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Synergistic Effect of Multiple Metals Present at Slightly Lower Concentration than the Australian Investigation Level Can Induce Phytotoxicity

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Abstract: An individual trace metal present in a soil at its ecological screening value or investigation level (trigger/threshold) is expected to cause phytotoxicity. However, phytotoxicity may be induced by a mixture of multiple metals, each present at a concentration lower than the corresponding investigation level. To investigate the accumulative impact of metals present below their individual investigation levels, three successive phytotoxicity trials were conducted in a greenhouse using the triticale plant CrackerJack (*Triticosecale rimpaui*), a cereal crop, in a sandy acidic soil treated jointly with Cd, Cu, and Zn at various rates. Seed germination and seedling growth were monitored. The metal rates in the first two trials were either too toxic or nontoxic. In the third trial, it was found that the mixture of Cd, Cu, and Zn at rates of 2.5, 97.5, and 188 mg kg^{-1}, respectively, did not affect seed germination, but caused a slight reduction in plant growth. Although metal concentrations used were lower than the Australian Ecological Investigation Level (Urban) for Cd, Cu, and Zn, which are 3.0, 100, and 200 mg kg^{-1}, respectively, the reduction occurred due to synergy. It was concluded that, to enhance the usefulness of environmental investigation limits, the synergistic effects of multiple metals present at levels slightly below the established limits must be considered.

Keywords: metal toxicity; phytotoxicity; soil contamination; sandy soil; trace metal; cereal crop

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1. Introduction

Adverse practices at trace metal mine sites, industrial areas, and agricultural lands can contaminate soils that eventually affect plants and humans [1–3]. The trace metals Cd, Cu, and Zn (CCZ) are commonly found in contaminated soils [2,4]. Metals including CCZ, when present in excess, can cause metabolic disorders in plants [5,6]. An investigation level is a screening or trigger value for a metal in soils, and is a calculated/predicted limit value (weight of metal/weight of soil) that is derived based on metal bioavailability, soil type, soil properties, input data (e.g., ecotoxicity data), calculation method, and/or protection goal (e.g., agro-system). The 'Ecological Investigation Levels (Interim Urban), Australia' (investigation levels) for Cd, Cu, and Zn are 3, 100, and 200 mg kg^{-1}, respectively [7]. Since these levels are influenced by pH and soil texture, they differ across national guidelines (Table 1).

Table 1. Critical limits for heavy metal in soils in some countries.

Country	Cd	Cu	Zn	Soil Condition
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	
Australian Ecological Investigation Limit (Urban) [7]	3	100	200	(based on sand and low pH)
European Directive 86/278/EEC [8]	1–3	50–140	150–300	6 < pH < 7
Austria (Carinthia) [9]	0.5	40	100	5 < pH < 5.5
	1	50	150	5.5 < pH < 6.5
	1.5	100	200	pH > 6.5
Germany [9]	1.5	60	200	
	1		150	Light soil with a clay content below 5% at 5 < pH < 6
Lithuania [9]	0.8	40	120	Sand, sandy loam
	1.1	60	200	Clay, clay loam
Portugal [9]	1	50	150	pH < 5.5
United Kingdom [9]	3	80	200	5 < pH < 5.5
	3	100	250	5.5 < pH < 6.0
	3	135	300	6 < pH < 7
	3	200	450	pH > 7

When concentration of a contaminant is found above investigation level, its impact on ecological values requires assessment and evaluation. Contaminated soils need remediation to reduce the associated health risks, enhance food security, and reduce land tenure problems [2] by decreasing metal mobility and bioavailability to levels below their recommended investigation levels [10].

Phytoavailability of metals depends on soil conditions such as soil type, pH, ionic strength, cation competition, and complexation by organic and inorganic ligands [11]. Cd, Cu, and Zn are highly soluble (available) in acidic soils [12,13]. Soil pH controls both the total and relative plant uptake of Cd, and its relative uptake by rice seedlings has been recorded at maximum for a pH range from 4.5 to 5.5 [5]. Accordingly, the national guidelines (Table 1) consider soil pH level in addition to Cd level. Metals accumulated by the plant have been found to correlate with free metal ion concentration in soil solution (Cd and Zn [14]; Cu (Kunhikrishnan et al., 2011). Uptake of metals, e.g., Cd, Cu, and Zn, by plants results in the reduction in concentration of these metals in the rhizosphere [15]. The depletion is followed by a replenishment of metals from their labile sources, i.e., soluble complexes and solid phase associations [15].

