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INSTITUTE OF CHEMISTRY AND TECHNOLOGY OF ENVIRONMENTAL PROTECTION


# KONTAMINACE ŽIVOTNÍHO PROSTŘEDÍ TĚŽEBNÍCH OBLASTÍ A MOŽNOSTI JEJICH FYTOREMEDIACE

ENVIRONMENTAL CONTAMINATION OF MINING AREAS AND THE POSSIBILITIES OF THEIR PHYTOREMEDIATION

**DIZERTAČNÍ PRÁCE**

DOCTORAL THESIS


**AUTOR PRÁCE** — **Ing. Václav Pecina**

AUTHOR

**ŠKOLITEL** — **doc. Mgr. Renata Komendová, Ph.D.**

SUPERVISOR



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# Zadání dizertační práce

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Zhodnocení kontaminace půdy v Mongolsku.
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Identifikace půdních aditiv vhodných pro zvýšení efektivity fytoremediace.
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Ing. Václav Pecina
student

doc. Mgr. Renata Komendová, Ph.D.
vedoucí práce

prof. Ing. Jiří Kučerík, Ph.D.
vedoucí ústavu

V Brně dne 1.9.2021

prof. Ing. Martin Weiter, Ph.D.
děkan

## ABSTRAKT

Znečištění životního prostředí těžkými kovy je globálním problémem eskalujícím v oblastech s těžbou nerostných surovin. Hledání řešení tohoto problému je předmětem výzkumu posledních dekád. Tato práce je koncipována jako soubor čtyř případových studií zaměřených na znečištění životního prostředí těžebních oblastí a fytoremediaci znečištěných půd. Závěry všech čtyř studií jsou diskutovány v kontextu situace v Mongolsku, kde je řešení této problematiky v iniciálním stádiu. Výsledky šetření znečištění životního prostředí těžebních oblastí ukázaly (I) nevhodnost transformace důlně-úpravárenského závodu a haldy v Příbrami na lesopark s ohledem na přetrvávající silné znečištění půdy těžkými kovy (As, Cd, Pb a Zn) znamenající potenciální zdravotní rizika pro návštěvníky a inhibici sazenic stromů i po realizaci rekultivačního projektu a (II) nízkou úroveň znečištění půdy těžebních oblastí v Mongolsku bez předpokládaných rizik pro obyvatele. Výsledky fytoremediačních studií potvrdily potenciál *Lupinus luteus* L. a *Festuca rubra* L. v kombinaci se specifickými aditivy (zejména biouhlem z vrbové štěpky, popelu z drůbežího peří a chalcedonitem) pro asistovanou fytostabilizaci znečištěných půd, příslušně pro Cd, Cr, Cu, Ni a Zn a Cr(VI), a tedy i potenciál pro využití při fytoremediaci půd v těžebních oblastech znečištěných těžkými kovy. S ohledem na nekontrolovanou pastvu v Mongolsku je pro snížení rizik šíření kontaminace a intoxikace nejen hospodářských zvířat nezbytná fytostabilizace hald a jejich znepřístupnění. Perspektivním druhem pro fytoremediaci v Mongolsku může být *Larix sibirica* Ledeb.; vhodným typem managementu těžebních odpadů asistovaná fytostabilizace s využitím potenciálně dostupného hnoje a štěpky. Budoucí výzkum by měl realizovat experimenty s identifikovanými druhy rostlin a aditivy a nalézt další vhodné lokální druhy schopné vytvořit souvislý vegetační pokryv.

## KLÍČOVÁ SLOVA

znečištění, těžké kovy, půda, těžba, fytostabilizace, Mongolsko, hodnocení zdravotního rizika

## ABSTRACT

Environmental pollution with heavy metals is a global problem escalating in areas with mining history. The search for solutions to this problem has been the subject of research for the past decades. This work is conceived as a set of four case studies focused on environmental pollution of mining areas and phytoremediation of polluted soils. The conclusions of all four studies are discussed in the context of the situation in Mongolia, where the investigation of this issue is at an initial stage. The results of the environmental pollution investigation in the mining areas showed (I) the unsuitability of the transformation of the mining-processing plant and the adjacent heap in Příbram into a forest park due to the persistent heavy pollution of the soil with the heavy metals (As, Cd, Pb and Zn) causing potential health risks for visitors and inhibition of the tree seedlings, even after the implementation of the reclamation project and (II) the low level of soil pollution of the mining areas in Mongolia with no expected risks to the local population. The results of the phytoremediation studies confirmed the potential of *Lupinus luteus* L. and *Festuca rubra* L. in combination with specific additives (mainly biochar from willow chips, poultry feather ash and chalcedonite, respectively) for aided phytostabilization of soils polluted with Cd, Cr, Cu, Ni and Zn, and Cr(VI), respectively, and thus the potential for use in phytoremediation of heavy metal-polluted soils in mining areas. Regarding uncontrolled grazing in Mongolia, phytostabilization of heaps and making them inaccessible is necessary to reduce the risks of spreading contamination and intoxication not only of livestock. Promising species for phytoremediation in Mongolia may be *Larix sibirica* Ledeb.; a suitable type of mining waste management aided phytostabilization focusing on the use of potentially available manure and wood chips. Future research should implement experiments with identified plant species and additives and find other suitable local species capable of creating a continuous vegetation cover.

