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Gradual Accumulation of Heavy Metals in an Industrial Wheat Crop from Uranium Mine Soil and the Potential Use of the Herbage

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Abstract: Testing the quality of heavy-metal (HM) excluder plants from non-remediable metalliferous soils could help to meet the growing demands for food, forage, and industrial crops. Field cultures of the winter wheat cv. JB Asano were therefore established on re-cultivated uranium mine soil (A) and the adjacent non-contaminated soil (C). Twenty elements were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) from soils and plant sections of post-winter seedlings, anthesis-state, and mature plants to record within-plant levels of essential and toxic minerals during ripening and to estimate the (re)use of the soil-A herbage in husbandry and in HM-sensitive fermentations. Non-permissible HM loads ($mg \cdot kg^{-1} \cdot DW$) of soil A in Cd, Cu, and Zn of 40.4, 261, and 2890, respectively, initiated the corresponding phytotoxic concentrations in roots and of Zn in shoots from the seedling state to maturity as well as of Cd in the foliage of seedlings. At anthesis, shoot concentrations in Ca, Cd, Fe, Mg, Mn, and Zn and in As, Cr, Pb, and U had fallen to a mean of 20% to increase to 46% during maturation. The respective shoot concentrations in C-grown plants diminished from anthesis (50%) to maturity (27%). They were drastically up/down-regulated at the rachis-grain interface to compose the genetically determined metallome of the grain during mineral relocations from adjacent sink tissues. Soil A caused yield losses of straw and grain down to 47.7% and 39.5%, respectively. Nevertheless, pronounced HM excluder properties made Cd concentrations of 1.6–3.08 in straw and 1.2 in grains the only factors that violated hygiene guidelines of forage (1). It is estimated that grains and the less-contaminated green herbage from soil A may serve as forage supplement. Applying soil A grains up to 3 and 12 in Cd and Cu, respectively, and the mature straw as bioenergy feedstock could impair the efficacy of ethanol fermentation by *Saccharomyces cerevisiae*.

Keywords: wheat crop; yield loss; Cd/Cu/Pb/Zn toxicity; grain fill regulation; forage supplement; biochar; biofuel fermentation; phytoextraction

1. Introduction

The worldwide loss of cropland to expanding urban communities and their infrastructure, to mining, and to areas of industrial heavy metal (HM) and radionuclide immissions is compounded by agricultural mismanagement, land erosion, increasing salinity [1,2], and the use of As, Cd, and U contaminated phosphate fertilizers [3–5]. Contemporarily, industrial and energy crops subsidized in favor of greenhouse gas-avoiding decarbonisations of the economy [6,7] displace food and forage production [2]. Land contaminated by mining and metallurgy with As, Cd, Cr, Cu, Ni, Pb, U, and Zn may gradually recover by the downwash of metal-clay complexes from the plough layer to subsoil strata (clay migration) within several decades [8,9]. Reducing the soils' *aqua-regia* HM load

down to 90%–50% by phytoextraction with plants takes hundreds to ten thousands of years [10–12]. An exclusion of non-remediable metalliferous soils from farming is therefore unacceptable.

With the production of 251,000 Mg uranium from 1946 to 1990, an area of 34 km^2 was depreciated in a densely populated East-German region with waste rock dumps, ore mill tailings, open pits, and subsurface mining activities [13,14]. During landscape restorations, contamination hot spots were sealed for long-term surveillance. The less contaminated sites were covered by up to 1 m of casing soil and afforested and turfed for (non)commercial use [9,15]. Local remnants of forgotten overburden soils elevated in As, Cd, Cu, Mn, Pb, U, and Zn were used to screen European crops for HM tolerance in pot and field cultures. Relative to HM excluder plants (bioconcentration factors < 1; [16]) such as bean, cereals, lupin, and maize, the herbage of potted HM sequestering plants (buckwheat, beet root species) contained up to 18-fold in trace metals. Herbage of 17 crops from uranium mine soils varied in the concentrations of As (32×), Cd (60×), Cu (5×), Ni (7×), Pb (7×), U (27×), and Zn (27×), and modified the cultivar-specific uptake of Cd by another 2.5 times [17].

The wide HM concentration spans of herbage formed in response to oversupply with soil minerals and nitrogen shrink drastically in field-grown seeds of cereals and legumes due to their strict indigenous mineral control [18–23]. Within the ears of wheat, the transfer of Zn to the grain is confined by two regulatory barriers. They are placed between the rachis and the grain, and between the grain's outer aleurone layer of three to four cells and the endosperm [24,25]. In pea plants, the more accidental metal concentrations in the herbage experienced drastic up- and down-regulations at the pod wall/seed coat interface. A further barrier was placed between the seed coat and the embryo, both of which were separated by a post-phloem-filled apoplastic space [19,26]. In this way, mineral concentration spans of 9–109× in Cu, Fe, Mn, Ni, and Zn within a set of tested field soils shrank to spans of 1.3–2× in whole peas and wheat grains [19,27]. This narrow span marked the target concentration point of the optimized and inherent seed metallome that ensures the vigorous and metal-stress-free development of the seedling. The strict HM excluder properties on the seed level result in food and forage-compatible seed crops even from soils with phytotoxic Cd, Cu, and Zn concentrations. Thus, cereals and legumes tend to reduce the critical Cd load of food and forage seeds to the permissible range of 0.1 and 1 $mg \cdot kg^{-1}$, respectively, fresh weight if grown on soils beyond the legislative limit of 3 $mg \cdot kg^{-1}$ [19,27,28]. In addition, seed crops allow for essential extensions of the permissible soil concentrations in As, Cd, Cu, Mn, U, and Zn, thus reducing pressure on the quality of soil remediation [19,29]. Nevertheless, the herbage of seed crops, which is in (potential) demand by husbandry, green chemistry, and bioenergy production is not under strict HM control. Therefore, its handling needs particular attention.

The global wheat production of 670 million metric tons, for example, in 2012 [30] yields 0.8-fold biomass in straw [31] by leaving the stubbles to the croplands' carbon cycle. Beyond the portion returned to agriculture and stock farming [32], 400 million tons of wheat straw per annum are expected to furnish the manufacturing of second-generation transportation fuel [33,34] to replace wheat grains and corn, which are presently used in bioethanol production [35]. A large spectrum of green-chemical precursors of lower MW can be derived from wheat straw by extraction and fast pyrolysis [36]. Its lignocellulose scaffold comprises 33%–40% cellulose in decay-resistant crystalline fibrils as well as 21%–26% hemicelluloses and 11%–23% lignin [32,35,37] connected by intimate structural links [38,39]. Apart from its content in HM, the key to its use as bioethanol feedstock is the cost-effective hydrolysis to its hydrocarbon monomers. The respective hexose sugars fermentable to ethanol or the gasoline surrogate, isobutanol, by microorganisms [40–42] are glucose as a cellulose monomer [43] and the glucose/galactose/mannose constituents of hemicelluloses [44]. Their major pentose sugars, D-xylose and L-arabinose, are not fermented [45]. Fungal cellulase mixes and beta-glucosidases [34,35,46] do not release more than 25% of the fermentable sugars that are theoretically present in the close lignocellulose complex of milled but untreated wheat straw [47]. In a hydrothermal pre-treatment, lignocellulose substrates such as timber, grasses, and straws are milled, amended with water, dilute mineral acids, alkali, or oxidizing agents and exposed to 25–260 °C at 1–12 MPa for minutes to

hours [38,47–50] to break the links between carbohydrates and lignin and between the carbohydrates themselves. Hydrolase treatments are then raising the yield of fermentable sugar monomers from 70% up to >90% [47,49]. Their fermentation by yeasts such as *Saccharomyces cerevisiae* and (recombinant) bacteria [49,51,52] results in ethanol outputs ranging from 62 to 111 $g \cdot kg^{-1}$ (equivalent to 78–140 $L \cdot Mg^{-1}$) of wheat or alfalfa straw at the lab scale [35,50,52,53]. The fermentations are impaired by microbial inhibitors derived from lignin and monomeric sugars during pre-treatment [53–56]. Furthermore, elevated Cd [57–59] and Cu [60] loads of the lignocellulose feedstock impair the vitality and efficacy of ethanologenic microorganisms.

With the objective of expanding farming to the non-remediable metalliferous soils from uranium mining, the fact that seeds respond much less to oversupply with (toxic) soil minerals than the associated herbage [18,20–23] was used. Thus, consecutive field cultures of wheat, rye, and pea plants were established on the aged and geologically related soils that formed concentration gradients from HM hot spots to non-contaminated sites. Concentrations of Cd, Cu, Pb, and Zn in grains of the winter wheat cv. JB Asano with 40%, 40%, 46%, and 66%, respectively, and 70%, 60%, 48%, 61%, respectively, remained below those of three high-performance wheat lines and one rye cv., respectively [27], but missed the low Cd values of pea seeds [19]. The HM load and the potential use of the associated herbage had not been reported. In this study, plant samples of field-grown JB Asano were drawn at the post-winter seedling state and at anthesis and maturity from the HM hot spot soil A and the non-contaminated reference soil C. The course of mineral macronutrient and (toxic) trace element concentrations was followed from the roots via different plant sections to the grains. The goal was to record the movements of essential and non-essential minerals among the plant sections during ripening as influenced by phytotoxic soil HM concentrations. The actual HM load in different parts of the flowering and mature herbage as well as in grains was then related to their permissible (re)use in nutrition and husbandry or to their applicability as an industrial crop in HM-sensitive fermentation processes.

