



# Muddying the unexplored post-industrial waters: Biodiversity and conservation potential of freshwater habitats in fly ash sedimentation lagoons

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## ABSTRACT

Deposits of fly ash and other coal combustion wastes are common remnants of the energy industry. Despite their environmental risks from heavy metals and trace elements, they have been revealed as refuges for threatened terrestrial biodiversity. Surprisingly, freshwater biodiversity of fly ash sedimentation lagoons remains unknown despite such lack of knowledge strongly limits the efficient restoration of fly ash deposits. We bring the first comprehensive survey of freshwater biodiversity, including nekton, benthos, zooplankton, phytoplankton, and macrophytes, in fly ash lagoons across industrial regions of the Czech Republic. To assess their conservation potential, we compared their biodiversity with abandoned post-mining ponds, the known strongholds of endangered aquatic species in the region with a shortage of natural ponds. Of 28 recorded threatened species, 15 occurred in the studied fly ash lagoons, some of which were less abundant or even absent in the post-mining ponds. These are often species of nutrient-poor, fishless waters with rich vegetation, although some are specialised extremophiles. Species richness and conservation value of most groups in the fly ash lagoons did not significantly differ from the post-mining ponds, except for species richness of benthos, zooplankton, and macrophytes, which were slightly lower in the fly ash lagoons. Although the concentrations of some heavy metals (mainly Se, V, and As) were significantly higher in the fly ash lagoons, they did not significantly affect species richness or conservation value of the local communities. The differences in species composition therefore does not seem to be caused by water chemistry. Altogether, we have shown that fly ash lagoons are refuges for threatened aquatic species, and we thus suggest maintaining water bodies during site restoration after the cessation of fly ash deposition. Based on our analyses of environmental variables, we discuss suitable restoration practices that efficiently combine biodiversity protection and environmental risk reduction.

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## 1. Introduction

The energy industry has long relied on coal combustion in thermoelectric power plants to generate electricity, which has strongly affected many regions. Deposits of solid wastes from coal combustion, dominated by fly ash, belong among the most apparent remainders of this industry (Gollakota et al., 2019; Tropek et al., 2015). In some industrial regions, these deposits pose potential environmental risks (Chmelová et al., 2021; Gollakota et al., 2019; Han et al., 2021). In Central Europe, where industry-related human activities have had a significant impact, the use of fly ash and other by-products in various industries has increased in recent years, which has led to a substantial decline in their deposition (Chmelová et al., 2021). However, the potential environmental consequences of fly ash deposition must be thoroughly examined to mitigate any negative impacts on biodiversity during the potential establishment of new deposits as well as during reclamation of abandoned deposits (Chmelová et al., 2021; Gollakota et al., 2019; Tropek et al., 2013).

Deposits of fly ash (i.e., fine glass-like particles of mineral residues) and other solid wastes from coal combustion (mainly harsher bottom ash, boiler slag, and desulphurisation residues) accompanied thermoelectric power plants, coal-combusting thermal plants, and some larger factories in Europe and other regions (Han et al., 2021; Tropek et al., 2015). Despite technological progress in fly ash deposition, various environmental risks associated with these deposits have been reported in previous studies (Borm, 1997; Rowe et al., 2002; Silva and da Boit, 2011). Fly ash is known to contain relatively high concentrations of heavy metals and other trace elements (Chmelová et al., 2021; Rausch et al., 1993), which can pollute the surrounding landscape through wind erosion or accidental leakage of deposited water (Han et al., 2021). Nevertheless, despite the undisputable environmental risks, fly ash deposits have been revealed as important secondary refuges for highly endangered terrestrial species (e.g., Tropek et al., 2013, 2017). Recent studies have proven that some of these post-industrial sites are strongholds for animal communities specialised for specific vanishing habitats, such as inland sand dunes, steppes and grasslands, riverine gravel beds, marshlands, and salt marshes (e.g. Bogusch et al., 2016; Tropek et al., 2013, 2016, 2017). Such findings have partly changed our view of these industrial barrens and their restoration, which should consider not only their environmental risks, but also their potential to protect threatened biodiversity (Chmelová et al., 2021; Tropek et al., 2015, 2016).

Surprisingly, virtually nothing is known about the biodiversity and conservation potential of freshwater habitats in fly ash sedimentation lagoons. This lack of information strongly limits the efficiency of approaches currently applied during the fly ash lagoon restoration. This is crucial because freshwater habitats, especially small and medium ponds, streams, and wetlands, rank high among the most threatened biotopes in Central Europe due to eutrophication, desiccation, salinisation, contamination by pesticides and pollutants, and degradation from industrialised agriculture and urbanisation (Chytrý et al., 2019; Reid et al., 2019; Stendera et al., 2012). Moreover, a substantial proportion of the threatened freshwater biodiversity is known to find secondary refuges in various post-industrial habitats, such as ponds in sand pits (e.g. Kolar and Boukal, 2020; Kolar et al., 2021a), clay quarries (e.g. Sroka et al., 2022), limestone quarries (e.g. Bobrek, 2020), and lignite spoil heaps (e.g. Kolar et al., 2017, 2021b; Tichanek and Tropek, 2015). These post-industrial water bodies often offer freshwater habitats with low nutrient content, no or few fish, and heterogeneous littoral habitats, which are all required by numerous endangered species. At least some fly ash lagoons provide habitats with very similar structure (Chmelová et al., 2021; Tropek et al., 2015), and we can therefore expect their colonisation by some threatened biota. Nevertheless, fly ash lagoons may differ from most of the other post-industrial freshwater habitats by higher concentrations of heavy metals, such as As, Cd, Cr, Cu, Ni, Pb, V, and Zn (e.g., Carreira et al., 2023; Izquierdo and Querol, 2012; Sushil and Batra, 2006), which can affect the fitness of some freshwater organisms (e.g. Courtney and Clements, 2002; Doig et al., 2015). On the

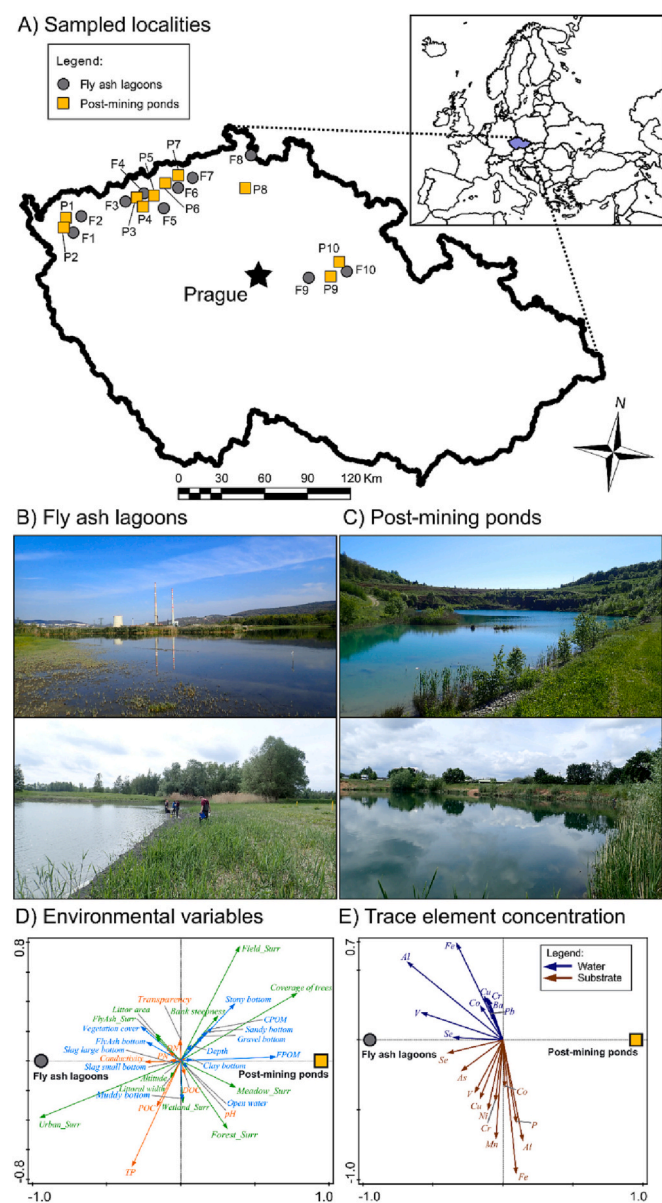
other hand, Chmelová et al. (2021) showed that at least part of the Central European fly ash deposits is not as affected by elevated concentration of heavy metals, as expected from the studies in other regions. In this case, their relatively smaller negative effect on the threatened species should not decrease the potential conservation value of the colonising freshwater communities, similar to some other post-industrial freshwater habitats (such as ponds in post-mining sites) mentioned above.