## KEYWORDS

pollution, heavy metals, soil, mining, phytostabilization, Mongolia, health risk assessment

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**PROHLÁŠENÍ**

Prohlašuji, že jsem disertační práci vypracovala samostatně a že všechny použité literární zdroje jsem správně a úplně citovala. Disertační práce je z hlediska obsahu majetkem Fakulty chemické VUT v Brně a může být využita ke komerčním účelům jen se souhlasem vedoucího práce a děkana FCH VUT.

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Ing. Václav Pecina

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OBSAH

# 1. ÚVOD

Práce se zaměřuje na kontaminaci životního prostředí těžkými kovy z těžby nerostných surovin a možnosti fytoremediace takto postižených oblastí. Znečištění životního prostředí těžkými kovy je globálním problémem, který eskaluje právě v oblastech s těžbou, kde představuje kritický faktor ohrožující zdraví obyvatel i dalších živých složek životního prostředí. Hledání řešení tohoto problému je významným předmětem výzkumu posledních dekád.

Tato práce je koncipována jako soubor čtyř případových studií zaměřených na danou problematiku: dvě studie jsou věnovány tématu znečištění životního prostředí těžebních oblastí a dvě fytoremediaci znečištěných půd. Pro větší přesah a širší environmentální i sociální přínos bylo jako nosné téma diskuze této práce vybráno hodnocení situace v Mongolsku, kde je řešení této problematiky teprve na počátku. Výsledky všech čtyř studií jsou proto diskutovány v kontextu situace v Mongolsku.

Těžební průmysl je pro Mongolsko základním pilířem hospodářského sektoru a mezi jeho nejvýznamnější komodity patří drahé kovy a uhlí. I přes významná environmentální a zdravotní rizika související s těžbou ale v této 18. největší zemi světa prakticky neexistují preventivní a nápravná opatření proti kontaminaci a doprovodný monitoring životního prostředí. Absence těchto mechanismů, které by napomohly ochraně prostředí a udržení nezávadnosti vodních zdrojů, které jsou v těchto podmínkách aridního klimatu kritické pro existenci života, představuje závažnou hrozbu. Navazujícím dílčím cílem této práce je proto zhodnocení kontaminace půd vybraných těžebních oblastí Mongolska a dosavadního povědomí o znečištění půdy na národní úrovni.

Aktivní revitalizace těžbou kontaminovaného životního prostředí je typická zejména pro rozvinutější země Evropy nebo Severní Ameriky. Jednou ze stále častěji praktikovaných metod je fytoremediace, která má vzhledem ke své nižší finanční náročnosti oproti konvenčním technickým a chemickým dekontaminačním postupům potenciál pro využití po celém světě. Na základě zkušeností s fytoremediací kontaminovaných půd a rekultivačních projektů ze střední Evropy je záměrem práce také zhodnotit možnosti a limity fytoremediace v Mongolsku.

Výsledkem práce je zhodnocení stavu znečištění životního prostředí cílových oblastí těžkými kovy, vyhodnocení souvisejícího rizika představovaného pro člověka, identifikace vhodných druhů a materiálů pro asistovanou fytoremediaci kontaminovaných půd a zhodnocení možnosti realizace a údržby zeleně na těžebních odpadech ve vztahu k těžkým kovům a kulturním specifikům Mongolska.

# 2. TEORETICKÁ ČÁST

## 2.1 Těžba a kontaminace životního prostředí

Těžba nerostných surovin může přispívat k sociálnímu a hospodářskému rozvoji regionů (Bhuiyan et al., 2010; Quadros et al., 2016), vytváří potřebná pracovní místa i v izolovaných komunitách (Betz et al., 2015) a může být stěžejním sektorem i pro ekonomiku celého státu (Byambaa a Todo, 2011; Regdel et al., 2012). Je ale také spojena s oblastmi, které trpí vysokou chudobou (Betz et al., 2015) a především významným zhoršením stavu životního prostředí (Tiwary, 2001; Bhuiyan et al., 2010; Pandey et al., 2016).

