

Review

Stability of the Inherent Target Metallome in Seed Crops and a Mushroom Grown on Soils of Extreme Mineral Spans

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Abstract: Extremes in soil mineral supply alter the metallome of seeds much less than that of their herbage. The underlying mechanisms of mineral homeostasis and the “puzzle of seed filling” are not yet understood. Field crops of wheat, rye, pea, and the mushroom *Kuehneromyces mutabilis* were established on a set of metalliferous uranium mine soils and alluvial sands. Mineral concentrations in mature plants were determined from roots to seeds (and to fungal basidiospores) by ICP-MS following microwave digestion. The results referred to the concentrations of soil minerals to illustrate regulatory breaks in their flow across the plant sections. Root mineral concentrations fell to a mean of 7.8% in the lower stem of wheat in proportions deviating from those in seeds. Following down- and up-regulations in the flow, the rachis/seed interface configured with cuts in the range of 1.6%–12% (AsPbUZn) and up-regulations in the range of 106%–728% (CuMgMnP) the final grain metallome. Those of pea seeds and basidiospores were controlled accordingly. Soil concentration spans of 9–109× in CuFeMnNiZn shrank thereby to 1.3–2× in seeds to reveal the plateau of the cultivar’s desired target metallome. This was brought about by adaptations of the seed:soil transfer factors which increased proportionally in lower-concentrated soils. The plants thereby distinguished chemically similar elements (As/P; Cd/Zn) and incorporated even non-essential ones actively. It is presumed that high- and low-concentrated soils may impair the mineral concentrations of phloems as the donors of seed minerals. In an analytical and strategic top performance, essential and non-essential phloem constituents are identified and individually transferred to the propagules in precisely delimited quantities.

Keywords: basidiospores; cereals; geochemistry; herbivores; nonessential minerals; pea; seed filling; seed:soil transfer; seed target metallome

1. Introduction

Extreme variations in the natural or anthropogenically influenced trace mineral stock of arable land are a challenge to non-endemic high-productivity seed crops rather than to locally-adapted landraces [1,2]. Insufficient Zn resources down to 2–3 mg kg^{−1} soil denote up to 50% of the cropland in India, China, and some countries in the Middle East [3]. Of the 14,900 Indian soil samples, 49% were deficient in Zn, 33 in B, 12 in Fe, 11 in Mo, 5 in Mn, and 3 in Cu [4]. Local crops experienced yield losses due to deficiencies in Zn (49%); B (31%); Mo (15%); Cu (14%); Mn (10%); and Fe (3%) [5]. Low soil concentrations of the plant- and nutritionally essential elements of CuFeMnNiZn (Block presentation of the single elements) and traces of IMoSe, in addition to their poor plant availability due to unfavorable sorption and redox conditions [6,7], as well as insufficiency in their uptake by some

crop genotypes [8,9] serving as staple food and feed are all factors causing mineral malnutrition and health problems in humans and livestock [10–12]. They also impair health and productivity of the crop itself [5,13,14]. Applications of micronutrient fertilizers are obligatory [9,12].

Land consumed by mining, industrial heavy-metal (HM) and radionuclide emissions, and applications of sewage sludge, husbandry sludges, and AsCdU-contaminated phosphate fertilizers as world-wide practices result in potential croplands with single or multiple metal toxicants [15–17] in concentrations outside permissible limits [7,18,19]. The late 20th century was faced with a world-wide annual release of 22,000 MgCd; 954,000 MgCu; 796,000 MgPb; and 1,372,000 MgZn [20]. Land contaminated by mining and metallurgy with AsCdCrCuNiPbZn may be gradually recovered with the downwash of metal-clay complexes from plough layer to subsoil strata (clay migration) [21] within several decades rather than by inefficient phytoextraction with plants [22–24]. Less contaminated cropland may be reserved for industrial, forage, or food crops whose hygienic deficits are non-critical [25,26] or negligible as in the case of grain crops with their strict indigenous HM control. Unlike the herbage, grains respond least to oversupply of soil minerals and nitrogen, and they stabilize their metallome inherently in a narrow range [27–29]. This was also reported for the FeZn content of soybean [30], rice [28], and pea [31], 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 [32], of amino acids and sugars in pea [33], and of starch and proteins in maize [34]. It is neither known how plants coordinate the uptake and shoot translocation of the required metal stock [35,36] nor is the “puzzle of seed filling” understood [37,38]. A “preset program of interacting genes and pathways” [34] then leads to the production of vigorous and stress-protected propagules almost irrespective of the extremes in the mineral stock of natural or contaminated soils [18,27].

The wealth of details describing the (putative) intervention of molecular genetics in uptake and translocation of minerals from soil to seed e.g., [39–41] do not fall within the scope of this paper. Instead, the mineral distribution within the mature plant, its uptake control, and the ability to distinguish (micro)nutrients from chemically similar non-essential toxicants will be discussed.

2. Bottlenecks in Plant Mineral Flow

On their passage across the plant, trace mineral concentrations in general fall gradually and are highest in the root and lowest in the seed. Heavy-metal loads in whole grains of four wheat cvs. grown on comparatively mineral-poor soils thus ranged in the order of 10% (for both CdCr); 17%–25% (MnNi); and 40%–70% (CuPbZn) of those in the roots [42–45]. This indicated that food-quality cereals can be potentially obtained even from seriously metalliferous soils [46].

To prevent cell damage by the reaction of transition metal cations such as CuFeMnZn with oxygen species in the root symplast [47], the metals pass as neutral nicotianamine (NA) complexes [48,49]. They are then unloaded into the xylem as free cations or recomplexed with common ligands such as carboxylates, NA, histidine, and phytochelatins in dicots; with carboxylates and 2'-desoxymugineic acid in monocots; or as arsenite > arsenate and selenate metalloids, respectively (for reviews, e.g., [50–52]). At the base of cereal grains, the xylem transport is discontinuous [53]. The minerals therefore pass over, after further putative recomplexations [50], at peduncle and rachis of cereals into the phloem [54,55] to supply sink tissues of the shoot, leaves, and seeds. In the seed coat of the wheat grain, aleurone cells form a layer of the three to four cells highest in the content of FeZn [56], contact the starchy endosperm, and catalyze the mobilization of the storage polymers enzymatically upon germination [57]. Accordingly, Zn flow into wheat grains was seen to be blocked by two barriers located between the rachis and grain and between the maternal and filial tissues of the grain itself [58]. In pea, xylem fluids are loaded into the phloem of leaves, stipules, and pod walls [59]. Connected with the pod wall over the funiculus, the seed coat is furnished with phloem-derived minerals, sucrose, and amino acids. The compounds are unloaded into the liquid-filled intercellular space (apoplast) between seed coat and embryo to be taken up by the latter [60].

