Plant harvest	Analysis	
(Wajid *et al.*, 2021)	Pearl millet variety (YBS–98)	Soil amender (Synthetic fertilizers and organic manure): ■ Poultry manure, ■ Cow manure ■ NPK	Trace Metals: ■ Pb ■ Ni ■ Cd ■ Mn ■ Zn ■ Fe ■ Cu	■ **Typology:** Soil ■ **Scale:** Botanical Garden ■ **Nr. of controls:** 1 ■ **Nr. of tested dosages:** 4 (NPK, poultry manure, cow manure, mix fertilizer respectively) ■ **Nr. of replicates per dosage:** ■ **Nr. of individuals per replicate:** 3 ■ **Size of reactor/container replicate:** 15 plastic (internal diameter of 42 cm and the height of 68 cm) ■ **Nr. of plants in one analyzed sample:** 8 **Collection of Soil and Livestock Manure:** The soil was taken from the plant nursery. Soil samples were air-dried and grounded fine. Later it was sieved to 2 mm and analyzed for physio-chemical parameters. The poultry manure and cow manure were taken from a poultry farm and dairy farm respectively. They were air-dried and kept in shadow at room temperature for subsequent chemical analysis. After drying they were also passed through a 2mm sieve before analysis.	Seeds were germinated but no further details on the germination process are reported.	15 plastic bags were taken and filled with a mixture of soil and different types of organic manure at 3:1 (7.5 kg soil and 2.5 kg manure) and let mineralize for 2 weeks and bags without manure treatments were filled with 10 kg soil. Two types of organic manure (poultry manure and cow manure) and chemical fertilizers (urea 0.55 g N/kg, superphosphate 0.51 g P/kg and sulfate of potash 0.26 g K/kg) were used in different combinations: ■ T_0: Control (Without chemical fertilizers/organic manure) ■ T1: Chemical fertilizer (NPK @ 0.66 + 0.51 + 0.26 g/kg) ■ T2: Poultry manure (PM @ 2.5 kg/pot) ■ T3: Cow manure (CM @ 2.5 kg/pot) ■ T4: Mix fertilizer [MF (PM @ 1.25 kg/pot, CM @ 1.25 kg/pot, NPK @ 0.66 + 0.51 + 0.26 g/kg)].	n/a	■ **Duration:** 3 months ■ **Procedure:** The sowing was done in the 1st week of July 2017. Eight seeds of pearl were grown in each plastic bag. A full dose of phosphorus and potash and half of the nitrogen was applied at the time of sowing, while the remaining half N was applied at the panicle development stage. Plants of all treatments were watered equally. Some plants at the end of germination were removed from each plastic bag for the proper growth of the remaining plants. ■ **Condition:**	Harvesting was done in the first week of October 2017. At harvest, pearl millet plants were separated into roots, shoots, panicles, and grains. The grains were separated by hand shelling. The root, shoot, and grains of plants were put in a brown paper envelope and dried in an oven at 72 °C for 2 days. **Collection of Post-harvest Soil Samples:** The soil sample was collected from each plastic bag with the help of the auger. 15 soil samples were taken from 0–30 cm of the soil profile. Soil samples were air-dried for several days and crushed with mortar and pestle and passed through a 2 mm sieve. The sieved samples of soil were stored in polythene bags and oven-dried at 72 °C for 2 days.	The digestion of soil and plant samples was done by the wet digestion method. Metal content in soil and pearl millet samples was analyzed by atomic absorption spectrophotometer. Precision and accuracy of analyses were guaranteed through repetitive samples against the National Institute of Standard Technology, Standard reference material (SRM 2709 for soil, CRM-NIST 1567a for cereals) for all metals. The glasswares were placed in 10% nitric acid overnight and rinsed several times with distilled water before using them to prevent them from contamination.	Bioaccumulation factor (BAF): **BAF = Concentration of metals in grains / Concentration of metals in soil** Translocation factor (TF): **TF = Concentration of metals in shoot / Concentration of metals in root** **TF = Concentration of metals in grains / Concentration of metals in shoot** The pollution load index (PLI): **PLI = Concentration of metals (mg/kg) in examined soil / Concentration of metals (mg/kg) in reference soil** Daily intake of metals (DIM) and health risk index: **DIM = (Concentration of metal in grains × Conversion factor × Daily intake of millet) / Average body weight** Health risk index: **HRI = Daily intake of metal / Oral reference dose**

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	Preparation of test replicates	Pre-growth of plants	Plants exposure	Plant harvest	Analysis	
(Beltrán *et al.*, 2021)	■ Lettuce (Lactuca sativa L.) ■ Radish (Raphanus sativus L.) ■ Tomato (Solanum lycopersicum L)	n/a	■ Atenolol ■ Carbamazepine ■ Triclosan alone and combined with perfluorooctanesulfonic acid (PFOS)	■ **Typology:** Soil and Hydroponic ■ **Scale:** Farm field for soil; Chamber for hydroponic ■ **Nr. of controls:** 1 for every 3 treatments for soil setup; 1 for each treatment for hydroponic setup ■ **Nr. of tested dosages:** 3 per specie for soil experiment ■ **Nr. of replicates per dosage:** 2 for every 3 treatments for soil setup; 9 for each treatment for hydroponic setup ■ Nr. of individuals per replicate: ■ **Size of reactor/container replicate:** 25 L capacity pots (35 cm diameter, 30 cm deep); AeroFlo-10 system for hydroponic with 50L of nutrient Sol. in a reservoir tank ■ **Nr. of plants in one analyzed sample:** 9 for radish, 2 for lettuce and 3 for tomato (for soil setup)	For hydroponic setup: The radish, lettuce, and tomato seeds were germinated in a hotbed for 7–10 days. **[Soiless Setup]:** A system called AeroFlo-10 was used to grow plants. When the plants had two cotyledons and roots were 2 cm long, they were moved to their growing chambers. Plants were left in each chamber, and perforated foam was used as a medium to support them. 50 L of nutrient solution (renewed weekly) was put in the reservoir tank to pump to the growing chambers by a pump.	Radish seeds: 9; lettuce seeds: 2; tomato seeds: 3. Every single one of them was sown and grown in a 25 L pot that had 20 kg of soil inside. **Soil:** The soil comprised 50% topsoil, 30% mulch, and 20% river sand, sieved to 6 mm and air-dried. Mineral nutrients with an NP-K ratio of 15-15-15 (i.e., 3.5 g for radish, 10 g for lettuce and 6 g for tomato) **Tap water:** ATN, CBZ and TCS were not found in the tap water for the first group. The second and third groups had their tap water fortified with ATN, CBZ, and TCS. The third group had their tap water fortified with PFOS. In a third group, the same. The PFOS concentration was chosen based on surveys of urban areas and surface water bodies.	n/a	■ **Duration:** 2 months ■ **Procedure: [soil setup]** The minerals nutrients are added to each pot every two months. Individual pots were irrigated every week to keep the soil moist, but not so much that contaminants would run off into the field. One group was irrigated with tap water; a second group was irrigated with TW fortified; a third group was irrigated with TW fortified and PFOS (10 g/L) added (TWF-PFOS group). All the pots of the same crop got the same amount of water. ■ **Conditions: (soilless growing chamber)** 17-27°C and 20-80% humidity. To get the right amount of light for the plants, LED lights (360 W, 13,000–20,000 lx, Orion 10) were used that were placed 85 cm above the ground.	■ **Soil setup:** Soil samples from each pot were collected with a tubular soil sampler. All soil samples were stored at - 20 °C until chemical analysis. The radish, lettuce and tomato plants from the three treatment groups in the soil experimental set were collected after 48, 50 and 150 days, respectively. ■ **Soilless Set:** The collection was done on day 21. No tomato fruits were collected here due to the insufficient time for the plants to develop any fruits and to the inherent limitations of the culture system to grow plants with large fruits. The different parts of the plants were rinsed with Milli-Q water, patted dry with a paper towel, the biomass recorded, ground, lyophilized and stored at - 20 °C for later chemical analysis.	The ECs, (ATN, CBZ and TCS) were extracted from soils by an ultrasonic solvent extraction method. The supernatants were collected and evaporated to dryness at 40 and later analyzed by LC-MS/MS. Quantification of ATN, CBZ and TCS was carried out by liquid chromatography-tandem mass spectrometry. The limits of detection and quantification for each EC and were calculated as the concentration that gave a peak with a signal-to-noise ratio of 3–10.	Bioaccumulation factors (BAFs) for the three plant species (soil & soilless): **BCF (roots, leaves, fruits) = Concentration of ECs in plant organs (roots, leaves, fruits) / Respective Concentration in Nutrient Solution and/or soil** The translocation factor (TF) values in both experimental sets were calculated: **TF = Concentration of each EC in aerial plant parts (leaves -L- or fruit -F-) / Concentration in roots (R)** The human health risks associated with the presence of ATN, CBZ or TCS in the edible plant organs were assessed for both experimental sets. The daily human exposure of the three ECs quantified in the edible parts of the three plant species was calculated as: **HE = C10^{-3} * I** Where; **HE** is human exposure (mg/day), **C** the average concentration in the edible plant part (ng/g, w.w., **I** was taken as daily intake (g/day)

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Di Carlo *et al.*, 2020)	■ Lolium perenne (perennial ryegrass)	■ Bauxite Residue (BR) amended with gypsum	Trace elements: ■ Aluminum (Al) ■ Arsenic (As) ■ Chromium (Cr) ■ Vanadium (V)	■ **Typology:** Soil ■ **Scale:** Growth chamber ■ **Nr. of controls:** 1 (6 pots) ■ **Nr. of tested dosages:** 5 ■ **Nr. of replicates per dosage:** 8 ■ **Nr. of individuals per replicate:** 20 ■ **Size of reactor/container replicate:** 6L ■ **Nr. of plants in one analyzed sample:** 500 seeds	n/a	BR samples were obtained from the field to evaluate various restoration strategies. The treatments comprised uncontaminated soil and unrehabilitated BR. The control soil was locally available topsoil that did not have the normal BR features. Field BR samples (0–10 cm or 10–20 cm) were air-dried, sieved to less than 2 mm, manually homogenized, and chemically characterized. pH and EC were measured in a 1:5 aqueous extract. Ca, Cr, K, Mg, Na and V concentrations were measured by ICP-OES following pseudo-total aqua-regia digestion and extraction with ammonium acetate (for Ca, Cr, K, Mg, Na) or magnesium chloride (for V) (extractable fraction). The exchangeable sodium percentage (ESP) is the ratio of extractable Na to extractable bases (Na, Ca, Mg, K).	■ **Duration:** 2 weeks ■ **Procedure:** The seeds were germinated and the seedlings were grown in an aerated nutrient solution. The seeds density per plant pot was augmented (from 40 to 500 seeds) to increase roots biomass as preliminary experiments had small root biomass (approximately 0.4 g), insufficient for trace element analysis. The RHIZOtest was carried out according to the ISO protocol (ISO 16198: 2015).	■ **Duration:** 21 days ■ **Procedure:** Test soil was put in contact with the plant's planar root mat, which was grown on a polyamide mesh. The planar root mat was connected to a nutrient solution jar, (with three filter paper wicks). There were two glass microfiber filters placed between the soil and roots (along with the 30-mm polyamide mesh) to avoid root contamination while still letting water flow between soils and roots. The preliminary experiments had the migration of BR particles through the 30-μm polyamide mesh. ■ **Condition:** 25 ± 3 °C temperature; 75 ± 5% relative humidity; 200–400 μmol photons $m^{-2} s^{-1}$ photosynthetically active radiation, except for the light hours: 12 h instead of 16 h to avoid any sunburn.	At the end of the exposure period, plants were harvested, thoroughly rinsed with deionized water, roots separated from the shoots, oven-dried (3 days at 50 °C), weighed and digested in ultrapure nitric acid before elements analysis by ICP-OES. A certified reference material (ERM®-CD281, rye-grass) was analyzed as well to ensure a satisfactory percentage of elements recovery.	Trace elements in both roots and shoots at the end of an exposure period were also measured. The net uptake of trace elements in the whole plants during that time was also calculated.	Two indices were calculated to estimate the risk of elements transfer in the food chain, as well as the phytoremediation potential of L. perenne. The transfer coefficient (TC) was calculated as the ratio of the element concentration in the plant over the element concentration in the soil. **$TC = C_{plant}/C_{soil}$** The translocation factor (TF) was calculated as the ratio between the element concentration in shoots over the element concentration in roots. **$TF = C_{shoot}/C_{Root}$,** where; **C_{Root}** is the concentration of an HM in the roots, **C_{Shoot}** is its concentration in the leaves (mg/ kg), and **C_{Soil}** is its concentration in the soil (mg/kg). Correlation analysis between the chemical properties of the treatments and the endpoints of the bioassays were computed by Pearson correlation coefficients or Spearman correlation coefficients for normally and not normally distributed variables, respectively

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Eid *et al.*, 2020)	Arugula (Eruca sativa Mill.)	Sewage sludge (From WWTP)(The sludge was mixed with the soil at the rates of 0, 10, 20, 30, 40 and 50 g kg^{-1})	Ten heavy metals (HMs) ■ Cadmium (Cd) ■ Cobalt (Co) ■ Chromium (Cr) ■ Copper (Cu) ■ Iron (Fe) ■ Manganese (Mn) ■ Molybdenum (Mo) ■ Nickel (Ni) ■ Lead (Pb) ■ Zinc (Zn)	■ **Typology:** Soil ■ **Scale:** Green house ■ **Nr. of controls:** 1 (6 pots) ■ **Nr. of tested dosages:** 5 ■ **Nr. of replicates per dosage:** 6 per treatment ■ **Nr. of individuals per replicate:** 20 ■ **Size of reactor/container replicate:** 6L ■ **Nr. of plants in one analyzed sample:** n/a	n/a	Samples of soil and sludge were air-dried for 2 weeks and then ground and sieved through a 2-mm sieve. The sludge was mixed with the soil at the rates of 0, 10, 20, 30, 40 and 50 g kg^{-1}. Each plastic pot was filled with 4 kg of a certain treatment.		■ **Duration:** 40 days ■ **Procedure:** 20 E. Sativa seeds were sown on 2 January 2018 in each pot and left to grow for 40 days in the greenhouse. Periodic watering (using tap water) was carried out to maintain a similar moisture level in each pot. ■ **Condition:** Natural light conditions.	After harvesting, the individual plants were separated into their root and leaf components, oven-dried (at 60°C), and homogenized by grinding in a metal-free plastic mill.The soil-sludge mixtures were air-dried after plant harvesting and sieved through a 2-mm sieve in preparation for analysis.	The organic matter (OM) content was estimated in the soil-sludge mixtures by loss-on-ignition at 550°C for 2 h. The digested plant and soil samples were filtered and diluted with double deionized water to 25 ml. Blank samples were used to demonstrate the accuracy of the digestion process and subsequent analyses.Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to measure the ten HMs in both the plant and soil samples. The HM detection limits (μg/l) were as follows: 6.0 for Ni; 2.0 for Co, Cr and Cu; 1.0 for Fe and Zn; 0.3 for Mn and Mo; and 0.1 for Cd and Pb.	The bioconcentration factor (BCF) estimates the capacity of a plant to accumulate an HM (Heavy metal) in its roots, while the translocation factor (TF) was used to determine the ability of a plant to translocate an HM from its roots to its leaves:**BCF =** C_{Root}/C_{Soil}, while **TF =** C_{Leaf}/C_{Root}, where;C_{Root} is the concentration of an HM in the roots, C_{Leaf} is its concentration in the leaves (mg/ kg), and C_{Soil} is its concentration in the soil (mg/kg). The **correlation coefficients (r)** were calculated between the BCF and each of the soil pH and soil OM, between the HMs in the plant tissues and the HMs in the soil, as well as the soil pH and OM.

