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Valuable Secondary Habitats or Hazardous Ecological Traps? Environmental Risk Assessment of Minor and Trace Elements in Fly Ash Deposits across the Czech Republic

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Abstract: Deposits of coal combustion wastes, especially fly ash, are sources of environmental and health risks in industrial regions. Recently, fly ash deposits have been reported as habitat surrogates for some threatened arthropods in Central Europe. However, the potential environmental risks of fly ash have not yet been assessed in the region. We analysed concentrations of 19 minor and trace elements in 19 lignite combustion waste deposits in the Czech Republic. We assessed their environmental risks by comparison with the national and EU legislation limits, and with several commonly used indices. Over 50% of the samples exceeded the Czech national limits for As, Cu, V, or Zn, whilst only V exceeded the EU limits. For some studied elements, the high-risk indices were detected in several localities. Nevertheless, the measured water characteristics, the long-term presence of fly ash, previous leaching by acid rains, and the low amount of organic matter altogether can infer low biological availability of these elements. We presume the revealed high concentrations of some heavy metals at some studied sites can be harmful for some colonising species. Nevertheless, more ecotoxicological research on particular species is needed for final decision on their conservation potential for terrestrial and freshwater biota.

Keywords: biodiversity conservation; coal combustion waste; energy industry; environmental pollution; human-made habitats; heavy metals

1. Introduction

Coal combustion in thermoelectric power plants still dominates power production in many regions, including Central Europe, despite a recent shift towards renewable and green energies. Among numerous other environmental risks, it generates enormous quantities of solid by-products dominated by fly ash (i.e., very fine glass-like particles of mineral residua carried in the flow of exhaust gases). Other waste types, such as harsher bottom ash, boiler slag, and desulphurisation residues, contribute only 25–30% of the total solid by-product weight [1].

For many decades, this solid waste (hereinafter called 'fly ash' because of the prevailing material) has been mixed with wastewater and deposited in sedimentation lagoons

near the power plants. Consequently, these lagoons were used for the gravitational settling of the particulate material and became common in many industrial and urban landscapes. However, technological progress in the late 1990s and early 2000s resulted in a more efficient separation of coal combustion dry by-products. This allowed for the utilisation of deposited dry wastes in the construction industry, reclamation of mining sites, and agriculture [2,3]. Except for smaller standby 0.5–1 ha emergency reservoirs, the sedimentation lagoons have thus been gradually drained and reclaimed [4], or left for spontaneous succession during the past two decades [5].

Several recent studies revealed that drained fly ash deposits left to successional development may constitute important secondary refuges for numerous highly endangered terrestrial arthropods in Europe (e.g., [6,7]). In some cases, fly ash deposits can act as strongholds for animal communities specialised for some vanishing habitats, such as inland sand dunes, steppe-like grasslands, riverine gravel beds, or marshlands and salt marshes [6–10]. At least some endangered species seem to benefit from the early successional habitats of fly ash deposits, despite the potentially high contents of heavy metals in fly ash [8,10]. For example, surveys of wild bees and wasps at five Czech fly ash deposits found 11 species previously considered to be nationally extinct and 33 critically endangered species [8,10]. Similarly, these human-made sites also harbour numerous highly endangered species of butterflies [5], beetles [5,11], spiders, and leafhoppers [8,10]. Last but not least, Central European fly ash deposits can also provide breeding habitats for threatened vertebrates such as the natterjack toad (*Epidalea calamita*) and the sand martin (*Ripariariparia*; [5]).

Even though fly ash deposits may harbour species of tremendous conservation value, they also pose considerable environmental risks. The minute size of fly ash particles facilitates aeolian erosion and the consequent pollution of adjoining environments [12–14]. Furthermore, fly ash deposits may have a negative impact on the threatened biodiversity and function as ecological traps owing to the higher concentrations of heavy metals, such as, Cd, Cr, Cu, Ni, Pb, U, V, and Zn [15–18]. Although the responses to toxic elements present in the environment are species-specific, both laboratory and field studies indicate that heavy metals can adversely affect the survival, growth, and reproduction of various organisms [19–24]. However, a full appraisal of the environmental risks posed by fly ash deposits requires further research, since the bioavailability of elements in the fly ash deposits may depend on specific physical, chemical, and biological factors [17,25,26].

While the conservation value of fly ash deposits highlighted above has been challenged on the grounds of their potential to act as ecological traps, no study has addressed this issue. Here, we present the first comprehensive survey of 19 selected minor and trace elements in sedimented fly ash at 19 lignite combustion waste deposits distributed throughout Bohemia, Czech Republic. To our knowledge, this study covered all remnants of non-reclaimed fly ash deposits in Bohemia at the time of our sampling. We sampled and analysed substrates from these fly ash deposits, and used the measured content of heavy metals and the available literature on heavy metal toxicity to assess potential impacts on biota. Our main aim was to assess the potential of fly ash deposits to act as ecological traps rather than biodiversity refuges, focusing on arthropods, the key group for which the fly ash deposits are known to hold high conservation value. Moreover, we examined the environmental risks of these fly ash deposits from a legal perspective, comparing their content of the potentially toxic elements with the national legal limits for different soil types in the Czech Republic, and with internationally recognised risk indices.

2. Materials and Methods

2.1. Study Localities

Deposited fly ash was sampled at 19 fly ash sedimentation lagoons associated with lignite-combusting thermal power plants located in several regions of Bohemia, Czech Republic, a relatively densely populated region with a long industrial history, and with numerous remnants of natural and semi-natural habitats (Figure 1). Lignite combusted in

all these power plants originated prevailingly from the Sokolov and North Bohemian lignite basins, both in the north-western Czech Republic. Energy industry development and the subsequent establishment of the associated sedimentation lagoons in this region occurred between the 1950s and 1970s (Table 1). Water bodies currently present at the studied fly ash deposits were left as remnants of the originally extensive system of sedimentation lagoons that have mostly been reclaimed since the 1990s (see Introduction). All studied sedimentation lagoons were equipped with a drainage system to circulate water between the power plant and the lagoon and to prevent polluted water from leaking into the surrounding environment. Except for the three localities with ongoing fly ash deposition (nos. 7, 12, and 19), all studied fly ash deposits were used only in emergency cases at the time of our sampling. Therefore, they were partly or fully overgrown by spontaneous succession, mainly with common reed (*Phragmites australis*). In six localities (nos. 5, 8, 15, 16, 17, and 18), technical reclamation projects (*sensu* [8,27]) have been initiated or even finished since the time of our sampling; the other localities are planned for restoration in the near future.