The permissible levels of Cd, Cu, and Zn in plants are 0.02, 10, and 0.60 mg kg^{-1}, respectively [16]. Each of Cd, Cu, and Zn, when present at a toxic level, causes different effects on plants. Excess amounts of these metals can create free radicals and oxidative stress that can harm biomolecules such as proteins, nucleic acids, lipids, and enzymes, resulting in physiological issues such as cell damage and inhibition of enzymatic activities [17,18]. Oxidative stress can inhibit plant growth and also cause cell death [18].

Excessive Cd can inhibit seed germination, reduce plant weight, root length and shoot height, reduce chlorophyll content or inhibit photosynthetic performance, and damage cells [19–21]. Cd has been found to induce oxidative stress in tomato leaves [22], whereas excessive Cu can reduce biosynthesis of chlorophyll and plant productivity by modifying photosynthesis process and nutrients [23]. Tiller, Merry [24] reported that excess Cu concentration in soils results in depressed plant growth, especially decreased germination and retarded seedling and root development. Talebi et al. [25] observed effects of Cd and Cu concentrations on the germination and growth of triticale over 10 days after each of these metals were applied individually at six levels (0, 50, 150, 300, 500, and 1000 mg/L), where it was observed that excess metals inhibited germination and growth of triticale. Excessive Zn in plants can reduce photosynthesis, root length and dry matter productivity [26,27], and eventually affect all physiological and biochemical mechanisms [28]. Brezoczki, Filip [29] observed the effects of Cd (1 to 16 mg/L), Cu (5 to 405 mg/L), and Zn (5 to 405 mg/L) individually on triticale seedling root and stem growth on day 3, 6, and 9. They found that the severity of toxicity generally had the order Cd > Cu > Zn. The critical values of these metals in Table 1 depict the same order.

Soil CCZ ions commonly enter the plant via the root, and then accumulate in roots and shoots at various concentrations. Joint toxic effects on the plant will depend on the metal species (Cd, Cu, or Zn) accumulated in the plant, location (root or shoot) of accumulation, and concentration. Co-occurrence of metals in excess may lead to synergism (increase in toxicity), antagonism (decrease in toxicity) or no effect in plants compared to a single metal [28]. The level of phytotoxicity can be affected by the interactions among Cd, Cu, Zn, and Pb present in the soil, as well as the length of exposure [6]. Oxidative stress was synergistic under combined application of Cd and Zn, where the later was at high level; the stress level was greater than it was for Cd or Zn in excess by themselves [22]. In a solution culture of single metals (Cd 5×10^{-7} M, Cu 10^{-5} M, or Zn 5×10^{-5} M), Wallace [30] observed no to some stress on plants; dry weight of trifoliate leaves was reduced by 0, 2, and 43% for Cd, Cu, and Zn, respectively. However, for a joint application of these three metals, the reduction was 46%. Additive, protective, and synergistic effects were involved. The authors suggested that even if the concentration of certain metals is too low to be visible, they can still have significant implications when combined with other trace element stresses. Versieren et al. [31] suggested that the effects of metal mixture can be stronger than single metals, and that Cd + Cu have synergistic effects, while Zn can reduce the synergistic effects when combined with Cd and/or Cu. When 2.5×10^{-5} M of Cd, Cu, and Zn were added to the solution culture individually, in pairs, or together, Cu, and Zn had an additive effect on reducing Cd concentrations in plant roots [32]. Kutrowska et al. [33] treated Indian mustard seedlings with CCZ in pairs, and observed that Zn decreased Cd accumulation in leaves while increasing the Zn level. The inhibition magnitude followed the order Cu + Cd > Cu + Zn, Cu > Cd > Zn > Zn + Cd. The first two pairs inhibited biomass production and decreased the seedlings' primary biomass by 38–54%. They observed various interactions: Cd + Cu showed weak synergy in roots and weak competition in favor of Cd in shoots, Cd + Zn showed competition in roots and synergy in favor of Cd, and Cu + Zn showed weak competition in favor of Zn and competition in shoots. During plant uptake, Cd can compete with H^+ even at low pH. It can also compete with Zn^{2+}, which actually displaces Cd^{2+} from soil sorption [12]. Sandy soils with acidic condition are vulnerable to metal contamination, as acidity increases mobility of CCZ [34] and sands have very low binding capacity for metals [35].

