Assessment of gentle remediation options for trace element-contaminated agricultural land under semi-controlled and field conditions

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Die Übereinstimmung dieses Exemplars mit dem Original der Dissertation zum Thema:

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"[...], the use of polluted lands will have to feature highly in modern agriculture."

P. C. Abhilash, 2016

To my family

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Abstract

The global soil resources are significantly threatened by pollution. In addition to the existing burden of contaminants in agricultural soils, the increasing anthropogenic input of metal(loid)s, further referred to as trace elements (TE), presents a major public health concern, since it endangers the food security of a rising human population. However, the growing demand for agricultural commodities will increase the pressure on fertile soils. In this context, steering the needed agricultural extensification towards arable TE-contaminated soils (TECS) could protect highly biodiverse or carbon stock land and, thereby, help reach global sustainability targets. The sustainable crop production on TECS requires effective and non-destructive measures to control relevant pollutant linkages. These are offered by gentle remediation options (GRO), the practical adoption of which is scarce across Europe as yet. This study provides different approaches of GRO applied to an agricultural soil in a characteristic post-mining region (Freiberg, Saxony (Germany)) in practical adoption (chapter 2) and under semi-controlled conditions (chapters 3 and 4). Due to severe topsoil contamination by metals (Cd, Pb, and Zn) and As, the pollutant linkages of concern at the study site are food-chain transfer, leaching to the groundwater, and tilling-related dust emissions. The overall aim was to find best management practices for coupling soil remediation with the production of marketable biomass. This was attempted via (i) *in situ* stabilization, alone (chapter 4.1) or combined with phytoexclusion (chapter 2), (ii) labile TE-phytoextraction (chapter 3), and (iii) (aided) phytostabilization (chapter 4.2).

Soil remediation by GRO was assessed with scientifically established (DGT, soil solution; chapter 4.1) and/or legally relevant chemical soil extractions (NH_4NO_3 -solution; chapters 2 to 4) and pH measurements. Additionally, earthworms served as ecotoxicological endpoints (chapter 4.1). Initial soils, earthworms, vegetative and generative biomass produced from each approach, as well as the investigated soil additives, including fertilizers, were microwave-assisted chemically digested (HNO_3 , H_2O_2 , *aqua regia*) prior to analysis. All environmental samples were analyzed for TE by inductively coupled plasma mass spectrometry (ICP-MS). Nutrient concentrations in soil additives and selected plant samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). The measured TE concentrations were evaluated against applicable European and/or national thresholds.

At field (chapter 2), the repeated fertilization with superphosphate and/or lime marl basically attenuated the chemical TE availability over a three years crop rotation of *Brassica napus*, *Triticum aestivum*, and *Hordeum vulgare*. In turn, the simultaneous phytoexclusion by low-accumulating cultivars (LAC) effectively decreased the Cd concentrations in cereal grains (by

averagely 21 % in wheat and 39 % in barley). However, straw metals' accumulation or grain As uptake partly revealed opposing trends among LAC and high-accumulating cultivars (HAC). As investigated under semi-controlled conditions (chapter 3), a sunflower (*Helianthus annuus* L. mutant inbred line M7 (R3B-F-U/R13M10A; test series R13F-MP), modified towards enhanced labile TE phytoextraction by chemical mutagenesis, proved less advantageous as pre-crop for winter wheat (*Triticum aestivum* L. cv. Tiger) than the regionally common winter oilseed rape (*Brassica napus* L. cv. Lorenz). This resulted from soil alkalization by rape, whereas sunflower mobilized more TE than it depleted from the rhizosphere. Within *in situ* stabilization approaches under semi-controlled conditions, a Fe-/Al-rich drinking water treatment residue (WTR), soil-applied at a rate of 1 % (*m/m*) prior to cultivation of wheat (*Triticum aestivum* L. cv. Tiger; chapter 4.1) or Szarvasi-1 (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1; chapter 4.2), decreased the chemical availability of As, Cd, and Pb by up to 77 % , 46 % , and 61 % , respectively. Thereby, it immobilized these hazardous TE increasingly effective over time and better than a Mn-rich WTR or lime marl. The bioassays with wheat and earthworms (*Dendrobaena veneta* L.) showed, however, that the habitat function for biocenoses benefited more from the Fe-/Al-rich WTR when it was applied at a lower application rate (0.5 % *m/m*). This resulted from dose-dependent P fixation and TE entries induced by the WTR, to which Szarvasi-1 appeared insensitive. Unlike As, the availability of Cd and Zn to biota in amended soils could not be predicted by any of the applied chemical methods due to endpoint-specific binding of competing cations to the biotic ligand (plant roots, earthworm tissue), and a preferential translocation of Zn over Cd *in planta*. Among all studied plants, the perennials Szarvasi-1 and cup plant (*Silphium perfoliatum* L.; chapter 4.2) best excluded the present mixture of TE in shoots, whereby the latter exhibited growth depression. However, only grain biomass of barley and rape, and partly of low-accumulating wheat, produced at the study site presented legally compliant animal feed based on European limit values for Cd and As.

Given the investigated measures' restricted efficacy to assure forage safety, a land-use change e.g. towards the perennial Szarvasi-1, which provides a continuous plant cover at low tillage and input requirements, possibly accompanied by the monitored reuse of an Fe- /Al-rich WTR, could most promisingly control all above-stated pollutant linkages. Revenues could be generated from energy conversion or valorization in the fibrous material sector. The waste recycling of WTR in TECS, though promising, requires proper characterization, eventual process optimization, and further studies regarding long-term stability to ensure legal compliance and environmental safety. Future research and breeding efforts regarding low Cd

cultivars in particular could greatly contribute to safe food or forage production at the majority of moderately contaminated sites.

Zusammenfassung

Die globalen Bodenressourcen sind in erheblichem Maße von Schadstoffbelastungen bedroht. Zusätzlich zu bereits bestehenden Belastungen landwirtschaftlich genutzter Böden gefährdet insbesondere der steigende anthropogene Eintrag von Metal(l)iden, nachfolgend als Spurenelemente (TE) bezeichnet, die Nahrungsmittelsicherheit einer wachsenden Weltbevölkerung und stellt damit ein wesentliches Gesundheitsrisiko dar. Zugleich steigt aufgrund wachsender Nachfrage nach landwirtschaftlichen Produkten der Nutzungsdruck auf fruchtbare Böden. In diesem Kontext könnte die Lenkung der notwendigen Ausdehnung landwirtschaftlicher Nutzflächen auf anbaufähige TE-belastete Böden (TECS) zum Schutz von Flächen mit großer biologischer Vielfalt oder hohem Kohlenstoffbestand, und damit zur Erreichung globaler Nachhaltigkeitsziele, beitragen. Für eine nachhaltige Biomasseerzeugung auf TECS bedarf es effektiver und zerstörungsfreier Maßnahmen zur Kontrolle relevanter Wirkungspfade der Schadstoffbelastung. Hierzu bieten sich sanfte Sanierungsverfahren (GRO) an, deren praktische Umsetzung innerhalb Europas jedoch bislang kaum erfolgt. Die vorliegende Arbeit beinhaltet verschiedene solcher Verfahren, die auf einen landwirtschaftlichen Boden in einem charakteristischen ehemaligen Bergbaugebiet (Region Freiberg, Erzgebirge, Sachsen) unter realen Freilandbedingungen (Kapitel 2) und unter kontrollierten freilandähnlichen Bedingungen (Kapitel 3 und 4) angewandt wurden. Aufgrund der starken Belastung des Oberbodens mit Schwermetallen (Cd, Pb und Zn) und As sind Schadstoffübergänge in die Nahrungskette und das Grundwasser sowie bearbeitungsbedingte Emissionen belasteter Stäube zu besorgen. Das übergeordnete Ziel bestand darin, optimierte Bewirtschaftungssysteme zur Koppelung von Bodensanierung und vermarktungsfähiger Biomasseerzeugung zu identifizieren. Dies wurde mittels (i) *in situ* Stabilisation, allein (Kapitel 4.1) oder in Verbindung mit Phytoexklusion (Kapitel 2), (ii) labiler TE-Phytoextraktion (Kapitel 3), und (iii) (unterstützter) Phytostabilisation (Kapitel 4.2) erprobt. Die bodensanierende Wirkung von GRO wurde anhand wissenschaftlich etablierter (DGT, Bodenlösung; Kapitel 4.1) und/oder gesetzlich relevanter chemischer Bodenextraktionen (NH_4NO_3 -Lösung; Kapitel 2 bis 4) sowie pH-Wertmessungen untersucht. Zusätzlich dienten Regenwürmer als ökotoxikologische Endpunkte (Kapitel 4.1). Die Analytik erfolgte für initiale Versuchssubstrate, Regenwürmer, die innerhalb der jeweiligen Versuchsansätze produzierte vegetative und generative Biomasse sowie für die untersuchten Bodenzusatzstoffe, einschließlich herkömmlicher Düngemittel, im Anschluss an mikrowellenunterstützten nasschemischen Aufschluss. Alle Proben wurden anhand von Massenspektrometrie mit induktiv gekoppeltem Plasma (ICP-MS) auf Spurenelemente

analysiert. Nährstoffanalysen in Bodenzusatzstoffen sowie ausgewählten Pflanzenproben (Kapitel 3 und Kapitel 4.1) wurden mittels optischer Emissionsspektrometrie mit induktiv gekoppeltem Plasma (ICP-OES) durchgeführt. Die gemessenen TE-Konzentrationen wurden gültigen europäischen und/oder nationalen Grenzwerten gegenübergestellt.

Im Freilandversuch (Kapitel 2) verringerte die wiederholte Düngung mit Superphosphat und/oder Kalkmergel über eine dreijährige Fruchtfolge von *Brassica napus*, *Triticum aestivum* und *Hordeum vulgare* im Wesentlichen die chemische TE-Verfügbarkeit im Boden. Die gleichzeitige Phytoexklusion mittels schwach akkumulierender Sorten (LAC) reduzierte indes effektiv die Konzentration von Cd im Getreidekorn (bei Weizen um durchschnittlich 21 % und bei Gerste um 39 %). Dabei wurden allerdings zum Teil gegenläufige Effekte zwischen schwach und stark akkumulierenden Sorten hinsichtlich der Schwermetallakkumulation im Stroh oder der As-Akkumulation im Korn beobachtet. Unter kontrollierten freilandähnlichen Bedingungen (Kapitel 3) zeigte eine, infolge chemischer Mutagenisierung verstärkt pflanzenverfügbare TE extrahierende, Sonnenblume (*Helianthus annuus* L. mutant inbred line M7 (R3B-F-U/R13M10A; test series R13F-MP) ungünstigere Wirkungen auf nachfolgend angebauten Winterweizen (*Triticum aestivum* L. cv. Tiger) als die in der Region übliche Getreidevorfrucht Winterraps (*Brassica napus* L. cv. Lorenz). Dies konnte auf Bodenalkalisierung durch Raps zurückgeführt werden, wohingegen Sonnenblumen mehr TE im Boden mobilisierten als durch sie akkumuliert wurden. Im Rahmen von Versuchsansätzen zur *in situ* Stabilisation unter kontrollierten freilandähnlichen Bedingungen verringerte ein Fe-/Al-reicher Wasserwerksschlamm (WTR), vor der Aussaat von Weizen (*Triticum aestivum* L. cv. Tiger; Kapitel 4.1) oder Szarvasi-1 (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1; Kapitel 4.2) in einer Aufwandmenge von 1 % (*m/m*) zum Boden appliziert, die chemische Verfügbarkeit von As, Cd und Pb um bis zu 77 %, 46 % beziehungsweise 61 %. Dabei wurden diese Schadstoffe mit zunehmender Expositionsdauer stärker und besser immobilisiert als durch einen simultan getesteten Mn-reichen WTR oder Kalkmergel. Die Bioassays mit Weizen und Regenwürmern (*Dendrobaena veneta* L.) zeigten jedoch, dass die Habitatfunktion für Biozönosen von dem Fe-/Al-reichen WTR stärker profitierte, wenn dieser in geringerer Konzentration appliziert wurde (0.5 % *m/m*). Dies war auf dosisabhängige P-Fixierung und TE-Einträge durch den WTR zurückzuführen, gegenüber derer sich Szarvasi-1 unempfindlich zeigte. Im Gegensatz zu As konnte die Bioverfügbarkeit von Cd und Zn in den beaufschlagten Böden durch keines der chemischen Verfahren adäquat bestimmt werden. Als Ursache sind die für die jeweiligen ökotoxikologischen Endpunkte spezifische Bindung konkurrierender Kationen an biotischen Liganden (Pflanzenwurzeln,

Gewebe des Regenwurms) sowie eine präferenzielle Translokation von Zn gegenüber Cd innerhalb der Pflanzen zu nennen. Unter allen untersuchten Pflanzen exkludierten die Perennierenden, Szarvasi-1 und Durchwachsene Silphie (*Silphium perfoliatum* L.; Kapitel 4.2), die Schadstoffmischung des Bodens in oberirdischen Kompartimenten am besten, wobei Letztere Wachstumsdepressionen aufwies. Konzentrationsbedingt (As, Cd) konnte lediglich auf der Versuchsfläche erzeugte Kornbiomasse von Gerste, Raps und teilweise von schwach akkumulierendem Weizen nach gültiger europäischer Rechtsordnung als Viehfutter eingesetzt werden.

Angesichts der begrenzten Wirksamkeit der geprüften Maßnahmen zur Gewährleistung der Futtermittelsicherheit könnte ein Landnutzungswandel, etwa in Richtung des perennierenden Szarvasi-1, das mit relativ geringem Einsatz von Ressourcen und Bodenbearbeitung eine geschlossene Pflanzendecke produziert, eventuell kombiniert mit überwachter Wiederverwertung eines Fe-/Al-reichen WTR, am ehesten alle eingangs dargelegten Schadstoffübergänge wirksam kontrollieren. Wirtschaftliche Erlöse könnten durch Energiekonversion oder Inwertsetzung der Biomasse im Faserstoffsektor generiert werden. Die Abfallverwertung von WTR in TECS bedarf, obgleich vielversprechend, zunächst gründlicher Charakterisierung, eventuellen Prozessoptimierungen und weiteren Studien zur Langzeitstabilität in Böden um Gesetzeskonformität und Umweltsicherheit zu garantieren. Für die überwiegende Mehrheit moderat belasteter Flächen sollten künftige Forschungs- und Züchtungsanstrengungen, insbesondere hinsichtlich schwach Cd-akkumulierender Sorten, einen wesentlichen Beitrag zur Lebens- und Futtermittelsicherheit leisten.

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Abbreviations

ANOVA	Analysis of variance
AV	Action value
BBodSchV	German Federal Soil Protection Ordinance
BCF	Bioconcentration factor
CaCl ₂	Calcium chloride
CaCO ₃	Calcium carbonate
CaO	Calcium oxide
C _{DGT}	Interfacial trace element concentration
CEC _{eff}	Effective cation exchange capacity
CEC _{pot}	Potential cation exchange capacity
CH ₄ N ₂ O	Urea
CoIA	Co-inertia Analysis
C _{org}	Organic carbon
C _{soln}	Soil solution trace element concentration
DGT	Diffusive gradients in thin films
DI	Deionized
DW	Dry weight
<i>E_h</i>	Redox potential
FAO	Food and Agriculture Organization of the United Nations
FP7	7 th Framework Programme for Research and Technological Development
GIS	Geographical information system
GPS	Global Positioning System
GRO	Gentle remediation options
H ₂ O ₂	Hydrogen peroxide
HAC	High-accumulating cultivar
HNO ₃	Nitric acid
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
K ₂ O	Potassium oxide
KBBE	Knowledge Based Bio-Economy
LAC	Low-accumulating cultivar
LfULG	Saxon State Office for Environment and Agriculture
LMWOA	Low molecular weight organic acid
LOD	Limit of detection
<i>n</i>	Number of replicates
NH ₄ NO ₃	Ammonium nitrate
(NH ₄) ₂ SO ₄	Ammonium sulfate
OTC	Open top chamber
<i>p</i> -value	Probability value

P ₂ O ₅	Phosphorus pentoxide
PCA	Principal Component analysis
PS	Phytosiderophores
<i>r</i>	Correlation coefficient
SD	Standard deviation
TE	Trace elements
TECS	Trace element-contaminated soils
TF	Translocation factor
TGW	Thousand grain weight
TME	Total metal(loid) extraction
TV	Trigger value
VAM	Vesicular-arbuscular mycorrhizae
WTR	(Drinking) Water treatment residue

1 Introduction

1.1 The contamination of agricultural soils with trace elements - a global challenge to be tackled at local scale

In a 2018 report, the Food and Agriculture Organization of the United Nations (FAO) identified soil pollution as a major threat to global soils and the ecosystem services they provide, with trace elements (TE) posing significant risks to biocenoses and food safety. Moreover, due to the lack of harmonized data, existing studies are believed to underestimate the global extent of this problem (Rodríguez-Eugenio et al., 2018). Given the high share of arable land being already contaminated and the need to supply the demands of an ever increasing population with limited land resources, sustainable crop production presents a challenging global task (Abhilash et al., 2016). Its urgency became very evident in the most recent estimates for the global cereal market in 2018/2019, published by the FAO (FAO, 2019), indicating excess demand in relation to production for the second time in this decade. The implied necessity for agricultural intensification and expansion, which is expected to globally account for 70 million hectares by 2050 as compared with 2005 (Alexandratos and Bruinsma, 2012), requires deliberate land-use policies in order not to compromise global environmental goals (Hunter et al., 2017). With this in mind, the safe use and/or restoration of trace element-contaminated soils (TECS) for agriculture could protect forests and other valuable habitats or carbon sinks from conversion to farmland. Therefore, Abhilash et al. (2016) regard TECS "as an untapped resource for environmental and agricultural sustainability".

1.1.1 Spatial relevance at European and regional scale

In the European Union, a first harmonized screening of agricultural topsoils at 22,000 locations revealed that 6.24 % or 137,000 km² require local assessment or remediation due to elevated TE concentrations (Tóth et al., 2016a). Large-scale TECS are often associated with historical or recent industrial and/or mining activities. In the European context, the Saxon Ore Mountain Mining Region, where the study area is located, stands out due to high topsoil concentrations of TE, in particular As and Pb (Tóth et al., 2016b). Based on detailed assessments by the Saxon authorities, land-use specific thresholds for As, Pb, and Cd regarding food or forage quality, laid down in the Federal Soil Protection Ordinance (BBodSchV, 1999), are exceeded at approximately 1,000 km² or 10 % of agricultural soils (SMI, 2013; see also Figure 1.1). Moreover, these are often accompanied by Zn

concentrations above the trigger value for expected impairment of plant growth (Barth et al.; unpublished data). For these reasons, As, Cd, Pb, and Zn present the targeted elements in this study. After more than 800 years of Ag, Pb, Cu, and Zn mining (Baumann et al., 2000), the former Freiberg mining district (Figure 1.1 and Figure 1.2), where the study area is located, now represents a post-mining and ore processing region (Hurst et al., 2002) with a characteristic, aged contamination of topsoils by multiple, basically chalcophile, elements. It is therefore classified as a priority region for high potential threat from TE within Europe (Tóth et al., 2016b).

Figure 1.1 Regions within Saxony, where land-use specific thresholds for trace element concentrations, laid down in the German Federal Soil Protection Ordinance (BBodSchV, 1999), are exceeded (adapted from LfULG (2014)).

1.1.2 Environmental relevance

Threats associated with elevated concentrations of TE in agricultural soils are related to their non-biodegradable nature, their migration e.g. to the groundwater, and their availability and toxicity to plants, microbes, or soil-dwelling species (Spurgeon, 2010). This toxicity is largely

species-dependent and varies among essential elements, such as Zn, being toxic only at levels of exposure exceeding an upper tolerance limit, and those elements with no known biological function like As, Cd, and Pb, which can be toxic already at low concentrations (Peralta-Videa et al., 2009). The symptoms of toxicity range from physiological abnormalities (like chloroses or necroses) over growth inhibition or reproductive disturbances to the mortality of the organism (Foy et al., 1978; Nahmani et al., 2007). According to Baker (1981), plants have evolved various mechanisms to tolerate a wide range of TE concentrations in soils. These comprise basically (i) the suppression or differential uptake and/or translocation of TE between roots, vegetative, and generative organs, and (ii) their internal detoxification, leading to the classification of plants into excluders or accumulators, respectively. A third type of response is presented by indicator plants, which accumulate TE proportional to soil concentrations, unless toxicity occurs.

Chaney (1980) undertook a classification of TE into groups based on their potential food chain risk via plant uptake, which has been modified by Rodríguez-Eugenio et al. (2018). Thereby, soil-borne Pb is regarded as relatively moderate risk to the food chain due to its strong sorption to the soil matrix and its low translocation to edible plant tissues. Zinc is readily plant-available but causes phytotoxicity at concentrations typically not harming human health, whereas the opposite applies to As and Cd, which therefore belong to the high risk group. Food crops dominate the human Cd exposure in the general population (Nordberg et al., 2018). Significant dose-response relationships are reported for adverse effects on kidneys and bone demineralization and fracture risk, which, in the most severe form, became known as the *itai itai* disease (Imura et al., 2019). Since Cd is further recognized as carcinogenic, uncritical exposure levels cannot be defined (Nordberg et al., 2018). For the same reason, existing thresholds for the most significant sources of human As exposure, being water, food, and air, are subject to scientific debates (Schmidt, 2014). Apart from cancer, As has been reported to cause a variety of severe functional disorders, as reviewed by Rasheed et al. (2016). Lead, also considered a carcinogen, can damage several organs, with the central nervous system and the gastrointestinal tract being most commonly affected (Murata et al., 2009; Jaishankar et al., 2014). Adverse effects of Zn are related to anemia or tissue lesions (Brevik, 2012). To date, the most research on TE accumulation and toxicity in major food crops has focused on rice due to its importance as a source for human As and Cd exposure (Clemens and Ma, 2016). Wheat, however, presents the largest primary commodity in the world with a global production of more than 700 million tons, grown on more land area than any other commercial crop (FAO, 2018). In Germany, winter wheat covers 51 % of the cereal

acreage (DESTATIS, 2019) and therefore presents a central research object of this thesis (chapters 2, 3, and 4.1).

It is generally approved, that plants (Kumpiene et al., 2014; Quintela-Sabarís et al., 2017), microbes (Hanauer et al., 2012; Touceda-González et al., 2017), and soil dwelling species (Peijnenburg et al., 1999; Lanno et al., 2004) basically respond to TE present in soluble or exchangeable forms in soil instead of total concentrations. Therefore, rendering TE less bioavailable is key to attenuating harmful effects on the food chain and the habitat function of contaminated agricultural soils for biocenoses.

1.1.3 Sustainable remediation options

The above mentioned is exactly what sustainable remediation addresses. It is regarded as "the elimination and/ or control of unacceptable risks in a safe and timely manner while optimizing the environmental, social, and economic value of the work" (Nathanail et al., 2017). Large-scale TECS inevitably require *in situ* remediation since *ex situ* measures are not applicable and/or too costly. Sustainable *in situ* techniques such as (i) the immobilization of TE through stabilizing agents, and (ii) phytoremediation using plants that stabilize, extract, or exclude TE, or a combination of both, are referred to as gentle remediation options (GRO) (Onwubuya et al., 2009). These techniques focus on effective risk management through site-specific control of pollutant linkages, i.e. pathways from sources to receptors, and on the realization of wider benefits (Cundy et al., 2013). The latter comprise the re-utilization of derelict land, economic returns through biomass generation as well as ecosystem services like nutrient cycling, carbon storage, regulation of water flow, or the restoration and maintenance of soil biological activity and fertility (Cundy et al., 2016). Together, these approaches constitute a paradigm of sustainable and profitable management of TECS, encompassing more than the mere decontamination, which used to present the core objective of remediation in its traditional understanding (Burgess et al., 2018).

A recent bibliometric analysis revealed a concentration of research on the use of phytotechnologies for remediation in countries with low Human Development Indices, where expensive conventional remediation is hardly financeable (Koelmel et al., 2015). By contrast, the practical application of GRO is still limited, particularly in Europe and at TECS (Cundy et al., 2016). In recent years, however, some successfully applied intermediate- and long-term studies have been published (Herzig et al., 2014; Greger and Landberg, 2015; Friesl-Hanl et al., 2017).

The GREENLAND project

The GREENLAND project (gentle remediation of trace element contaminated land), which has been financially supported by the European Commission within the Seventh Framework Programme for Research (FP7-KBBE- 266124, Greenland), aimed to overcome the perceived or existing shortfalls of GRO to promote their wider application in practical land management across Europe. For this purpose, most promising GRO were examined on tailings, marginal lands, (post-)industrial, sludge-amended, and agricultural soils co-contaminated in various mixtures and degrees by TE such as Cd, Zn, Cu, Ni, Cr, Pb, and As. A European network of thirteen long-term field experiments presented the core of the investigations, complemented by further investigations at greenhouse- and lab-scale. In addition to the project-based investigations reported in this thesis, another long-term field experiment using poplars and willows in short rotation coppice has been conducted within the study area, the major outcomes of which are reported elsewhere (Cundy et al., 2013; Kumpiene et al., 2014; Kidd et al., 2015; Quintela-Sabarís et al., 2017; Touceda-González et al., 2017).

1.2 The study site

The Saxon authorities hold a basic data inventory on soil TE concentrations for the whole administrative territory. Areas with known or suspected contamination, due to geogenic preconditions and historical mining activities, have been investigated more in detail. The areas concerned are inter alia located downwind of the former smelters, like the study site, where, or with substrate of which, all investigations in this thesis have been conducted. It constitutes an agricultural site east of the former smelter Muldenhütten (Figure 1.2), the emissions of which caused, in addition to local mineralizations and heaps, severe topsoil contamination with As, Cd, Pb, and Zn (LfULG, 2001; Barth and Forberg, 2015). According to the Saxon State Office for Environment and Agriculture (LfULG), the mineral topsoil on site contains *aqua regia*-extractable concentrations of As, Cd, Pb, and Zn above the 95th percentile of area-wide investigations (Figure 1.2 c)). Moreover, the ammonium nitrate (NH₄NO₃)-soluble, hence potentially plant-available, concentrations of As, Cd, and Pb are such that pre-harvest analyses of locally produced food or forage are officially recommended to examine whether compliance with legal thresholds is given (LDS, 2012). In the frame of a permanent soil monitoring program, LfULG operates a monitoring station in direct vicinity

(BDF 43; GPS coordinates: 13° 23' 53" E, 50° 54' 11" N). The data recorded there provided the basis for a preliminary risk assessment.

Figure 1.2 Location of the study site (b) within the region of Freiberg in spatial proximity to (a) mineralizations and former smelters (blue lines: quartz-sulfide-associations, green lines: baryte-fluoride-associations, C: smelters, !: sampling points of mineral topsoils). The purple rectangle (a) corresponds to the satellite imagery section (b); note the different orientation. Maps of the pseudo-total concentrations of As, Cd, Pb, and Zn (mg kg⁻¹ DW⁻¹) and corresponding percentiles (left of the respective Figure legend) in mineral topsoils (c); the location of the study site is indicated by a white circle. The maps a) and c) are adapted from LfULG (2001).

According to Barth et al. (unpublished data), the long-term study of seepage water at the monitoring station, obtained with suction cups from the unsaturated zone, revealed, that Cd, Pb, and Zn are currently not present in concentrations that pose a risk to the groundwater. Starting from a high level in the 1990s, a general decline of As in seepage water was recorded over the past two decades. Occasionally observed peaks were associated with heavy precipitation events. In 2011, when the study site was established, concentrations of 28 and 67 $\mu\text{g/l}$ in depths of 40 and 80 cm below ground, respectively, were measured. These high, but for this specific site comparably moderate, concentrations do not inevitably imply a groundwater contamination according to BBodSchV (1999), since neither depth nor method of sampling comply with the legal regulations for the risk assessment of the soil-to-groundwater transfer pathway. However, phases of bare soil should be best possibly avoided on site to minimize leaching. A considerable bulk deposition of As, Cd, and Pb, determined by the Bergerhoff method (VDI, 2012), has been detected, whereby peaks correlated with tilling under dry soil conditions. Due to excess accumulation of these elements in traditionally cultivated crops, the growth of legally compliant biomass for food purposes (EC, 2014) is precluded on site. Moreover, at partially low yields, possibly caused by TE toxicity, sampled crops were frequently unsuitable for animal consumption (EC, 2002). Thereby, marked differences occurred in specific crops between different years of sampling. To summarize, the pollutant linkages on site comprise the soil-to-plant/biota transfer of As, Cd, Pb, (and Zn), the soil-to-groundwater transfer of As, and the susceptibility of the contaminated soil to wind erosion.

1.3 Objectives

The investigations presented in this thesis supported the work packages I ("Sustainable management adapted to TECS and deployment of GRO at field scale") and IV ("Improving GRO through plant selection and modifications in soil TE bioavailability") of the GREENLAND project.

The basic objective was to assess whether the relevant pollutant linkages on site could be controlled by the application of GRO while best possibly preserving the current cultivation system of the farmer, yet moving it towards a more sustainable site management. Thereby, in a regional context, but going beyond, this thesis aims to contribute to one of the outstanding questions regarding sustainable crop production from TECS formulated by Abhilash et al.

(2016): "What are the key sustainability challenges for the crop production from multiple and heavily contaminated sites?"

1.3.1 *Scope of this thesis*

The research questions addressed in this thesis are:

- I. How can conventional soil-applied fertilizers contribute to the reduction of plant-available metals and As? (chapter 2)
- II. To what extent can non-invasive measures like the selection of preceding crops (chapter 3) or TE-excluding cultivars of annual crops (chapter 2) affect the accumulation of metals and As in vegetative and generative plant tissues?
- III. Can results obtained from greenhouse studies be verified under heterogeneous field conditions in practical application? (chapter 2)
- IV. Can residuals from drinking water treatment, applied to soil instead of being disposed, simultaneously stabilize metals and As in soil and mitigate their bioavailability to plants and soil-dwelling organisms? (chapters 4.1 and 4.2)
- V. How do selected species of annual and perennial plants from the *Poaceae*, *Asteraceae*, and *Brassicaceae* family comparatively perform in a soil heavily contaminated with metals and As? (chapter 4.2)

1.4 Structure of the thesis

This thesis is cumulatively composed and subdivided into six chapters. Following this introduction, chapters 2 to 4 present a compilation of publications; two of these have been published in peer-reviewed journals (chapter 3.1 and chapter 4.1) while the remaining two are under consideration for publication in peer-reviewed journals (chapter 2.1 and chapter 4.2). Taking into account the recent scientific literature related to the research questions, chapter 5 presents a synthesis with general conclusions from the previous chapters. Future perspectives are pointed out in chapter 6. A list of scientific publications is included behind the list of references.