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	Preparation of test replicates	Pre-growth of plants	Plants exposure	Plant harvest	Analysis	
(Margenat *et al.*, 2020)	Lettuce (Lactuca sativa)	Organic amendments: ■ Sewage sludge (SS) from a wastewater treatment plant (WWTP). ■ The organic fraction of municipal solid waste (OFMSW) from a composting plant. (a mixture of pruning waste from the nearby area and organic waste from the Hospital Universitari Vall d'Hebron kitchen.) ■ Swine manure (SM)	Trace elements: ■ Cu; Zn; B; Co; Sr; Mn; Cd; Ba; Cr; Mo; Hg; As; Ni; Pb Antibiotics: ■ 8-hydroxyquinoline ■ Azithromycin ■ Chlortetracycline ■ Ciprofloxacin ■ Enrofloxacin ■ Lincomycin ■ Ofloxacin ■ Oxytetracycline ■ Sulfacetamide ■ Sulfadiazine ■ Sulfamethazine ■ Sulfamethizole ■ Sulfamethoxazole ■ Sulfapyridine ■ Sulfathiazole ■ Tetracycline [The ABs were selected based on their occurrence in organic fertilizers and wastewater.]	■ Typology: Soil ■ **Scale:** Glass greenhouse ■ **Nr. of controls:** 1 with chemical fertilization (with 5 repetitions each control) ■ **Nr. of tested dosages:** 3 (Dose 1- half the optimal N dose, dose 2- optimal N dose as the reference nitrogen dose, and dose 3- twice the optimal N dose) ■ **Nr. of replicates per dosage:** 3 (with 5 repetitions each replicate) ■ **Nr. of individuals per replicate:** n/a ■ Size of reactor/container replicate: 2.5 L amber glass pots (15 cm diameter, 20 cm high) ■ **Nr. of plants in one analyzed sample:** n/a	n/a	2.3 kg of soil sieved to 2 mm was deposited in 60 2.5L amber glass pots (15 cm diameter, 20 cm height) with an inverted bottle shape bottom outlet linked to drainage tubing (0.5 cm diameter). All treatments received the same amount of organic fertilizer supplied in each pot (100 kg of N per ha). This experiment used dirt from a farm in the Llobregat River Delta. The soil had a pH of 8.5, a texture of loam-clay, and an electrical conductivity of 0.24 dS/m. The total organic carbon content was 1.27 percent and the nitrogen level was 0.09 percent (Kjeldahl). On average, 33 mg/kg Olsen phosphorus was found in the soil, with 344 mg/kg K, 7014 mg Ca2, Mg2, and Na cations.	n/a	■ **Duration:** 57 days total (duration of greenhouse cultivation from October 8 until harvesting on December 4, 2018.) ■ **Procedure:** A variety of Batavia lettuce (Lactuca sativa L.) was sown in the pots. Drip irrigation with a reservoir of primary rainwater mixed with groundwater was employed. NH4NO3, P2O5, and K2O are reagent grade compounds, thus no trace elements such as heavy metals are foreseen. ■ **Condition:**	After the experiment, each mesocosm's lettuce leaf length and number were measured.	Trace elements (TE) from soil, antibiotics (AB) from soil and organic fertilizers, and AB from lettuce were isolated using UPLC-MS/MS. After the experiment, each mesocosm's lettuce leaf length and number were measured. -In situ measurements of chlorophyll and leaf weight A chlorophyll meter measured it. The lipid extraction was done in the lab.	Human health risk associated with the consumption of lettuces is amended with the aforementioned organic fertilizers. The potential risk to human health associated with the consumption of Trace elements in vegetables was assessed using the hazard quotient (HQ) **HQ = EDI / RfD** Where RfD is the reference dose, i.e., the maximum tolerable daily intake (g/kg bw/day) of a given metal without causing significant damage, and EDI is the estimated daily intake (g/kg bw/day), determined as follows: **EDI = DI. C_M / BW**

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Gredelj *et al.*, 2020)	Radicchio (Red chicory plants (Cichorium inybus L. var. foliosum Hegi), Chioggia type	n/a	Per- and polyfluoroalkyl substances (seven carboxylic and two sulfonic acids) Short-chain PFAAS: ■ Perfluorobutanoic acid ■ Perfluoropentanoic acid ■ Perfluorobutane sulfonic acid ■ Perfluorohexanoic acid; ■ Perfluoroheptanoic acid. Long-chain PFAAS: ■ Perfluorooctanoic acid; ■ Perfluorononanoic acid; ■ Perfluorooctane sulfonic acid; ■ Perfluorodecanoic acid;	■ **Typology:** Soil ■ **Scale:** Greenhouse ■ **Nr. of controls:** 6 (5 plant pots and 1 blank (no-plant) with loam agricultural soil and clean tap water) ■ **Nr. of tested dosages:** 2 (100 ng/gdw - 200 ng/gdw) ■ **Nr. of replicates per dosage:** 6 ■ **Nr. of individuals per replicate:** n/a ■ **Size of reactor/container replicate:** Round plastic pots (Φ = 25 cm) of 10 L nominal volume ■ **Nr. of plants in one analyzed sample:**n/a	n/a	Plastic pots were filled with spiked soil and left to settle for 10 days. Nine PFAAs were spiked into agricultural soil at nominal concentrations of 100 or 200 ng/gdw, and spiked irrigation water at nominal quantities of 1, 10, and 80 mg/L in each of twelve treatments. Soil spiking was done in phases, one for each treatment, with 8 cycles of PFAA matrix spike followed by 4 cycles of carrier solution solely for the control soil treatments. Each treatment combines PFAA exposure from irrigation water and pre-contaminated, spiked soil.	■ **Duration:** 4 weeks ■ **Procedure** Plants were grown from seeds in the soil in peat nursing pots. The most uniform-looking transplants were transferred to pots after they had formed 3-4 true leaves.	■ **Duration:** The growth period lasted 87 days (from transplanting) ■ **Procedure:** Water was only watered on the top soil to avoid direct contact with plants. The bottom pot holes were sealed with PFAS-free duct tape to prevent leaching. During the experiment, nutrient solution (Hoagland's solution) was provided three times with irrigation water. The health of chicory plants was maintained by periodic insect and fungal infection treatments. 6 mL each of solutions A and B and 1 mL of 45 percent phosphoric acid were added as nutrients. ■ **Condition:** The soil temperature ranged from 12.9°C to 34.3°C (average 22.3°C) and the greenhouse air temperature from 10.6°C to 57.5°C (average 26.0°C).	3 fully mature chicory plants were harvested per each treatment and split into roots, leaves and heads. Leaves and heads were washed with distilled water and stored in sealed plastic bags at -20 °C until the extraction. Roots were thoroughly washed under a water spray for 5 min each to remove all remaining soil and were air-dried before the extraction (water loss was accounted for by weighing).	The symmetrical halves of each box were used to construct PFAA concentration samples. Weighing samples and drying them at 65°C for 72 hours yielded the dry matter content. Before extraction, a whole-pot composite sample was collected and stored at 4°C. A cylindrical plastic sediment corer was utilized to collect vertical PFAA samples from agricultural soil. Three PFAA-rich water and/or soil treatments were chosen (i.e., with only contaminated irrigation water and clean soil, only spiked soil and clean irrigation water, and their combination). To fit the pot's top and bottom, each soil core was cut into two 10 cm sections. Each treatment comprised three pots, two with chicory and one without.	The Bioconcentration Factor (BCF) is the ratio of each PFAA's concentration in chicory roots, leaves, heads (and shoots) to the concentration in soil. ■ **BCF =** PFAA concentration in plant compartment / PFAA concentration in soil. ■ Roots concentration factor (RCF) **RCF =** C_{root} / C_{soil} ■ Leaves concentration factor (LCF) **LCF =** C_{leaves} / C_{soil} ■ Heads concentration factor (HCF) **HCF =** C_{heads} / C_{soil} ■ Shoots concentration factor (SCF) **SCF =** C_{shoots} / C_{soil} ■ Shoots concentrations $C_{shoots} = m_{head} * c(PFFA)_{head} + m_{leaves} * c(PFFA)_{leaves} / m_{head} + m_{leaves}$ m_{head}, m_{leaves} is mass of head and leaves respectively.

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Turull *et al.*, 2019)	Lettuces (Lactuca sativa L. cv. Batavia)	Amended agricultural peri-urban soils: ■ wood-based biochar at two rates (3% and 6%, w/w); ■ Compost at one rate (30% w/w).	Mercury (Hg)	■ **Typology:** Soil ■ **Scale:** Greenhouse ■ **Nr. of controls:** 1 ■ **Nr. of tested dosages:** 3 ■ **Nr. of replicates per dosage:** 5 ■ **Nr. of individuals per replicate:** n/a ■ **Size of reactor/container replicate:** 2.5L (17 × 15.5 cm) cylindrical pot ■ **Nr. of plants in one analyzed sample:** 1 seedling per pot	n/a	The soil sample used was taken from an agricultural site located in the peri-urban area of Barcelona (Spain). The sample was obtained from a mixture of 5 × 10 sub-samples taken from an area of 100 m2 with a depth soil horizon of 0-25 cm. Air-dried soil was sieved (<2 mm) to homogenize the sample. Afterward, the soil sample was mixed with wood-based biochar at two rates and compost at one rate. Pots filled with 2 kg of air-dried soil. The time of incorporation of the amendments in soil was 72 h before planting the seedlings.	n/a	■ **Duration:** 48 days ■ **Procedure:** Seedlings were planted in each pot filled with air-dried soil. Plants were irrigated manually every day with Tarssan nutritive solution (50–75 mL per pot, depending on the humidity). ■ **Condition:** During plant growth, the temperature and the amount of light was controlled. Temperature: 18-23 °C Light: 16h light and 8-hour dark	After 48 days of growth, when lettuce reached commercial size, the leaves and roots were harvested separately, and the fresh weight (fw) was determined along with the length of both. Then, leaves and roots were washed off with deionized water to remove any surface contamination and were dried in an oven at 55 °C	DGT manufactured in-house devices with polyacrylamide gel using both open and restricted diffusive layers (ODL and RDL, respectively) were used to determine organic and inorganic Hg labile species in soils. All the Hg analyses were performed using an Advanced Mercury Analyzer, model AMA-254. Water/soil mass phase ratio was calculated for Agriculture soil (AS), AS with 3% w/w of biochar (BC3), AS with 6% of biochar (BC6) and AS with 30% of biochar (BC30).	Bioconcentration factor (BCF) **BCF = Concentration $_{(Hg)}$ in roots / Concentration $_{(Hg)}$ in soil**

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Neu *et al.*, 2018)	■ Winter wheat (Triticum aestivum L. cv. Tiger)	Water treatment residues (WTR), are based on (hydroxides of Fe, and Mn. ■ WTR_A: Carbonate-enriched sludge (from Fe rich groundwater treatment) from Wittkoppenberg waterworks in Germany ■ WTR_B: Mn-rich sludge from Oborniki, Poland ■ LM amendment tested in parallel (in phytoremediation field) to Pot experiment.	Trace elements: ■ Cadmium (Cd) ■ Lead (Pb) ■ Zinc (Zn) ■ Arsenic (As)	■ **Typology:** Soil ■ **Scale:** Pot based ■ **Nr. of controls:** 1 ■ **Nr. of tested dosages:** 3 (in total 4 including control with unspiked nutrient solution) ■ **Nr. of replicates per dosage:** 6 per exposure (24 in total) ■ **Nr. of individuals per replicate:** 16■ **Size of reactor/container replicate:** Glass container filled with aluminum foil ■ **Nr. of plants in one analyzed sample:** 16 **Soil:** TE-contaminated agricultural topsoil (0-20 cm) Phytoremediation soil (Cont) from Freiberg, Saxony. Uncontaminated topsoil (Ref) from a nearby farm had TE concentrations within the region's background. An elemental comparison was made using this soil. These samples were taken at 420m elevation, 630mm annual precipitation and 8°C temperature.	n/a	The soil was homogenized, sieved to 4 mm, and steam-treated before use in the pot experiment. In the soil, WTR was administered at 0.5 and 1.0% by dry weight. WTRA treatments were referred to as A-0.5 (0.5%) and A-1 (1%). (B-0.5 and B-1). The field trial rate of 0.4 kg m-2 lime marl was used. All additives were sieved to 63 mm and carefully mixed with the soil. The substrates were put in 13-l white polyethylene vessels in four duplicates and irrigated with DI water to 70% field capacity for 7 days.	■ **Duration:** ■ **Procedure**: An initial number of 22 seeds per pot were sown in October 2013. No further details are provided. ■ **Condition:** During wintertime, pots were arranged outside in a sand bed for vernalization.	■ **Duration:** ■ **Procedure:** In early spring, the number of seedlings was reduced to 16 plants per pot, corresponding to plant densities in the field. Pots were randomly set up in greenhouses with filtered ambient air. The soils were fertilized with amounts corresponding to 90 kg ha-1 N (CH4N2O), 18 kg ha-1 P (P fertilizer with 40% P2O5), and 100 kg ha-1 K (K fertilizer with 60% K2O). A second N fertilization was done with (NH4)2SO4 corresponding to 42 kg ha-1 N during bolting. Water content was maintained close to field capacity by daily watering with (de-ionized (DI)) water.	When the flag leaf was fully developed, six leaves per pot were sampled across subjacent leaf levels to assess TE and nutrient status of the plants. During harvest, biomass was separated into grain, straw, and roots (captured by sieving). Fresh soil samples were sieved to < 2 mm as required by German legislation (BBodSchV 1999). Aliquots were air-dried for use in the earthworm experiment and chemical analyses of soil other than DGT (diffusive gradients in a thin film).	After the amendments were digested with aqua regia, the element concentrations of each one were determined Using aqua regia (DIN ISO 11466: 1997) and NH4NO3 standard techniques, the pseudo-total and plant-available element fractions of Con and Ref were analyzed (DIN ISO 19730: 2008). Before and after harvest, the soil's pH was analyzed (DIN ISO 10390: 2005). The remaining fresh soil samples were analyzed with DGT in separate steps for metals (chelex gel) and As (Fe oxide gel). To access the TE in soil solution (Csoln), the water-saturated soils were centrifuged and the filtered supernatant was analyzed by ICP-MS. **Plant** material was washed with DI (de-ionized) water and subsequently dried at 60 °C to constant weight. Samples were finely ground.	TE (Trace elements) bioavailability in soil, TE tissue concentration, and biomass of plants Treatment effects on element concentrations and plant biomass production were evaluated using one-way analysis of variance (ANOVA), followed by pairwise comparison using the Bonferroni post hoc test for adjustment of probabilities. Statistical analyses were performed using PASW Statistics 21 (SPSS, Inc., Somers, NY, USA). The dimensionless indicator for the extent of TE resupply from labile pools of the solid phase to the soil solution R was calculated as the ratio between CDGT and Csoln.

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Namiki *et al.*, 2018)	■ Hayadori-2 (Hordeum distinction L.), ■ Gold dent (Zea mays L.) ■ Fukuyutak (Glycine max Merrill and Phaseolus vulgaris L.) ■ Irodori (Brassica oleracea L. var. capitata) ■ Yokattana (Brassica rapa L. var. peruviridis) ■ Satoyutak (Chrysanthemum coronarium L.) ■ Sun valley (Lactuca sativa L.) ■ Jakkoh gold (Allium wakegi Araki) ■ Top seller (Apium graveolens L. var. dulce) ■ Magnet (Solanum lycopersicum Mill. and Capsicum grossum L. ■ Sharp-1 (Cucumis sativus L.) ■ Ebisu (Cucurbita maxima Duch.) ■ Summers (Spinacia oleracea L. and Beta vulgaris L. var. cicla)	n/a	■ Dinotefuran ■ Imidacloprid Clothianidin ■ Thiacloprid ■ Fosthiazate ■ Metalaxyl ■ Fenobucarb ■ Procymidone ■ Flutolanil ■ β-HCH ■ Tolclofos-methyl ■ Dieldrin	■ **Typology:** Soil ■ **Scale:** Greenhouse ■ **Nr. of controls:** ■ **Nr. of tested dosages:** ■ **Nr. of replicates per dosage**: 4 ■ **Nr. of individuals per replicate:** n/a ■ **Size of reactor/container replicate:** 600 mL ■ **Nr. of plants in one analyzed sample:**	Seeds of 16 species were germinated in a growth chamber (Koito Kogyo, Tokyo, Japan)	The organic chemicals were dissolved and mixed to a concentration of 50 mg/L in acetone. One liter of the mixture was mixed with 278 g of Celite® powder, and the acetone was allowed to evaporate for 4 hours at room temperature in a draft chamber. Since compounds became volatilized when the acetone evaporated. These organic chemicals were applied to a clean Andosol (soil composition, loam; pH [H_2O], 5.5; cation exchange capacity, 33.8 cmol/kg; organic carbon, 52.1 g/kg; and water-holding capacity [WHC], 747.1 mL/kg soil). Plastic pots were filled with prepared soil (450 g of uncontaminated soil mixed with 5 g of Celite®)	■ **Duration:** 7 days ■ **Procedure**: Plants of 16 species seeds were sown in nursery soil. ■ **Condition:** 20°C under a 14: 10 hr. light: dark cycle. 7–28 days, the seedlings were transplanted into pots and raised in the same Condition for 21 days.	■ **Duration:** 21 days ■ **Procedure:** The seedlings were transplanted into pots and raised in the same Condition for 21 days. Growth periods and plant densities were chosen to obtain approximately equal amounts of biomass so that the root dry weights were 1–2 g per species. The soil moisture was maintained at 50–70% water holding capacity (WHC) ■ **Condition:** Same as in pre-growth	21 days after transplanting, shoots and roots were harvested. The roots were washed in running tap water and sonicated in distilled water for 5 min to remove soil particles. For each sample, the fresh weight of shoots and roots was measured, and then cut finely, mixed, and divided into two subsamples. One subsample was dried at 70°C to measure the moisture content, and the other was used to measure organic chemical contents.	Analyzing organic chemicals in soil and the soil solution required two types of testing: liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the dinotefuran and clothianidin, and gas chromatography-mass spectrometry (GC-MS/MS) for the fenobucarb procymidone, flutolanil, and tolclofos-methyl in the purified samples. The ß-HCH and dieldrin in the purified extracts were measured by GC-high resolution MS. The assess the content of dinotefuran, imidacloprid, clothianidin, and thiacloprid as well as Fosthiazate, metalaxyl, fenobucarb, and flutolanil in the purified samples LC-MS/MS was employed. Procymidone was quantified by GC-ECD and GC-FPD measured tolclofos-methyl. The ß-HCH and dieldrin extracts were measured similarly to soil extracts. tested	Two kinds of BCFs to compare the uptake and translocation of plants, root concentration factor (RCF), shoot concentration factor (SCF). **RCF = C_{root} / $C_{soil\ solution}$** **LCF = C_{leaf} / $C_{soil\ solution}$** where C_{shoot}, C_{root}, and $C_{soil\ solution}$ is the concentration in the shoot, root, soil solution, respectively. Data of log KOW of the chemicals against the RCF or SCF were plotted to examine the relationships between the chemical properties and BCFs.