Těžba nerostných surovin způsobuje úplnou destrukci původních přírodních ekosystémů (Mudrák et al., 2010; Jain et al., 2015; Obrázek 1), vede k drastickým strukturálním a biologickým změnám (Quadros et al., 2016), a přetváření zemského povrchu spojené s vytvářením hald těžebního odpadu a hlubokými terénními depresemi fatálně ovlivňuje krajinu jako celek (Szarek-Łukaszewska a Grodzińska, 2007; Mudrák et al., 2010; Rout et al., 2015; McIntyre et al., 2016). V souvislosti s těžbou dochází ke změně výšky hladiny podzemní vody a celkovému ovlivnění vodního režimu v krajině (Motyka a Postawa, 2013; Jain et al., 2015; McIntyre et al., 2016). Mimoto těžební aktivity typicky doprovází také zhutnění a eroze půdy, stejně jako nárůst její acidifikace (Quadros et al., 2016). Povrchová těžba je dokonce považována za jednu z nejhorších disturbancí způsobovaných člověkem (Alday et al., 2011).

Další negativní dopady představuje především znečištění životního prostředí. To může být spojeno přímo s těžebními aktivitami, jako primárním zdrojem znečištění, nebo s těžebním odpadem, jako sekundárním zdrojem znečištění (Mendez a Maier, 2008). Během povrchové těžby dochází ke znečištění ovzduší vlivem emisí prachu a dalších částic uvolňovaných do atmosféry (Jain et al., 2015; Rout et al., 2015; Quadros et al., 2016). Vlivem zvětrávání hlušiny uložené na haldách může docházet ke kontinuální kontaminaci životního prostředí i několik století po ukončení těžby (Stefanowicz et al., 2014).

Globálně nejvíce řešenou problematikou v kontextu kontaminace životního prostředí těžbou nerostných surovin je pravděpodobně znečištění půdy (např. Brumbaugh et al., 2013; Pandey et al., 2016; Li et al., 2018) a vody (např. Inam et al., 2011; McIntyre et al., 2016; Quadros et al., 2016). Za kritické polutanty jsou považovány zejména těžké kovy (Thorslund et al., 2012), v současné době v odborné literatuře označovány také jako potenciálně toxické prvky, stopové prvky, rizikové prvky nebo pouze (polo)kovy.

Například těžba uhlí, ať už povrchová, nebo podpovrchová, produkuje velký objem hlušiny. V hlušině jsou běžně obsaženy minerály doprovázející uhelné sloje, jako jsou galenit (PbS), clausthalit (PbSe), greenockit (CdS) nebo sfalerit (ZnS). Jejich zvětráváním se mohou uvolňovat významné koncentrace těžkých kovů, které tyto minerály tvoří (Bhuiyan et al., 2010; Reza et al., 2015; Li et al., 2018). Samotné uhlí je obohaceno dalšími prvky, zejména bismutem, dále ale také například arzenem, kadmiem a rtutí (Rout et al., 2013). Při těžbě drahých nebo těžkých kovů je riziko kontaminace životního prostředí těžkými kovy, ať už při těžbě, zvětrávání hlušiny nebo zpracování rudy, ještě významnější (Ettler et al., 2006; Mihaljevič et al., 2006; Stefanowicz et al., 2014).

***Obrázek 1**. Kontrast původní podoby říčního aluvia (v pravé části snímku) s remodelací krajiny spojenou s postupující těžbou zlata z rozsypů kolem řeky Sharyn Gol, Mongolsko (Autor fotografie: Ing. Václav Pecina).*

Dalším rizikovým kontaminačním faktorem spojovaným s těžbou nerostných surovin je transport vytěženého materiálu. Během něj je produkováno enormní množství suspendovaných částic, které mohou přispívat k šíření znečištění (Rout et al., 2013; Rout et al., 2015; Tang et al., 2017). V případě uhlí jsou potenciálně toxické látky zahrnující těžké kovy dále uvolňovány i jeho spalováním, a tak je i jeho finální využívání považováno za významný zdroj znečištění (Batjargal et al., 2010; Kosheleva et al., 2010; Chung a Chon, 2014; Pandey et al., 2016).

S těžbou uhlí a jeho spalováním jsou přímo spojovány například Pb, As, Hg, Co, Cu, Cr a Ni (Rout et al., 2015; Liang et al., 2017; Tang et al., 2017).

Těžké kovy se v životním prostředí vyskytují přirozeně a řada z nich je esenciální pro život mnoha organismů. Mezi tyto esenciální těžké kovy patří například Cr, Cu, Mn, Ni a Zn (Nagajyoti et al., 2010). V nadměrných koncentracích mají ale i tyto potřebné prvky toxické účinky (Gall et al., 2015). Za rizikovější jsou, nicméně, považovány neesenciální těžké kovy, jako například As, Cd, Hg a Pb (Qadir et al., 2005), které jsou vysoce toxické i při nízkých koncentracích (Wu et al., 2016). Kromě potenciální toxicity představují jeden z nejvážnějších problémů znečištění životního prostředí také díky své perzistenci a snadné bioakumulaci (Ali et al., 2013; Gall et al., 2015; Wu et al., 2016; Cheng et al., 2017; Sarwar et al., 2017; Tang et al., 2018). Mezi nejčastěji sledované těžké kovy v rámci vědeckých studií patří Cd, Cr, Cu, Ni, Pb a Zn, méně často se objevují také například As, Co, Fe, Hg a Mn. Těžké kovy mohou být v případě biodostupnosti v prostředí absorbovány rostlinami a houbami nebo přímo přijímány zvířaty a člověkem, případně jsou přijímány v rámci potravních řetězců (Ali et al., 2013; Stefanowicz et al., 2014; Gall et al., 2015; Wu et al., 2016; Li et al., 2018).