A trace metal present in a soil at its investigation level is expected to cause phytotoxicity. However, we hypothesized that multiple metals, each present at a slightly lower level than its corresponding investigation level, will affect plant growth. No published scientific information was found addressing this topic. Predicting investigation level of Cu content of soil for plants is extremely complex [5]. It is also a fact that validation studies of metal limit values used by jurisdictions across the world for environmental and ecosystem protection is rare [36]. The general aim of this research was to study phytotoxicity of a soil treated with CCZ at various levels around their corresponding investigation levels. Moreover, a soil, which is marginally contaminated with CCZ, was also required for subsequent research studies (not covered in this communication) on bioremediation of metal phytotoxicity using biochar and compost. It should be noted that the subsequent research studies involved a CCZ desorption trial, and a greenhouse rhizosphere trial using CCZ-contaminated soil and triticale plant.

A decision was made to treat a soil with CCZ, since a contaminated soil that had only these three metals at expected levels could not be found in the environment. Hence, the specific objective of this study was to identify a suitable set of CCZ rates close to their corresponding investigation levels, which would allow germination of plant seeds yet marginally affect their growth.

2. Materials and Methods

2.1. Soil Collection

An uncontaminated sandy acidic soil (uSoil) was collected from Cleland Conservation Park (Google map coordinate 34°58′19.8″ S, 138°42′28.9″ E), Mt Lofty, South Australia. The area has a native tree cover and receives about 1000 mm of rain annually [37]. The soil was collected in the summer from the top 10 cm of the profile. The soil was sieved to less than 4 mm on site. Since the moisture content was low (0.72%), no further drying of the soil was required. This was later sieved to less than 2 mm prior to use.

2.2. Soil Properties

The properties of uSoil are listed in Table 2. Briefly, it is a sandy soil (sand 89.05%; clay 1.97%) that has low pH (3.72), low CEC (3.27 cmol (+) kg^{-1}), and low contents of Cd, Cu, and Zn (<0.1, 1.95 and 7.92 mg kg^{-1} (dry weight), respectively).

Table 2. Chemical properties of uncontaminated sandy soil (<2 mm fraction) collected from Cleland Conservation Park, Mt Lofty, South Australia.

Soil	pH	EC	CEC	OC	N	P	K	S	Ca	Mg	Na
		dS m^{-1}	cmol (+) kg^{-1}	%	%	%	%	%	%	%	%
uSoil *	3.72	5.77	3.27	2.29	0.15	<0.01	0.13	0.01	0.03	0.04	<0.01
Soil	Al	As	B	Cd	Cu	Fe	Mn	Mo	Pb	Zn	
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	
uSoil	7053.21	<0.1	1.53	<0.1	1.95	1404.37	9.86	<0.5	29.14	7.92	

* uSoil: uncontaminated soil.

2.3. Treating of Soil with Metals and Incubation

The recommended investigation levels of 3, 100 and 200 mg kg^{-1} for Cd, Cu, and Zn, respectively [7], were used as reference rates. The uSoil and a multi-metal solution containing enough water to raise moisture content to 50% of water holding capacity (100% WHC = 35.54 g mL^{-1}) were manually mixed in a strong plastic bag and left for 24 h. It was assumed that the metals were distributed in various soil compartments by this period [38]. Analytical grade chemicals $CdSO_4$, $CuSO_4$, and $ZnSO_4.7H_2O$ from Sigma Aldrich were used for treating the soil. Ultra-pure water (Type 1: 18.2 MΩ-cm; ELGA PURELAB Classic) was used throughout the experiment.

Three successive trials were conducted to test phytotoxicity [39] and identify CCZ rates (Table 3). For each trial, uSoil was used as the control treatment. The CCZ rates were varied from very low to high during the three plant trials. The CCZ rates are shown in Table 3. The relative germination, shoot weight, and relative shoot weight were recorded.

Since the CCZ rates used in Trial 1 Trial led to an inconclusive result (discussed in Results section later), the uSoil was treated again at different CCZ rates for Trial 2 (Trial Table 3). Based on the result in the first two trials, the uSoil was finally treated for Trial 3 Trial with Cd, Cu, and Zn at 2.5, 97.5, and 188 mg kg^{-1}, respectively. The CCZ rates are discussed in Section 3.