2. Materials and Methods

2.1. Wheat Cultivar

The winter wheat cultivar JB Asano is an A-quality crop denoted by a high 1000-seed fresh weight (FW) and an average raw protein content. An 11-ha field culture was established in fall, 2013 in Ronneburg district (Germany).

2.2. Soils

With the local inclusion of aged metalliferous overburdened soil from uranium mining into non-contaminated cropland, a gradient of geologically related soils with diminishing HM concentrations had been formed (refer to Table 1 for mineral concentrations) in the late 1960s. The clay-loam soil was derived from Permian limestone, mottled sandstone, and dolomite [61]. Plots of around 120 m^2 were marked at the positions A (HM hot spot) and C (not impaired by mining) in September 2013. Shaping the test plots ensured homogeneity of $\leq$15% in the soil content of Ca and Mg and the main toxicants, As, Cd, Cu, and Zn, to minimize the effects of metal uptake competition. The soil mineral content was confirmed by the random collection of six soil samples per location (3 kg each) from the 20-cm plough layer in early May 2014. All the samples were analyzed independently. The soil samples A and C (sieve 0.8 mm) of pH_{aqu} 7.26 and 6.20 contained 3.53% and 4% in C_{org}, respectively.

Table 1. Evolution of the mineral concentrations in roots, shoots, and grains (mg·kg^{-1}·dry weight (DW)) of wheat cv. JB Asano grown on the metalliferous soil (A) and the reference soil (C) from the seedling state via anthesis to maturity. In parentheses, the concentrations of the individual elements in the soil A- to soil C-grown plant tissues are expressed as quotients from high:low values. Note the recommended concentration limits for arable soil, herbage, and food grains.

Element	Soil		Seedling 25 February		Anthesis 22 May		Maturity 18 July		Grains	Limits	
			Root	Shoot	Root	Shoot	Root	Shoot		Soil [a]	Herbs [b,c]
Macronutrients											
Ca	A	6650	1718	4660	2010 [d]	1626	3380	3230	287	-	-
	C	2400	1324	3906 [e]	1707 [e]	2020	2443	1308	257 [e]		
		(2.77)	(1.3)	(1.19)	(1.18)	(1.24)	(1.38)	(2.47)	(1.12)		
K	A	2270	24,420	32,355	5840	13,205	5910 [d]	10,940 [d]	4275	-	-
	C	2065	21,495 [e]	34,470 [e]	7730	16,710	5222 [e]	8600 [e]	4310 [e]		
Mg	A	3660	1160	2950	680	1016	1060	1230	1114	-	-
	C	5110	860	1713	669 [e]	965 [e]	576	433	1116 [e]		
	-	(1.4)	(1.35)	(1.72)	(1.02)	(1.05)	(1.84)	(2.84)	(1)		
N	A	68.1	ND	ND	ND	ND	11,000	3920	18,000	-	-
	C	87.5	ND	ND	ND	ND	ND	ND	ND		
P	A	670	2733	5190	530	1170	784	273	2606	-	-
	C	863	2783 [e]	4410 [e]	722	2220	486	356 [e]	3143		
	-	(1.29)	(1.02)	(1.18)	(1.36)	(1.9)	(1.61)	(1.3)	(1.21)		
Micronutrients											
(Cd)	A	**40.4**	56.9	13.1	25.5	1.6	29.4 [d]	3.08	1.2	3	0.05–0.4
	C	1.38	1.2	0.5	0.864	0.097	0.610 [d]	0.100 [d]	0.075	*0.3*	
	-	(29.3)	(47.4)	(26.2)	(29.5)	(16.5)	(48.2)	(30.8)	(16)	-	
Cu	A	**261**	45.5	13.5	37.2 [d]	3.83	65.6	2.80 [d]	4.94	100	2–20
	C	50	11	9.1	13.5 [d]	7.35 [d]	11.6 [d]	8.94	3.98	*50–100*	
	-	(5.22)	(4.14)	(1.48)	(2.76)	(1.92)	(5.66)	(3.19)	(1.24)	-	
Fe	A	18,510	635	200	666 [d]	27.6	2410	33 [d]	24.6	-	-
	C	31,750	498 [e]	188 [e]	2620	66.4	1840	43.0 [e]	36		
	-	(1.72)	(1.28)	(1.06)	(3.93)	(2.41)	(1.31)	(1.3)	(1.46)		
Mn	A	**1780**	56.7	64.5	65 [d]	16.8	217	19.1 [d]	20	40–1000	14–30
	C	**1540**	37.3	29.8	149	18.9 [e]	119	6.47	18.4 [e]		
Ni	A	43	5.22	1.74	5.38 [d]	1.09 [d]	11.8	0.605	2.09	100	0.1–3
	C	37.9	1.28	0.749	4.19 [e]	0.492	3.18 [d]	0.596 [d,e]	0.392	*40–50*	
Zn	A	**2890**	1570	649	787	276	1203	887	126	300	10–100
	C	208	102	42	49.4	19.3	32.7	7.18	27	*200–500*	
	-	(13.9)	(15.4)	(15.5)	(15.9)	(14.3)	(36.8)	(124)	(4.67)	-	

Table 1. *Cont.*

Element	Soil		Seedling 25 February		Anthesis 22 May		Maturity 18 July		Grains	Limits	
			Root	Shoot	Root	Shoot	Root	Shoot		Soil [a]	Herbs [b,c]
Non-essential elements											
As	A	**140**	6.54	1.4	6.95 [d]	0.275	20.2	0.752	0.109	20	0.01–1
	C	29	0.893	0.281	3.68	0.11	2.27	0.07	0.02	*30–40*	
	-	(4.83)	(7.32)	(4.98)	(1.89)	(2.5)	(8.9)	(10.7)	(5.45)	-	
Cr	A	16.4	1.62	0.79	0.92	0.072	4.2	0.108 [d]	0.075	100	0.1–1
	C	30.7	1.02 [e]	1.18 [e]	5.54	0.655 [d]	4.48 [d,e]	2.91	0.026	*150–200*	
Pb	A	148	4.96	1.56	5.64 [d]	0.11	20.3	0.303 [d]	0.045	100	0.1–6
	C	54	1.07	0.48	6.43 [e]	0.322 [de]	4.48 [d]	0.211 [d,e]	0.012 [e]	*250–300*	
U	A	**29.9**	4.83	0.615	3.36	0.031	8.49	0.337	0.006		
	C	6.7	0.421	0.092	1.25	0.023 [e]	0.844 [d]	0.004 (84.3)	0.001 [e]	23	0.002–0.015
	-	(4.46)	(11.5)	(6.68)	(2.69)	(1.35)	(10.1)	-	(6)		

[a] Heavy-metal concentration limits of cropland recommended for Europe [62,63] and China ([64]; the latter are given in italics). Bold face font: Unusually elevated soil concentrations; [b] Normal plant heavy-metal concentrations [62,65]; [c] Legislative contamination limits for food grains after [66] (and expanded values for forage grains and herbage after [5] in parentheses): Cd, 0.1 (1); As, 0.5 (2); Pb, 0.2 (10; green forage, 40 $mg \cdot kg^{-1} \cdot DW$); [d] Values for roots and shoots, respectively, within a horizontal line **fail to differ** significantly at $p \leq 0.05$ from the preceding ones; [e] Mineral concentrations of plant tissues from gradient soils A and C **fail to differ** significantly at $p \leq 0.05$. N, sum of NH_4-N + NO_3-N in soil and as Kjeldahl-N ($mg \cdot kg^{-1} \cdot DW$) in herbage. ND, not determined. Phytotoxicity thresholds ($mg \cdot kg^{-1} \cdot DW$) in cereals and vegetables: As, 20; Cd, 5–10; Cr(III), 5–20; Cr(VI), 1–2; Cu, 14–25; Ni, 11–30; Pb, 20–35; Zn, 150–220 [67–69].

2.3. Processing Plant Samples

Duplicate sets of 70–100 plants each were randomly drawn from both 120-m^2 plots on 25 February (post-winter-seedlings, 8 cm tall), 22 May (anthesis state), and 18 July 2014 (maturity). After intensive rinsing, the size and the dry weight (DW) details were determined prior to and after the dissection into roots, stems, leaves and sheaths, rachis, glumes, and grains. The duplicate samples milled for analyses (Pulverisette 14; Fritsch GmbH, Idar-Oberstein, Germany) were prepared from root and shoot tissues carefully washed with deionized water (at least 10 g in DW) and from seeds (at least 20 g in air DW).

2.4. Outwash of Straw Minerals during Simulated Hydrothermal Pre-Treatment

The leaves and upper stems of mature soil A-grown straw were milled (sieve 1.2 mm). A portion of 2 g·DW was suspended in 20 mL of bideionized water, acidified from pH 7.3 to 3.9 with 32% HCl, and autoclaved at 121 °C for 10 min. After discarding a supernatant of 8 mL, the wet solids were washed in another 20 mL of bideionized water (total dilution 1:15 *w*/*w*) and then dried.

2.5. Mineral Concentrations in Soils and Plant Tissues

Mineral concentrations were determined for duplicate aqua regia-extracted 5 g samples of soils (sieve 0.8 mm), whereas 0.3 g samples of milled plant tissues were microwave-digested in HNO_3 (4 mL) and H_2O_2 (1 mL) (Mars Xpress; CEM GmbH, Kamp-Lintfort, Germany) and diluted to 150 mL with bideionized water. The solutions were analyzed by Inductively Coupled Plasma Mass Spectrometry (Thermo, X series ICP-MS). The resulting detection limits (in $mg \cdot kg^{-1} \cdot DW$) were as follows: 0.0005, Th; 0.002, Cd, Co, Cs, U; 0.005, Cr; 0.01, As, Mn, Pb; 0.02, Cu, Sr; 0.03,Ba, Ni, Zn; 0.04, Fe; 0.1, Al, Mg; 0.4, K, Na; 1, P; and 3, Ca.