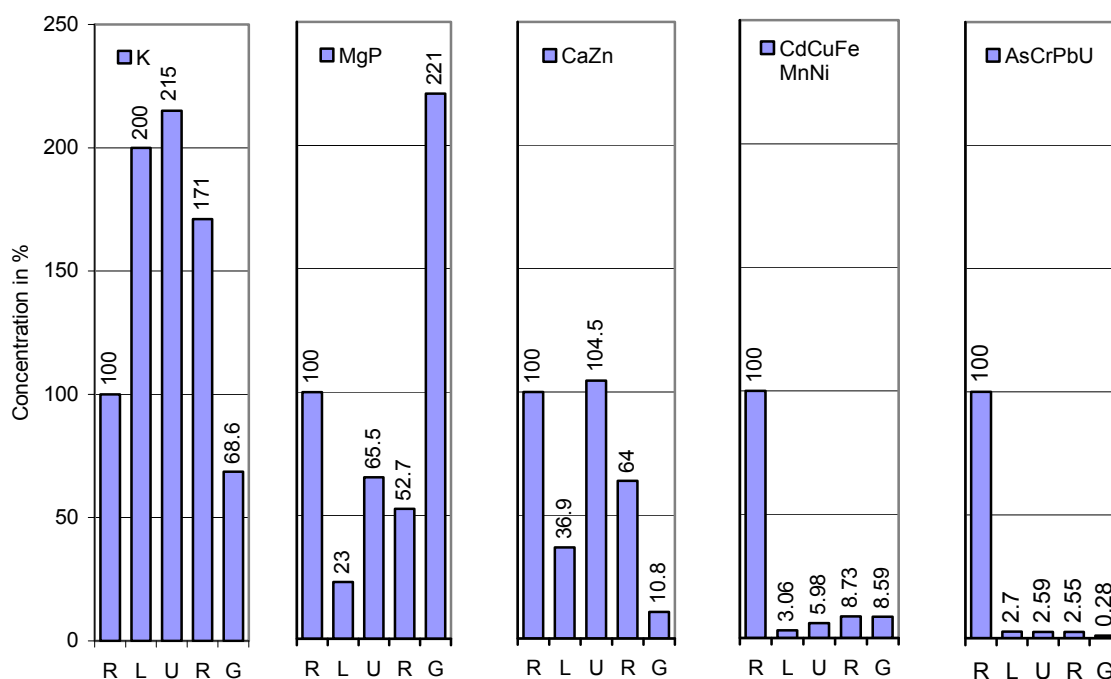


Figure 1. Variation in concentrations of minerals from the root (R; 100%) via the lower part (L; 12 cm) of the mature wheat stem to upper stem (U), rachis (R), and grain (G). Values are arithmetic means for sets of minerals with similar modes of passage (refer to Table 1).

Table 1. Up- and down-regulated mineral concentrations (mg kg^{-1} DW) across the mature plants of wheat cv. Asano grown on the high-contaminated uranium gradient soil A. (), quotients calculated from mineral concentrations of the actual to the preceding column expressed in percent.

Element	Root	Lower stem	Upper stem	Rachis	Whole grain
K	5910	11,820 ^a (200)	12,690 (107)	10,103 (80)	4052 ^a (40)
Mg	1060	352 ^a (33)	965 ^a (274)	631 ^a (65)	1162 ^a (184)
P	784	102 ^a (13)	314 ^a (308)	359 (114)	2612 ^a (728)
Ca	3,380	989 ^a (29)	4,095 ^a (414)	1,190 ^a (29)	346 ^a (29)
Zn	1,203	536 ^a (45)	1,059 ^a (198)	1,117 (105)	138 ^a (12)
Cd	29.4	2.29 ^a (7.8)	4.26 ^a (186)	2.34 ^a (55)	1.55 ^a (66)
Cu	65.6	1.43 ^a (2.2)	3.05 ^a (213)	2.86 (94)	6.43 ^a (225)
Fe	2,410	16.6 ^a (0.7)	15 (91)	35.7 ^a (238)	24.6 ^a (69)
Mn	217	4.95 ^a (2.3)	10.8 ^a (218)	18.8 ^a (174)	19.9 (106)
Ni	11.8	0.28 ^a (2.4)	0.615 ^a (220)	2.51 ^a (408)	2.09 ^a (83)
As	20.2	0.215 ^a (1.1)	0.745 ^a (347)	0.920 ^a (123)	0.100 ^a (11)
Pb	20.3	0.374 ^a (1.8)	0.176 (47)	0.289 (164)	0.044 ^a (15)
U	8.49	0.571 ^a (6.7)	0.360 (63)	0.185 (51)	0.003 ^a (1.6)

^a Values within a horizontal line differ significantly at $p \leq 0.05$ ($n = 2-4$) from preceding ones.

In order to locate bottlenecks in the plant mineral flow, several seed crops were consecutively grown on an 11-hectare gradient of geologically related soils C_{org} 3.5%–4% with a wide span of diminishing HM concentrations. The gradient had been formed by the inclusion of metalliferous overburden soil from uranium mining into non-contaminated cropland some decades ago [18,27]. Mineral concentrations exceeding the permissible limits for cropland reached 156 mg kg^{-1} DW in As, 41 mg kg^{-1} DW in Cd, 283 mg kg^{-1} DW in Cu, 2130 mg kg^{-1} DW in Mn, 150 mg kg^{-1} DW in Pb, 41 mg kg^{-1} DW in U, and 3005 mg kg^{-1} DW in Zn within the central gradient soil A. Figures 1 and 2 and the associated Tables 1 and 2 illustrate the breaks in the nutrient flow for minerals with similar modes of passage across mature plants of wheat and pea. Figure 3 and Table 3 illustrate striking similarities with the conditions in crop plants in the mineral flow across basidiomes of the

wood-degrading mushroom *Kuehneromyces mutabilis* as a lower-plant saprobe. Mushrooms grew on leafwood sections embedded in sand, soil A, and a garden soil C_{org} 8.9%. Available mineral resources in Ca, K, and the essential trace minerals were up to 96%–99.9% located in the bed soils [61].