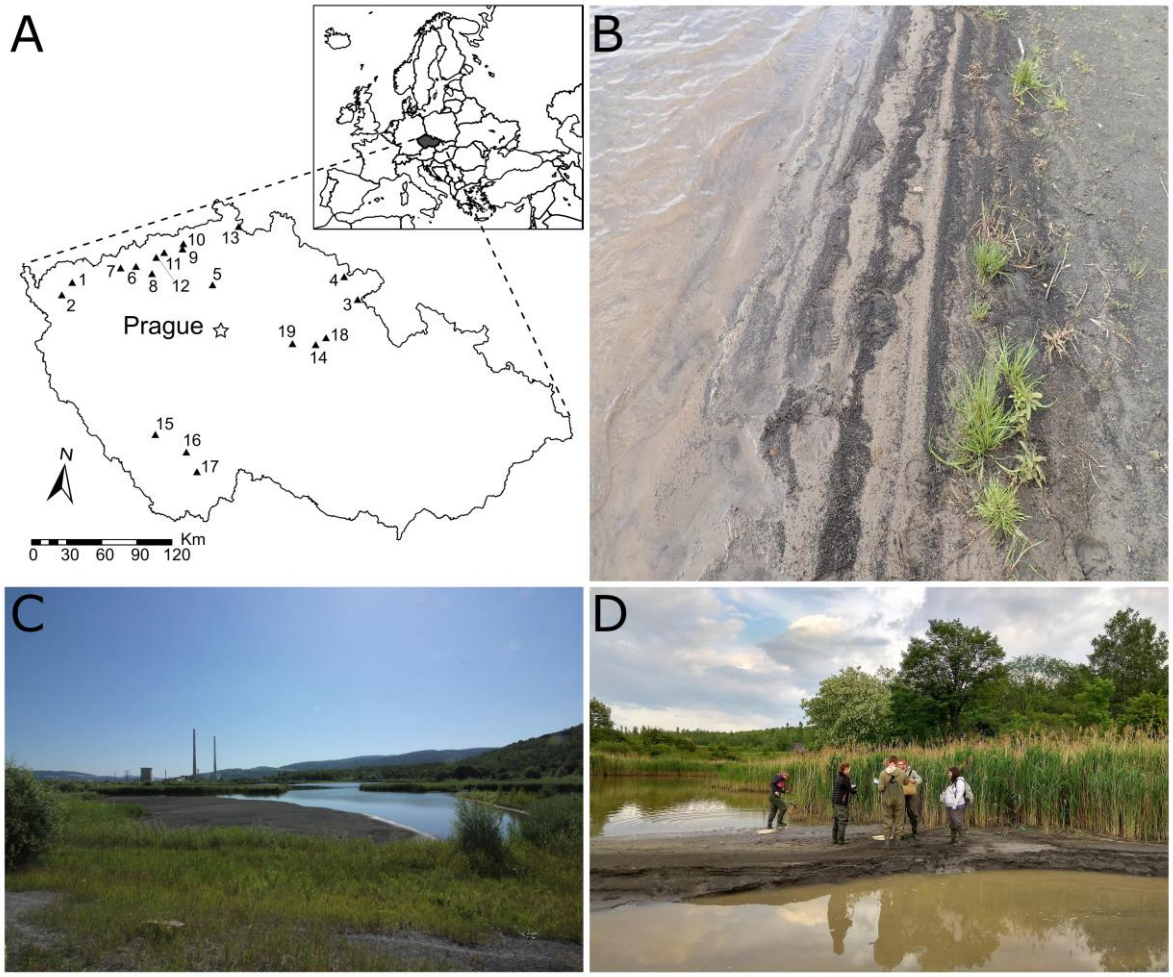

Figure 1. Studied fly ash deposits in the Czech Republic. (**A**) Map of the studied localities (numbered black triangles; see Table 1 for details on individual fly ash deposits). (**B**) Detail of the sedimented fly ash in locality no. 2. (**C**) Heterogeneity of habitats in locality no. 9. (**D**) Sampling of the sediments in locality no. 11.

Table 1. Characteristics of the studied fly ash lagoons in the Czech Republic. NA = data not available.

Locality	GPS Coordinates	Altitude (m a.s.l.)	Lagoon Area (ha)	Year of Establishment	Change of Deposition Technology	pH of Water	Conductivity ($\mu S \cdot cm^{-1}$)	LOI * (%)
1	50.2602° N, 12.7219° E	465	6.2	1967	1996	8.53	1256	30.3
2	50.1518° N, 12.6221° E	390	16	1961	1997	8.44	958	54.9
3	50.4269° N, 16.1514° E	375	2.2	1969	1990s	8.58	322	4.3
4	50.5918° N, 15.9584° E	450	0.07	1957	1998	8.69	708	6.9
5	50.4007° N, 14.3952° E	205	2	1961	1998	8.23	483	9.7
6	50.4541° N, 13.4491° E	310	27.5	1992	2000s	8.26	1431	3.4
7	50.4267° N, 13.2685° E	370	2.5	1968	1998	8.35	3741	5.2
8	50.4215° N, 13.6529° E	260	17.8	1977	1997	8.26	2520	4.3
9	50.6425° N, 13.9754° E	155	16.3	1974	1997	NA	NA	5.5
10	50.6832° N, 13.9760° E	255	1.6	1961	1992	8.38	695	3.2
11	50.5944° N, 13.7620° E	190	10.7	1968	1998	8.51	940	25.9
12	50.5489° N, 13.6765° E	250	63.6	1951	1999	8.58	2159	11.2
13	50.8897° N, 14.6319° E	355	2.6	1972	2000s	7.99	939	7.8
14	50.0440° N, 15.7142° E	220	6	1953	1997	8.87	551	31.6
15	49.1957° N, 13.9694° E	495	0.002	1954	2002	8.11	2178	8.4
16	49.0951° N, 14.3597° E	408	1	1967	2009	8.12	3076	23.7
17	48.9550° N, 14.5149° E	430	5.6	1962	1996	9.03	321	19.4
18	50.1059° N, 15.8276° E	235	9.2	1960	2000s	NA	797	3.2
19	50.0299° N, 15.4350° E	225	0.5	1978	1998	NA	NA	5.0

* LOI—percentage loss on ignition, i.e., organic matter content in sediment (% LOI; 550 °C, 2 h).