2.6. Nitrogen Compounds

Duplicate soil samples (1–4 g·DW) were extracted with 0.1 M·KCl solution (1:10, w/v) at 25 °C for 1 h on an overhead shaker. The centrifuged supernatants (14,000 g, 5 min) were used to determine the NH_4^+ content with Aquamerck ammonium test solution (Merck, Darmstadt). The nitrate content was spectrophotometrically quantified at 210 nm (Helios Beta; Unicam UV–Vis; Cambridge, U.K.) against a blank sample reduced with copper-coated zinc granules [70] with maximum deviations of ±8% for soil replicates. Organic N in 250 mg of milled plant samples was determined with a modified Kjeldahl method by using 5 mL each of H_2O_2 (30%) and H_2SO_4 (96%) in the presence of 0.5 g Kjeldahl catalyst (Fluka).

2.7. Statistical Treatments

SPSS 8.0 software was used to calculate the standard deviations (SD) of duplicate to quadruplicate results and linear correlations, and to perform one-way analyses of variance.

3. Results

3.1. Development of the Mineral Load in Herbage from the Seedling State to Maturity

The arable soil from HM hot spot A and the non-contaminated reference soil C showed geogenically determined differences in the concentrations of Ca, Mg, and all the trace elements whereas mineral fertilizing avoided notable deviations in N, P, and K. Soil A was elevated in uranium and the gangue minerals, As, Cd, Cu, Mn, and Zn while soil C safely matched the guidelines set for cropland (Table 1). The post-winter seedlings of cv. JB Asano denoted by a 100-plant root and shoot DW of 2.14 and 13.74 g, respectively, discriminated between macronutrients and trace minerals in their modes of uptake. Compared to the mineral load of the soils A and C, concentrations in roots of the respective seedlings reached values of 3–4 times in P, 11 times in K, and 0.2–0.55 times in Ca and Mg. Additionally, shoot concentrations surpassed those in roots by another 1.6–3 times (Table 1).

Deviating from the uptake mode of macronutrients, concentrations of trace elements in the seedling roots reached only 2%–24% of those in the soils A and C. They further diminished upon their transfer into the shoot (Table 1). The elevated root concentrations of 50%–140% to those in soils thereby reached the highly soluble traces of Cd and Zn [62].

At anthesis, 100-plant sets grown on the soils A and C came with 7.72 and 13.4 g in roots, respectively, and 88.3 and 172 g in shoots to DWs, respectively, which did not essentially change during further maturation, although the shoots increased by 130%. The root concentrations of Ca, Fe, Mn, Ni, and the non-essentials As, Cr, Pb, and U progressively increased from the post-winter seedling state to maturity both in soil A and soil C-grown plants to means of 292% and 304%, respectively. Anthesis-state roots fell to means of 46% (soil A) and 69% (soil C) in the essentials (Cd), Cu, K, Mg, P, and Zn due to the preceding rapid vegetative growth. Exploited differently as sink tissues of minerals for the developing grains, the elements enriched in maturing soil-A grown roots decreased to a mean of 64% and continued to fall in soil C-grown roots to 50% by supplying the 2.5-fold higher grain mineral demand in the latter case (Tables 1 and 2). Accordingly, shoot concentrations of Ca, (Cd), Fe, Mg, Mn, Zn, and the non-essentials As, Cr, Pb, and U of soil-A grown plants fell to a mean of 20% to those in seedlings at anthesis to increase once more to 46% during maturation. Concentrations of Cu, K, Ni, and P continuously declined to 23% (Table 1).

This progressive decline to 27% of the concentrations in seedlings also denoted all Table 1 elements of soil C-grown shoots, with Cu and Cr recovering moderately during maturation. The development of the concentration spans given for soil, root, and herbage samples (values in parentheses, Table 1) also deserves attention. Relative to those of the greatly diverging soils, the concentration spans of roots, shoots, and especially those of grains predominantly tended to decline in the case of macro- and micronutrients. They remained arbitrary for non-essential elements.

Table 2. Production of grains and straw by wheat cv. JB Asano grown on the metalliferous (A) and the non-contaminated (C) soil. Gravimetric values are given in fresh weight (FW) ± SD (rest water around 16% per DW).

Soil	No. of Grains Per 100 Plants	Grain FW Per 100 Plants (in Grams)	1000-Seed FW (in Grams)	Straw Per 100 Plants (in Grams)	Straw (Mg) Per Mg of Grains
A	1840 ± 132	96 ± 8	52.37 ± 0.32	95 ± 5.4	0.984 ± 0.10
C	4100 ± 78	243 ± 4.5	59.24 ± 0.52	199 ± 6.4	0.818 ± 0.04

Grain production on soil C amounted to 7.23 Mg·ha^{-1} in 2014.

3.2. Grain and Straw Yield at Maturity

Determined on the basis of randomly drawn 100-plant sets, the number and FW of grains from soil-A grown plants reached 47.7% and 39.5%, respectively, of the values for soil C-grown plants. The 1000-seed FWs from both locations differed significantly by 12% (Table 2). The straw: grain relation amounted to 0.984 for soil A- and 0.818 for soil C-grown plants cut at the root collar. A grain yield of 7.23 Mg·ha^{-1} in 2014 on soil C would thus correspond with a harvest of 5.9 Mg of straw. Accordingly, straw production on soil A would come to 2.8 Mg·ha^{-1} (Table 2).

3.3. Course of Mineral Concentrations Across the Sections of Mature Soil A- and Soil C-Grown Wheat

Complementing the individual concentration data of Table 3, Figure 1 unites several groups of elements with a similar mode of passage and illustrates their way from root to grain of soil A-grown wheat plants. The plotting of potassium deposits from the root via stem and rachis to the mature grain resulted in a bell-shaped curve (Figure 1a). It illustrates the reduction of excessive K concentrations in the upper herbage by a factor of 3 to make grains correspond with those from other soil A-grown wheat cvs. (Table 3). Deviating from this mode, Mg, N, and P also reached relative maxima in the upper stem and rachis. Nevertheless, a drastic up-regulation of the Mg, N, and P transfer by 177%,

600%, and 726%, respectively, at the rachis/grain interface was required to obtain minerally balanced grains (Table 3; Figure 1a). From the high concentrations of Ca and Zn in upper stem and rachis, only 7% and 11%, respectively, gained access to grains of a normal composition. The poor transfer from the high-concentrated root to the lower stem at percentages of 0.7%–12% was typical of the essential and the non-essential trace elements (Table 3; Figure 1a). Denoted by relative enrichments in the upper stem and rachis, the current concentrations were broken down to 3.2 (U) to 87% (Cr) at the rachis-grain interface to compose the final grain mineral load. Cu and Mn experienced final up-regulations to 173% and 110%, respectively (Table 3).

Table 3. The course of mineral concentrations (mg·kg^{-1}·DW) across the plant sections of wheat cv. JB Asano in the states of maturity (18 July) and anthesis (22 May). The plants were grown on uranium gradient soil A.