To fill this critical knowledge gap, we present the first comprehensive study of freshwater communities, including macrophytes, phytoplankton, zooplankton, benthos, and nekton, from fly ash sedimentation lagoons. Moreover, we evaluated their potential to harbour threatened freshwater species, which is crucial for their efficient restoration. In Central Europe, the region heavily affected by the coal-related industry, we compared the fly ash lagoons with post-mining ponds already known to serve as valuable secondary refuges for freshwater biodiversity in the region (e.g., Harabiš et al., 2013; Kolar et al., 2021b; Poláková et al., 2022; Tichanek and Tropek, 2015; Vojar et al., 2016). Similar to the known biodiversity refuges in terrestrial habitats in fly ash deposits (e.g., Bogusch et al., 2016; Tropek et al., 2013, 2017), we expected a high conservation potential of the studied fly ash lagoons for freshwater biodiversity. Nevertheless, we expected differences among functional groups of organisms, as we can expect a higher sensitivity of substrate-related groups (macrophytes and benthos) to the different chemistry of the fly ash substrate, including higher concentrations of heavy metals. In general, we expected the occurrence of some declining species typical for waters with specific chemistry (such as high conductivity and extreme pH), but also the presence of some threatened species requiring sparsely vegetated habitats in oligotrophic waters without agricultural pollution. We have also analysed the effects of the heavy metal pollution on biodiversity and community composition, with a special focus on populations of threatened species. Additionally, we have explored the influence of habitat characteristics and other environmental variables on the conservation potential of post-industrial habitats, as such knowledge is crucial for the efficient planning of restoration of these post-industrial habitats.

## 2. Methods

### 2.1. Study sites and design

Our research was performed in Western, Northern and Eastern Bohemia, Czech Republic, Central Europe (Fig. 1), the regions with a common history of lignite mining and other industries, accompanied by the construction of coal combusting power plants during the 20th century (Chmelová et al., 2021). For surveying freshwater biodiversity, we selected 10 fly ash lagoons (Fig. 1, Table S1), i.e. most of the remaining non-reclaimed remnants of these post-industrial sites in the country (Chmelová et al., 2021). Unfortunately, any natural small and medium-sized standing waters are virtually missing in the region, as the ponds are used for intensive aquaculture (Chytrý et al., 2019; Krivánek et al., 2012). Therefore, we used freshwater bodies created during the mining of fine substrates (i.e., sand, gravel, or clay) as controls to assess the conservation potential of fly ash lagoons, because these post-mining habitats were repeatedly proven to be key biodiversity strongholds in the studied regions (e.g., Dolný and Harabiš, 2012; Harabiš et al., 2013; Kolar et al., 2021b; Poláková et al., 2022; Tichanek and Tropek, 2015; Vojar et al., 2016). Because of their artificial origin, they differ from fly ash lagoons mainly in the water and bottom substrate chemistry, especially in the content of some minor and trace elements from combusted coal (Carreira et al., 2023; Chmelová et al., 2021). From the numerous post-mining ponds in the study regions, we selected 10 post-mining ponds (Fig. 1, Table S2) that were close in distance and similar in size and successional stage to each of the 10 selected fly ash lagoons. At each sampled pond of both types, we selected three sampling sites in the littoral zone (Fig. 2). Where possible, these sampling sites were

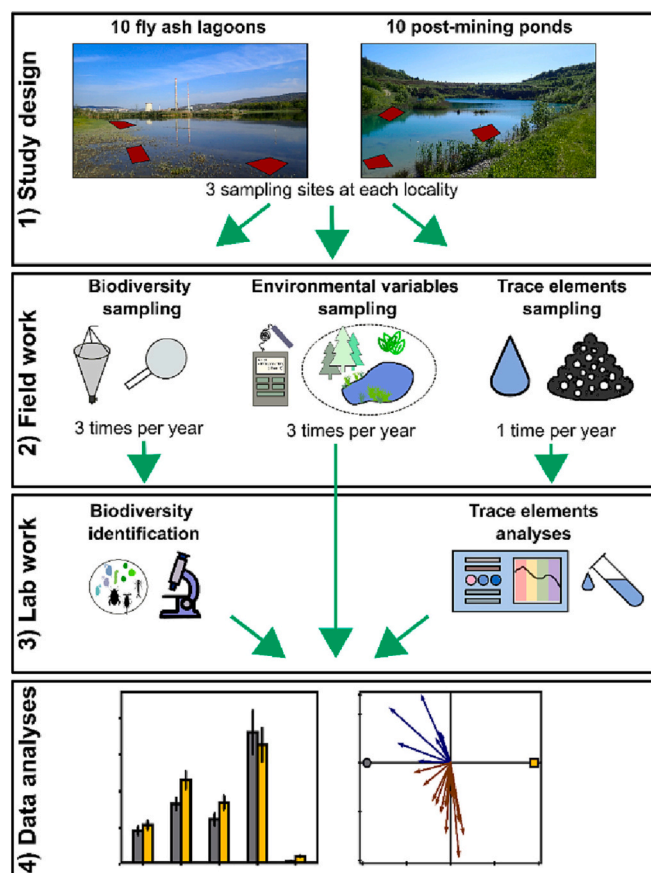


**Fig. 1. Overview of the studied freshwater ponds.** (A) Map of the study area with positions of the samples freshwater bodies. Freshwater habitats of (B) fly ash lagoons and (C) post-mining ponds. Constrained ordination (RDA) diagrams visualising differences of fly ash lagoons and post-mining ponds in (D) environmental variables (green – characteristics of ponds and surrounding habitats; orange – water characteristics; blue – microhabitat characteristics) and in (E) concentrations of heavy metals and trace elements in bottom substrate (brown) and water (blue); only the elements best fitting to the model are visualised.

comprised of a site with a bare bottom, a site with dense littoral vegetation, and a site with intermediate characteristics. The sampling sites were at least 20 m apart to reduce the effects of disturbance during sampling. All methodological approaches applied to each studied pond are visualised in a flowchart (Fig. 2).

## 2.2. Environmental variables sampling and chemical analyses

We analysed 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) in water and substrate from all studied ponds (Fig. 2). From each sampling site, we took 125 ml of water before sampling biodiversity and environmental variables (to avoid stirring the



**Fig. 2. Flowchart of the methodological approaches used in this study.**

bottom substrate), mixed it in a clean container, and froze 125 ml of the mixed sample for chemical analyses. Water samples were filtered through 0.4  $\mu\text{m}$  pore size glass-fibre filters (MN-5, Macherey Nagel), acidified with the addition of 1 % concentrated suprapur nitric acid, and analysed by inductively coupled plasma mass spectrometer (ICP-MS; Agilent 8800 ICP-QQQ, Agilent Technologies Inc., Tokyo, Japan). We also took 125 ml samples of substrate, which were freeze-dried, gently grinded using mortar and pestle to disintegrate ash aggregates, and sieved through a 2 mm mesh to exclude stones and larger organic debris in the samples. The substrate 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 the protocols in Kopáček et al. (2001). An inductively coupled plasma mass spectrometer (ICP-MS; Agilent 8800 ICP-QQQ, Agilent Technologies Inc., Tokyo, Japan) was used to determine the total concentrations of the 19 trace elements. The values of the analysed trace elements in individual ponds are shown in Table S4.

Additionally, each sampled pond was characterised by several environmental variables considered potentially important for aquatic communities (Fig. 2): altitude (m), bank steepness ( $^{\circ}$ ), relative area of the littoral zone (%), average width of the littoral zone (m), proportion of surrounding habitats (forest, field, meadow, wetlands, urban areas, and deposited fly ash; %), and cover of trees along the shoreline (%). At each pond, we measured water conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ) and water pH using YSI ProDSS probe (YSI Inc.) and water transparency (cm) using Sneller's tube. To quantify water eutrophication, we analysed the amount of dissolved organic carbon (DOC,  $\text{mg}\cdot\text{l}^{-1}$ ), particulate organic carbon (POC,  $\text{mg}\cdot\text{l}^{-1}$ ), dissolved nitrogen (DN,  $\text{mg}\cdot\text{l}^{-1}$ ), particulate nitrogen (PN,  $\text{mg}\cdot\text{l}^{-1}$ ), and total phosphorus (TP,  $\text{mg}\cdot\text{l}^{-1}$ ). POC, DOC, DN, and PN were analysed by elemental analyses using TOC-L analyser (Shimadzu, Japan) using a high-temperature ( $680^{\circ}\text{C}$ ) catalytic



oxidation method with an infrared gas analyser (NDIR) for C detection, a chemiluminescence detector for N detection, and a CHNS Elemental Analyser vario MICRO cube (Elementar Analysensysteme GmbH, Germany). For analyses of DOC and DN, the samples were filtered through 0.45 µm glass fibre filters (MN-GF5, Germany). TP was analysed together with the water samples (see above). Each sampling site was characterised by water depth (cm), proportion of bottom substrate (sand, stones, gravel, clay, mud, fly ash, and small and large slag particles; %), and proportional of fine (FPOM, %) and coarse (CPOM, %) particulate organic matter. The values of all environmental variables in individual ponds are shown in Table S4.