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Puschenreiter *et al.*, 2017)	Common wheat (Triticum aestivum cv. Tamaro)	Four soils originating from different locations namely; From Arnoldstein ■ ARN A ■ ARN D From Banská Štiavnica (SK), Slovakia. ■ SK From Redlschlag (REDL), Austria ■ REDL	Phytosiderophores (PS)	■ **Typology:** Soil ■ **Scale:** Greenhouse ■ **Nr. of controls:** ■ **Nr. of tested dosages:** ■ **Nr. of replicates per dosage: 5** ■ **Nr. of individuals per replicate:** ■ **Size of reactor/container replicate:** 6L; 34(inner diameter of plant pot) ■ **Nr. of plants in one analyzed sample:** n/a	■ **Duration:** 3d The seeds were surface sterilized with 6% (v/v) H_2O_2 for 10 min. seeds germinated in a nutrient sol. containing 600 μM $CaCl_2$ and 2 μM H_3BO_3. The floating tub filled with the aerated solution of 600 μM $CaCl_2$ and 2 μM H_3BO_3 contained the cylindrical pots closed with a nylon mesh size of 30um at the bottom.	Four soils were used. All soils were air-dried, passed through a 2-mm sieve and stored under dry and dark conditions until further use. Soils were incubated for 24 h in darkness at 20 °C with the soil contact solution for equilibration at 70% of MWHC.	■ **Duration:** 7d ■ **Procedure:** Plants were grown in different conditions: Parts were grown in a complete nutrition solution (sufficient Fe supply; +Fe) and the other half was grown in the same solution (deficient Fe supply; -Fe). The nutrient solutions were renewed every third day. ■ **Condition:** Temperature: 27°C/20°C day/night Light: 16 h photoperiod at 500 μmol m^{-2} s^{-1}	■ **Duration:** 10 days ■ **Procedure:** The plant containers were transferred onto soil discs (3–4 mm thick, 40.5 mm diameter) loaded with 4.5 g soil (dw). During the soil stage, a filter paper wick was used to apply the following nutrient solution: 50 μM KH_2PO_4, 2000 μM KNO_3, 2000 μM Ca $(NO_3)_2$, and 1000 μM $MgSO_4$. This solution was changed every two days ■ **Condition:** Same as for pre-growth	Plants were separated from the soil after exposure. Before the hydroponic and soil stages, deoxymugineic acid (DMA) release rates were calculated. Plants were harvested after 4 hours and the hydroponic solution was filtered via 0.45 mm syringe filters	5 replicates of each treatment were collected after two weeks of hydroponic growth to measure exudation rates and plant nutritional status before soil contact. One-fifth of the collected hydroponic solution was combined with 0.5 mL of an internal standard solution that contained 10 μM 13-C DMA. The DMA measurement was conducted by liquid chromatography-electrospray ionization tandem mass spectrometer (LC-ESI–MS/MS). The remaining hydroponic solution was frozen (-20C) before DOC was measured using a TOC analyzer.	Release rates of total Carbon and DMA were determined in the roots and shoots of the plants.

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	Preparation of test replicates	Pre-growth of plants	Plants exposure	Plant harvest	Analysis	
(Vittori Antisari *et al.*, 2015)	Tomato (Lycopersicon esculentum Mill.)	n/a	Metal oxide nanoparticles: ■ Cer-oxid (CeO_2) ■ Iron (II, III) oxide (Fe_3O_4) ■ Tin (IV) oxide (SnO_2) ■ Titanium dioxide (TiO_2) Metallic nanoparticles: ■ Silver (Ag) ■ Cobalt (Co) ■ Nickel (Ni)	■ **Typology:** Soil ■ **Scale:** Greenhouse ■ **Nr. of controls:** 1 ■ **Nr. of tested dosages:** 7 ■ **Nr. of replicates per dosage**: 6 (pots) ■ **Nr. of individuals per replicate:** n/a ■ **Size of reactor/container replicate:** 5L ■ **Nr. of plants in one analyzed sample:** n/a	n/a	The spiked solutions contained 20 g/ml Ag-, CeO2-, Co-, Fe3O4-, Ni-, SnO2-, and TiO2-NP solutions. For all NPs except silver, ultrasonic vibration (100 W, 40 kHz) was utilized for 30 minutes to disperse them in deionized water. Then, before watering the plant, 20 g/ml of NP elements were put into the soil near the root. The experiment did not employ fertilizer. Pots were filled with (5Kg of soil) containing model soil made of natural soil and peat (1:4 v/v).To ensure drainage, a 4 cm layer of quartz and feldspar sand was added to the pot.	n/a	■ **Duration:** 130 days (March 26 to August 4, 2012). This period corresponds to the vegetative cycle of tomato ■ **Procedure:** The seedlings (about 10 cm high) were placed in pots. A total of 48 pots (6 pots for each NPs) were placed in a randomized block; after 2 weeks of adaptation, the seedlings were spiked with spiked solutions once per week, twice from the 13th week, to simulate a chronic exposure to NPs supplied with irrigation. For the control test, only water was supplied. ■ **Conditions:** Photoperiod 11.5/13 h winter/summer. The maximum temperature in the greenhouse was set at 28 °C.	**Plant sample** After the growing cycle (130 days), each tomato plant was divided into shoots and roots The above-ground plant was washed with deionized water and oven-dried at 60 °C until constant weight to determine the dry mass and water content. The fruits were also collected, washed, frozen at -80 °C and lyophilized. **Soil sample:** Three tomato plants' soil was sampled: A 12 cm column of soil was sampled using a Plexiglas cylinder. The soil column had 4 layers, each 3 cm deep, with sand at the bottom. The rhizosphere soil samples were acquired by shaking the roots after soil drying and carefully collecting the aggregate remaining adhering to the roots. The roots were dried in the oven.	**Vegetal tissue analysis:** Using a modified US Environmental Protection Agency approach, dry tissues of various tomato organs were crushed and digested in a microwave using nitric acid and oxygen peroxide (USEPA 2009). Inductively coupled plasma spectrometry was used to determine the nutritional and metal content of leaves, stems, fruits, and roots. It was tested on reagent blanks and international reference materials (BCR-CRM 062) before use. Every ten samples of standard solutions (0.5 mg/L Ag) were also examined for quality control/assurance. **Soil analysis:** Three tomato plants' soil was tested. A 12 cm column of soil was sampled using a Plexiglas® cylinder. The soil column had four strata, each 3 cm deep, with sand at the bottom. The rhizosphere soil samples were air-dried. ICP-OES determined the metal content.	Translocation Index: The translocation index (TI) was calculated to synthesize the capability of the species to translocate nutrients and pollutants from roots to shoots: **TI =DML / (DMR + DMS + DML) *100** and **TI =DMS / (DMR + DMS + DML) *100** where DMR, DML and DMS are the element concentrations as a function of dry matters of roots, leaves and stem, respectively.

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	**Preparation of test replicates**	**Pre-growth of plants**	**Plants exposure**	**Plant harvest**	**Analysis**	
(Shtangeeva *et al.*, 2014)	Wheat (Triticum aestivum L.)	■ Chicken manure ■ Energen (natural stimulator)	Antimony (Sb)	■ **Typology:** Soil ■ **Scale:** Greenhouse ■ **Nr. of controls:** 1 ■ **Nr. of tested dosages:** 5 ■ **Nr. of replicates per dosage:** 3 (randomized) ■ **Nr. of individuals per replicate:** ■ **Size of reactor/container replicate:** Ceramic pots (20 cm top diameter) ■ **Nr. of plants in one analyzed sample:** 20 seedlings per pot	Seeds of wheat Triticum aestivum L. were germinated on wet filter paper for 5 days, and then uniformed germinated.	Ceramic pots were filled with soil (7 kg of soil in a pot). The soil was classified as Ferric Podzol with a sandy loam texture.Half of the plants were grown in Sb-free soil, and the other half was grown in soil spiked with 15 mg kg-1 of Sb as Sb (OH)2NO3Sb-free and Sb-spiked soils were divided into three parts. To the first part of pots, 100 mg kg-1 of dry chicken manure was added, and to the second part of pots, 20 mg kg-1 of Energen was added. The doses are recommended by the fertilizer's producers for this type of soil and this plant species.	n/a	■ **Duration:** 17 days ■ **Procedure:** Uniformed germinated seedlings were transferred to ceramic pots. There were ~20 seedlings in a pot.During the experiment, the soil pH value was 6.3±0.2. Soil water content was measured at the beginning of the experiment by soil moisture sensor 10HS. During the experiment, the soil water content was checked every day. To maintain the mean level of soil moisture (25%), the pots were watered daily by adding 300 mL of water per pot. Before seedlings were transferred to pots, soil samples (initial soil) were taken from all pots. ■ **Condition:** Information on light and temperature conditions not reported	Plants (together with the rhizosphere soil) were collected within 1, 6, 12, and 17 days after the transfer of seedlings to the soil.At the end of the experiment, the soil was also taken from the bottom of the pots to check for possible leaching of Sb to deeper soil layers. After sampling, the soil was air-dried up to constant weight. Plants were carefully washed with deionized water just after sampling, separated into roots and leaves, and also dried under room temperature to constant weight.	The ICP-OES and ICP-MS techniques were applied to determine the concentrations of macro- and trace elements in the plant and soil material. The accuracy of the measured concentrations was verified by determining the same elements in the certified reference materials (CRMs), tomato leaves 1573 and 1573a (National Institute for Science and Technology, USA), and marine sediment reference material PACS-2 (National Research Council, Canada). The results of the analysis of the CRMs showed a good agreement with certificated values (differences did not exceed 5-7%)	Bioaccumulation through correlation and cluster analysis statistically

REFERENCE	PLANTS TESTED	FERTILIZERS TESTED	CHEMICALS TESTED	EXPERIMENTAL DESIGN	EXPERIMENTAL STEPS						OUTPUTS
					Seeds preparation and germination	Preparation of test replicates	Pre-growth of plants	Plants exposure	Plant harvest	Analysis	
(Macherius *et al.*, 2012)	■ Barley (Hordeum vulgare)■ Meadow Fescue (Festuca pratense)■ Carrot (Daucus carota ssp. sativus) [4 cultivars of carrots: Napoli, Amager Rothild, Nutri-Red]	Sewage sludge	■ Galaxolide, HHCB (Polycyclic musk compounds 1,3,4,6,7,8 hexahydro-4,6,6,7,8-hexamethylcyclopenta-[g]-2-benzopyran);■ Tonalide, AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene);■ Triclosan, Antibacterial compound (5- chloro-2-(2,4-dichlorophenoxy) phenol)	■ **Typology:** Soil ■ **Scale:** Greenhouse ■ **Nr. of controls:** n/a ■ **Nr. of tested dosages:** 3 ■ **Nr. of replicates per dosage:** n/a ■ **Nr. of individuals per replicate:** n/a ■ **Size of reactor/container replicates** Each plant pot with 175 mm inner diameter and 210 mm high. ■ **Nr. of plants in one analyzed sample:** 5-6 in the case of all carrot types, 10 for barley, and 20 for meadow fescue ■ **Direct Measures:** The concentration of the target substances in the soils was examined before seeding (day 0) and after 49 and 119 days of plant cultivation.	Seeds were germinated and all pots were kept at 14 °C during germination.	**Soil:** The sandy soil used in the trials was air-dried and sieved to 4 mm before being mixed with a commercial slow-release fertilizer (3 g kg1 soil). The actual mineral composition was unknown. All chemicals were 95% pure and utilized for soil spiking and as reference compounds for analytical analyses. **Spiking Procedure**: The test substances from a stock solution made in acetone were spiked into 50 mL of acetone and added to the soil of each pot filled with 4 kg (dry weight) of soil. All was mixed thoroughly manually to adjust concentrations of 10 mg kg-1(dry weight) for HHCB, AHTN, and triclosan. **Condition:** Three days were required after spiking to allow leftover acetone to evaporate from the soil. The xenobiotic amounts used were based on calculated worst-case concentrations.	n/a	■ **Duration:** 119 days (entire cultivation period) ■ **Procedure:** After spiked, the pots were seeded. The incubation temperature was 14°C. It was then irrigated with water to maintain a water content of around 70% of the soil's water retention capacity, which was around 11%. Between the spiked pots were non-spiked carrot (cultivar Napoli) and barley plants to see if significant amounts of the examined xenobiotics were transmitted directly from the soil to leaf tissue. Most exposed plants developed slower than non-spiked controls, but they made up for it during cultivation. ■ **Condition:** A 16-hour day with 20°C Day and 14°C night temperature was set after germination The lighting was 350 mol/m2/day PPF with SON-T lamps equal to 30 mol/m2/day.	Plant components were harvested over two months depending on seed freshness and carrot and meadow fescue growth. The same pot supplied root and leaf samples. The roots were washed with tap water. Carrots were peeled (depth of 2 mm). The plants were dried in an oven for 3 days, at 40°C -50°C. Control and exposed plant materials were dried separately to avoid cross-contamination. For two weeks at room temperature, the dried plant samples were wrapped in paper bags and the soil in glass jars. Ultracentrifuge milling coarsely chopped and crushed dry samples A modified QuEChERS extraction technique was used to obtain these samples for GC-MS analysis. Ethyl	1 l of each extract was injected into a 6890GC-5973MSD-system for GC-MS analysis. Analyses of 3 parallel extractions were averaged and quantified using external standards. The soil concentration of target substances was measured before sowing, 49 and 119 days later. The total root xenobiotic concentrations were estimated as the sum of root peel and root core concentrations.	Bioconcentration Factor **BCF=** concentration in dry plant tissue / concentration in dry soil

1.5 Fertilizers

Fertilizers are in the broadest context any substance, natural or manmade, that is applied to soil or plants tissues to deliver nutrients to the plants or to increase the chemical and physical qualities of the soil to help plant growth, and production and quantity directly or indirectly. There are several sources of fertilizer, both natural and man-made (Scherer *et al.*, 2009).

Chemically fertilizers can be classified into mineral fertilizers, organic fertilizers and synthetic soil conditioners. Mineral fertilizers are composed of inorganic or synthetic organic compounds. Organic fertilizers are animal waste products (stable manure, slurry manure), plant decomposition products (compost, peat), or waste treatment materials (composted garbage, sewage sludge). Synthetic soil conditioners are substances whose primary purpose is to enhance the physical qualities of soils, such as friability and air movement. Whereas categories classified based on their nutritional content include conventional fertilizers containing a single main nutrient, compound fertilizers made up of a combination of main and micronutrients and micronutrient fertilizers which in comparison to macronutrient fertilizers include nutrients that plants require in trace amounts, dosages ranging from 1 to 500 g ha^{-1} a^{-1}. Finally, fertilizers can be classed as solid or liquid fertilizers, as well as soil or foliage fertilizers, the latter of which is supplied solely by spraying on an existing plant population (Kiiski *et al.*, 2016).

1.5.1 Fertilizer Regulations concerning Digestate and compost

The European Parliament Regulation (EC) No 2003/(2003), which almost entirely covers fertilizers derived from mined or chemically synthesized inorganic materials, has partly harmonized the internal market for fertilizers. However, not all fertilizing products are covered by this legalization. It does not recognize a clear framework to address the new concerns including environmental and material safety in organic fertilizer. Cadmium, uranium, and other potentially harmful elements are components of phosphorites, which means that mined mineral phosphate fertilizers may include potentially dangerous materials. Contaminants in EU fertilizers, such as cadmium, may pose a risk to human, animal, and plant health, as well as to the environment due to their accumulation in the environment and entry into the food chain. Concerning the preceding, the New EU 1009/2019 regulation will replace (EC) No 2003/2003 and will take effect on 16 July 2022. It will be fully binding and immediately applicable to member states including Italy. The present EU regulations do not apply to so-called “national

fertilizers" which are placed on the market by member states in conformity with their national law. Certain member states have comprehensive national legalization, whereas some others do not. The following summarizes the important elements of the new EU Regulation (2019)/1009:

- Opening the single market for bio-based fertilizers: The agreement on the regulation of the fertilizing product will facilitate the entry of new and innovative organic fertilizers into the EU single market by establishing the prerequisites for their entry.