V oblastech s těžbou černého uhlí se udává jako hlavní riziko zejména vystavení emisím kontaminovaného prachu (Rout et al., 2015). V oblastech s těžbou a zpracováním rud je vzhledem ke komplexnímu znečištění životního prostředí věnována pozornost obvykle všem jeho složkám s ohledem na riziko kontaminace zdrojů vody nebo zemědělských produktů. V dlouhodobém horizontu může riziko představovat především kontinuální příjem těžkých kovů vlivem jejich vysoké koncentrace v prostředí, který může vést k řadě negativních dopadů na lidské zdraví (Gall et al., 2015; Wu et al., 2016). Ty zahrnují například poškození centrální a periferní nervové soustavy, poškození jater, ledvin, plic a mohou vést až k úmrtí intoxikovaného člověka (Rout et al., 2013). Některé kovy, jako například As, Cd, Cr, Hg, Ni a Pb, jsou uvažovanými nebo prokázanými karcinogeny (Koedrith et al., 2013).

## 2.2 Migrace těžkých kovů v životním prostředí

Zvýšený obsah těžkých kovů v prostředí lokalit s těžbou uhlí nebo rud byl zaznamenán celou řadou studií (např. Rout et al., 2015; Cheng et al., 2017; Drahota et al., 2018; Tang et al., 2018). Těžba těchto nerostných surovin může způsobit velkoplošné znečištění těžkými kovy a zasáhnout tím městské oblasti, zemědělskou i lesní půdu, a to jak v bezprostředním okolí dolů, tak v regionálním meřítku, především prostřednictvím atmosférické depozice (Liang et al., 2017; Pecina et al., 2022). Těžbou emitované pevné částice (Obrázek 2) představují základní médium pro disperzi polutantů v dotčených oblastech (Rout et al., 2013). Resuspenzí se kontaminované prachové a další částice mohou uvolňovat také z hald těžebního odpadu a dál se šířit přirozeně atmosférickým prouděním nebo uměle prostřednictvím dopravy (Tang et al., 2017).

***Obrázek 2***. *Prašnost způsobená dopravou v povrchovém uhelném dole Shariin Gol, Mongolsko (Autor fotografie: Ing. Václav Pecina).*

Disperze větrem nicméně není jediný způsob šíření znečištění z těžby. Výše zmíněné haldy fungují jako lokální bodový zdroj znečištění, který může v dlouhodobém měřítku představovat významné riziko. Synergické působení vody, slunečního záření, tepla, mikroorganismů a vegetace vede ke zvětrávání těžebního odpadu a promývání hald dešťovými srážkami ke kontinuálnímu uvolňování těžkých kovů do půdy (Li et al., 2018). Na výsledný obsah těžkých kovů v půdě a jejich další šíření má vliv celá řada faktorů. Do souvislosti jsou dávány zejména s organickou hmotou, kde jsou vázány na huminové látky (organické koloidy), nebo s jílovými minerály (anorganické koloidy) (Gall et al., 2015; Tang et al., 2018). V kontextu toxicity pro živé organismy není důležitý ale jen samotný obsah těžkých kovů v půdě, ale také jejich biologická dostupnost, která závisí na dalších chemických, fyzikálních a biologických faktorech (Ernst, 1996; Tang et al., 2018). Jako jeden z nejdůležitějších faktorů je udáváno převládající pH (Wong, 2003).

Výše zmíněná těžbou indukovaná acidifikace prostředí definovaná převládajícím kyselým pH je jedním z často zkoumaných problémů dávaných do souvislosti s těžkými kovy a jejich migrací. Kyselé důlní vody jsou typickým doprovodným jevem těžby uhlí a rud po celém světě a představují závažný environmentální problém (Harris et al., 2003; Bhuiyan et al., 2010; Reza et al., 2015; Pandey et al., 2016). Jsou spojovány především s odpadním materiálem z těžby, kde je přítomný pyrit ($FeS_2$) a další sulfidy, jejichž oxidací kyselé důlní vody vznikají (Bhuiyan et al., 2010). Kyselé prostředí stimuluje rozpouštění dalších přítomných minerálů a umožňuje tak vysokou migraci uvolněných prvků, a tedy i těžkých kovů, v prostředí (Medveď et al., 2004; Thorslund et al., 2012; Reza et al., 2015). Kyselé důlní vody tak představují problém rostoucí půdní acidity, zvýšené koncentrace, mobility a biodostupnosti těžkých kovů a poškození vegetace (Bhuiyan et al., 2010).