*The first manuscript (chapter 2.1), entitled "Management of trace element-contaminated agricultural land by *in situ* stabilization combined with phytoexclusion over a three years crop rotation", informs about potentials and drawbacks of GRO deployed under practical and*

heterogeneous field conditions. The latter are identified as important drivers for observed discrepancies from specific studies performed at greenhouse-scale. Based on plant and soil assays, the large and non-invasive potential of cultivating excluder species and cultivars to attenuate food chain transfer of certain TE is emphasized as compared with *in situ* stabilization using fertilizers (phosphate and lime marl) made of primary raw materials.

*The second manuscript (chapter 3.1), entitled " Trace elements bioavailability to winter wheat (*Triticum aestivum* L.) grown subsequent to high biomass plants in a greenhouse study", addresses the potential of crop rotation management to attenuate TE accumulation in wheat as a globally and regionally important staple crop. Results from soil and plant assays with winter oilseed rape and a chemically mutagenized sunflower are presented, revealing that rhizosphere effects of these pre-crops superimpose their capabilities to bring about the intended labile TE phytoextraction.*

*The third manuscript (chapter 4.1), entitled "Trace elements bioavailability to *Triticum aestivum* and *Dendrobaena veneta* in a multielement-contaminated agricultural soil amended with drinking water treatment residues" (WTR), informs about the high capabilities of these secondary raw materials, dominated either by Fe/Al or by Mn (hydr)oxides, to reduce bioavailable TE in soil. Thereby, investigated physiological and chemical responses of biota allow for recommendations on beneficial application rates. The suitability of chemical biomimetic surrogates (DGT, soil solution, and NH_4NO_3 -solution) in residual-treated soils co-contaminated by metals and As is critically discussed based on the biotic ligand model.*

*The fourth manuscript (chapter 4.2), entitled "(Aided) phytostabilization of trace elements using cup plant (*Silphium perfoliatum* L.), or tall wheatgrass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) and soil-applied drinking water treatment residues", informs about the suitability of these perennial non-food energy or fibre plants to be grown alternative to annual crops in the study soil, and whether WTR amendments can aid the phytostabilization towards compliance of the biomass for forage purposes. Thereby, the chapter emphasizes the importance of harvest management and enables, in conjunction with chapter 4.1, an evaluation of the intermediate-term stability of WTR-treated soils.*

2 Field assessment of conventional fertilizers and cultivars of annual plants

2.1 Management of trace element-contaminated agricultural land by *in situ* stabilization combined with phytoexclusion over a three years crop rotation

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Abstract

This study assessed *in situ* stabilization combined with phytoexclusion in practical application on agricultural land contaminated strongly, and spatially heterogeneous, with metals (Cd, Pb, and Zn) and As. Single and combined lime marl and phosphate treatments were consecutively planted with two cultivars each of rape, wheat, and barley differing in trace elements (TE) accumulation. The effects on soil acidity, NH_4NO_3 -soluble, and straw and grain TE concentrations were evaluated. The combined fertilizer treatment most effectively reduced metals mobility, but neither amendment mitigated plant TE status, which correlated more with pseudo-total than NH_4NO_3 -soluble TE in soil. The cultivar choice reduced grain Cd by 39 % or 21 % in barley or wheat, respectively, simultaneously decreased grain Zn, but conversely affected As uptake in wheat grains. The lack of correlations between grain TE concentrations suggests the potential for breeding cultivars with low Cd and As accumulation without causing Zn malnutrition. The cereals had relatively low yields, particularly on highly polluted areas, and only rape and barley grains unexceptionally suited for animal consumption. Agricultural measures and climatic conditions influenced TE mobility. The cultivars' TE uptake varied less than in greenhouse studies, stressing the importance of field studies for an adequate estimation of phytoexclusion potentials.

Keywords: heavy metals and arsenic, lime and phosphate, genotypic differences

Introduction

The need to feed a growing global population and increasing shares of arable land that are contaminated with trace elements (TE) require management options for safe crop production (Abhilash et al., 2016). In the Saxon Ore Mountains (Germany), a characteristic post-mining region, approximately 1,000 km² of topsoils under agricultural use exceed land use specific thresholds for metals and As concentrations due to geochemical preconditions and atmospheric deposition during centuries of ore mining until 1991 (SMI, 2013). On these sites, proper land management requires the adaptation of agricultural practices in order to simultaneously warrant the control of pollutant linkages, safe biomass production, and economic returns for farmers (Kidd et al., 2015). The choice of pollutant-excluding genotypes and/or cultivars within species of major staple crops constitutes a non-invasive and cost-friendly option. The Saxon authorities conduct periodic greenhouse-studies to provide information about TE accumulation in regionally adapted cultivars, which have revealed a considerable potential of phytoexclusion by cultivar choice (BfUL/LfL, 2002-2018). However, the effectiveness is typically limited to certain elements and might differ in the field. Furthermore, the farmers' cultivar choice is basically driven by yield potential and pest resistance and does not focus adequately on TE phytoexclusion (Kidd et al., 2015). Therefore, the authorities in Saxony officially recommend additional measures for agricultural soils contaminated with metals and As: the optimization of soil pH towards a defined range depending on land-use, soil texture, and humus content, and a moderate P fertilization at the beginning of the vegetation period are suggested (BfUL, 2017). Though containing TE themselves (Kratz et al., 2016), legal obstacles rarely impede the application of phosphates or liming agents.

However, studies have yielded contrasting results regarding advantageous effects of both amendment types on TE bioavailability. The immobilization of TE by materials containing phosphate is primarily based on its tendency to precipitate with metals, resulting in stable solid phases (Kumpiene, 2010). Based on greenhouse studies, P fertilization could mitigate (Serfling and Klose, 2008) or augment (Friesl et al., 2006; Brackhage et al., 2015) As mobility in soil or its accumulation in cereals. Phosphate amendments could effectively immobilize Pb (Park et al., 2011; Seshadri et al., 2017), whereas the bioavailable Cd may remain unchanged (Gao et al., 2010), decrease (Seshadri et al., 2017) or increase (Grant et al., 2010), at a simultaneous decrease of Zn concentrations in biota. Liming agents have been widely used for *in situ* stabilization of metal-contaminated soils via pH-dependent increases of positive surface charges or precipitation reactions (Kumpiene 2010). At moderate soil

acidity, the latter may also reduce bioavailable As through the formation of As-Ca complexes (Porter et al., 2004). In turn, if liming increases soil pH above the point of zero charge, desorption of As from oxidic surfaces will enhance bioavailable concentrations (Hartley and Lepp, 2008). In any case, sustained immobilization requires frequently repeated lime applications (Seoane and Leirós, 2001), whereas lasting decreases of Cd available to wheat were found following P fertilization (Qayyum et al., 2017).

Despite well-known mechanisms and the vast potential of gentle remediation options (GRO), there is still a lack of practical adoption in contaminated land management (Cundy et al., 2016). Therefore, in 2011, the field trial reported here was established in collaboration with the owner of an agricultural site heavily contaminated with metals (Cd, Pb, and Zn) and As. The overall aims were to assess the potential of (i) *in situ* stabilization using phosphate and lime marl fertilizers frequently used in the region and (ii) regionally adapted low-accumulating cultivars within a local crop rotation of winter oilseed rape, winter wheat, and spring barley for reducing the transfer of Cd, As, and Pb into the food chain.

Materials and Methods

Experimental set-up

The experimental site was located on agricultural land near Freiberg, Saxony, which is severely contaminated with metals (Cd, Pb, Zn) and As (Kidd et al. 2015; GPS coordinates: 13° 23' 52" E, 50° 54' 17" N). The soil is a Stagnosol derived from loess and paragneiss, composed of 35 % sand, 51 % silt, and 14 % clay. Due to past emissions from adjacent smelters (Figure 2.5), the contamination concentrates in the upper 25 cm but transitions between horizons are uneven (Barth and Forberg, 2015). An area of approximately 34,000 m² of temporarily fallow land was sub-divided into eight plots of about 4,000 m² each (Figure 2.2). On these plots, four fertilizer treatments were established and repeated annually (Unt: untreated, LM: 0.4 kg lime marl m⁻², P: 0.04 kg superphosphate m⁻² using P 40 (containing 40 % P₂O₅), LM+P: 0.4 kg lime marl m⁻² + 0.04 kg P 40 m⁻²). Each treatment was consecutively planted with two cultivars of *Brassica napus* L. (cv. Visby (hybrid cultivar) and cv. Lorenz (line cultivar), 2011/2012), *Triticum aestivum* L. (cv. Türkis as low Cd-accumulating cultivar (LAC) and cv. Tiger as high Cd-accumulating cultivar (HAC), 2012/2013), and *Hordeum vulgare* L. (cv. Salome (LAC) and cv. Marthe (HAC), 2013/2014). The information on cultivar-specific Cd-accumulation in cereal grains were derived from annual screenings conducted by the Saxon authorities (BfUL/LfL, 2002-2018).

Detailed information about soil and plant sampling and monthly records of precipitation during the investigation period (Table 2.3) are presented in the electronic supplementary material. Prior to any treatments, five composite samples (~ 1 kg) were taken from each plot in September 2011 to characterize the study site. For simultaneous sampling with crops during harvests in 2012 to 2014 the number of samples was reduced to four. Unless otherwise specified, entry points were placed in the root zone of sampled individuals, which were subsequently divided into straw and grains. Aliquots were dried at 60 °C to constant weight for chemical analyses. The farmer conducted mineral fertilization, sowing, pest management, and an unscheduled ploughing of the whole study site due to soil compaction in 20 to 25 cm below ground prior to spring barley cultivation in 2014. Based on each crop's N and K demands, he applied $\text{CH}_4\text{N}_2\text{O}$, $(\text{NH}_4)_2\text{SO}_4$, and a K fertilizer containing 60% K_2O equally for all treatments. During the mechanical harvests, the grain yields per treatment were determined by weighing.

Chemical analyses

Pseudo-total and potentially plant-available concentrations in soil were analyzed using standard procedures of *aqua regia* (DIN ISO 11466:1997) and NH_4NO_3 (DIN ISO 19730:2008) extractions. Elemental concentrations of fertilizers were determined following digestion with *aqua regia*. Soil pH and organic carbon (C_{org}) were analyzed according to DIN ISO 10390:2005 and DIN EN 15936:2012–11, respectively. Plant samples were finely ground and digested using HNO_3 and H_2O_2 (DIN EN 13805:2014). Chemical analyses were validated with blanks and standard reference material BCR 281, rye grass (EU-JRC) for plant analyses, and WEPAL-ISE 979 (Rendzina soil) for *aqua regia* and NH_4NO_3 extraction. Microwave assisted acid digestions were conducted using MARS5 (CEM Corp., Matthews, United States). Elements in extraction solutions were measured by ICP-MS (PQ exCell, Thermo Fisher Scientific Inc, UK).

Geostatistical and Statistical analyses

Contour maps of pseudo-total TE concentration were created using the kriging interpolation method with ArcGIS 10.1.

Soil and plant chemical data were checked for normal distribution of residuals using the Shapiro-Wilk test, and for homoscedasticity with the Levene's test. Treatment effects were analyzed for statistical differences with one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test using PASW statistics 21 (SPSS, Inc., Somers, NY, USA). In the event

that data failed to meet the homoscedasticity prerequisite, the significant differences were tested using Welch's ANOVA and Games-Howell *post hoc* test. Data were log-transformed if necessary to meet the requirement of normal distribution of residuals. The relationships between variables were determined via Pearson's correlation analysis. A significance level of $p < 0.05$ was used throughout the study.

The chemical data were organized in two multivariate data sets, one containing soil pH, C_{org} , P and pseudo-total and potentially plant-available TE concentrations, and the other containing plant TE concentrations. The data were grouped by years (i.e. crops) for Principal Component Analyses (PCA) of each data set followed by a Co-inertia Analysis (CoIA, Dolédec and Chessel 1994) using R software (version 3.5.1, The R Foundation, Vienna, Austria) to unravel co-structure between both data sets, as described in Quintela-Sabarís et al. (2017). The RV-coefficient, i.e. the correlation coefficient between both data sets, was calculated and its significance determined by Monte Carlo random permutation tests. Values below the limits of detection (LOD) were removed from the dataset.

Relative changes in TE mobility (i.e. the percentage of NH_4NO_3 -soluble to pseudo-total TE in soil (Figure 2.3)) and bioconcentration factors (BCF; i.e. the ratio between total TE concentration in aboveground plant tissue / pseudo-total TE in soil (Figure 2.4)) were calculated to improve the assessment of treatment effects.

Results

Elemental composition of soil and plant samples

Soil properties and TE concentrations

The regional background levels of 12, 0.41, 43, and 84 mg kg⁻¹ DW⁻¹ (*aqua regia*) of As, Cd, Pb, and Zn, respectively, for loess-derived arable topsoils (Kardel et al., 2015) are exceeded by a multiple at the study site. Likewise, all samples exceeded national action or trigger values for TE concentrations in agricultural soils regarding crop quality (Table 2.1). Elemental concentrations of fertilizers complied with national thresholds (DüMV, 2012) except for Cd and Ni concentrations in P 40 (Table 2.5). The initial site characterization revealed a spatially heterogeneous soil contamination with maximum pseudo-total loads of metals and As in the northwestern section (Figure 2.2). Relatively low levels of C_{org} were found in the (south)western part of the study site, whereas soil acidity was evenly distributed (Table 2.1).

Table 2.1 NH_4NO_3 -soluble TE concentrations ($\text{mg kg}^{-1} \text{DW}^{-1}$), pH, and C_{org} in untreated (Unt) and treated (lime marl: LM, phosphate: P, lime marl and phosphate: LM+P) topsoils separated by cultivars (LAC or hybrid cultivar: A, HAC or line cultivar: B). 2011 represents initial conditions prior to any treatment (trt.) or planting, whereas winter oilseed rape was cultivated in the year (yr.) 2012, winter wheat in 2013 and spring barley in 2014. Values are means $\pm$ SD ($n = 5$ (2011) or $n = 4$ (2012 - 2014)). Different letters indicate significant differences between treatments per year ($p < 0.05$). Trigger and Action values are derived from the German Federal Soil Protection Ordinance (BBodSchV, 1999).

yr.	trt.	cv.	As	Cd	Pb	Zn	pH	C _{org}
2011	Unt	A	1.2 ± 0.3 ab	0.6 ± 0.3	2.6 ± 1.6	4.6 ± 3.1	6.0 ± 0.2	2.7 ± 0.4 d
	LM		1.7 ± 0.2 b	1.2 ± 0.5	3.8 ± 1.9	11.9 ± 5.7	5.9 ± 0.4	2.3 ± 0.1 abcd
	P		1.4 ± 0.2 ab	0.8 ± 0.3	4.2 ± 2.4	5.8 ± 2.8	5.9 ± 0.2	2.3 ± 0.1 bcd
	LM+P		0.9 ± 0.5 a	1.1 ± 0.2	2.5 ± 1.2	8.7 ± 4.2	6.0 ± 0.1	1.8 ± 0.3 ab
	Unt	B	1.0 ± 0.3 a	0.9 ± 0.1	3.2 ± 1.8	6.8 ± 1.1	5.9 ± 0.1	2.0 ± 0.4 abc
	LM		1.4 ± 0.4 ab	1.0 ± 0.3	2.6 ± 1.4	9.4 ± 4.4	5.9 ± 0.2	2.0 ± 0.1 abc
	P		1.4 ± 0.2 ab	0.7 ± 0.2	2.2 ± 1.5	6.2 ± 2.3	6.0 ± 0.2	2.4 ± 0.2 cd
	LM+P		0.8 ± 0.3 a	0.7 ± 0.2	1.2 ± 0.3	7.6 ± 7.2	6.1 ± 0.1	1.6 ± 0.3 a
2012	Unt	A	0.8 ± 0.2 ab	0.9 ± 0.2	4.2 ± 0.7 b	6.4 ± 1.8 b	5.5 ± 0.1 a	2.9 ± 0.1 c
	LM		1.2 ± 0.1 c	0.9 ± 0.2	2.4 ± 0.8 ab	6.1 ± 2.5 ab	5.8 ± 0.1 bc	2.2 ± 0.2 b
	P		0.9 ± 0.1 bc	0.7 ± 0.2	3.1 ± 0.9 ab	4.0 ± 1.7 ab	5.9 ± 0.0 bc	2.0 ± 0.2 ab
	LM+P		1.0 ± 0.2 bc	0.6 ± 0.2	1.2 ± 0.5 a	2.4 ± 1.1 a	6.1 ± 0.1 c	1.7 ± 0.2 a
	Unt	B	0.5 ± 0.2 a	0.9 ± 0.2	2.4 ± 1.6 ab	5.7 ± 2.2 ab	5.7 ± 0.1 ab	1.8 ± 0.1 ab
	LM		0.9 ± 0.1 abc	0.7 ± 0.2	1.4 ± 0.4 a	4.7 ± 1.5 ab	6.0 ± 0.1 bc	2.0 ± 0.2 ab
	P		1.0 ± 0.2 bc	0.8 ± 0.3	2.5 ± 0.9 ab	6.0 ± 2.7 ab	5.8 ± 0.2 ab	2.1 ± 0.4 ab
	LM+P		0.9 ± 0.2 abc	0.7 ± 0.2	1.4 ± 0.7 a	4.5 ± 1.7 ab	5.9 ± 0.2 bc	1.7 ± 0.1 a
2013	Unt	A	0.6 ± 0.5 abc	1.3 ± 0.5 ab	6.4 ± 3.1 b	13.0 ± 8.9 ab	5.7 ± 0.2 a	2.4 ± 0.3 b
	LM		1.0 ± 0.3 c	1.3 ± 0.3 ab	3.7 ± 1.2 ab	15.3 ± 8.0 ab	5.9 ± 0.2 ab	2.4 ± 0.1 b
	P		0.7 ± 0.5 abc	1.2 ± 0.5 ab	6.2 ± 3.3 ab	11.1 ± 8.8 ab	5.8 ± 0.2 a	2.4 ± 0.3 b
	LM+P		0.7 ± 0.2 abc	0.7 ± 0.2 a	1.4 ± 0.7 a	5.8 ± 1.6 a	6.3 ± 0.2 b	2.1 ± 0.2 ab
	Unt	B	0.5 ± 0.3 ab	1.7 ± 0.3 b	6.7 ± 2.7 b	17.7 ± 7.2 b	5.6 ± 0.2 a	1.7 ± 0.2 a
	LM		0.9 ± 0.3 bc	1.3 ± 0.3 ab	3.1 ± 0.9 b	15.1 ± 6.1 b	5.9 ± 0.1 ab	2.1 ± 0.1 ab
	P		0.8 ± 0.3 abc	1.6 ± 0.3 b	5.6 ± 1.2 ab	17.1 ± 7.1 b	5.8 ± 0.1 a	2.3 ± 0.3 b
	LM+P		0.4 ± 0.4 a	1.1 ± 0.4 ab	2.6 ± 1.5 a	7.2 ± 3.4 ab	6.1 ± 0.1 ab	1.6 ± 0.3 a
2014	Unt	A	0.8 ± 0.1 ab	0.3 ± 0.1 ab	1.0 ± 0.2 ab	1.4 ± 0.6 ab	6.4 ± 0.2 a	2.9 ± 0.4 c
	LM		1.2 ± 0.1 b	0.5 ± 0.1 b	1.1 ± 0.2 a	2.5 ± 0.7 b	6.5 ± 0.1 abc	2.3 ± 0.2 ab
	P		0.8 ± 0.1 ab	0.2 ± 0.1 a	0.8 ± 0.4 ab	0.8 ± 0.6 a	6.6 ± 0.2 abc	2.5 ± 0.3 ab
	LM+P		1.1 ± 0.1 ab	0.3 ± 0.1 a	0.4 ± 0.2 a	1.0 ± 0.4 a	6.8 ± 0.1 c	2.4 ± 0.2 ab
	Unt	B	0.7 ± 0.1 a	0.3 ± 0.1 ab	0.7 ± 0.2 b	1.4 ± 0.9 ab	6.5 ± 0.1 ab	2.0 ± 0.2 a
	LM		1.1 ± 0.1 ab	0.2 ± 0.1 a	0.4 ± 0.1 ab	0.9 ± 0.4 a	6.8 ± 0.1 c	2.4 ± 0.2 ab
	P		0.9 ± 0.1 ab	0.3 ± 0.1 a	0.8 ± 0.3 b	1.2 ± 0.6 ab	6.6 ± 0.1 abc	2.4 ± 0.3 ab
	LM+P		0.9 ± 0.0 ab	0.2 ± 0.0 a	0.3 ± 0.1 a	0.9 ± 0.1 a	6.7 ± 0.1 bc	2.1 ± 0.2 a
Action value			0.04*/0.1					
Trigger value			0.4**		0.1	2**		

*if bread wheat or accumulating vegetables are cultivated

**possible impairment of plant growth

The mobility of all targeted elements varied depending on the year and season of sampling and the crops cultivated throughout the respective vegetation period (Table 2.1). In 2011, the NH_4NO_3 -soluble concentrations of all TE correlated significantly with pseudo-totals (Table 2.6). With the implementation of fertilizer treatments this continued to apply for As only in all years, represented by similar weightings of pseudo-total and mobile As on the Co-inertia axes (Figure 2.1 a) to c) - III). Contrastingly, the NH_4NO_3 -soluble metals concentrations became more strongly pH-dependent, shown by opposing weightings to those of pH on the horizontal Co-inertia-axes. The samples obtained during all harvests contained significantly less NH_4NO_3 -soluble As than those from autumn 2011. The NH_4NO_3 -soluble metal concentrations were highest in 2013 and lowest in 2014, where soil pH was significantly elevated compared to the previous years throughout the study site (Table 2.1). Lime marl-containing treatments enhanced soil pH slightly but in parts significant and significantly lowered percentages of mobile Cd and Pb compared to the control treatment (Unt) in all years except sub-plot A of LM in 2014 (Figure 2.3). The combined treatment LM+P was more effective than LM and, furthermore, exclusively decreased NH_4NO_3 -soluble Pb significantly compared to Unt (Table 2.1). The P fertilization effected an approximation of P levels throughout the study site due to lower initial levels in the P and LM+P treatments compared to Unt and LM (Table 2.4). Significantly elevated percentages of mobile related to pseudo-total As were observed in the single P treatment compared to Unt in all years.

Accumulation of TE in plant tissues

The level of accumulated TE differed significantly among crop species and tissues. In straw, rape enriched the most Cd and Pb, wheat the most As and Zn, and barley the least Cd (Table 2.2), whereas wheat allocated the most As and Cd and barley the least Cd and Zn in grains. All of the crops' straw samples exceeded European thresholds for TE in animal feed (EC, 2002) due to elevated Cd and As concentrations (Table 2.2). Rape and barley grains complied with all limits, whereas 16 % of wheat grain samples passed that for As and 94 % of the HAC and 44 % of the LAC that for Cd.

Particularly cereals exhibited significant cultivar-specific TE accumulation (Table 2.7). The LAC of barley accumulated averagely 39 % and 25 % less Cd and Zn, respectively, in grains than the HAC, which, at higher translocation factors ($\text{TF: TE}_{\text{grain}}/\text{TE}_{\text{straw}}$), enriched less in straw.

Table 2.2 Concentrations (mg kg⁻¹ DW⁻¹) of TE in tissues of winter oilseed rape (*Brassica napus* L. cv. Visby (A) and cv. Lorenz (B)), winter wheat (*Triticum aestivum* L. cv. Türkis (A) and cv. Tiger (B)), and spring barley (*Hordeum vulgare* L. cv. Salome (A) and cv. Marthe (B)) cultivated on untreated (Unt) and treated (lime marl: LM, phosphate: P, lime marl and phosphate: LM+P) soils. Values are means $\pm$ SD ($n = 4$). Different letters indicate significant differences between treatments within a species ($p < 0.05$).

treatment	cv.	As		Cd		Pb		Zn		As		Cd		Pb		Zn	
winter oilseed rape - straw										winter oilseed rape - grain							
Unt	A	11.9 ± 4.0	ab	13.0 ± 1.6	ab	11.7 ± 5.7		57.5 ± 12.4	ab	0.3 ± 0.1	a	0.6 ± 0.1	a	0.8 ± 0.6		44.9 ± 0.9	cd
LM		16.4 ± 3.9	ab	17.9 ± 2.7	b	17.8 ± 12.4		69.7 ± 17.0	b	0.4 ± 0.1	a	0.8 ± 0.1	bc	0.5 ± 0.2		47.5 ± 3.0	d
P		6.6 ± 0.8	a	9.2 ± 2.1	a	10.5 ± 3.5		36.8 ± 12.1	a	0.4 ± 0.1	a	0.6 ± 0.1	a	0.2 ± 0.1		39.8 ± 1.2	ab
LM+P		10.4 ± 2.9	ab	14.0 ± 5.5	ab	13.0 ± 10.4		54.3 ± 16.6	ab	0.9 ± 0.3	b	1.0 ± 0.1	c	0.4 ± 0.2		45.7 ± 1.3	d
Unt	B	9.7 ± 2.8	ab	9.0 ± 2.0	a	8.4 ± 1.9		46.0 ± 11.7	ab	0.2 ± 0.1	a	0.6 ± 0.1	a	0.4 ± 0.2		41.6 ± 1.1	abc
LM		18.5 ± 8.6	b	10.9 ± 5.9	ab	23.4 ± 18.1		60.9 ± 23.4	ab	0.2 ± 0.0	a	0.7 ± 0.1	ab	0.6 ± 0.4		41.1 ± 1.4	ab
P		8.5 ± 3.0	ab	10.5 ± 2.0	ab	9.0 ± 4.0		52.7 ± 10.6	ab	0.6 ± 0.2	ab	0.7 ± 0.0	ab	0.4 ± 0.2		42.0 ± 1.9	bc
LM+P		14.2 ± 3.1	ab	9.1 ± 1.8	a	12.7 ± 4.3		49.0 ± 8.5	ab	1.5 ± 0.1	c	0.7 ± 0.0	a	0.6 ± 0.3		38.1 ± 0.5	a
winter wheat - straw										winter wheat - grain							
Unt	A	14.1 ± 2.6	a	3.6 ± 1.0		5.4 ± 0.6		65.5 ± 17.0	a	1.9 ± 0.2	bc	1.0 ± 0.1	a			32.5 ± 1.7	a
LM		27.2 ± 5.8	bc	4.1 ± 0.9		6.5 ± 2.2		88.4 ± 16.0	ab	2.2 ± 0.1	c	1.3 ± 0.2	ab			34.8 ± 3.7	a
P		17.0 ± 4.1	ab	3.0 ± 1.0		5.3 ± 2.1		63.9 ± 20.3	a	1.7 ± 0.3	bc	1.0 ± 0.3	a			34.7 ± 1.3	a
LM+P		15.9 ± 3.6	ab	5.5 ± 3.4		6.2 ± 5.2		88.8 ± 41.9	ab	1.6 ± 0.2	abc	1.2 ± 0.5	ab			40.2 ± 5.0	ab
Unt	B	29.5 ± 11.6	bc	3.8 ± 0.6		4.7 ± 1.5		78.7 ± 12.2	ab	1.9 ± 0.6	abc	1.2 ± 0.2	ab	< LOD		46.6 ± 5.6	bc
LM		34.4 ± 5.8	c	5.0 ± 0.6		6.7 ± 0.8		153.8 ± 42.1	b	2.0 ± 0.7	bc	1.5 ± 0.2	ab			58.2 ± 4.1	d
P		28.0 ± 4.8	bc	5.2 ± 1.3		4.5 ± 0.9		102.2 ± 22.8	ab	1.2 ± 0.4	ab	1.7 ± 0.2	b			52.7 ± 2.1	cd
LM+P		27.2 ± 6.0	bc	4.8 ± 0.3		5.5 ± 1.0		108.5 ± 62.1	ab	1.0 ± 0.2	a	1.3 ± 0.2	ab			53.1 ± 6.5	cd
spring barley - straw										spring barley - grain							
Unt	A	6.8 ± 0.6	a	1.9 ± 0.4	ab	4.8 ± 0.2	a	35.1 ± 7.4	a	1.0 ± 0.2	a	0.2 ± 0.0	a	0.3 ± 0.1		28.1 ± 1.5	a
LM		12.9 ± 1.0	ab	2.8 ± 0.2	bc	8.1 ± 0.5	a	58.0 ± 9.4	ab	1.1 ± 0.3	ab	0.2 ± 0.1	ab	0.3 ± 0.1		30.0 ± 3.1	ab
P		9.9 ± 2.4	a	1.8 ± 0.4	a	7.7 ± 1.1	a	39.4 ± 15.1	a	1.0 ± 0.2	ab	0.2 ± 0.1	ab	0.4 ± 0.1		29.0 ± 3.5	a
LM+P		18.6 ± 7.6	b	2.9 ± 0.8	c	14.4 ± 6.3	b	65.3 ± 6.8	b	0.8 ± 0.2	a	0.3 ± 0.1	abc	0.1 ± 0.1		29.8 ± 1.6	a
Unt	B	8.0 ± 1.6	a	1.2 ± 0.0	a	3.6 ± 0.7	a	37.3 ± 3.8	a	1.1 ± 0.1	ab	0.4 ± 0.1	abc	0.3 ± 0.2		34.9 ± 6.4	ab
LM		11.4 ± 1.2	ab	1.3 ± 0.3	a	4.5 ± 0.4	a	48.2 ± 7.5	ab	1.1 ± 0.0	b	0.4 ± 0.0	bcd	0.4 ± 0.2		39.7 ± 2.8	ab
P		12.5 ± 2.5	ab	1.4 ± 0.4	a	4.8 ± 1.0	a	37.2 ± 7.3	a	1.1 ± 0.1	ab	0.5 ± 0.1	cd	0.5 ± 0.1		42.8 ± 10.8	b
LM+P		10.8 ± 2.5	a	1.2 ± 0.3	a	6.5 ± 0.6	a	41.7 ± 15.7	a	0.8 ± 0.1	a	0.5 ± 0.1	d	0.3 ± 0.1		40.0 ± 6.9	ab

EU-threshold
animal feed (DW;
EC (2002))

2.27

1.14

11.36

2.27

1.14

11.36

The cultivar-specificities are illustrated by the arrow tips in the CoIA results (Figure 2.1 c) – IV), being displaced to different plot sections representing Cd or Zn tissue concentrations (Figure 2.1 c) – III), respectively. The LAC of wheat allocated on average 32 % and 21 % less Zn and Cd in grains, respectively, less Zn, Cd, and As in straw but more As (21 %) in grains compared to the HAC (Table 2.2, Figure 2.1 b) - IV). Both wheat cultivars showed higher TF of Cd (A: 0.3 ± 0.07 , B: 0.3 ± 0.05) compared to those of As (A: 0.12 ± 0.04 , B: 0.05 ± 0.03), which differed significantly among the cultivars. Significant cultivar-specificities regarding bioconcentration (Figure 2.4) of Cd and Zn in rape (hybrid cv. > line cv.) or of Pb in cereal straw (HAC < LAC) were only partly verified using absolute tissue concentrations (Table 2.7). Several correlations were found among TE in plants, whereby the grain TE concentrations were uncorrelated among each other except for the LAC of barley, where grain Zn correlated inversely with grain As and Pb (Table 2.8).