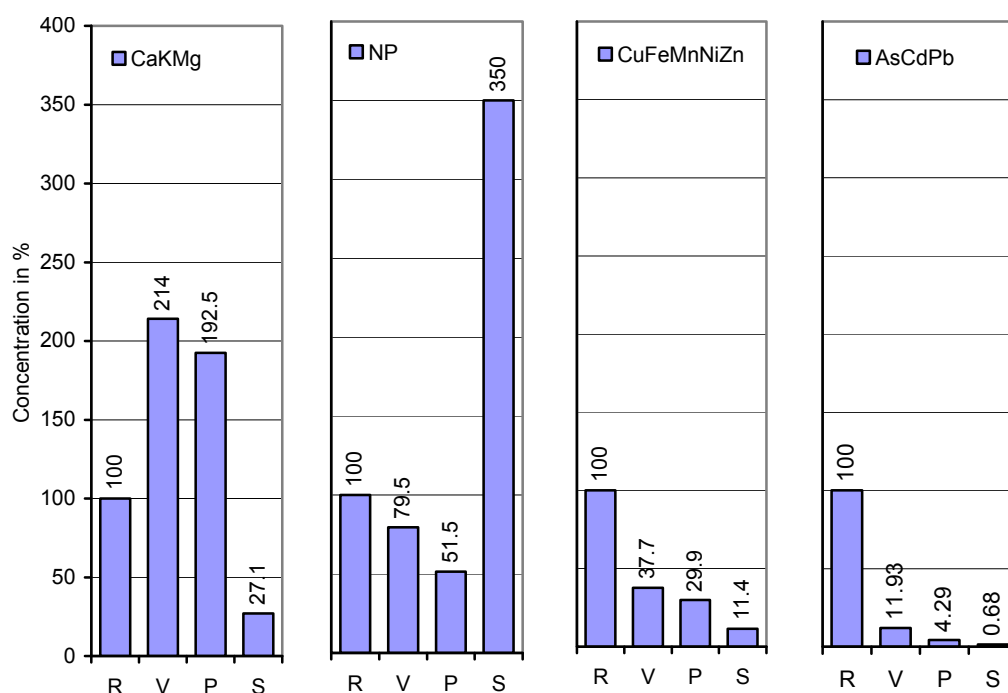


Figure 2. Variation in concentrations of minerals from roots (R) of straw-type pea plants (100%) via upper vine (V) and pod (P) to the whole seed (S). Values are arithmetic means for sets of macronutrients, essential traces, and toxicants with similar modes of passage (refer to Table 2). Adapted from primary data of Gramss and Voigt [18].

Table 2. Up- and down-regulated mineral concentrations (mg kg^{−1} DW) across the mature plant of pea cv. Rocket grown on the high-contaminated uranium gradient soil A. (), quotients calculated from mineral concentrations of the actual to the preceding column expressed in percent.

Element	Root	Whole upper vine	Pod wall	Whole seed
Ca	12,300	18,800 (153)	15,115 (80)	932 (6.2)
K	8,390	11,190 (133)	18,350 (164)	10,090 (55)
Mg	2,400	6600 (275)	6290 (95)	1120 (18)
P	986	580 (59)	414 (71)	3,406 (823)
Cu	63.9	12.2 (19)	23.4 (192)	8.14 (35)
Fe	1,145	109 (10)	72 (66)	49.8 (69)
Mn	160	123 (77)	44 (36)	11.8 (27)
Ni	3.83	1.82 (48)	2.33 (128)	0.95 (40)
Zn	684	311 (35)	160 (51)	70.2 (44)
As	7.45	0.833 (11)	0.106 (13)	0.049 (46)
Cd	36.7	6.18 (17)	2.81 (45)	0.302 (11)
Pb	7.24	0.548 (8)	0.273 (50)	0.040 (15)

All values within horizontal lines differ significantly at $p \leq 0.05$ ($n = 2-4$) from preceding ones. Adapted from primary data of Gramss and Voigt [18].

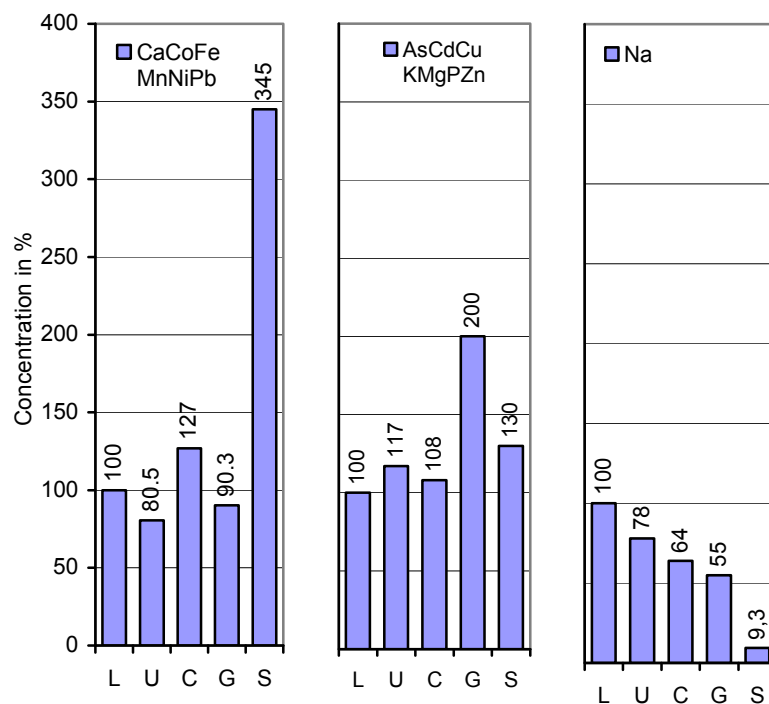


Figure 3. Variation in concentrations of minerals from the lower stipe (L) of *Kuehneromyces mutabilis* basidiomes (100%) via upper stipe (U), cap plectenchyma (C), and gills (G) to basidiospores (S). Values are arithmetic means for sets of minerals with similar modes of passage (refer to Table 3). Adapted from Gramss and Voigt [61].

Table 3. Up- and down-regulated mineral concentrations (mg kg^{-1} DW) across sporulating basidiomes of the mushroom *K. mutabilis* grown on trunk sections of European beech embedded into non-contaminated soil C_{org} 8.9%. (), quotients calculated from mineral concentrations of the actual to the preceding column expressed in percent.