2.4. Chemical Analysis

Total N was determined by dry combustion of 0.2 g of dried and ground sample at 1100 °C in a LECO TruMac CNS Analyzer [40]. Organic carbon (OC) was determined by dry combustion of 0.2 g of dried, ground and post-acidified sample at 1100 °C in a LECO TruMac CNS Analyzer [41]. The pre-weighed sample was acidified on Ni boat liner placed within a ceramic crucible; this was followed by oven drying overnight. The LECO

TruMac CNS Analyzer uses IR technology to determine analyte content. Total elements were measured by ICP-OES after microwave digestion of 0.1 g of sample with 4 mL 69% HNO_3 and 1 mL 33% H_2O_2. pH (1:10) was measured electrometrically in a slurry, with smartCHEM-LAB Laboratory Analyzer; EC was measured in the filtrate using the same equipment. Deionized water was used in the chemical analyses.

Table 3. Trial 1, 2 and 3: germination and shoot growth of triticale plant in soils with various metal treatments.

Treatment	Metal Rate			Relative Germination	Shoot Weight (Variance)		Relative Shoot Weight	Comment on Phytotoxicity	Comment on Metal Rate	Decision about Metal Rate
	mg kg^{-1}			%	g		%			
	Cd	Cu	Zn							
Investigation level *	3	100	200					Expected phytotoxicity		
Trial-1 (5 days)										
T1a (Control)	0	2	7.9	100%	0.0380^{II}	(1.6×10^{-6})	100%	Not phytotoxic	Very low	
T1b (+ve Control)	0.5	9.8	29.7	123%	0.0519^{IV}	(4.6×10^{-7})	137%	Not phytotoxic (Cu & Zn acted as nutrient)	Very low	Ignore
T1c (+ve Control)	2.5	48.8	111.4	123%	0.0467^{III}	(6.4×10^{-6})	123%	Not phytotoxic (Cu & Zn acted as nutrient)	Low	Ignore
T1d	12.5	243.8	417.7	115%	0.0143^{I}	(3.1×10^{-6})	38%	Phytotoxic	High	Ignore
T1e	62.5	1218.8	1566.2	0%	0%	(0)	0%	Very phytotoxic	Very high	Ignore
Trial-2 (5 days)										
T2a (Control)	0	2	7.9	100%	0.0473^{II}	(8.7×10^{-6})	100%	Not phytotoxic	Very low	
T2b	5	97.5	187.9	100%	0.0386^{I}	(7.1×10^{-7})	82%	Phytotoxic	Cd rate high	Reduce Cd a little
T2c	7.5	146.3	264.5	100%	0.0345^{I}	(4.4×10^{-5})	73%	Phytotoxic	Cd rate high	Ignore
Trial-3 (32 days)										
T3a (Control)	0	2	7.9	100%	0.2176^{II}	(1.6×10^{-5})	100%	Not phytotoxic	Very low	
T3b	2.5	97.5	187.9	100%	0.1894^{I}	(2.8×10^{-7})	87%	Slightly phytotoxic	Little lower than EIL level	Accept

Color: data reflecting phytotoxicity are highlighted with light red; the rest are highlighted with blue. * Australian Environment Investigation Level (Urban) [7]. $^{I–IV}$ Treatments in each trial with the same roman superscript belong to the same statistically significant group according to Tukey post hoc analysis (One-way ANOVA) in trial 1 and 2, and *t*-test in Trial 3.

2.5. Trial 1, 2 and 3

Pots were used in the first two trials, and rhizotron [42] in the third trial. The uSoil had a very low pH and high Al level (Table 2), and thus was expected to have high Al toxicity. Hence, a triticale plant (*Triticosecale rimpaui*, CrackerJack variety from Heritageseeds, Australia; Triticale) was selected for its high tolerance for acidity and Al toxicity. Triticale is one of the major cereals in the world. It is an amphiploid cereal crop developed by crossing wheat and rye [25].

The experiments were conducted in a greenhouse. Each pot (plastic, d = 12.5 cm) contained 200 g (dry weight equivalent) and each rhizotron (200 mm × 400 mm × 6 mm) contained 491 g (dw equivalent) soil. The rhizotron was made from transparent Perspex. Inorganic fertilizer P and K (40 and 50 mg kg^{-1} of dry equivalent soil) solution made with KH_2PO_4 and deionized water was added to the treated soil. The soil moisture content was maintained at 70% of the water-holding capacity. The pots/rhizotrons were weighed every two days, followed by addition of the required makeup water. The treated and fertilized soil was manually mixed prior to placement in a pot or rhizotron, followed by the sowing of eight Triticale seeds. There were four pots/rhizotrons per set of CCZ rates. The pot/rhizotron was covered with a thin plastic film to limit evaporation until the seedlings grew over the container brim. The pots/rhizotrons were placed in a greenhouse, where the temperature was controlled between 17.5 °C and 25 °C. The rhizotron was rested vertically and wrapped with aluminum foil to keep the roots in the dark. The irrigation was conducted from the top and front panel holes using a plastic syringe.