- Safety and quality standards: The new legalization will establish rigorous standards for the safety, quality and labeling of all fertilizers to be traded across the EU. Before applying the CE mark, manufacturers must demonstrate that their products satisfy those standards.

- EU fertilizing products are classified into distinct product function categories (PFC), each of which should have its own set of safety criteria tailored to its intended purpose

- Component materials for EU fertilizing products are classified into distinct categories, each of which should have its processing criteria and control procedures. It should be allowed to sell an EU fertilizing product made of several component materials from several component material categories (CMC), provided that each component material conforms with the standards of the component material category to which it belongs.

- Introducing new contaminants limits values in fertilizers.

Compost is classified as CMC3 in Annex II of the new regulation, whereas non-energy crop digestate is classified as CMC5.

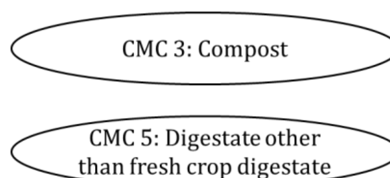


Figure 1.1 - Component material category (CMC); CMC3 and CMC5

PFCs are also subject to specific labeling criteria, which include fertilizers, soil improvers, growth medium, liming products, and bio-stimulants. Although the new fertilizer regulation specifies seven PFCs and eleven CMCs, only PFC1, PFC3, CMC3 and CMC5 are relevant to this study. Only a summary and crucial facts are provided for each of these categories for the reader's convenience. (*Figure 1.2*).

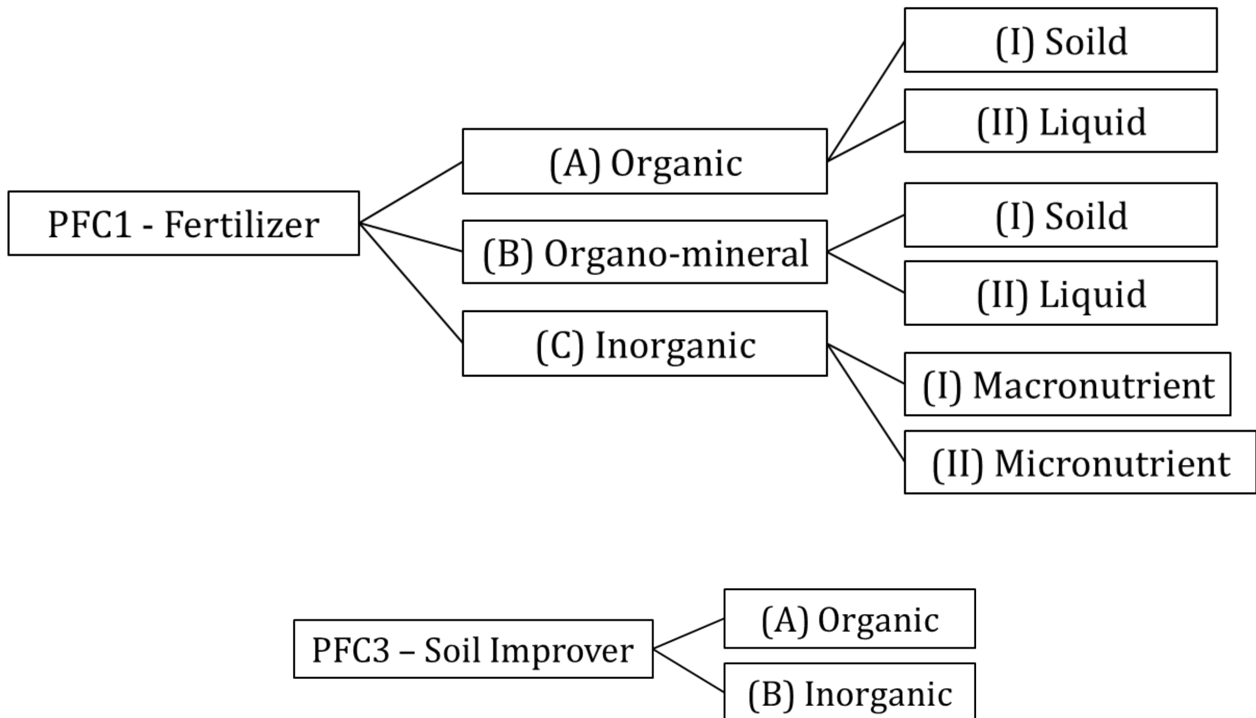


Figure 1.2 -.PFC1 as fertilizer and PFC3 as a soil improver

1.5.2 Criteria for Product function category 1 and 3

In the new regulation, the annexes define the quality standards for certain raw materials used in the production of fertilizers, soil improvers, and growing media. The specific requirements and criteria for compost and digestate products are based on technical guidelines (Saveyn *et al.*, 2014)(End of Waste Criteria for Biodegradable Waste Subjected to Biological Treatment).

Table 1.2 - Limit values of contaminants (metals, pathogens) in organic fertilizer PFC 1(A) and PFC 3(A)

Criteria	**PFC1(A) (I)/(II)**	**PFC 3(A)**
	Organic fertilizer	**Organic soil improver**
Cd (mg kg^{-1} dm)	1,5	2
Cr VI / Cr (mg kg^{-1} dm)	2	2
Hg (mg kg^{-1} dm)	1	1
Ni (mg kg^{-1} dm)	50	50
Pb (mg kg^{-1} dm)	120	120
Cu (mg kg^{-1} dm)	300	300
Zn (mg kg^{-1} dm)	800	800
As (mg kg^{-1} dm)	40	40
$C2H_5N_3O_2$ (g kg^{-1} dm)	absent	-
Salmonella spp.	absent	absent
E. Coli / Enterococcaceae (CFU g^{-1})	< 1000	< 1000

Along with standards for the manufacturing process and product quality, only separately collected organic waste is authorized as input material for composting and anaerobic digestion. An overview criterion of contaminants such as metals and pathogens is given in *Table 1.2* and is applicable for both PFC1 (Organic fertilizer category) and PFC3 (Organic soil improver).

The nutrient content in solid (category-I), as well as liquid (category-II) organic fertilizer for PFC 1(A), may contain only one declared primary nutrient. *Table 1.3* describes the required criteria for these nutrients.

Table 1.3 - Criteria requirements for the nutrients for organic fertilizer

	PFC1(A) (I)	**PFC1(A) (II)**
Criteria	**Solid**	**Liquid**
Corg	≥ 15 %	≥ 5 %
Nitrogen (N)	≥ 2,5 %*	≥ 2 %
Phosphorus (P_2O_5)	≥ 2 %*	≥ 1%
Potassium (K_2O_4)	≥ 2 % *	≥ 2 %
SUM (NPK)	(1/1/1) ≥ 4%	(1/1/1) ≥ 3%

* As a minimum, if only one of the basic nutrients is present (NPK)

Similarly, the content of dry matter and composition in the soil improver PFC 1(A) are listed in *Table 1.4*.

Table 1.4 - Criteria requirements for soil improvers

PFC3(A)	
Criteria	**value**
Dry matter	≥ 20 %
Corg	≥ 7,5 %
Composition	▪ An organic soil improver shall consist of 95% of material solely biological origin ▪ including peat, leonardite, lignite and humic substances obtained from them ▪ but excluding other materials which are fossilized or embedded in geological formations.

1.5.3 Process Requirements for CMC 3 and CMC 5

Only certain input materials are allowed for the CMC3 “compost” and CMC5 “digestate,”. Separated bio-waste, including animal by-product (ABP) category 2 and 3 materials and residues from the food processing industry, can be used as appropriate input materials. Sewage sludge and mixed municipal garbage are excluded as input materials. However, to use the ABP as input material for composting and anaerobic digestion (AD) the requirements of Regulation (EC) No 1069/(2009) have to be fulfilled. The precise process criteria for composting and anaerobic digestion are described in annex II of the legalization.

Table 1.5 - Process criteria for compost and digestate

Input material	Bio-waste, source-separated, ABP cat 2 and 3, excluding sewage sludge and mixed municipal waste Plus, a liquid or non-liquid microbial or non-microbial extract made out of compost; and Unprocessed and mechanically processed residues from food production industries, except ABPR materials
Process criteria for digestate	a) Thermophilic at 55 °C/24 h/hydraulic retention time of 20 days b) Thermophilic at 55 °C incl. pasteurization step 70 °C-1h c) Thermophilic at 55 °C followed by composting d) Mesophilic at 37-40 °C incl. pasteurization step 70 °C-1 h e) Mesophilic at 37-40 °C followed by composting
Process criteria for compost	70 °C ≥ 3 days 65 °C ≥ 5 days 60 °C ≥ 7 days 55 °C ≥ 14 days

In addition to process criteria, specific safety and environmental criteria concerning organic pollutants, impurities (glass, metals, and plastics) and stability are also required for the compost and digestate and are listed in *Table 1.6*.

Table 1.6 - Environmental and safety criteria for a component material category

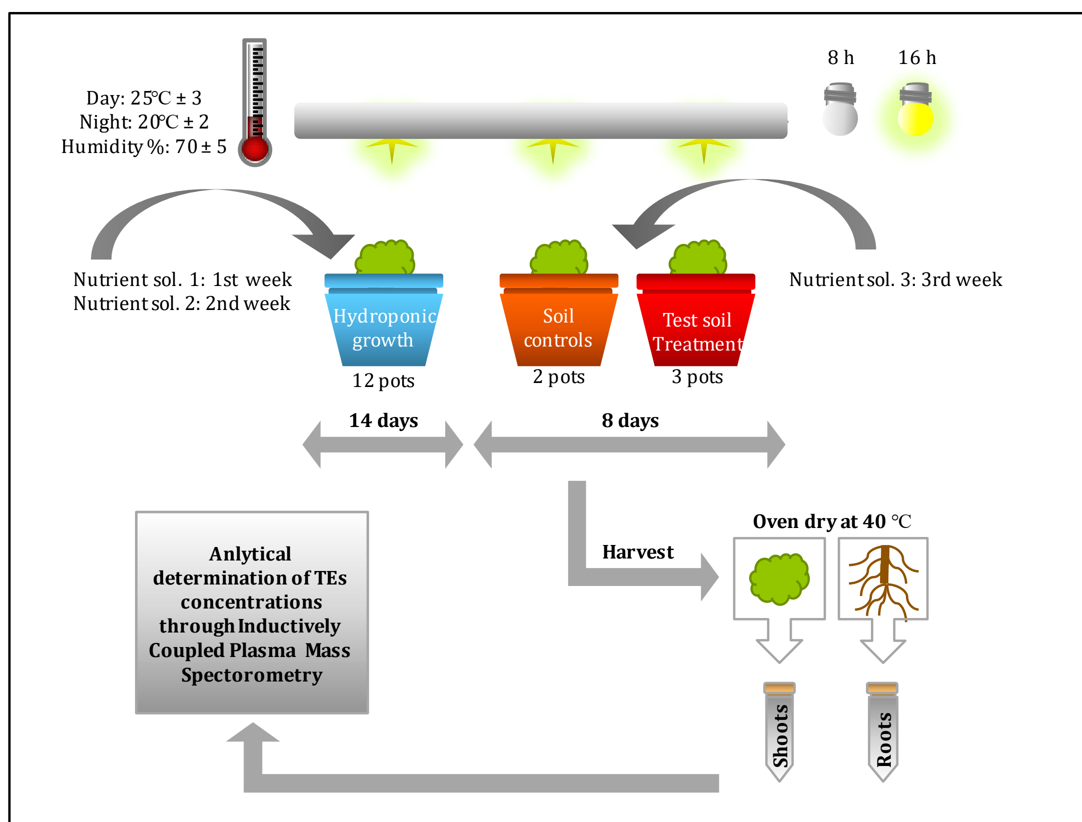
Criteria	Compost (CMC 3)	Digestate (CMC 5)
PAH_{16} (mg kg^{-1} dm)	6	6
Weed seeds (Seed L^{-1})	-	-
Impurities (% dm)	≤ 0,5[a]	≤ 0,5[a]
Stability	-	-
O2 uptake rate (OUR) (mmol O_2 OM^{-1} * h)	25	25
Residual Gas potential (liter biogas g^{-1} volatile solids) / organic acids (mg l^{-1})/Rotting degree	III/-/-	-/≤0,25/-

[a] Not more than 3 g Kg^{-1} dry matter of macroscopic impurities above 2 mm in any of the following forms: glass, metal or plastics.

Part -II Scientific Article

This part is named "scientific article" since it contains the abstract of the scientific study, a brief introduction, the materials and methods, and finally the results of the research experiment (RHIZOtest Bioassay) and conclusion.

Abstract



A graphic abstract illustrating the RHIZOtest Bioassay scheme.

This study investigates the uptake and transport of pollutants in tomato plants exposed to organic fertilizer (digestate). The new EU Regulation No. 1009/2019 will replace EU Regulation (EC) No. 2003/2003 on July 16, 2022, and will allow the incorporation of new and innovative organic fertilizers, including liming materials such as organic soil improvers and digestates derived from resources other than fresh crops. Thus, this research investigation examined the environmental bioavailability of trace elements (TEs), notably metals, to tomato plants using the RHIZOtest bioassay, in which plants were exposed to control and test the soil. The test soil was a combination of ordinary soil and digestate from the Organic Fraction of Municipal Solid Waste (OFMSW). The results suggested that when exposed plants were compared to control plants, their root biomass increased significantly ($p < 0.05$). The amounts of Cd (0.3 mg kg^{-1} d.w.), Cr, Cu, Ni, and Pb (5 mg kg^{-1} d.w.) in the control and treatment groups were equivalent to or less than the limit of quantification (LOQ). Despite the fact that the concentrations in the shoots were greater, there was a statistically significant difference ($p < 0,05$) in the mean Zn

concentrations in the roots of the plants. This indicates that the accumulation of Zn in shoots was facilitated. The decreased flux value (ng m^{-2} s^{-1}) also demonstrated that zinc was deposited in the plants' shoots. This was not true, however, for the remaining trace elements. While the results indicate that the risk of increased zinc accumulation in tomato shoots is quite low, additional studies with a greater number of replicates may be required to substantiate this observation and conduct efficient analysis, thereby improving standards and providing efficient modeling for uptake in plants.

Keywords: Plant uptake; Contaminants; Fertilizers; RHIZOtest; Rhizosphere; Translocation; Trace elements; Bioavailability.

Riassunto

Questo studio indaga l'assorbimento e il trasporto di inquinanti nelle piante di pomodoro esposte al fertilizzante organico (digestato). Il nuovo Regolamento UE n. 1009/2019 sostituirà il Regolamento UE (CE) n. 2003/2003 il 16 luglio 2022 e consentirà l'incorporazione di fertilizzanti organici nuovi e innovativi, compresi materiali calcificanti come ammendanti organici e digestati derivati da risorse diverse dalle colture fresche. Pertanto, questa ricerca ha esaminato la biodisponibilità ambientale di oligoelementi (TE), in particolare metalli, alle piante di pomodoro utilizzando il biotest RHIZOtest, durante il quale le piante sono state esposte al controllo e al terreno di prova. Il terreno di prova era una combinazione di terreno ordinario e digestato dalla frazione organica dei rifiuti solidi urbani (OFMSW). I risultati hanno evidenziato che la biomassa radicale delle piante esposte è aumentata in modo significativo ($p < 0{,}05$) rispetto a quanto rilevato sulle piane di controllo. Le quantità di Cd (0,3 mg kg^{-1} s.s.), Cr, Cu, Ni e Pb (5 mg kg^{-1} s.s.) nei gruppi di controllo e di trattamento erano equivalenti o inferiori al limite di quantificazione (LOQ). Nonostante il fatto che le concentrazioni nei germogli fossero maggiori, c'era una differenza statisticamente significativa ($p < 0{,}05$) nelle concentrazioni medie di Zn nelle radici delle piante. Ciò indica che l'accumulo di Zn nei germogli è stato facilitato. La diminuzione del valore di flusso (ng m^{-2} s^{-1}) ha anche dimostrato che lo zinco si è depositato nei germogli delle piante. Questo fenomeno non si è, tuttavia, verificato per gli oligoelementi rimanenti. Mentre i risultati indicano che il rischio di aumento dell'accumulo di zinco nei germogli di pomodoro è piuttosto basso, potrebbero essere necessari ulteriori studi con un numero maggiore di repliche per convalidare questa osservazione e condurre analisi efficienti, migliorando così gli standard e fornendo una modellazione efficiente per l'assorbimento nelle piante.