Element	Lower stipe	Upper stipe	Cap plectenchyma	Gills	Basidiospores
Ca	176	114 ^a (65)	382 ^a (335)	137 ^a (36)	277 ^a (202)
Fe	87	80.5 (93)	91 (113)	133 ^a (146)	199 ^a (150)
Mn	47.5	42 (88)	46 (110)	28.5 ^a (62)	73.5 ^a (260)
Ni	1.40	1.45 (104)	2.30 ^a (159)	1.85 ^a (80)	13 ^a (703)
Pb	0.210	0.105 ^a (50)	0.180 ^a (171)	0.090 ^a (50)	0.555 ^a (620)
As	0.069	0.061 (88)	0.088 ^a (144)	0.112 ^a (127)	0.109 (97)
Cd	0.630	1.08 ^a (171)	0.300 ^a (28)	1.23 ^a (410)	0.850 ^a (69)
Cu	36	33.5 (93)	52 ^a (155)	71.5 ^a (138)	24 ^a (34)
K	26,125	28,800 (110)	26,940 (94)	34,180 ^a (127)	8,157 ^a (24)
Mg	1,130	890 ^a (79)	1,340 ^a (151)	1,580 (118)	1,140 ^a (72)
P	2,980	3,920 ^a (132)	3,280 ^a (84)	9,610 ^a (293)	5,150 ^a (54)
Zn	31	45.5 ^a (147)	33 ^a (73)	78.5 ^a (238)	75 ^a (96)
Na	987	774 ^a (78)	636 ^a (82)	539 ^a (85)	92 ^a (17)

^a Values within a horizontal line differ significantly at $p \leq 0.05$ ($n = 2-4$) from preceding ones. Adapted from primary data of Gramss and Voigt [61].

Displayed by the bell-shaped curves, concentrations in K (Figure 1 and Table 1) and CaKMg (Figure 2 and Table 2) essentially surpassed those in the roots and were drastically cut down to rest concentrations of 6.2%–55% on passage from rachis/pod wall to the seeds. In contrast, resources of the macronutrients MgP (Figure 1) and NP (Figure 2) persisted below those in the roots but rose

to 184%–823% from rachis/pod to seeds. This mode of passage also applied to CaCoFeMnNiPb in *K. mutabilis* which enriched at rates of 150% (Fe) to 700% (Ni) at the gill/basidiospore interface.

In the heterogeneous group AsCdCuKMgPZn of the mushroom, most intermediate concentrations exceeded those in the lower stipe. Although cut down at the gill/spore interface to 24%–97% (Figure 3 and Table 3), AsCdMgPZn unlike CuK continued to exceed the concentrations in the lower stipe significantly.

The common feature of the micronutrients CuFeMnNiZn and the toxicants AsCdCrPbU in the crop plants was their poor transfer from root to shoot and their subsequent predominant decline (Figures 1 and 2 and Tables 1 and 2). Deviating from this mode, (As)CuFeMnNiZn were enriched in wheat from lower stem to rachis in preparation for their high demand in grains.

A comparison of the resulting seed and basidiospore metallomes with those of analogous crops from uranium mine gradient soils and with common hygiene guidelines for forage comprises Table 4. The concentration spans represented by the wheat cvs. Akteur, Brilliant, and Bussard and the rye cv. Visello (“cereals”; [27]) matched with those of CaKMgP in wheat Asano, in the basidiospores (Tables 1 and 3; [61]), and with MgP in pea. They were surpassed by CaK of pea (Table 2; [18]). The essential micronutrients CuFeMnNiZn of wheat and pea (Tables 1 and 2) also matched with the limits drawn by Table 4 cereals and corresponded, apart from the elevated value of Zn in wheat, with the spans of “normal plant concentrations.” Basidiospores were normal in Zn but elevated in FeNi > Mn > Cu whereby CuFeMn are co-factors of laccase and manganese peroxidase enzymes that are untypical of herbs [62]. Concentrations of AsCdPb in seeds and basidiospores (Tables 1–3) met the Table 4 ranges in cereals. While AsPb resources were negligible and concurred with the standards of food hygiene, Cd surpassed the respective limits.

In the mineral costume of the mature plant, the stock of trace elements in the lower stem of wheat had dropped to a mean of 7.8% relative to the root (Table 1). Whereas AsCdPbUZn concentrations thereby exceeded, and those of CuFeMnNi fell short of the target level in the grain (Table 1), all the minerals but PbU were enriched in the direction of the upper stem (by a mean of 176%) and the rachis (157%). They were then cut down at the rachis/grain interface to 1.6%–12% (AsPbUZn) or up-regulated to 106% (Mn) and 225% (Cu) within a common mean of 65.4%. Interventions into the mineral flow are thus by far more drastic at the interface of the root and the lower stem but do not yet reflect the proportions in the mature seed. Mineral excesses (CaFeKZn) and deficits (CuMgP) expressed in the upper 12-cm sections of the stem (Table 1) vary greatly with the soil fertilization regime [63,64]. In confining its impact on the narrow and preset target metallome of the seed, the rachis/grain interface plays a unique and astonishingly versatile part. The metallomes of pea seeds and even those of fungal basidiospores were controlled accordingly (Tables 2 and 3).

3. The Adopted Seed Metallome in Correspondence with the Geochemical Environment

Data sets of Table 4 reflect the cultivation of three wheat and single rye and pea cvs. on three metalliferous uranium mine gradient soils. Reference crops were grown on garden soil and alluvial sand, which represented HM loads at the lower range of arable soils (Table 4; [18,27]). Concentrations of essential and non-essential minerals of the seeds varied much less than those in the soils. Respective concentration spans of 9–35× in CuFeMn(Ni) shrank to 1.3–2× in the seeds. Wide variations in Zn (109 to 2.13×) and Cd (475 to 15.9×) were more efficiently out-regulated by pea than by cereals. Potential health problems linked with the repressed uptake of As(Cd)Pb [38,65,66] and amplified by the concerted action of other HM [19] were subsequently minimized and excluded (Table 4). The grain deposition in BaCs evoking concern by the presence of their radioisotopes deviated from this general mode. The concentration spans in seeds surpassed those in different sets of soils permanently [18].

Table 4. Wide concentration spans () in sets of HM-contaminated and non-contaminated arable soils diminish drastically in seeds. The resulting seed mineral loads correspond with the dietary pretensions of (livestock) herbivores and human and indicate uptake repression to AsCdPb toxicants. All values are given in mg kg^{−1} DW. Adapted from primary data of Gramss and Voigt [18,27].