### 2.3. Biodiversity sampling

Freshwater biodiversity in the 20 ponds was surveyed during three visits (Fig. 2; July 2018, September 2018, and May 2019) to cover the regional phenology of the freshwater communities. To representatively sample ecological and taxonomic diversity of the communities, we applied several standardised sampling approaches (Klečka and Boukal, 2011): vegetation and bottom sweeping, light trapping, box trapping, sweeping with a plankton net, standardised water sampling for phytoplankton, electrofishing, and macrophyte recording (see Table S3 for focal groups and sampling periods of particular sampling methods). To avoid unnecessary sampling artifacts, the same team members applied particular sampling methods to all ponds and visits.

Vegetation was swept with a kitchen strainer (18 cm diameter, 900 µm mesh size) to sample larger phytophilous and more active invertebrates and their larvae. At each sampling site, we swept macrophyte vegetation, larger objects (stones, rocks, etc.), and bottom substrate for 5 min and stored the swept material in a vial with 80 % ethanol. A light trap with a LED light (four 4 mm-wide entrance holes per trap constructed after Ditrich and Čihák, 2017) per sampling site was used to sample invertebrates with nocturnal activity. The light traps were set up at dusk and removed the next morning. When pulling the traps out of the water, we covered the entrance holes and poured the content through a small sieve (250 µm mesh size). All individuals were preserved in a vial with 80 % ethanol. Benthic fauna were sampled with a box trap (50 × 50 cm surface area) placed in shallow undisturbed water close to the shore at each sampling site. An approximately 5 cm bottom substrate layer in the box trap was washed through a net (280 µm mesh size) and, after removing large objects, it was stored in 96 % ethanol. Zooplankton and phytoplankton were sampled with towed plankton nets (mesh size 100 µm and 20 µm, respectively), which were tossed three times at each sampling site and slowly dragged approximately 5 m towards the shore. The net content was then placed in a vial and fixed with 4 % formaldehyde. Phytoplankton was sampled from ca 500 ml water taken below the surface (0–15 cm) of open water ca 1 m from the shore and fixed in the Lugol solution. Aquatic and semi-aquatic macrophytes were recorded (cover of individual species in the littoral vegetation, %) in each sampled pond. Fish were collected, recorded, and released during electrofishing by a battery Grassl electrofisher (Hans Grassl GmbH); each sampling site was surveyed for 10 mins. Additional fish specimens were added from the vegetation sweeping.

Animals captured by all sampling methods, except electrofishing, were manually sorted from the samples under a dissecting stereomicroscope in the laboratory. According to their life strategies and capturing efficiency, particular groups were processed only from samples collected using particular methods (Table S3). Phytoplankton from the water samples was sedimented and decanted, identified, counted in Burkner's chamber, and recalculated as cell numbers per 1 ml. Phytoplankton from the net samples was checked for rare and large species which were not detected in the water samples. All the specimens from each focal group were identified to the lowest possible taxonomic level by a specialist. The conservation status categories (NT – nearly threatened; VU – vulnerable; EN – endangered; CR – critically endangered) were obtained from the latest red lists of each group for the Czech

Republic (Farkač et al., 2005; Hejda et al., 2017). For all analyses, the identified species were assigned to the following functional groups: *nekton* (actively swimming animals living in the water column and/or on emergent vegetation; i.e. Coleoptera, Heteroptera, and fish); *benthos* (animals occupying the bottom and substrate; i.e. Clitellata, Diptera, Ephemeroptera, Odonata, and Trichoptera); *zooplankton* (animals drifting in the water column; i.e. Acari, Cladocera, Cyclopoida, and Ploima); *phytoplankton* (primary producers floating in the water column; i.e. Bacillariophyceae, Chlorophyta, Chrysophyceae, Cryptophyceae, Dinophyceae, Euglenophyta, Raphidophyceae, Streptophyta, Xanthophyceae, and Cyanobacteria); and *macrophytes*. A list of all species recorded in individual ponds, together with the identification and nomenclature literature can be found in Table S4.

### 2.4. Statistical analyses

Only specimens identified to the species level were used in the analyses. All data were pooled across the sampled sites within each sampled pond as the sum of individuals or as the mean of the measured environmental variables. All ordination analyses were performed using CANOCO 5 (ter Braak and Šmilauer, 2012), and all other analyses were run using R v. 4.1.0 (R Core Team, 2021).

Differences of the fly ash lagoons and the post-mining ponds in their habitat characteristics, water and substrate physico-chemical features, and trace element concentrations were tested by separate redundancy analyses (RDAs) with the habitat type as a categorical explanatory variable and with all habitat and chemical characteristics as continuous response variables. All response data were log-transformed and centred. The RDAs significances were tested using Monte Carlo test with 999 permutations, the pond pairs were treated as blocks.

To analyse differences in *species richness* (i.e., the number of all identified species) and *conservation value* (i.e., the number of individuals per pond weighted by their red-list status following Tropek et al., 2010) of each functional group between fly ash lagoons and post-mining ponds, we applied generalized linear mixed-effect models (GLMMs; *glmmTMB* package, Brooks et al., 2017) with gaussian, negative binomial, or truncated generalized Poisson distribution depending on the model residuals (*DHARMA* package, Hartig, 2018). The pond pairs were treated as a random effect, and habitat type as a fixed effect. Consequently, we tested the influence of environmental variables and trace elements on species richness and conservation value of the functional groups using RDAs with interactive forward selection (with all data centred and log-transformed, the habitat type treated as a covariate, pond pairs treated as blocks, and tested using Monte Carlo tests with 999 permutations). Because the RDA analyses were insignificant (see Results), we examined their correlations among all groups' species richness and conservation values using Pearson's correlations to better understand the relationships among the biodiversity indicators of the studied communities.

Differences in the community composition of individual functional groups between fly ash lagoons and post-mining ponds were first visualised by principal component analyses (PCAs), with centred and log-transformed data (except macrophytes which were only centred). Consequently, differences in community composition between the pond types were analysed using canonical correspondence analyses (CCAs, log-transformed data, tested by Monte Carlo tests with 999 permutations), with habitat type as a categorical explanatory variable and the abundances of individual benthos, nekton, zooplankton, phytoplankton, and macrophyte species as response variables.

## 3. Results

Altogether we recorded 18 species of fish (1 CR), 344 species of invertebrates (8 species of Ephemeroptera: 1 RE, 1 VU; 22 species of Odonata: no red-listed species; 58 species of Diptera: 1 VU; 26 species of Trichoptera: 1 EN, 2 VU, 6 NT; 33 species of Oligochaeta and Hirudinea:

3 NT; 59 species of Coleoptera: 1 CR, 2 VU, 1 NT; 22 species of Heteroptera: 2 EN, 1 VU; 82 species of zooplankton: no red-listed species; and 34 species of Acari: no red list available), 27 species of macrophytes (1 VU, 4 NT), and 384 species of phytoplankton (no red list available; Table S4). Of the 773 recorded species, 556 species occurred in fly ash lagoons and 605 in post-mining ponds, whereas from the 28 recorded red-listed species 15 occurred in fly ash lagoons and 26 in post-mining ponds (Table S4).

The RDA revealed differences in numerous habitat characteristics and environmental variables between fly ash lagoons and post-mining ponds (pseudo- $F = 3.4$ ,  $P = 0.007$ , 19.31 % explained variability; Fig. 1d). As expected, the fly ash lagoons were surrounded by more urban areas and dried deposited fly ash, whereas the post-mining ponds were surrounded by more fields, forests, and meadows. Water of the fly ash lagoons had higher conductivity and two out of six measures of nutrient level (POC and TP). The fly ash lagoons were also shallower and covered by more emergent vegetation and larger littorals. The post-mining ponds had steeper banks with narrower littoral zones and more trees. As expected, bare substrate of the fly ash lagoons consisted mainly of ash and slag, while bottom of the post-mining ponds was more covered with both types of organic detritus (FPOM and CPOM; Fig. 1d). The two habitat types differed significantly in trace element concentrations in water and substrate (RDA, pseudo- $F = 2.7$ ,  $P = 0.004$ , 14.65 % explained variability; Fig. 1e). Most trace elements showed higher concentrations in water of the fly ash lagoons with the greatest differences for Se, V, and Al, whereas the substrate concentrations mostly did not differ, except for the higher concentrations of Se and As in fly ash lagoons (Fig. 1e).