Parole chiave: Assorbimento delle piante; Contaminanti; Fertilizzanti, RHIZOtest; Rizosfera, Traslocazione; Oligoelementi; Biodisponibilità.

2 Introduction

Biological impacts are not proportional to the overall concentration of a pollutant in the soil, as established by laboratory and field research. Rather than that, an organism (in this case, referring to plants and/or crops) reacts solely to the percentage that is physiologically available to it (bioavailable). This is especially true in soils when contaminants interact with the soil matrix in such a way that the uptake and thus the accumulation is no longer obtainable by the organism or are present in an inaccessible form. The bioavailable portions of pollutants are determined by soil qualities and a variety of time-dependent processes, as well as by the biological receptors. The conservative method of exposure assessment, as it is often presented in regulatory contexts, assumes that the complete concentration of a contaminant in a soil or soil material is available for accumulation by organisms, including humans, and hence overestimates the risks. As a result, risk assessment may be optimized by employing a technique that is based on estimated exposure, which represents the accessible, effective concentration of the contaminants, and on existing underlying toxicity data (ISO 17402, 2011). Innovative methodologies for evaluating the uptake and bioavailability of contaminants in soils to plants cultivated on agricultural land fertilized with different fertilizing products, which may show the presence of pollutants, must be developed and verified. Plant Uptake and bioavailability of contaminants are required for the assessment of food chain contamination and phytotoxicity, i.e. toxicological bioavailability. (Peijnenburg *et al.*, 1997).

Fertilizers, as reported in several publications, were a critical component of the green revolution, resulting in a large rise in fertilizer output and use. While they supply crops with macronutrients and micronutrients are also high in heavy metals, radioactive compounds, and other pollutants, and so constitute a significant source of toxins in the soil and environment over time. For example, inorganic fertilizer application can have a detrimental effect on soil health by hardening the soil surface, lowering the pH of the soil, inhibiting microbial activities, adversely changing the physical and chemical characteristics of the soil, and therefore indirectly harming crop output. Several of the most frequently encountered problems as a result of widespread fertilizer use, such as soil acidification, salinization, groundwater contamination, eutrophication, crop yield reduction, greenhouse gas emissions, and air pollution, resulting in the degradation of natural resources, impeding sustainable food

production (Ju *et al.*, 2009). Generally, three key nutrients nitrogen (N), phosphorus (P), and potassium (K) account for the majority of the fertilizer sector, as these nutrients are required for crop yield. Nitrogen and phosphorus are regarded as the building blocks of any agricultural production system. In comparison to nitrogen fertilizer, which is produced chemically by reacting nitrogen from the atmosphere with hydrogen via natural gas, phosphate and potassium fertilizers are mostly produced through digestion and mining thus depleting the natural sources. Excessive nitrogenous fertilizer application frequently leads to a variety of losses, including leaching and volatilization, which not only affects nutrient use efficiency but also poses an environmental hazard. Nitrate is the primary pollutant found in water bodies where nitrogen fertilizers are applied in excess. In another study, the author reported that phosphorus is the second most abundant major nutrient taken up by plants via fertilizers. The primary issue with P fertilizers is their extremely poor usage efficiency, with a small amount of about 10 to 15% of applied fertilizer being used by the crop plant, while the rest stays in the soil or finds its way into water bodies, generating a variety of environmental concerns (Lun *et al.*, 2018). Furthermore, cadmium (Cd) is prevalent in phosphate fertilizers made from rock phosphate, and increased Cd accumulation has a detrimental effect on soil health. These pollutants may undergo chemical transformations, resulting in the formation of new compounds that may be harmful to the environment. In this framework, heavy metals (HMs) are readily absorbed by crops and tend to accumulate in the bodies of plants and animals. Furthermore, soil characteristics and management influence the fate of pollutants in determining the uptake by living organisms. The uptake of pollutants and their transport in the soil–water system is influenced by soil parameters such as texture, pH, organic matter, moisture content, temperature, and heavy metals.

The negative environmental effects posed by the use of conventional fossil-fuel-derived fertilizers may be mitigated by the extended use of organic or organic-based fertilizers. Organic fertilizers include animal excreta such as liquid manure, and slurry (farmyard manure). Green manures, mulch, as well as organic residual fertilizers and growing media such as composted biowaste, sewage sludge, growing media (peat), and fermentation residues (digestate), are also organic fertilizers. Among them, digestate has been recognized for its unique fertilizing properties due to its high nutrient content (N, P, K) and their availability, as well as its potential as a soil amendment and long-term significance in sustaining the economy.

Digestate is the by-product of anaerobic digestion (decomposition under low oxygen conditions) of a biodegradable feedstock. Anaerobic digestion (AD) yields two major by-products: digestate and biogas. Digestate is produced by both acidogenesis and methanogenesis, and each has distinct properties. These properties are a result of both the initial feedstock source and the procedures themselves (Peng & Pivato, 2019).

The agronomic use of digestate from OFMSW is allowed in compliance with the new European regulation of 2019 applicable in July 2019. Numerous studies have been performed in connection with the operation of Aerobic digestion (AD) plants to validate the digestate appropriateness for agricultural usage. One of the related studies evaluated the legal status of digestate from OFMSW with the help of statistical analysis for the quality assessment of the feedstock to the AD plant. The study determined the differences between the two digestate typologies (OFMSW versus AGRO) through statistical analysis. Upper confidence limits for the means (level of significance $\alpha = 0.05$) were found to be compliant with the legal requirements Furthermore the authors of the study concluded that digestate can be a good substitute for inorganic fertilizers (Beggio *et al.*, 2019).

Nonetheless, organic fertilizers such as manure, agricultural residues, digestate from the organic fraction of municipal solid waste (OFMSW), and the food processing sector, among others, can act as a sink for various heavy metals, disease-causing pathogens, and other contaminants, wreaking havoc on soil and water resources. If used on agricultural land, these contaminants may enter the food chain and be consumed by humans and animals, posing a risk to both humans and the environment.

Numerous studies have been undertaken to investigate the uptake of contaminants in soil from the source of fertilizing chemicals to plants under diverse exposure conditions (hydroponics, semi-soil, standardized sand, and natural soils) employing different test species (including cucumber, tomato, soybean, radish as some to mention). These methodologies, however, are not standardized, which hinders the scientific community from using the same approach for diverse plant species, jeopardizing previously established aims, findings, and outcomes. Therefore, the experimental technique used in this study is based on the standardized procedure as described in the iso standard (ISO 16198, 2015). The fundamental advantage of this approach is that it allows assessment of the environmental bioavailability of trace elements

to plants, either as concentrations in shoots and roots or, more comprehensively, as net uptake flux in plants.

The primary aim of this preliminary study is to analyze the uptake of metals (Cd, Cu, Ni, Pb and Zn) to tomato plants (cultivar Lycopersicon esculentum) under standard soil and test soil exposure (i.e., soil + digestate).

3 Material and Methods

3.1 Experimental Design

The entire experimental activity was carried out consistently by the standard ISO-16198:2015. The RHIZOtest consisted of two phases: a first hydroponic phase during which seeds were germinated in an aerated nutrient solution and a second exposure (or contact) phase during which the seedlings were placed in contact with the test soil. The root mat developed was not directly in contact with the soil but mediated by a polyamide mesh. A list of apparatus used in this bioassay is provided in the appendix table (See **Appendix A** *Table A- 1*).

3.1.1 Treatment and Replicates

Due to the time frame and limited availability of the apparatus necessary for the experiment, the number of treatments (i.e., dose) was kept to one treatment only, in addition to the control. The total number of experimental units (i.e., plant pots) to be prepared was estimated using Equation. *3.1* as indicated by the standard ISO-16198:2015(E).

$$n_p = \left[(n_s * n_r) + n_c\right] * f \qquad 3.1$$

Where:

- n_p is the total number of plant pots that must be prepared for each plant species;
- n_s is the number of soil or soil material tested;
- n_r is the number of replicates (minimum 5)
- n_c is the number of plant pots that serve as a control of the preculture period (minimum 5);
- f is the security factor (minimum 1,2).

A total of five replicates were performed for the hydroponic phase (hydroponic controls) and a total of five replicates were for the test phase (control soil: n=2, treated soil: n=3). The number of replicates adhered to the standard criterion for the minimum number of replicates (ISO 16198, 2015)

Two extra plant pots were added to replace the ones which may have been damaged or sick plants. This resulted in the preparation of 12 plant pots following the standard procedure for one treatment.

3.1.2 Nutrient Solutions

Three distinct nutrient solutions were prepared for both phases of the bioassay (i.e., pre-culture period, test culture period). *Table 3.1* shows how three nutritional solutions were used at various steps of the experiment. The pre-culture phase consisted of seed germination and hydroponic seedling pre-growth, whereas the test culture phase comprised plant growth. The nutrient solution's composition is listed in the appendix table. (See ***Appendix A*** *Table A-2).*
To minimize needless over-preparation of the nutrient solution, the required amount of solution was estimated in mg L^{-1}. The appendix table reports the determined quantity in mg L^{-1} for each nutrient solution (See ***Appendix A*** *Table A-3*).

The calculated amount of chemicals for the preparation of nutrient solutions was measured through analytical balance (Sartorius BP210S) and was poured into the flask with 1L of demineralized water each and stirred using a hot plate magnetic stirrer (IKA RCT Classic) at room temperature of 25°C.

Table 3.1 - Nutrient solution employed at various steps of the bioassay.phases

	Seed germination	Seedling pre-growth in hydroponics	Plant growth period
0 – 7 days	Nutrient Sol. 1	-	-
7 – 14 days	-	Nutrient Sol. 2	-
14 – 22 days	-	-	Nutrient Sol. 3

3.1.3 Control Soil and Test soil

A laboratory-prepared standard soil was utilized in the experiment as control soil. The control soil was prepared according to the protocol indicated in section 7.2.3.2 of ISO 11269-2 (ISO 11269-2, 2013). In contrast, the test soil was a mix of standard soil and substrate (i.e., digestate). Before application to the soil and subsequent mixing, the digestate was dried at 40°C. The digestate dosage (i.e., concentration) per pot was 0.06g per 14 g of soil. This dosage was determined using the yearly digestate application rate (i.e., 0.4 kg TS m^{-1}). This application rate was derived from the maximum nitrogen application rate of 340 KgN ha^{-1} y^{-1} in non-vulnerable areas and the average of the mean nitrogen concentrations reported for OFMSW, i.e., 110 gN kg^{-1} TS. Additionally, soil mixing processes based on "good practices" result in a soil-digestate mixing layer of 0.2m and a soil bulk density of 1.25 kg dm^{-3} (Beggio *et al.*, 2021).

Digestate is a nutrient-rich substance produced by anaerobic digestion (AD) of food waste or the organic fraction of municipal solid waste (OFMSW) and it may be used as a fertilizer (Peng & Pivato, 2019). The input feedstock to AD producing the used digestate is also referred to as "*Frazione Organica Rifiuti Solidi Urbani*" (FORSU) in the Italian language.

The digestate was collected from the output of four anaerobic digesters operating in parallel under wet thermophilic conditions (TS 10%, 55 °C) with a hydraulic retention time of 21 days, treating 120,000 tons of biowaste collected each year from various municipalities in the Veneto region North-East Italy. The information on the characteristics of the feedstock used for the production of digestate can be found in the appendix table (see ***Appendix*** *A Table A-4)*

3.1.4 Plant specie

The experimental plant was a tomato (Cultivar Lycopersicon esculentum Mill.). The seeds were purchased from an Italian seed company and were not treated with herbicides or pesticides. The tomato plant seeds complied with European Union (EU) seed commercialization requirements and regulations, and they are registered in the EU plant variety database.

3.2 RHIZOtest Bioassay

The RHIZOtest was carried out in November 2021 in the laboratory of the local waste management agency, *Società Estense Servizi Ambientali* (SESA), Monselice, Padua, Italy, at geographical coordinates 45.2235° North and 11.7496° East. The RHIZOtest was performed by the ISO standard (ISO 16198, 2015). There were two endpoints of the bioassay, a) the concentration of trace elements in shoots and roots at the end of the test culture period and b) the net uptake flux of trace elements in the whole plants during the test culture period. *Figure 3.1* illustrates the experiment's flow in a graphical form.

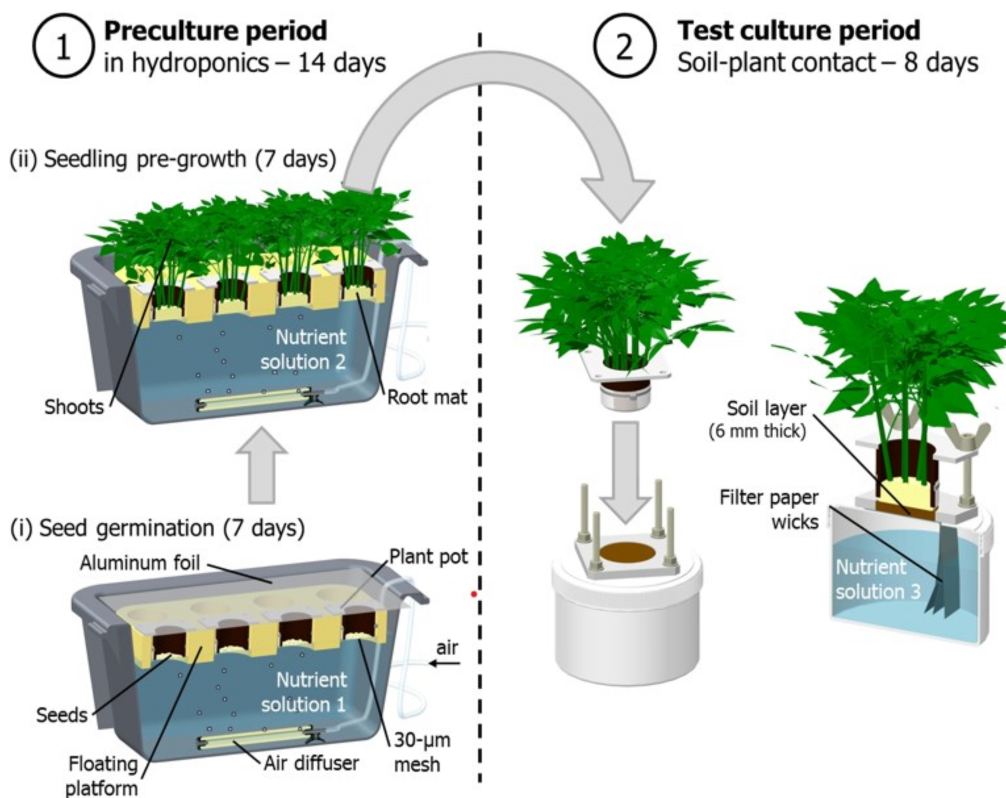


Figure 3.1 - RHIZOtest short procedureaccording to ISO 16198:2015. Retrieved from ("The RHIZOtest - (http://www.metrhizlab.com/),")

3.2.1 Preculture Period (Seed Germination and Seedlings pre-growth)

The preculture stage, which lasted 14 days, included seed germination and seedlings pre-growth under hydroponics to achieve adequate plant biomass and a dense, planar root mat. Throughout the two weeks, the bubbling device was used to aerate the nutritional solutions

with an air diffuser placed in the solution holding container. The plant assembly was assembled before seeding as illustrated on the left side of *Figure 3.2*. The plant pot assembly is designed to support the entire plant for the whole duration of the experiment. The plant pots allowed for the development of a planar and thick root mat while physically separating the plants from the nutrient solution in the container during exposure. The plant pots are made out of a cylinder that is connected to an upper plate at the top and closed at the bottom with a polyamide mesh secured with an adjustable clamp. The mash with the pore size of 30 μm was fixed tightly.

After assembling the pots, 40 seeds were placed on the polyamide mesh surface of each of the five plant pots that served as controls for the preculture phase. Following sowing, the plant pots are passed via the floating platform (12 plant pots) that has been set on top of the 6L of nutrition solution 1 in the tank as shown in the graphical illustration on the right side of *Figure 3.2*.