Element		Concentration spans () in soil and seed, number of test soils (refer to Figure 4)			Balanced dietary pretensions to herb forage		Usual heavy-metal ranges	
		Wheat (3 cvs., 3 soils)	Rye (4 soils)	Pea (5 soils)	Monogastric livestock	Ruminants	Arable soils	Herbage
Ca	Soil	7235–2296 (3.15)	7235–2296 (3.15)	7546–1646 (4.6)			–	
	Seed	538–281 (1.9)	411–330 (1.25)	1256–847 (1.48)	2400–9000 ^a	2000–15,000 ^a		–
K	Soil	5130–4086 (1.26)	5130–4086 (1.26)	2264–1296 (1.75)			–	
	Seed	6535–4289 (1.52)	8006–4806 (1.67)	10,335–9139 (1.13)	1500–10,000 ^a	5000–10,400 ^a		–
Mg	Soil	3237–2252 (1.44)	3237–2252 (1.44)	5595–945 (5.9)			–	
	Seed	1707–1241 (1.38)	1267–996 (1.27)	1236–945 (1.31)	400–1300 ^a	1000–2100 ^a		–
P	Soil	873–713 (1.22)	873–713 (1.22)	2792–307 (9.1)			–	
	Seed	5290–4206 (1.26)	4052–3086 (1.31)	3902–1974 (1.98)	1700–7000 ^a	1600–5900 ^a		–
Cu	Soil	283–15 (18.9)	283–9 (31.4)	261–7.5 (34.8)			2–40 (30) ^c	
	Seed	10.7–14.2 (1.33)	8.21–5.09 (1.61)	8.14–5 (1.63)	3–10 (250–800) ^a	7–11 (25–100) ^a		2–20 (LC 10) ^d
Fe	Soil	23,390–10,170 (2.30)	23,390–5445 (4.30)	41,630–4370 (9.53)			–	
	Seed	39.6–64.3 (1.62)	28–37 (1.32)	49.8–72.3 (1.45)	40–100 (500–3000) ^a	15–50 (500–1000) ^a		–
Mn	Soil	2130–515 (4.14)	2130–380 (5.61)	1780–126 (14.1)			40–1000 (550)	
	Seed	26.4–33.5 (1.27)	22–16.3 (1.35)	8.55–20.5 (2.40)	2–60 (400–2000) ^a	14–40 (1000) ^a		14–30
Ni	Soil	40.8–11.8 (3.46)	40.8–4.58 (8.91)	54.3–3.9 (13.9)			3–50 (30)	
	Seed	1.41–0.180 (7.83)	0.811–0.069 (11.8)	0.626–1.21 (1.93)	0.05–0.2 (50–300) ^a	0.3–0.5 (50) ^a		0.1–3 (LC 1)
Zn	Soil	3005–48 (62.6)	3005–36 (83.5)	2890–26.4 (109)			10–80 (90)	
	Seed	190–35.8 (5.31)	207–24.8 (8.35)	33–70.2 (2.13)	35–100 (500–1000) ^a	20–55 (300–500) ^a		10–100 (LC 50)
As	Soil	156–6.34 (24.6)	156–3.53 (44.2)	152–2.3 (66)			1–20 (6)	
	Seed	0.433–<0.080 (>5.41)	<0.080 all	0.044–0.062 (1.41)	2 (50) ^b	2 (50) ^b		0.01–1 (L 0.5; LC 0.7)
Cd	Soil	41.3–0.231 (179)	41.3–0.209 (198)	40.4–0.085 (475)			0.1–0.6 (0.35)	
	Seed	3.13–0.033 (94.8)	1.72–0.018 (95.6)	0.333–0.021 (15.9)	1 (0.5) ^b	1 (0.5) ^b		0.05–0.4 (L/LC 0.1)
Pb	Soil	150–20.5 (7.32)	150–16.5 (9.1)	148–9 (16.4)			2–80 (35)	
	Seed	0.057–0.140 (2.46)	0.093–0.033 (2.82)	0.040–0.019 (2.11)	30 (30) ^b	30 (30) ^b		0.1–6 (L 0.2; LC 0.4)

^a Recommended mineral concentration spans and (maximum tolerable levels in forage) according to McDowell [10]; ^b Legislative limits for toxicants in forage after [16] and (maximum tolerable levels) according to McDowell [10]; ^c Concentration ranges of non-contaminated soils after Schachtschabel *et al.* [7]; (), after Bowen [67]; ^d Normal plant heavy-metal concentrations after Auermann *et al.* [68] and Schachtschabel *et al.* [7]; L, legislative limits for food grains [69]; LC, tolerance limits of Chinese standards for food-quality wheat grains according to Huang *et al.* [19]. In cvs. of wheat/rye, correlations between HM concentrations in soils and grains were recorded for BaCsSr/BaZn ($r \leq 0.05$) and CaCdNiZn/CdCuSr ($r \leq 0.33$). Concentrations of AsCoCrFeMnPbThU did not correlate [27].

Owing to the flexibility of the rachis/pod to seed interface, seed crops compensated for the drastic variations in the soil mineral load by down-regulation of the seed:soil transfer factors (TFs) on high-concentrated soils and their up-regulation on low-concentrated soils to reach a narrow and preset plateau concentration span (Figure 4). Viable seeds were thus equipped with an optimum but not the maximum of essential minerals [18,27] in the range of “normal plant concentrations” (Table 4; [7,68]) rather than with non-essential toxicants. This ensures the metal-stress-free development of the germling until it starts interacting with the soil solution. On several test soils, the seed metallomes of most crop plants surpassed the variability ranges of $\leq 2\times$ in CdZn (Table 4; Figures 4 and 6).