Na rozdíl od většiny dalších kontaminantů nejsou těžké kovy biologicky rozložitelné, mohou pouze změnit svou chemickou formu a v půdě díky své perzistenci mají potenciál přetrvat a působit velmi dlouho (Ali et al., 2013; Wu et al., 2016; Cheng et al., 2017). V závislosti na výše zmíněných faktorech se mění jejich chemická speciace, mobilita a biodostupnost, které ovlivňují jejich rizikovost a toxicitu (Batjargal et al., 2010; Gall et al., 2015). Největší riziko představuje výměnná frakce, která je biologicky dostupná. Dále jsou tvořeny frakce oxidů Fe a Mn, uhličitanová frakce a frakce organické hmoty, které jsou potenciálně biologicky dostupné. Nejmenší riziko představuje zbytková frakce, která není biologicky dostupná (Batjargal et al., 2010; Tang et al., 2018).

## 2.3 Transfer těžkých kovů v potravním řetězci

Těžké kovy z antropogenních zdrojů vstupují do půd a podzemních vod. Pokud jsou v biologicky dostupné formě, mohou se snadno dostat do živých organismů celou řadou expozičních cest, načež se dál šíří v rámci potravních řetězců s potenciálem negativně ovlivňovat biotu (Ali et al., 2013; Stefanowicz et al., 2014; Gall et al., 2015). Toto zjištění vyvolalo celosvětově rostoucí zájem veřejnosti o problematiku zdravotních rizik představovaných znečištěním potravin, především zemědělských produktů a ryb (Reza et al., 2015; Zhang et al., 2015; Cheng et al., 2017) nebo hub (Komárek et al., 2007; Pecina et al., 2022). Tento zájem je reflektován rostoucím množstvím studií, které se zabývají touto tematikou, zejména v kontextu těžkých kovů v půdě, sedimentech a vodě těžebních oblastí, které jsou kontaminaci silně exponovány a zároveň bývají součástí zemědělské produkce (Rout et al., 2013; Reza et al., 2015; Sarwar et al., 2017; Tang et al., 2017). Pochopení transferů těžkých kovů v rámci potravních řetězců je zásadní pro zhodnocení jejich ekotoxikologických rizik a vyhodnocení jejich potenciálních rizik pro člověka (Liu et al., 2019).

Těžké kovy přítomné v půdě jsou přijímány rostlinami, skrze které se stávají dostupnými pro bezobratlé živočichy a spásače (Gall et al., 2015). Prostřednictvím přenosu v potravním řetězci pak mohou být akumulovány až v člověku (Wu et al., 2016; Liu et al., 2019). Rostoucí riziko spočívá také v tom, že jejich koncentrace často roste během jejich přechodu na vyšší trofickou úroveň, což je označováno jako biomagnifikace (Ali et al., 2013; Liu et al., 2019). Intoxikace savců probíhá především prostřednictvím požívání kontaminované rostlinné potravy, případně také půdy nebo vody (Obrázek 3). Míra akumulace těžkých kovů v jejich tělech závisí na vlastnostech jednotlivých prvků a jejich obsahu ve vegetaci a půdě, věku zvířete a na tom, jak rychle projde daný kov gastrointestinálním traktem (Gall et al., 2015; Liu et al., 2019).

***Obrázek 3**. Přítok řeky Sharyn Gol (Mongolsko) kontaminovaný uhelným prachem. Řeka Sharyn Gol protéká zemědělskou oblastí a její voda se využívá k zavlažování polí i napájení zvířat (Autor fotografie: Ing. Václav Pecina).*

Potrava a voda jsou nejběžnějšími zdroji příjmu těžkých kovů u člověka, a to především proto, že jsou zemědělské plodiny často pěstované na znečištěných půdách (Gall et al., 2015; Hoang et al., 2021). Kromě příjmu těžkých kovů ingescí dochází ale také k příjmu skrze inhalaci a dermální absorpci (Wuana a Okieimen, 2011; Luo et al., 2012), například prostřednictvím prachu (Tang et al., 2017) nebo styku s kontaminovanou půdou.

## 2.4 Charakteristika těžkých kovů

### 2.4.1 Arzen

Ve vyšších koncentracích se As přirozeně vyskytuje především v oblastech tvořených sirnými rudami (Wu et al., 2016). Vůbec nejrozšířenějším minerálem obsahujícím As je arsenopyrit (FeAsS), který je běžným doprovodným minerálem v mnoha rudách drahých kovů a uhlí (Lide, 2005; Reimann et al., 2009, Dani, 2010). Je ale také běžný jako příměs v dalších sulfidových minerálech, jako jsou pyrit, galenit a sfalerit (Salminen et al., 2005). Jejich zvětráváním se může přirozeně dostávat do půdy a dále se šířit životním prostředím. Antropogenně je uvolňován především prostřednictvím spalování fosilních paliv, těžby a používáním některých insekticidů a pesticidů (Bissen et al., 2003; Qadir et al., 2005; Vukašinović-Pešić et al., 2005; Dani, 2010; Syu et al., 2015; Wu et al., 2016; Sarwar et al., 2017). Spojován je i přímo s těžbou, zpracováním a spalováním uhlí (Shi et al., 2013); jeho průměrný obsah v uhlí je přibližně 13 mg/kg (Bissen et al., 2003). Jeho přirozený obsah v půdách se pohybuje od 0,1 do 40 mg/kg při průměru 5 mg/kg (Vukašinović-Pešić et al., 2005).