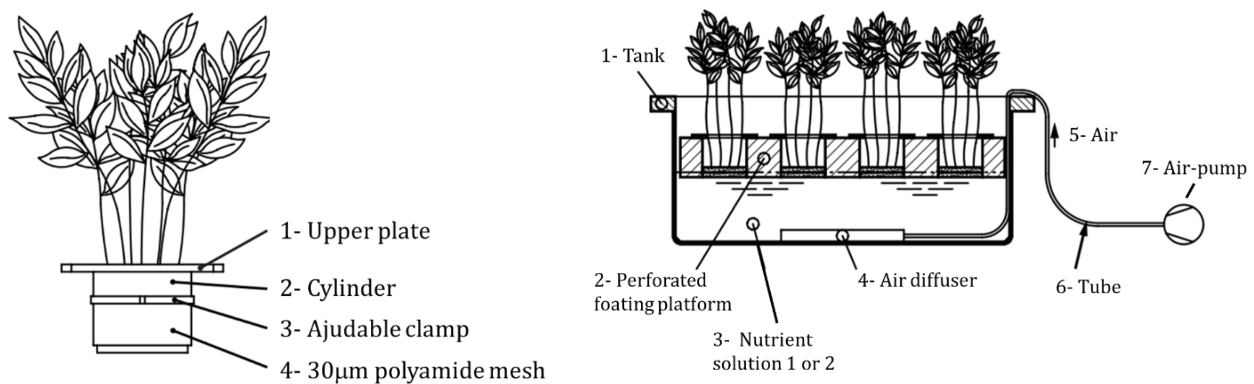


Figure 3.2 - Pot assembly for preculture phase.
plant pot (left), floating platform (right), retrieved from (ISO 16198, 2015)

Nutrient solution 1 was composed of 600 μmol·dm^{-3} $CaCl_2$ and 2 μmol·dm^{-3} H_3BO_3. The entire assembly of the plant pots on the floating platform in the nutrient solution tank was placed inside the incubator by covering the tank with an aluminum sheet to create a dark environment for the first 4 days of germination. The aluminum foil was removed when the functional photosynthetic organ, for example, green pigmentation on cotyledons or leaves, developed. Seedlings are then grown over nutrient solution 1 until the end of the first week.

After 7 days seedlings are grown for one additional week at the top surface of 6L of the nutrient solution 2. the nutrient solution 2 was prepared by adding the nutrients in the following order and concentration: 500 μmol·dm^{-3} KH_2PO_4; 2000 μmol·dm^{-3} KNO_3; 2000 μmol·dm^{-3} $Ca(NO_3)_2$;

1000 µmol·dm^{-3} $MgSO_4$; 0,2 µmol·dm^{-3} $CuCl_2$; 10 µmol·dm^{-3} H_3BO_3; 2 µmol·dm^{-3} $MnCl_2$; 1 µmol·dm^{-3} ZnSO4; 0,05 µmol·dm^{-3} Na_2MoO_4 and 100 µmol·dm^{-3} NaFe(III)EDTA.

Every third day, the nutrition solution 2 was renewed. The position of the plant pots on the floating platform was randomized at each renewal. Throughout these 14 days in hydroponics, nutrient solutions were aerated using in-tank air diffusers.

3.2.2 Test Culture Period

After the pre-culture period was carried out in hydroponic solution, the pre-culture phase was completed, and five plant pots with homogenous plant biomasses were selected and rinsed under a stream of demineralized water to serve as control and test plants during the test culture phase (2 replicates exposed to control soil and 3 to control soil amended with digestate).

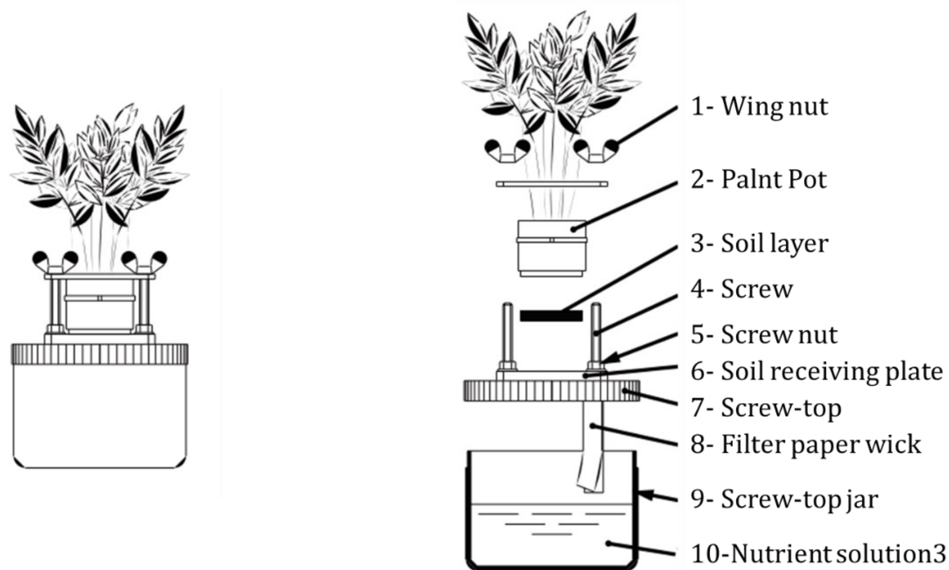


Figure 3.3 - Assembly Setup for Test culture phase
; individual components (left) full fitted plant pot (right), retrieved from (ISO 16198, 2015)

Each plant container was put in contact with the soil layer placed on the top of the soil-receiving plate for 8 days. The amount of soil put on each soil-receiving plate deviated from the quantity specified in the standard. Thus, instead of 9 g of fresh control soil or test soil (i.e., control soil amended with digestate), the mass of the soil was modified to 6 g placed down on each soil-receiving plate to achieve a soil layer thickness of approximately 6 mm and a soil density of approximately 1.2 g cm^{-3}. *Figure 3.3* illustrates the schematic of the assembly setup for the test culture period. It consists of two modules and three paper wicks wedged in between; a) contact assembly that uses fastenings (i.e., screws and screws nut) to firmly hold the plant pot over the

soil layer and a 0,5 dm^3 screw-top jar filled with the nutrient solution 3. This assembly is designed to enable close contact between the root mat and the entire surface area of the soil layer and it allows the filter paper wicks to stay fully soaked throughout the test culture period. The root mat in contact with the soil has a surface area of 12.6cm^2. Nutrient solution 3 was prepared by adding the nutrients in the following order and concentration: 50 $\mu mol \cdot dm^{-3}$ KH_2PO_4; 2000 $\mu mol \cdot dm^{-3}$ KNO_3; 2000 $\mu mol \cdot dm_{-3}$ Ca $(NO_3)_2$, and 1000 $\mu mol \cdot dm^{-3}$ $MgSO_4$. The nutrient solution was changed every other day, except on the last day of the test culture period. Each renewal included randomization of the soil-plant contact assembly. The wicks were inserted through the screw top in such a way that they remained damp at all times, allowing the soil layer to remain at 100% water holding capacity (WHC) during the test. No wick replacement was required during the test because it remained undamaged. The complete RHIZOtest experiment was performed under the regulated climatic conditions (16 h day, 200–400 µmol of photons$\cdot m^{-2} \cdot s^{-1}$, 75% relative humidity and a temperature of 25 °C; 8 h night, 70% relative humidity and a temperature of 20 °C). The screw-top jars were washed in hot water, then in a volume fraction of 10% HNO_3, followed by thorough rinsing with demineralized water before and at the end of the test.

3.2.3 Harvests of plants

At the end of the Test Culture period, the plants of each replicate were harvested. One following the pre-culture phase, and another following the test culture phase. The soil-receiving systems were isolated from the plant containers. The polyamide sheet was removed from the bottom of the plant pots, and the plants were washed under a stream of demineralized water (shoots and roots included). Additionally, the root mat was properly washed and cleaned to minimize contamination with dirt particles less than 30µm (i.e., smaller than the pore diameter of the polyamide mesh). Finally, the plants were taken from their containers with care. Shoots were separated from roots by cutting, and seed husks were removed to the extent feasible, however owing to the dense root mat, seed husks were not entirely removed to prevent damaging the roots, and the small amount that remained attached was pooled with the root sample. Each replicate's roots and shoots were put in a separate sample container, which was labeled appropriately in advance to avoid sample mixing. After that, the plant samples were placed in an incubator and dried at 40°C for four days to obtain a stable mass. Following that, plant samples were weighed to a precision of 1 mg. Additionally, the plants were preserved in the incubator until grinding. To minimize contamination during the harvest, laboratory gloves were worn and separate zirconium oxide scissors and blades were employed.

3.3 Plant and soil analysis

3.3.1 Concentration and fluxes in plants

For the analysis of plant biomass, and concentration in its parts (i.e., Shoots and roots) an inductively coupled plasma spectroscopy (ICP-MS) was used. The amounts of trace elements in the shoots and roots of the samples were analyzed (first endpoint of bioassay). The flux of contaminants such as trace elements (Cd, Cr, Cu, Ni, Pb, and Zn) taken up by plants (roots and shoots pooled together) throughout the test culture period was determined using *Equation. 3.2* (second endpoint of bioassay).

$$F_p = \frac{Q_p}{S * t} \qquad 3.2$$

$$Q_P = [(C_{t,s} * m_{t,s} - C_{c,s} * m_{c,s}) + (C_{t,r} * m_{t,r} - C_{c,r} * m_{c,r})] \qquad 3.3$$

Where:

- F_p is the flux of trace elements to the plants during the test culture period (ng m^{-2} s^{-1});
- Q_p is the quantity of trace elements accumulated by plants during the test culture period, in (ng);
- S is the surface area of the root mat in contact with soil (0,0126 m^2);
- t is the duration of the test culture period (s);
- $C_{t,s}$ is the trace element concentration in shoots at the end of the test culture period (µg g^{-1}) (dry biomass);
- $m_{t,s}$ is the dry biomass of shoots at the end of the test culture period (g);

- $C_{c,s}$ is the mean trace element concentration in shoots of control plant pots at the end of the pre-culture period (µg g^{-1}) (dry biomass);

- $m_{c,s}$ is the mean dry biomass of shoots of control plant pots at the end of the preculture period (g);

- $C_{t,r}$ is the trace element concentration in roots at the end of the test culture period (µg g^{-1}) (dry biomass);

- $m_{t,r}$ is the dry biomass of roots at the end of the test culture period (g);

- $C_{c,r}$ is the mean trace element concentration in roots of control plant pots at the end of the pre-culture period (µg g^{-1}) (dry biomass);

- $m_{c,r}$ is the mean dry biomass of roots of control plant pots at the end of the preculture period (g).

3.3.2 Statistical analysis

Statistical analysis and consequent graphical representation were performed in Microsoft excel 2019 to compare and evaluate the mean of the control samples with the treatment samples (i.e., control roots, shoots vs root and shoots exposed to treated soil and similarly control soil vs test soil). Student's t-Test for two-sample ANOVA was used by assuming the equal variances of the two compared samples to determine whether there is enough statistical evidence to claim the calculated parameters mean is equal at the population level. The 95% confidence interval ($\alpha = 0.05$) was calculated for two datasets (i.e., control and treatment) to accept or reject the null hypothesis.

Additionally, two types of bioconcentration factors were defined in this study: a) shoot concentration factor (SCF) and b) root concentration factor (RCF). These factors were used to analyze the results and characterize the uptake. SCF and RCF values were determined for control and treatment samples, respectively.

SCF was defined as the ratio between the mean TE concentration in shoots C_{shoots} (mg Kg^{-1} d.w.) and mean TE concentration in soil C_{soil} (mgKg^{-1} d.w.).

$$SCF = \frac{C_{shoots}}{C_{soil}} \quad 3.4$$

Whereas RCF was calculated as the ratio between the mean TE concentration in roots C_{roots} (mg Kg^{-1} d.w.) and mean TE concentration in soil C_{soil} (mg Kg^{-1} d.w.).

$$RCF = \frac{C_{roots}}{C_{soil}} \quad 3.5$$

Shoot-roots concentration was defined as translocation factor (TF), which describes the translocation of contaminants (metals) from roots to shoots of the plant. It was calculated as the ratio between mean TE concentration in shoots C_{shoots} (mg Kg^{-1} d.w.) and mean TE concentration in roots C_{roots} (mg Kg^{-1} d.w.).

$$TF = \frac{C_{shoots}}{C_{roots}} \qquad 3.6$$

Concentrations values below the limit of quantification (<LOQ) were considered equal to the LOQ, which represents the safest option for the evaluation of the risk.

4 Results and Discussion

The data output following the chemical analysis and statistical analysis are depicted graphically as well as presented in the table to better interpret and comprehend it. The results are described and discussed in the following sections of this chapter.

4.1 Plant Biomass

Although variation in root and shoot biomass is not an endpoint of the RHIZOtest, the current experiment demonstrates that using soil enriched with digestate from OFMSW has a significant impact on root Lycopersicon esculentum Mill plants, despite adequate macronutrient availability (i.e., Ca, K, Mg, N, P, S) through nutrient solutions throughout the experiment. Since the validity of the bioassay notably depends on a significant increase in shoot and root biomasses between the end of the preculture period and the end of the test culture period. Therefore, the biomass of the plants at the end of the preculture period was also analyzed and the average dry weight of shoots (0.38 ± 0,03) and roots (0.8 ± 0,06) were compared to those after exposure. It was found that there was an increase in the biomass of the shoots n roots after the test culture period, proving the bioassay test valid.

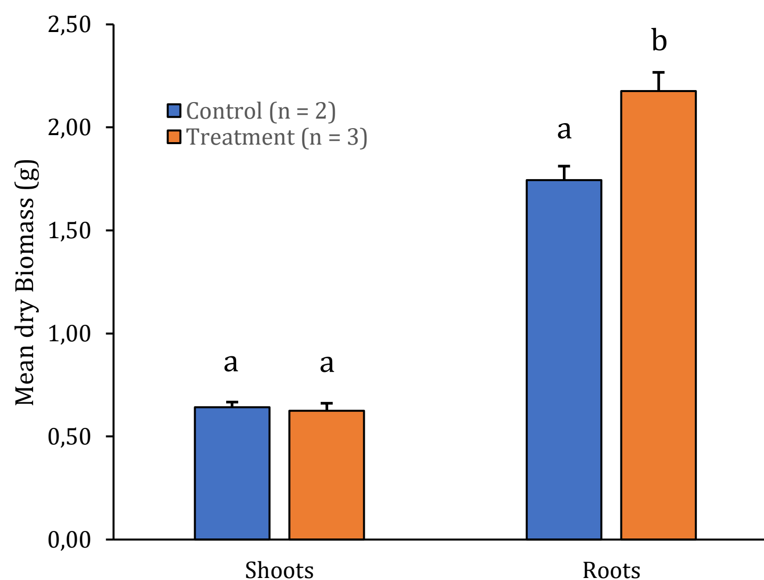


Figure 4.1 - Mean dry weight (g) of roots and shoots of RHIZOtest plants after exposure. Error bars represent the standard error (SE) of the mean (n = 2 for control; n=3 for treatment). Samples followed by the same letters do not show significant differences (p>0.05) from the control

Figure 4.1 depicts the average root and shoot weights along with their standard errors (dry weight (g) ± SE). The average dry weight of control shoots (0,64g ± 0,03) was marginally greater than the average dry weight of shoots exposed to amended soil (0,63g ± 0,04). In contrast, the average dry weight of the roots of plants exposed to amended soil (2,18g ± 0,09) was greater than that of the roots of control plants (1,74g ± 0,07). This was further confirmed by statistical analysis, which revealed a significant difference ($p < 0,05$) in root biomass between plants exposed to amended soil and control plants. This can be justified by the usage of digestate as a fertilizer and by the fact that digestate includes numerous vital nutrients, including nitrogen (N), phosphorus (P), and potassium (K); all of which are required for the development and growth of plants. Therefore, the digestate has a positive effect on the growth of the plants.

The conducted t-Test did not demonstrate significant differences in shoots of exposed plants ($p > 0,05$). Although neither control nor treated plants revealed evidence of stress, except for one or two plants that had mildly evident stress symptoms (notably, chlorosis).

4.2 Trace Element Concentrations

Table 4.1 summarizes the mean concentrations of trace elements (TEs) in the rhizosphere of tomato plants (roots, shoots, and soil). Asterisk (*) denotes a difference from the control that is statistically significant.

*Table 4.1 - Concentrations of TEs in Tomato plant shoots and roots. of control (mean ± standard error; n=2) and treatment after exposure (mean ± standard error; n=3). Values indicated with * are statistically significant (p < 0.05)*

		Shoots		Roots		Soil	
TEs	**Unit**	**Control**	**Treatment**	**Control**	**Treatment**	**Control**	**Treatment**
Cd	mg kg^{-1} d.w.	< LOQ	< LOQ	< LOQ	<LOQ	< LOQ	< LOQ
Cr	mg kg^{-1} d.w.	< LOQ	< LOQ	< LOQ	<LOQ	14,50 ± 1,50	13,67 ± 0,67
Cu	mg kg^{-1} d.w.	< LOQ	5,00	< LOQ	< LOQ	26,00 ± 1,00	22,67 ± 1,67
Ni	mg kg^{-1} d.w.	< LOQ	< LOQ	< LOQ	< LOQ	20,50 ± 0,50	19,33 ± 0,88
Pb	mg kg^{-1} d.w.	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Zn	mg kg^{-1} d.w.	12,50 ± 0,50	14,00 ± 0,50	21,00 ± 1,00*	14,67 ± 0,50*	27,00 ± 6,00	19,67 ± 0,88

The elemental content of Cd in the shoots of both control and treated plants was less than LOQ (0.3 mg kg^{-1} d.w.). Additionally, Cr, Cu, Ni, and Pb concentrations were discovered to be equivalent to or less than LOQ (5 mg kg^{-1} d.w.). Thus, because no degree of variation was achieved, these data for the extracted concentrations were not subjected to statistical analysis.