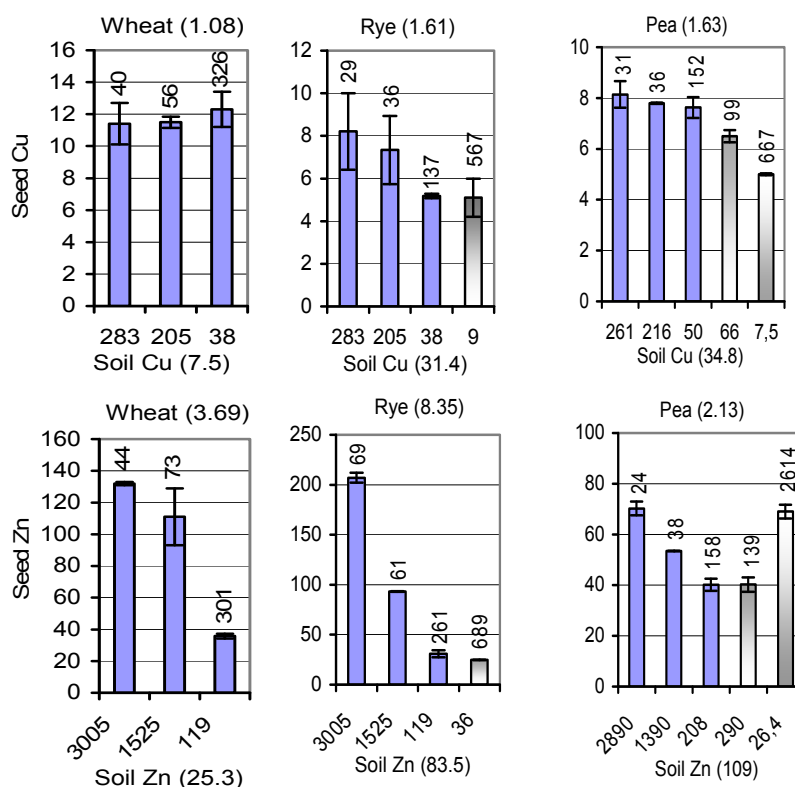


Figure 4. Concentration spans (factors in parentheses) in the CuZn stock (mg kg⁻¹ DW) of the soils diminish drastically in the seeds of wheat, rye, and pea due to proportionate up-regulations of the seed:soil transfer factors (values heading the columns $\times 10^{-3}$) on lower concentrated soils. Soils, left to right: Uranium gradient soils ($3\times$) C_{org} 3.5%–4%; soil C_{org} 8.9%; alluvial sand C_{org} 1.97%. Concentrations in CuZn represented by the bars are given in mg kg⁻¹ DW. Adapted from primary data of Gramss and Voigt [18,27].

Pea expressively reached the concentrations of the apparent target metallome on the organic- and extremely mineral-poor alluvial sand (C_{org} 1.97%). In the absence of the dominating humic-acid ligands which are notorious for the organic-rich soils, alluvial sand was dominated by the highly plant-available free metal cations and metal-organic-acid complexes of highest water-solubility [18,27]. Failures to reach the target seed concentration may thus correlate with low plant availabilities of minerals in the respective soils.

Approaching plateau concentration spans in seed crops with their cultivar-specific variation is evidently the result of polygenic interactions [70,71]. The concentrations in the nutritionally essential FeZn range by factors 2–2.6 $\times$ in bean, 3.3–4 $\times$ in rice, 2 $\times$ in wheat, and around 1.5 $\times$ in maize seeds [72,73]. Respective differences, e.g., in wheat, may result from long-term adaptations to the local geochemistry rather than to closer relationships with diploid and tetraploid high FeZn wheat species such as ssp. *boeoticum* and *dicoccoides* (178/159 mg kg⁻¹ DW in Zn) [74]. Comparable long-term adaptations are strongly suggested by the seed’s Ni content of up to 4800 mg kg⁻¹ from the Ni

hyperaccumulator *Alyssum lesbiacum* endemic to serpentine soils [75]. Moreover, the inherent degree of Ni hyperaccumulation in different *A. lesbiacum* accessions varied with the Ni content of their local soils [76] indicating that the regional within-species variations as studied for the shoot Zn of plants are actually in part an evolutionary trait [77].

It is then not surprising that mineral concentrations needed by herbivores (Table 4) [10,13] have also been adapted to the order of those considered normal by plants [7,68] and adequate for seed germination in the given geochemical environment [27,78]. Seed concentrations in KMgP rather than in Ca corresponded with the need of monogastric animals (horse, swine, chicken) and ruminants (beef and dairy cattle, sheep). Pretensions in CuFeMnNiZn coincided, too. Of the non-essential toxicants AsCdPb, non-tolerable concentrations of Cd in cereals were confined to soils reaching 40 mg kg^{-1} , whereas AsPb levels in seeds remained marginal (Table 4). According to the illustration for Cu (Figure 5), concentrations in wheat, rye, and pea seeds as well as the pretensions of herbivores matched predominantly with the lower range of Cu in herbs grown on non- and severely contaminated soils [10,18,27]. They thereby surpassed the putative minimum demand for Cu required for vigorous plant growth [13].

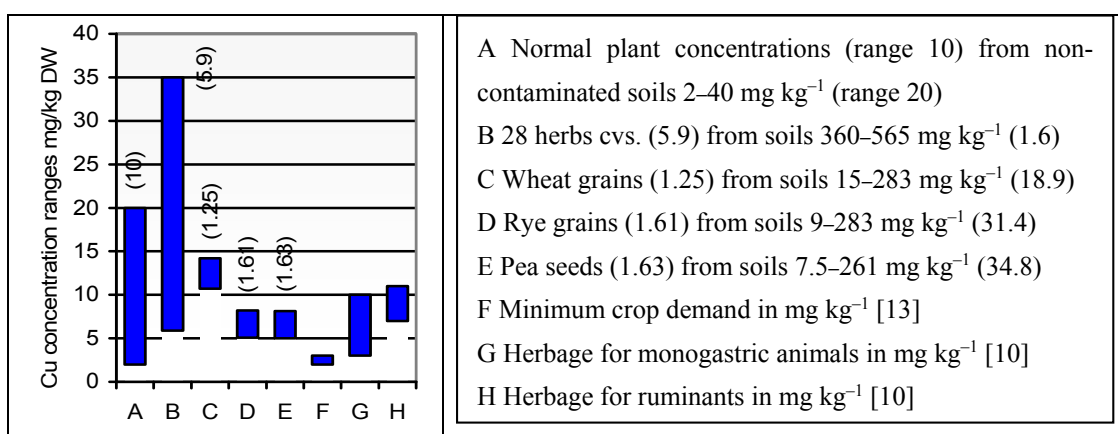


Figure 5. Normal shoot Cu concentrations of crops and rangeland plants adapted to the geochemical environment of non-contaminated soils (A) rise in the presence of elevated soil Cu (B). In contrast, seeds of wheat, rye, and pea observe their adopted mineral target stock by compensating for widest soil mineral fluctuations with proportionate seed:soil TF adaptations (C–E). Their mineral content surpasses the putative minimum crop demand (F) but concurs exactly with pretensions to the forage for herbivores (G, H). (), concentration spans. Refer to Table 4 for the ecological position of further nutritionally relevant elements. Adapted from primary data of Gramss and Voigt [18,27].