The fertilizer treatments hardly affected TE bioaccumulation (Figure 2.4). Phosphorus-containing treatments insignificantly reduced the BCF of metals and As in rape straw (P) or that of As in wheat straw and grains (LM+P) compared to Unt (Figure 2.4). By contrast, the LM-containing treatments appeared to enhance plant metals concentrations (Table 2.2), whereby significant increases in BCF compared to Unt were only obtained for Cd and Zn accumulation in wheat tissues. However, these effects mostly referred to the spatial heterogeneity of the pollution level, as confirmed by correlation analyses (see the following section).

Relations between soil and plant chemical data

The CoIA showed significant but moderate relationships between soil and plant chemical data in case of winter oilseed rape (Figure 2.1 a) - II; $RV = 0.33$) and spring barley (Figure 2.1 c) - II; $RV = 0.39$). By contrast, only few correlations were found between TE concentrations in soil and those in wheat shoots, unless separated by cultivars (Table 2.8).

Mostly, TE tissue concentrations correlated more strongly with pseudo-total than with NH_4NO_3 -soluble TE (Table 2.8). Moreover, all species' grain BCF correlated inversely with pseudo-total TE in soil (Table 2.9). Significant inverse correlations between C_{org} in soil and grain As and Cd concentration in rape and barley (each $r = -0.37$) were verified using the corresponding BCF ($r = -0.4$ and -0.38 , respectively). Significant positive correlations between soil pH and grain As and Cd concentrations in rape were not accompanied by correlations with BCF, and are therefore determined by the heterogeneous site conditions.

Figure 2.1 Results of Co-inertia Analyses (CoIA) performed on the data for (a) 2012 - winter oilseed rape (*Brassica napus* L. cv. Visby (A) and cv. Lorenz (B)), (b) 2013 - winter wheat (*Triticum aestivum* L. cv. Türkis (A) and cv. Tiger (B)), and (c) 2014 - spring barley (*Hordeum vulgare* L. cv. Salome (A) and Marthe (B)). I - Correlation circles depicting the projections of the PCA axes from soil and plant data onto the CoIA axes. II - Histogram of Monte Carlo permutation tests distinguishing a random distribution of RV values (bars) from the actual RV-coefficient (black diamond). III - Projection of the coefficients of combinations of soil (left) or plant (right) variables to defined Co-inertia axes. Respectively, pseudo-total or mobile element concentrations are denoted with a lowercase "t" or "m", and straw or grain concentrations with a lowercase "s" or "g". IV - CoIA results separated for cultivar A (top) and B (bottom); both graphs originate from the same analysis. Each arrow corresponds to a soil sample and its corresponding plant sample. The beginning of the arrow is the position of the sample described by the soil data set; the tip of the arrow is the position of the sample described by the plant data set. The arrow length is indicative for differences between each sample's soil and plant data: the shorter the arrow, the stronger both datasets are related. Treatments are differentiated by arrow colors: black-Unt, blue - LM, green - P, red - LM+P.

The pseudo-total P concentrations correlated significantly inversely with As status of rape grains and barley straw (each $r = -0.38$; illustrated by opposing weightings in Figure 2.1 a) and c) - III), and positively with concentrations of As in wheat grains (each $r = 0.46$) and Cd ($r = 0.5$) and Zn ($r = 0.46$) in rape straw. The grouping of LM and LM+P treatments in the CoIA apart from Unt and P in case of the LACs of rape and barley reflects the spatial heterogeneity of the contamination level and presents no treatment effect (Figure 2.1 a) and c) - IV).

Biomass production

The grain yields (Table 2.10) of cereals were much below regional averages of $> 0.6 \text{ kg m}^{-2} \text{ DW}^{-1}$ obtained in the respective years (Saxon State Statistical Office, 2018). The wheat cultivars varied largely due to growth retardation of the LAC, particularly in heavily contaminated areas of treatments LM and LM+P, as observed already in early growth stages (Figure 2.5 c) and d)). The cereals' HACs were superior to the LACs, except for equal yields of both barley cultivars in Unt. Contrastingly, the regional average of $0.37 \text{ kg m}^{-2} \text{ DW}^{-1}$ for rape harvested in 2012 was almost reached by both cultivars of treatment LM+P, and exceeded by cultivar A of treatment LM.

Discussion

Species- and cultivar-specific response to varying levels of soil contamination

The pseudo-total TE concentrations encountered at the study site clearly exceeded regional backgrounds (Kardel et al., 2015) or European levels (Tóth et al., 2016b) and varied largely due to their origin from adjacent chimney stacks as former emission sources for subsequent atmospheric deposition. Usually, trace element-contaminated sites are characterized by a certain spatial heterogeneity of the contamination level or its variation in depth. Both can limit the efficiency of GRO via impacts on plant yield or TE uptake (Mench et al., 2006; Lamé, 2011). Accordingly, the cereals' yields were below the regional average, indicating toxicity. By contrast, winter oilseed rape appeared to tolerate varying pollution levels, as found before (Marchiol et al., 2004; Neu et al., 2018b). Remarkably, the LACs of cereals had particularly low yields. Phytoexclusion is considered a tolerance mechanism for avoidance of TE induced stress in plants (Rizwan et al., 2016). Therefore, based on lower grain metals accumulation by the cereals' LACs, a higher tolerance and no negative yield response might have been expected. However, both LACs were cultivated on sub-plots with maximum pseudo-total

loads of multiple contaminants. Furthermore, in case of barley the lower Cd and Zn concentrations in grains were accompanied by higher levels in straw compared to the HAC. The differential partitioning of TE between plant compartments among cultivars corresponds with the findings for wheat Cd accumulation by Kubo et al. (2016) and Shi et al. (2015), who attributed it to differences in phloem remobilization from vegetative to generative organs. The antagonistic accumulation of As and Cd in wheat tissues is related to different transporters involved in plant As or Cd xylem and phloem loading (Ma et al., 2008; Mendoza-Cózatl et al., 2011). The significantly higher translocation factors ($TF_{\text{grain}}/TF_{\text{straw}}$) of Cd compared to As in both wheat cultivars correspond with the findings of Shi et al. (2015), whose results further imply that the rachis is essential for differential As and Cd transport to grains. The authors supposed that the selection of cultivars with low Cd and As accumulation in grains is possible since both were not related, as found in this study. Likewise, the lack of relationship between grain Cd and Zn concentrations among wheat and barley cultivars suggests that breeding for reduced Cd concentration in major staple crops, which is still in its infancy (Rizwan et al., 2016), could be attained without the side-effect of Zn malnutrition (Gao et al., 2011).

The obtained differences between grain Cd accumulation in LACs and HACs of cereals were lower than in greenhouse studies using the same varieties (barley: 39 % vs. 46 %, wheat 21 % vs. ~ 42 %; (BfUL/LfL, 2002-2018). This stresses the importance of field studies to adequately estimate phytoexclusion potentials for minimizing Cd transfer into animal feed or the human diet as an ongoing environmental issue (Rizwan et al., 2016; Rodríguez-Eugenio et al., 2018). However, even on this severely contaminated soil, all grains of rape and barley and more than half of the LAC's wheat grain samples complied with the European threshold for Cd in animal feed (EC, 2002). By contrast, the valorization of all species' straw biomass was limited to the bioenergy sector, where certain restrictions, e.g. regarding TE concentration of the output materials (digestate, ashes), have to be considered (Bert et al., 2017), or it should remain on site for humus formation and carbon sequestration.

The inverse correlations between BCF and pseudo-total TE in soil reflect saturation processes in plants leading to lower values at higher pollution levels (McLaughlin et al., 2011).

Seasonal and year-to-year differences in TE mobility

Lower percentages of mobile As in soil samples obtained during harvests in the summer months may reflect a higher redox potential (Masscheleyn et al., 1991) in the Stagnosol compared to the initial sampling in September 2011, where more precipitation and/or the lack of vegetation cover caused higher levels of soil moisture. The high NH_4NO_3 -soluble TE

concentrations in 2013 were possibly due to heavy precipitation in May and June, which caused a 100-year-flood event in the region. Although no decrease in bulk soil pH was found compared to 2012, rhizosphere acidification as a result of root exudation of low molecular weight organic acids in response to water-logging, as found before for a *Poaceae* (Schreiber et al., 2012), may have induced TE solubilization. In turn, variations of soil properties in depth presumably effected the low potentially plant-available concentrations of metals and the high soil pH in 2014. Since both occurred also at the untreated sub-plots, they could not result from additive effects of lime marl fertilization, but from the entrance of carbonate-rich material from a CaCO_3 -enriched loamy horizon in a depth of ~ 25 cm belowground (Barth and Forberg, 2015) in the course of ploughing.

Influencing factors for *in situ* immobilization in practical field application

The presence of multiple contaminants competing for sorption sites can hamper the efficiency of stabilizing agents (Kumpiene et al., 2008). However, in a complementary pot experiment the same lime marl treatment effected a distinct increase in soil pH and a simultaneous decrease of labile metals fractions (Neu et al., 2018a), whereas only marginal changes were obtained in the present field study. This corresponds with the findings of Friesl et al. (2006), who attributed differing results between pot and field experiments with stabilizing agents to inhomogeneous application and varying soil moisture in the field. Oliver et al. (1998) found smaller decreases in Cd accumulation by wheat following liming in soils with high level or aged contamination than in low contaminated or spiked soils. Yet, LM-containing treatments effected a significant reduction in the percentage of mobile related to pseudo-total metals in this study. The combined application with phosphate (LM+P) was the most effective, particularly regarding the immobilization of Pb, which supports previous findings (Brown et al., 2005; Seshadri et al., 2017). However, the amendments rarely affected plant TE concentrations, which remained more influenced by pseudo-total than NH_4NO_3 -soluble TE in soil. Plant-scale heterogeneity of the contamination level, prevailing at the study site not least due to uneven, wavy transitions between differentially polluted soil horizons (Barth and Forberg, 2015), has been found to considerably affect TE bioconcentration and yield (Millis et al., 2004). Furthermore, root density and depth in relation to soil moisture and the vertical extent of amendment effects are crucial for plant response to *in situ* stabilization measures (Mench, 1998; Friesl et al., 2006). Accordingly, since the contamination at the study site is located basically in the plough layer, high precipitation and soil moisture, as occurred during

bolting and grain filling of wheat in 2013, may have induced high local root activity effecting TE uptake similar or higher than in a simultaneous greenhouse study (Neu et al., 2018a).

Phosphate fertilizers present a major anthropogenic source of Cd, and other TE, in agronomic systems (Grant et al., 2010). Recently, Kratz et al. (2016) found that many straight P fertilizers sold in Germany exceeded the national threshold of 50 mg Cd kg⁻¹ P₂O₅ (DüMV, 2012), as did the superphosphate used in this study. Thus, the positive correlations between soil P status and Cd or Zn tissue concentrations might be related to fertilizer inputs, or to secondary effects on root growth or mycorrhizal association (Mench, 1998; Grant et al., 2010), making a frequent fertilization of amounts exceeding plant demands questionable. The elevated proportion of mobile related to pseudo-total As in the P treatment and the positive correlation between soil P and As concentration in wheat grains might confirm the findings of Brackhage et al. (2015), who attributed As mobilization by high P fertilization to competing exchange/adsorption on soil mineral phases. Under simulated water-logging conditions, which naturally prevailed temporarily during wheat cultivation in this study, this process had been followed by the reduction of mobilized As (V) to As (III), effecting increased bio-accumulation in aboveground tissues of another regional winter wheat cultivar (Brackhage et al., 2015).

Conclusions

This study proved the potential of cultivar choice to reduce TE transfer into the food chain in practical application. Particularly strong, however partly antagonistic, effects were obtained regarding the accumulation of Cd and As in wheat and barley tissues. Nevertheless, among the biomass produced on site only grains of rape and barley unexceptionally complied with the European legislation on animal feed. The combined enhanced lime marl and phosphate fertilization most effectively reduced metals mobility, but neither amendment mitigated plant TE status, which remained more closely related with pseudo-total than NH₄NO₃-soluble TE in soil. Agricultural measures and climatic conditions strongly affected TE mobility. Also, cultivar differences in TE uptake were lower than in greenhouse studies, stressing the importance of field assessments. Considering the relatively low cereal yields and the measures' limited efficacy to assure forage safety, biomass production for energetic or material use present more appropriate types of land use on such severely contaminated agricultural land.

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Supplementary Information

Study site and treatments

Figure 2.2 Spatial distribution (kriging-interpolated contour maps) of pseudo-total concentrations ($\text{mg kg}^{-1} \text{DW}^{-1}$) of As and metals (Cd, Pb, and Zn) at the study site. The arrangement of treatments (Unt: untreated, LM: $0.4 \text{ kg lime marl m}^{-2}$, P: $0.04 \text{ kg P } 40 \text{ m}^{-2}$, LM+P: $0.4 \text{ kg lime marl m}^{-2} + 0.04 \text{ kg P } 40 \text{ m}^{-2}$; "A" represents low-accumulating cultivars and "B" high-accumulating cultivars of wheat and barley based on cultivar screenings of grain Cd uptake, whereas for rape "A" represents a hybrid and "B" a line cultivar) is exemplified at the contour map showing As concentrations.

Sampling procedures

Composite samples were obtained with an auger from the 0 - 20 cm topsoil by five entry points each around a sampling location determined by a randomized design according to Huesemann (1994).

The sampling of cereals was conducted immediately prior to mechanical harvests. In the case of winter oilseed rape, the entrance of the planting for randomized sampling would have caused yield losses due to the break of pods. Therefore, the wide of one thresher (about 4.8 m) was mechanically harvested in the midst of each treatment over its entire length. Within this area entry points were placed in the root zone of sampled straw. Grain samples were captured in four replicates from the harvested composite sample, which was previously filled into big bags, weighted and used for calculation of the yield per treatment.

Table 2.3 Monthly amounts of precipitation (mm m^{-2}) recorded at an adjacent permanent soil monitoring site^{a)}.

month	precipitation
09 - 2011 ^{b)}	67.2
10 - 2011	42.4
11 - 2011	0.0
12 - 2011	58.8
01 - 2012	92.2
02 - 2012	35.0
03 - 2012	17.2
04 - 2012	27.8
05 - 2012	39.0
06 - 2012	87.2
07 - 2012 ^{c)}	84.2
08 - 2012	50.8
09 - 2012	42.0
10 - 2012 ^{b)}	23.0
11 - 2012	52.4
12 - 2012	56.0
01 - 2013	66.8
02 - 2013	27.8
03 - 2013	22.6
04 - 2013	23.6
05 - 2013	128.8
06 - 2013	182.4
07 - 2013	33.8
08 - 2013 ^{c)}	41.0
09 - 2013	60.0
10 - 2013	48.0
11 - 2013	37.8
12 - 2013	14.2
01 - 2014	19.6
02 - 2014	7.8
03 - 2014	28.0
04 - 2014 ^{b)}	24.4
05 - 2014	91.4
06 - 2014	32.4
07 - 2014	93.4
08 - 2014 ^{c)}	37.4

^{a)} the site is located 30 meters uphill from the southern boundary of the study site^{b)} sowing of winter oilseed rape (2011), winter wheat (2012), or spring barley (2014)^{c)} harvest of winter oilseed rape (2012), winter wheat (2013), or spring barley (2014)

Table 2.4 Pseudo-total phosphorus concentrations ($\text{mg g}^{-1} \text{DW}^{-1}$) at the study site per treatment (Unt: untreated, LM: $0.4 \text{ kg lime marl m}^{-2}$, P: $0.04 \text{ kg P } 40 \text{ m}^{-2}$, LM+P: $0.4 \text{ kg lime marl m}^{-2} + 0.04 \text{ kg P } 40 \text{ m}^{-2}$; "A" represents low-accumulating cultivars and "B" high-accumulating cultivars of wheat and barley based on cultivar screenings of grain Cd uptake, whereas for rape "A" represents a hybrid and "B" a line cultivar). Values are means $\pm$ SD ($n = 4$).

		2011	2012	2013	2014
Unt	A	2.1 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1
	B	1.7 ± 0.2	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.2
LM	A	2.0 ± 0.1	2.0 ± 0.1	1.9 ± 0.2	1.9 ± 0.1
	B	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
P	A	1.6 ± 0.2	1.6 ± 0.1	1.8 ± 0.2	1.8 ± 0.1
	B	1.6 ± 0.2	1.7 ± 0.1	1.7 ± 0.1	1.9 ± 0.2
LM+P	A	1.6 ± 0.2	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.3
	B	1.5 ± 0.2	1.5 ± 0.1	1.4 ± 0.3	1.6 ± 0.1

Decision basis for the amount of applied fertilizers

For the given soil (texture: loamy sand, $\text{pH}(\text{CaCl}_2)$: 5.5, and humus content: $> 4.1\%$; see Barth and Forberg (2015)) the Saxon State Office for Environment, Agriculture and Geology (LfULG) recommends an application of $0.12 \text{ kg CaO m}^{-2}$ for four years to accomplish and maintain an optimal pH level, also in terms of metals immobilization (BfUL, 2017). The LM fertilizer contains $\sim 30\%$ CaO, thus, an annual amount of 0.4 kg m^{-2} has been applied.

Prior to the establishment of the field trial, the P status of $7.1 \text{ mg } 100 \text{ g}^{-1}$ soil was within the optimal range. Based on the recommendations for As contaminated soils (BfUL, 2017) the P fertilization should be applied at the beginning of the vegetation period and meet the crops' demands to enable the substitution of As during plant uptake. The requirements of wheat, barley, and rape range between $0.004 - 0.005 \text{ kg P m}^{-2}$ (Landwirtschaftskammer NRW, 2015). The applied P 40 fertilizer contains 17.5% P. Thus, with the application of 0.04 kg m^{-2} a fertilization of $0.007 \text{ kg P m}^{-2}$ has been conducted, which exceeds the maximum expected removals to enable an additional immobilization of the present metals.

Table 2.5 Trace element concentrations (mg kg⁻¹ DW⁻¹) in fertilizers (superphosphate: P 40 (40 % P₂O₅), lime marl: LM). Values are means ± SD (*n* = 3). For interpretation, legal thresholds* for trace elements (mg kg⁻¹ DW⁻¹ unless otherwise specified) were added in the table according to the German Fertilizer Ordinance (DüMV, 2012).

	P 40			LM		
Cr	169.8	±	4.1	11.6	±	1.1
Ni	133.0	±	4.4	73.5	±	5.0
As	6.7	±	0.3	8.9	±	1.0
Cd	27.2	±	0.8	0.5	±	0.1
Tl	0.8	±	0.0	0.2	±	0.0
Pb	3.0	±	0.2	17.1	±	2.1
Zn	n.a.**			5.4	±	0.7

*As: 40; Cd: 1.5 or (for fertilizers > 5 % P₂O₅ (FW)) 50 mg kg⁻¹ P₂O₅⁻¹; Ni: 80 ; Pb: 150 , Tl: 1

**values for common superphosphates sold in Germany are 469 ±136 mg Zn kg⁻¹ DW⁻¹ (Kratz et al., 2016)

Table 2.6 Pearson correlation coefficients between NH₄NO₃-soluble TE concentrations in soil (denoted with a lowercase "m") and *aqua regia* extractable (AR) concentrations of the respective element, soil pH or C_{org}. The significance of the correlation is represented by *(*p* < 0.05) or **(*p* < 0.01).

		AR	pH	C _{org}		AR	pH	C _{org}
As _m	2011	0.46**	-0.22	0.48**	2012	0.62**	0.26	0.16
Cd _m		0.36*	-0.71**	-0.25		0.04	-0.77**	0.39*
Pb _m		0.45**	-0.76**	0.14		0.33	-0.83**	0.64**
Zn _m		0.90**	-0.15	-0.25		0.42*	-0.78**	0.41*
As _m	2013	0.72**	-0.05	0.66**	2014	0.61**	0.33	0.05
Cd _m		-0.15	-0.88**	0.02		0.21	-0.70**	-0.24
Pb _m		-0.45*	-0.91**	0.13		0.37*	-0.81**	0.18
Zn _m		0.02	-0.73**	0.01		0.04	-0.67**	-0.18

Table 2.7 Results of the one-way ANOVA for cultivar-dependent variation in TE tissue concentrations. Straw or grain tissue are denoted with a lowercase "s" or "g", respectively. The explained variation (Expl. Var.) is given in percent and *F*-values with significance level (ns = not significant; *: *p* < 0.05; **: *p* < 0.01; ***: *p* < 0.001).

	winter oilseed rape			winter wheat			spring barley		
	Expl. Var.	<i>F</i> -value		Expl. Var.	<i>F</i> -value		Expl. Var.	<i>F</i> -value	
As _s	0.4	0.1	ns	41.1	21.0	***	2.5	0.8	ns
Cd _s	18.0	6.6	*	11.2	3.8	ns	60.0	45.0	***
Pb _s	0.3	0.1	ns	0.3	0.1	ns	36.2	17.0	***
Zn _s	0.5	0.1	ns	19.4	7.2	*	9.7	3.2	ns
As _g	0.4	0.1	ns	15.3	5.4	*	1.9	0.6	ns
Cd _g	12.3	4.2	ns	25.4	10.2	**	56.1	38.4	***
Pb _g	1.0	0.3	ns	-	-	-	11.0	3.7	ns
Zn _g	33.3	15.0	***	74.1	85.7	***	54.6	36.1	***

Figure 2.3 Percentage $((\text{NH}_4\text{NO}_3\text{-soluble} / \text{pseudo-total TE concentration}) \times 100)$ of mobile As and metals (Cd, Pb, and Zn) in soil following different fertilizer treatments (Unt: untreated, LM: $0.4 \text{ kg lime marl m}^{-2}$, P: $0.04 \text{ kg P } 40 \text{ m}^{-2}$, LM+P: $0.4 \text{ kg lime marl m}^{-2} + 0.04 \text{ kg P } 40 \text{ m}^{-2}$). Data from 2011 represent initial conditions (untreated, unplanted), whereas data from the following years represent soils at harvest date of winter oilseed rape (2012; *Brassica napus* L. cv. Visby (A) and cv. Lorenz (B)), winter wheat (2013; *Triticum aestivum* L. cv. Türkis (A) and cv. Tiger (B)), and spring barley (2014; *Hordeum vulgare* L. cv. Salome (A) and cv. Marthe (B)). For wheat and barley, "A" represents low-accumulating cultivars and "B" high-accumulating cultivars based on cultivar screenings of grain Cd uptake, whereas for rape "A" represents a hybrid and "B" a line cultivar. Values are means $\pm$ SD ($n = 4$).

Figure 2.4 Bioconcentration factors (BCF; concentration_{shoot} / concentration_{soil}) of As and metals (Cd, Pb, and Zn) in straw and grain biomass of winter oilseed rape (*Brassica napus* L. cv. Visby (A) and cv. Lorenz (B)), winter wheat (*Triticum aestivum* L. cv. Türkis (A) and cv. Tiger (B)), and spring barley (*Hordeum vulgare* L. cv. Salome (A) and cv. Marthe (B)) following different fertilizer treatments ((Unt: untreated, LM: 0.4 kg lime marl m⁻², P: 0.04 kg P 40 m⁻², LM+P: 0.4 kg lime marl m⁻² + 0.04 kg P 40 m⁻²). For wheat and barley, "A" represents low-accumulating cultivars and "B" high-accumulating cultivars based on cultivar screenings of grain Cd uptake, whereas for rape "A" represents a hybrid and "B" a line cultivar. The Pb concentrations in wheat grains were below the limit of detection. Values are means $\pm$ SD ($n = 4$).

Table 2.8 Pearson correlation coefficients between tissue concentrations of TE in winter oilseed rape (*Brassica napus* L. cv. Visby (A) and cv. Lorenz (B)), winter wheat (*Triticum aestivum* L. cv. Türkis (A) and cv. Tiger (B)), and spring barley (*Hordeum vulgare* L. cv. Salome (A) and cv. Marthe (B)) and the respective element concentrations in soil (AR: *aqua regia* extractable, AN: NH_4NO_3 -soluble). Plant straw or grain are denoted with a lowercase "s" or "g", respectively. The significance of the correlation is represented by *($p < 0.05$) or **($p < 0.01$). The grain Pb concentrations of winter wheat were below the limit of detection.

winter oilseed rape	A	AR	AN	As _s	As _g	Cd _s	Cd _g	Pb _s	Pb _g	Zn _s	Zn _g	B	AR	AN	As _s	As _g	Cd _s	Cd _g	Pb _s	Pb _g	Zn _s	Zn _g
	As _s	0.69**	0.19	1	-0.05	0.74**	0.39	0.85**	-0.20	0.72**	0.34	As _s	0.43	0.07	1	0.19	-0.01	0.38	0.94**	0.64**	0.22	-0.14
	As _g	0.00	0.08		1	-0.21	0.73**	-0.12	-0.01	-0.28	0.21	As _g	-0.25	0.36		1	-0.22	0.38	0.09	0.40	-0.18	-0.65**
	Cd _s	0.58*	0.28			1	0.35	0.39	-0.30	0.87**	0.53*	Cd _s	0.09	0.50			1	-0.12	-0.19	-0.33	0.90**	-0.06
	Cd _g	0.60*	-0.23				1	0.07	0.10	0.31	0.69**	Cd _g	0.37	-0.41				1	0.44	0.58*	-0.07	0.06
	Pb _s	0.24	-0.31					1	-0.22	0.40	-0.07	Pb _s	0.31	-0.25					1	0.57*	0.03	0.06
	Pb _g	0.19	0.41						1	-0.15	-0.03	Pb _g	0.20	-0.31						1	-0.17	-0.17
	Zn _s	0.39	0.48							1	0.44	Zn _s	0.21	0.53*							1	-0.21
	Zn _g	0.32	0.21								1	Zn _g	-0.20	0.08								1
winter wheat	A	AR	AN	As _s	As _g	Cd _s	Cd _g	Pb _s	Pb _g	Zn _s	Zn _g	B	AR	AN	As _s	As _g	Cd _s	Cd _g	Pb _s	Pb _g	Zn _s	Zn _g
	As _s	0.63**	0.81**	1	0.51*	0.21	0.43	0.26	-	0.43	0.00	As _s	0.45	0.62*	1	0.16	0.17	0.16	-0.37	-	0.42	0.17
	As _g	0.16	0.48		1	-0.38	-0.21	0.05	-	-0.24	-0.49	As _g	0.53*	0.15		1	-0.07	-0.12	0.20	-	0.22	0.05
	Cd _s	0.26	0.13			1	0.89**	-0.06	-	0.76**	0.23	Cd _s	0.13	0.07			1	0.71**	-0.19	-	0.15	0.27
	Cd _g	0.36	0.32				1	-0.15	-	0.81**	0.25	Cd _g	0.21	0.15				1	-0.24	-	0.01	0.23
	Pb _s	0.21	-0.02					1	-	0.04	0.28	Pb _s	0.39	-0.33					1	-	0.29	0.38
	Pb _g	-	-						-	-	-	Pb _g	-	-					-	-	-	-
	Zn _s	-0.20	0.01							1	0.44	Zn _s	0.72**	0.38							1	0.83**
	Zn _g	0.27	-0.14								1	Zn _g	0.67**	0.27								1
spring barley	A	AR	AN	As _s	As _g	Cd _s	Cd _g	Pb _s	Pb _g	Zn _s	Zn _g	B	AR	AN	As _s	As _g	Cd _s	Cd _g	Pb _s	Pb _g	Zn _s	Zn _g
	As _s	0.53*	0.64**	1	-0.23	0.71**	0.77**	0.92**	-0.38	0.63**	0.22	As _s	0.32	0.34	1	0.27	0.36	0.52*	0.50*	-0.14	0.44	0.42
	As _g	-0.19	-0.09		1	-0.45	-0.30	-0.31	0.16	-0.63**	-0.57*	As _g	-0.10	-0.26		1	0.38	-0.25	-0.30	-0.37	-0.01	-0.24
	Cd _s	0.67**	0.25			1	0.49	0.74**	-0.29	0.68**	0.24	Cd _s	0.15	0.14			1	0.26	-0.08	-0.13	0.46	-0.01
	Cd _g	0.48	0.03				1	0.67**	-0.30	0.40	0.24	Cd _g	0.42	-0.23				1	0.45	0.49	0.27	0.09
	Pb _s	0.39	-0.41					1	-0.41	0.57*	0.25	Pb _s	0.20	-0.35					1	-0.10	0.04	0.05
	Pb _g	-0.60*	0.03						1	-0.51*	-0.52*	Pb _g	0.03	-0.29						1	-0.13	0.12
	Zn _s	0.68**	0.37							1	0.62*	Zn _s	0.71**	0.21							1	0.42
	Zn _g	0.55*	0.21								1	Zn _g	0.49	0.28								1

Table 2.9 Pearson correlation coefficients between bioconcentration factors (BCF) of TE in tissues of winter oilseed rape (*Brassica napus* L. cv. Visby (A) and cv. Lorenz (B)), winter wheat (*Triticum aestivum* L. cv. Türkis (A) and cv. Tiger (B)), and spring barley (*Hordeum vulgare* L. cv. Salome (A) and cv. Marthe (B)) and the respective *aqua regia* extractable element concentrations in soil. Plant straw or grain are denoted with a lowercase "s" or "g", respectively. The significance of the correlation is represented by * ($p < 0.05$) or ** ($p < 0.01$). The grain Pb concentrations of winter wheat were below the limit of detection.