The Zn content in the shoots of treated plants (i.e., exposed to control soil amended with digestate) was greater than in the shoots of control plants. However, since the p-value exceeds the predefined significance interval ($\alpha = 0,05$), the concentration of Zn in the shoots is not statistically significant. In contrast to shoots, the statistical analysis showed a significant difference ($p < 0,05$) at the 95% confidence level ($\alpha = 0,05$) between the mean of the Zn concentration in the roots of control and treatment plants.

In comparison, only Zn concentrations were found higher in plants than other trace elements, which may also be due to the nature of the digestate, which contains a higher amount of Zn (82 mg Kg^{-1} d.w. Zn). Additionally, the soil was also analyzed for the TEs concentrations after exposure for both the control and testing soil (i.e. control soil with digestate).

The outcomes are reported graphically in *Figure 4.2*. Cd and Pb were not included in the bar charts as they were found less than LOQ, hence t-Test for these values was not possible.

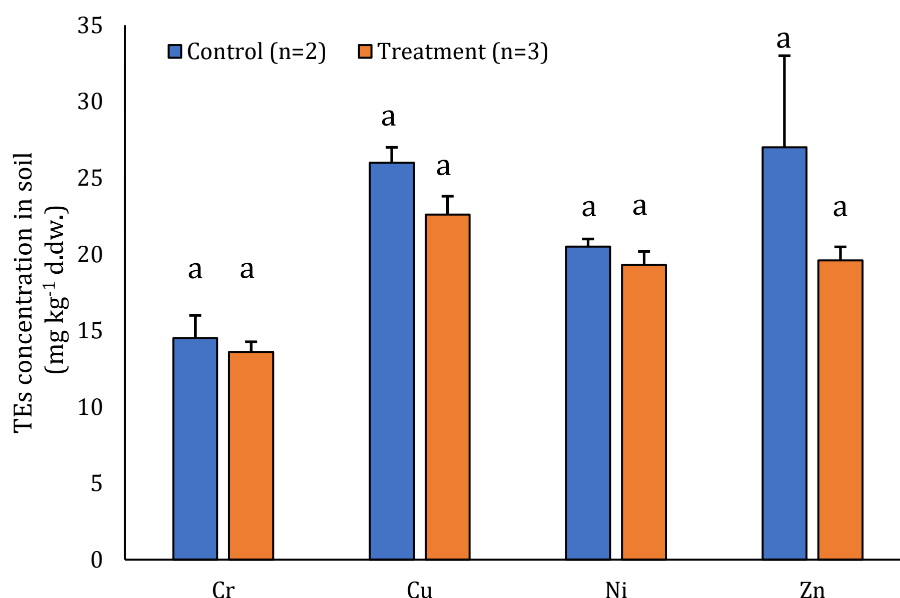


Figure 4.2 - (Trace) elements extractible concentration (mg kg^{-1} d.w.) of soil matrix Error bars indicate the standard error (SE) of the mean (n = 2 for control; n=3 for treatment). Samples followed by the same letter do not indicate significant differences (p>0.05). Cd and Pb were not included since under LOQ

The extracted concentrations were also evaluated statistically with a two-sample t-Test assuming the same variance. The results were not statistically significant ($p > 0,05$) for Cr, Cu, Ni, and Zn concentrations in control soil and test soil.

As shown by the results in *Table 4.1*, Zn concentrations were higher in control roots than in treated root samples, which may imply that Zn is less bioavailable in soils amended with digestate than in control soil. This could indicate that the digestate reduced the bioavailability of Zn, possibly due to organic matter complexation or potentially due to higher transfer from roots to shoots.

Bioavailability is reliant on the speciation of contaminants (here heavy metals). Thus, a higher concentration of an ionic form (dissolved in the liquid phase) results in increased uptake. By adding digestate, we introduce organic matter that has the tendency to complicate the ionic fraction of Zn (i.e., Zn with complex organic matter molecules), because these organic matter molecules are characterized by lower uptake potential. Nevertheless, increasing the number of replicates as well as digestate dosages will aid in a better understanding of the outcomes, and hence of uptake and bioavailability.

Higher Zn concentrations in control roots and lower concentrations in treated root samples may imply that Zn is less bioavailable in soils amended with digestate than in control soil as shown by the results in. This could indicate that the digestate reduced the bioavailability of Zn, possibly due to organic matter complexation or potentially due to higher transfer from roots to shoots Bioavailability is reliant on the element's speciation (contaminants). Thus, a higher concentration of an ionic form (dissolved in the liquid phase) results in increased uptake. By adding digestate, we introduce organic matter that has the tendency to complicate the ionic fraction of Zn (i.e., Zn with complex organic matter molecules), since organic matter molecules are characterized by lower uptake potential. Regardless more analysis need to be done by increasing the number of replicates and the dosages of digestate

4.3 Net Uptake Flux

The uptake flux was computed for both control and digestate-exposed plants following the exposure period. *Figure 4.3* displays the trace element net uptake fluxes in the RHIZOtest plants.

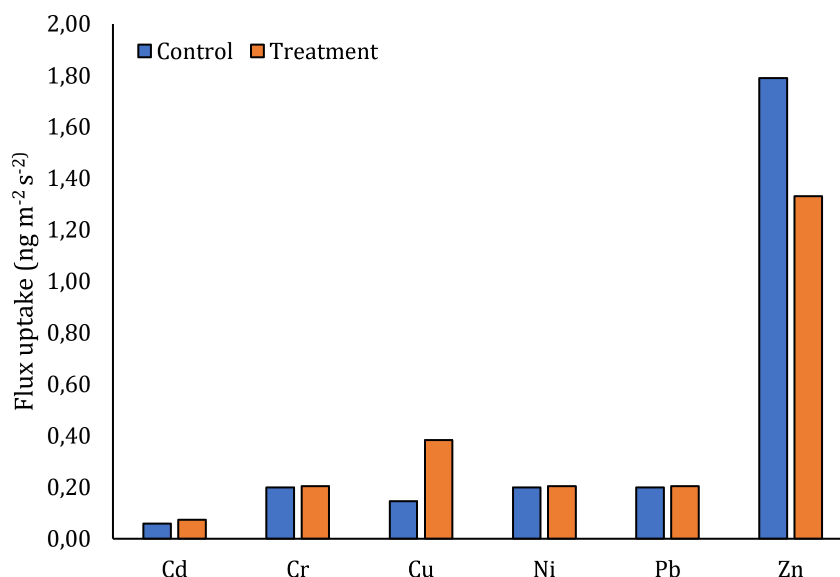


Figure 4.3 - Net uptake flux (ng m^{-2} s^{-1}) for trace elements in the RHIZOtest plants

The net uptake flux of trace elements Cd and Cu was moderately higher in digestate-treated plants than in control plants, but not of Cr, Ni, or Pb. In the case of Zn, the net uptake flux in digestate-treated RHIZOtest plants was lower than in control plants, corresponding to a decrease in bioavailability. As a result, decreased net Zn uptake flux confirms lower bioavailability.

4.4 Bio-concentration Factor and Translocation Factor

Two types of bioconcentration factor (BCF) were calculated, shoots concentration factor (SCF) and roots concentration factor (RCF), Furthermore, the translocation factor (TF) is also calculated and reported in *Table 4.2.* No such indices were calculated for macronutrients (Ca, Mg, K) which are essential elements for plants and animals, not known to pose an ecotoxicological risk.

Table 4.2 - Bioconcentration(shoots, roots) and Translocation factor for trace elements in the RHIZOtest plants (control and exposed to treatment)

	SCF		RCF		TF	
TEs	**Control**	**Treatment**	**Control**	**Treatment**	**Control**	**Treatment**
Cd	--	--.	--	--	--	--
Cr	0,34	0,37	0,34	0,37	--	--
Cu	0,19	0,22	0,19	0,22	--	--
Ni	0,24	0,26	0,24	0,26	--	--
Pb	--	--	--	--	--	--
Zn	0,46	0,71	0,78	0,36	0,60	0,95

The TF of plants grown in control soil and plants are grown in digestate-treated soil was calculated.TF > 1 means that the element has high mobility and translocate from the roots to the shoots. Since the shoot and root concentrations value for the elements was less than LOQ, it was treated as equal to LOQ for the computation. As a result, Cd, Cr, Cu, Ni, and Pb were assigned TF = 1. However, it was ruled invalid since the concentrations of these components in both the shoots and the roots were equal.

Since we are interested in the risk related to the translocation of HMs from soil to other edible parts of the plants. The trend for the bioconcentration factor in the case of Zn was observed and it was found out that digestate amendment leaded to the higher SCF, lower RCF and higher TF (SCF=0.71; RCF= 0,36; TF = 0,95) with respect to the control (SCF=0,46; RCF=0,78; TF=0.60). As it is a relative measure, thus by looking at SCF and RCF values, we may expect a higher occurrence of Zn in the shoots and lower in roots of treated plant samples.

However all the values are < 1, this could be due to the fact that dosage of digestate was very low and hence resulted in low uptake of Zinc.

4.5 Conclusion

Our extraction test results revealed that the competitive metal was Zn, which accumulated in the shoots of the plants exposed to treatment. Other contaminants, such as Cd, Cr, Ni, and Pb, were hardly affected in the plant experiment, and there was no concentration increase in the shoots of the tomato plant, implying that increased transfer of these potentially toxic elements to the food chain is apparently not enhanced in plants exposed to digestate from OFMSW. Long-term experiments and a larger number of replicates, however, may be necessary to corroborate this finding. Furthermore, further treatments may improve the effectiveness of the bioassay for pollutant uptake comparison purposes. Additionally, there is a need to investigate similar behavior for uptake and transport using this test in the field. Among the key determinants of their uptake and bioaccumulation in some or all sections of crop plants are soil type and its composition. Increasing the number of replicates will also help reduce standard errors in the bioassay's output data, hence improving standards and enabling more effective modeling of plant uptake.

Part-III Data

Part III contains all supporting material and raw data that were generated as a result of the bioassay and are used for computational and statistical analysis. Additionally, it includes images of the laboratory experiment.

Appendices

A Experimental Design

Table A- 1 - Apparatus used in the Bioassay

Pre-culture		
Equipment	**Quantity**	**Remarks**
Plant part	12	Composed of 1 cylinder + 1 upper plate
Tank	2	x2 for nutrient solution replacements
Floating platform	2	x2 for nutrient solution replacements
Air pump	1	
Ceramic diffusor	2	
Air pipe	1	Lenght according to the growth chamber space (from 50 cm to 1 m)
Slip collar pliers	1	
Tes culture		
Equipment	**Quantity**	**Remarks**
Soil part	5	Composed of 1 lid + 1 lower plate + 2 screws + 4 screw nuts + 2 wing nuts + jar
Extra jar	10	x2 for nutrient solution replacements
Consumables	**Quantity**	
Mesh	12	
Slip collar	12	
Paper wick	15	3 wicks per device

Table A-2 - Composition of nutrient solutions

Nutrients	Nutrient Sol. 1 [μmol dm^{-3}]	Nutrient Sol. 2 [μmol dm^{-3}]	Nutrient Sol. 3 [μmol dm^{-3}]
$CaCl_2$	600	-	-
H_3BO_3	2	10	-
KH_2PO_4	-	500	50
KNO_3	-	2000	2000
Ca (NO_3)	-	2000	2000
MG (SO_4)	-	1000	1000
$CuCl_2$	-	0.2	-
$MnCl_2$	-	2	-
$ZnSO_4$	-	1	-
Na_2MoO_4	-	0.05	-
NaFe (III)EDTA	-	1000	-

Table A-3 - Determined quantity for each nutrient solution for preparation

Nutrients	Nutrient Sol. 1 [mg L^{-1}]	Nutrient Sol. 2 [mg L^{-1}]	Nutrient Sol. 3 [mg L^{-1}]
$CaCl_2$	66		-
H_3BO_3	0.12	0.62	-
KH_2PO_4	-	68.05	6.80
KNO_3	-	202.20	202.20
Ca (NO_3)	-	328.18	328.18
MG (SO_4)	-	10.37	120
$CuCl_2$	-	0.03	-
$MnCl_2$	-	0.25	-
$ZnSO_4$	-	0.18	-
Na_2MoO_4	-	0.01	-
NaFe (III)EDTA	-	367.71	-

Table A-4 - Characteristics of the feedstock (digestate) used for test soil

Description	Method	Value	Unit
Residual Moisture	UNI 10780:1998 app. C.2.	4.6	[%]
Residual at 105° C	CNR IRSA 2 Q64 Vol 2 1984	30.8	[%]
Organic Substance (from calculation)	UNI 10780:1998 app. E	68	[s.s.]
Total Nitrogen	UNI 10780:1998 app. J.1	2.3	[s.s. N]
Phosphorus	UNI EN 16173 2012 + UNI EN 16170 2016	1.03	[% s.s. P]
Potassium	UNI EN 16173 2012 + UNI EN 16170 2016	1.41	[% s.s. K]
Cadmium	UNI EN 16173 2012 + UNI EN 16170 2016	<0.3	[mg/kg s.s. Cd]
Mercury	UNI EN 16173 + UNI EN 16175-2	<0.05	[mg/kg s.s. Hg]
Nickel	UNI EN 16173 2012 + UNI EN 16170 2016	<5	[mg/kg s.s. Ni]
Lead	UNI EN 16173 2012 + UNI EN 16170 2016	<5	[mg/kg s.s. Pb]
Copper	UNI EN 16173 2012 + UNI EN 16170 2016	18	[mg/kg s.s. Cu]
Zinc	UNI EN 16173 2012 + UNI EN 16170 2016	82	[mg/kg s.s. Zn]
Chromium VI	UNI 10780:1998 app. B CrVI	<0.2	[mg/kg s.s. Cr VI]

B Concentration Values Before Exposure

Table B- 1 – Concentration, wet weight (TQ), dry weight (TS) and percentage mass of shoots after hydroponic growth

	Cd	Cr	Cu	Ni	Pb	Zn	TQ	TS (g)	TS
Unit	(mg/kg d.w.)						(g)		%
CS1	< 0,3	< 5	12	< 5	< 5	30	5,66	0,50	9%
CS2	< 0,3	< 5	13	< 5	< 5	33	5,72	0,38	7%
CS3	< 0,3	< 5	14	< 5	< 5	36	4,93	0,37	7%
CS4	< 0,3	< 5	14	< 5	< 5	36	4,97	0,34	7%
CS5	< 0,3	< 5	15	< 5	< 5	39	5,42	0,33	6%

Table B- 2 – Concentration, wet weight (TQ), dry weight (TS) and percentage mass of roots after hydroponic growth

	Cd	Cr	Cu	Ni	Pb	Zn	TQ	TS	TS
Unit	(mg/kg d.w.)						(g)		%
CR1	< 0,3	< 5	14	< 5	< 5	49	0,86	0,24	28%
CR2	< 0,3	< 5	25	< 5	< 5	72	0,70	0,19	27%
CR3	< 0,3	< 5	18	< 5	< 5	54	0,80	0,50	62%
CR4	< 0,3	< 5	25	< 5	< 5	64	0,56	0,17	31%
CR5	< 0,3	< 5	14	< 5	< 5	38	0,76	0,32	43%

Table B- 3 - TEs concentrations in standard soil (Experiment control)

	Cd	Cr	Cu	Ni	Pb	Zn
	(mg/kg d.w.)					
S1	< 0,3	< 5	14	< 5	< 5	49
S2	< 0,3	< 5	25	< 5	< 5	72

C Concentration Values After Exposure

Table C- 1 - TEs concentrations in test soil after exposure

	Cd	Cr	Cu	Ni	Pb	Zn
	(mg/kg d.w.)					
SD1	< 0,3	< 5	18	< 5	< 5	54
SD2	< 0,3	< 5	25	< 5	< 5	64
SD3	< 0,3	< 5	14	< 5	< 5	38

Table C- 2 - Concentration, wet weight (TQ), dry weight (TS) and percentage mass of shoots after exposure to test soil

	Cd	Cr	Cu	Ni	Pb	Zn	TQ	TS	TS
	(mg/kg d.w.)						(g)		%
T1S	< 0,3	< 5	5	< 5	< 5	14	9,10	0,62	7
T2S	< 0,3	< 5	5	< 5	< 5	13	8,36	0,57	
T3S	< 0,3	< 5	5	< 5	< 5	15	10,20	0,69	

Table C- 3 - Concentration, wet weight (TQ), dry weight (TS) and percentage mass of roots after exposure to test soil

	Cd	Cr	Cu	Ni	Pb	Zn	TQ	TS	TS
	(mg/kg d.w.)						(g)		%
T1R	< 0,3	< 5	< 5	< 5	< 5	16	6,51	2,00	31
T2R	< 0,3	< 5	< 5	< 5	< 5	14	7,27	2,23	
T3R	< 0,3	< 5	5	< 5	< 5	14	7,48	2,30	

Table C- 4 - Concentration, wet weight (TQ), dry weight (TS) and percentage mass of shoots after exposure to control soil

	Cd	Cr	Cu	Ni	Pb	Zn	TQ	TS	TS
	(mg/kg d.w.)						(g)		%
C1S	< 0,3	< 5	< 5	< 5	< 5	12	9,84	0,67	7
C2S	< 0,3	< 5	< 5	< 5	< 5	13	9,10	0,62	

Table C- 5 - Concentration, wet weight (TQ), dry weight (TS) and percentage mass of roots after exposure to control soil

	Cd	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**	**TQ**	**TS**	**TS**
	(mg/kg d.w.)						(g)		%
C1R	< 0,3	< 5	< 5	< 5	< 5	22	5,46	1,68	31
C2R	< 0,3	< 5	5	< 5	< 5	20	5,90	1,81	

D Flux, Bio-accumulation and Translocation Factor

Table D- 1 - The flux of trace elements taken up in plants during the test culture (both control and plant exposed to treatment)

Net-flux uptake (ng $m^{-2}s^{-1}$)		
TEs	**Control**	**Treatment**
Cd	0,059	0,074
Cr	0,199	0,204
Cu	0,145	0,384
Ni	0,199	0,204
Pb	0,199	0,204
Zn	1,789	1,332

Table D- 2 - Bioconcentration factor and Translocation factor for control plants

TEs	SCF	RCF	TF
Cd	1,00	1,00	1,00
Cr	0,34	0,34	1,00
Cu	0,19	0,19	1,00
Ni	0,24	0,24	1,00
Pb	1,00	1,00	1,00
Zn	0,46	0,78	0,60

Table D- 3 - Bioconcentration factor and Translocation factor for Treatment (Plants exposed to soil with digestate)

TEs	SCF	RCF	TF
Cd	1,00	1,00	1,00
Cr	0,37	0,37	1,00
Cu	0,22	0,22	1,00
Ni	0,26	0,26	1,00
Pb	1,00	1,00	1,00
Zn	0,71	0,36	0,95

E Statistical Analysis.