4. Discrimination of Essential and Non-Essential Elements Masked by Chemical Similarities

As reviewed by Gramss and Voigt [18], plants express hyperaccumulator and metal tolerance properties in adaptation to elevated soil concentrations of essential (CuNiZn) and non-essential (AsCdCrPb) trace minerals [35,36,75,79]. They do not seem to identify those of physiological insignificance in order to block up their acquisition via transmembrane transport proteins. Proteins acting as ligands are imperfect in the steric selection of metal cations [80]. It is thus the relative affinity of the metal cation itself that regulates the formation of protein complexes in the stability range $\text{CuZn} > \text{NiCo} > \text{Fe}^{2+}\text{Mn} > \text{CaMg}$ [81]. It has not yet been stated whether the respective affinities stand at least in part for quantitative differences in the non-selective (root) uptake of essential and non-essential trace minerals. Specific transporters for non-essential metals such as CdPb are not known. These metals pass cell membranes via ZIP family (Zinc/iron permease), DMT1 (Divalent metal transporters), and P-type ATPase transport proteins, preventing essential elements from reaching their physiological optima [82]. Arsenite (AsO_3^{3-}) passes aquaglyceroporins with glycerol, urea, and water [82], whereas arsenate (AsO_4^{3-}) as phosphate (PO_4^{3-}) analog uses the phosphate transporters

Pht1;1 and Pht1;4, e.g., in *Arabidopsis thaliana* [83]. Selective uptake of Cd versus $\text{Fe}^{2+}\text{MnZn}$ by the ZIP family transporter IRT1 is complicated as its electron configuration and the preference for phytochelators and histidine ligands coincide with those of Zn [38]. In addition, Cd shares its preference for thiols with further non-essential metals that can then pass as unsplit complexes via amino acid and cation/anion transporters [84]. Moreover, the ubiquitous membrane-voltage-independent non-selective cation channels (VI-NSCCs) give room to the passive and non-selective transmembrane flow of $\text{Cs}^+\text{Hg}^+\text{K}^+\text{Na}^+\text{NH}_4^+$ and the divalent cations of CaCdMgMnPbZn [85]. Root uptake of $\text{Fe}^{2+}\text{Mn}^{2+}$ by wheat as a strategy-II plant proceeds by the immediate uptake via ZRT- and IRT-like proteins (ZIPs) or as Fe(III)-phytosiderophores by yellow-stripe-like (YSL) transporters [86]. Is then the plant prepared to perceive the ingress of a particular (non-essential) element and to denote its presence with an element-specific and unique signal?

Metal cations such as Cd^{2+} induce NADPH oxidase activity in contacted cell membranes and a rise in the production of reactive oxygen species (ROS) [87] that damage cellular components such as lipids, proteins, carbohydrates, and DNA [88–91]. ROS incited signals expressed in formation and modulation of mitogen-activated protein kinases (MAPKs), Ca^{2+} /calmodulins, nitric oxide, and the common plant hormones alter nuclear gene expression and the formation of cation-neutralizing chelants to control the cellular redox status [92–95].

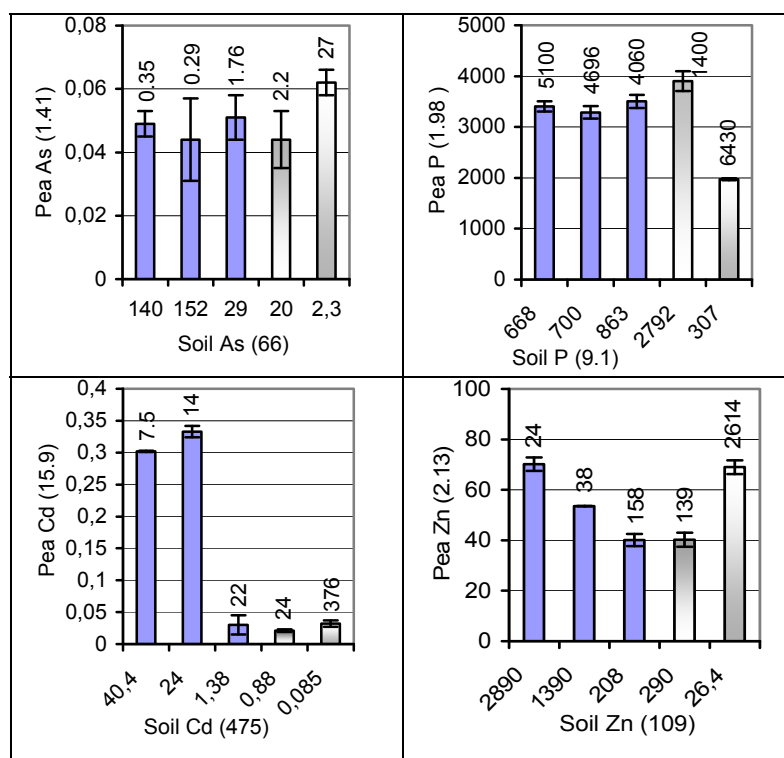


Figure 6. Incorporation of the chemically similar elements As/P and Cd/Zn in pea seeds. Up-regulations of the seed:soil TFs (values heading the columns $\times 10^{-3}$) on low-concentrated soils (mg kg^{-1} DW) indicate the active acquisition of both essential and non-essential elements. Differences in TFs and in the quotients of TF: soil concentrations indicate the safe discrimination of chemically similar elements. Concentrations in AsCdPZn represented by the bars are given in mg kg^{-1} DW. Adapted from primary data of Gramss and Voigt [18].

Nevertheless, similar plant signaling responses specifically attributable to the presence of a unique (non-essential) element are poorly documented. In rice roots, Cd^{2+} and Cu^{2+} induced identical MAP kinase activities via distinct ROS-generating systems [96]. In roots of alfalfa seedlings, Cd^{2+} and Cu^{2+} provoked different cellular signaling mechanisms by the induction of distinct MAPK variants [97].

Cu exposed to *Arabidopsis thaliana* roots led by activation of NADPH oxidases and Fenton reactions to the production of H_2O_2 that triggered signaling activities by MAPK and oxylipin. In contrast, oxidative signaling was the sole response by root exposure to Cd [90]. *Arabidopsis thaliana* grown on an agar medium amended with 100 μM CdPb contained 0.6/0.15 μM of the toxicants. The rates of up-/down-regulated genes amounted to 65/338 and 19/76 in the Cd and Pb treatment, respectively, with half the genes responding likewise to both Pb and Cd treatment [98]. In *Deschampsia cespitosa* roots exposed to Ni, concentrations of cytokinins and abscisic acid did not change whereas the content of nicotianamine increased. Exposure to Cd caused increase in phytochelatin and abscisic acid but reduction in the content of cytokinins [95].