The germination count was conducted on the fifth day in the case of Trials 1 and 2, or the seventh day in the case of Trial 3. The shoot samples were collected on the fifth day in the case of Trial 1 and 2, or in the 4th week in the case of Trial 3. The study period (4 weeks) of Trial 3 was matched to that of the rhizosphere trial that was conducted later, as noted after the general aim above. The shoots were dried at 70 °C prior to measuring their dry weight. Relative germination % and relative shoot weight % were calculated based on germination and shoot weight, respectively, of the control.

2.6. Data Analysis

The mean shoot weights were compared using one-way analysis of variance (ANOVA) in conjugation with Tukey post hoc test for Trial 1 and 2 [43]. However, having only two treatments, the *t*-test was used to compare the mean shoot weights in the case of Trial 3. The statistical analyses were conducted at the significance level of 0.05. IBM SPSS 20 software was used for the statistical analyses.

3. Results and Discussion

3.1. Trial 1 and 2

In Trial 1, CCZ rates were lower in treatments T1b and T1c than the corresponding investigation levels, while they were higher in treatments T1d and T1e (Table 3). In Trial 2, Cu, and Zn rates were slightly lower in treatment T2b than their corresponding investigation levels, but higher in treatment T2c. Cd rate was higher in both treatments.

The relative germination in the first two trials was 123%, 123%, 100%, and 100% for treatments T1b, T1c, T2b, and T2c, respectively (Table 3). In Trial 1, the control treatment T1a had a low germination rate (6.5 seedlings on average from 8 seeds), thereby leading to relative (to control) germination >100% in T1b and T1c.

The relative shoot growth in the two trials were 137%, 123%, 38%, 82%, and 73% for T1b, T1c, T1d, T2b, and T2c, respectively (Table 3). The CCZ rates applied in treatments T1d, T2b, and T2c inhibited shoot growth due to excess metal content [25]. The CCZ rates applied in treatments T1b and T1c were too low to inhibit shoot growth, therefore, these rates were deemed unsuitable. The CCZ rate applied in treatment T1d was unacceptable due to very low shoot growth. Treatment T2c caused a moderate inhibiting effect on shoot growth and a strong inhibiting effect on root growth; this is visible in Figure 1. Hence, the rates in this treatment were also unacceptable.

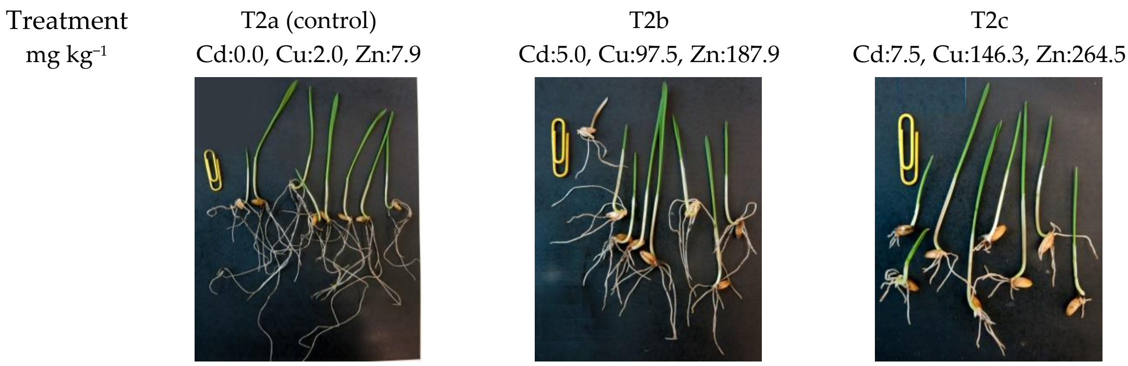

Figure 1. Germination and growth of Triticale in one of the replications in the control and treated soil on the fifth day in Trial 2.