Element	State	Root	Lower Stem	Upper Stem	Leaves	Rachis	Whole Grains	Glumes	Reference Grains [d]
K	Matur	5910	11,820 [a]	12,690	12,915	10,103	4275 [a]	5890 [a]	4690–6540
	Anth	*5840*	*7270* [a,b]	*14,540* [a]	*15,336*	*11,030* [a]	*Lacking*	*15,076* [a,b]	
Mg	Matur	1060	352 [a]	965 [a]	2915 [a]	631 [a]	1114 [a]	903 [a]	1520–1710
	Anth	*680* [b]	*204* [a,b]	*800* [a]	*1620* [a,b]	*723* [a]		*958* [a,b]	
N	Matur	11,000	3000 [a]	4000	5000	3000 [a]	18,000 [a]	4000 [a]	19,800–30,000
P	Matur	784	102 [a]	314 [a]	270	359	2606 [a]	403 [a]	4750–5290
	Anth	*530* [b]	*417* [a,b]	*1347* [a,b]	*691* [a,b]	*2523* [a,b]		*3138* [a,b]	
Ca	Matur	3380	989 [a]	4095 [a]	6920 [a]	1190 [a]	287 [a]	1390 [a]	457–538
	Anth	*2012* [b]	*307* [a,b]	*710* [a,b]	*3304* [a,b]	*525* [a,b]		*526* [b]	
Zn	Matur	1203	536 [a]	1059 [a]	1353	1117	126 [a]	346 [a]	132–190
	Anth	*787* [b]	*69* [a,b]	*286* [a,b]	*454* [a,b]	*179* [a,b]		*83* [a,b]	
Cd	Matur	29.4	2.29 [a]	4.26 [a]	4.11	2.34 [a]	1.20 [a]	1.69 [a]	1.37–3.13
	Anth	*25.5*	*1.78* [a,b]	*2.32* [a,b]	*1.61* [a,b]	*0.435* [a,b]		*0.480* [b]	
Cu	Matur	65.6	1.43 [a]	3.05 [a]	4.44 [a]	2.86 [a]	4.94 [a]	2.28 [a]	11.4–12.3
	Anth	*37.2* [b]	*1.53* [a]	*4.07* [a]	*4.29*	*4.46* [b]		*5.60* [a,b]	
Fe	Matur	2410	16.6 [a]	15.2	75.7 [a]	35.7 [a]	24.6	25.7	39.6–50.3
	Anth	*666* [b]	*14.7* [a]	*22.4* [a]	*34.9* [a,b]	*29.5*		*34.6*	
Mn	Matur	217	4.95 [a]	10.8 [a]	45.6 [a]	18.1 [a]	20	16.5	26.4–30.8
	Anth	*65* [b]	*7.1* [a,b]	*14.7* [a,b]	*24.9* [a,b]	*9.7* [a,b]		*13.6* [a]	
Ni	Matur	11.8	0.280 [a]	0.615 [a]	0.340 [a]	2.51 [a]	2.09 [a]	0.715 [a]	1.09–1.41
	Anth	*5.38* [b]	*0.303* [a]	*1.40* [a,b]	*0.283* [a]	*2.83* [a]		*3.81* [a,b]	
As	Matur	20.2	0.215 [a]	0.745 [a]	1.27 [a]	0.920 [a]	0.109 [a]	0.775 [a]	0.080–0.433
	Anth	*6.95* [b]	*0.159* [a]	*0.155* [b]	*0.500* [a,b]	*0.072* [a,b]		*0.040* [b]	
Cr	Matur	4.2	0.050 [a]	0.065	0.172 [a]	0.086 [a]	0.075	0.164	0.025–0.036
	Anth	*0.920* [b]	*0.068* [a]	*0.057*	*0.077* [b]	*0.1*		*0.078*	
Pb	Matur	20.3	0.374 [a]	0.176	0.52	0.289	0.045 [a]	0.135	0.057–0.098
	Anth	*5.64* [b]	*0.061* [a,b]	*0.059*	*0.151* [b]	*0.139*		*0.138*	
U	Matur	8.49	0.571 [a]	0.36	0.323	0.185 [a]	0.006 [a]	0.090 [a]	0.016–0.099
	Anth	*3.36* [b]	*0.032* [a,b]	*0.025* [b]	*0.044* [b]	*0.012* [b]		*0.008* [b]	
	Biomass in grams (DW ± SD) given for 100-plant sets of roots, whole shoots, and grains								
	Matur	7.25 ± 0.79	40.2 ± 4.2 for whole stem		18.7 ± 2.3	4.94 ± 0.29	82.1 ± 7.5	17 ± 1.1	80.8 ± 4.9
	Anth	7.72 ± 0.74	37.7 ± 3.6		35.2 ± 3.8 [c]	4.16 ± 0.25	-	11.2 ± 0.7 [c]	88.3 ± 5.3 shoot

[a] Values within a horizontal line differ significantly at $p \leq 0.05$ from preceding ones; [b] Tissue mineral concentrations at anthesis differ significantly at $p \leq 0.05$ from those in the state of maturity; [c] Biomass at anthesis differs significantly at $p \leq 0.05$ from that in the state of maturity. "Leaves" comprise internodal sheaths; [d] Soil-A grown wheat grains of the cultivars Bussard, Brilliant, and Akteur [27]. Matur, at maturity (18 July); Anth, anthesis (22 May).

Figure 1b demonstrates that the curves denoting the mineral flow for the different groups of elements in mature soil *C*-grown plants resembled those of the soil A-crops. The topographic deviations of Figure 1b to those of Figure 1a were attributed to the fact that the root mineral concentrations, which represented the 100% reference points, were 2.3 times higher in soil A- than in soil C-grown plants (Table 3 versus Table 4). At the same time, soil A- and soil C- grown plants tried to hold the mineral resources of their grains within a narrow and identical range (Table 1). This is best demonstrated in the curves of the poorly regulable Zn. The soil A-supported concentration of 1117 mg·kg^{-1} in the rachis was (insufficiently) broken down to 11% (126 mg·kg^{-1}) in the grain (Table 3). Due to the 'normal' Zn content in soil C, a drastic up-regulation of 286% was necessary to derive acceptable 27 mg·kg^{-1} in the grain from the poor resources in the upper shoot (Table 4).

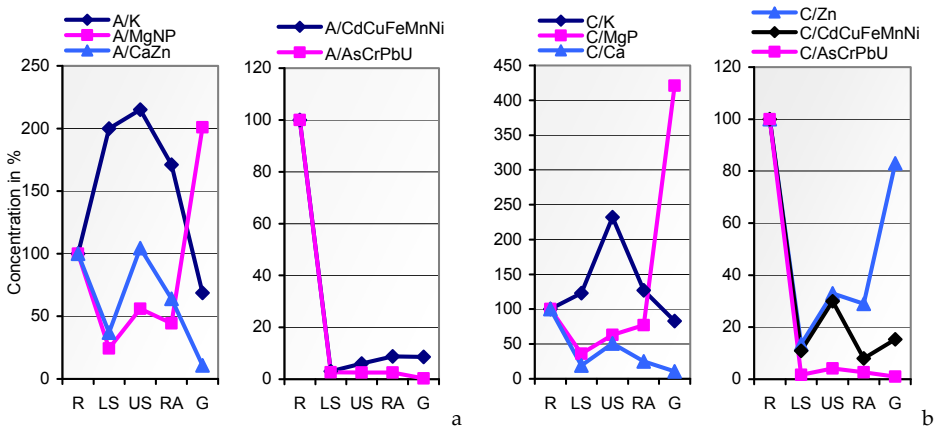

Figure 1. Variation in concentrations of minerals from the root (R; 100%) via the lower part (12 cm) of the mature wheat stem (LS) to upper stem (US), rachis (RA), and grain (G) in plants of cv. JB Asano grown on the metalliferous soil A (**a**) and the non-contaminated soil C (**b**). Values are arithmetic means for sets of minerals with similar modes of passage. Compare Tables 3 and 4 for significant differences within the flow of the individual elements.

Table 4. The course of the mineral concentrations (mg·kg^{-1}·DW) across the plant sections of wheat cv. JB Asano in the states of maturity (18 July) and anthesis (22 May). The plants were grown on the non-contaminated reference soil C (compare Table 3).

Element	State	Root	Lower stem	Upper Stem	Leaves	Rachis	Whole Grains	Glumes
K	Matur	5222	6400 ^{a}	12,120 ^{a}	10,460	6650 ^{a}	4310 ^{a}	4230
	Anth	*7730* ^{b}	*9684* ^{a,b}	*19,235* ^{a,b}	*20,685* ^{b}	*11,340* ^{a,b}	*Lacking*	*16,540* ^{a,b}
Mg	Matur	576	128 ^{a}	344 ^{a}	935 ^{a}	249 ^{a}	1116 ^{a}	477 ^{a}
	Anth	*669* ^{b}	*285* ^{a,b}	*827* ^{a,b}	*1523* ^{a,b}	*820* ^{a,b}		*1130* ^{a,b}
P	Matur	486	241 ^{a}	321 ^{a}	332	540 ^{a}	3143 ^{a}	591 ^{a}
	Anth	*722* ^{b}	*1140* ^{a,b}	*2946* ^{a,b}	*1.870* ^{a,b}	*3530* ^{a,b}		*3612* ^{b}
Ca	Matur	2443	464 ^{a}	1242 ^{a}	2915 ^{a}	611 ^{a}	257 ^{a}	862 ^{a}
	Anth	*1707* ^{b}	*364* ^{a,b}	*1316* ^{a}	*4238* ^{a,b}	*443* ^{a,b}		*1224* ^{a,b}
Zn	Matur	32.7	4.41 ^{a}	10.8 ^{a}	5.70 ^{a}	9.44 ^{a}	27 ^{a}	7.02 ^{a}
	Anth	*49.4* ^{b}	*7.39* ^{a,b}	*23.5* ^{a,b}	*16.7* ^{a,b}	*32.7* ^{a,b}		*39.8* ^{a,b}
Cd	Matur	0.61	0.158 ^{a}	0.116 ^{a}	0.064 ^{a}	0.05	0.075	0.038 ^{a}
	Anth	*0.864* ^{b}	*0.120* ^{a}	*0.078* ^{a,b}	*0.115* ^{a,b}	*0.034* ^{a}		*0.055*
Cu	Matur	11.6	2.49 ^{a}	14.9 ^{a}	9.65 ^{a}	1.49 ^{a}	3.98 ^{a}	6.87 ^{a}
	Anth	*13.5* ^{b}	*2.86* ^{a}	*7.71* ^{a,b}	*8.76* ^{a}	*4.47* ^{a,b}		*13.4* ^{a,b}
Fe	Matur	1840	11.2 ^{a}	20.8 ^{a}	91 ^{a}	33.8 ^{a}	36	73.3 ^{a}
	Anth	*2620* ^{b}	*24.1* ^{a,b}	*42.5* ^{a,b}	*108* ^{a}	*49.4* ^{a,b}		*92.5* ^{a,b}
Mn	Matur	119	3.42 ^{a}	5.18 ^{a}	9.51 ^{a}	11.2 ^{a}	18.4 ^{a}	8.22 ^{a}
	Anth	*149* ^{b}	*7.59* ^{a,b}	*20.7* ^{a,b}	*26.7* ^{a,b}	*12.1* ^{a}		*18.3* ^{a,b}
Ni	Matur	3.18	0.114 ^{a}	0.232 ^{a}	1.28 ^{a}	0.249 ^{a}	0.392 ^{a}	1.24 ^{a}
	Anth	*4.19* ^{b}	*0.289* ^{a,b}	*0.235*	*0.540* ^{a,b}	*0.484* ^{b}		*1.38* ^{a}
As	Matur	2.27	0.036 ^{a}	0.084 ^{a}	0.095	0.083	0.020 ^{a}	0.066 ^{a}
	Anth	*3.68* ^{b}	*0.054* ^{a}	*0.040* ^{b}	*0.230* ^{a,b}	*0.030* ^{a,b}		*0.054*
Cr	Matur	4.48	0.145 ^{a}	0.404 ^{a}	7.99 ^{a}	0.175 ^{a}	0.026 ^{a}	5.94 ^{a}
	Anth	*5.54* ^{b}	*0.398* ^{a,b}	*0.130* ^{a,b}	*1.35* ^{a,b}	*0.124* ^{a}		*0.460* ^{a,b}
Pb	Matur	4.48	0.065 ^{a}	0.16	0.390 ^{a}	0.121	0.012 ^{a}	0.336 ^{a}
	Anth	*6.43* ^{b}	*0.159* ^{a}	*0.083*	*0.512* ^{a}	*0.105* ^{a}		*0.724* ^{a,b}
U	Matur	0.844	0.003 ^{a}	0.002	0.007	0.004	0.001	0.005
	Anth	*1.25* ^{b}	*0.010* ^{a,b}	*0.007*	*0.047* ^{a,b}	*0.006* ^{a}		*0.016* ^{a,b}
	Biomass in grams (DW ± SD) given for 100-plant sets of roots, grains (and whole shoots: 169.2 ± 6.4 at maturity; 172 ± 7.3 at anthesis).							
	Matur	13.2 ± 0.6	95 ± 5.3 for whole stem		38.3 ± 1.9	9.25 ± 0.25	207.8 ± 4.5	26.6 ± 2.7
	Anth	13.4 ± 1.1	86.2 ± 5.8		59.4 ± 3.6 ^{c}	7.30 ± 0.70	-	19.1 ± 2.4