The GLMMs revealed significantly higher species richness in the post-mining ponds than in the fly ash lagoons for benthos (Fig. 3a,  $\chi^2_1 = 5.65$ ,  $p = 0.018$ ), zooplankton ( $\chi^2_1 = 6.08$ ,  $p = 0.014$ ), and macrophytes ( $\chi^2_1 = 43.82$ ,  $p < 0.001$ ; Fig. 2a), whereas there were no significant differences in species richness for nekton ( $\chi^2_1 = 1.13$ ,  $p = 0.288$ ) and phytoplankton ( $\chi^2_1 = 0.26$ ,  $p = 0.607$ ). The conservation value was significantly higher in post-mining sites for macrophytes (Fig. 3b,  $\chi^2_1 = 12.05$ ,  $p < 0.001$ ), with no significant differences for nekton ( $\chi^2_1 = 0.02$ ,  $p = 0.897$ ) and benthos ( $\chi^2_1 = 2.39$ ,  $p = 0.122$ ; Fig. 2b). The conservation values of phytoplankton and zooplankton were not analysed because an insufficient number of red-listed species was recorded. The RDAs revealed no significant effects of environmental variables (pseudo- $F <$

0.1,  $p = 1$ ) or trace element concentrations (pseudo- $F < 0.1$ ,  $p = 1$ ) on species richness and conservation value of the studied functional groups' communities.

The intercorrelation matrix (Fig. S1) showed that the biodiversity indicators (species richness and conservation value) of nekton had a negative or neutral relationship with those of most of the other studied groups. The strongest negative correlations were detected with phytoplankton and zooplankton for nekton species richness, and with benthos for the nekton conservation value. The strongest positive correlations were found between species richness of phytoplankton and zooplankton, between species richness of zooplankton and benthos, and between species richness of benthos and macrophytes.

Regarding the composition of the studied communities, the PCAs showed a relatively distinct separation of phytoplankton communities (along the second ordination axis; Fig. 4d) and macrophytes (along the first ordination axis; Fig. 4e) between the pond types, and a less apparent separation of the other functional groups' communities (Fig. 4a-c). The pond pairs were often widely distant in the ordination diagrams for all studied groups, indicating highly different freshwater communities between the fly ash lagoons and post-mining ponds in the same geographic area. Consistently, CCAs showed significant differences in community composition between fly ash lagoons and post-mining ponds for all functional groups (Fig. 5): nekton (pseudo- $F = 1.9$ ,  $p = 0.01$ , 8.63 % explained variability; Fig. 5a), benthos (pseudo- $F = 1.4$ ,  $p = 0.007$ , 4.29 % explained variability; Fig. 5b), zooplankton (pseudo- $F = 1.6$ ,  $p = 0.007$ , 5.71 % explained variability; Fig. 5c), phytoplankton (pseudo- $F = 2.2$ ,  $p = 0.003$ , 1.20 % explained variability Fig. 5d), and macrophytes (pseudo- $F = 1.4$ ,  $p = 0.007$ , 4.29 % explained variability; Fig. 5e).

Focusing on the red-listed species, two species of nekton, eleven species of benthos, and four species of macrophytes were affiliated to the post-mining ponds, whilst three species of nekton, two species of benthos, and no macrophyte species were affiliated to the fly ash lagoons (Fig. 5). Several other species showed no apparent preferences for the habitat type. More specifically, most nekton arthropods found mainly in the fly ash lagoons (such as the beetle *Halipidius confinis*, CR, and the hemipterans *Sigara semistriata*, VU, and *Glaenocoris propinqua*, EN) are known to prefer large unpolluted waters, ideally without fish. *Halipidius confinis* is also typical for waters with higher conductivity. The benthic caddisflies found mostly in the fly ash lagoons (*Agrypnia pagetana*, VU, and *Holocentropus picicornis*, NT) are known to prefer densely vegetated

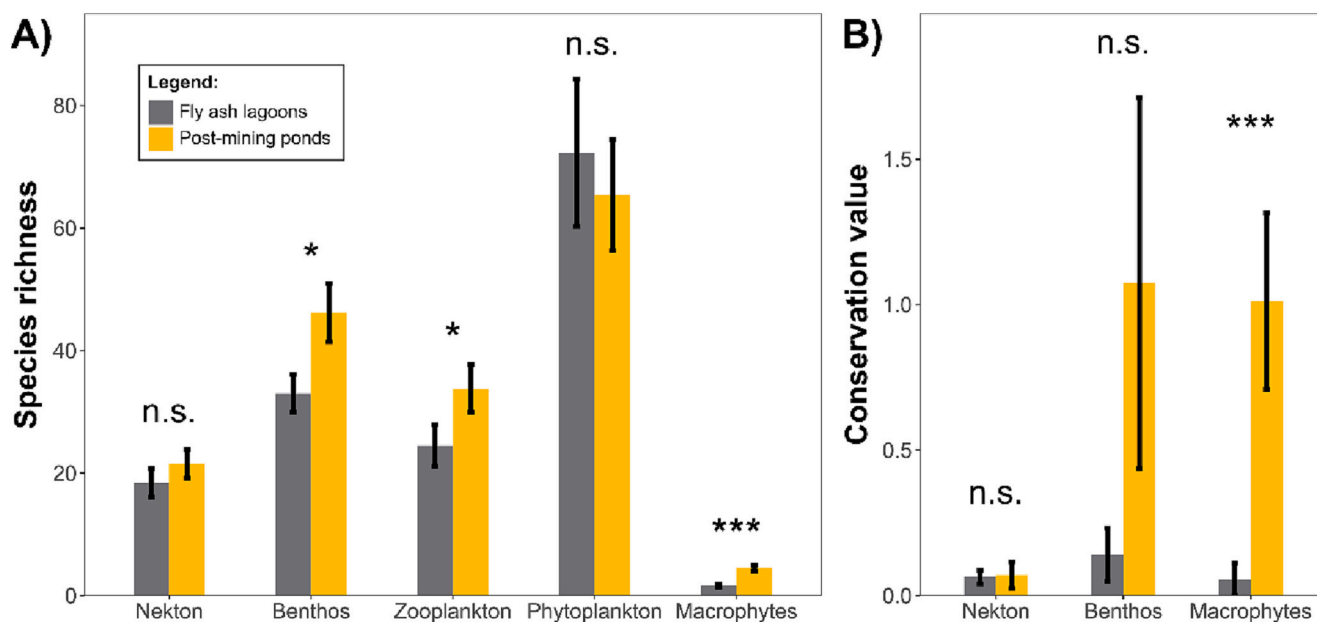
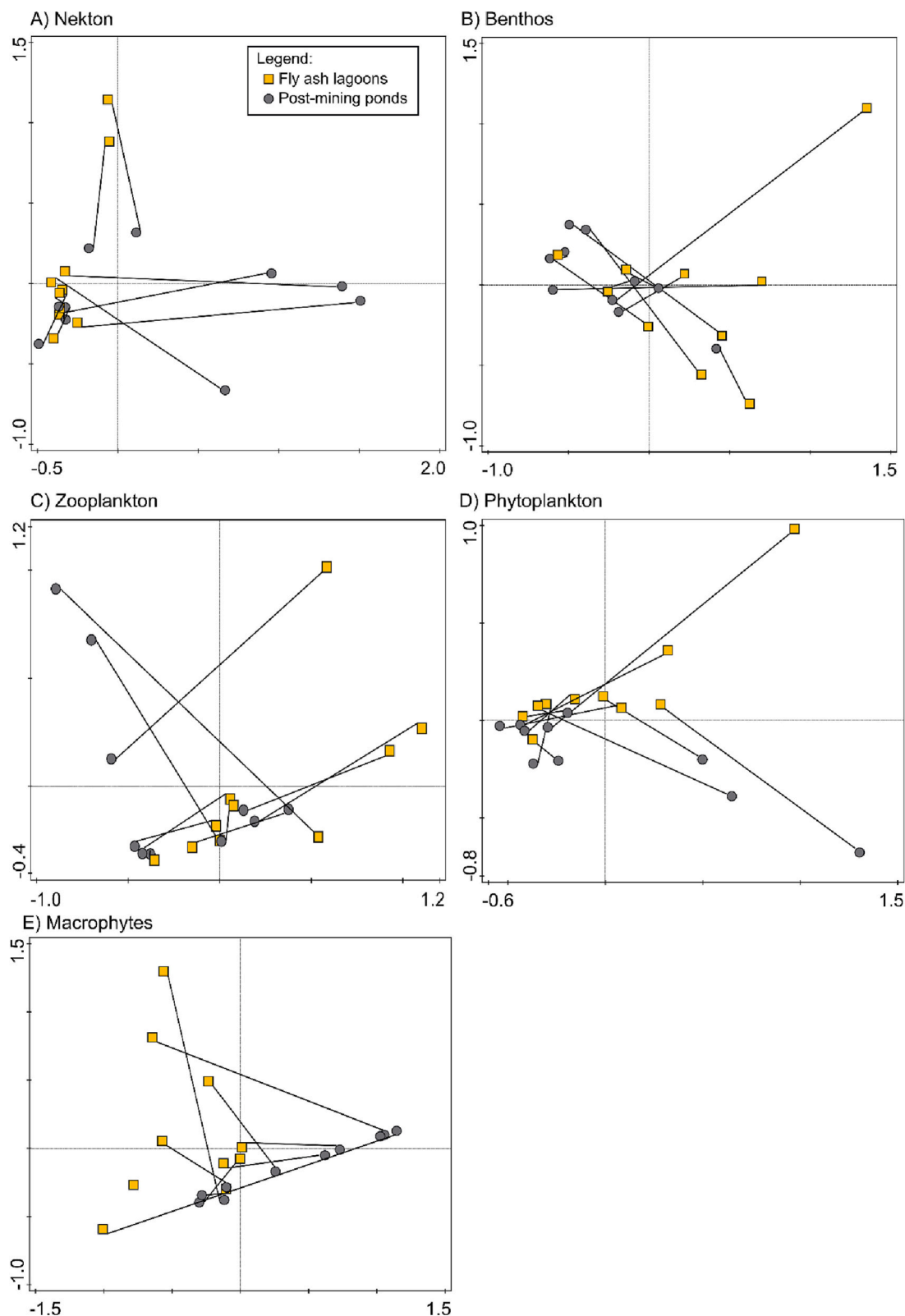