The treatment T2b caused a slight negative effect on the shoot growth. Cu and Zn rates were slightly lower in this treatment than the corresponding investigation levels, but Cd rate was higher, which was assumed to cause significant inhibition effect of the root growth (Figure 1). Such inhibition could pose a risk to a proper study of remediation of rhizosphere soil. Hence, to reduce negative effects on shoot growth, and particularly root growth, a slightly lower rate than the corresponding investigation level was considered for Cd. Therefore, for Trial 3, the metal rates investigated were Cd:2.5, Cu:97.5, and Zn:188.0 mg kg^{-1} (T3b, Table 3).

3.2. Trial 3

In Trial 3, the relative germination was 100% and the relative shoot growth was 87% for treatment T3b. A single metal present in a soil at its investigation level is expected to affect plant growth. However, this trial shows that presence of CCZ at slightly lower levels than their corresponding investigation levels can marginally affect plant growth. Conclusively, the combined adverse effect of multiple metals on plant growth is higher than an individual metal [44]. Moreover, bioavailability of CCZ in a sandy texture with acidic conditions is expected to be high [34]. The adverse effect on seedlings can be caused by heavy metal-induced free radicals and oxidative stress that can harm plant biomolecules leading to physiological problems such as cell damage and inhibition of enzymatic activities [17,18]. Since in Trial 3 treatment T3b marginally affected plant growth but not germination, it was deemed acceptable as a media for subsequent metal desorption and rhizosphere studies. This study reveals that soil with CCZ levels slightly below investigation levels can be phytotoxic, even though it is not considered to be so according to investigation levels. To enhance the practicality of environmental investigation limits, it is essential to consider the synergistic effects of multiple metals present at levels slightly below the set limits.

4. Conclusions

Three greenhouse trials were conducted to determine a combination of CCZ rates that would have a minimal effect on the growth of Triticale plants without impacting germination. Finally, the set of rates Cd:2.5, Cu:97.5, and Zn:188.0 mg kg^{-1} (T3b) was found to satisfy this aim. Each rate in this set is slightly lower than its corresponding investigation level, i.e., Cd:3, Cu:100, and Zn:200 mg kg^{-1}. We conclude that multiple metals, even if each is present at slightly below investigation level, can cause mild phytotoxicity. Treatment T3b was suitable for subsequent bioremediation studies. It is necessary to address the synergistic effects of multiple metals present at levels slightly below the set limits to enhance the practicality of environmental investigation limits. Future studies are recommended to encompass a broader selection of crops, soils, soil conditions, and metal combinations at levels slightly below the set limits to further improve the environmental investigation limits.

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References

1. Bohra, A.; Sanadhya, D.; Chauhan, R. Heavy Metal Toxicity and Tolerance in Plants with Special Reference to Cadmium: A Review. *J. Plant Sci. Res.* **2015**, *31*, 51–74.
2. Wuana, R.A.; Okieimen, F.E. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecol.* **2011**, *2011*, 1–20. [CrossRef]
3. Pinto, M.M.S.C.; Marinho-Reis, A.P.; Almeida, A.; Freitas, S.; Simões, M.R.; Diniz, M.L.; Pinto, E.; Ramos, P.; Ferreira da Silva, E.; Moreira, P.I. Fingernail trace element content in environmentally exposed individuals and its influence on their cognitive status in ageing. *Expo. Health* **2019**, *11*, 181–194. [CrossRef]
4. Bolan, N.; Kunhikrishnan, A.; Thangarajan, R.; Kumpiene, J.; Park, J.; Makino, T.; Kirkham, M.B.; Scheckel, K. Remediation of heavy metal(loid)s contaminated soils—To mobilize or to immobilize? *J. Hazard. Mater.* **2014**, *266*, 141–166. [CrossRef]
5. Kabata-Pendias, A. *Trace Elements in Soils and Plants*; CRC Press: Boca Raton, FL, USA, 2010.
6. Påhlsson, A.-M.B. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants: A literature review. *Water Air Soil Pollut.* **1989**, *47*, 287–319. [CrossRef]
7. NEPC. *National Environment Protection (Assessment of Site Contamination) Measure 1999: Schedule B (1)—Guideline on the Investigation Levels for Soil and Groundwater*; National Environment Protection Council: Canberra, Australia, 1999; p. 16.
8. Grobelak, A.; Napora, A. The chemophytostabilisation process of heavy metal polluted soil. *PLoS ONE* **2015**, *10*, e0129538. [CrossRef]
9. Council of the European Communities. Council directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (86/278/EEC). *Off. J. Eur. Communities* **1986**, *L181*, 1–13.
10. Hudcová, H.; Vymazal, J.; Rozkošný, M. Present restrictions of sewage sludge application in agriculture within the European Union. *Soil Water Res.* **2019**, *14*, 104–120. [CrossRef]