^{a} Values within a horizontal line differ significantly at $p \leq 0.05$ from preceding ones; ^{b} Tissue mineral concentrations at anthesis differ significantly at $p \leq 0.05$ from those in the state of maturity; ^{c} Biomass at anthesis differs significantly at $p \leq 0.05$ from that in the state of maturity. "Leaves" comprise internodal sheaths. Matur, at maturity (18 July); Anth, anthesis (22 May).

3.4. Putative Contributions of Sink Tissues to Grain Fill

The abating resources of minerals in the herbage from anthesis to maturity may be indicative of their gradual translocation to the emerging grains. According to Table 1, the concentrations of whole soil A-grown anthesis-state shoots surpassed those of the mature ones in K (1.2×), P (4.3×), Cu (1.4×), and Ni (1.8×). The concentrations of all the other elements ranged by factors of 0.09× (U) to 0.9× (Mg) below the mineral loads of the mature herbage. The high surplus in P was observed for all sections of the anthetic plant (Table 3, Figure 2a). Its fall to the level of the mature plant accompanied a drastic rise in grain P. This was apparently promoted by contributions of 38% from the sink tissues of all shoot sections to grain fill (Figure 2a). The relocations of K from the leaves and glumes amounted to 80% of the demand in grains. Their content of Cu and Ni could have originated from sink tissues of the whole herbage (to the amounts of 27.6% and 27.7%, respectively). In the case of Fe and Zn, like the majority of elements, the anthetic tissues had not yet reached the concentrations of the mature herbage (Tables 1 and 3, Figure 2a) and were therefore devoid of early disposable mineral reserves in this state of development. Although their temporary role as sink tissues shall not be negated, they joined the grains as net importers of the respective elements during maturation to serve, like the roots themselves, as the final repository or dead-end sink of the minerals not required for grain fill (Table 1).

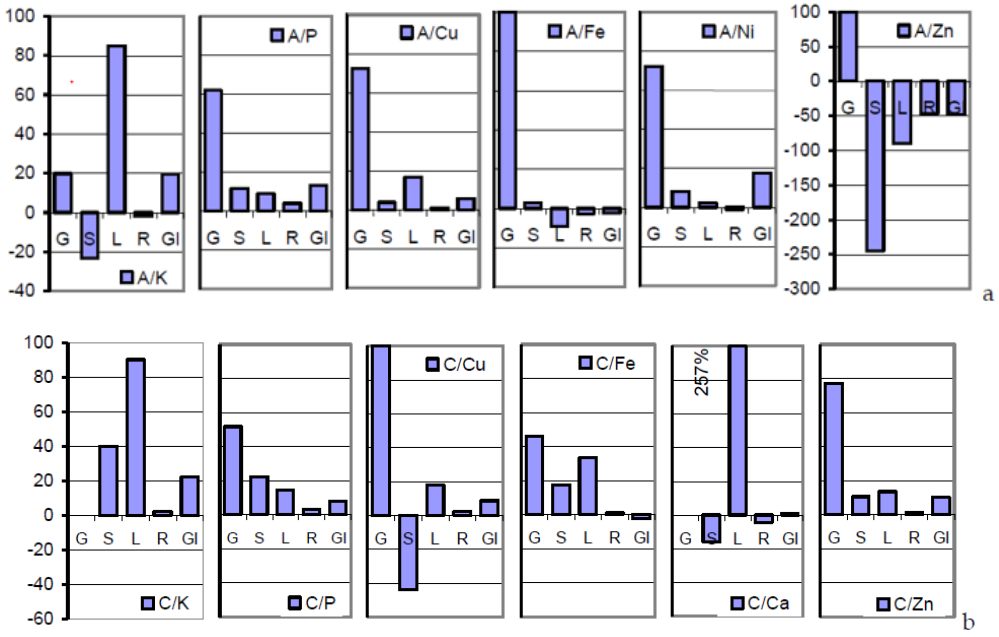

Figure 2. Putative contribution of sink tissues of the anthesis-state plant to the mineral transfer into the maturing grains of wheat cv. JB Asano from metalliferous soil A (**a**) and non-contaminated soil C (**b**). Values expressed in percent. G, grains; S, stem; L, leaves and sheaths; R, rachis; Gl, glumes. Values indicated for "G" mark the putative direct and sink-tissue independent mineral transfer to the grain. Standard deviations range 0.025–0.075× value.

The mineral composition of soil C was not basically different from that of soil A, but it lagged the phytotoxic Cd, Cu, and Zn load (Table 1). The surplus concentrations of Ca (1.5×), Fe (1.5×), K (1.9×), Mg (2.2×), Mn (2.9×), P (6.2×), and Zn (2.7×) in soil C-grown anthetic whole shoots had disappeared in the mature herbage (Table 1) by the putative contribution of most of the plant sections to grain fill (Figure 2b). Additionally, even the roots yielded diminishing concentrations of all minerals (Table 1) to the high production of grains (Table 2). Confined to the herbage, relocations exceeded, or contributed to, the mineral demand in grains with 155% in K, 39% in Mg, 48.2% in P, 237% in Ca, −15.1% in Cu, 54.2% in Fe, 55% in Mn, and 35.2% in Zn.

3.5. Distribution of Heavy-Metal Toxicants and Macronutrients in Soil-A Grown Herbage

Figure 3 demonstrates that most of the critical HM toxicants were held to 83% in As, Cd, Pb, and Zn and to 59%, 36%, and 98% in Cr, Cu, and U, respectively by the whole straw and kept off from the grain. As determined by concentration and biomass, 45%–53% of the toxicants Cd, Cu, Pb, and Zn were associated with the stems and another 31%–40% with flag leaves and sheaths (Figure 4).

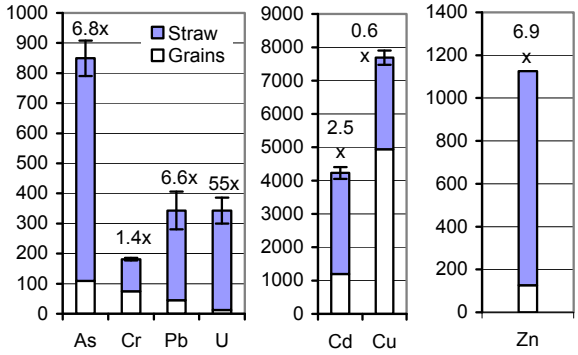

Figure 3. Absolute amounts (mg; Zn in g) and proportions (values heading the columns) of the heavy-metal toxicants in soil-A grown straw (0.984 Mg) and in the associated grains of cv. JB Asano (1 Mg) at maturity. Refer to soil-A values in Table 1. The dark columns sections do not refer to the zero axis. Error bars show the ± SD values for straw.

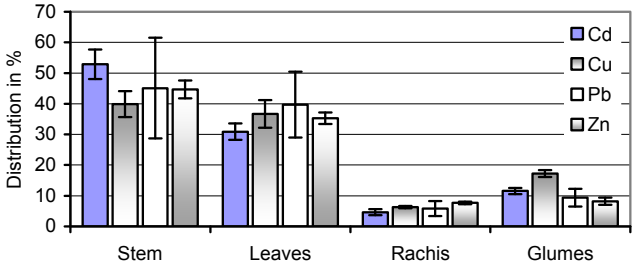

Figure 4. Approximate distribution (%) of Cd, Cu, Pb, and Zn in straw sections of wheat cv. JB Asano grown on the metalliferous gradient soil A. Error bars show ± SD.

The respective order of HM extraction from the 20 cm plough layer of soil A is given in Table 5. The values are specified for roots with the adhering 12-cm stubbles, for the harvestable rest straw, and for grains. The whole plants' HM load ranged from 0.02‰ (As, Pb) to 0.2‰–0.4‰ (Cd, Zn) of the total content in soil A. The percentage of (N), P, and K retention by stubbles in both test soils did not predominantly exceed the 10-% threshold mark (Table 6).