2.2. Sample Collection and Chemical Analyse

At each fly ash deposit, we collected one 0.125 L core of the sedimented fly ash from an undisturbed bottom of the lagoon between 14–18 May 2018. We took ca. 5 cm of the surface layer approximately 1 m from the shore in an open water area without macrophytes and organic detritus. We assumed that a single core at each locality was sufficient since the sediments should be highly homogenised owing to repeated blending by the long-term water circulation. The samples were subsequently stored at −18 °C until further analyses. At all but two localities, we measured water pH and conductivity ($\mu S \cdot cm^{-1}$) using a portable YSI multimeter (type 556 MPS, YSI Environmental, Yellow Springs, OH, USA; mean values in Table 1).

Before analyses, all samples were freeze-dried, gently grinded using mortar and pestle to disintegrate ash aggregates, and sieved through 2 mm mesh to exclude stones and larger organic debris in the samples. Samples were then grinded to a fine powder using a laboratory mixer mill (MM 200, Retsch, Haan, Germany) and digested in triplicates with nitric and perchloric acid for 12 h following protocols in [28]. An inductively coupled plasma mass spectrometer (ICP-MS; Agilent 8800 ICP-QQQ, Agilent Technologies Inc., Tokyo, Japan) was used to determine total concentrations of 19 essential or potentially toxic minor and trace elements, Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, P, Pb, Se, Sr, V, and Zn, following protocols in [28]. The term 'heavy metals' hereinafter refers to the metals with a specific density over 5 $g \cdot cm^{-3}$ (i.e., As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn) that can adversely affect the environment and living organisms (*sensu* [29]). Although Hg also belongs to the commonly analysed heavy metals, our methods were unable to reliably determine its content in our samples (cf. [30]). Nevertheless, Hg is almost completely volatilised during the combustion of coal and its proportion in ash particles

is extremely low [31]. Organic matter content in the samples was estimated by loss on ignition (LOI; 550 °C, 2 h; Table 1).

2.3. Risk Assessment of Fly Ash Deposits

Our risk assessment analyses focused primarily on the nine heavy metals (As, Cd, Co, Cr, Cu, Ni, Pb, V, and Zn) with their limit values implemented in the national legislations and/or commonly used for risk assessments. To assess the environmental risks of the fly ash deposits in our study, we compared the total concentrations of the nine heavy metals in our samples with the concentrations in the Czech national limits for 'common agricultural soils' and 'light sandy agricultural soils' (Regulation no. 153/2016). These limits represent the thresholds restricting certain forms of land management in agricultural soils to minimise health and environmental risks.

We used two different indices to assess the degree of heavy metals contamination in the studied fly ash: the *contamination factor* (CF) and the *pollution load index* (PLI). While CF estimates the relative contamination level of a particular trace element to its reference value, PLI compares the level of metal pollution in the sediments as a complex mixture of contaminants [32–34]. At each locality, we determined the individual CF values of the nine heavy metals using their background concentrations available from >200 unpolluted agricultural localities across the Czech Republic [35]. For this purpose, we calculated the CF_X of a particular heavy metal X as the ratio of its concentration in the sediment to the mean of its natural background value in the unpolluted agricultural localities ($CF_X = C_{sediment}/C_{background}$). Following Tomlinson et al. [33], $CF_X < 1$ indicates low contamination, CF_X = 1–3 moderate contamination, CF_X = 3–6 considerable contamination, and $CF_X > 6$ very high contamination levels. PLI of each site was calculated as the geometric mean of all nine CF_X values, i.e., $PLI = (CF_{As} \times CF_{Cd} \times CF_{Co} \times CF_{Cr} \times CF_{Cu} \times CF_{Ni} \times CF_{Pb} \times CF_{V} \times CF_{Zn})^{1/9}$. Values of $PLI \geq 1$ indicate existing pollution, whereas $PLI < 1$ indicates no pollution [33].

We then assessed the environmental risks of As, Cd, Cr, Cu, Ni, Pb, and Zn following the internationally recognised criteria of the consensus-based limits developed for these heavy metals ([36]; Co and V were omitted from these analyses because their limits have not been established). These limits provide a unifying synthesis of several environmental risk indices based on the known effects of contaminant mixtures in sediments. The *threshold effect concentration* (TEC; [36]) indicates the element concentration below which no harmful effects on organisms are expected. The *probable effect concentration* (PEC; [36]) indicates the element concentration above which harmful effects are expected to occur frequently. We compared the measured concentrations of the individual heavy metals with the limit values of TEC and PEC from MacDonald et al. [36]. To evaluate the combined effects of multiple contaminants in each sediment sample, we calculated the *mean PEC quotient* [36], defined as the arithmetic mean of the ratios between the concentration C of each element in the sediment sample and its PEC value at the given locality, i.e., mean PEC quotient = $(C_{As}/PEC_{As} + C_{Cd}/PEC_{Cd} + C_{Cr}/PEC_{Cr} + C_{Cu}/PEC_{Cu} + C_{Ni}/PEC_{Ni} + C_{Pb}/PEC_{Pb} + C_{Zn}/PEC_{Zn})/7$. Mean PEC quotients < 0.5 indicate non-toxic sediments.