11. Ashworth, D.J.; Alloway, B.J. Complexation of copper by sewage sludge-derived dissolved organic matter: Effects on soil sorption behaviour and plant uptake. *Water Air Soil Pollut.* **2007**, *182*, 187–196. [CrossRef]
12. Stacey, S.P.; McLaughlin, M.J.; Hettiarachchi, G.M. Fertilizer-Borne Trace Element Contaminants in Soils. In *Trace Elements in Soils*; Hooda, P.S., Ed.; John Wiley & Sons Ltd.: Wiltshire, UK, 2010; pp. 135–154.
13. Chaney, R.L.; Broadhurst, C.L.; Centofanti, T. Phytoremediation of Soil Trace Elements. In *Trace Elements in Soils*; Hooda, P.S., Ed.; John Wiley & Sons Ltd.: Wiltshire, UK, 2010; pp. 311–352.
14. Hamon, R.; Lorenz, S.; Holm, P.; Christensen, T.; McGrath, S. Changes in trace metal species and other components of the rhizosphere during growth of radish. *Plant Cell Environ.* **1995**, *18*, 749–756. [CrossRef]
15. Tack, F.M.G. Trace elements: General soil chemistry, principles and processes. In *Trace Elements in Soils*; Hooda, P.S., Ed.; Wiley Online Library: Wiltshire, UK, 2010; pp. 9–37.
16. WHO. *Permissible Limits of Heavy Metals in Soil and Plants*; World Health Organization: Geneva, Switzerland, 1996.
17. Wu, X.; Cobbina, S.J.; Mao, G.; Xu, H.; Zhang, Z.; Yang, L. A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. *Environ. Sci. Pollut. Res.* **2016**, *23*, 8244–8259. [CrossRef] [PubMed]
18. Loix, C.; Huybrechts, M.; Vangronsveld, J.; Gielen, M.; Keunen, E.; Cuypers, A. Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. *Front. Plant Sci.* **2017**, *8*, 1867. [CrossRef]
19. Hayat, M.T.; Nauman, M.; Nazir, N.; Ali, S.; Bangash, N. Environmental hazards of cadmium: Past, present, and future. In *Cadmium Toxicity and Tolerance in Plants*; Hasanuzzaman, M., Ed.; Academic Press: London, UK, 2019; pp. 163–183.
20. Gratão, P.L.; Monteiro, C.C.; Rossi, M.L.; Martinelli, A.P.; Peres, L.E.P.; Medici, L.O.; Lea, P.J.; Azevedo, R.A. Differential ultrastructural changes in tomato hormonal mutants exposed to cadmium. *Environ. Exp. Bot.* **2009**, *67*, 387–394. [CrossRef]
21. Vardhan, K.H.; Kumar, P.S.; Panda, R.C. A review on heavy metal pollution, toxicity and remedial measures: Current trends and future perspectives. *J. Mol. Liq.* **2019**, *290*, 111197. [CrossRef]
22. Cherif, J.; Mediouni, C.; Ammar, W.B.; Jemal, F. Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (*Solarium lycopersicum*). *J. Environ. Sci.* **2011**, *23*, 837–844. [CrossRef] [PubMed]
23. Adrees, M.; Ali, S.; Rizwan, M.; Ibrahim, M.; Abbas, F.; Farid, M.; Zia-ur-Rehman, M.; Irshad, M.K.; Bharwana, S.A. The effect of excess copper on growth and physiology of important food crops: A review. *Environ. Sci. Pollut. Res.* **2015**, *22*, 8148–8162. [CrossRef]
24. Tiller, K.G.; Merry, R.H. Copper pollution of agricultural soils. In *Copper in Soils and Plants*; Loneragan, J.F., Robson, A.D., Graham, R.D., Eds.; Academic Press: New York, USA, 1981.
25. Talebi, S.; Kalat, S.N.; Darban, A.S. The study effects of heavy metals on germination characteristics and proline content of Triticale (*Triticoseale wittmack*). *Int. J. Farming Allied Sci.* **2014**, *3*, 1080–1087.
26. Hammerschmitt, R.K.; Tiecher, T.L.; Facco, D.B.; Silva, L.O.S.; Schwalbert, R.; Drescher, G.L.; Trentin, E.; Somavilla, L.M.; Kulmann, M.S.S.; Silva, I.C.B.; et al. Copper and zinc distribution and toxicity in 'Jade'/'Genovesa' young peach tree. *Sci. Hortic.* **2020**, *259*, 108763. [CrossRef]