Table 5. The approximate order of heavy-metal extractions (g·ha^{-1}) by cv. JB Asano and their proportions (‰) to the total content in the 20-cm plough layer of soil A. Compare Table 1 for total soil concentrations.

Element	Roots and Stubbles	Grains	Harvestable Rest Straw	In the Whole Plant (‰)
As	4.43	0.266	1.72	0.023
Cd	7.19	2.93	6.53	0.206
Cr	0.923	0.183	0.240	0.041
Cu	14.7	12.1	6.18	0.063
Pb	4.51	0.110	0.586	0.018
U	2.04	0.015	0.593	0.044
Zn	461	308	1927	0.466

Basic data (Mg·ha^{-1} DW): whole straw, 2.40 ± 0.15; grains, 2.44 ± 0.22; roots, 0.215 ± 0.023; stubbles (12 cm), 0.377 ± 0.020.

Table 6. The approximate order of N, P, and K extraction (kg·ha^{-1}) by cv. JB Asano from soils A and C. Compare Tables 1–4 for primary data. In parentheses, the percentages of minerals in roots/stubbles to those of the harvestable crop. In brackets [ ], the mineral concentrations of the herbage (kg·Mg^{-1}·DW).

Soil	Crop	Mg·ha^{-1} ± SD (DW)	Uptake of Macronutrients in kg·ha^{-1}		
			K	P	N
A	Grains Harvestable straw	2.44 ± 0.22 2.023 ± 0.12	10.430 [4.27] 22.130 [10.9]	6.360 [2.61] 0.550 [0.27]	43.920 [18.0] 7.930 [3.92]
Sum			32.560 (100%)	6.910 (100%)	51.850 (100%)
A	Roots Stubbles 12 cm	0.215 ± 0.023 0.337 ± 0.020	1.270 [5.91] 3.977 [11.8]	0.169 [0.79] 0.039 [0.12]	2.365 [11.0] 1.130 [3.35]
Sum			5.247 (16.1%)	0.208 (3%)	3.495 (6.7%)
C	Grains Harvestable straw	6.18 ± 0.13 5.06 ± 0.19	26.640 [4.31] 43.520 [8.60]	19.400 [3.14] 1.800 [0.36]	ND -
Sum			70.160 (100%)	21.200 (100%)	-
C	Roots Stubbles 12 cm	0.395 ± 0.018 0.714 ± 0.024	2.060 [5.22] 4.570 [6.40]	0.192 [0.49] 0.172 [0.24]	ND -
Sum			6.630 (9.5%)	0.364 (1.7%)	-

ND, not determined.

3.6. Leaching Heavy Metals from Mature Straw During Simulated Hydrothermal Pre-Treatment

The mature soil-A grown straw composed of upper stem and leaf tissues lost half of its mineral load during autoclaving at 121 °C combined with a 1:15 (*w*/*w*) dilution step at pH 3.9. The concentrations fell to 31%–46% (mean 39.0) in Ca, K, Mg, P, and in As, Mn, Sr, and Zn; to 49%–60% (mean 52.5) in Ba, Cd, Co, Cs, Na, and Th; and to 68%–87% (mean 76.4) in Al, Cr, Cu, Fe, Ni, Pb, and U. The phytotoxic elements diminished from (mg·kg^{-1}·DW) 4.11 to 2.20 in Cd; 5.21 to 3.52 in Cu; and 1278 to 533 in Zn.

4. Discussion

4.1. Impact of Soil Heavy Metals on Performance and Mineral Flow of the Developing Wheat Crop

Non-permissible HM levels of soil A [62–64] were accountable for phytotoxic concentrations (Table 1; [68,69]) of Cd, Cu, and Zn in roots and of Zn in shoots of cv. JB Asano from the seedling state to maturity, as well as of Cd in the foliage of seedlings. Cadmium concentrations in the herbage violated the hygiene standards of forage (1 mg·kg^{-1}; [5]), whereas the high Zn load may be tolerable for monogastric livestock (500–1000 mg·kg^{-1}) rather than for ruminants (300–500 mg·kg^{-1}; [71]). The strict indigenous HM control of seeds [19,20,28,72] at least held the grains obtained from a soil of 40 mg·kg^{-1} Cd to the permissible range of forage (Table 1). The roots, herbage, and seeds grown on the non-contaminated soil C persisted in the range of 'normal plant HM concentrations' (Table 1; [62,65]) and yielded food quality grains. In reference to these, phytotoxic effects of soil A reduced straw and grain production to 47.7% and 39.5%, respectively (Table 2). Both the quantity of grains produced as well as their inherent attempts to gain the optimum but not maximum in (organics and) minerals essential for survival and germination [27,73] coined the mineral flow across the herbage.

In the sum of macronutrients and trace elements except Zn, soil-A grains reached around 94% and 90%, respectively, of the soil *C*-grown seeds but were loaded up with Zn at the expense of Fe (derived from Table 1). The soil A-grown straw reached 96.4% of the soil *C*-grown biomass in the sum of trace elements except Zn. It dominated in the sum of macronutrients with 147% the soil-*C* grown straw whose partial depletion resulted from the higher rates of mineral relocations from sink tissues to the 2.5-fold higher grain biomass.

The high demand of grains in soil C treatment depleted the minerals in root and shoot, whereas the few saturated soil A grains blocked mineral drain from the respective sink tissues (Tables 1 and 2)

and depreciated them to dead-end sinks of the less essential elements in the course of maturation. The gradual (seed fill-initiated) decline of the mineral load from root to seed is common to mature cereals obtained from non-contaminated soils [74–77]. However, a declining mineral load in growing plants may also be uptake-dependent. In rice, the acquisition of Ca, K, Mg, N, and P peaked at panicle initiation (i.e., at 56% of the plants' development) to fall in a bell-shaped curve to zero at maturity [78]. Similarly, the foliages of alfalfa and annual ryegrass showed diminishing mineral resources in later growth stages [79,80]. Maillard et al. [81] recorded growth cessation in leaves of cereals, further crops, and trees at around 40% of their life span and initiation of senescence followed by a drain of nutrients at midlife. Ageing goldenrod (*Solidago gigantea* Ait.) preferentially translocated KP to the perennial rhizomes [82].

In the search for mineral uptake competitions in cv. JB Asano from the two variably composed soils A and C, we calculated the percentages of the elements per kg of the whole plant tissue ($mg \cdot kg^{-1}$ DW) to their total content in soil. Fixing soil A percentages at factor 1, the soil uptake rates of Ca, Cd, K, and P by soil *C*-grown tissues coincided with those from soil A (Figure 5). Unlike Cu and Cr, Mg and most of the traces were taken up at drastically lower rates from the resources in soil C. Attempts to level the Cu and Mg content of soil A- and soil *C*-grown grains during seed fill in the inherently narrow span (Table 1; [27,72]) may have caused the relatively elevated and reduced respective uptake of these elements, whereas variations in the uptake rates of other elements, for example, by mechanisms of uptake competition, shall not be derived from the data. The respective conclusions for a single organism should solely be drawn from lab-scale tests on defined media rather than on polymetallic soils. Thus, the impact of elevated HM concentrations in soil on the grain content of macronutrients cannot be postulated.

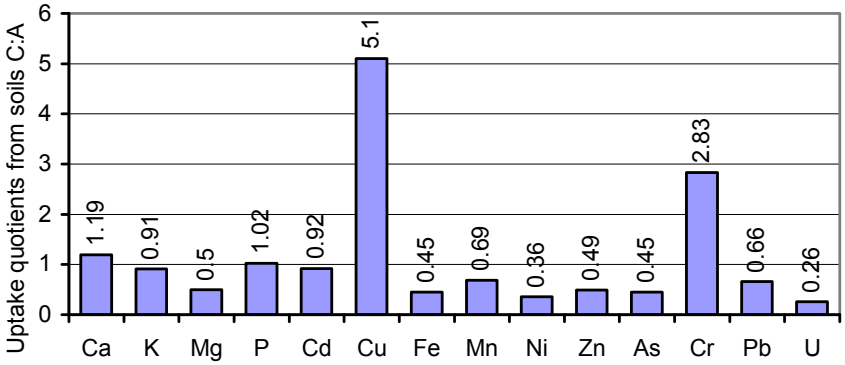

Figure 5. Quotients of the percentages the whole plants (root, shoot, and grains) incorporated from the total soil content of the respective mineral. Values of soil-*C* grown plants are divided by those of the soil-A grown plants which represent factor 1. Standard deviations range from 6%–13% of the values.