Fig. 3. Differences in (A) species richness and (B) conservation value of individual functional groups of organisms between fly ash lagoons and post-mining ponds. Means and standard errors of the mean are visualised. Significances of individual GLMMs: n.s. = non-significant; \* =  $p < 0.01$ ; \*\*\* =  $p < 0.0001$ .



**Fig. 4.** Unconstrained ordination (PCA) diagrams of the community composition of the functional groups of organisms in fly ash lagoons (grey circles) and post-mining ponds (yellow squares). The lines connect the pond pairs (see Methods for details of the study design). First and second ordination axes are visualised.

habitats with detritus-rich bottoms. Most nekton and benthic red-listed species found mostly in the post-mining ponds (such as the mayfly *Ephemera glaucops*, RE, the caddisfly *Cyrrus crenaticornis*, EN, and the fish *Leucaspius delineatus*, CR) are known to prefer densely vegetated freshwater habitats in the later succession stages with clean water. Some

of the red-listed species without any obvious preference for the pond type are known to prefer relatively extreme water conditions. For example, the clitellate *Vejdovskyella intermedia* (NT) is typical for alkaline waters, the hemipteran *Sigara iactans* (EN) for acidic waters, and the plant *Najas marina* (NT) typically grows in waters rich in minerals,

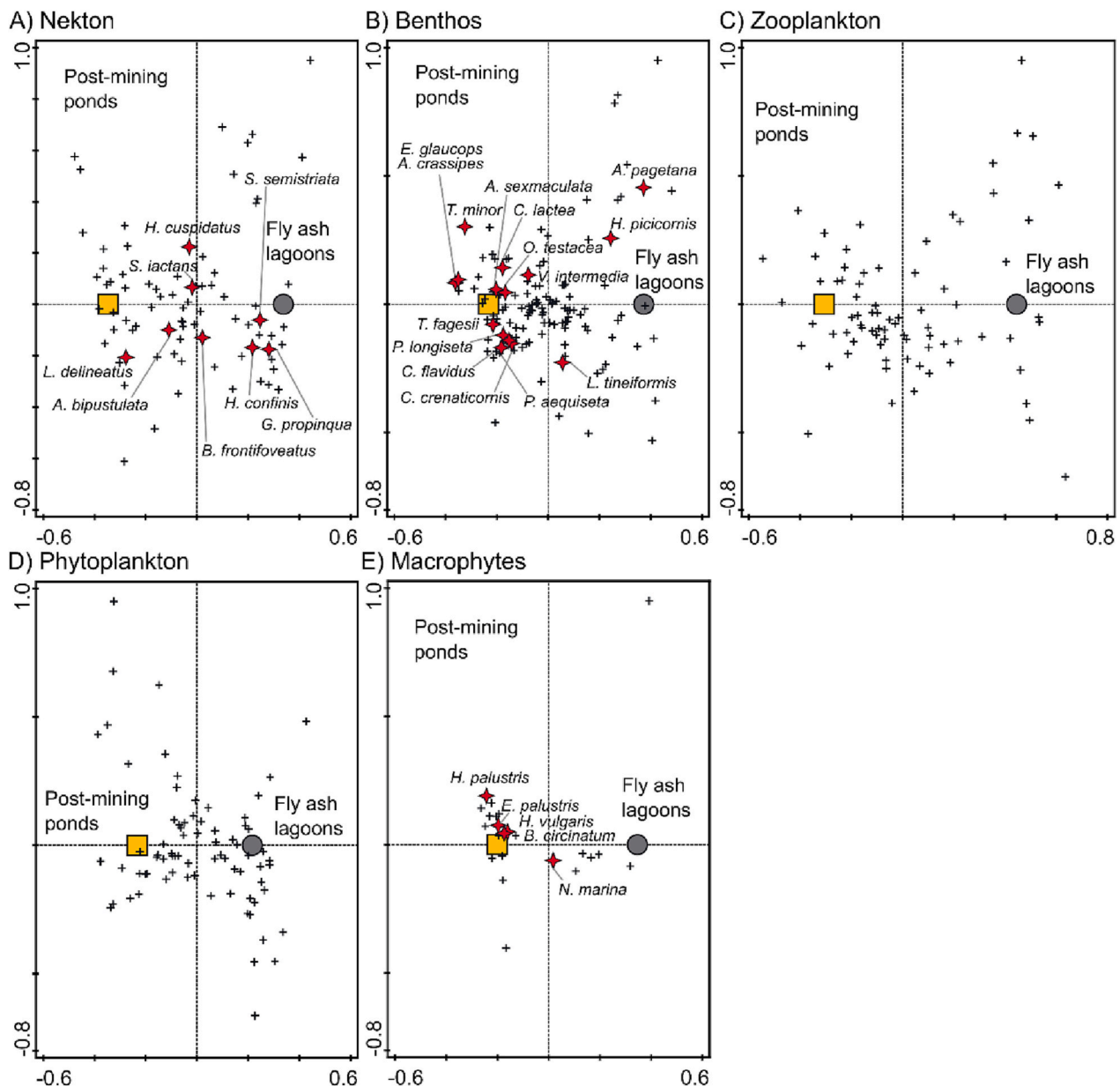


Fig. 5. Constrained ordination (CCA) diagrams visualising the affinities of individual species of freshwater organisms to fly ash lagoons and post-mining ponds. The symbols distinguish between the red-listed (red stars) and common (black crosses) species of each functional group. First and second ordination axes are visualised.

particularly calcium.

## 4. Discussion

### 4.1. Conservation potential of fly ash lagoons

Our study confirms the expected conservation potential of fly ash lagoons for freshwater biodiversity. The occurrence of numerous threatened species from various groups, including the critically endangered beetle *Halipedium confinis*, the endangered heteropterans *Glaenocoris propinqua* and *Hesperocoris linnaei*, and numerous vulnerable and nearly threatened species from various freshwater groups (Fig. 5; Table S4). Moreover, some of these red-listed species were recorded in multiple specimens and/or in multiple fly ash lagoons, which indicates the existence of established populations rather than incidental occurrences. These findings clearly demonstrate that freshwater habitats in fly ash lagoons deserve attention, especially during their restoration.

As is typical for freshwater communities of other post-industrial sites

(reviewed by Kolar et al., 2021b), colonising species often require habitat conditions which have become rare or even no longer present in the surrounding agricultural or industrial landscapes (as in much of Central Europe: Chytrý et al., 2019; Rodwell et al., 2013). In line with our expectations, the fly ash lagoons in our study were preferred by threatened species typical for saltmarshes and other waters with higher conductivity (e.g., the critically endangered beetle *Halipedium confinis*, and a nearly threatened plant *Najas marina*), and species requiring oligotrophic waters unpolluted by agricultural chemistry and with low fish density (e.g. the heteropterans *Glaenocoris propinqua* and *Sigara semistriata*, and the mite *Piona carnea*). Nevertheless, some species recorded mainly in the fly ash lagoons in our study (e.g. threatened caddisflies *Agrypnia pagetana* and *Holocentropus picicornis*) prefer unmanaged and non-intensively managed oligotrophic and mesotrophic ponds and fishponds with dense emergent vegetation, i.e. a historically very common habitat which has virtually disappeared in Central Europe recently (Chytrý et al., 2019; Krivánek et al., 2012).