Finally, we compared CFs of the nine heavy metals using a one-way ANOVA with the log-transformed data, and used a post-hoc Tukey HSD test to assess significant differences ($p > 0.05$) among the environmental risks of individual heavy metals. We also analysed differences of the individual heavy metal concentrations against their national limits, and TEC and PEC values using one-sided Wilcoxon tests to evaluate if their concentrations were significantly ($p < 0.025$) higher than the national limits and PECs (i.e., posed significant environmental risks), or lower than TECs (i.e., posed low environmental risks). All analyses were run in R v. 3.6.2 [37].

3. Results

Concentrations of all 19 minor and trace elements analysed in the 19 fly ash deposits are listed in Table 2 (together with the used Czech national limits, and the environmental risk limits and thresholds) and visualised in Figure 2. Concentrations in the fly ash sediment

samples varied substantially both among the elements and among the sites. The most abundant elements were Al, Ca, and Fe, while the least abundant elements were Cd and Ag. Some elements were more correlated with redox sensitive Fe (As, Co, Cr, Cu, Mn, Zn), while some heavy metals correlated more with Ca (Ba, Co, Ni, Sr, V; Figure A1), which could affect their biological availability. Concentrations of all heavy metals exceeded the national limits for common agricultural soils or light sandy agricultural soils in one or more localities despite mostly non-significant differences in their median values (Table 2): As (12 localities for the common soils limits/13 for the light sandy soils limits), Cd (3/4), Co (2/7), Cr (0/6), Cu (10/13), Ni (7/9), Pb (1/1), V (12/14), and Zn (10/10). However, only the median concentration of V significantly exceeded the limits for both soil types, while the median concentrations of As, Cu, and Zn significantly exceeded the limits for light sandy agricultural soils only (Figure 2C,D and Table 3). Most of the localities were relatively poor in terms of organic detritus, with only 3–26% of organic matter content in the sediments; organic matter constituted more than 30% of the sediment at three sites only (Table 1).

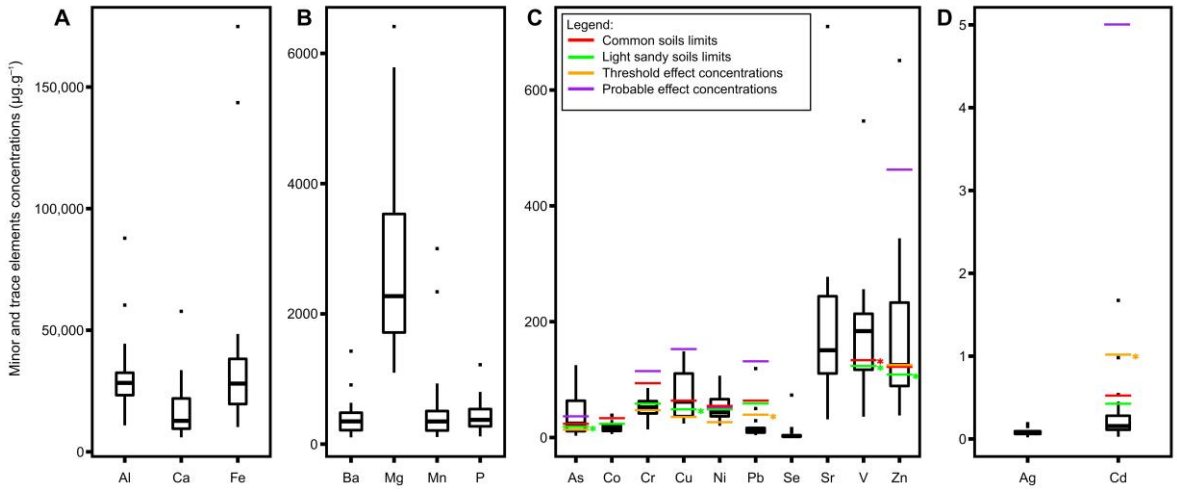

Figure 2. Concentrations of the studied minor and trace elements in the sediments at 19 fly ash deposits in the Czech Republic. Note the different scale of *y* axes in panels (**A**–**D**). Medians with interquartile range (box plot), 95% confidence intervals (whiskers), and outliers (black points) are visualised. Horizontal lines represent the Czech national limits for particular contaminants in common agricultural soils (red) and light sandy agricultural soils (green), the threshold effect concentrations TEC (orange), and the probable effect concentrations PEC (purple). Asterisks (typeset in the respective colour next to the corresponding horizontal line) denote significant differences ($p < 0.025$) of individual element concentrations relative to their national limit values, or to the TEC and PEC indices.

When evaluating the overall environmental risks of particular fly ash deposits, we found the concentration of at least one heavy metal exceeding the national limits for agricultural soils at each locality (Table 2). Locality no. 7 exceeded the limits for common agricultural soils in the concentrations of six heavy metals, while three other localities (nos. 1, 3, and 5) exceeded these limits in the concentrations of five heavy metals. Moreover, locality no. 5 exceeded the limits for light sandy soils in the concentrations of eight heavy metals.

Contamination factors varied significantly among the nine heavy metals (one-way ANOVA: $F_{8,162} = 14.9$, $p < 10^{-4}$; Figure 3). Mostly low relative contamination levels (CF < 1) were detected for Pb (16 localities) and Cd (14 localities). Contamination factors of the other heavy metals were mostly moderate to considerable (CF = 1–6). Very high contamination factors (CF > 6) in the fly ash sediments were detected for As (four localities), Cd (one), Cu (five), V (one), and Zn (one). The empirical pollution load index (PLI) of the nine heavy metals ranged from 0.82 to 2.97 (Table 4), indicating sediment pollution (PLI $\geq$ 1) in 17 out of the 19 studied localities and strong sediment pollution (PLI > 2) at four localities (nos. 1, 5, 7, and 15).

Table 2. Minor and trace elements concentrations ($\mu g \cdot g^{-1}$) in fly ash lagoon sediments at each locality, the Czech national limits in common soils and light sandy soils ($mg \cdot kg^{-1} = \mu g \cdot g^{-1}$ DM; Regulation no. 153/2016), the threshold effect concentrations (TEC, $\mu g \cdot g^{-1}$, taken from [36]), and the probable effect concentrations (PEC, $\mu g \cdot g^{-1}$, taken from [36]) representing consensual limits for assessing the substrate environmental risks, and the environmental reference values ($\mu g \cdot g^{-1}$, taken from [35]). Mean PEC quotient represents the combined effects of multiple contaminants at each locality; values <0.5 indicate no toxicity. See Methods for details on individual limits and risk indices.