Arzen nemá pro člověka význam jako stopový prvek (Dani, 2010), naopak je pro něj silně karcinogenní (Wu et al., 2016). Chronické otravy arzenem jsou spojené především s požíváním kontaminované vody (Ahsan et al., 2006; Kapaj et al., 2006) a v posledních letech v souvislosti s těžbou také s požíváním kontaminované rýže pěstované v těžebních oblastech (Liu et al., 2015; Hoang et al., 2021). Významnost jeho rizika demonstruje také skutečnost, že má nejvyšší hodnoty potenciální karcinogenity ze všech substancí zmiňovaných v regulacích týkajících se pitné vody (Reimann et al., 2009) a obecně se řadí na první místo v národních i mezinárodních seznamech nebezpečných látek (Dani, 2010). Akumuluje se ve tkáních, jako jsou vlasy a nehty (Vukašinović-Pešić et al., 2005) a může způsobit rakovinu kůže, jater a plic (Ray a Ray, 2009; Syu et al., 2015). S otravou arzenem jsou spojovány také kardiovaskulární choroby, neurologická onemocnění, poruchy endokrinního systému a neoplastické poruchy (Bissen et al., 2003; Ahsan et al., 2006).

Arzen se přirozeně vyskytuje ve většině rostlin (Kabata-Pendias a Pendias, 2001), i když je jeho obsah obvykle přirozeně velmi nízký (Reimann et al., 2009). V prostředí je relativně mobilní (Vukašinović-Pešić et al., 2005), a proto je pro rostliny snadno absorbovatelný a může v nich docházet k jeho snadné akumulaci (Syu et al., 2015). Jeho mobilitu v půdě ovlivňuje především její oxidačně-redukční potenciál a pH (Bissen et al., 2003); na rozdíl od jiných těžkých kovů je As mobilnější při vyšším pH (Hartley et al., 2009). Tyto podmínky ovlivňují jeho adsorpci zejména na oxidy a hydroxidy železa a další chování v prostředí (Liu et al., 2015). Fytotoxicita arzenu silně závisí také na dalších půdních vlastnostech. Kupříkladu v těžkých půdách může nastat 90% redukce rostlinného růstu při obsahu As 1000 mg/kg půdy, zatímco v lehkých půdách je úměrně toxický obsah již 100 mg/kg (Kabata-Pendias, 2010). Mezi symptomy intoxikace rostlin tímto polokovem patří změna barvy listů a kořenů, zvlnění listů, buněčná plazmolýza a již zmíněná redukce růstu (Kabata-Pendias a Pendias, 2001).

### 2.4.2 Kadmium

Kadmium se v životním prostředí vyskytuje přirozeně ve velmi nízkých koncentracích (Arnold et al., 2006; Faiz, 2009 Wu et al., 2016). Z geologického pohledu se nejčastěji vyskytuje jako náhrada za Cu, Hg, Pb nebo Zn v sulfidových minerálech, zejména ve sfaleritu, v menší míře smithsonitu ($ZnCO_3$) a v jiných minerálech (Salminen et al., 2005; Birke et al., 2014). Do životního prostředí se dostává přirozeně zvětráváním matečných hornin, nicméně k významnému zvýšení jeho obsahu v půdě přispívají antropogenní aktivity. Mezi antropogenní zdroje Cd v prostředí se řadí především těžba nerostných surovin, spalování uhlí, hutě nebo doprava (Qadir et al., 2005; Arnold et al., 2006; Järup a Åkesson, 2009; Ray a Ray, 2009; Birke et al., 2014; Wu et al., 2016; Sarwar et al., 2017; Pecina et al., 2021a). Jeho přirozený obsah v půdách se pohybuje od 0,06 do 10 mg/kg, obvykle je ale v rozmezí 0,2 až 0,5 mg/kg (Birke et al., 2014).

Kadmium nemá pro člověka význam jako stopový prvek (Arnold et al., 2006; Divrikli et al., 2006) a již ve velmi nízkých koncentracích pro něj může být toxické (Wu et al., 2016; El Rasafi et al., 2022). Hlavním zdrojem příjmu Cd je u nekouřící populace potrava (Järup a Åkesson, 2009; Birke et al., 2014; El Rasafi et al., 2022). Kadmium se akumuluje v mnoha tkáních a orgánech, ale nejvíce zatížené bývají ledviny a játra (Arnold et al., 2006; Divrikli et al., 2006). Chronický příjem tohoto kovu může vést k onemocněním, jako jsou rakovina plic, prostaty a ledvin, plicní adenokarcinom, proliferační léze prostaty, poškození kostí, dysfunkce ledvin a hypertenze (Järup a Åkesson, 2009; Ray a Ray, 2009; Ali et al., 2013; Birke et al., 2014; Wu et al., 2016).