In general, the freshwater communities in the studied fly ash lagoons



showed comparable or slightly lower species richness and conservation values than the post-mining ponds used as the control habitats in our study. Post-mining ponds have been repeatedly shown as crucial regional refuges for numerous endangered freshwater species (e.g., Harabiš et al., 2013; Kolar and Boukal, 2020; Poláková et al., 2022; Vojar et al., 2016). Although with lower or comparable species richness, freshwater communities of fly ash lagoons harbour some threatened species that do not colonise the post-mining ponds. Therefore, fly ash lagoons can be considered efficient supplements of key habitats for declining populations of many aquatic species in agricultural or industrial regions with a shortage of natural freshwater habitats.

#### 4.2. Potential risks of heavy metals for biodiversity

Concentrations of some heavy metals were higher in fly ash lagoons than in post-mining ponds, which was reflected in the higher tissue concentrations of some heavy metals found in several fish and invertebrate species (Carreira et al., 2023). Nevertheless, we found no significant effects of heavy metal concentrations on species richness or conservation value of the studied communities. This could be related to the fact that the higher concentrations were mostly far below the risk limits (Carreira et al., 2023; Chmelová et al., 2021) and/or that a large part of the heavy metals in fly ash are immobile and biologically unavailable, at least for some recorded freshwater species (Jankowski et al., 2006; Worms et al., 2006). Moreover, various aquatic arthropods and other invertebrates, i.e. the groups with most of the threatened species recorded in fly ash lagoons in this study, have physiological (Van Straalen and Donker, 1994; Zvereva et al., 2003), behavioural (Gillet and Ponge, 2003), or life-history (Eeva et al., 2004; Van Straalen and Donker, 1994) adaptations to cope with high concentrations of heavy metals and other pollutants. The hypothesis that the heavy metal concentrations in the studied fly ash lagoons are not harmful to the communities is supported by the high abundances of some recorded threatened species, indicating that their local populations are viable. Similar results were shown in the previous studies of terrestrial communities in fly ash deposits (e.g. Tropek et al., 2013, 2017). Altogether, it seems that the higher concentrations of some heavy metals in some of the fly ash lagoons do not diminish their potential as habitat surrogates, for at least some declining aquatic biodiversity. The significant differences in community composition of all studied groups indicate that they are colonised by a different spectrum of species than the other artificial habitats. Therefore, only sensitive restoration of both fly ash deposits and post-mining ponds can help maintain regional biodiversity, especially in regions with a shortage of natural freshwater habitats. The potential negative effects of heavy metals on the survival and development of many organisms (e.g. Besser and Leib, 2007; Cempel and Nikel, 2006; Doig et al., 2015; Horvat et al., 2007; Rowe, 2014) still needs to be considered when evaluating the conservation potential of any polluted site, at least until the real impacts from fly ash deposits are known. More research on the bioaccumulation of heavy metals and other trace elements in freshwater food webs is needed (Carreira et al., 2023).

We expected that the high concentrations of some heavy metals would act as an environmental filter, because the above-mentioned adaptations to cope with polluted environments are often species specific (e.g. Buckland-Nicks et al., 2014; Heikens et al., 2001). Following this, sensitive species cannot establish viable populations in polluted habitats, which are then successfully colonised only by the adapted species (Clements, 1994; Courtney and Clements, 2002). This is supported by the significant differences in community composition of all groups between the two pond types in our study, as well as in some other studies on polluted freshwater habitats (Clements, 1994; Courtney and Clements, 2002). It also seems noticeable that many endangered species recorded in the fly ash deposits were arthropod predators. Unlike vertebrates, where apex predators tend to be most affected by high environmental concentrations of heavy metals (Ali et al., 2019; Badry et al., 2019), aquatic arthropod predators are less affected than other groups

(Liess et al., 2017). Moreover, two of the three groups (benthos and macrophytes) with significantly lower species richness in the fly ash lagoons than in the post-mining ponds depend more closely on the physico-chemical features of the fly ash substrate, which may have been suboptimal for these groups. Although more research is needed, such intergroup differences can imply substantial environmental filtering. Therefore, we assume that the fly ash lagoons have been successfully colonised by species with suitable adaptations to cope with heavy metal pollution or other unusual environmental conditions at these sites. When adapted to such conditions for many other species, the colonising specialists can further benefit from lower competition, predation, or parasitism caused by the lack of species sensitive to the extreme water chemistry.

#### 4.3. Implications for restoration of fly ash lagoons

Efficient restoration of any post-industrial site should balance contradictory societal and environmental aspects. Unfortunately, the common practice of the fly ash deposit reclamation only considers environmental risks, and squanders the high potential of many sites for biodiversity conservation (Prach and Hobbs, 2008; Tropek et al., 2015). Considering the results of this study, fly ash deposits represent a challenge for restoration ecology because of their substantial negative environmental risks due to the high levels of heavy metals (Haynes, 2009; Rowe et al., 2002; Sherrard and Greeley, 2015) coupled with the high conservation potential for numerous threatened species (Chmelová et al., 2021; Tropek et al., 2013, 2015). Our study clearly indicates that preserving freshwater habitats in sedimentation lagoons during the fly ash deposits restoration can be beneficial for regional biodiversity. If restored respectfully to the specific needs of such aquatic communities (see below), these habitats can have the great potential for colonisation by threatened species from different freshwater groups. This would increase the already known conservation potential of the terrestrial habitats in these post-industrial barrens.

Any successful restoration of fly ash deposits must first minimise the main environmental risks (Haynes, 2009): wind erosion (Tropek et al., 2016) and leaks of contaminated waters (Han et al., 2021). Nevertheless, maintaining the water reservoir, even in a small area of the restored deposit, will reduce any wind erosion of fly ash to the surrounding landscape. In addition, the presence of water in the deposit can promote the dissolution of heavy metals from fly ash and their mobility into the deeper substrate layers, thus decreasing the environmental risks of the restored site (Chmelová et al., 2021). A functional water system is almost always present in fly ash deposits, and its technical improvements that decrease or avoid the leakage risks are already an integral part of any restoration project (Haynes, 2009).

The (re)creation of suitable habitats for the target (threatened) species or communities should be implemented in successful restoration plans for post-industrial sites, including for fly ash lagoons. Based on our results, together with studies from other post-industrial freshwater habitats (e.g. Kolar et al., 2021b; Tichanek and Tropek, 2015; Vojar et al., 2016), the introduction of nutrient-rich materials into the restored lagoons and their surroundings should be avoided, because the recorded threatened species are mostly sensitive to water eutrophication. It is also important to maintain the restored waters without fish or with low fish abundances. Some of the recorded threatened species are extremophiles specialised for freshwaters with extreme chemistry, particularly increased conductivity or acidity. Therefore, the unusual chemistry of fly ash lagoons may contribute to regional biodiversity and should not always be adjusted, especially because other public interests (such as the use of lagoons for leisure activities or aquaculture; Řehouňková et al., 2016) are highly unlikely in these post-industrial ponds. To maintain or even increase the diversity of freshwater communities in fly ash lagoons, their restoration should aim to support heterogeneous habitats with respect to the general rules known for freshwater habitats in sand pits, mining ponds, or spoil heaps, at least until more results from the fly ash



lagoons are available. These general rules include elimination of total shading by trees on the shoreline, and establishment of shallow banks with a combination of dense littoral and emergent vegetation and open substrate (e.g. Kolar et al., 2021b; Vojar et al., 2016). Given the unique water and substrate chemistry, together with the occurrence of extremophilic species, the efficient restoration of fly ash lagoons should benefit from future research on their biodiversity and its response to different restoration tools and approaches.

## 5. Conclusions

This study confirms the conservation potential of fly ash lagoons for freshwater biodiversity. Although the species richness and conservation value were comparable or slightly lower than those in post-mining ponds, the presence of threatened species (some less abundant or not found in the control ponds) indicates that the fly ash lagoons can serve as supplements to known secondary refuges for declining aquatic species. These post-industrial sites possess unique water and substrate chemistry that can contribute to regional biodiversity by harbouring of specialised species, including the threatened ones. While higher concentrations of heavy metals were observed in fly ash lagoons, their impact on species richness and conservation value was not significant, likely due to adaptations of the colonising freshwater species to cope with pollution. The restoration of fly ash lagoons should minimise the environmental risks and support the suitable habitats for threatened species. This included the avoidance of eutrophication and of high fish abundance. By implementing appropriate restoration strategies, fly ash lagoons have the potential to play a vital role in biodiversity conservation, provide valuable habitats for threatened species, and contribute to fulfilling regional ecological restoration efforts.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.165803>.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data used in this study are available in supplementary files.

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## CRedit authorship contribution statement

R.T., V.K., D.S.B., and E.C. conceptualised the research; all authors

participated in the investigation, either during field sampling (V.K., E.C., B.M.C., P.H., A.L.-D., O.L.-S., Z.M., Š.O., M.P., L.V., D.S.B., and R.T.), focal groups identification (V.K., M.B., M.Č., T.D., P.H., L.H., F.H., O.L.-S., Z.M., Š.O., M.P., V.P., V.S., J.Š., P.S., and L.V.), or chemical analyses (J.J. and J.B.); V.K., E.C., and Š.O. curated the data; V.K. and R.T. performed the analyses; V.K. and R.T. led the writing and wrote the first draft; all authors contributed the writing by reviewing and editing the drafts.