The random plant uptake of minerals which are termed non-essential at the current evolutionary state may be excused for their cell membrane passage in chemical resemblance to, or in company of, essential elements [38,82,84,85]. Several data, however, contradict the impression that plants discriminate minerals insufficiently. In Chinese cabbage (*Brassica chinensis* L.) treated with NH_4Cl , shoot concentrations in (metallo) proteins (N_{org}) and in the protein-associated group of (Cd)CoCuMnNiZn rose concomitantly by factors of 3.4–3.7. Uptake of non-essentials such as AlBaCrLiPbTiUV diminished or failed to surpass the factor 1.2 $\times$. This was due to their predominant lack in affinities to proteinaceous ligands and their relative dilution in a biomass propagated by the nitrogen treatment [99]. Seeds determined to persist in the current geochemical environment were actively equipped with out-regulated, optimized, and tolerable concentrations of both essential and non-essential minerals including the toxicants AsCdPb (Figures 1, 2 and 4; Tables 1, 2 and 4; [18,27]. Of the arsenate (As(V)) entering via phosphate transporters [83], >90% passed the xylem of the hyperaccumulator *Pteris vittata* as arsenite (As(III)) [79] and could not be distinguished from phosphate by the respective aquaporin transporters [100]. Nevertheless, data from Figure 6 show that seed:soil TFs in As and P given for pea differed in the absolute values heading the columns; these absolute values divided by the respective soil concentrations also differed drastically.

Both in wheat and pea, the differences in the passages of As/P from roots to the rachis/pod interfaces were less pronounced but terminated in drastic and mineral-specific down-/up-regulations upon seed filling (Figures 1 and 2; Tables 1 and 2). The respective passages of Cd/Zn also ended with individual differences in their rates of cut-down to the seeds. Soil-A-grown grains of the wheat cvs. Akteur, Brilliant, Bussard, Asano, and the rye cv. Visello then contained 78/61/96/89/120 times less Cd than Zn [27].

As discussed by Gramss and Voigt [18], the issue of why plants incorporate non-essentials actively remains subject to speculation. Experience with the respective pathways could help plants such as couch grass (*Agropyron repens* L.) tolerate and colonize regions of normally lethal toxic imissions around metal smelters within a few decades [101]. Clemens [102] explains the current rating of chemically similar elements as essential or non-essential with evolutionary adaptations of living organisms to those of higher abundance in the earth's crust. This could imply that the physiological activities of "non-essential" elements situated in the nanomolar range might have been overlooked. Maintaining ruminants on feeds < 4 $\mu g\ kg^{-1}$ DW of Cd may result in growth depression and higher mortality rates in their offspring. Diets of 14 $\mu g\ kg^{-1}$ led to higher weight gain [103]. Respective experiments indicating the essentiality of low CdPb doses for health and development of animals go back to the nineteen seventies [104].

5. Conclusion

Soil concentrations ranging 9–109 $\times$ in CuFeMnNiZn shrank to spans of 1.3–2 $\times$ in seeds to mark the order of their adopted target mineral load (Table 4; Figure 4) and the stability of their inherent metallome in the presence of soil mineral extremes. This regulatory process comprised the initiation of the phloem-to-seed mineral transfer at anthesis and its sensing and termination by unknown back-coupling (feedback) mechanisms as soon as the target mineral load had been reached. The big green-plant seeds of cereals and peas and the 7- μm basidiospores of the lower-plant saprobe *K. mutabilis*

(Tables 1–3) were concerned likewise. Approaches to the genetically fixed seed target metallome were based on proportionate up-regulations of the seed:soil mineral transfer factors on low-concentrated soils, and their down-regulation on high-concentrated soils (compare Figures 4 and 6). Non-essential elements were actively incorporated in the same way. Mineral concentrations in the phloem- (and cytoplasmic-stream) draining tissues of rachis, pea pod, and mushroom gill as well as in the remaining sections of the plants, deviated considerably and irregularly from those of the propagules' target metallome (Tables 1–3). This may also bear true for the phloem composition whose dependence on changes in the soil mineral stock, the rate of soil water supply, and the plant's transpiration rate are not documented. Phloem extracted from wheat plants by aphids, a treatment known as aphid stylectomy, showed increases in MgZn to 190% and reductions in K to 50% in samples taken 1–2 to 17–21 day after anthesis. Concentrations of Fe did not change [105]. The total concentrations in phloem samples persisted below those in mature grains (Table 4) [105]. In the phloem of five pea cvs., concentrations of 22 amino acids were ranged 1.4–3 times [106]. Contemplating these variations, one can not presume that the percentage proportions between phloem constituents are congruent with, or immediately determine those, of the fed grain. This means grain fill could not end with the non-selective incorporation of the constituents introduced, e.g., by 10 mL of phloem. Seed filling out of the phloem may thus imply the identification of its essential and non-essential minerals (as well as organics) and their selective and individual transfer to the propagule to constitute its target composition. The extreme numerical variation in the seed:soil transfer factors (Figures 4 and 6); [18,27] of plants from mineral-rich and poor soils may therefore be pretended, *i.e.*, the “transfer factors” phloem:soil could be much smaller. The huge numerical transfer factors may then be reached when the required absolute amounts, e.g., of As and Cu (Figures 4 and 6) are selectively transferred from the variably concentrated phloem of plants from rich and poor soils to the seed. In explaining this analytical and strategic top performance, gene-RNA interferences (RNAi) [40,107] alone may contribute to shape the seed metallome, but they may not represent the unknown principle which is active in the coordination of the mineral homeostasis. It is concluded that seed crops with their stable metallome can be obtained from severely mineral-poor and metalliferous soils whose permissible HM loads can even be considerably expanded in AsCdCuMnUZn [18,108]. This implies that seed crops reduce the pressure on the quality of detoxification of non-remediable metalliferous croplands. In optimizing the plant-soil partnership, actively selected and bred food and forage crops should be provided whose uptake/excluder profile, including the Zn/Cd proportion, should be certified for clay-loam and sandy soils under standard conditions. The quality of the resulting crop must be substantiated in a local case study.

Author Contributions: In this cooperative work, Gerhard Gramss primarily conducted the agricultural part, whereas Klaus-Dieter Voigt performed the mineral analyses. The script was elaborated in mutual consultation.

Conflicts of Interest: The authors declare no conflict of interest.

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