Kadmium je pro rostliny neesenciální prvek (Arnold et al., 2006), nicméně je jimi snadno absorbováno, a díky tomu je v nich často akumulováno ve vysokých koncentracích (Kabata-

Pendias, 2010). K jeho efektivní absorpci dochází zejména při nízkém půdním pH, které patří mezi hlavní faktory ovlivňující mobilitu Cd (Birke et al., 2014). Ve srovnání s jinými těžkými kovy má kadmium nejvyšší mobilizační potenciál (Kubier et al., 2019), což jej řadí mezi nejrizikovější těžké kovy. Vysoká mobilita mu umožňuje nejen rychlejší absorpci živými organismy v půdě (El Rasafi et al., 2022), ale také rychlejší vyplavování z půdy do podzemní vody, kde představuje další hrozbu (Kubier et al., 2019). Vysoký obsah kadmia v půdě je pro rostliny rizikový s ohledem na jeho silnou fytotoxicitu i při nízkých koncentracích (Kabata-Pendias, 2010; Birke et al., 2014; El Rasafi et al., 2022). Po absorpci rostlinou inhibuje její fotosyntézu, způsobuje retardaci růstu a poškození kořenů, chlorózu listů a hnědočervené zbarvení okrajů listů nebo žilnatiny (Kabata-Pendias a Pendias, 2001; Nagajyoti et al., 2010; El Rasafi et al., 2022).

### 2.4.3 Měď

Měď se přirozeně vyskytuje v elementární kovové formě i jako složka mnoha minerálů, mezi které patří například chalkopyrit ($CuFeS_2$), azurit ($Cu_3(CO_3)_2(OH)_2$) a malachit ($Cu_2CO_3(OH)_2$) (Lide, 2005). Hojně rozšířena je i jako příměs v dalších minerálech, například biotitu nebo pyroxenech a amfibolech (Salminen et al., 2005; Albanese et al., 2015). Díky těmto geologickým předpokladům je široce rozšířená také v životním prostředí (Faiz, 2009). K obsahu tohoto kovu v prostředí významně přispívá také člověk. Měď má mnoho industriálních aplikací, její emise jsou spojovány s těžebními aktivitami, spalováním uhlí, pesticidy a hnojivy a dopravou (Qadir et al., 2005; Divrikli et al., 2006; Wei et al., 2010; Wu et al., 2011; Ali et al., 2013; Shi et al., 2013; Albanese et al., 2015; Brtnický et al., 2020). Její přirozený obsah v půdách se nejčastěji pohybuje mezi 20 a 30 mg/kg, poměrně časté jsou ale i její hodnoty pod 10 mg/kg (Kabata-Pendias a Mukherjee, 2007).

Měď je pro člověka esenciálním prvkem (Fraga, 2005, Bost et al., 2016), který obvykle nepředstavuje hrozbu pro lidské zdraví (Taylor et al., 2020). Přijímána je především v potravě a vodě (Kabata-Pendias a Mukherjee, 2007). Nicméně, nadměrný příjem mědi a její zvýšený obsah v organismu může mít negativní zdravotní dopady s projevy toxicity (Bost et al., 2016; Taylor et al., 2020). Chronická otrava mědí primárně ovlivňuje a poškozuje játra, protože jsou prvním místem, kde se tento kov v lidském těle ukládá po vstupu do krve (Gaetke a Chow, 2003; Fraga, 2005). Otrava mědí se proto projevuje především vývojem jaterní cirhózy, dochází ale i k poškozením renálního tubulu, mozku a dalších orgánů (Gaetke a Chow, 2003; Ali et al., 2013; Taylor et al., 2020).

Měď je pro rostliny esenciálním mikronutrientem (Divrikli et al., 2006; Shabbir et al., 2020) hrající klíčovou roli zejména při tvorbě chlorofylu a fotosyntéze (Rehman et al., 2019). Nicméně, ve vyšších koncentracích může také působit toxicky (Kabata-Pendias a Pendias, 2001). Příjem mědi rostlinou je závislý především na pH; s klesajícím pH dochází k růstu její biodostupnosti a akumulaci v rostlině (Shabbir et al., 2020), která se omezuje především na kořeny (Rehman et al., 2019). Význam má ale také obsah organické hmoty, zinku, dusíku a fosforu (Rehman et al., 2019; Shabbir et al., 2020). Při nadměrné akumulaci tohoto kovu dochází k inhibici velkého množství enzymů, ovlivnění rostlinné biochemie, a tím i fotosyntézy, dále inhibuje respiraci a fixaci dusíku, a negativně tak ovlivňuje vegetativní růst (Fernandes a Henriques, 1991; Kabata-Pendias a Mukherjee, 2007; Rehman et al., 2019). Ve srovnání s jinými těžkými kovy, jako jsou Cd, Ni a Zn, je měď více rhizotoxická, a není tolik toxická pro zvířata a člověka (Michaud et al., 2008). Symptomy intoxikace mědí jsou například tmavě zelené listy přecházející do indukované Fe-chlorózy nebo deformace kořenového systému (Kabata-Pendias a Pendias, 2001; Michaud et al., 2008).