Locality	Ag	Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	P	Pb	Se	Sr	V	Zn	Mean PEC Quotient
1	0.15	32,524	115.9	483	25,626	0.98	20	52.5	133.2	21,516	2345	220	41.4	709	16.6	73	277	256	339	0.97
2	0.09	28,335	32.4	349	11,051	0.11	19	54.3	118.2	17,949	1710	456	31.9	319	8.6	2	241	221	241	0.50
3	0.07	44,444	22.3	516	33,604	0.29	30	74.2	80.6	48,481	3355	439	83.6	380	10.9	2	247	153	87	0.56
4	0.08	10,861	23.6	104	5950	1.67	6	13.9	32.6	10,185	2272	171	20.1	119	118.8	1	31	36	242	0.47
5	0.10	28,329	124.8	358	9438	0.45	21	85.5	102.8	28,054	1719	348	61.3	476	15.1	10	151	256	125	1.00
6	0.09	87,843	8.6	374	9718	0.11	11	52.1	46.3	28,199	3575	227	40.4	373	28.9	3	216	121	82	0.33
7	0.06	26,628	72.8	909	12,416	0.26	38	56.6	99.1	143,591	4100	3002	106.8	659	16.4	15	155	184	183	0.88
8	0.05	25,608	18.5	210	12,733	0.16	11	41.2	31.9	29,738	2230	334	43.5	266	12.6	1	132	125	68	0.33
9	0.05	32,484	2.6	153	7892	0.03	17	51.9	41.0	24,030	1588	198	70.4	204	4.1	0	80	130	91	0.36
10	0.09	36,061	29.2	1427	57,760	0.22	29	30.9	61.8	35,041	5786	679	47.2	263	11.9	4	710	223	225	0.45
11	0.08	32,240	10.8	218	9559	0.14	20	68.9	58.8	23,639	1627	233	87.5	360	8.9	4	100	188	69	0.48
12	0.02	22,699	27.8	180	28,656	0.03	11	41.9	28.9	16,969	1736	162	61.9	281	7.4	2	138	547	38	0.40
13	0.09	60,325	22.6	481	23,129	0.16	30	82.4	62.7	37,362	6413	533	94.9	554	18.3	2	260	189	98	0.60
14	0.07	14,215	6.2	295	16,060	0.10	10	27.7	24.0	19,054	5350	394	22.8	340	10.6	1	138	49	344	0.27
15	0.18	23,707	119.5	226	19,896	0.54	12	71.7	122.6	174,889	3493	932	36.5	1219	49.5	2	112	84	651	1.11
16	0.07	31,838	10.4	367	9414	0.27	16	34.9	149.0	15,364	1216	173	35.2	523	8.1	2	205	187	102	0.38
17	0.08	31,837	11.4	636	12,983	0.12	15	44.8	125.9	39,054	1963	484	37.2	802	8.4	2	274	206	202	0.41
18	0.05	18,690	59.4	121	9097	0.11	11	43.7	25.2	20,240	1096	108	36.6	182	7.8	7	65	89	134	0.50
19	0.06	22,871	67.9	276	20,741	0.16	16	44.1	52.0	41,672	2988	2339	47.5	458	9.8	1	109	113	95	0.59
Mean	0.08	32,186	41.4	404	17,670	0.31	18	51.2	73.5	40,791	2872	602	53.0	447	19.6	7	192	177	180	
(SD)	(0.04)	(17,269)	(40.3)	(316)	(12,430)	(0.40)	(9)	(18.9)	(41.4)	(43,214)	(1577)	(765)	(25.0)	(261)	(26.1)	(16)	(146)	(110)	(145)	
Median	0.08	28,335	23.6	349	12,733	0.16	16	51.9	61.8	28,054	2272	348	43.5	373	10.9	2	151	184	125	
Common soil	-	-	20.0	-	-	0.5	30	90.0	60.0	-	-	-	50.0	-	60.0	-	-	130	120	
Light sandy soil	-	-	15.0	-	-	0.4	20	55.0	45.0	-	-	-	45.0	-	55.0	-	-	120	105	
TEC	-	-	9.8	-	-	1.0	-	43.4	31.6	-	-	-	22.7	-	35.8	-	-	-	121	
PEC	-	-	33.0	-	-	5.0	-	111.0	149.0	-	-	-	48.6	-	128.0	-	-	-	459	
Reference value	-	-	11.7	-	-	0.3	12	37.3	19.2	-	-	-	24.1	-	25.3	-	-	44.6	71.6	

Table 3. Results of one-sided Wilcoxon tests comparing the concentrations of heavy metals in fly ash deposits across the 19 study sites in the Czech Republic to their Czech national limit values for common agricultural soils (CS) and light sandy agricultural soils (LS), and to probable effect (PEC) and threshold effect concentrations (TEC; see Methods for details). Asterisks mean significant differences ($p < 0.025$; in bold), *n.s.* mean non-significant differences ($p > 0.025$).

Heavy Metal	CS	LS	PEC	TEC
As	$p = 0.08$ *n.s.*	$p =$ **0.006** *	$p = 0.48$ *n.s.*	$p = 1.0$ *n.s.*
Ba	$p = 0.99$ *n.s.*	-	-	-
Cd	$p = 0.99$ *n.s.*	$p = 0.99$ *n.s.*	$p = 1.0$ *n.s.*	$p <$ **0.0001** *
Co	$p = 1.0$ *n.s.*	$p = 0.85$ *n.s.*	-	-
Cr	$p = 1.0$ *n.s.*	$p = 0.81$ *n.s.*	$p = 1.0$ *n.s.*	$p = 0.94$ *n.s.*
Cu	$p = 0.13$ *n.s.*	$p =$ **0.01** *	$p = 0.99$ *n.s.*	$p = 1.0$ *n.s.*
Ni	$p = 0.49$ *n.s.*	$p = 0.25$ *n.s.*	$p = 0.39$ *n.s.*	$p = 1.0$ *n.s.*
Pb	$p = 0.99$ *n.s.*	$p = 0.99$ *n.s.*	$p = 1.0$ *n.s.*	$p =$ **0.002** *
V	$p = 0.04$ *n.s.*	$p =$ **0.01** *	-	-
Zn	$p = 0.11$ *n.s.*	$p = 0.04$ *n.s.*	$p = 1.0$ *n.s.*	$p = 0.89$ *n.s.*