## References

- Ali, H., Khan, E., Ilahi, I., 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *J. Chemother.* 2019 <https://doi.org/10.1155/2019/6730305>.
- Badry, A., Palma, L., Beja, P., Ciesielski, T.M., Dias, A., Liehagen, S., Janssen, B.M., Sturaro, N., Eulaers, I., Jaspers, V.L.B., 2019. Using an apex predator for large-scale monitoring of trace element contamination: associations with environmental, anthropogenic and dietary proxies. *Sci. Total Environ.* 676, 746–755. <https://doi.org/10.1016/j.scitotenv.2019.04.217>.
- Besser, J.M., Leib, K.J., 2007. *Toxicity of Metals in Water and Sediment to Aquatic Biota*. Integr. Investig. Environ. Eff. Hist. Min. Animas River Watershed, San Juan County, Color, p. 14.
- Bobrek, R., 2020. High biodiversity in a city Centre: Odonatofauna in an abandoned limestone quarry. *Eur. J. Environ. Sci.* 10, 107–114. <https://doi.org/10.14712/23361964.2020.12>.
- Bogusch, P., Macek, J., Janšta, P., Kubík, Š., Řezáč, M., Holý, K., Malenkovský, I., Banár, P., Mikát, M., Astapenkova, A., Heneberg, P., 2016. Industrial and post-industrial habitats serve as critical refugia for pioneer species of newly identified arthropod assemblages associated with reed galls. *Biodivers. Conserv.* 25, 827–863. <https://doi.org/10.1007/s10531-016-1070-5>.
- Borm, P., 1997. Toxicity and occupational health hazards of coal fly ash (CFA). A review of data and comparison to coal mine dust. *Ann. Occup. Hyg.* 41, 659–676. [https://doi.org/10.1016/S0003-4878\(97\)00026-4](https://doi.org/10.1016/S0003-4878(97)00026-4).
- ter Braak, C.J.F., Šmilauer, P., 2012. *Canoco Reference Manual and user's Guide: Software for Ordination, Version 5.0*.
- Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9, 378–400. <https://doi.org/10.32614/rj-2017-066>.
- Buckland-Nicks, A., Hillier, K.N., Avery, T.S., O'Driscoll, N.J., 2014. Mercury bioaccumulation in dragonflies (Odonata: Anisoptera): examination of life stages and body regions. *Environ. Toxicol. Chem.* 33, 2047–2054. <https://doi.org/10.1002/etc.2653>.
- Carreira, B.M., Kolár, V., Chmelová, E., Jan, J., Adašević, J., Landeira-Dabarca, A., Vebrová, L., Poláková, M., Horká, P., Otáhalová, Š., Musilová, Z., Borovec, J., Tropek, R., Boukal, D.S., 2023. Bioaccumulation of heavy metals at post-industrial freshwater sites varies predictably between habitats, elements and taxa: a power law approach. *Sci. Total Environ.* this issue.
- Cempel, M., Nikel, G., 2006. Nickel: a review of its sources and environmental toxicology. *Pol. J. Environ. Stud.* 15, 375–382.
- Chmelová, E., Kolar, V., Jan, J., Carreira, B.M., Landeira-Dabarca, A., Otáhalová, Š., Poláková, M., Vebrová, L., Borovec, J., Boukal, D.S., Tropek, R., 2021. Valuable secondary habitats or hazardous ecological traps? Environmental risk assessment of minor and trace elements in fly ash deposits across the Czech Republic. *Sustain.* 13 <https://doi.org/10.3390/su131810385>.
- Chytrý, M., Hájek, M., Kočí, M., Pešout, P., Roleček, J., Sádlo, J., Šumberová, K., Sychra, J., Boublík, K., Douda, J., Grulich, V., Härtel, H., Hédli, R., Lustyk, P., Navrátilová, J., Novák, P., Peterka, T., Vydrová, A., Chobot, K., 2019. Red list of habitats of the Czech Republic. *Ecol. Indic.* 106, 105446 <https://doi.org/10.1016/j.ecolind.2019.105446>.
- Clements, W.H., 1994. Benthic invertebrate community responses to heavy metals in the Upper Arkansas River Basin, Colorado. *J. North Am. Benthol. Soc.* 13, 30–44. <https://doi.org/10.2307/1467263>.
- Courtney, L.A., Clements, W.H., 2002. Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: an experimental approach. *Freshw. Biol.* 47, 1766–1778. <https://doi.org/10.1046/j.1365-2427.2002.00896.x>.
- Ditrich, T., Čihák, P., 2017. Efficiency of subaquatic light traps. *Aquat. Insects* 38, 171–184. <https://doi.org/10.1080/01650424.2017.1359305>.
- Doig, L.E., Schiffer, S.T., Liber, K., 2015. Reconstructing the ecological impacts of eight decades of mining, metallurgical, and municipal activities on a small boreal lake in northern Canada. *Integr. Environ. Assess. Manag.* 11, 490–501. <https://doi.org/10.1002/ieam.1616>.
- Dolný, A., Harabiš, F., 2012. Underground mining can contribute to freshwater biodiversity conservation: allogenic succession forms suitable habitats for dragonflies. *Biol. Conserv.* 145, 109–117. <https://doi.org/10.1016/j.biocon.2011.10.020>.
- Eeva, T., Sorvari, J., Koivunen, V., 2004. Effects of heavy metal pollution on red wood ant (*Formica s. str.*) populations. *Environ. Pollut.* 132, 533–539. <https://doi.org/10.1016/j.envpol.2004.05.004>.
- Farkač, J., Král, D., Škorpič, M., 2005. Red list of threatened species in the Czech Republic Invertebrates. In: *Agentura ochrany přírody a krajiny ČR. Praha*. 1–760.