	Soil TE					
	winter oilseed rape		winter wheat		spring barley	
	A	B	A	B	A	B
BCF As _s	0.56*	0.21	0.23	0.65**	0.07	-0.15
BCF Cd _s	-0.11	-0.50	0.01	0.31	-0.15	-0.58*
BCF Pb _s	-0.03	0.13	-0.47	-0.12	0.15	-0.38
BCF Zn _s	-0.10	-0.05	-0.33	0.64**	0.27	-0.03
BCF As _g	-0.20	-0.41	-0.73**	0.13	-0.75**	-0.58*
BCF Cd _g	-0.23	-0.81**	-0.02	0.35	-0.42	-0.52*
BCF Pb _g	0.07	-0.12	-	-	-0.67**	-0.12
BCF Zn _g	-0.80**	-0.86**	-0.54*	0.48	-0.79**	-0.34

Table 2.10 Grain yields in kg m⁻² DW⁻¹ of winter oilseed rape (*Brassica napus* L. cv. Visby (A) and cv. Lorenz (B)), winter wheat (*Triticum aestivum* L. cv. Türkis (A) and cv. Tiger (B)), and spring barley (*Hordeum vulgare* L. cv. Salome (A) and cv. Marthe (B)) following different fertilizer treatments (Unt = untreated, L = lime, P = superphosphate, L+P = lime and superphosphate). For wheat and barley, "A" represents low-accumulating cultivars and "B" high-accumulating cultivars based on cultivar screenings of grain Cd uptake, whereas for rape "A" represents a hybrid and "B" a line cultivar.

	winter oilseed rape		winter wheat		spring barley	
	A	B	A	B	A	B
Unt	0.30	0.33	0.18	0.39	0.20	0.20
LM	0.38	0.32	0.07	0.33	0.23	0.27
P	0.31	0.32	0.28	0.33	0.23	0.27
LM+P	0.34	0.35	0.08	0.42	0.27	0.29

Figure 2.5 Plant development during the vegetation period of winter oilseed rape (*Brassica napus* L. cv. Visby and Lorenz; a) in May 2012 and b) during harvest in July 2012), winter wheat (*Triticum aestivum* L. in May 2013; cv. Türkis is depicted on the right side and cv. Tiger on the left side, respectively, of pictures c) and d)), and spring barley (*Hordeum vulgare* L.; cv. Salome is depicted on the right side and cv. Marthe on the left side of picture e) in June 2014 and vice versa on picture f) in July 2014). Wooden piles delimit the cultivars and fertilizer treatments.

3 Exploring pre-crop effects of annual oilseeds

3.1 Trace elements bioavailability to winter wheat (*Triticum aestivum* L.) grown subsequent to high biomass plants in a greenhouse study

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Abstract

Multielement-contaminated agricultural land requires the adaptation of agronomic practices to meet legal requirements for safe biomass production. The incorporation of bioenergy plants with, at least, moderate phytoextraction capacity into crop rotations with cereals can affect trace elements (TE) phytoavailability and, simultaneously, constitute economic revenues for farmers outside the food or forage sector. Hence, in a crop rotation pot study sunflower (*Helianthus annuus* L.), modified for high biomass and TE accumulation by chemical mutagenesis, was compared to winter oilseed rape (*Brassica napus* L.) as pre-crop. On two agricultural soils with different TE loads, the crops' potential for phytoextraction and for impacts on TE uptake by subsequent winter wheat (*Triticum aestivum* L.) was studied. The results showed that rape tolerated high-level mixed contamination with metals (Cd, Pb, and Zn) and As more than sunflower. In both soils, labile metals concentration increased and soil acidity remained high following sunflower. Furthermore, enhanced grain As accumulation in subsequent wheat was observed. By contrast, soil acidity and Cd or Zn accumulation of subsequent wheat decreased following rape. In the short term, moderate phytoextraction was superimposed by nutrient use or rhizosphere effects of pre-crops, which should be carefully monitored when designing crop rotations for contaminated land.

Keywords: heavy metals and arsenic, winter oilseed rape, enhanced sunflower

Introduction

Globally, past and present ore mining and processing activities have led to extensive contamination of topsoils with trace elements (TE). In particular, agricultural production within or adjacent to mining areas, like an approximate 100,000 ha of topsoils in the Saxon Ore Mountains (Germany), has to be adapted to meet requirements for safe production of biomass (SMI, 2013; Kidd et al., 2015). It should, therefore, be accompanied by a risk management approach, which ideally includes soil remediation as a secondary objective. Phytomanagement options comprise techniques to control relevant pollutant linkages within a reasonable time frame and may reduce TE bioavailability (Kidd et al., 2015). Phytoextraction, where plants are used to extract phytoavailable TE from soils, was found to be a feasible option (Keller and Hammer, 2004; Herzig et al., 2014). It has been conducted with herbaceous TE accumulating plants (Keller and Hammer, 2004; Bani et al., 2007), fast-growing trees (Fischerová et al., 2006; Van Slycken et al., 2013; Puschenreiter et al., 2013) or annual plants (Grispen et al., 2006; Kolbas et al., 2011; Herzig et al., 2014). Its efficiency is limited (i) to the root zone, (ii) by the plants' tolerance toward phytoavailable TE in soil, and (iii) by the produced biomass. On contaminated agricultural land, frequent inclusion of annual biomass plants with a moderate phytoextraction capacity into crop rotations with cereals could be attractive to assure economic benefits for farmers within the traditional cultivation system. Hitherto, most studies focused on potential TE removal and effects on labile TE in soil (Marchiol et al., 2004; Meers et al., 2005; Herzig et al., 2014), whereas only few assessed effects of such plants on TE status of subsequent crops, which could be increased (Quartacci et al., 2009; Puschenreiter et al., 2013), decreased (Khoshgoftarmanesh and Chaney, 2007; Kidd et al., 2015), or remain unaffected (Gao et al., 2010). Apart from TE removal, plant physiological processes exert influence on soil chemical properties and TE availability to other plants within cropping systems (Luo et al., 2008; Quartacci et al., 2009; Tang et al., 2012). Thereby, depending on the plants' strategies for nutrient acquisition, the nature of root exudates, or plant-associated microbes, the rhizosphere can become acidified but also alkalinized (Kidd et al., 2009; Sessitsch et al., 2013).

From an agronomic perspective oilseeds, like rape and sunflower, represent beneficial preceding crops for the subsequent cultivation of monocots like winter wheat. Both favor soil structure, biological activity and humus balance and, thus, partially compensate for tillage and fertilization measures (Thüringer Ministerium für Landwirtschaft Naturschutz und Umwelt, 2006, 2008). Cultivars or mutants of rape (Marchiol et al., 2004; Grispen et al., 2006) or sunflower (Kolbas et al., 2011; Herzig et al., 2014) have been successfully tested for

phytoextraction purposes under field conditions and resulting biomass might be safely valorized in the bioenergy sector (Grispen et al., 2006; Herzig et al., 2014). Thereby, inbred lines of sunflower, modified for high biomass and accumulation of TE by chemical mutagenesis, were shown to be particularly effective (Herzig et al., 2014). Hence, we compared a commercial cultivar of rape and a non-genetically modified sunflower for effects on soil acidity and TE availability, and on TE accumulation in subsequent wheat, which, as major staple crop, is typically cultivated following rape in local crop rotations.

Materials and Methods

Soils and plants

Topsoil material (Con), contaminated with metals (Cd, Pb, and Zn) and As, was obtained from an agricultural site in Saxony, Germany (GPS coordinates: 13°23'52"E, 50°54'17"N). Uncontaminated topsoil material (Ref) was collected from another agricultural site (GPS coordinates: 13°16'49"E, 50°57'47"N) with TE concentrations reflecting regional background levels (Kardel et al., 2015). Detailed information on sampling sites and sampling design are provided in the supplementary information. The main soil characteristics are presented in Table 3.1.

Commercial etched seeds of winter oilseed rape (*Brassica napus* L. cv. Lorenz) and winter wheat (*Triticum aestivum* L. cv. Tiger) were obtained from a rural services and trade cooperative for comparability with field experiments. Seeds of sunflower (*Helianthus annuus* L.), improved for biomass production and aboveground Zn, Cd, and Pb accumulation on moderately contaminated soils by chemical mutagenesis (Nehnevajova et al., 2007) (mutant inbred line M7 (R3B-F-U/R13M10A; test series R13F-MP)), were obtained from Phytotech Foundation, reproduced and rinsed with 3 % H₂O₂, repeatedly rinsed with deionized (DI) water and heated (45 °C for 60 minutes). While sowing, all seeds were mycorrhized with 300 l ha⁻¹ "INOQSpezial" from INOQ GmbH (Schneba, Germany) to avoid variations due to spontaneous inoculation under semi-controlled conditions.

Table 3.1 Soil^a characteristics; information in italics are derived from Barth & Forberg (2015). Regarding the soil-to-plant pathway action values and trigger values according to BBodSchV (1999) are specified if bread wheat or accumulating vegetables are cultivated (*) and for possible impairment of plant growth (**).

		Ref	Con	Trigger value	Action value
pH (H ₂ O)	-	6.1	5.6		
C _{org}	g kg ⁻¹	<i>11</i>	<i>21.6</i>		
CEC _{pot}	cmol _c kg ⁻¹	<i>15.8</i>	<i>21.5</i>		
Skeleton	g kg ⁻¹	2.2	2		
Sand	g kg ⁻¹	90	350		
Silt	g kg ⁻¹	770	510		
Clay	g kg ⁻¹	140	140		
Field capacity	g kg ⁻¹	220	220		
As aqua regia	mg kg ⁻¹	41	868	200	
As NH ₄ NO ₃	mg kg ⁻¹	0.02	0.8	0.4**	
Cd aqua regia	mg kg ⁻¹	0.7	14.4		
Cd NH ₄ NO ₃	mg kg ⁻¹	0.01	0.6		0.04*/0.1
Pb aqua regia	mg kg ⁻¹	78	1635		
Pb NH ₄ NO ₃	mg kg ⁻¹	0.01	1.9	0.1	
Zn aqua regia	mg kg ⁻¹	80	437		
Zn NH ₄ NO ₃	mg kg ⁻¹	0.4	2.6	2**	

^aRef uncontaminated reference soil, Con contaminated soil

Experimental set-up

Prior to use in the pot experiment soil material was homogenized, sieved < 4 mm and treated with steam for partial sterilization (Tanaka et al., 2003). Soils were filled into 13-l polyethylene vessels in four replicates. All treatments, including pots without preceding culture, were grouped together and daily watered with DI water to 70 % of field capacity until the end of the experiment. For all cultures, the final number of plants per pot corresponded to plant densities in agricultural practice (see supplementary information). Nine seeds per pot of rape were sown in September 2012. During winter time, pots were arranged outside in a sand bed under natural snow cover. After vernalization, in spring 2013 seedlings were reduced to six per pot and randomly arranged in open top chambers (OTC; Figure 3.3a). In early May 2013, one seed per pot of sunflower was sown. At harvest of preceding cultures substrates were sieved again < 4 mm to remove roots and kept moist at 4 °C until sowing of wheat. In October 2013, 22 seeds per pot of winter wheat were sown and arranged outside as described for rape. After vernalization, seedlings were reduced to 16 per pot. These pots were randomly set-up in greenhouses under semi-controlled conditions (Figure 3.3b); the greenhouse was removed by day, unless during rainfall events. According to agricultural practice, fertilization was conducted with commercial fertilizers pre-sowing for sunflowers and with lateral shoot

development of rape and wheat in amounts corresponding to 90 kg N ha⁻¹ (as CH₄N₂O), 18 kg P ha⁻¹ (P fertilizer with 40 % P₂O₅) and 100 kg K ha⁻¹ (K fertilizer with 60 % K₂O). A second N-fertilization was conducted with (NH₄)₂SO₄ corresponding to 42 kg ha⁻¹ N with beginning longitudinal growth. During harvest, plants were separated into roots, straw, and grains. In addition, six leaves per pot of wheat were sampled at maturity for assessment of nutrient status.

Soil and plant analysis

For analysis of pseudo-total and potentially plant-available element fractions standard procedures of *aqua regia* (DIN ISO 11466:1997) and NH₄NO₃ (DIN ISO 19730:2008) were conducted. Soil pH was analyzed initially and after harvest of each crop in H₂O slurry at 1:5 soil/solution ratio (v/v) (DIN ISO 10390:2005). Pseudo-total TE concentrations were determined for initial soils. Potentially plant-available TE concentrations were determined (i) at the beginning of the experiment, (ii) at harvest of preceding crops, and (iii) at harvest of winter wheat. Samples were washed with DI water, dried at 60 °C to constant weight, finely ground and digested at 180 °C with 3 ml HNO₃ and 2 ml H₂O₂ (DIN EN 13805:2014). Chemical analyses were validated by including blanks and standard reference material BCR 667, rye grass (EU-JRC), for plant analyses, and WEPAL-ISE 979 (Rendzina soil) for *aqua regia* and NH₄NO₃ extraction. The limit of detection (LOD) was calculated as the nine-fold standard deviation of blanks. Analyses of TE were performed by ICP-MS (PQ exCell, Thermo Fisher Scientific Inc, UK).

Total metal(loid) extraction (TME) was calculated according to Equation (1) and bioconcentration factors (BCF) as the ratio between shoot metal(loid) concentration and pseudo-total metal(loid) concentration in soil.

$$\sum_{i=1}^4 [\text{shoot TE concentration}] \times \text{weight} [\text{shoot biomass per pot}] \quad (1)$$

Treatment effects were analyzed for statistical differences ($p < 0.05$) with one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test using PASW statistics 21 (SPSS, Inc., Somers, NY, USA). Data were log-transformed when necessary to meet the requirements of homoscedasticity (Levene's test) and normal distribution of residuals (Shapiro-Wilk test).

Results

Biomass production and trace elements accumulation of pre-crops

Biomass production of sunflower and rape did not differ, except for soil Con, where sunflowers showed inhibited, and partially stunted, growth ($p < 0.05$; Table 3.2). Root accumulation of TE differed with respect to the substrate used. In Con, sunflower accumulated less Cd and Zn ($p < 0.05$), and insignificantly less As and Pb than rape. At low TE levels in Ref, this reversed toward higher levels of As and Pb at, by trend, lower accumulation of Cd and Zn. By contrast, sunflower exhibited translocation factors (TE_{shoot}/TE_{root} ; data not shown) for all TE of a multiple of rape, leading to higher aboveground tissue concentrations ($p < 0.05$; Table 3.2) and BCF ($p < 0.05$; Table 3.5) in both soils. Exceptions were concentrations of Cd (Ref) and As (Con) similar to rape. Consequently, TME (Table 3.6) of rape was lower than that of sunflower in Ref, whereas rape showed higher TME (except for Pb) than sunflower in Con, due to inhibited growth of the latter.

Table 3.2 Shoot (a) and root (b) biomass ($\text{g pot}^{-1}\text{DW}^{-1}$) and trace element accumulation ($\text{mg kg}^{-1}\text{DW}^{-1}$) of winter oilseed rape (R; *Brassica napus* L. cv. Lorenz) and sunflower (S; *Helianthus annuus* L. mutant inbred line M7). Values are means $\pm$ SD ($n = 4$), different letters indicate significant differences between treatments at $p < 0.05$.

soil ^a	crop	biomass	As	Cd	Pb	Zn
(a)						
Ref	R	26.8 $\pm$ 1.1 b	0.1 $\pm$ 0.0 a	0.8 $\pm$ 0.0 a	0.1 $\pm$ 0.0 a	42.0 $\pm$ 3.3 a
	S	22.8 $\pm$ 3.9 b	0.6 $\pm$ 0.2 b	1.1 $\pm$ 0.2 a	< LOD	79.7 $\pm$ 9.2 c
Con	R	27.9 $\pm$ 2.4 b	13.7 $\pm$ 2.4 c	11.3 $\pm$ 0.8 b	2.9 $\pm$ 0.5 b	58.6 $\pm$ 3.2 b
	S	8.7 $\pm$ 3.5 a	20.6 $\pm$ 6.8 c	17.9 $\pm$ 2.1 c	17.4 $\pm$ 2.6 c	141.1 $\pm$ 23.1 d
(b)						
Ref	R	3.5 $\pm$ 0.8 b	2.0 $\pm$ 0.3 a	1.4 $\pm$ 0.2 a	3.1 $\pm$ 0.3 a	38.3 $\pm$ 3.0 a
	S	1.5 $\pm$ 0.8 a	5.6 $\pm$ 0.9 b	1.3 $\pm$ 0.1 a	5.7 $\pm$ 0.6 b	32.2 $\pm$ 4.1 a
Con	R	3.2 $\pm$ 0.4 b	485 $\pm$ 50 c	100 $\pm$ 17 c	348 $\pm$ 56 c	218 $\pm$ 30 c
	S	0.8 $\pm$ 0.5 a	461 $\pm$ 63 c	28 $\pm$ 4 b	335 $\pm$ 56 c	139 $\pm$ 17 b

^aRef uncontaminated reference soil, Con contaminated soil

Pre-crop effects on soil acidity and potentially plant-available concentration of trace elements

In both soils, the pH increased in the rape treatment ($p < 0.05$; Table 3.3), whereas it remained unaffected (Con) or slightly increased (Ref; $p < 0.05$), compared to relatively acid initial conditions, in the sunflower treatment. The subsequent wheat cultures aligned differences by trend, but the pH remained lower (Ref; $p < 0.05$) following sunflower (S+W) compared to rape (R+W).

Table 3.3 Soil^a acidity at harvest of winter oilseed rape (R; *Brassica napus* L. cv. Lorenz) and sunflower (S; *Helianthus annuus* L. mutant inbred line M7) in 2013 and subsequent wheat (*Triticum aestivum* L. cv. Tiger) in 2014 (respectively, R+W and S+W) or wheat cultivated without preceding crop (W) related to initial pH. Values represent means $\pm$ SD ($n = 4$). Different letters indicate significant differences at $p < 0.05$.

	Ref			Con		
Initial	6.06	$\pm$ 0.03	a	5.56	$\pm$ 0.03	a
R	6.42	$\pm$ 0.03	de	6.05	$\pm$ 0.02	c
S	6.24	$\pm$ 0.06	b	5.63	$\pm$ 0.05	a
W	6.36	$\pm$ 0.02	cd	5.89	$\pm$ 0.08	b
R+W	6.45	$\pm$ 0.02	e	6.04	$\pm$ 0.05	c
S+W	6.31	$\pm$ 0.02	bc	5.99	$\pm$ 0.03	bc

^aRef uncontaminated reference soil, Con contaminated soil

Potentially plant-available concentrations of TE increased in both Con and Ref following cultivation of sunflower (Figure 3.1). Analyses of Pearson correlation coefficients showed dependences between NH_4NO_3 -soluble TE in soil at harvest of preceding cultures and pH and TME to a different extent in Ref and Con.

Table 3.4 Pearson correlation coefficients between NH_4NO_3 -soluble trace elements in soil^a and soil acidity and total metal(loid) extraction (TME including roots) after harvest of preceding crops.

	As		Cd		Pb		Zn	
	Ref	Con	Ref	Con	Ref	Con	Ref	Con
pH (H ₂ O)	-0.62 $p < 0.01$	-0.44 $p = 0.26$	-0.52 $p = 0.02$	-0.41 $p = 0.04$	-0.64 $p < 0.01$	-0.38 $p = 0.06$	-0.72 $p < 0.01$	-0.69 $p < 0.01$
TME	0.7 $p = 0.06$	-0.61 $p = 0.11$	-0.14 $p = 0.75$	-0.93 $p < 0.01$	-0.39 $p = 0.34$	-0.83 $p = 0.01$	0.79 $p = 0.02$	-0.78 $p = 0.02$

^aRef uncontaminated reference soil, Con contaminated soil

Data is highlighted with bold font, if significant ($p < 0.05$)

More precise, labile TE in Ref were predominantly negatively correlated to soil pH. This effected a positive correlation between labile Zn and Zn removal, which was highest for

sunflower at simultaneous increase of labile Zn in Ref. Vice versa, in Con, labile Cd and Zn were negatively correlated to soil pH as well, but also mutually negative correlations with TME were found for metals (Table 3.4). This resulted from enhanced labile Cd, Pb, and Zn (Figure 3.1) at simultaneous growth impairment of sunflowers (Table 3.2) leading to lower TME, and vice versa for rape.

After harvest of subsequent wheat, in Con, metals mobility increased in S+W treatments ($p < 0.05$) compared to W and R+W, whereas increases were significant compared to W only in Ref (Figure 3.1). Differences between R+W and W were insignificant, except for a decrease of labile As and an increase of labile Pb in Con, and an increase of labile Cd in both soils (all $p < 0.05$).

Figure 3.1 NH_4NO_3 -soluble trace element concentration in soils *Ref* (uncontaminated reference soil) and *Con* (contaminated soil) at harvest date of winter oilseed rape (R) and sunflower (S) in 2013, and of wheat in 2014 without (W) or with succession to preceding crops (respectively, R+W and S+W). Values are means $\pm$ SD ($n = 4$); different letters indicate significant differences at $p < 0.05$.

Yield response of *Triticum aestivum* L. to pre-crops

The pre-crops did not affect biomass production of wheat (Table 3.7). However, between the soils, a trend toward lower grain yields in Con was observed. This was accompanied by reduced thousand grain weight, which was most pronounced ($p = 0.05$) in R+W treatments (Ref: 45.2 ± 1.1 g, Con: 41.2 ± 2.9 g).

Pre-crop effects on trace elements phytoavailability to *Triticum aestivum* L.

Differences in TE (Figure 3.2) uptake of wheat plants were found between W and +W treatments, with similar effects in Ref and Con. Thereby, As concentration in grains increased in S+W treatments, whereas As uptake by straw decreased compared to W. The increase in grains was significant for S+W toward W and R+W in Con ($p < 0.01$), and in Ref compared to W only ($p < 0.05$). The decrease in straw was largest for R+W in Ref ($p < 0.01$) and Con ($p < 0.05$), without differences between R+W and S+W. Regarding Cd uptake, vegetative and generative tissue responded similarly to the treatments. In Ref ($p < 0.05$) and Con ($p < 0.01$), grain Cd status decreased in R+W compared to W, whereas the decrease was insignificant if related to S+W. Yet, permissible levels for Cd in animal feed of the European Commission were passed, also by grains, in Con (Figure 3.2; EC 32/2002). Lead accumulation was low in aboveground biomass except for straw in Con, where R+W accumulated less than S+W ($p < 0.05$). The uptake of Zn was less in R+W compared to S+W and W but differed significantly only in case of Con for both grain ($p < 0.01$) and straw ($p < 0.05$).

Concentrations of Fe and P (Ref: $p < 0.05$) were enhanced, whereas Ca (Ref: $p < 0.05$) and Mn ($p < 0.05$), or Mg (S+W: $p < 0.05$) were reduced in mature leaves of wheat from +W treatments in both soils (Table 3.8).

Figure 3.2 Trace element concentrations in straw and grains of *Triticum aestivum* L. cv. Tiger without preceding crop (W) and after cultivation of winter oilseed rape (R+W) or sunflower (S+W) in soils *Ref* (uncontaminated reference soil) and *Con* (contaminated soil). Values are means $\pm$ SD ($n = 4$); different letters indicate significant differences at $p < 0.01$ (ab) and at $p < 0.05$ (cd). Dashed lines represent European thresholds for contaminants in animal feed (EC, 2002).

Discussion

General potential of the selected pre-crops for phytoextraction

For a given high biomass crop's suitability for phytoextraction, at least moderate BCF are required (Puschenreiter et al., 2013; Kidd et al., 2015). In this study, the tested rape cultivar had $BCF > 1$ only for Cd in Ref, which dropped with increasing total soil Cd in Con to < 1 (Table 3.5), as found elsewhere (Puschenreiter et al., 2013). Sunflower showed $BCF > 1$, at higher levels than rape, in both soils for Cd, but for Zn in Ref only. Thus, the phytoextraction potential, particularly of sunflower, for Cd was moderate, slightly lower for Zn and low for As and Pb, as found before (Puschenreiter et al., 2013). However, in the highly multielement-contaminated soil Con sunflowers showed partially stunted growth, which indicates toxicity

and limits TME. Initial soil acidity (Sutradhar et al., 2014) and labile Zn (Herzig et al., 2014) of Con (Table 3.1) were within the tolerable range for sunflower, but high concentrations of Cd can cause stunting, particularly if exposed to contaminated substrate in early growth stages (Nehnevajova et al., 2012). Stunting due to As toxicity was found to be alleviated by inoculation with arbuscular mycorrhizae (Ultra et al., 2007) but at levels of exposure to As far below those presented by Con. By contrast, oilseed rape is known to tolerate high loads of various metals (Marchiol et al., 2004) and As (Cox et al., 1996).

Pre-crop effects on soil acidity and trace elements phytoavailability

Root exudation or other plant-driven processes can result in acidification (Montiel-Rozas et al., 2016) or alkalinization (Pearse et al., 2006) of the rhizosphere, which is not necessarily accompanied by a simultaneous effect on pH in the bulk soil (Chiang et al., 2006; Hoefer et al., 2015). The species and cultivar dependent ability of plants to excrete root exudates, like low molecular weight organic acids (LMWOAs), is a well-known process for (i) nutrient and metal acquisition and (ii) response to stress induced by excess TE exposure (Kidd et al., 2009; Montiel-Rozas et al., 2016). In this study, soil pH remained at relatively low levels following cultivation of sunflower when compared to rape, where it increased. The ability of *B. napus* to increase rhizosphere pH has been found before (Pearse et al., 2006). The potential of sunflowers to excrete LMWOAs or decrease rhizosphere pH as a response to Cd supply (Chiang et al., 2006) or Fe-deficiency (Alcantara et al., 1990) has been studied for different inbred lines and found to be under genetic control. By contrast, a long-term increase of soil pH was shown during a phytoextraction field study with selected sunflower mutant lines (Herzig et al., 2014). Effects of crop rotations on soil acidity are known to affect TE phytoavailability (Basta et al., 2005) to wheat (Oliver et al., 1993). Thereby, lowered TE uptake by wheat in R+W treatments of Con might result from TME and increase of pH, whereas the latter must have dominated in Ref because rape extracted less TE than sunflower. By contrast, the remaining increase of labile TE in the soil after harvest of wheat in S+W treatments compared to R+W might, in addition to remaining differences in soil acidity, suggest a certain stability of root exudates over the period of a crop rotation, as found before (Quartacci et al., 2009; Kidd et al., 2015). This was supported by the shoot ionome of S+W, further indicating that persistent changes in the rhizosphere outweighed effects of TME on TE available to subsequent wheat plants. Similarly, higher tissue concentrations of Cd and Zn have been found in a barley culture following repeated croppings of a *Salix smithiana* clone than after successive barley croppings (Puschenreiter et al., 2013). Elevated As concentration

in grains of S+W may result from rhizosphere acidification and related co-dissolution of phytoavailable As from Fe-(hydr)oxides (Fitz and Wenzel, 2002). Apart from Fe status, enhanced P concentrations in mature leaves of +W treatments (Table 3.8) suggest an enrichment of fertilizer-born P during the crop rotations compared to W. Phosphorus fertilization has been found to enhance As accumulation in wheat grains, indicating competition for binding sites in soil (Brackhage et al., 2015). Thus, in case of S+W both effects might have cumulated to increased grain As levels (Ref: $r = 0.73$ at $p = 0.01$ for the correlation between leaf P and grain As). Despite a reduction of Ca and Mn concentration in +W treatments, effected by fertilization restricted to NPK (Table 3.8), nutrition remained within the span observed for vegetative biomass elsewhere (Weih et al., 2016) and no inverse correlations, e.g. between Ca or Mn and leaf Cd status or its translocation within the plant (data not shown), were found that pointed to interferences. In contrast to the shoot ionome of R+W treatments elevated NH_4NO_3 -soluble concentrations of Cd or Pb in +W treatments might, besides rhizosphere processes discussed above, derive from the decomposition of TE-rich root residues, which were not captured by sieving through the 4 mm mesh. Lower NH_4NO_3 -soluble concentrations of As in R+W treatments may in turn be due to low capacity of both species for carboxylate exudation (Pearse et al., 2006), which is assumed to promote As mobilization and phytoavailability (Fitz and Wenzel, 2002).

Conclusion

In this study, rape tolerated high phytoavailable loads of various TE more than sunflower and indicated a potential for soil alkalinization. By contrast, higher TE bioconcentration of sunflower was accompanied by the maintenance of relatively acid conditions, especially in the contaminated soil. This effected enhanced NH_4NO_3 -soluble concentrations of Cd, Pb, and Zn, persisting until harvest of the subsequent wheat culture. Obviously, rhizosphere effects of sunflower outweighed phytoextraction effects within the studied crop rotation. In both soils, pre-cropping of rape reduced Cd and Zn uptake in subsequent wheat shoots, whereas sunflower pre-cropping increased As accumulation in wheat grains compared to W. Exposed to high-level mixed contamination, sunflower pre-cropping further induced enhanced Pb and Zn accumulation in subsequent wheat straw. We conclude that moderate phytoextraction effects may, in the short term, be superimposed by nutrient use or rhizosphere effects associated with different pre-crops, which should be carefully monitored when designing crop rotations for contaminated soils. At the high level of soil contamination tested, and at

greenhouse scale, crop rotation effects were too small to enable biomass valorization, also of wheat grains, for other than energetic purposes.

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Supplementary Information

Sampling sites and soil samples collection

The agricultural site where the contaminated soil (Con) was sampled is located within the Ore Mountain Mining Region in Freiberg, Saxony. Area-wide contamination of topsoils is of anthropogenic origin, resulting from atmospheric deposition from chimney stacks during decades of metallurgic industry. Soil material was obtained from the control treatment (no mineral fertilization) of a phytoremediation field trial on in situ stabilization combined with phytoexclusion (Kidd et al., 2015). The agricultural site where the uncontaminated reference soil (Ref) was collected, represents background concentrations of TE in the region (Kardel et al., 2015). Both soils are classified as stagnosols, derived from loess and paragneiss and both sampling locations are characterized by an altitude of ~ 400 m, > 630 mm average annual precipitation, and ~ 8 °C average annual temperature (Barth and Forberg, 2015). Topsoil material (0 - 20 cm) was sampled according to a randomized design (Huesemann, 1994; Kumpiene et al., 2014) from an area of ~ 0.008 km² with an unpainted spade. Due to spatial heterogeneity, each soil material was thoroughly homogenized by multiple, systematic mixing prior to use in the pot experiment. The pots were filled with each 7,300 g (Ref) or 7,250 g (Con) of dry-weight soil.