The mineral loads arrested in the sections of root, lower/upper stem, and rachis of the mature soil A-grown plant deviated drastically from those of the grain in a manner that is poorly accessible to interpretations. Severe incisions into the flow of all the minerals except K occurred at the root/lower stem interface (Table 3; Figure 1a). The concentrations predominantly rising in the upper shoot were once more dramatically down- or up-regulated at the extremely versatile rachis/grain interface in more or less successful attempts to realize the inherent seed target metallome of the cultivar [19–21,72]. Unlike the herbage, grains respond least to changes in agronomic conditions and the supply with soil minerals [83–85] and nitrogen [18,23], and they stabilize their metallome inherently in a narrow range [21,23,27]. This was also reported for the Fe and Zn content of soybean [22], rice [21], and pea plants [20], although the respective concentrations in vegetative tissues rose drastically. Similarly, variable regimes of fertilization had little impact on the seed content of fatty acids and cellulose in peanut [86], of amino acids, sugars, and phenolics in pea plants [19,87], and of starch and proteins in maize [88]. Raising the N_{org} content in shoots of Chinese cabbage (*Brassica chinensis* L.) to the 3.4- to

3.7-fold of water-treated plants by NH_4Cl application resulted in near-linear increases in the sum of the metalloprotein-associated transition metals, (Cd), Co, Cu, Mn, Ni, and Zn but not of Fe to 3.6- to 3.7-fold. Contributions of the individual elements ranged from 2.5× (Co, Cu, Ni) to >4× (Cd, Mn, Zn). Concentrations in Ca and Mg with their structural, metabolic, and enzyme regulatory functions [89,90] reached 2-fold. Concentrations in the sum of Fe, K, Na, V, and a group of non-essential metals did not exceed 1.1- to 1.2-fold as the individual contributions of Cr, Fe, and V diminished (to 0.45–0.75×) whereas those of Al, Ba, K, Li, Pb, Sr, Ti, and U slightly increased (to 1–1.8×) [91]. The protein (N_{org}) content in grains from soil A ranged below that of the comparable reference grains (Table 3) and could be partly responsible for their lower mineral load. Peterson et al. [92] postulate the uptake promotion of Ca, Cu, Fe, Mg, Mn, P, S, and Zn but not of Cl and K by rising grain protein.

Table 4 and Figure 1b document the congruity in the mode of mineral distribution and management in soil A- and soil C-grown plants. Therefore, the grains coincided with the widest in the content of macronutrients as well as in Cu and Fe (Table 1) as shown by the narrow concentration spans around the inherent target level (values in parentheses). The corresponding concentration spans in soils and wheat grains amounted to, for example, 3.15 and 1.49 in Ca, respectively, 7.5 and 1.08 in Cu, respectively, 25 and 3.69 in Zn, respectively, but 32 and 24, respectively, in the less compensable Cd [27].

In wheat grown on Tunisian mine tailings, soil, shoot, and grain concentrations varied by 27, 49, and 13× in Pb, respectively; 420, 43, and 30× in Cd, respectively; and 97, 35, and 2.4× in Zn, respectively [83]. The field pea cv. Rocket responded to soil concentration spans of macro- and micronutrients in the range of 4.6 (Ca) to 109 (Zn) with variations of 1.31–2.40×, and to 475× in soil Cd with 15.9× in seeds [19]. Hundreds of spelt wheat genotypes were grown at six locations in Turkey over three years. Notwithstanding large differences in their individual mineral uptakes, the median-value variations in grain protein and Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn across all accessions ranged from as little as 1.16× (K) to 2.02× (Ca) [85].

Higher resources in anthetic than in mature plant sections indicate mineral relocations from the gradually senescing sink tissues of the post-midlife herbage [81] to the phloem-fed grains, superimposed by rapidly diminishing mineral uptake rates from the soil [78]. Flag leaves with internodal sheaths and glumes were the sink tissues with the highest mineral concentrations (Tables 3 and 4; Figures 2 and 6). Sucrose and potassium are the major osmotic species [93] to move the phloem sap via differences in the hydrostatic pressure from the donor tissues to the grains [94].

It is part of the plant's strategic and analytical top performance to identify the individual organic and mineral phloem constituents and to translocate them in delimited quantities to the grain in realizing its genetically pre-determined composition [72]. The cytoplasm strands in sieve tubes actively capture the single phloem constituents in congruence with the current plant metabolic state [90]. The Figure 6a–c illustrate the order of surplus concentrations of K, P, but not Zn, across anthetic to mature sections of soil A-grown plants. Interestingly, the plant safely distinguishes between both the rachis-borne structures of grains and glumes in their roles as sink and source tissues.

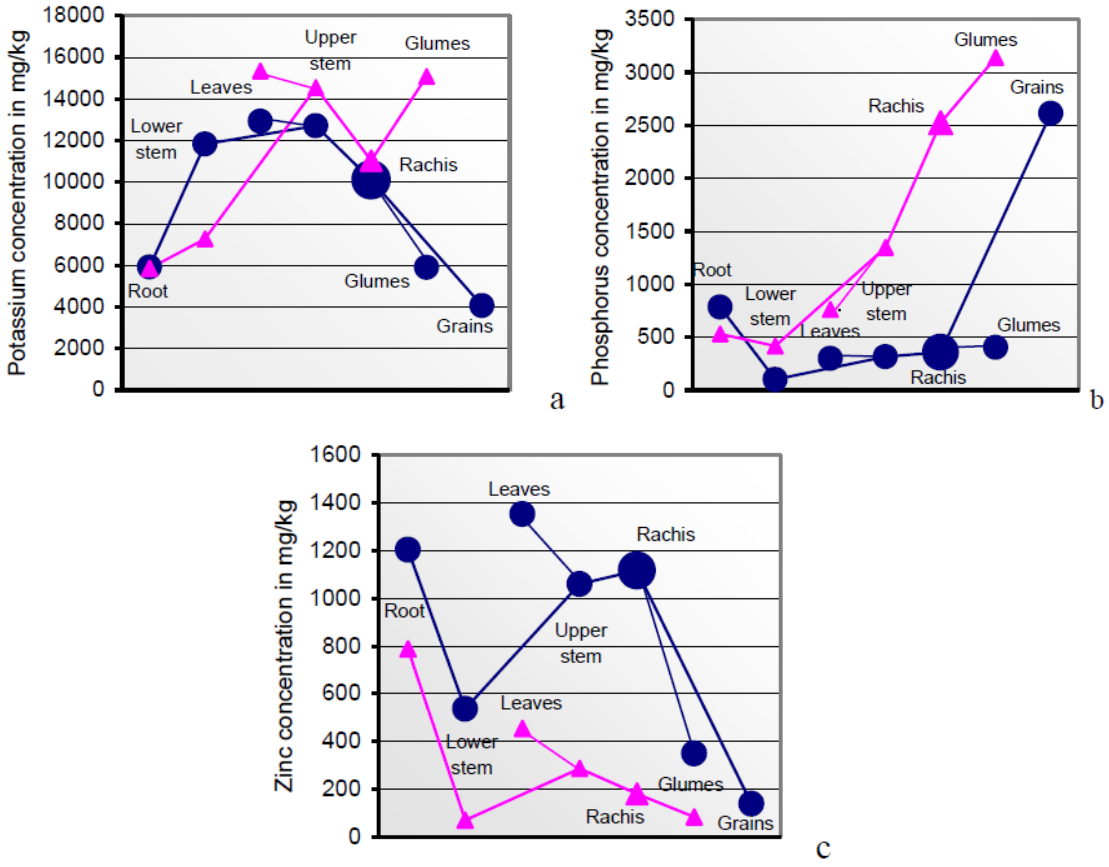

Figure 6. Course of K (**a**); P (**b**); and Zn (**c**) concentrations from roots to mature grains of whole wheat plants cv. JB Asano analyzed at anthesis (bright curves) and maturity (dark curves). Note the role of leaves and glumes as sink tissues and the concentration differences between both rachis-borne structures of grains and glumes in the plants grown on the metalliferous soil A. Compare Table 3 for significant differences within the flow of the individual elements.

4.2. Applicability of the Herbage from the Metalliferous Soil A

Compared to the herbage from soil C, the Cd, Cu, and Zn phytotoxicity of soil A reduced the harvestable biomass in cv. JB Asano to 40% and the total uptake of K and P from soil proportionally to 46% 33%, respectively (Table 6). N, P, and K concentrations of the harvestable straw from both soils (Table 6) corresponded the widest with the values reported for K (6.7–11.6 kg·Mg^{-1}·DW); P (0.38–1.3); and N (5) [32,95–97]. Contaminations of 1.69–4.24 mg·kg^{-1} DW in Cd and up to 1353 in Zn of the mature soil-A grown straw (Tables 1 and 3) exclude its use as bedding material or roughage supplement to domestic animals [5,71]. This rule must not apply to the premature herbage. The Cd load of the (post-)winter herbage as well as of the soil itself is a serious hazard to winter grazing and thereby soil ingesting local herbivores (Table 1; [12,98,99]). Nevertheless, the rapidly growing green matter in the pre-tillering to the post-anthesis state shows a temporary and significant decline in the critical toxicants As, Cd, Cr, Pb, U, and Zn (Table 1) and could potentially serve as a roughage supplement for the domestic livestock. This is supported by the facts that wheat herbage in the predominantly leafy and anthesis states varied in crude protein (24.4% and 11.8% of DW, respectively), hemicellulose (17.9 and 31.5, respectively), cellulose (18 and 27.3, respectively), lignin (1.9 and 4, respectively), and thus in digestibility (74.7 and 68.3, respectively) [100]. Similarly, the metabolizable energy (ME) in the herbage cut at flowering, milk or dough stage, and maturity was 9, 8, and 7–8 MJ·kg^{-1}·DW in oats and 8, 8, and 6–7 MJ·kg^{-1}·DW in wheat, respectively [101].