27. Song, C.; Yan, Y.; Rosado, A.; Zhang, Z.; Castellarin, S.D. ABA alleviates uptake and accumulation of zinc in grapevine (*Vitis vinifera* L.) by inducing expression of ZIP and detoxification-related genes. *Front. Plant Sci.* **2019**, *10*, 872. [CrossRef]
28. Alengebawy, A.; Abdelkhalek, S.T.; Qureshi, S.R.; Wang, M.-Q. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics* **2021**, *9*, 42. [CrossRef]
29. Brezoczki, V.M.; Filip, G.M. The heavy metal ions (Cu^{2+}, Zn^{2+}, Cd^{+}) toxic compounds influence on triticale plants growth. In *IOP Conference Series: Materials Science and Engineering*; IOP Publishing: Bristol, UK, 2017.
30. Wallace, A. Additive, protective, and synergistic effects on plants with excess trace elements. *Soil Sci.* **1982**, *133*, 319–323. [CrossRef]
31. Versieren, L.; Evers, S.; De Schamphelaere, K.; Blust, R.; Smolders, E. Mixture toxicity and interactions of copper, nickel, cadmium, and zinc to barley at low effect levels: Something from nothing? *Environ. Toxicol. Chem.* **2016**, *35*, 2483–2492. [CrossRef]
32. Wallace, A.; Romney, E.M. Synergistic trace metal effects in plants. *Commun. Soil Sci. Plant Anal.* **1977**, *8*, 699–707. [CrossRef]
33. Kutrowska, A.; Małecka, A.; Piechalak, A.; Masiakowski, W.; Hanć, A.; Barałkiewicz, D.; Andrzejewska, B.; Zbierska, J.; Tomaszewska, B. Effects of binary metal combinations on zinc, copper, cadmium and lead uptake and distribution in Brassica juncea. *J. Trace Elem. Med. Biol.* **2017**, *44*, 32–39. [CrossRef] [PubMed]
34. Kuo, S.; Baker, A.S. Sorption of copper, zinc, and cadmium by some acid soils. *Soil Sci. Soc. Am. J.* **1980**, *44*, 969–974. [CrossRef]
35. Rosen, V.; Chen, Y. The influence of compost addition on heavy metal distribution between operationally defined geochemical fractions and on metal accumulation in plant. *J. Soils Sed.* **2014**, *14*, 713–720. [CrossRef]
36. Hooda, P.S. Regulatory Limits for Trace Elements in Soils. In *Trace Elements in Soils*; Hooda, P.S., Ed.; John Wiley & Sons Ltd.: Wiltshire, UK, 2010; pp. 293–309.
37. Bureau of Meteorology. Climate Data Online. Available online: http://www.bom.gov.au/climate/data (accessed on 26 January 2023).
38. Hogg, D.S.; McLaren, R.G.; Swift, R.S. Desorption of copper from some New Zealand soils. *Soil Sci. Soc. Am. J.* **1993**, *57*, 361–366. [CrossRef]
39. Organisation for Economic and Cultural Development. *Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test*; OECD: Paris, France, 2006; p. 21.

40. LECO. *LECO Trumac CN Analyser—Carbon/Nitrogen in Soil and Plant Tissue (Organic Application Note)*; LECO Corporation: St Joseph, MI, USA, 2012.
41. LECO. *Non-Carbonate Carbon Method*; LECO Corporation: St Joseph, MI, USA, 2013; p. 1.
42. Prendergast-Miller, M.; Sohi, S. Investigating biochar impacts on plant roots and root carbon. In Proceedings of the Soil Organic Matter Conference, Cote d'Azur, France, 19–23 September 2010.
43. Jones, D.; Rousk, J.; Edwards-Jones, G.; DeLuca, T.; Murphy, D. Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biol. Biochem.* **2011**, *45*, 113–124. [CrossRef]
44. Kumar, A.; CSIRO, Urrbrae, SA, Australia. Personal communication, 2021.