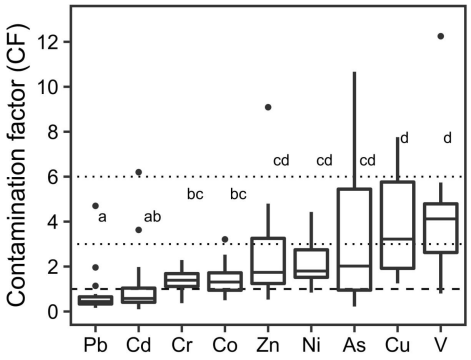

Figure 3. Contamination factors (CF) of the nine selected heavy metals at 19 fly ash deposits in the Czech Republic. Medians with interquartile range (box plot), 95% confidence intervals (whiskers), and outliers (black points) are visualised. Individual letters (a, b, c, d) indicate significant differences (Tukey HSD; $p < 0.05$) among the individual elements. Dashed lines indicate the limit of low contamination (CF = 1), and dotted lines indicate the limits of high (CF = 3) and very high (CF = 6) contamination levels.

Table 4. Contamination factors CF of the nine heavy metals and the resulting pollution load index (PLI) of the complex mixture of contaminants in the fly ash deposits in the Czech Republic (see Methods for details). CF < 1 indicates low contamination; CF = 1–3 moderate contamination; CF = 3–6 considerable contamination; and CF > 6 very high contamination. PLI $\geq$ 1 indicates existing pollution, whereas PLI < 1 indicates no pollution.

Locality	As	Cd	Co	Cr	Cu	Ni	Pb	V	Zn	PLI
1	9.90	3.63	1.69	1.41	6.94	1.72	0.65	5.74	4.73	2.97
2	2.77	0.41	1.59	1.45	6.16	1.32	0.34	4.96	3.36	1.70
3	1.90	1.09	2.53	1.99	4.20	3.47	0.43	3.44	1.21	1.86
4	2.02	6.20	0.50	0.37	1.70	0.84	4.70	0.80	3.38	1.52
5	10.67	1.67	1.74	2.29	5.36	2.54	0.60	5.74	1.74	2.62
6	0.73	0.41	0.92	1.40	2.41	1.68	1.14	2.71	1.14	1.21
7	6.22	0.97	3.21	1.52	5.16	4.43	0.65	4.12	2.56	2.55
8	1.58	0.58	0.94	1.11	1.66	1.80	0.50	2.80	0.95	1.16
9	0.22	0.10	1.41	1.39	2.14	2.92	0.16	2.92	1.28	0.82
10	2.49	0.81	2.40	0.83	3.22	1.96	0.47	5.00	3.15	1.79
11	0.93	0.50	1.70	1.85	3.06	3.63	0.35	4.22	0.96	1.42
12	2.38	0.11	0.95	1.12	1.50	2.57	0.29	12.25	0.53	1.08
13	1.93	0.60	2.48	2.21	3.27	3.94	0.72	4.25	1.37	1.91
14	0.53	0.37	0.79	0.74	1.25	0.95	0.42	1.11	4.80	0.88
15	10.21	1.98	1.02	1.92	6.39	1.52	1.96	1.88	9.09	2.86
16	0.88	0.99	1.29	0.93	7.76	1.46	0.32	4.20	1.43	1.42
17	0.97	0.45	1.22	1.20	6.56	1.54	0.33	4.62	2.83	1.45
18	5.08	0.39	0.94	1.17	1.31	1.52	0.31	2.00	1.87	1.20
19	5.81	0.58	1.31	1.18	2.71	1.97	0.39	2.54	1.33	1.49
No. of LocalitiesCF < 1	6	14	6	4	0	2	16	1	3	
CF = 1–3	7	3	12	15	8	13	2	7	10	
CF = 3–6	2	1	1	0	6	4	1	10	5	
CF > 6	4	1	0	0	5	0	0	1	1	
PLI $\geq$ 1										17

Median concentrations of the seven heavy metals with known PEC values did not significantly exceed these values (one-sided Wilcoxon test, $p > 0.025$; Figure 2 and Table 2). However, concentrations in some particular localities exceeded the PEC value for As (six localities), Ni (seven localities), and Zn (one locality), indicating potentially harmful local concentrations of these heavy metals. On the other hand, all local concentrations of Cd, Cr, Cu, and Pb were below the respective PEC values. Mean PEC quotients >0.5 estimated for 9 out of 19 localities (47%) suggested that the sediments at these sites were potentially harmful for organisms (Table 2). Median concentrations of five heavy metals (As, Cr, Cu, Ni, and Zn) were not significantly lower than their TEC values; only Cd and Pb concentrations were significantly lower, and their respective local concentrations were below their TEC values in all but one (no. 4) and two localities (nos. 4 and 15), respectively, indicating no harmful effects from these heavy metals (Table 3).

4. Discussion

4.1. Overall Levels of Heavy Metals in the Fly Ash Deposits

Our comprehensive survey of the 19 fly ash deposits from thermal power plants distributed across the Czech Republic identified the potential environmental risks from some analysed minor and trace elements in the sedimented fly ash. Over 50% of the studied fly ash deposits exceeded the Czech national limits for concentrations of As, Cu, V, and Zn in agricultural soils. Contamination factors of these four heavy metals together with Cd were very high at 5–26% of the localities. Interestingly, the Czech national limits for most of the heavy metals included in this study are substantially stricter than the national limits of neighbouring countries, such as Poland (Regulation no. 165/2002 of the Polish legislation) and Slovakia (Regulation no. 220/2004 of the Slovak legislation), as well as the respective EU guidelines [38]. In fact, none of the heavy metals except V (with median concentration 22% above the EU guidelines) would be classified as a potential risk in any of our localities according to the EU limits.