Returning the mature soil A-grown herbage to the cropland may be less problematic. In the sum of As, Cd, Cr, Cu, Pb, and U, and Zn (individually), 33.8 and 461 g ha^{-1}, respectively, came to roots and stubbles, 9.85 and 1927, respectively, to the entire harvestable rest straw, and, for comparison, 15.6 and 308, respectively, to the grains (Table 5; Figure 3). Processing the straw to biochar [102,103] may prevent the excessive propagation of mineral-immobilizing and nitrogen-binding microorganisms in alkaline soils while its positive effects on crop production of temperate zone soils are contested [104]. Apart from mineral losses by volatilization during straw combustion at 400–800 °C [96], 3.08 mg·kg^{-1} DW of Cd in the mature soil-A grown straw (Table 1) would account for only 0.03‰–0.06‰ of the ash content reportedly ranging 4.65%–9.9% per·DW [32,35,96,105]. In proportion to the macronutrients, Cd came to 11,280 mg·per·kg·P, to 282 per·kg·K, and to 786 per·kg·N (refer to Table 1). Comparable values for the root biomass amounted to 37,500 in P, 4975 in K, and 2673 in N. Ashed soil-A grown herbage is therefore no NPK fertilizer. Commercial high-purity mineral P fertilizers of 7–33 mg·Cd per·kg·P_2O_5 constitute 16–75 mg·Cd·per·kg·P and are surpassed by values of 220–570 mg·Cd·per·kg·P in other products [8,106].

One can accept the use of the Cd-contaminated wheat straw in rates of 15%–45% as bulking material in the vermicomposting of sewage sludge (e.g., [107]). Renouncing its use as a soil structure-improving material, however, does not really contribute to soil remediation. Consecutive wheat crops of 1070 years (Zn), 2400 (Cd), and 28,400 years (Pb) were necessary to capture 50% of the HM load of soil A by the whole plant (including roots) under the conditions of stable uptake rates (Table 5). This corresponds with similar calculations [10–12]. True soil remediation effects may come from the gradual downwash of mineral-clay complexes from the plough layer to subsoil strata [8,9]. Uranium waste rock deposits restored and turfed 30–35 years ago were initially treated with liquid manure and husbandry composts which released metal cation ligands and motile organic colloids. Across 34 test plots, the re-distribution of As, Cd, Co, Cr, Cu, Ni, Pb, U, and Zn resulted in mean concentrations of 108, 158, and 187 mg·kg^{-1} DW in the 0–3-cm sward layer, the 4–20-cm (plough) layer, and the 21–50-cm subsoil, respectively. In test plots not impaired by soil compaction and pseudogley formation, the ratings from the 0–3 cm sward layer, the 4–20 cm (plough) layer, and the 50–80 cm subsoil came to, for example, 42, 66, and 190 mg·kg^{-1}·DW, respectively, in As; 3.6, 4.8, and 23.5, respectively, in Cd; 31, 41, and 111, respectively, in Pb; and 459, 725, and 4104, respectively, in Zn with means of 76, 116, and 577, respectively, across all the elements [9,108].

The HM load of the soil A-grown herbage does not interfere with the extraction of green chemicals [36,109]. However, the fungi used in the preparatory delignification of the biomass feedstock for bioethanol production face the full toxicity of internal HM. The resulting oxidative stress leads to the damage of cellular biomolecules such as DNA, lipids, and proteins by reactive oxygen species and may result in cell death [110–112] and restrictions in mycelial growth, enzymatic activity, and the rate of substrate conversion [113–115]. Toxicity effects of Cd as pronounced in liquid media drastically diminish in the presence of soils, organic matter, and high concentrations of dissolved salts [116]. In the solid-state fermentation of rice straw with the basidiomycete *Phanerochaete chrysosporium*, Cd halved the activities of lignin peroxidase at 4 mg·kg^{-1} DW and of manganese peroxidase at 6 mg·kg^{-1}; it also halved the degradation of lignin at 16 mg·kg^{-1}, and of cellulose and hemicellulose at about 32 mg·kg^{-1} [115]. The weight loss of straw incubated with *P. chrysosporium* was optimum at Pb concentrations of 30 mg·kg^{-1}, but proceeded, too, at 400 mg·kg^{-1} [117]. The colonization of non-sterile soil by the basidiomycete *Pleurotus ostreatus* reached limits at concentrations of 50–100 mg·kg^{-1} DW in Hg and 100–500 mg·kg^{-1} in Cd [113]. An A-type soil lot of 41 mg·kg^{-1}·DW in Cd, 156 mg·kg^{-1} in Pb, and 3320 mg·kg^{-1} in Zn enabled normal mycelial growth and fructification by the lignicolous basidiomycetes *Hypholoma fasciculare*, *Kuehneromyces mutabilis*, and *P. ostreatus* [118]. Lignocellulose fermenting fungi should therefore tolerate the full but moderate HM load of the soil-A grown wheat straw, whereas drastic HM dilution steps precede its use as feedstock in current bioethanol fermentation experiments at lab scale.

The preferred Simultaneous Saccharification and Fermentation (SSF) technology includes the hydrothermal pre-treatment at elevated temperature and atmospheric pressure predominantly in solid-liquid proportions of 1:10 (*w*/*w*) followed by the washing and drying of the solids. In the subsequent breakdown of cellulose and hemicellulose by commercial hydrolase enzymes and the concomitant microbial transformation of the liberated fermentable hexose sugars to ethanol, solid-liquid proportions of 1:10 to 1:20 are reported in the incubation media (e.g., [35,37,48,52]). According to Le et al. [119], the retention of minerals in the hydrothermally pre-treated lignocellulose is dependent on pH, temperature, and treatment time. Initial pH values of 2–4 made Ca, K, Mg, Mn, P, and Zn levels fall to less than 20% by DW whereas Al, Cu, Fe, and Si concentrations did not change. These data match the widest with those obtained by experimental autoclaving of soil A-grown straw at 121 °C which was combined with a 1:15 dilution step at pH 3.9 (Section 3.6). The treatment rendered the concentrations of the phytotoxicants Cd, Cu, and Zn non-critical.

The enzymatic hydrolysis of the pre-treated lignocellulose structures could promote release of the residual HM resources into the liquid incubation medium. Baker's yeast (*Saccharomyces cerevisiae*) as the preferred ethanologenic organism experienced 5% and EC50 growth inhibition in solution at 0.11 and 1.12 mg·L^{-1}, respectively, in Cd; 55 and 96 mg·L^{-1}, respectively, in Mn; 59 and 117 mg·L^{-1}, respectively, in Ni; and 65 and 163 mg·L^{-1}, respectively, in Zn during 12 h of incubation [57]. The yeast exposed to 10 mg·L^{-1} Cd was temporarily inhibited to regain an almost normal growth rate [120] apparently owing to its biosorption capacity in the order of 45 mg Cd^{2+} per gram of biomass within 2 h [121]. Exposure to Cu reduced the viability of the yeast cells to 88% at 0.32 mg·L^{-1} within 1 h and to 11% at 2.54 mg·L^{-1} within 5 min [60]. Incubation at 30 °C for 24 h reduced the number of viable yeast cells from 18 × 10^9 per gram to 5–12 × 10^9 in the presence of 25 mg·L^{-1} Pb, and to 4–8 × 10^9 in the presence of 10 mg·L^{-1} Cd [58]. Adding 50 μM Cd (5.6 mg·L^{-1}) to a glucose/yeast extract medium reduced ethanol production by *S. cerevisiae* from 25 to 2 g·L^{-1} within 10 h. Ethanol formation was not impaired even at 1 mM·Cd in the medium if its uptake was competitively repressed by Ca^{2+} supplements at the molar ratio of 50–100:1 [59]. The equal distribution of Cd, Cu, Pb, and Zn in stems and leaves of the soil A-grown straw (Figure 4) makes speculations about a selective harvest of less-contaminated and therefore more valuable portions of the herbage illusory. At the same time, wet pre-treatment removes the majority of its potential HM toxicants.

Critical Cd and Cu concentrations may be obtained during the fermentation of ground whole-wheat grains in aqueous slurries. The suspensions may contain 10%–17% dry matter (*w*/*w*) at lab scale [122,123] and around 25% in practice [124]. The combined effects of Cd and Cu concentrations reaching 3.13 and 12.3 mg·kg^{-1}·DW, respectively, in several soil A-grown wheat cvs. (Table 3) could strain HM tolerance and vitality of yeast cells to the utmost. Their productivity could be impaired by the progressive acquisition of HM even from low concentrations in the incubation media during the 48–72-h batch fermentation process [125].

5. Conclusions

The geologically related soils A and C differed little in their macronutrient content, however, phytotoxic effects incited by the elevated Cd, Cu, and Zn load of soil A reduced the straw and grain yield in cv. JB Asano to 47.7% and 39.5%, respectively. The combined excluder properties and the strict HM delimitations during seed fill prevented the widest of an enrichment in the crop of the high-concentrated soil contaminants, As, Cd, Cu, U, and Zn. Cadmium concentrations of 1.6 mg·kg^{-1}·DW at anthesis, 3.08 in the mature straw, and 1.2 in grains were the only ones to violate the forage hygiene guidelines of 1 mg·kg^{-1}. Unlike green matter and grains, the mature straw should therefore not be used in husbandry and biochar production. Caution is recommended for the use of grains rather than of straw with Cd and Cu concentrations beyond 3 and 12 mg·kg^{-1} DW, respectively, as bioethanol feedstock. Cd and Cu toxicities could lead to productivity losses in the fermentation of alcohol by *S. cerevisiae*. With that said, a higher Mn, Ni, Pb, and Zn load would be tolerated by the yeasts [57,58] and accepted by forage hygiene guidelines (Table 1 legend). Therefore, the straw did not

lose its applicability as a roughage supplement in husbandry and as bioethanol feedstock if its As, Mn, Pb, and U content would essentially increase. This means that for alkaline A-type clay-loam soils, the acceptable load in As, Mn, Pb, and U—but not in the phytotoxic Ni and Zn—could rise beyond the Table 1 values if quasi-industrial grain crops are grown.

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