Table E- 1 - t-Test: Two-sample assuming equal variances for the concentration of Zn in control shoos and treatment shoots

	Control shoots	**Treatment Shoots**
Mean	12,50	14
Variance	0,50	1
Observations	2,00	3
Pooled variance	0,83	
Hypothesized mean difference	0,00	
Degree of freedom	3,00	
t Stat	-1,80	
P(T<=t) one-tail	0,08	
t Critical one-tail	2,35	
P(T<=t) two-tail	0,17	
t Critical two-tail	3,18	

Table E- 2 - t-Test: Two-sample assuming equal variances for the concentration of Zn in control roos and treatment roots

	Control roots	**Treatment roots**
Mean	21,00	14,67
Variance	2,00	1,33
Observations	2,00	3
Pooled variance	1,56	
Hypothesized mean difference	0,00	
Degree of freedom	3,00	
t Stat	5,56	
P(T<=t) one-tail	0,01	
t Critical one-tail	2,35	
P(T<=t) two-tail	0,01	
t Critical two-tail	3,18	

Table E- 3 - Two-sample t-test assuming different variances for the concentration of Zn in control soil and treatment soil

	Control Soil	**Treatment Soil**
Mean	27,00	19,67
Variance	72,00	2,33
Observations	2,00	3,00
Hypothesized mean difference	0,00	
Degree of freedom	1,00	
t Stat	1,21	
P(T<=t) one-tail	0,22	
t Critical one-tail	6,31	
P(T<=t) two-tail	0,44	
t Critical two-tail	12,71	

Bibliography

Akenga, P., Gachanja, A., Fitzsimons, M.F., Tappin, A. & Comber, S. 2021. Uptake, accumulation and impact of antiretroviral and antiviral pharmaceutical compounds in lettuce. *Science of The Total Environment*, **766**, 144499, (At: https://www.sciencedirect.com/science/article/pii/S004896972038030X. Accessed: 13/5/2021).

Arnot, J.A. & Gobas, F.A. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environmental Reviews*, **14**, 257–297, (At: http://www.nrcresearchpress.com/doi/10.1139/a06-005. Accessed: 9/11/2021).

Beggio, G., Bonato, T., Schievano, A., Garbo, F., Ciavatta, C. & Pivato, A. 2021. Agricultural application of digestates derived from agricultural and municipal organic wastes: a health risk-assessment for heavy metals. *Journal of Environmental Science and Health, Part A*, **56**, 1409–1419, (At: https://www.tandfonline.com/doi/full/10.1080/10934529.2021.2002628. Accessed: 1/4/2022).

Beggio, G., Schievano, A., Bonato, T., Hennebert, P. & Pivato, A. 2019. Statistical analysis for the quality assessment of digestates from separately collected organic fraction of municipal solid waste (OFMSW) and agro-industrial feedstock. Should input feedstock to anaerobic digestion determine the legal status of digestate? *Waste Management*, **87**, 546–558, (At: https://linkinghub.elsevier.com/retrieve/pii/S0956053X19301114. Accessed: 1/4/2022).

Beltrán, E.M., Fernández-Torija, C., Pablos, M.V., Porcel, M.Á., García-Hortigüela, P. & González-Doncel, M. 2021. The effect of PFOs on the uptake and translocation of emerging contaminants by crops cultivated under soil and soilless conditions. *Ecotoxicology and Environmental Safety*, **215**, 112103, (At: https://www.sciencedirect.com/science/article/pii/S0147651321002141. Accessed: 6/5/2021).

Dal Ferro, N., Pellizzaro, A., Fant, M., Zerlottin, M. & Borin, M. 2021. Uptake and translocation of perfluoroalkyl acids by hydroponically grown lettuce and spinach exposed to spiked solution and treated wastewaters. *Science of The Total Environment*, **772**, 145523, (At: https://www.sciencedirect.com/science/article/pii/S004896972100591X. Accessed: 7/5/2021).

Di Carlo, E., Boullemant, A. & Courtney, R. 2020. Ecotoxicological risk assessment of revegetated bauxite residue: Implications for future rehabilitation programmes. *Science of The Total Environment*, **698**, 134344, (At: https://linkinghub.elsevier.com/retrieve/pii/S0048969719343359. Accessed: 21/2/2022).

Eid, E.M., Shaltout, K.H., Abdallah, S.M., Galal, T.M., El-Bebany, A.F. & Sewelam, N.A. 2020. Uptake Prediction of Ten Heavy Metals by Eruca sativa Mill. Cultivated in Soils Amended with Sewage Sludge. *Bulletin of Environmental Contamination and Toxicology*, **104**, 134–143, (At: http://link.springer.com/10.1007/s00128-019-02746-3. Accessed: 5/5/2021).

Gredelj, A., Nicoletto, C., Valsecchi, S., Ferrario, C., Polesello, S., Lava, R., Zanon, F., Barausse, A., Palmeri, L., Guidolin, L. & Bonato, M. 2020. Uptake and translocation of perfluoroalkyl acids (PFAA) in red chicory (Cichorium intybus L.) under various treatments with pre-contaminated soil and irrigation water. *Science of The Total Environment*, **708**, 134766, (At: https://linkinghub.elsevier.com/retrieve/pii/S0048969719347576. Accessed: 5/5/2021).

Henry, H.F., Burken, J.G., Maier, R.M., Newman, L.A., Rock, S., Schnoor, J.L. & Suk, W.A. 2013. Phytotechnologies – Preventing Exposures, Improving Public Health. *International journal of phytoremediation*, **15**, 889–899, (At: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3954606/. Accessed: 9/11/2021).

ISO 11269-2. 2013. *Soil quality - Determination of the effects of pollutants on soil flora. Effects of contaminated soil on the emergence and early growth of higher plants.* BSI. (At: https://doi.org/10.3403/30197451U.).

ISO 16198. 2015. *Soil quality - Plant-based test to assess the environmental bioavailability of trace elements to plants.* BSI. (At: https://doi.org/10.3403/30234130U.).

ISO 17402. 2011. *Soil quality - Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials.* BSI. (At: https://doi.org/10.3403/30236055U.).

Ju, X.-T., Xing, G.-X., Chen, X.-P., Zhang, S.-L., Zhang, L.-J., Liu, X.-J., Cui, Z.-L., Yin, B., Christie, P., Zhu, Z.-L. & Zhang, F.-S. 2009. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. *Proceedings of the National Academy of Sciences*, **106**, 3041–3046, (At: https://pnas.org/doi/full/10.1073/pnas.0813417106. Accessed: 5/4/2022).

Kiiski, H., Dittmar, H., Drach, M., Vosskamp, R., Trenkel, M.E., Gutser, R. & Steffens, G. 2016. Fertilizers, 2. Types. In: *Ullmann's Encyclopedia of Industrial Chemistry* (ed. Wiley-VCH Verlag GmbH & Co. KGaA), pp. 1–53. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

Liu, Q., Liu, Y., Dong, F., Sallach, J.B., Wu, X., Liu, X., Xu, J., Zheng, Y. & Li, Y. 2021. Uptake kinetics and accumulation of pesticides in wheat (Triticum aestivum L.): Impact of chemical and plant properties. *Environmental Pollution*, **275**, 116637, (At: https://www.sciencedirect.com/science/article/pii/S0269749121002153. Accessed: 13/5/2021).

Lun, F., Liu, J., Ciais, P., Nesme, T., Chang, J., Wang, R., Goll, D., Sardans, J., Peñuelas, J. & Obersteiner, M. 2018. Global and regional phosphorus budgets in agricultural systems and their implications for phosphorus-use efficiency. 18.

Macherius, A., Eggen, T., Lorenz, W.G., Reemtsma, T., Winkler, U. & Moeder, M. 2012. Uptake of Galaxolide, Tonalide, and Triclosan by Carrot, Barley, and Meadow Fescue Plants. *Journal of Agricultural and Food Chemistry*, **60**, 7785–7791, (At: https://doi.org/10.1021/jf301917q. Accessed: 10/5/2021).

Margenat, A., You, R., Cañameras, N., Carazo, N., Díez, S., Bayona, J.M. & Matamoros, V. 2020. Occurrence and human health risk assessment of antibiotics and trace elements in Lactuca sativa amended with different organic fertilizers. *Environmental Research*, **190**, 109946, (At:

https://www.sciencedirect.com/science/article/pii/S0013935120308410. Accessed: 10/5/2021).

Mousavi, S.A., Dalir, N., Rahnemaie, R. & Schulin, R. 2021. Phosphate and methionine affect cadmium uptake in valerian (Valeriana officinalis L.). *Plant Physiology and Biochemistry*, **158**, 466–474, (At: https://www.sciencedirect.com/science/article/pii/S0981942820305830. Accessed: 14/5/2021).

Namiki, S., Otani, T., Motoki, Y., Seike, N. & Iwafune, T. 2018. Differential uptake and translocation of organic chemicals by several plant species from soil. *Journal of Pesticide Science*, **43**, 96–107, (At: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6140680/. Accessed: 7/5/2021).

Neu, S., Müller, I., Brackhage, C., Gałązka, R., Siebielec, G., Puschenreiter, M. & Dudel, E.G. 2018. Trace elements bioavailability to Triticum aestivum and Dendrobaena veneta in a multielement-contaminated agricultural soil amended with drinking water treatment residues. *Journal of Soils and Sediments*, **18**, 2259–2270, (At: https://doi.org/10.1007/s11368-017-1741-1. Accessed: 10/5/2021).

Newman, M.C. 2014. *Fundamentals of Ecotoxicology*. 4th ed. CRC Press, Boca Raton. (At: https://doi.org/10.1201/b17658.).

Peijnenburg, W.J.G.M., Posthuma, L., Eijsackers, H.J.P. & Allen, H.E. 1997. A Conceptual Framework for Implementation of Bioavailability of Metals for Environmental Management Purposes. *Ecotoxicology and Environmental Safety*, **37**, 163–172, (At: https://linkinghub.elsevier.com/retrieve/pii/S0147651397915396. Accessed: 24/2/2022).

Peng, W. & Pivato, A. 2019. Sustainable Management of Digestate from the Organic Fraction of Municipal Solid Waste and Food Waste Under the Concepts of Back to Earth Alternatives and Circular Economy. *Waste and Biomass Valorization*, **10**, 465–481, (At: http://link.springer.com/10.1007/s12649-017-0071-2. Accessed: 25/2/2022).

Pullagurala, V.L.R., Rawat, S., Adisa, I.O., Hernandez-Viezcas, J.A., Peralta-Videa, J.R. & Gardea-Torresdey, J.L. 2018. Plant uptake and translocation of contaminants of emerging concern in soil. *Science of The Total Environment*, **636**, 1585–1596, (At: https://linkinghub.elsevier.com/retrieve/pii/S0048969718315602. Accessed: 5/5/2021).

Puschenreiter, M., Gruber, B., Wenzel, W.W., Schindlegger, Y., Hann, S., Spangl, B., Schenkeveld, W.D.C., Kraemer, S.M. & Oburger, E. 2017. Phytosiderophore-induced mobilization and uptake of Cd, Cu, Fe, Ni, Pb and Zn by wheat plants grown on metal-enriched soils. *Environmental and Experimental Botany*, **138**, 67–76, (At: https://linkinghub.elsevier.com/retrieve/pii/S0098847217300795. Accessed: 21/2/2022).

Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). 2009.

Regulation (EC) No 2003/2003 of 13 October 2003 relating to fertilizers. 2003. (At: http://data.europa.eu/eli/reg/2003/2003/oj.).

Regulation (EU) 2019/1009 of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. 2019. (At: http://data.europa.eu/eli/reg/2019/1009/oj.).

Rehman, A., Arif, M.S., Tufail, M.A., Shahzad, S.M., Farooq, T.H., Ahmed, W., Mehmood, T., Farooq, M.R., Javed, Z. & Shakoor, A. 2021. Biochar potential to relegate metal toxicity effects is more soil driven than plant system: A global meta-analysis. *Journal of Cleaner Production*, **316**, 128276, (At: https://linkinghub.elsevier.com/retrieve/pii/S0959652621024914. Accessed: 13/10/2021).

Saveyn, H., Eder, P., & Institute for Prospective Technological Studies. 2014. *End-of-waste criteria for biodegradable waste subjected to biological treatment (compost & digestate): technical proposals.* Publications Office, Luxembourg. (At: http://dx.publications.europa.eu/10.2788/6295. Accessed: 17/3/2022).

Scherer, H.W., Mengel, K., Kluge, G. & Severin, K. 2009. Fertilizers, 1. General. In: *Ullmann's Encyclopedia of Industrial Chemistry* (ed. Wiley-VCH Verlag GmbH & Co. KGaA), p. a10_323.pub3. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

Shtangeeva, I., Niemelä, M. & Perämäki, P. 2014. Effects of soil amendments on antimony uptake by wheat. *Journal of Soils and Sediments*, **14**, 679–686, (At: http://link.springer.com/10.1007/s11368-013-0761-8. Accessed: 27/5/2021).

Su, Y.H. & Liang, Y.C. 2011. Transport via xylem of atrazine, 2,4-dinitrotoluene, and 1,2,3-trichlorobenzene in tomato and wheat seedlings. *Pesticide Biochemistry and Physiology*, **100**, 284–288, (At: https://linkinghub.elsevier.com/retrieve/pii/S0048357511000915. Accessed: 24/2/2022).

The RHIZOtest - (http://www.metrhizlab.com/). (At: https://rhizotest.cirad.fr/en/the-rhizotest/methodology. Accessed: 8/4/2022).

Turull, M., Fontàs, C. & Díez, S. 2019. Conventional and novel techniques for the determination of Hg uptake by lettuce in amended agricultural peri-urban soils. *Science of The Total Environment*, **668**, 40–46, (At: https://linkinghub.elsevier.com/retrieve/pii/S0048969719307429. Accessed: 5/5/2021).

Vittori Antisari, L., Carbone, S., Gatti, A., Vianello, G. & Nannipieri, P. 2015. Uptake and translocation of metals and nutrients in tomato grown in soil polluted with metal oxide (CeO2, Fe3O4, SnO2, TiO2) or metallic (Ag, Co, Ni) engineered nanoparticles. *Environmental Science and Pollution Research*, **22**, 1841–1853, (At: https://doi.org/10.1007/s11356-014-3509-0. Accessed: 26/5/2021).

Wajid, K., Ahmad, K., Khan, Z.I. & Nadeem, M. 2021. Pattern of Trace Metal Uptake in Pearl Millet as a Result of Application of Organic and Synthetic Fertilizers. *International Journal of Environmental Research*, **15**, 33–44, (At: https://doi.org/10.1007/s41742-020-00287-w. Accessed: 6/5/2021).