Decision basis for the number of seedlings per pot

In order to avoid heat stress in greenhouses 13 l white polyethylene vessels with a surface area of 0.045 m² were used for the cultivation of all plants. A plant density of 350 seeds per m², as recommended for wheat (Thüringer Ministerium für Landwirtschaft Naturschutz und Umwelt, 2015), corresponds to 16 individuals per pot. Based on preliminary studies, the 13 l

pots assure adequate growth conditions for one sunflower. In regional agricultural practice, the recommended plant density of winter oilseed rape is six times that of sunflower on an area basis ($\sim 75,000$ individuals of sunflowers (Thüringer Ministerium für Landwirtschaft Naturschutz und Umwelt, 2006) and $\geq 450,000$ individuals of winter oilseed rape (Thüringer Ministerium für Landwirtschaft Naturschutz und Umwelt, 2013) (line breed) per hectare). The culture, especially line breeds like the selected cultivar "Lorenz", tolerates high variations in row and plant spacing (Thüringer Ministerium für Landwirtschaft Naturschutz und Umwelt, 2013; Kuai et al., 2016). Due to limited surface area of the chosen pots a relatively narrow plant spacing of ≥ 10 cm was realized. However, no stress symptoms like reduction of maturity rate or lodging were observed.

Water supply

The field capacity of both soils was initially assessed by free percolation: an excess of water was slowly applied to dry weight soil, via storage in a sampling cylinder on filter paper in a water bath. When moisture appeared on the sealed top of the soil surface, water was allowed to release. The soils were assumed to be at field capacity when no further droplets formed at the bottom of the filter after free percolation, and were re-weighed. During the pot experiment, water supply was adjusted to 70 % of field capacity, whereby water loss (= reduction of pot weight) due to evapotranspiration was compensated on a daily basis in the morning hours. In events of high temperatures and solar radiation during the summer months, losses were compensated twice per day.

Table 3.5 Bioconcentration factors ($\text{conc}_{\text{shoot}} / \text{conc}_{\text{soil (aqua regia)}}$) for trace elements of winter oilseed rape (R; *Brassica napus* L. cv. Lorenz) and sunflower (S; *Helianthus annuus* L. mutant inbred line M7) grown in uncontaminated soil (Ref) or contaminated soil (Con). Values are means $\pm$ SD ($n = 4$). Different letters indicate significant differences at $p < 0.05$.

		As				Cd				Pb				Zn			
Ref	R	0.002	±	0.001	a	1.11	±	0.12	b	0.002	±	0.001	a	0.53	±	0.05	c
	S	0.015	±	0.004	b	1.44	±	0.19	c	< LOD				1.00	±	0.11	d
Con	R	0.016	±	0.003	b	0.78	±	0.05	a	0.002	±	0.000	a	0.13	±	0.01	a
	S	0.024	±	0.008	b	1.24	±	0.16	bc	0.011	±	0.002	b	0.32	±	0.06	b

Table 3.6 Total metal(loid) extraction (mg pot⁻¹) of winter oilseed rape (R; *Brassica napus* L. cv. Lorenz) and sunflower (S; *Helianthus annuus* L. mutant inbred line M7) grown in uncontaminated soil (Ref) or contaminated soil (Con). Values are means $\pm$ SD ($n = 4$). Different letters indicate significant differences at $p < 0.05$.

Soil	Crop	As		Cd		Pb		Zn	
Ref	R	0.003 $\pm$ 0.001	a	0.022 $\pm$ 0.001	a	0.003 $\pm$ 0.001	a	1.14 $\pm$ 0.11	a
	S	0.013 $\pm$ 0.005	b	0.023 $\pm$ 0.003	a	< LOD		1.8 $\pm$ 0.3	b
Con	R	0.36 $\pm$ 0.1	c	0.31 $\pm$ 0.04	c	0.08 $\pm$ 0.02	b	1.66 $\pm$ 0.15	ab
	S	0.22 $\pm$ 0.08	b	0.16 $\pm$ 0.05	b	0.15 $\pm$ 0.05	b	1.24 $\pm$ 0.44	a

Table 3.7 Biomass (g DW⁻¹ pot⁻¹) of wheat plants (*Triticum aestivum* L. cv. Tiger) grown without preceding crop (W) and after cultivation of winter oilseed rape (R+W) or sunflower (S+W) in uncontaminated soil (Ref) or contaminated soil (Con). Values are means $\pm$ SD ($n = 4$). Differences between treatments and soils were insignificant ($p > 0.05$).

	Ref				Con			
	grain		straw		grain		straw	
W	19.70 $\pm$ 2.36		14.33 $\pm$ 0.62		17.45 $\pm$ 2.92		13.22 $\pm$ 1.87	
R+W	20.10 $\pm$ 1.50		15.53 $\pm$ 1.54		17.58 $\pm$ 1.18		13.66 $\pm$ 0.63	
S+W	19.07 $\pm$ 2.56		15.14 $\pm$ 1.74		17.60 $\pm$ 1.94		16.07 $\pm$ 3.09	

Table 3.8 Concentrations of macronutrients (mg g⁻¹ DW⁻¹) in mature leaves of wheat (*Triticum aestivum* L. cv. Tiger) grown without preceding crop (W) and after cultivation of winter oilseed rape (R+W) or sunflower (S+W) in uncontaminated soil (Ref) or contaminated soil (Con). Values are means $\pm$ SD ($n = 4$). Different letters indicate differences at $p < 0.05$.

		Al		Ca		Fe		Mg		Mn		P	
Ref	W	0.01 $\pm$ 0.01	a	4.8 $\pm$ 0.3	b	0.1 $\pm$ 0.0		1.7 $\pm$ 0.1	b	0.5 $\pm$ 0.0	b	3.5 $\pm$ 0.2	a
	R+W	0.05 $\pm$ 0.02	b	3.5 $\pm$ 0.6	a	0.2 $\pm$ 0.1		1.5 $\pm$ 0.3	ab	0.1 $\pm$ 0.1	a	4.4 $\pm$ 0.3	b
	S+W	0.03 $\pm$ 0.02	ab	2.7 $\pm$ 0.8	a	0.3 $\pm$ 0.1		1.0 $\pm$ 0.2	a	0.1 $\pm$ 0.1	a	5.5 $\pm$ 1.1	b
Con	W	0.03 $\pm$ 0.01		4.3 $\pm$ 0.3		0.05 $\pm$ 0.03		1.5 $\pm$ 0.2	b	0.4 $\pm$ 0.1	b	3.5 $\pm$ 0.4	
	R+W	0.04 $\pm$ 0.02		3.7 $\pm$ 0.5		0.08 $\pm$ 0.02		1.3 $\pm$ 0.2	ab	0.1 $\pm$ 0.0	a	4.3 $\pm$ 0.5	
	S+W	0.05 $\pm$ 0.01		3.7 $\pm$ 0.4		0.06 $\pm$ 0.01		1.1 $\pm$ 0.1	a	0.1 $\pm$ 0.0	a	4.2 $\pm$ 0.7

Figure 3.3 Rapeseed (back row) and sunflower (front row), arranged randomly in open top chambers (OTC) with rapeseed pods protected by perforated bags against uncontrolled loss of seeds (a) and winter wheat, arranged randomly in greenhouses (b); for wheat, white polyethylene vessels were chosen in order to avoid overheating in the small greenhouses.

4 The potentials of drinking water treatment residues and/or perennial plants

4.1 Trace elements bioavailability to *Triticum aestivum* and *Dendrobaena veneta* in a multielement-contaminated agricultural soil amended with drinking water treatment residues

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Abstract

Purpose: The *in situ* stabilization of multielement-contaminated agricultural soils has limited effectiveness when using common single amendments. This study examined the use of drinking water treatment residues (WTR), based on (hydr)oxides of Fe, Al, or Mn, as a cost-effective solution to optimize the immobilization of metals (Cd, Pb, Zn) and As.

Materials and methods: Trace elements (TE) bioavailability was assessed under semi-controlled conditions in a pot study cultivating winter wheat (*Triticum aestivum* L. cv. Tiger) until maturity. An Fe-based WTR and a Mn-based WTR, applied at rates of 0.5 % and 1 % (m/m), were related to effects of lime marl (LM) application. Additionally, a bioassay with earthworms (*Dendrobaena veneta*) was conducted. Both bioassays were compared with measurements of NH₄NO₃-soluble, diffusive gradients in thin films (DGT)-available and soil solution TE concentrations, representing well-established surrogates for mimicking the bioavailable element fractions in soil.

Results and discussion: The application of the Fe-based WTR reduced As accumulation in vegetative wheat tissues (by up to 75 %) and earthworms (by up to 41 %), which corresponded with the findings from soil chemical analyses and improved plant growth and earthworm body weight. However, As concentrations in cereal grains were not affected, Cd or Pb accumulation by wheat were not mitigated and Zn uptake was enhanced. By contrast, the Mn-based WTR effected the greatest reduction in Pb uptake, and lowered Cd transfer to wheat grain (by up to 25 %). Neither the NH₄NO₃-soluble nor DGT-available concentrations matched with Cd and Zn accumulation in plants or earthworms, indicating interferences due to competition for binding sites according to the biotic ligand model.

Conclusions: The results obtained in this study suggest that a bioassay with key species prior to field application should be mandatory when designing *in situ* stabilization options. The application of WTR to an agricultural soil strongly affected TE bioavailability to plants and earthworms. Low application rates tended to improve biomass production of biota. Higher application rates involved risks (e.g. P fixation, TE inputs) and none of the amendments tested could immobilize all targeted elements.

Keywords: Bioassay • Fe-oxide • In situ stabilization • Lime marl • Mn-oxide

Introduction

Centuries of ore mining and processing have led to extensive contamination of topsoils with trace elements (TE) at many sites worldwide. The long history of mining in the Saxon Ore Mountains (Erzgebirge, Germany) has led to large-scale contamination with TE including As, Cd, Pb, and Zn, endangering current agricultural food and animal feed production (SMI, 2013; BfUL, 2015). Thus, efforts are needed to manage this pollutant linkage. Since conventional remediation techniques are not viable for treating trace element contaminated soils (TECS) over a large area, gentle remediation options (GRO) offer alternative solutions for their sustainable management (Onwubuya et al., 2009; Cundy et al., 2015). The decontamination of multielement-contaminated soils by phytoextraction turned out to be infeasible in a reasonable time frame, even if supported by mobilizing agents (Nowack et al., 2006; Van Nevel et al., 2007). Thus, recent approaches concentrated on reducing the labile pool of TE. Thereby, *in situ* stabilization by use of soil amendments may enable simultaneous soil remediation and maintenance of agricultural production (Kumpiene, 2010; Bolan et al., 2014).

The remediation of TE with partially antagonistic behavior (e.g. Cd, Pb, Zn, and As) requires the use of appropriate multifunctional stabilizing agents. Oxides and hydroxides of Fe, Mn, or Al are known to immobilize a wide range of metal(oids) in soils via the provision of sorption sites for TE, coprecipitation or formation of secondary minerals or inner-sphere complexes with TE (Kumpiene, 2010; Tiberg et al., 2016). These oxides and hydroxides are enriched to high concentrations in drinking water treatment residues (WTR) during the production of potable water. Land application of WTR for remediation purposes is therefore seen as a potentially important alternative to disposal of this byproduct since it is available for free or at low costs. However, in addition to macronutrients, WTR can also contain considerable amounts of TE, depending on the origin and geochemical background of the pre-treatment water (Ippolito et al., 2011). Nonetheless, this approach has shown significant potential, as demonstrated by Nielsen et al. (2011), who showed that a single application of WTR (consisting mainly of ferrihydrite) stabilized As in pore water for a period of three years under field conditions. By contrast, liming agents have to be applied periodically in order to maintain soil pH at a level enabling the effective immobilization of metal cations (Bolan et al., 2014).

Wheat is one of the most widely cultivated crops worldwide and has the potential to take up considerable amounts of TE (Jamali et al., 2009; Kidd et al., 2015). During uptake, the presence of macronutrients or other TE in the soil, due to either natural abundance or through

additives (e.g. from WTR application), may affect the accumulation by roots as well as translocation of TE within the plant (Foy et al., 1978; Siedlecka, 1995; Pigna et al., 2010). In addition, the abundance and diversity of soil invertebrates may be diminished in TECS. Uptake of TE by earthworms is mainly controlled by passive uptake kinetics from soil pore water, with some influence of soil acidity (Spurgeon, 2010). However, at mixed contamination, pronounced interactions between elements have been observed (e.g., Cd and Zn; Qiu et al. 2011).

Therefore, assessment tools of long-term TE bioavailability to key species in WTR-treated soils are of crucial importance. Basta et al. (2005) showed that conventional single or sequential extraction methods are unable to mimic bioavailability in soil-residual systems. Several studies have found that DGT is a better predictor of phytoavailability, in cases where plant uptake was limited by diffusive transport to roots and not saturated (Degryse et al., 2009; Muhammad et al., 2012; Puschenreiter et al., 2013). The DGT technique accounts for the depletion of TE at the interface of soil and roots or organisms, and the depletion-induced resupply of TE from labile pools of the solid phase to the soil solution (Zhang et al., 2001). This technique has also been found to mimic TE uptake by earthworms and human bioaccessibility (Bade et al., 2012), which in turn could be reduced by the use of WTR regarding As (Sarkar et al., 2007). Yet, DGT was not applied to soils treated with WTR, where an influx of various elements is likely to affect plant uptake of TE without simultaneous effects on DGT fluxes (Degryse et al., 2009). The objectives of this study are therefore (i) to test the potential of WTR based on (hydr)oxides of Fe, Al, or Mn for immobilizing Cd, Pb, Zn, and As in an agricultural soil with bioassays (*Triticum aestivum* L. cv. Tiger and *Dendrobaena veneta*); and (ii) to test the suitability of a conventional (NH_4NO_3) chemical soil extraction and DGT to assess TE bioavailability in WTR-treated soils.

Materials and Methods

Soils and biota

Topsoil material (0-20 cm) was taken from an agricultural site contaminated with TE (Cd, Pb, Zn, and As). The soil (Con) was obtained from the control treatment of a phytoremediation field trial in Freiberg, Saxony (Kidd et al. 2015; GPS coordinates: 13° 23' 52" E, 50° 54' 17" N). Uncontaminated topsoil material (Ref) was obtained from an agricultural site nearby (GPS coordinates: 13° 16' 49" E, 50° 57' 47" N), where TE concentrations were within the range of background levels for the region (Kardel et al., 2015). This soil was used as a reference

regarding element concentration and biomass production of biota. Soils Ref and Con are classified as Stagnosols, derived from loess and paragneiss, and were sampled at locations with an altitude of 420 m, 630 mm average annual precipitation, and 8 °C average annual temperature (Barth and Forberg, 2015).

Chemically sterilized commercial seeds of *Triticum aestivum* L. cv. Tiger were inoculated with vesicular-arbuscular mycorrhizae (VAM; *Rhizophagus irregularis*) using 300 l ha⁻¹ INOQSpezial (INOQ GmbH, Schnega, Germany) in order to minimize variability under semi-controlled conditions. Compost earthworms (*Dendrobaena veneta*) were chosen as the soil invertebrates for assessment.

Two different WTR were compared; amendment WTR_A is a carbonate-enriched sludge from Wittkoppenberg waterworks in Germany, where Fe-rich groundwater was treated (Müller, 2000; Marschner et al., 2008), and amendment WTR_B is a Mn-rich sludge from Oborniki, Poland (Siebielec et al., 2013). The LM amendment was tested in the phytoremediation field trial parallel to this pot study. General characteristics of all amendments are presented in Table 4.1.

Experimental set-up

Prior to use in the pot experiment, the soil material was homogenized, sieved to < 4 mm, and treated with steam. The WTR amendments were freeze-dried and applied to the soil at rates of 0.5 % and 1 % by dry weight. The WTR_A treatments will hereafter be referred to as A-0.5 (0.5 % application) and A-1 (1 % application) and analogous for WTR_B (B-0.5 and B-1). Lime marl was added at a rate of 0.4 kg m⁻², corresponding to the field trial. All amendments were sieved to < 63 µm and thoroughly homogenized with the soil material. The substrates were watered with de-ionized (DI) water to 70 % of field capacity for seven days and placed in 13 l white polyethylene vessels in four replicates. An initial number of 22 seeds per pot were sown in October 2013. During wintertime, pots were arranged outside in a sand bed for vernalization. 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 DI water. When the flag leaf was fully developed, six leaves per pot were sampled across subjacent leaf levels in order 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 for chemical analyses of soil other than DGT.

The earthworm experiment followed the method used by (Siebielec, 2010), and was conducted in three replicates per treatment. After incubation at 20 ± 2 °C at daytime with moisture equal to field water capacity, 0.5 kg soil portions were transferred to 1 l glass jars. The initial weight of earthworms was recorded prior to putting five individuals into each jar. The jars were stored at 15 °C and soil moisture was maintained at field water capacity. Earthworms were removed from the soil after four weeks, and mortality and weight were recorded.

Chemical analyses

Detailed information on the chemical analyses described in the following sections is provided in the supplementary information. All chemicals were of analytical grade and extractions were made up with DI water ($18 \text{ M}\Omega \text{ cm}^{-1}$, E-pure system). The validity of chemical analyses was confirmed by including blanks and standard reference material BCR 281, rye grass (EU-JRC) for plant analyses, and WEPAL-ISE 979 (Rendzina soil) for *aqua regia* and NH_4NO_3 extraction. Microwave assisted acid digestions were conducted using the system MARS5 (CEM Corp., Matthews, United States) unless otherwise stated. Measurements of TE in extraction solutions were performed by ICP-MS (PQ exCell, Thermo Fisher Scientific Inc, UK). Nutrients and effective cation exchange capacity (CEC_{eff}) were measured by ICP-OES (IRIS Intrepid II XSP, Thermo Fischer Scientific).

Amendment and soil analysis

Element concentrations of the amendments were determined following digestion with *aqua regia*. For characterization of Con and Ref, pseudo-total and potentially plant available element fractions were analyzed using standard procedures of *aqua regia* (DIN ISO 11466:1997) and NH_4NO_3 (DIN ISO 19730:2008). Effective cation exchange capacity (CEC_{eff}) was assessed according to section 3.2.1.8 of BMEL (2014). Soil pH was analyzed initially and after harvest as described in DIN ISO 10390:2005. Remaining fresh soil samples were analyzed with DGT according to Zhang et al. (2001) in separate steps for metals (chelex-gel) and As (Fe-oxide-gel). To assess the concentration of TE in soil solution (C_{soln}), the remaining water saturated soils were centrifuged according to Muhammad et al. (2012)

and the filtered supernatant was analyzed by ICP-MS. The interfacial TE concentration (C_{DGT}) was calculated according to Zhang et al. (2001), using a diffusion coefficient of $5.69 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for As according to Fitz et al. (2003). The dimensionless indicator for the extent of TE resupply from labile pools of the solid phase to the soil solution R (Harper et al., 2000; Zhang et al., 2001) was calculated as the ratio between C_{DGT} and C_{soln} .

Plant and earthworm analysis

Plant material was washed with DI water and subsequently dried at 60 °C to constant weight. Samples were finely ground and digested using HNO_3 and H_2O_2 (DIN EN 13805:2014). Earthworms were cleaned and kept on moist filter papers in glass vessels for three days for full depuration. The filter papers were replaced once per day to prevent secondary contamination of earthworms by their excretions. The earthworms were washed with DI water, dried on paper towels, and subsequently lyophilized. They were digested using HNO_3 and H_2O_2 .

Statistical analyses

The pot experiment was conducted in a completely randomized design. 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).

Results

Soil characteristics and changes in trace elements mobility

The main soil characteristics are shown in Table 4.1. Soil pH values did not change significantly after seven days of incubation compared to the initial level of pH 5.6 (moderately acid), except for a decrease in treatment B-1. By contrast, CEC_{eff} increased in A-1 and B-0.5 (Figure 4.1). Until harvest, pH increased in treatments LM, A-0.5, and A-1 (each $p < 0.01$) to weakly acid conditions (Figure 4.1), whereas WTR_B did not affect pH (B-0.5) or slightly enhanced it (B-1; $p < 0.01$).

Table 4.1 General soil and amendment characteristics.

		soil				amendment		
		Ref	Con			LM	WTR _A	WTR _B
pH (H ₂ O)	-	6.1	5.6	pH (H ₂ O)	-	9.1	7.9	7.9
<i>C_{org}</i>	<i>g kg⁻¹</i>	<i>11</i>	<i>21.6</i>	C	<i>g kg⁻¹</i>	121	20	9
<i>CEC_{pot}</i>	<i>cmol_c kg⁻¹</i>	<i>15.8</i>	<i>21.5</i>	<i>C_{org}</i>	<i>g kg⁻¹</i>	NA	11	3
Skeleton	<i>g kg⁻¹</i>	2.2	2	Ca	<i>g kg⁻¹</i>	205	15	16
<i>Sand</i>	<i>g kg⁻¹</i>	<i>90</i>	<i>350</i>	Fe	<i>g kg⁻¹</i>	4.7	354	18
<i>Silt</i>	<i>g kg⁻¹</i>	<i>770</i>	<i>510</i>	Al	<i>g kg⁻¹</i>	5	22	6
<i>Clay</i>	<i>g kg⁻¹</i>	<i>140</i>	<i>140</i>	Mn	<i>g kg⁻¹</i>	0.4	6	72
Field capacity	<i>g kg⁻¹</i>	220	220	P	<i>g kg⁻¹</i>	< DL ^a	3.3	1.6
As aqua regia	<i>mg kg⁻¹</i>	41	868.4	As	<i>mg kg⁻¹</i>	8.9	57.2	13
As NH ₄ NO ₃	<i>mg kg⁻¹</i>	0.02	0.8	Cd	<i>mg kg⁻¹</i>	0.5	1	4
Cd aqua regia	<i>mg kg⁻¹</i>	0.7	14.4	Pb	<i>mg kg⁻¹</i>	17.1	28	3.6
Cd NH ₄ NO ₃	<i>mg kg⁻¹</i>	0.01	0.6	Cr	<i>mg kg⁻¹</i>	11.6	3.5	235.8
Pb aqua regia	<i>mg kg⁻¹</i>	77.7	1635.4	Ni	<i>mg kg⁻¹</i>	73.5	43	435
Pb NH ₄ NO ₃	<i>mg kg⁻¹</i>	0.01	1.9	Zn	<i>mg kg⁻¹</i>	5.4	80	677.3
Zn aqua regia	<i>mg kg⁻¹</i>	80.0	437.3	Tl	<i>mg kg⁻¹</i>	0.2	0.02	1.8
Zn NH ₄ NO ₃	<i>mg kg⁻¹</i>	0.4	2.6	Cu	<i>mg kg⁻¹</i>	NA	5.5	49.4
AV Cd (mg kg ⁻¹ NH ₄ NO ₃)		0.04 ^b / 0.1						
TV Pb (mg kg ⁻¹ NH ₄ NO ₃)		0.1						
TV As (mg kg ⁻¹ aqua regia)		200						
TV As (mg kg ⁻¹ NH ₄ NO ₃)		0.4 ^c						
TV Zn (mg kg ⁻¹ NH ₄ NO ₃)		2 ^c						

Information in italics are derived from Barth and Forberg (2015)

Ref uncontaminated soil, *Con* untreated contaminated soil, *LM* lime marl, *WTR_A* Fe-based drinking water treatment residue, *WTR_B* Mn-based drinking water treatment residue

^a0.25 g kg⁻¹

^bAction value (AV) according to BBodSchV (1999) if bread wheat or accumulating vegetables are cultivated

^cTrigger values (TV) according to BBodSchV (1999) for expected impairment of plant growth

Figure 4.1 Initial pH (pH_i) and CEC_{eff} (CEC_i cmol_c kg⁻¹) and pH at harvest (pH_h) in untreated (Con) and treated soils (LM lime marl, A-x and B-x Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in % (m/m)). Values are means ± SD (n = 4).

Soil Con was characterized by high labile concentrations of As, Cd, Pb, and Zn, which exceed German trigger or action values for agricultural soils (BBodSchV 1999; Table 4.1). At the end of the pot experiment, all utilized methods (NH_4NO_3 , C_{DGT} , and C_{soln}) indicated a reduction of TE mobility by both WTR beyond the effect of pH-modification (Figure 4.2, Table 4.4). The pH values increased at a similar rate in LM and A-1, but (with the exception of Zn) TE mobility decreased the most in A-1 (As: by $\leq 85\%$, Cd: by $\leq 80\%$, Pb: by $\leq 62\%$). Arsenic mobility still decreased by $\sim 60\%$ in treatment A-0.5, where Cd and Pb were also immobilized effectively. In treatment B-0.5 TE mobility showed the lowest decrease, accompanied by a negligible change of pH compared to Con. Both WTR similarly reduced labile Zn, with higher doses being more effective. However, no treatment reduced mobile TE to the level of Ref. Along with a high total concentration of Ni in WTR_B , an increase of NH_4NO_3 -soluble Ni within the tolerable range (BBodSchV, 1999) was observed in both treatments (Table 4.8).

The order and significance of treatment effects on the reduction of TE mobility varied with respect to the analytical method used (Figure 4.2, Table 4.4). The potential resupply of depleted TE from the solid phase to the soil solution as assessed by the indicator R ($C_{\text{DGT}}/C_{\text{soln}}$) tended to increase in treated soils when compared to Con (Table 4.5).

Figure 4.2 Trace elements mobility in treated soils (LM lime marl, A-x and B-x Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in % (m/m)) at the end of the pot experiment expressed as % of untreated soil (Con). Values are means $\pm$ SD ($n=4$).

Plant biomass production, nutritional status, and earthworm performance

Insignificant ($p > 0.05$) changes in biomass production of wheat plants were observed with the different treatments. The supply of LM or low levels of WTR enhanced grain yield, but higher application rates of both WTR reversed this trend (Figure 4.3a). This was accompanied by changes in the nutritional status recorded by leaf analyses (Table 4.6). While Con and Ref had a P leaf concentration of $3.5 \text{ g kg}^{-1} \text{ DW}^{-1}$ the P status decreased by 38 % in A-0.5 and by 50 % in A-1. The concentration of Ca in leaves was significantly lower in all treatments but LM compared to Con.

Overall, the earthworms showed little mortality (Figure 4.3b). Moderate mortality occurred in Con, LM, and B-1, where earthworms were also characterized by a loss of body weight. Compared to these treatments, weight loss was significantly lower in treatment A-0.5 ($p < 0.05$).

Figure 4.3 Variability in (a) grain and straw biomass production of wheat (*Triticum aestivum* L. cv. Tiger) and (b) weight loss per earthworm (*Dendrobaena veneta*) expressed as percentage of initial weight from untreated (Con) and treated soils (LM lime marl, A-x and B-x Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in % (m/m)). Values are means $\pm$ SD ($n = 4$). Average earthworm, grain (black) and straw biomass (gray) in uncontaminated soil (Ref) are represented by dashed lines. Significant differences ($p < 0.05$) are indicated by an asterisk. The number of perished worms is indicated within the respective bars.

Changes in chemical elements accumulation in biota

As shown in Figure 4.4, the amendments altered TE concentration in plant and earthworm tissue when compared to Con. Samples treated with WTR_A showed a strong decrease of As transfer into straw (40 % in A-0.5 and 48 % in A-1 ($p < 0.05$); see also Table 4.7) and leaves

(68 % and 75 % ($p < 0.01$)) as well as into earthworm tissue (26 % ($p < 0.05$) and 41 % ($p < 0.01$)). Root uptake of Pb decreased in A-0.5 ($p < 0.05$) and A-1 ($p < 0.01$), whereas uptake of Cd increased in A-0.5 ($p < 0.05$) (see also Figure 4.5a). In grains, both WTR_A treatments enhanced Zn concentration ($p < 0.01$), whereas WTR_B lowered the Cd status (B-0.5 by 23 % and B-1 by 25 % ($p < 0.05$)). Treatment B-1 reduced Pb concentration in straw, leaves (both $p < 0.01$), and earthworm tissue ($p < 0.05$) as well as leaf As ($p < 0.01$). Treatment LM only reduced root uptake of Pb ($p < 0.05$). However, the TE status of biota in treated soils did not reach levels as low as in Ref, except for Zn (grain, leaves) and Pb (leaves). Besides the TE listed above, enhanced Ni concentrations were found in biota exposed to WTR_B treatments (Table 4.8).

Figure 4.4 Concentration of As (a), Cd (b), Pb (c) and Zn (d) in plant compartments of *Triticum aestivum* L. cv. Tiger and in tissue of *Dendrobaena veneta* from untreated (Con) and treated soils (LM lime marl, A-x and B-x Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in % (m/m)). Values are means ± SD (n = 4). Means of Ref are represented by dashed lines. Asterisks represent significant differences between treatments and Con, * for $p < 0.05$; ** for $p < 0.01$.

Correlation of tissue concentrations in *Triticum aestivum* and *Dendrobaena veneta* with soil chemical analyses

Table 4.2 and Table 4.3 show the Pearson correlation coefficients between TE concentration in soil, biomass production, and TE concentration in plants and earthworms. Thousand grain weight (TGW) of wheat was inversely correlated with As (NH_4NO_3), Cd (C_{DGT}), and Zn ($C_{\text{DGT}} > C_{\text{soln}}$) in soil. Shoot biomass or shoot TE removal did not correlate with soil chemical characteristics (data not shown). Correlations were found between labile As concentrations in the soil and As uptake into straw ($r \leq 0.79$; $p < 0.01$) or leaves ($r \leq 0.86$; $p < 0.01$), and between NH_4NO_3 -soluble Pb concentration and Pb uptake into roots ($r = 0.77$; $p < 0.01$) or leaves ($r = 0.59$; $p < 0.01$). For all other combinations, the correlation was weak, inverse, or insignificant. Overall, NH_4NO_3 provided predictability equal to or better than C_{DGT} , whereas C_{soln} was a better predictor than C_{DGT} for Pb.

Earthworm biomass was inversely correlated with soil As (NH_4NO_3), Cd ($C_{\text{DGT}} > \text{NH}_4\text{NO}_3$), Pb (NH_4NO_3) and Zn (C_{soln}). In contrast to the other TE, As concentration in earthworm tissue correlated well ($r \leq 0.86$; $p < 0.01$) with labile As in soil measured by all kinds of chemical analyses.

Correlations between TE accumulation and biomass production of biota

Cadmium uptake into plant roots ($r = 0.53$; $p < 0.05$), As uptake into vegetative tissue (straw: $r = 0.63$; $p < 0.01$, leaves: $r = 0.70$; $p < 0.01$), and Pb uptake into all aboveground plant compartments (straw: $r = 0.57$; $p < 0.05$, leaves: $r = 0.57$; $p < 0.05$, grain: $r = 0.52$; $p < 0.05$) correlated with the accumulation of the respective TE in earthworm tissue. Earthworm biomass correlated with TGW ($r = 0.67$; $p < 0.01$) and grain dry weight ($r = 0.50$; $p < 0.05$).