Moreover, the combined effects of mixed contaminants (PLI) based on nine heavy metals revealed that nearly all sites can be classified as polluted (PLI > 1), with four of them as strongly polluted (PLI > 2). The consensus-based probable effect concentration index (PEC) showed that the levels of As, Ni, and Zn were potentially harmful in 1–7 localities. Altogether, these results imply potential strong environmental risks of the sediments in the fly ash deposits across the country. However, the mean PEC quotient suggested none or limited toxicity at 53% of the localities, and the concentrations of most heavy metals were very low at several studied deposits. These results show that the environmental risks of the fly ash deposits may be potentially high but need to be considered on a case-by-case basis and utilise multiple indices of potential risks from a range of international sources. In addition to the potential for on-site biodiversity conservation discussed below, this issue is also relevant for the secondary usage of fly ash, such as for the construction industry (e.g., [39]), for extraction of rare earth elements [40], or for enhancing of soil properties in agriculture [41].

The average concentrations of the environmentally significant heavy metals in our study (As, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, V, and Zn) were generally comparable to the values detected in fly ash deposits in the USA, Turkey, India, and Australia [42–45]. Concentrations of heavy metals in fly ash can vary significantly among sites as they depend on numerous factors, especially the characteristics of combusted lignite and the combustion technology [17]. However, the mobility of elements from fly ash follows general underlying patterns, regardless of the composition and characteristics of the ash [17]. Therefore, it is not surprising that the heavy metal concentrations observed in our study are similar to those found in other studies. The only apparent exception was Mn, which occurred in our samples at unusually high concentrations (Table 2) in comparison with the above-mentioned studies, even though similar concentrations were reported in industrial regions of Serbia and the USA [46,47]. Although Mn is often considered among the least

toxic heavy metals to birds and mammals [48], it is still potentially toxic in excessive concentrations [49–51].

4.2. Toxicity of the Heavy Metals to Colonising Organisms and Its Implications

Previous field studies and laboratory experiments showed that some heavy metals included in our study (especially As, Cd, Cu, Mn, Pb, and Zn) may have lethal or sublethal effects for arthropods [52,53]. They can directly affect individuals and populations [53–56], as well as indirectly modify their environment [57–60]. Nevertheless, the total heavy metal concentrations in the environment often provide only an indirect indicator of environmental stress for particular taxa. For example, moderate concentrations of As, Cd, Cu, Ni, and Pb were reported to increase the immune response of geometrid moths and ants, even in non-adapted communities [54,55]. This response was suppressed at higher concentrations (e.g., Ni > 300 $\mu g \cdot g^{-1}$, Cu > 207 $\mu g \cdot g^{-1}$), indicating a potentially higher risk of infections in more polluted environments [54,55,61]. Nevertheless, concentrations of the potentially most harmful heavy metals in the fly ash were far below these levels in our study, as well as at other contaminated localities where a wide range of viable invertebrate populations were observed [58,62–64]. By contrast, the concentration of As at six of our study localities exceeded 60 $\mu g \cdot g^{-1}$ (Table 2), a value known to be phytotoxic [65]. Altogether, based on these results, we expect the observed heavy metal concentrations to have substantial negative effects on some but not all taxa found in the fly ash lagoons.

Additionally, the biological availability of individual elements is often limited. This makes an assessment of the environmental risks difficult when considering only absolute concentrations of heavy metals. The potential of heavy metals to enter the food chain depends greatly on their fixation in the fly ash particles and on their properties when in contact with water in solutions [66]. The biological availability of heavy metals in fly ash under mildly alkaline pH (such as in our study deposits; Table 1) has been repeatedly shown as highly limited [67–70]. Despite not having detailed data, we can expect the heavy metals in our samples were probably present in heterogeneous forms and associations due to the mildly alkaline pH [71], as reflected by heterogeneous correlations among the elements in our sites (Figure A1). Some heavy metals, especially As oxyanionic forms [72], can be dissolved or released from fly ash during microbial mineralisation of organic matter using redox sensitive Fe and Mn oxyhydroxides (e.g., [73]). This might be more relevant for substrates with higher contents of organic matter accumulated over time, and with less stable Fe oxyhydroxides. Although there was a generally low amount of organic matter in our samples, the highly positive correlations of P with As and Fe (Figure A1) imply that Fe could be present in forms of oxides. At higher pH, these oxyanionic Fe species might be released into the environment [17].

Aside from the described chemical speciation of these elements, numerous other chemical, physical, or biological processes can modulate the potential penetration of particular heavy metals into the food chain [74]. As our data provide only one temporal snapshot, we can only infer the long-term dynamics of heavy metal concentrations from other evidence. The decades of exposure to leaching during historical (1970s till early 1990s) acid rains that dramatically decreased pH in Central European industrial landscapes, together with the mobility of dissolved heavy metals to deeper substrate layers, suggest that the pollution potential of the Czech fly ash deposits may have been greatly reduced or even lost [45,75]. On the other hand, due to the low amount of organic matter in most of our fly ash samples, the pH is not expected to fluctuate to extreme values in the past three decades. The relatively high conductivity at some localities (Table 1) could imply the opposite patterns, but we have no data on other substances that could have been dissolved in the water. Altogether, most of the analysed heavy metals are probably stable and unlikely to enter the food chain, despite their frequently high concentrations.

Although the concentrations of one or more heavy metals were potentially risky at each of our study sites, invertebrate populations are known to permanently inhabit even heavily polluted sites (e.g., [7,58,76]). This indicates that invertebrates can at least partly

cope with heavy metal toxicity. Various studies reported the development of physiological [77,78] or behavioural adaptations [58]. These may include shorter life cycles and higher reproductive effort [79], or smaller colony sizes and reproduction trade-offs [80]. These mechanisms are often species-specific (e.g., [81]) and can differ even among closely related species (e.g., [62,82]). Consequently, communities at otherwise similar polluted and unpolluted localities may differ in the proportions of tolerant and susceptible species to heavy metals [58,64,78,79].