- Gillet, S., Ponge, J.-F., 2003. Changes in species assemblages and diets of Collembola along a gradient of metal pollution. *Appl. Soil Ecol.* 22, 127–138. [https://doi.org/10.1016/S0929-1393\(02\)00134-8](https://doi.org/10.1016/S0929-1393(02)00134-8).
- Gollakota, A.R.K., Volli, V., Shu, C.-M., 2019. Progressive utilisation prospects of coal fly ash: a review. *Sci. Total Environ.* 672, 951–989. <https://doi.org/10.1016/j.scitotenv.2019.03.337>.
- Han, D., Xu, L., Wu, Q., Wang, S., Duan, L., Wen, M., Li, Z., Tang, Y., Li, G., Liu, K., 2021. Potential environmental risk of trace elements in fly ash and gypsum from ultra-low emission coal-fired power plants in China. *Sci. Total Environ.* 798, 149116 <https://doi.org/10.1016/j.scitotenv.2021.149116>.
- Harabis, F., Tichanek, F., Tropek, R., 2013. Dragonflies of freshwater pools in lignite spoil heaps: restoration management, habitat structure and conservation value. *Ecol. Eng.* 55, 51–61. <https://doi.org/10.1016/j.ecoleng.2013.02.007>.
- Hartig, F., 2018. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R Package Version 0.2.0. <https://CRAN.R-project.org/package=DHARMA>.
- Haynes, R.J., 2009. Reclamation and revegetation of fly ash disposal sites – challenges and research needs. *J. Environ. Manag.* 90, 43–53. <https://doi.org/10.1016/j.jenvman.2008.07.003>.
- Heikens, A., Peijnenburg, W.J.G., Hendriks, A., 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. *Environ. Pollut.* 113, 385–393. [https://doi.org/10.1016/S0269-7491\(00\)00179-2](https://doi.org/10.1016/S0269-7491(00)00179-2).
- Hejda, R., Farkač, J., Chobot, K., 2017. Red list of threatened species of the Czech Republic. *Invertebrates Příroda* 36, 1–612.
- Horvat, T., Vidaković-Cifrek, Ž., Oreščanin, V., Tkalec, M., Pevalek-Kozlina, B., 2007. Toxicity assessment of heavy metal mixtures by *Lemma minor* L. *Sci. Total Environ.* 384, 229–238. <https://doi.org/10.1016/j.scitotenv.2007.06.007>.
- Izquierdo, M., Querol, X., 2012. Leaching behaviour of elements from coal combustion fly ash: an overview. *Int. J. Coal Geol.* 94, 54–66. <https://doi.org/10.1016/j.coal.2011.10.006>.
- Jankowski, J., Ward, C.R., French, D., Groves, S., 2006. Mobility of trace elements from selected Australian fly ashes and its potential impact on aquatic ecosystems. *Fuel* 85, 243–256. <https://doi.org/10.1016/j.fuel.2005.05.028>.
- Klecka, J., Boukal, D.S., 2011. Lazy ecologist's guide to water beetle diversity: which sampling methods are the best? *Ecol. Indic.* 11, 500–508. <https://doi.org/10.1016/j.ecolind.2010.07.005>.
- Kolar, V., Boukal, D.S., 2020. Habitat preferences of the endangered diving beetle *Graphoderus bilineatus*: implications for conservation management. *Insect Conserv. Divers.* 13, 480–494. <https://doi.org/10.1111/icad.12433>.
- Kolar, V., Tichanek, F., Tropek, R., 2017. Effect of different restoration approaches on two species of newts (Amphibia: Caudata) in central European lignite spoil heaps. *Ecol. Eng.* 99, 310–315. <https://doi.org/10.1016/j.ecoleng.2016.11.042>.
- Kolar, V., Vlašánek, P., Boukal, D.S., 2021a. The influence of successional stage on local odonate communities in man-made standing waters. *Ecol. Eng.* 173, 106440 <https://doi.org/10.1016/j.ecoleng.2021.106440>.
- Kolar, V., Tichanek, F., Tropek, R., 2021b. Evidence-based restoration of freshwater biodiversity after mining: experience from central European spoil heaps. *J. Appl. Ecol.* 1365-2664, 13956. <https://doi.org/10.1111/1365-2664.13956>.
- Kopáček, J., Borovec, P., Hejzlar, J., Porcal, P., 2001. Spectrophotometric determination of iron, aluminum, and phosphorus in soil and sediment extracts after their nitric and perchloric acid digestion. *Commun. Soil Sci. Plant Anal.* 32, 1431–1443. <https://doi.org/10.1081/CSS-100104203>.
- Křivánek, J., Němec, J., Kopp, J., 2012. Rybníky v České republice. Pro Ministerstvo zemědělství ČR vydal Consult, Praha.
- Liess, M., Gerner, N.V., Kefford, B.J., 2017. Metal toxicity affects predatory stream invertebrates less than other functional feeding groups. *Environ. Pollut.* 227, 505–512. <https://doi.org/10.1016/j.envpol.2017.05.017>.
- Poláková, M., Straka, M., Poláček, M., Němejcová, D., 2022. Unexplored freshwater communities in post-mining ponds: effect of different restoration approaches. *Restor. Ecol.* 30, 1–10. <https://doi.org/10.1111/rec.13679>.
- Prach, K., Hobbs, R.J., 2008. Spontaneous succession versus technical reclamation in the restoration of disturbed sites. *Restor. Ecol.* 16, 363–366. <https://doi.org/10.1111/j.1526-100X.2008.00412.x>.
- R Core Team, 2021. R: A Language and Environment for Statistical Computing.
- Rausch, H., Fliszár-Baranyai, R., Sándor, S., László-Sziklai, I., Török, S., Papp-Zenplén, É., 1993. Distribution of toxic elements in fly-ash particulates. *Sci. Total Environ.* 130–131, 317–330. [https://doi.org/10.1016/0048-9697\(93\)90086-L](https://doi.org/10.1016/0048-9697(93)90086-L).
- Řehounková, K., Čížek, L., Řehounek, J., Šebelíková, L., Tropek, R., Lencová, K., Bogusch, P., Marhoul, P., Máca, J., 2016. Additional disturbances as a beneficial tool for restoration of post-mining sites: a multi-taxa approach. *Environ. Sci. Pollut. Res.* 23, 13745–13753. <https://doi.org/10.1007/s11356-016-6585-5>.
- Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol. Rev.* 94, 849–873. <https://doi.org/10.1111/brev.12480>.
- Rodwell, J.S., Janssen, J.A., Gubbay, S., Schaminée, J.H., 2013. Red List Assessment of European Habitat Types. A feasibility study. Rep. to DG Environ. Eur. Comm. p. 78.
- Rowe, C.L., 2014. Bioaccumulation and effects of metals and trace elements from aquatic disposal of coal combustion residues: recent advances and recommendations for further study. *Sci. Total Environ.* 485, 490–496.
- Rowe, C.L., Hopkins, W.A., Congdon, J.D., 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: a review. *Environ. Monit. Assess.* 80, 207–276.
- Sherrard, R.M., Neil, E.C., Greeley, M.S., 2015. How toxic is coal ash? A laboratory toxicity case study. *Integr. Environ. Assess. Manag.* 11, 5–9. <https://doi.org/10.1002/ieam.1587>.
- Silva, L.F.O., da Boit, K.M., 2011. Nanominerals and nanoparticles in feed coal and bottom ash: implications for human health effects. *Environ. Monit. Assess.* 174, 187–197. <https://doi.org/10.1007/s10661-010-1449-9>.
- Sroka, P., Bojková, J., Kolar, V., 2022. Mayfly *Ephemera glaucops* (Ephemeroptera, Ephemeridae) recorded in the Czech Republic after almost a century. *Biodivers. Data J.* 10 <https://doi.org/10.3897/BDJ.10.e90950>.
- Stendera, S., Adrian, R., Bonada, N., Cañedo-Argüelles, M., Huguency, B., Januschke, K., Pletterbauer, F., Hering, D., 2012. Drivers and stressors of freshwater biodiversity patterns across different ecosystems and scales: a review. *Hydrobiologia* 696, 1–28. <https://doi.org/10.1007/s10750-012-1183-0>.
- Sushil, S., Batra, V.S., 2006. Analysis of fly ash heavy metal content and disposal in three thermal power plants in India. *Fuel* 85, 2676–2679. <https://doi.org/10.1016/j.fuel.2006.04.031>.
- Tichanek, F., Tropek, R., 2015. Conservation value of post-mining headwaters: drainage channels at a lignite spoil heap harbour threatened stream dragonflies. *J. Insect Conserv.* 19, 975–985. <https://doi.org/10.1007/s10841-015-9814-1>.
- Tropek, R., Cerna, I., Straka, J., Cizek, O., Konvicka, M., 2013. Is coal combustion the last chance for vanishing insects of inland drift sand dunes in Europe? *Biol. Conserv.* 162, 60–64. <https://doi.org/10.1016/j.biocon.2013.03.027>.
- Tropek, R., Kadlec, T., Karesová, P., Spitzer, L., Kocárek, P., Malenovský, P., Baňar, P., Tuf, I.H., Hejda, M., Konvicka, M., 2010. Spontaneous succession in limestone quarries as an effective restoration tool for endangered arthropods and plants. *J. Appl. Ecol.* 47, 139–147. <https://doi.org/10.1111/j.1365-2664.2009.01746.x>.
- Tropek, R., Rauch, O., Kovář, P., 2015. Fly ash deposits and depots of fine substrates. In: Řehounková, J., Řehounková, K., Tropek, R., Prach, K. (Eds.), *Ecological Restoration of Areas Disturbed by Mining and Industrial Landfills*. Calla, České Budějovice, pp. 158–191.
- Tropek, R., Cerna, I., Straka, J., Kocarek, P., Malenovsky, I., Tichanek, F., Sebek, P., 2016. In search for a compromise between biodiversity conservation and human health protection in restoration of fly ash deposits: effect of anti-dust treatments on five groups of arthropods. *Environ. Sci. Pollut. Res.* 23, 13653–13660. <https://doi.org/10.1007/s11356-015-4382-1>.
- Tropek, R., Cizek, O., Kadlec, T., Klecka, J., 2017. Habitat use of *Hipparchia semele* (Lepidoptera) in its artificial stronghold: necessity of the resource-based habitat view in restoration of disturbed sites. *Pol. J. Ecol.* 65, 385–399. <https://doi.org/10.3161/15052249PJE2017.65.3.006>.
- Van Straalen, N.M., Donker, M.H., 1994. Heavy metal adaptation in terrestrial arthropods: physiological and genetic aspects. In: *Proc. Sect. Exp. Appl. Entomol. Netherlands Entomol. Soc.*
- Vojar, J., Doležalová, J., Solský, M., Smolová, D., Kopecký, O., Kadlec, T., Knapp, M., 2016. Spontaneous succession on spoil banks supports amphibian diversity and abundance. *Ecol. Eng.* 90, 278–284. <https://doi.org/10.1016/j.ecoleng.2016.01.028>.
- Worms, I., Simon, D.F., Hassler, C.S., Wilkinson, K.J., 2006. Bioavailability of trace metals to aquatic microorganisms: importance of chemical, biological and physical processes on biouptake. *Biochimie* 88, 1721–1731. <https://doi.org/10.1016/j.biochi.2006.09.008>.
- Zvereva, E., Serebrov, V., Glupov, V., Dubovskiy, I., 2003. Activity and heavy metal resistance of non-specific esterases in leaf beetle *Chrysomela lapponica* from polluted and unpolluted habitats. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 135, 383–391. [https://doi.org/10.1016/S1532-0456\(03\)00115-7](https://doi.org/10.1016/S1532-0456(03)00115-7).