Table 4.2 Pearson correlation coefficients between NH_4NO_3 -soluble (NH_4NO_3) or DGT-available (C_{DGT}) or soil solution (C_{soln}) TE concentrations in contaminated soil (untreated and treated) and thousand grain weight (TGW) or TE concentrations in plant compartments of wheat (*Triticum aestivum* L. cv. Tiger); values in parentheses represent the significance of the correlation (p value).

	TGW	As root	As straw	As leaf	As grain
As NH_4NO_3	-0.56 (< 0.01)	-0.23 (0.28)	0.79 (< 0.01)	0.86 (< 0.01)	-0.53 (< 0.01)
As C_{DGT}	-0.39 (0.07)	-0.39 (0.85)	0.75 (< 0.01)	0.78 (< 0.01)	-0.54 (< 0.01)
As C_{soln}	-0.40 (0.06)	-0.11 (0.63)	0.48 (0.02)	0.65 (< 0.01)	-0.40 (0.06)
	TGW	Cd root	Cd straw	Cd leaf	Cd grain
Cd NH_4NO_3	-0.40 (0.06)	-0.61 (< 0.01)	-0.55 (< 0.01)	-0.05 (0.80)	0.05 (0.78)
Cd C_{DGT}	-0.64 (< 0.01)	-0.74 (< 0.01)	-0.65 (< 0.01)	-0.07 (0.76)	-0.15 (0.50)
Cd C_{soln}	-0.11 (0.63)	-0.33 (0.12)	-0.11 (0.61)	0.16 (0.48)	0.01 (0.97)
	TGW	Pb root	Pb straw	Pb leaf	Pb grain
Pb NH_4NO_3	-0.31 (0.15)	0.77 (< 0.01)	-0.04 (0.87)	0.59 (< 0.01)	-0.32 (0.14)
Pb C_{DGT}	-0.34 (0.11)	0.19 (0.37)	-0.11 (0.62)	0.24 (0.27)	-0.25 (0.26)
Pb C_{soln}	-0.07 (0.76)	0.48 (0.02)	-0.42 (0.04)	0.03 (0.87)	-0.51 (0.02)
	TGW	Zn root	Zn straw	Zn leaf	Zn grain
Zn NH_4NO_3	-0.65 (0.77)	-0.40 (0.06)	-0.37 (0.08)	-0.20 (0.35)	-0.23 (0.30)
Zn C_{DGT}	-0.46 (0.03)	-0.11 (0.62)	-0.40 (0.06)	-0.25 (0.25)	-0.41 (0.05)
Zn C_{soln}	-0.44 (0.04)	0.53 (< 0.01)	-0.65 (< 0.01)	-0.50 (0.01)	-0.58 (< 0.01)

If significant ($p < 0.05$), data are highlighted with bold font

Table 4.3 Pearson correlation coefficients between NH_4NO_3 -soluble (NH_4NO_3) or DGT-available (C_{DGT}) or soil solution (C_{soln}) TE concentrations in contaminated soil (untreated and treated) and biomass of earthworms (*Dendrobaena veneta*) expressed as percentage of initial weight and TE concentrations in earthworm tissue; values in parentheses represent the significance of the correlation (p value).

	biomass	As tissue
As NH_4NO_3	-0.65 (< 0.01)	0.86 (< 0.01)
As C_{DGT}	-0.42 (0.08)	0.80 (< 0.01)
As C_{soln}	-0.45 (0.06)	0.81 (< 0.01)
	biomass	Cd tissue
Cd NH_4NO_3	-0.50 (0.03)	-0.29 (0.23)
Cd C_{DGT}	-0.62 (< 0.01)	-0.47 (0.05)
Cd C_{soln}	-0.33 (0.18)	-0.54 (0.02)
	biomass	Pb tissue
Pb NH_4NO_3	-0.48 (0.04)	0.25 (0.31)
Pb C_{DGT}	-0.25 (0.33)	0.08 (0.75)
Pb C_{soln}	-0.31 (0.22)	-0.11 (0.66)
	biomass	Zn tissue
Zn NH_4NO_3	-0.21 (0.40)	0.30 (0.23)
Zn C_{DGT}	-0.34 (0.17)	0.27 (0.28)
Zn C_{soln}	-0.47 (< 0.05)	-0.05 (0.83)

If significant ($p < 0.05$), data are highlighted with bold font

Discussion

Characteristics of WTR influencing their immobilization efficiency for TE and implications for the use in agricultural soils

The efficiency of Fe-oxide-containing soil additives for As immobilization has been examined by numerous studies (e.g., Basta et al. 2005; Kumpiene et al. 2008). Moore et al. (2000) found an Fe/As molar ratio of two and higher to be the most effective. The treatments with WTR_A represent an Fe/As molar ratio of 2.7 (A-0.5) and 5.5 (A-1), respectively, with increasing stabilization efficiency for As indicated by the soil chemical analyses and concentration in biota. The stimulation of Zn uptake by wheat in WTR_A treatments supports the findings for Fe-oxide treatments by Mench et al. (2006). The results indicate that WTR_A, consisting of Fe- and Al-compounds, reduced oxyanion availability in soils with subsequent P limitation in crops, as suggested by Sarkar et al. (2007). Frequent P fertilization in agricultural soils may, in turn, lead to the mobilization of As due to a competition between P and As for sorption sites. However, according to these authors As was long-term stabilized even in WTR-treated sandy soils fertilized with triple super phosphate due to the formation of inner-sphere complexes. Within weakly to moderately acid soil conditions, Mn-oxides were reported to immobilize TE like Cd, Pb, and Zn more effectively than Fe-hydroxides (Mench et al., 1999, 2006). Accordingly, WTR_B most successfully decreased Cd (B-0.5) and Pb (B-1) in the soil solution, and exclusively reduced Cd status of wheat grains (B-0.5 and B-1). Both WTR reduced labile TE in a moderately acid soil under semi-controlled conditions. However, if WTR are used in agricultural soils under field conditions, higher fluctuations in pH (liming) and E_h (heavy rainfall events and irrigation) are likely to hamper effective surface sorption of cations and anions (Kumpiene et al., 2008; Bolan et al., 2014).

Depending on the geochemical origin of the processed natural water, WTR may contain high amounts of potentially toxic elements (Ippolito et al., 2011). Measured concentrations of As, Cd, and Pb in WTR used in this study corresponded with ranges reported by other authors, whereas concentrations of Zn, Cr, and Ni in WTR_B were relatively high (Ippolito et al., 2011; Nielsen et al., 2011). No enhanced concentrations of As, Cd, Pb, or Zn in treated soils or biota were found that could be related to an introduction by WTR, indicating strong sorption onto (hydr)oxide surfaces. However, labile (NH₄NO₃) concentrations of Ni were significantly enhanced in WTR_B treatments ($p < 0.05$), albeit at a moderate level.

Effects of WTR on plants and earthworms

A marked dose responsive decrease of P in leaf tissue was recorded in WTR_A treatments. Hence, a strong fixation of P parallel to that of As in soil by Fe-oxides might be confirmed (Sarkar et al., 2007). Nevertheless, A-0.5 showed a non-significant increase of plant dry-matter yield compared to Con. For a higher application rate (A-1) the increase, particularly of grain biomass, reversed almost to the level of Con. Hence, alleviated TE toxicity might be counteracted by a deficiency in P, as found earlier in plants grown on soils treated with Fe- or Al-based WTR (Müller and Pluquet, 2000; Ippolito et al., 2011). Earthworm mortality was prevented and body weight was enhanced by WTR_A, but as with plants, a higher application rate also impaired the positive response. However, the results obtained in this study suggest that reduced As bioavailability improves the health of earthworms, which in turn implies improvement of soil functionality (Nahmani et al., 2007). In the case of WTR_B, plant biomass and earthworm body weight and mortality responded negatively to a higher application rate as well. This may be attributed to enhanced Ni uptake into plant roots and grains in case of B-1 and by earthworms ($p < 0.05$) in both treatments, pointing to oxidative stress or inhibition of nutrient uptake (Maleri et al., 2007; Chen et al., 2009).

Factors limiting the predictability of TE status of biota by analytical methods

The predictability of As concentration in vegetative plant organs and earthworm tissue was satisfactory ($r \sim 0.8$) by the selected methods. For oligochaete worms, uptake routes for As are typically related to the soil pore water compartment and further affected by soil characteristics, in particular acidity (Peijnenburg et al., 1999). Despite the strong reduction of available As in soil, root uptake was not lowered by WTR_A treatments, but translocation to shoots (Table 4.7). Although leaf P status simultaneously decreased, the molar ratio of P/As was still significantly higher when compared to the other treatments. The influence of P on the mitigation of As uptake and translocation in durum wheat (*Triticum durum* L.) and winter wheat (*Triticum aestivum* L.) has been intensively studied by various authors, who also found most of As being retained in the roots (Pigna et al., 2009; Brackhage et al., 2015). No significant changes in leaf Fe-status were identified, from which further conclusions could be drawn about its role in As translocation within the plant. In addition to phosphate transporters, the pathway of silicic acid was found to be relevant for the accumulation of arsenous acid by plants (Zhao et al., 2010). This study did not focus on interactions with Si. However, Si should be considered in future studies. If phytoavailable, it might influence As and Cd

(Rizwan et al., 2012) uptake and/or translocation in treatments with WTR, which can contain considerable amounts of Si (Nielsen et al., 2011).

Root uptake of Pb was correlated with Pb concentrations measured in NH_4NO_3 - and soil solution (C_{soln}). The latter was superior to C_{DGT} , which might be due to overestimation of metal concentrations in roots since the fraction bound to the apoplast was not removed before measuring root TE concentrations. Furthermore, Pb translocation from plant roots to shoots is known to be limited by selective cellular uptake mechanisms (Nowack et al., 2006). The weak correlations obtained with chemical extractants or C_{DGT} also comply with the findings of Senila (2014).

For soils at or near an equilibrium state, significant correlations are typically found between Cd and Zn concentrations extracted with NH_4NO_3 or DGT and their concentration in plants (Gryschko et al., 2005; Senila et al., 2012). By contrast, the observed correlations were either inverse or insignificant, which corresponds with the findings from Huynh et al. (2010), who attributed it to plant removal from the labile pool. This was indicated by high concentrations of Zn in plant tissue found in this study as well. Since root uptake of Zn correlated best with C_{soln} , Zn uptake was not limited by diffusion, which was supported by a considerable resupply to the soil solution (Table 4.5). According to the biotic ligand model (Spurgeon, 2010), the uptake of Cd as a non-essential element for plants and organisms like earthworms is determined by the presence of competing cations like Ca^{2+} and Zn^{2+} as well as protons (Oste et al., 2001). Strong deviations were found between the ratio of Cd/Zn in roots and earthworms and Cd/Zn ratio in the available pool in soil. More precise, a relative enrichment of Cd in plants (Figure 4.5a) and earthworms (Figure 4.6) was found with decreasing Cd/Zn ratio in soil. Cadmium concentration in roots could be explained by a linear regression with pH ($R^2 = 0.45$, $p < 0.001$), while a multiple linear fitting including available Zn improved the correlation ($R^2 = 0.56$, $p < 0.001$). Thereby, higher concentrations of H^+ and/or available Zn, represented by Con, B-0.5, and B-1, lowered root uptake of Cd possibly due to preferential binding of Zn and protons to root membranes and transfer via specific transporters. Within the plants, an inhibition of Cd translocation additionally modified the Cd/Zn ratio towards a relative enrichment of Zn, especially in the WTR_A treatments (Figure 4.5b). In a chelant buffered hydroponic study, Green et al. (2003) found that high ionic Zn^{2+} activities did not mitigate root uptake of Cd, but decreased its translocation to shoots. Consequently, when competing cations impact accumulation by plants and earthworms and further alter translocation within plants, neither chemical extractants nor DGT could serve as appropriate biomimic surrogates (Degryse et al., 2009).

Figure 4.5 Concentration ratios between Cd and Zn of plant tissue related to that in soil solution (C_{soln}) (a) and of aboveground tissues related to root tissue (b) in untreated (Con) and treated soils (LM lime marl, A-x and B-x Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in % (m/m)).

Conclusions

The application of WTR strongly affected TE bioavailability in soil, TE tissue concentration, and biomass of plants and earthworms in this study. However, none of the treatments was able to mitigate the transfer of all targeted elements. The Fe-based WTR_A was the most effective at reducing As accumulation, whereas the Mn-based WTR_B was the most successful at reducing Pb and Cd (plants) accumulation in biota. In both cases, the biota responded negatively to higher application rates. Thus, the use of WTR for *in situ* stabilization of TE contaminated agricultural soils can be quite effective, but it also holds risks related to nutrient limitation or input of potentially toxic elements. In this study, the transfer of TE to cereal grains, which is most relevant in practice, could not be deduced from chemical soil analyses. Likewise, observed reductions of TE concentration in leaves or earthworms, which might be assessed by short-term tests, were not indicative of grain TE status. The predictability of Cd and Zn bioavailability can be weak in soils with high labile shares of both elements and/or soils treated with WTR, where an influx of various elements causes interactions during uptake. Therefore, such amendments should be tested in a bioassay with a set of key species prior to application at field scale.

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Supplementary Information

Detailed information on chemical analyses

Amendment and soil analysis

Prior to microwave-assisted acid digestion with aqua regia (3 ml concentrated HNO_3 (ROTIPURAN® 65 %, p.a.) and 9 ml concentrated HCl (ROTIPURAN® 37 %, p.a.)) soil samples were air-dried, sieved to < 2 mm, and subsequently ground to < 150 μm as described in (DIN ISO 11466:1997). Extractions were performed using 0.5 g soil in a closed vessel microwave digester at 160 °C, 25 minutes ramp time and 20 minutes hold time. After cooling down samples were transferred into 50 ml volumetric flasks, filled up with de-ionized (DI) water (> 18 $\text{M}\Omega\text{ cm}^{-1}$) and analyzed with ICP-MS. The extraction procedure was conducted analogously for both WTR. Mean recovery rates for acid extractable As, Cd, Pb, and Zn in ISE 979 (Rendzina soil) were 107 %, 100 %, 108 %, and 106 %, respectively.

Ammonium nitrate (NH_4NO_3) soluble TE concentrations were determined using the standard procedure (DIN ISO 19730:2008). Air dried, sieved to < 2 mm soil was extracted with 1 M NH_4NO_3 solution at a liquid-to-solid ratio of 2.5 l kg^{-1} for 120 minutes in an end-over-end shaker at 20 °C. Extraction solutions were filtered through 0.45 μm membrane filters and subsequently analyzed with ICP-MS. Mean recovery rates for NH_4NO_3 -soluble As, Cd, and Zn in ISE 979 (Rendzina soil) were 102 %, 95 %, and 108 %, respectively.

For analysis with **diffuse gradients in thin films (DGT)**, fresh soil samples were sieved < 2 mm and stored at 0 – 7 °C. The procedure aims at the determination of the soils resupply capacity of depleted metals and arsenic to the soil solution and was conducted according to Zhang et al. (2001) in separate steps for heavy metals (chelex-gel) and arsenic (Fe-oxide-gel). For that purpose, each 50 grams of soil were filled into 50 ml centrifuge tubes, adjusted to 100 % water holding capacity and equilibrated for 24 h. Subsequently, approximately 3 g each were placed onto the DGT devices. These were kept in a water saturated atmosphere in closed plastic boxes, which were stored in an incubator at 20 °C. After 24 h of incubation, the soil was removed from the devices by means of rinsing with DI water. The exact time (hh:mm:ss) of placement and removal of soil were noted for each sample. Then the devices were opened to obtain the resin gels. Chelex gels were eluted by 10 ml of 1 M HNO_3 and Fe-oxide gels were eluted with 10 ml of 0.25 M H_2SO_4 and analyzed with ICP-MS. To assess the concentration of TE in soil solution (C_{soln}), the remaining water saturated soils were centrifuged at 614xg for 10 minutes according to Muhammad et al. (2012). The supernatant

was collected with a syringe and filtered through 0.45 μm membrane filters into 10 ml vials. The filtered supernatant was analyzed with ICP-MS. C_{DGT} was calculated according to Zhang (2003). The authors do not provide diffusion coefficients for As. Therefore, a value of $5.69 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ was assumed according to Fitz et al. (2003), who obtained this result with a diffusion cell from duplicate experiments. The distribution coefficient R was calculated as the ratio between C_{DGT} and C_{soln} .

Effective cation exchange capacity (CEC_{eff}) was assessed with 0.5 M NH_4Cl at a soil/solution ratio of 2.5:100 (m/v) according to section 3.2.1.8 of BMEL (2014). The suspension was shaken for 120 minutes, kept at 20 °C overnight, shaken again for 30 minutes and passed through a 0.45 μm membrane filter. The filtered supernatant was analyzed with ICP-OES.

Soil pH was determined using H_2O according to (DIN ISO 10390:2005). Air-dried, sieved to < 2 mm soil was mixed with DI water ($> 18 \text{ M}\Omega \text{ cm}^{-1}$) at a ratio of 1:5 (v/v) in a 50 ml PE tube. The suspensions were placed in an end-over-end shaker for 60 minutes and left standing for an additional 60 minutes before measurement.

Plant and earthworm analysis

Plant samples were dried at 60 °C to constant weight and ground to < 0.25 mm. Extractions were performed using 0.2 g plant material digested with 3 ml concentrated HNO_3 (ROTIPURAN® 65 %, p.a.) and 2 ml concentrated H_2O_2 (ROTIPURAN® 30 %, p.a.) at 180 °C, 25 minutes ramp time and 20 minutes hold time. After cooling down, samples were transferred to 20 ml volumetric flasks and filled up with DI water ($> 18 \text{ M}\Omega \text{ cm}^{-1}$). Metal concentrations were measured with ICP-MS and nutrient concentrations with ICP-OES. Mean recovery rates for TE in BCR 281, rye grass (EU-JRC) for As, Cd, Pb, and Zn were 101 %, 94 %, 103 %, and 98 %, respectively.

Microwave pressurized digestion coupled with ICP-MS technique was used for the quantitative analysis of trace elements in **earthworms**. CEM Mars Xpress microwave oven is equipped with 40 – position rotor with 75 ml digestions mid – pressure (32 bars) vessels. Digestion was carried out with the use of 8 ml concentrated HNO_3 (J. T. Baker, 68.5 – 70 %, Instra – Analyzed) and 2 ml of H_2O_2 (Chemlab, 35 %, analytical reagent). Then samples were digested at 180 °C for five minutes (ramping time 25 minutes), then at 190 °C for five

minutes (ramping time five minutes) and finally at 200 °C for five minutes (ramping time five minutes). After cooling down, samples were transferred into 50 ml volumetric flasks and filled up with 0.055 $\mu\text{S cm}^{-1}$ ultrapure water. To ensure the control of contamination, simultaneously the procedure was performed for blank samples and for certified reference materials to meet the quality control criteria.

Supplementary Tables and Figures

Table 4.4 Trace element mobility in treated soils^a at the end of the pot experiment expressed as % of untreated contaminated soil (Con). Values are means $\pm$ SD ($n = 4$). Different letters represent significant differences at $p < 0.05$.

	NH ₄ NO ₃			C _{dgt}		C _{soln}	
As							
Con	100 ± 3	d		100 ± 6	c	100 ± 10	e
LM	87 ± 2	c		105 ± 14	c	87 ± 1	d
A-0.5	39 ± 1	b		48 ± 5	b	35 ± 4	b
A-1	23 ± 1	a		25 ± 3	a	15 ± 2	a
B-0.5	89 ± 4	c		110 ± 8	c	117 ± 7	e
B-1	84 ± 2	c		98 ± 13	c	68 ± 11	c
Cd							
Con	100 ± 2	e		100 ± 4	c	100 ± 7	d
LM	67 ± 1	b		72 ± 4	b	48 ± 1	c
A-0.5	74 ± 1	c		65 ± 9	b	37 ± 4	b
A-1	54 ± 2	a		43 ± 2	a	22 ± 2	a
B-0.5	89 ± 2	d		89 ± 12	bc	51 ± 7	c
B-1	75 ± 1	c		79 ± 15	bc	54 ± 11	c
Pb							
Con	100 ± 4	e		100 ± 14	c	100 ± 6	c
LM	60 ± 1	c		106 ± 3	c	95 ± 9	c
A-0.5	61 ± 1	c		55 ± 13	ab	66 ± 12	b
A-1	39 ± 2	a		38 ± 5	a	38 ± 6	a
B-0.5	70 ± 1	d		109 ± 14	c	96 ± 15	c
B-1	48 ± 1	b		64 ± 13	b	95 ± 14	c
Zn							
Con	100 ± 6	d		100 ± 8	c	100 ± 6	c
LM	47 ± 4	a		75 ± 17	bc	48 ± 2	a
A-0.5	90 ± 15	cd		61 ± 9	ab	55 ± 4	a
A-1	61 ± 1	b		63 ± 12	ab	43 ± 2	a
B-0.5	82 ± 3	c		81 ± 13	bc	74 ± 14	b
B-1	65 ± 2	b		42 ± 5	a	85 ± 7	b

^aLM lime marl, A-x and B-x Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in % (m/m)

Table 4.5 Trace element resupply in untreated^a and treated soils^b from labile pools of the solid phase R (C_{DGT}/C_{soln}). Values are means $\pm$ SD ($n = 4$). Different letters represent significant differences at $p < 0.05$.

	R (As)		R (Cd)		R (Pb)		R (Zn)	
<i>Ref</i>	<i>n.d</i>		<i>n.d</i>		<i>n.d</i>		<i>0.24 ± 0.20 ab</i>	
<i>Con</i>	0.13	± 0.01 a	0.40	± 0.20	0.27	± 0.08	0.32	± 0.13 ab
<i>LM</i>	0.17	± 0.04 ab	0.50	± 0.04	0.35	± 0.13	0.50	± 0.10 b
<i>A-0.5</i>	0.19	± 0.04 ab	0.58	± 0.05	0.22	± 0.08	0.36	± 0.08 ab
<i>A-1</i>	0.23	± 0.02 b	0.65	± 0.07	0.39	± 0.27	0.47	± 0.09 b
<i>B-0.5</i>	0.14	± 0.05 a	0.58	± 0.04	0.27	± 0.09	0.35	± 0.04 ab
<i>B-1</i>	0.18	± 0.03 ab	0.53	± 0.15	0.26	± 0.17	0.21	± 0.10 a

^a*Ref* uncontaminated soil, *Con* untreated contaminated soil^b*LM* lime marl, *A-x* and *B-x* Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where *x* is the application rate in % (*m/m*)**Table 4.6** Concentrations of macronutrients in mature leaves ($g\ kg^{-1}\ DW^{-1}$) of plants grown on untreated^a and treated soils^b. Values are means $\pm$ SD ($n = 4$). Different letters represent significant differences at $p < 0.01$.

	Ca		Fe		Mg		Al		Mn		P	
<i>Ref</i>	4.78	± 0.34 c	0.08	± 0.03	1.73	± 0.09 ab	0.01	± 0.01	0.47	± 0.03	3.49	± 0.21 b
<i>Con</i>	4.29	± 0.27 bc	0.07	± 0.05	1.52	± 0.15 ab	0.03	± 0.01	0.39	± 0.06	3.51	± 0.44 b
<i>LM</i>	3.47	± 0.33 ab	0.18	± 0.09	1.47	± 0.11 ab	0.03	± 0.02	0.40	± 0.03	3.78	± 0.45 b
<i>A-0.5</i>	3.37	± 0.45 a	0.07	± 0.02	1.43	± 0.14 ab	0.05	± 0.02	0.45	± 0.07	2.18	± 0.12 a
<i>A-1</i>	3.01	± 0.21 a	0.09	± 0.02	1.29	± 0.08 a	0.02	± 0.01	0.38	± 0.05	1.75	± 0.08 a
<i>B-0.5</i>	3.29	± 0.08 a	0.10	± 0.05	1.22	± 0.06 a	0.04	± 0.02	0.34	± 0.03	3.77	± 0.90 b
<i>B-1</i>	3.29	± 0.32 a	0.06	± 0.06	1.40	± 0.13 ab	0.03	± 0.02	0.44	± 0.04	3.99	± 0.29 b

^a*Ref* uncontaminated soil, *Con* untreated contaminated soil^b*LM* lime marl, *A-x* and *B-x* Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where *x* is the application rate in % (*m/m*)**Table 4.7** Translocation factors (TF) from root to shoot (S/R) and shoot to grain (G/S) of plants grown on untreated^a and treated soils^b. Values are means ($n = 4$). Different letters represent significant differences at $p < 0.01$.

	As		Cd		Pb		Zn	
	S/R	G/S	S/R	G/S	S/R	G/S	S/R	G/S
<i>Ref</i>	0.04	a	0.38	b	0.90	c	0.38	b
<i>Con</i>	0.17	c	0.04	a	0.28	b	0.24	a
<i>LM</i>	0.17	c	0.04	a	0.22	ab	0.18	a
<i>A-0.5</i>	0.07	ab	0.08	a	0.16	a	0.22	a
<i>A-1</i>	0.07	ab	0.09	a	0.22	ab	0.19	a
<i>B-0.5</i>	0.11	abc	0.04	a	0.19	ab	0.25	a
<i>B-1</i>	0.12	bc	0.03	a	0.20	ab	0.22	a

^a*Ref* uncontaminated soil, *Con* untreated contaminated soil^b*LM* lime marl, *A-x* and *B-x* Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where *x* is the application rate in % (*m/m*)

Table 4.8 Concentration of Ni ($\text{mg kg}^{-1} \text{ DW}^{-1}$) in untreated^a and treated soils^b and biota. Values are means $\pm$ SD ($n = 4$). Different letters represent significant differences at $p < 0.05$.

	NH_4NO_3		root		grain		earthworm	
<i>Ref</i>	0.006 ± 0.001	<i>a</i>	$< \text{DL}^*$		0.27 ± 0.04	<i>b</i>	0.87 ± 0.12	<i>a</i>
<i>Con</i>	0.028 ± 0.002	<i>d</i>	$< \text{DL}$		0.13 ± 0.01	<i>a</i>	0.87 ± 0.11	<i>a</i>
<i>LM</i>	0.009 ± 0.001	<i>b</i>	$< \text{DL}$		$< \text{DL}$		0.84 ± 0.19	<i>a</i>
<i>A-0.5</i>	0.014 ± 0.001	<i>c</i>	$< \text{DL}$		$< \text{DL}$		0.72 ± 0.07	<i>a</i>
<i>A-1</i>	0.005 ± 0.001	<i>a</i>	$< \text{DL}$		$< \text{DL}$		0.71 ± 0.08	<i>a</i>
<i>B-0.5</i>	0.065 ± 0.007	<i>e</i>	$< \text{DL}$		0.23 ± 0.07	<i>b</i>	1.47 ± 0.16	<i>b</i>
<i>B-1</i>	0.088 ± 0.008	<i>f</i>	8.84 ± 0.29		0.85 ± 0.26	<i>c</i>	1.82 ± 0.41	<i>b</i>

^a*Ref* uncontaminated soil, *Con* untreated contaminated soil^b*LM* lime marl, *A-x* and *B-x* Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where *x* is the application rate in % (*m/m*)^{*} 0.11 mg kg^{-1} **Figure 4.6** Concentration ratio between Cd and Zn in earthworm tissue related to the ratio in soil solution (C_{soln}) in untreated (*Con*) and treated soils (*LM* lime marl, *A-x* and *B-x* Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where *x* is the application rate in % (*m/m*)).

4.2 (Aided) phytostabilization of trace elements using cup plant (*Silphium perfoliatum* L.), or tall wheatgrass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) and soil-applied drinking water treatment residues

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Abstract

Under semi-controlled conditions, the phytostabilization potential of two perennials, cup plant (*Silphium perfoliatum* L.) and tall wheatgrass (*Elymus elongatus* subsp. ponticus cv. Szarvasi-1), was investigated in an agricultural soil heavily contaminated with metals (Cd, Pb, and Zn) and As. Both crops excluded trace elements (TE) in shoots. As compared with annuals, cup plant exhibited exceptionally low shoot Cd and Zn concentrations, but, unlike Szarvasi-1, proved unsuitable for the investigated purpose due to growth depression. Thus, drinking water treatment residues (WTR), dominated by Fe/Al (WTR_A) or Mn (WTR_B) and soil-applied at rates of 0.5 % and 1 % (*m/m*), were compared with 0.4 kg m⁻² lime marl (LM) for aided phytostabilization with Szarvasi-1. The effects on NH₄NO₃-soluble TE suggested stronger TE stabilization than actually observed in plant shoots. Therein, the strongest reductions were attained with WTR_A for As and Cd (up to 35 % and 25 %, respectively) and with WTR_B for Pb (up to 71 %). The effects differed among common harvest dates, being lower in autumn, where less biomass concentrated more TE than in summer. Yet, the cultivation of Szarvasi-1, combined with the application of an Fe-/Al-rich WTR, could link waste recycling with a resource-saving biomass production providing mechanical and biochemical TE stabilization.

Keywords: in situ stabilization • perennial crops • harvest management

Introduction

Impelled by climate change and the need for a sustainable use of resources, researchers and farmers worldwide have begun to cultivate novel non-edible, low-input, drought-adapted, and perennial high-biomass plants for multiple valorization purposes (Jones, 2017). Cultivating such plants on contaminated arable land can combine the aim to overcome the land-use conflict between food and fuel supply with phytoremediation and the provision of ecosystem services (Abhilash et al., 2016). Therefore, a shift towards biomass production for energetic or material use is officially stipulated on soils heavily contaminated with metals (Cd, Pb, and Zn) and As in the Saxon Ore Mountain Mining Region, where the study area is located (SMI, 2013). Moreover, the German Renewable Energies Act (EEG, 2012) compelled stakeholders to complement maize, the use of which became restricted in biogas facilities. Among various studied perennials, regional agricultural associations identified the cup plant (*Silphium perfoliatum* L.) and a Hungarian breeding of tall wheatgrass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) as promising candidates, which attract increasing attention among stakeholders in the Central European bioenergy sector (Schoo et al., 2017; Bernas et al., 2019). As compared with Maize, Mast et al. (2014) found that Szarvasi-1 could provide higher specific methane yield, whereas the cup plant basically attained equal dry matter yields. Bernas et al. (2019) highlighted the low environmental burden, expressed in carbon dioxide equivalents, of long-term (~ 10 years) Szarvasi-1 cultivation compared with maize and two other perennial grasses.