Heavy metal toxicity may establish an environmental filter allowing the colonisation of polluted fly ash deposits only by a subset of the local biodiversity. At least some threatened insect species are undoubtedly able to colonise Central European fly ash deposits despite their pollution by heavy metals, as shown by several recent studies [6–10]. Unfortunately, detailed data on the composition of communities in fly ash deposits are virtually unavailable, including the sites sampled in this study. Therefore, the real effects of heavy metals on communities at these sites remain unclear, as well as the potential drivers of such effects.

Although the concentrations of some metals exceeded the risk limits at numerous localities, they rarely reached potentially harmful levels. Therefore, we presume that the availability of early successional habitats may outweigh the potential stress from pollution by heavy metals for at least some taxa present in the fly ash deposits. In particular, the related environmental stress may provide a competitive advantage or release from predation for some competitively weak species in the community. However, these habitats may also attract individuals of species that cannot efficiently cope with heavy metals, for example females of flying insects looking for oviposition sites. This could turn the fly ash lagoons into population sinks or even ecological traps for some species [83]. This study cannot resolve these issues as it focuses on the net concentrations and not on the biological availability of the individual heavy metals at the studied sites. Detailed data on the community composition of arthropods at the studied sites are not available and would require intensive research in the future. Therefore, only indirect evaluations, such as in this study, are possible at the moment. The crucial question whether fly ash deposits function as biodiversity reservoirs or as ecological traps can only be answered with future species-specific ecotoxicological studies combined with the assessment of potential benefits mediated by altered biotic interactions. Our unique dataset has provided the first step in this direction by evaluating individual sites and identifying the general trends necessary for the restoration projects. Based on these indirect measures, together with the available evidence on already established viable populations of numerous rare and threatened arthropods, we are convinced that proper restoration of most Central European fly ash deposits should maintain or enhance conditions necessary for protection of these rare species.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

Appendix A

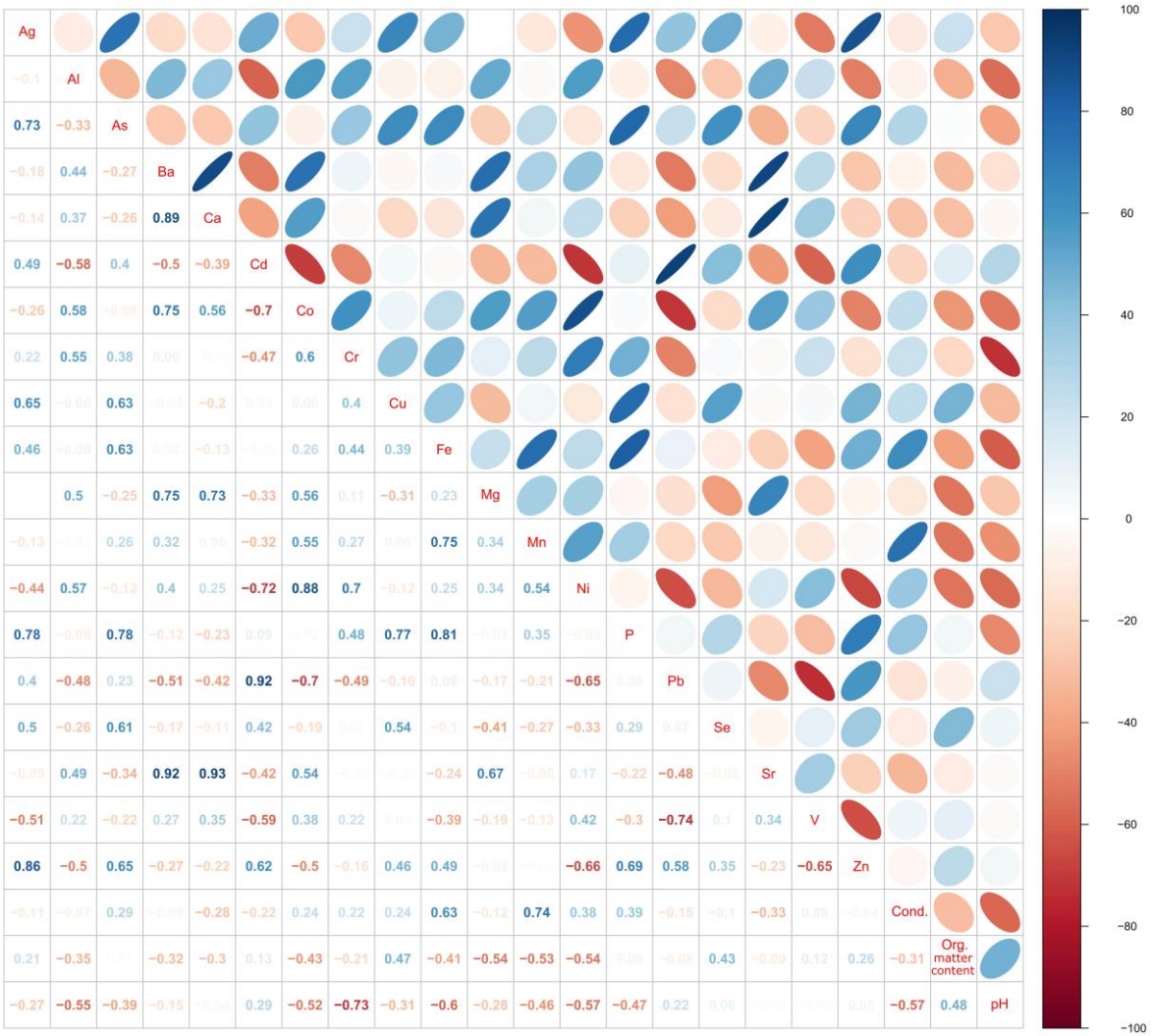

Figure A1. Pearson correlations of all concentrations of trace elements, conductivity (Cond), organic matter content, and pH values. Values below the diagonal are the Pearson correlation coefficients. Ellipse slope and colour intensity illustrate the sign and strength of the correlation (colour code in %). The figure was prepared using the *Hmisc* (v. 4.3-1; [84]) and *corrplot* (v. 0.84; [85]) packages in R [37].

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