Yet, to date few studies examined both plants' tolerance and accumulation of trace elements (TE), particularly (i) under semi-controlled conditions in soils naturally contaminated with metals and As, or (ii) in comparison to annual crops. At moderate mixed contamination, cup plant studied under field conditions had substantial biomass production and relatively low shoot Cd, Pb, and Zn accumulation compared to other herbaceous plants (Mayerová et al., 2017). Zhang et al. (2010) reported similar findings from a greenhouse study using spiked soil, where cup plant tolerated a wide range of Cd concentrations, while elevated Zn concentrations induced growth depression. By contrast, in hydroponic studies, excess Cd exposure inhibited and Zn stimulated physiological processes of Szarvasi-1 (Sipos et al., 2013), which also tolerated moderate Pb concentrations (Vashegyi et al., 2011). Based on these findings it has been concluded that both cultures were appropriate for the phytostabilization of certain TE.

Aided phytostabilization of metals and As requires the use of multifunctional agents. Drinking water treatment residues (WTR), rich in (hydr)oxides of Fe, Al, and/or Mn, can combine

liming capacities with effective and lasting sorption of As (Makris et al., 2006; Ippolito et al., 2011; Nielsen et al., 2011). Since WTR are complimentary waste by-products generated to huge amounts during potable water production, their use for soil remediation is considered as a sustainable alternative to disposal. However, care should be taken regarding the availability of nutrients and the entry of potentially toxic elements (Neu et al., 2018a).

This study examined the phytostabilization potential of cup plant and Szarvasi-1, cultivated to maturity, in an agricultural soil strongly polluted with metals and As. The data were compared with those of selected annual crops cultivated analogously. A Fe- and a Mn-dominated WTR, applied to the soil prior to the cultivation of Szarvasi-1, were studied regarding intermediate-term potentials to stabilize soil TE and mitigate their transfer into harvestable biomass.

Methods

Experimental set-up

Topsoil (Con) from an agricultural site in Saxony, Germany (GPS coordinates: 13°23'52''E, 50°54'17''N), contaminated with metals (Cd, Pb and Zn) and As, and topsoil (Ref) from an agricultural site nearby (GPS coordinates: 13°16'49''E, 50°57'47''N), which represents regional background TE concentrations (Kardel et al., 2015), were sieved < 4 mm and treated with steam. Both soils are Stagnosols, derived from loess and paragneiss. Soils Ref and Con were initially characterized by a CEC_{pot} (cmol_c kg⁻¹) of 15.8 and 21.5, and an organic carbon content of 11.0 and 21.6 g kg⁻¹, respectively (Barth and Forberg 2015).

Seedlings of cup plant (*Silphium perfoliatum* L.) were purchased from N.L. Chrestensen Erfurter Samen- und Pflanzenzucht GmbH. Commercial seeds of tall wheatgrass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1), further referred to as Szarvasi-1, were purchased from Screw-Press GmbH in Reut, Germany, and sterilized with electron beam at Fraunhofer Institute for Organic Electronics, Electron Beam and Plasma Technology in Dresden, Germany. As a starter culture, one individual each of cup plant was planted into 0.5 l pots containing Con or Ref in sufficient replicates in June 2012. Likewise, two seeds each of Szarvasi-1 were placed in sufficient replicates into 0.25 l pots containing Con or Ref in June 2012, and inoculated with vesicular-arbuscular mycorrhizae (*Rhizophagus irregularis*) using 30 ml m⁻² INOQ_{Spezial} (INOQ GmbH, Schnega, Germany). All pots were set-up in greenhouses, daily watered with de-ionized (DI) water to maintain moisture close to field capacity, and kept outside under natural snow cover during winter time. In early spring 2013, four replicates of each starter culture were transplanted into 17 l pots with a surface area of

0.075 m² containing the respective substrate. At that time, cup plants cultivated in Con already showed chloroses and stunted growth compared to Ref. Therefore, additive treatments were limited to Szarvasi-1. Four replicates each of its starter culture were transplanted to equal amounts of Con substrate previously amended analogous to Neu et al. (2018a) with 0.4 kg m⁻² lime marl (LM), or with 0.5 % and 1 % (*m/m*), respectively, of drinking water treatment residues (WTR), one of which was dominated by Fe-oxides (WTR_A), and the other by Mn-oxides (WTR_B). The characteristics of the amendments are described in Table 4.12. All soils were fertilized in April 2013 with amounts corresponding to 9 g m⁻² N (CH₄N₂O), 1.8 g m⁻² P (P fertilizer containing 40 % P₂O₅), and 10 g m⁻² K (K fertilizer with 60 % K₂O). In May, another N rate was applied using (NH₄)₂SO₄ adequate to 4.2 g m⁻² N. In accordance with the agricultural practice, Szarvasi-1 was harvested in July and in October 2013 at full panicle each and cup plant by the end of September 2013 with beginning seed ripening.

In the frame of another study, a regionally adapted cultivar of winter oilseed rape (*Brassica napus* L. cv. Lorenz) and a sunflower, modified towards enhanced growth and metal accumulation by chemical mutagenesis (*Helianthus annuus* L. mutant inbred line M7 (R3B-F-U/R13M10A; test series R13F-MP; Nehnevajova et al. (2007))), were cultivated analogously, except for pot size and culture-specific divergent sowing and harvest dates, as described in detail in Neu et al. (2018b). Likewise, a regionally adapted cultivar of winter wheat (*Triticum aestivum* L. cv. Tiger) was cultivated in parallel to the above mentioned plants in greenhouses, as described in Neu et al. (2018a).

Chemical analyses

Pseudo-total TE concentrations were analyzed for initial soils using *aqua regia* (DIN ISO 11466:1997). Potentially plant-available TE were analyzed initially and at the second harvest using NH₄NO₃ extraction (DIN ISO 19730:2008). Soil pH was analyzed in H₂O slurry at 1:5 soil/solution ratio (*v/v*) (DIN ISO 10390:2005). Plant samples were divided into roots (not obtained during the first harvest of Szarvasi-1) and shoots, washed with DI water, dried at 60 °C to constant weight, finely ground and digested at 180 °C with 3 ml HNO₃ and 2 ml H₂O₂ (DIN EN 13805:2014). Chemical analyses were validated by blanks and standard reference material (plants: BCR 667, soil: WEPAL-ISE 979). The limit of detection (LOD) was calculated as the nine-fold standard deviation of blanks. Analyses of TE were performed by ICP-MS (PQ exCell, Thermo Fisher Scientific Inc, UK).

Statistical analyses

Statistical differences ($p < 0.05$) between treatments were examined with one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test using PASW statistics 21 (SPSS, Inc., Somers, NY, USA). In the event that data did not comply with the homoscedasticity assumption (Levene's test), the significant differences were analyzed using Welch's ANOVA and Games-Howell *post hoc* test. Data were log-transformed when necessary to meet the requirement of normal distribution of residuals (Shapiro-Wilk test).

Results

Availability of TE in soil as affected by plant growth and amendments

The cultivation of both cultures effected a slight enhancement of soil acidity (Ref) and tended to increase TE mobility (Table 4.9) in unamended soils compared with the initial conditions. Thereby, cup plant affected mobile TE in Con more strongly than Szarvasi-1.

Table 4.9 NH_4NO_3 -soluble TE concentrations ($\text{mg kg}^{-1} \text{DW}^{-1}$) and soil pH (H_2O) in initial soils, and after growth of cup plant (*Silphium perfoliatum* L.) or Szarvasi-1 (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) in uncontaminated soil (Ref), untreated contaminated (Con) or treated contaminated soil (LM: lime marl, A-x- and B-x: Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in percentage (m/m)). Values are means $\pm$ SD ($n = 4$). Different letters indicate significant differences ($p < 0.05$) between the treatments for each soil.

		As	Cd	Pb	Zn	pH
initial soils	Ref	0.020 ± 0.002 c	0.01 ± 0.001 a	0.01 ± 0.002 a	0.41 ± 0.05	6.06 ± 0.03 b
	Con	0.77 ± 0.01 c	0.57 ± 0.03 bc	1.94 ± 0.14 bc	2.62 ± 0.16 bc	5.56 ± 0.03 a
cup plant	Ref	0.01 ± 0.002 a	0.02 ± 0.004 b	0.02 ± 0.005 b	0.44 ± 0.09	5.97 ± 0.07 b
	Con	1.11 ± 0.09 e	0.76 ± 0.11 d	2.91 ± 0.76 d	3.49 ± 0.90 d	5.55 ± 0.22 a
Szarvasi-1 (2 nd harvest)	Ref	0.016 ± 0.001 b	0.02 ± 0.002 b	0.02 ± 0.002 b	0.44 ± 0.05	5.79 ± 0.10 a
	Con	1.10 ± 0.03 e	0.67 ± 0.05 cd	2.45 ± 0.20 cd	2.60 ± 0.37 bc	5.62 ± 0.07 a
	LM	1.04 ± 0.02 e	0.51 ± 0.04 ab	1.70 ± 0.17 b	1.53 ± 0.30 a	5.85 ± 0.09 b
	A-0.5	0.48 ± 0.03 b	0.58 ± 0.02 bc	1.76 ± 0.09 b	3.14 ± 0.25 cd	5.67 ± 0.04 ab
	A-1	0.26 ± 0.00 a	0.42 ± 0.01 a	1.01 ± 0.04 a	2.17 ± 0.06 ab	5.85 ± 0.02 b
	B-0.5	0.92 ± 0.03 d	0.67 ± 0.02 cd	1.87 ± 0.11 bc	2.94 ± 0.17 bcd	5.57 ± 0.02 a
	B-1	0.90 ± 0.04 d	0.60 ± 0.01 bc	1.49 ± 0.02 ab	2.57 ± 0.09 ab	5.62 ± 0.04 a
		0.4 ^a	0.04/0.1 ^b	0.1 ^b	2 ^a	

^a Trigger values for expected impairment of plant growth laid down in the Federal Soil Protection Ordinance (BBodSchV 1999)

^b Action values for soil-to-plant transfer; the lower value for Cd applies if bread wheat or accumulating vegetables are cultivated (BBodSchV 1999)

Among stabilizing agents, WTR_A applied to soil prior to cultivation of Szarvasi-1 at a rate of 1 % (A-1) most successfully reduced mobile As, Cd, and Pb compared to Con, on average by 76 %, 37 %, and 59 %, respectively. Lime marl effected the strongest reduction (41 %) of mobile Zn despite equal amelioration of soil pH, which remained unaffected by the other treatments but proved to control metals availability (Table 4.11). The application of WTR_A at a rate of 0.5 % (A-0.5) significantly increased labile Zn. Otherwise, except for insignificantly higher labile Zn and equal Cd concentrations following WTR_B applied at a rate of 0.5 % (B-0.5), or no effects of B-1 on labile Zn and of LM on labile As, the TE mobility consistently decreased in treated soils compared to Con. However, apart from As concentrations in A-1 and Zn concentrations in LM below the German trigger values regarding soil-to-plant transfer (BBodSchV, 1999; Table 4.9), no compliance with any threshold was achieved.

Accumulation of TE in plants grown on unamended soils

Despite lower bioconcentration factors (BCF; TE shoot/TE soil, see Table 4.13), both cultures accumulated significantly more TE except Zn in shoots when cultivated in Con than in Ref (Table 4.10). Unlike stunted growth of cup plant in soil Con, the biomass of Szarvasi-1 showed no substrate-induced differences in both harvests, the first of which provided significantly more biomass than the second. In soil Ref Szarvasi-1 accumulated significantly less Pb and more Cd and Zn in shoots than cup plant, irrespective of the harvest. In soil Con the same applied for Cd, whereas no differences were obtained for Pb and Zn. In both soils, Szarvasi-1 accumulated less or more As than cup plant when harvested in summer or autumn, respectively. At significantly higher Pb accumulation in roots from both soils, Szarvasi-1 translocated less to shoots than cup plant, and conversely for Zn.

Accumulation of TE in Szarvasi-1 grown on amended soils

As compared with Con, all WTR treatments significantly reduced root As and Pb concentrations without affecting root-to-shoot translocation. Treatment A-1 solely reduced shoot As concentrations in both harvests significantly, effecting compliance of the summerly harvested biomass with the European threshold for As in animal feed (EC, 2002). Thereby, the extent of the reduction over Con remained stable at ~ 35 % for As and at 25 % for Cd ($p > 0.05$) between the two harvests, whereas that of the other treatments increased in the second harvest for all TE. Treatment B-0.5 continuously induced the lowest Pb accumulation at a maximum reduction of 71 % in the second harvest. By contrast, the LM treatment

significantly enhanced shoot As and Pb in the summerly harvest and, unlike all WTR treatments, had no impact on TE status in autumn.

Table 4.10 Trace element concentrations ($\text{mg kg}^{-1} \text{ DW}^{-1}$) in shoots and roots and dry weight (g) per individual of cup plant (*Silphium perfoliatum* L.) or Szarvasi-1 (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) grown on uncontaminated soil (Ref), untreated contaminated (Con) or treated contaminated soil (LM: lime marl, A-x- and B-x: Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in percentage (m/m)). Values are means $\pm$ SD ($n = 4$). Different letters indicate significant differences ($p < 0.05$) between the treatments for each plant/harvest.

		As	Cd	Pb	Zn	DW
		shoot				
cup plant	Ref	0.6 ± 0.1 a	0.03 ± 0.02	0.8 ± 0.3	15.9 ± 2.5	65.5 ± 4.5 b
	Con	4.1 ± 0.3 b	0.5 ± 0.3	2.8 ± 1.6	22.0 ± 10.6	9.9 ± 6.2 a
Szarvasi-1 (1 st harvest)	Ref	0.4 ± 0.0 a	0.4 ± 0.0 a	0.3 ± 0.1 a	23.8 ± 2.2 a	8.4 ± 0.5
	Con	2.0 ± 0.2 c	3.7 ± 0.6 bc	1.5 ± 0.2 c	29.5 ± 5.1 ab	7.7 ± 1.0
	LM	2.8 ± 0.4 d	3.1 ± 0.7 bc	2.2 ± 0.4 d	26.8 ± 2.9 ab	8.9 ± 0.9
	A-0.5	2.5 ± 0.5 cd	4.2 ± 0.9 c	1.6 ± 0.3 c	35.3 ± 4.8 b	9.4 ± 1.1
	A-1	1.3 ± 0.2 b	2.8 ± 0.4 b	1.2 ± 0.1 bc	30.8 ± 2.1 ab	8.7 ± 1.0
	B-0.5	2.4 ± 0.3 cd	3.4 ± 0.7 bc	0.9 ± 0.1 b	25.2 ± 4.1 a	8.3 ± 1.5
	B-1	2.5 ± 0.4 cd	3.3 ± 0.4 bc	1.2 ± 0.1 bc	27.3 ± 5.8 ab	8.2 ± 0.4
Szarvasi-1 (2 nd harvest)	Ref	1.3 ± 0.2 a	0.6 ± 0.2 a	0.2 ± 0.1 a	29.9 ± 4.7	2.3 ± 0.3
	Con	5.7 ± 0.8 c	5.3 ± 1.7 b	3.1 ± 1.2 c	39.2 ± 10.7	2.5 ± 0.3
	LM	5.8 ± 0.6 c	3.9 ± 0.9 b	2.1 ± 0.7 bc	29.1 ± 1.0	2.2 ± 0.7
	A-0.5	4.3 ± 0.5 bc	4.1 ± 0.3 b	1.4 ± 0.8 ab	40.8 ± 6.3	2.1 ± 1.3
	A-1	3.6 ± 0.4 b	4.0 ± 1.3 b	1.5 ± 0.5 ab	50.6 ± 24.7	2.8 ± 0.7
	B-0.5	4.9 ± 0.2 bc	4.8 ± 1.7 b	0.9 ± 0.4 ab	40.7 ± 4.9	2.8 ± 0.6
	B-1	5.0 ± 1.9 bc	4.6 ± 0.9 b	1.3 ± 0.3 ab	33.4 ± 10.8	2.3 ± 0.8
		root				
cup plant	Ref	1.4 ± 0.1 a	0.2 ± 0.1 a	1.2 ± 0.1 a	30.4 ± 3.1 a	30.5 ± 3.0 b
	Con	105.7 ± 11.3 b	36.4 ± 9.1 b	71.6 ± 19.8 b	147.5 ± 14.4 b	4.5 ± 2.5 a
Szarvasi-1 (2 nd harvest)	Ref	1.8 ± 0.6 a	1.8 ± 0.6 a	2.7 ± 0.9 a	25.1 ± 6.9 a	0.7 ± 0.2 ab
	Con	124.7 ± 18.1 c	37.2 ± 5.6 bc	232.7 ± 11.2 c	115.6 ± 8.9 b	0.5 ± 0.0 a
	LM	136.8 ± 27.7 c	34.0 ± 3.7 bc	287.9 ± 72.0 c	126.0 ± 16.5 b	1.0 ± 0.1 b
	A-0.5	61.0 ± 8.5 b	38.2 ± 3.3 bc	131.9 ± 29.3 b	119.7 ± 9.9 b	0.6 ± 0.3 a
	A-1	50.3 ± 15.1 b	28.3 ± 6.7 b	118.9 ± 40.0 b	104.1 ± 19.8 b	0.6 ± 0.2 ab
	B-0.5	79.2 ± 18.7 b	44.9 ± 7.6 c	127.2 ± 17.9 b	96.5 ± 8.1 b	0.5 ± 0.1 a
	B-1	81.7 ± 12.4 b	33.9 ± 3.1 bc	139.0 ± 41.1 b	98.9 ± 16.3 b	0.6 ± 0.2 a
EU-threshold animal feed (DW; EC (2002))		2.27	1.14	11.36		

The shoot As and Pb concentrations correlated with those in soil and roots (Table 4.11), whereas no relations were obtained for Cd and Zn. However, the root Cd/Zn ratio correlated inversely with pH ($r = -0.77$; $p < 0.001$), effecting highest values in treatment B-0.5 and lowest in A-1 and LM (Figure 4.8). Overall, shoot dry weight appeared to be adversely

affected by TE status. The opposite applied for root dry weight, which in turn correlated inversely with soil metal concentrations (Table 4.11).

Table 4.11 Pearson correlation coefficients between TE in soil, roots, or shoots sampled during the second harvest of Szarvasi-1 (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) and soil pH or the respective tissue's TE concentrations (conc.) or dry weight (DW); asterisks represent the significance of the correlation (p value): * $p < 0.05$, ** $p < 0.01$.

	pH	root		shoot	
		conc.	DW	conc.	DW
As _{soil}	-0.37	0.77**	0.09	0.70**	-0.10
Cd _{soil}	-0.91**	0.65**	-0.44*	0.38	0.04
Pb _{soil}	-0.59**	0.38	-0.17	0.47*	-0.02
Zn _{soil}	-0.80**	-0.24	-0.61**	0.14	0.15
As _{root}	0.12	1	0.41*	0.63**	-0.06
Cd _{root}	-0.58**	1	-0.12	0.37	0.30
Pb _{root}	0.35	1	0.59**	0.58**	-0.06
Zn _{root}	0.36	1	0.55**	-0.28	0.09
As _{shoot}	-0.10		0.23	1	-0.82**
Cd _{shoot}	-0.39		-0.21	1	-0.45**
Pb _{shoot}	0.06		0.22	1	-0.16
Zn _{shoot}	0.05		-0.37	1	-0.42**

If significant ($p < 0.05$), data are highlighted with bold font

Comparative assessment of TE accumulation in perennial and annual plants

When cultivated in Con, the perennials accumulated significantly less As and Zn in shoots than annuals. The Cd and Pb concentrations in Szarvasi-1 resembled (first harvest) or exceeded (second harvest) those of wheat (Figure 4.7). In accordance with the breeding objectives, the highest shoot metal concentrations were obtained in sunflowers, while rape had the highest root uptake of As, Cd, and Zn (Figure 4.9). These rankings partially differed in soil Ref, whereas cup plant exhibited significantly the lowest shoot Cd concentrations in both soils.

Figure 4.7 Trace element concentrations (mg kg⁻¹ DW⁻¹) in shoots of Szarvasi-1 (*Elymus elongatus* L. supsp. *ponticus* cv. Szarvasi-1) at the first (h1) and second (h2) harvest, cup plant (*Silphium perfoliatum* L.), and in the annuals winter oilseed rape (*Brassica napus* L. cv. Lorenz), sunflower (*Helianthus annuus* L. mutant inbred line M7), and winter wheat (*Triticum aestivum* L. cv. Tiger). Values are means ± SD (n = 4). Different letters represent significant differences (p < 0.05). Dashed lines represent thresholds set by the European Commission for trace elements in animal feed.

Discussion

Phytostabilization capacity of cup plant and Szarvasi-1

When defined according to the United States Environmental Protection Agency (US EPA, 2000), the absence of decreased potentially plant-available TE in soil following the cultivation of cup plant or Szarvasi-1 compared to initial conditions suggests no capability of phytostabilization. However, the establishment of a continuous vegetation cover by TE-tolerant plants, which preferably exclude TE in their harvestable tissues (Vangronsveld et al., 2009), to prevent erosion or leaching of TE is also accepted as phytostabilization (Lombi et al., 1998). Both perennials retained most of accumulated TE in roots and had bioconcentration

factors (BCF: TE_{shoot}/TE_{soil}) much below 1 (Table 4.13). They can therefore be considered TE excluders, which limits TE spreading during biomass valorization and increases its acceptance among operators (Bert et al., 2017).

However, unlike the TE-tolerant Szarvasi-1, cup plant was intoxicated by a certain or the mixture of plant-available TE present at high concentrations in soil Con, as found before for sunflower (Neu et al., 2018b), which also belongs to the *Asteraceae* family. This confirms the findings obtained for cup plant by Zhang et al. (2010) at levels of sole Zn exposure similar to those under study. The authors obtained higher Cd and Zn shoot concentrations and $BCF > 1$ for cup plant, which may result from spiked soil used as growth medium. Similarly, Mayerová et al. (2017) reported higher values for Cd, Pb, and Zn in a strongly acidic contaminated soil (pH ~ 4). Based on a hydroponic study, cup plant accumulated more As than various *Poaceae*, which were cultivated in a potting mix containing the same level of As, but did not respond with higher tissue concentrations or growth inhibition to increased levels of exposure (Rofkar et al., 2007).

Effects of the harvest regime on TE accumulation in perennial grasses

Mayerová et al. (2017) studied different perennials from the *Poaceae* family, which includes Szarvasi-1. Coinciding with the results obtained in this study, their harvestable biomass was lower in the second than in the first harvest in a year while containing significantly higher TE concentrations. Since Szarvasi-1 was sampled at full panicle each, growth dilution effects likely induced a large part of these differences, whereas limited translocation *in planta* has been discussed to explain low TE concentrations of *Arrhenatherum elatius* during seed development in summer (Deram et al., 2006). In this study, the lower As accumulation in the first harvest of Szarvasi-1 compared to cup plant coincided with the findings of Rofkar et al. (2007). By contrast, higher As concentrations in shoots from the second harvest, exceeding those of cup plant, likely refer in parts to lower biomass. However, the shoot As concentrations differed more between both harvests than those of metals. Tall wheatgrass exposed to metals has been found to excrete low molecular weight organic acids (LMWOAs) (Yang et al., 2001), which in turn could have increased bioavailable metals and As to different extents (Nworie et al., 2017), inter alia via the dissolution of Fe-oxides. Enhanced release of LMWOAs over time may be assumed as a strategy for P and Fe acquisition (Bolan et al., 1994), since no repeated fertilization has been conducted after the first harvest.

Potentials and limitations of aided phytostabilization using WTR

The results obtained with the investigated amendments confirm the findings of Neu et al. (2018a) regarding the concentration-dependent superior effectiveness of WTR against lime marl in reducing bioavailable TE in soil. A particularly strong immobilization of As in soil by the Fe-rich WTR_A confirms the generally accepted affinity of Fe- or Al-based WTR for oxyanions (Makris et al., 2005, 2006; Ippolito et al., 2011). However, corresponding effects on As uptake in Szarvasi-1 were only obtained when applied at a rate of 1 %. The observed trend towards stable or increased stabilization between both harvests suggests the potential of lasting remedial success, which has been documented for an Fe-rich WTR in a multiannual field study (Nielsen et al., 2011). Unlike observations reported for wheat (Neu et al. 2018a), the Mn-rich WTR_B did not affect shoot Cd and reduced shoot Pb concentrations in both harvests at the lower instead of the higher application rate. The lack of correlations between soil and shoot Cd or Zn concentrations confirms previous findings pointing to interferences during uptake (Neu et al., 2018a). Thereby, unlike wheat, Szarvasi-1 enriched Cd relative to Zn in roots under more acidic soil conditions (B-0.5) compared to treatments with increased soil pH and more effectively stabilized Cd and Zn concentrations (LM, A-1). At a coincident enrichment of Zn over Cd in shoots, Szarvasi-1, however, kept the Cd/Zn ratio in a more narrow range.

Conclusions

In contrast to Szarvasi-1, cup plant appeared non-tolerant to high-level mixed soil contamination. However, its exceptionally low accumulation of Cd makes it a potential candidate for phytostabilization at moderately Cd-contaminated sites. In agricultural soils, where the use of annual crops for animal consumption is basically impeded by excess As concentrations, Szarvasi-1, eventually combined with an adequately high application of an Fe- or Al-rich WTR, may provide legally compliant biomass. However, in the present study this attainment depended on the harvest regime and was hampered by excess Cd concentrations, which were not alleviated by neither of the studied amendments. Yet, the cultivation of perennials tolerating and excluding TE, like Szarvasi-1, combines low input requirements with the provision of a continuous plant cover for mechanical TE stabilization. It further promotes the acceptance of the biomass among stakeholders in the forage, energy, or fibrous material sector. Thus it can present a more sustainable alternative to annual crops with similar or higher TE accumulation in heavily contaminated agricultural soils.

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Supplementary Information

Table 4.12 Characteristics of soil amendments (LM: lime marl, WTR_A and WTR_B: Fe-based and Mn-based drinking water treatment residue, respectively).

		LM	WTR _A	WTR _B
pH (H ₂ O)		9.1	7.9	7.9
C		121	20	9
C _{org}		NA	11	3
Ca		205	15	16
Fe	g kg ⁻¹	4.7	354	18
Al		5	22	6
Mn		0.4	6	72
P		< LOD ^a	3.3	1.6
As		8.9	57	13
Cd		0.5	1	4
Pb		17.1	28	3.6
Cr	mg kg ⁻¹	11.6	3.5	236
Ni		74	43	435
Zn		5.4	80	677
Tl		0.2	0.02	1.8
Cu		NA	5.5	49

^a0.25 g kg⁻¹

Table 4.13 Concentration of pseudo-total TE (mg kg⁻¹ DW⁻¹) in soil and bioconcentration factors (BCF: TE shoot/TE soil) in plant shoots from unamended soils. Values are means ± SD (*n* = 4). Different letters indicate significant differences (*p* < 0.05) between plants/harvests.

		soil TE	BCF					
			cup plant		Szarvasi-1 1 st cut		Szarvasi-1 2 nd cut	
Ref	As	41 ± 2	0.02 ± 0.003	b	0.01 ± 0.00	a	0.03 ± 0.00	c
	Cd	0.7 ± 0.02	0.05 ± 0.02	a	0.53 ± 0.06	b	0.78 ± 0.25	b
	Pb	78 ± 2	0.01 ± 0.004	b	0.004 ± 0.001	a	0.003 ± 0.001	a
	Zn	80 ± 13	0.20 ± 0.02	a	0.30 ± 0.04	b	0.38 ± 0.06	b
Con	As	868 ± 23	0.005 ± 0.0004	b	0.002 ± 0.0002	a	0.01 ± 0.001	c
	Cd	14.4 ± 0.4	0.03 ± 0.02	a	0.25 ± 0.05	b	0.37 ± 0.12	b
	Pb	1635 ± 57	0.002 ± 0.001		0.001 ± 0.0002		0.002 ± 0.001	
	Zn	437 ± 8	0.05 ± 0.02		0.07 ± 0.01		0.09 ± 0.02

Figure 4.8 Concentration ratios between Cd and Zn in tissues of Szarvasi-1 (*Elymus elongatus* L. supsp. *ponticus* cv. Szarvasi-1) related to that in soil (NH_4NO_3 -solution) (a) and in shoots related to roots (b) in untreated (Con) and treated soils (LM: lime marl, A-x and B-x: Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in % (m/m)).

Figure 4.9 Trace element concentrations ($\text{mg kg}^{-1} \text{DW}^{-1}$) in roots of the perennials Szarvasi-1 (*Elymus elongatus* L. supsp. *ponticus* cv. Szarvasi-1) and cup plant (*Silphium perfoliatum* L.), and in those of the annuals winter oilseed rape (*Brassica napus* L. cv. Lorenz), sunflower (*Helianthus annuus* L. mutant inbred line M7), and winter wheat (*Triticum aestivum* L. cv. Tiger). Values are means $\pm$ SD ($n = 4$). Different letters represent significant differences ($p < 0.05$).

5 Synthesis

5.1 The potential of crop management practices to mitigate food chain transfer of trace elements

Performance of regional species and cultivars of annual plants under field conditions

Crop species and cultivars suitable for food, animal feed, or bioenergy production on contaminated arable land are current objects of research (Kidd et al., 2015; Chen et al., 2016; Rizwan et al., 2016; Mayerová et al., 2017; He et al., 2017), since such sites are needed to meet the demands of a rising global population (Abhilash et al., 2016). For the above-mentioned valorization options the TE concentration in the produced biomass is to be minimized to achieve compliance with legal thresholds and acceptance among end-users (EC, 2002; Bert et al., 2017).

Since the commercially available cultivars of annual plants change rapidly, the access of farmers to regionally adapted excluder genotypes and/or cultivars depends on the provision of appropriate information. These should ideally be provided by the authorities in contaminated regions based on screening programs, as practiced in Saxony (BfUL/LfL, 2002-2018; Kidd et al., 2015; BfUL, 2017). In many countries such information are missing. This applies even more to data obtained from practical adoption of GRO on TECS, which are scarce, particularly across Europe (Cundy et al., 2016). Moreover, screenings of excluder genotypes/cultivars mostly focus on Cd as an element of concern (Greger and Löfstedt, 2004; Kidd et al., 2015; Rizwan et al., 2016; He et al., 2017). Hitherto, the effects on other TE such as As (Shi et al., 2015; Zvobgo et al., 2018) or Pb (Liu et al., 2015), particularly in multielement-contaminated soils (Friesl-Hanl et al., 2009; Gao et al., 2011), have been less well studied for major food crops other than rice.

These issues were addressed in chapter 2 of this thesis. It was demonstrated that field-grown cereal cultivars varied considerably in TE accumulation. Thereby, official results from greenhouse studies regarding grain Cd uptake were confirmed, albeit at a lower level of variation between high- and low-accumulating cultivars. Moreover, differential effects on vegetative and generative organs and reverse impacts on As uptake in wheat grains had to be noted. Low-Cd cultivars of cereals concurrently exhibited relatively low Zn accumulation, suggesting a conflict between safe and nutritious food production. However, as already found by Gao et al. (2011) and Shi et al. (2015), grain Cd and Zn or As were not correlated. This implies, that the breeding of Cd excluders could be attained at simultaneous exclusion of As

and biofortification with Zn, which presents an important effort against malnutrition (Cakmak and Kutman, 2018).

The species- and cultivar-specific TE accumulation is summarized in Figure 5.1. For this purpose, data from vegetative and generative tissues of field-grown winter oilseed rape, winter wheat, and spring barley, presented in chapter 2, were aggregated to shoot concentrations and subjected to a Principal Component Analysis (PCA) using R software (version 3.5.1, The R Foundation, Vienna, Austria). The axes of the first and the second Principal Component (PC) explained 57.3 % and 33.3 %, respectively, of the variation in the dataset. As illustrated by its position on the graph of individuals (Figure 5.1 a)), barley accumulated the least of all elements, thus presenting a favorable species for crop production on TECS. The lower metals accumulation in barley than in wheat under comparable soil conditions confirms previous findings (Adams et al., 2004; Brunetti et al., 2012). However, the growth of both *Poaceae* species was inhibited in the strongly polluted soil under study, restricting their suitability to moderate contamination levels that can be well tolerated (Brunetti et al., 2012).

Figure 5.1 Results of a Principal Component Analysis (a) performed on TE concentrations in shoots of winter oilseed rape (*Brassica napus* L. cv. Visby (A) and cv. Lorenz (B)), winter wheat (*Triticum aestivum* L. cv. Türkis (A) and cv. Tiger (B)), and spring barley (*Hordeum vulgare* L. (cv. Salome (A) and cv. Marthe (B)) grown under field conditions in untreated contaminated soil. The B-cultivars of cereals represent high-Cd-accumulating cultivars according to official greenhouse studies. The plot of variables (TE) on the components (b) is colored according to each element's contribution to the component.

Wheat or rape were characterized by relatively high shoot accumulation of Zn and As or Cd and Pb, respectively (Figure 5.1 a)). This induced the corresponding, untypical, grouping of these elements on the graph of variables (Figure 5.1 b)), where a more close positioning of Cd and Zn would have been expected since they are cognate elements. Thus, depending on the mixture of elements present in a contaminated soil and the targets pursued with the cultivated

biomass, both plant species could serve different purposes. In case of wheat, however, this notably depends on cultivar choice.

The observed uptake patterns might be attributed to the fact that *Brassicaceae* and *Poaceae* fundamentally differ regarding strategies for nutrient acquisition upon deficiency. Both release specific root exudates to the rhizosphere, where these in turn interact with specific micro-organisms (Marschner et al., 1989; Dakora and Phillips, 2002). *Brassicaceae*, as most non-graminaceous plants, predominantly excrete protons and release reducing and chelating compounds (Strategy I plants), whereas graminaceous plants like *Poaceae* release phytosiderophores (PS) and have a high-affinity uptake system for ferrated PS (Strategy II plants). However, due to the low specificity of the released compounds, a concomitant mobilization of metals and As occurs in contaminated soils (Römheld and Marschner, 1986; Römheld and Awad, 2000; Fitz and Wenzel, 2002). The relatively high accumulation of Zn and As in the studied wheat (*Triticum aestivum* L. cv. Tiger) is possibly due to larger release of PS, which can vary considerably in amount and chemical nature even among cereal cultivars (Römheld and Marschner, 1990; Cakmak et al., 1996; Shenker et al., 2001). As compared with other cereal species, (bread) wheat has been classified as Fe-efficient due to high PS exudation (Römheld and Marschner, 1990; Römheld and Awad, 2000). However, TE acquisition by plant roots and subsequent translocation to shoots is governed by several mechanisms. In chapter 4 we demonstrated that these differ among graminaceous plants cultivated in the same growth media. Thereby, the presence of various elements for interaction in antagonistic or synergistic ways further add to the complexity in multielement-contaminated soils (Siedlecka, 1995; Siedlecka and Krupa, 1999; Mendoza-Cózatl et al., 2011; Puschenreiter et al., 2013; Brackhage et al., 2015; Neu et al., 2018a).

Performance of mutagenized and novel non-food energy or fiber plants under semi-controlled conditions

In addition to the field assay, two further crop management options were investigated under semi-controlled conditions (chapters 3 and 4). The mutual target was to assess whether and how phytoremediation options could enable the sustainable production of biomass in the present multielement-contaminated soil.

The first approach focused on labile TE phytoextraction, also referred to as bioavailable contaminant stripping (Fitz et al., 2003; Herzig et al., 2014). Hitherto, little research has been dedicated to the investigation of pre-crops that could attenuate TE accumulation in subsequent cereals like wheat (Oliver et al., 1993; Khoshgoftarmanesh and Chaney, 2007; Gao et al.,

2010; Greger and Landberg, 2015) or barley (Puschenreiter et al., 2013) from contaminated soils. Existing studies focused on Cd only except for Puschenreiter et al. (2013), who additionally investigated Zn. None of the studies examined a broader spectrum of TE present in typical (post-)mining regions (Tóth et al., 2016b).

These issues were addressed in chapter 3 of this thesis. Winter oilseed rape, commonly cultivated prior to cereals in the study area, was compared with a sunflower (*Helianthus annuus* L. mutant inbred line M7 (R3B-F-U/R13M10A; test series R13F-MP; Nehnevajova et al. 2007), characterized by enhanced growth and metal accumulation as a result of chemical mutagenesis, regarding pre-crop effects on subsequent wheat. Previously, such enhanced sunflowers had been successfully cultivated for labile Zn phytoextraction, the effectivity of which, however, was assessed by soil chemical analyses instead of biological endpoints (Herzig et al., 2014). In contrast to a study in saline soils, where a non-enhanced sunflower mitigated Cd uptake in post-cultivated wheat grains (Khoshgoftarmanesh and Chaney, 2007), our results showed an increment of TE accumulated in subsequent wheat compared to individuals cultivated following rape. Instead of labile TE phytoextraction, rhizosphere processes, particularly antagonistic effects on soil acidity, seemed to be the main drivers for these differential impacts (chapter 3.1). This confirms earlier findings from an approach cultivating *Salix smithiana* prior to barley (Puschenreiter et al., 2013). Based on enhanced available TE and relatively low pH in soil, it was concluded that root exudates of enhanced sunflower or willow, likely enabling their accumulation efficiency, mobilized more TE in the short term than could be depleted from the labile pool (Puschenreiter et al. 2013; chapter 3.1). In such cases, longer phytoextraction periods might be required before target crops, in which TE accumulation is to be minimized, are cultivated (Kidd et al., 2015). However, successful examples with sunflower (Khoshgoftarmanesh and Chaney, 2007) or willow (Greger and Landberg, 2015) lead to the conclusion that these processes largely depend on soil conditions and plant genotype/cultivar.

The second management approach examined the cultivation of two novel perennial non-food plants alternative to annual crops, which are currently grown in the study area. With regard to the relevant pollutant linkages at the study site (chapter 1.2), perennial plants would generally bring about two advantages. First, phases of bare soil, favoring wind erosion and leaching of TE, would be avoided. Second, fertilization and tillage operations could be reduced (Culman et al., 2010; Pimentel et al., 2012), which further minimizes the emission of contaminated dust.

Both the cup plant and a Hungarian breeding of tall wheatgrass called Szarvasi-1 (chapter 4.2) can be valorized as fodder or as energetic and industrial feedstock. Recently, they have gained in significance among farmers in the region (Biomass Association Freiberg, 2013) and among stakeholders from the Central European bioenergy sector (Mast et al., 2014; Schoo et al., 2017; Bernas et al., 2019), since they combine low input requirements compared to annuals (Csete et al., 2011; Gansberger et al., 2015; Bernas et al., 2019) with phytostabilization capabilities, as identified in few existing studies (Zhang et al., 2010; Vashegyi et al., 2011; Sipos et al., 2013; Mayerová et al., 2017). However, these studies were based on single TE exposure and conducted under artificial conditions, except for that of Mayerová et al. (2017). Moreover, none of the studies represented contamination levels as high as in the study area investigated in this thesis.

Therefore, chapter 4.2 addressed the suitability of these promising crops to be cultivated in a heavily contaminated soil under more realistic, semi-controlled, experimental conditions. Unlike Szarvasi-1, cup plant did not tolerate a certain or the mixture of TE present at high concentrations in the study soil. However, its exceptionally low Cd accumulation could enable successful phytostabilization at more moderately Cd-contaminated sites (Zhang et al., 2010). The phytoexclusion potential of Szarvasi-1 strongly depended on the harvest regime, since considerably higher TE concentrations were obtained in the second harvest compared to the first, as found for other perennial grasses (Mayerová et al., 2017). A sustainable management could therefore be to use only the summerly harvest for animal consumption, whereas the second harvest in autumn could be valorized in the energy or fiber industry.

For a species comparison between annual and perennial plants, data from the greenhouse studies described in chapters 3 and 4 were aggregated to shoot concentrations and subjected to a PCA. The PC axes 1 and 2 explained 83 % and 12.3 % of the variation in the dataset (Figure 5.2). As illustrated by their position on the graph of individuals (Figure 5.2 a)), the perennials excluded TE in shoots whereas sunflower enriched metals, as had been intended by chemical mutagenesis (Nehnevajova et al., 2007; chapter 3.1). Rape and wheat ranged in midfield regarding metal uptake, while, as in the field study, wheat accumulated the most As.

The TE status in vegetative and generative tissues, respectively, in relation to regulatory thresholds, as reported in chapters 2 to 4, requires adaptations during harvest management. If neither grain nor straw biomass is suitable for animal consumption, which can be assessed by a pre-harvest control routine as offered by the authorities in Saxony (BfUL, 2015; Kidd et al., 2015), whole plant harvest is recommended, instead of threshing, for subsequent energy

conversion. As a potential alternative to the latter, straw biomass not suitable for animal consumption could be returned to soil for humus formation. However, preliminary studies are advisable to prevent adverse effects on the TE mobilization in soil and growth or TE accumulation in post-cultivated crops (Habiby et al., 2014; Wang et al., 2015).

Figure 5.2 Results of a Principal Component Analysis (a) performed on TE concentrations in shoots of winter oilseed rape (*Brassica napus* L. cv. Lorenz), winter wheat (*Triticum aestivum* L. cv. Tiger), sunflower (*Helianthus annuus* L. mutant inbred line M7), cup plant (*Silphium perfoliatum* L.), and tall wheatgrass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1; first (1) and second (2) harvest) grown under semi-controlled conditions in untreated contaminated soil. The plot of variables (TE) on the components (b) is colored according to each element's contribution to the component.

In summary, the field and pot experiments suggest that species and cultivar choice, as well as harvest management, hold a large and non-invasive potential to sustainably produce and valorize biomass even from soils heavily co-contaminated with metals and As. The investigated perennials and barley cultivars proved more suitable to minimize toxic pollutants transfer into phytoproducts than the other annuals under study. The occasional inclusion of accumulating plants into crop rotations with the aim of reducing TE availability to subsequent target crops can effect just the opposite and requires further research (see chapter 6).

5.2 The *in situ* stabilization efficacy under differential experimental conditions

Phosphates and liming agents

The *in situ* stabilization of soils contaminated by metals and As, which partly exhibit antagonistic properties considering sorption or complexation processes with constituents of

the soil matrix, is challenging and techniques are not yet sophisticated (Lee et al., 2011; Komárek et al., 2013).

At moderate soil acidity, as prevailed in the contaminated soil under study, common fertilizers like phosphates and liming agents can, in principle, simultaneously immobilize the targeted contaminants Cd, Pb, Zn, and As (Porter et al., 2004; Kumpiene, 2010; Seshadri et al., 2017; Qayyum et al., 2017). This was examined in chapter 2 of this thesis under real field conditions, meaning that the site owner/farmer conducted all agricultural measures according to the regional agricultural practice. On site, the contamination levels varied considerably in space and depth. This is basically typical of TECS (Mench et al., 2006; Burgos et al., 2006; Lamé, 2011). However, as found earlier (Millis et al., 2004), it substantially affected plant growth and TE accumulation, thus aggravated risk assessment, and required complex statistical approaches, such as the applied CoIA, to differentiate treatment effects from heterogeneous pre-conditions. As hypothesized, the combined soil application of phosphate and lime marl decreased labile metals concentrations more effectively than single applications. However, neither treatment decreased As mobility. Presumably due to inhomogeneous incorporation and varying soil moisture (Friesl et al., 2006; Komárek et al., 2013), lime marl treatments reduced soil acidity and labile metals much less than in corresponding studies under semi-controlled conditions (chapter 4). Moreover, plant TE concentrations remained more strongly correlated with pseudo-total instead of labile TE in treated soils. Given the poor effects the studied fertilizer treatments had compared to non-invasive measures like species and cultivar choice (chapter 2, chapter 5.1), we concluded that particularly the application of P as a scarce resource in amounts exceeding plant demands needs to be reconsidered, since its chemical composition causes further TE entry into soils (Grant et al., 2010; Kratz et al., 2016; Table 5.1).

Drinking water treatment residuals

Multifunctional agents suitable for the *in situ* stabilization of soils co-contaminated with metals and As are (hydr)oxides of Fe, Al, or Mn (Kumpiene et al., 2008; Komárek et al., 2013), since they exhibit extraordinary sorption properties due to a particle size-mediated high specific surface area and great stability at typical soil pH values (Sposito, 2008). Ideally, their application should be combined with liming agents to maintain/create moderate pH conditions for optimal binding of metals and As in soil (Warren and Alloway, 2003; Hartley et al., 2004; Lee et al., 2011). Thereby, the use of industrial by-products is desirable to couple remediation efforts with waste recycling. Worldwide, huge amounts of WTR are generated as by-products

during drinking water purification that unite all of the above-mentioned criteria (Ippolito et al., 2011; Bai et al., 2014; Alvarenga et al., 2018). However, large quantities are still being disposed to landfills. Sustainable ways of handling and reuse are therefore current objects of research (Ahmad et al., 2016), including the potential advantages and hazards of WTR applied to agricultural soils (Dassanayake et al., 2015; Howells et al., 2018; RoyChowdhury et al., 2018). Recently investigated benefits of land-applied WTR comprised P capture within catchment areas (Ippolito, 2015) and the remediation and recovery of contaminated soils through TE immobilization (Manzano et al., 2016; Garau et al., 2017; chapter 4.1). Significant improvements of soil organic matter status and water holding capacity have been reported earlier (Bugbee and Frink, 1985; Ulén et al., 2012) but plant growth inhibition due to strong P sorption by Al/Fe has been identified as a major drawback in agricultural soils (Bugbee and Frink, 1985; Lombi et al., 2010). In Germany, land application in agriculture and forestry currently represents a minor reuse option compared to wastewater treatment or industrial use, due to legal restrictions arising from potentially toxic elements present in WTR (DüMV, 2012; Wiedemann, 2017). Their elemental composition can vary largely depending on the utilized coagulant and the chemistry of the processed water (Lombi et al., 2010; Ippolito et al., 2011; chapter 4.1). As illustrated in Table 5.1, the use of both WTR investigated in the present thesis would be impeded by the German fertilizer ordinance (DüMV, 2012) due to excess As (WTR_A) or Cd, Ni, and Tl concentrations (WTR_B). However, the use of the P fertilizer, obtained from a regional agricultural cooperative, would equally be prohibited due to noncompliant levels of Cd and Ni, which presents no singularity (Kratz et al., 2016).

Table 5.1 Overview of mean TE concentrations (mg kg⁻¹ DW⁻¹; *n* = 3) in the studied fertilizers (lime marl: LM, superphosphate: P 40; it contains 40 % P₂O₅) and drinking water treatment residues (Fe-based: WTR_A, Mn-based: WTR_B) related to legal thresholds for fertilizers or soil additives (DüMV, 2012).

	LM	P 40	WTR _A	WTR _B	threshold
As	9	7	57	13	40
Cd	1	27	1	4	1.5 ^b
Pb	17	3	28	4	150
Cr	12	170	4	236	(-) ^c
Ni	74	133	43	435	80
Zn	5	n.a. ^a	80	677	-
Tl	0.2	0.8	0.02	1.8	1

^a superphosphates sold in Germany typically contain 469 ± 136 mg Zn kg⁻¹ DW⁻¹ (Kratz et al., 2016)

^b for fertilizers > 5 % P₂O₅ (FW) the threshold is 50 mg kg⁻¹ P₂O₅⁻¹

^c a threshold of 2 mg kg⁻¹ DW⁻¹ is defined for Cr^{VI} instead of total Cr

Data are highlighted with bold font, if legal thresholds are exceeded

The above implies that beneficial land-application of WTR in agriculture requires careful selection and appropriate dosage to assure adequate crop harvests and quality. To date, almost no studies have investigated the response of wheat to WTR-amended TECS (Garau et al. 2014; chapter 4.1). Moreover, no research has been dedicated to dose-dependent impacts of WTR on earthworms in such environments. Recently, the first study examined the performance of *Eisenia fetida* and its WTR-borne element assimilation in an amended uncontaminated soil without evidence of harmful effects at realistic application rates (Howells et al., 2018). However, soil dwelling species other than *Eisenia fetida/andrei* have long been, and are still, underrepresented in ecotoxicological studies, especially considering (amended) soils naturally contaminated with TE (Nahmani et al., 2007). Furthermore, it is well accepted that conventional single or sequential extractions often fail to predict TE bioavailability in residual-treated soils (Basta et al., 2005). However, virtually no studies exist using more comprehensive assessment tools, like DGT, in WTR-amended soils.

The aforementioned issues were addressed in chapter 4 of this thesis under semi-controlled conditions. Therein, chemical methods (NH_4NO_3 -solution, DGT, soil solution) were accompanied by bioassays, in which wheat (*Triticum aestivum* L. cv. Tiger) and earthworms (*Dendrobaena veneta*; chapter 4.1), or Szarvasi-1 (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1; chapter 4.2) were exposed to a soil co-contaminated with metals and As, that had previously been treated with lime marl or WTR dominated either by Fe- or Mn- (hydr)oxides. Basically, the assays with wheat and Szarvasi-1, the first of which covered a period of nine and the second of seven months, rendered surprisingly coherent results on the *in situ* stabilization efficiency. The Fe-rich WTR_A applied at a rate of 1 % maximally decreased potentially plant-available As, Cd, and Pb in wheat- or Szarvasi-1-cultivated soils, namely by 77 % or 76 %, 46 % or 37 %, and 61 % or 59 %, respectively. By contrast, lime marl, applied in amounts of 0.4 kg m⁻², most efficiently immobilized soil Zn in the plant assays (respectively, by 39 % or 41 %). In disregard of certainly existing plant impacts, the above stated data suggest, together with increasingly attenuated plant TE accumulation over the respective control between the first and the second harvest of Szarvasi-1 (chapter 4.2), that the immobilization capacity of the WTR_A-amended soils still increased after several months. These results are particularly important against the background of potential threats to the groundwater arising from excess leaching of As at the study site (see chapter 1.2), which could possibly be controlled by Fe-/Al-rich WTR in the long term. This assumption is supported by Nielsen et al. (2011), who effectively minimized As leaching from a polluted

soil over a duration of three years, even under temporarily waterlogging conditions, by an Fe-rich WTR applied at a rate of 2.5 % (*m/m*).

The biomass and tissue As concentrations of wheat and earthworms were well correlated with bioavailable As in soil and, therefore, responded most positively to WTR_A-treatments (chapter 4.1). This was not observed in case of Szarvasi-1, which showed no significant response in biomass production to any of the treatments (chapter 4.2). Unlike As, the availability of Cd and Zn to biota could not be predicted by any of the chemical surrogates tested. This could be attributed to endpoint-specific binding of competing cations (Cd^{2+} , Zn^{2+} , H^{+}) to the biotic ligand (roots, earthworm tissue), and a preferential transport of Zn from roots into aboveground plant tissues (chapters 4.1 and 4.2). The measurements with DGT and in soil solution indicated that no diffusion limitations prevailed for Zn in the highly contaminated soil under study (chapter 4.1), which is, however, a prerequisite for the predictability of plant-uptake by the DGT-measured fluxes (Degryse et al., 2009).

In a PCA conducted with shoot TE concentrations (Figure 5.3), all wheat samples grouped distinctly apart from those of Szarvasi-1, since the former generally accumulated more As, Cd, and Zn. On the other hand, Szarvasi-1 partly exhibited higher Pb concentrations, particularly when treated with lime marl, to which it responded contrary to wheat, where the concentrations decreased compared to the control. In summary, the choice of plant species surpassed the potential of the investigated soil additives to attenuate food chain transfer of As and Cd, both classified as high risk elements (Rodríguez-Eugenio et al., 2018). Since Szarvasi-1 moreover exhibited a higher tolerance and can be grown less resource-intensive (Csete et al., 2011) than annuals, its cultivation can be recommended in soils highly contaminated with the given mixture of TE. The biomass response of wheat and earthworms to WTR-amended soils clearly reflected, that application rates above 0.5 % involved environmental risks via the creation of P deficiency (WTR_A) or excess exposure to potentially toxic elements (WTR_B), such as Ni (chapter 4.1). However, even this application rate might necessitate higher than normal P supplementation or other measures, which help compensate for eventual deficiency over time (chapter 4.1).

Figure 5.3 Results of a Principal Component Analysis (a) performed on TE concentrations in shoots of winter wheat (*Triticum aestivum* L. cv. Tiger; depicted to the right of axis PC 1) and tall wheatgrass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1; first harvest, depicted to the left of axis PC 1) grown under semi-controlled conditions in untreated (Con) and treated contaminated soil (LM: lime marl, A-x- and B-x: Fe-based (WTR_A) and Mn-based (WTR_B) drinking water treatment residue, where x is the application rate in percentage (m/m). The plot of variables (TE) on the components (b) is colored according to each element's contribution to the component.

6 Future perspectives

This thesis demonstrates that excluder cultivars of cereals can help attenuate food chain transfer of TE, especially through reductions in grain Cd accumulation. However, the breeding of Cd-excluding cultivars, e.g. of bread wheat as a major staple crop, is still in its infancy, since the value of identified molecular markers involved in Cd accumulation for the selection of excluder phenotypes could not yet be verified (Guttieri et al., 2015). Hence, the constant demand for new marketable cultivars requires more research on the identification of early predictors for low grain Cd accumulation. This would enable breeders to consider relevant traits, besides others like yield, composition, or resistance to specific biotic or abiotic stressors, within the relatively small time frame prior to the next sowing (Liu et al., 2018). Once established, such traits should become mandatory for the release of new cultivars, as e.g. practiced in Canada (Grant et al., 2008). Moreover, while the independency of Cd minimization from biofortification with Fe and Zn can be deemed certain (Guttieri et al., 2015; Liu et al., 2018), more research is needed on the possibilities of simultaneous selection and/or breeding for low As and Pb cultivars.

It is shown, that labile TE phytoextraction can, in the short-term, be superimposed by rhizosphere effects, which may unintentionally enhance TE availability to subsequent target crops. In order to produce the desired attenuating effect by including phytoextracting plants into crop rotations, further research is needed regarding (i) long-term studies and (ii) eventual countermeasures to trap excessively mobilized TE in the rhizosphere of accumulating plants. Additionally, relevant traits should be explored in order to identify plant species, genotypes/cultivars, or mutants that specifically take up TE from pools in soil utilized by roots of cereals to be grown subsequently. Such characteristics are likely pivotal to apparently contradicting results obtained e.g. for pre-crop effects of *Salix viminalis* (Greger and Landberg, 2015) and *Salix smithiana* (Puschenreiter et al., 2013).

It is further pointed out, that Fe-/Al- rich WTR can strongly immobilize As, Cd, and Pb in soil, hence mitigate leaching to the groundwater (Nielsen et al., 2011), and improve the habitat function of contaminated soils for biocenoses, e.g. via reduced As bioavailability. However, as reviewed by Komárek et al. (2013), some drawbacks have been reported, which raise concern about the long-term stability of the applied or transformed oxides and co-precipitated or adsorbed TE in treated soils under field conditions. Therefore, the execution of long-term studies with naturally and aged contaminated soils, including ecotoxicological assays, is vital to assess whether and under which environmental conditions a beneficial reuse in contaminated land management can be warranted. Based on the findings presented in this thesis, the crucial point in terms of a sustainable use is to choose WTR sources and application rates, which balance the immobilization of TE with P availability, while avoiding excess entry of other potentially toxic elements into the soil. As attempted in the past for P-deficient WTR-treated soils by Oladeji et al. (2007), future research should include different agriculturally relevant P-sources and their effects on the P storage capacity of soils. Moreover, as recently examined with banded instead of broadcast placement methods in urban soils (Tay et al., 2017), different incorporation techniques of P fertilizers applicable in agricultural practice should be investigated to assure sufficient bioavailability to plants in the presence of WTR. This is crucial for ensuring economically viable yields while saving scarce P resources. A conceivable alternative would be to mobilize soil P resources via silicon (Si) application (Neu et al., 2017). However, any Si source should be tested in preliminary studies to prevent adverse effects on plant-available As, since it competes, just as P, with Si for adsorption sites in soil (Seyfferth and Fendorf, 2012). Microbial inoculants, e.g. with TE-resistant bacteria (Ghosh et al., 2015), could render P more plant-available in a sustainable way. Depending on

the P-mobilizing strategy, however, care must be taken to avoid collateral effects on metals and As (Mailloux et al., 2009; Jones and Oburger, 2011).

Finally, it is demonstrated that the Hungarian breeding of tall wheatgrass, Szarvasi-1, presents a perennial non-food crop that tolerates high-level co-contamination of metals and As while concentrating less of these elements in shoots than several annual crops. According to own surveys among operators of combustion or anaerobic digestion plants throughout Saxony, biomass originated from phytotechnologies is most likely to be processed in such installations when TE concentrations are as low as possible (Bert et al., 2017). Therefore, Szarvasi-1 would gain better acceptance as a feedstock for bioenergy production than e.g. the straw of all investigated annual crops, for which energy conversion would currently present the only economically viable valorization option. However, the legal status of phytomass produced on TECS, defining whether it is considered waste or biomass, is not yet clarified at European or national level (Bert et al., 2017). Thus, the initial question "What are the key sustainability challenges for the crop production from multiple and heavily contaminated sites?" (Abhilash et al., 2016) must also be conceived from its end. This urgently requires that these regulatory gaps be filled to generate planning security for farmers and operators of biomass power plants, and to enable the use of heavily contaminated land in accordance with sustainability criteria laid down in the Renewable Energy Directive (EU, 2001, 2015; EC, 2009).

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List of publications

Research articles

- Neu S, Müller I & Dudel EG (2020):** Management of trace element-contaminated agricultural land by *in situ* stabilization combined with phytoexclusion over a three years crop rotation. *International Journal of Phytoremediation*; doi: 10.1080/15226514.2020.1726869.
- Neu S, Müller I & Dudel EG (submitted to *Environmental Science and Pollution Research*):** (Aided) phytostabilization of trace elements using cup plant (*Silphium perfoliatum* L.), or tall wheatgrass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) and soil-applied drinking water treatment residues.
- Xue K, Van Nostrand JD, Zhou J, Neu S, Müller I, Giagnoni L, Renella G (in review: *Soil Biology and Biochemistry*):** Changes in microbial functional diversity and activities of a trace element polluted soil managed with willow short rotation coppice.
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- Quintela-Sabarís C, Marchand L, Kidd P, Friesl-Hanl W, Puschenreiter M, Kumpiene J, Müller I, Neu S, Janssen J, Vangronsveld J, Dimitriou I, Siebielec G, Galazka R, Bert V, Herzig R, Cundy AB, Oustrière N, Kolbas A, Galland W, Mench M (2017):** Assessing phytotoxicity of trace element-contaminated soils phytomanaged with gentle remediation options at ten European field trials. *Science of the Total Environment* 599-600:1388-1398; doi:10.1016/j.scitotenv.2017.04.187.

- Kidd P, Mench M, Álvarez-López V, Bert V, Dimitriou I, Friesl-Hanl W, Herzig R, Janssen JO, Kolbas A, Müller I, **Neu S**, Renella G, Ruttens A, Vangronsveld J, Puschenreiter M (2015): Agronomic practices for improving gentle remediation of trace element-contaminated soils. *International Journal of Phytoremediation* 17:1005-1037; doi:10.1080/15226514.2014.1003788.
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Talks and poster presentations

- Müller I, Klose R & **Neu S** (talk): Phytomanagement of arable TECS using cultivar variation and amendments – results from pot and field trials in Saxony (Germany). ICOTBE 2017, 16th to 20th July 2017, Zürich, Switzerland.
- Neu S** & E. Gert Dudel (poster presentation & contribution to conference proceedings): Akkumulation und *in vivo* Stressindikation von $n\text{SiO}_2$ und $n\text{CeO}_2$ in Winterweizen. Clustermeeting NanoCare 4th to 5th Mai 2017, Eggenstein-Leopoldshafen, Germany.
- Neu S**, Hoppe M, Fries E & E. Gert Dudel (poster presentation & contribution to conference proceedings): Verbleib und Langzeitwirkung von SiO_2 - und CeO_2 -Nanomaterialien im System Boden – Pflanze. Clustermeeting NanoCare 3rd to 4th Mai 2016, Frankfurt am Main, Germany.
- Neu S** & I. Müller (talk): Gentle remediation options (GRO) at large-scale contaminated agricultural sites in Saxony, Germany. XII Symposium on Trace elements in the environment, 17th – 19th November 2014, Puławy, Poland.
- Neu S** & I. Müller (talk): Gentle remediation options (GRO) for the management of large-scale contaminated agricultural sites in Saxony, Germany. 4th International Conference on Managing Urban Land – Tailored and Sustainable Redevelopment towards Zero Brownfields, 14th – 16th October 2014, Frankfurt am Main, Germany.
- Neu S** & I. Müller (poster presentation & contribution to conference proceedings): Sanfte Sanierungsverfahren für den Umgang mit großflächig schadstoffbelasteten Flächen in Sachsen. Praxisbeispiele aus dem EU-Projekt GREENLAND (FP7). 5th Saxonian-Thuringian Soil Protection Days 2013 in Altenburg, Thuringia.

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Ich bestätige, dass ich die Promotionsordnung der Fakultät Umweltwissenschaften der TU Dresden anerkenne.

Dresden, 24.06.2019

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