### 2.4.4 Rtuť

Rtuť se v životním prostředí přirozeně vyskytuje ve velmi nízkých koncentracích (Sasmaz et al., 2016; Wu et al., 2016). Její nejvýznamnější rudou je cinabarit (HgS) (Kabata-Pendias a Pendias, 2001; Lide, 2005), geologicky je spojována také s eklogity nebo uhlím (Salminen et al., 2005). Její přirozený obsah v půdách nepřekračuje 0,4 mg/kg (Sasmaz et al., 2016), průměr je odhadován na 0,06 mg/kg (Wang et al., 2012). Nicméně, vzhledem k častému znečištění půdy antropogenními aktivitami není snadné hodnotu determinovat (Kabata-Pendias a Mukherjee, 2007). Mezi významné antropogenní aktivity spojované s emisemi rtuti patří průmysl, spalování uhlí, zavlažování odpadními vodami, těžba zlata a doprava (Nagajyoti et al., 2010; Zheng et al., 2010; Azevedo a Rodriguez, 2012; Liu et al, 2012; Sasmaz et al., 2016; Sarwar et al., 2017). Především spalování uhlí je globálně udáváno jako jeden z nejdůležitějších zdrojů znečištění tímto prvkem (Shiyab et al., 2009; Liu et al, 2012; Wang et al., 2020).

Rtuť nemá žádnou biologickou funkci v lidském těle (Sharma a Agrawal, 2005; Duruibe et al., 2007). K jejímu příjmu obvykle dochází v kontaminované půdě nebo vodě (Wu et al., 2016) a nebezpečná je zejména vzhledem k vysoké toxicitě, mobilitě a bioakumulaci (Moreno-Jiménez et al., 2006). Sasmaz et al. (2016) ji dokonce udává jako jeden z nejtoxičtějších těžkých kovů. Chronické nebo akutní vystavení rtuti může způsobit například nadledvinovou dysfunkci, poškození centrálního nervového systému, deformaci DNA, mentální retardaci, hypotyreózu a degradaci nervových vláken; mimoto se jedná o karcinogenní prvek (Divrikli et al., 2006; Kabata-Pendias a Mukherjee, 2007; Ray a Ray, 2009; Wu et al., 2016).

V rostlinách rtuť nemá žádnou známou biologickou funkci, naopak je pro ně vysoce toxická (Moreno-Jiménez et al., 2006; Shiyab et al., 2009). Biodostupnost tohoto kovu v půdě je pro rostliny a další organismy poměrně nízká, protože je obvykle silně vázána na organickou hmotu (Moreno-Jiménez et al., 2006; Sasmaz et al., 2016; Kumari et al., 2020; Pecina et al., 2021b). Pokud dojde k její akumulaci rostlinou, ukládá se převážně do kořenů, odkud může být šířena do jejích dalších částí (Moreno-Jiménez et al., 2006; Sasmaz et al., 2016). Zvýšené koncentrace rtuti v rostlině mohou způsobovat oxidační stres, narušení strukturní integrity membrán, redukuje příjem živin a omezuje fotosyntézu, transpiraci a příjem vody (Shiyab et al., 2009; Azevedo a Rodriguez, 2012; Wang et al., 2012). Symptomy intoxikace mohou být například krnění sazenic a kořenového systému a chloróza listů (Kabata-Pendias a Pendias, 2001).

### 2.4.5 Olovo

Olovo se přirozeně vyskytuje v životním prostředí a je součástí celé řady běžných minerálů, mezi které patří například galenit (PbS), anglesit ($PbSO_4$) a cerusit ($PbCO_3$) (Lide, 2005; Salminen et al., 2005; Kabata-Pendias a Mukherjee, 2007). Do životního prostředí se dostává jak přirozenými cestami, tak antropogenními, mezi které se řadí především doprava, průmysl, těžba nerostných surovin, spalování fosilní paliv nebo pesticidy (Al-Khashman, 2004; Lide, 2005; Qadir et al., 2005; Sharma a Dubey, 2005; Kabata-Pendias a Mukherjee, 2007; Ali et al., 2013; Wu et al., 2016; Sarwar et al., 2017). Antropogenní příspěvky olova do půd jsou tak významné, že se stalo jedním z nejběžnějších a nejvýznamnějších anorganických polutantů v přírodě, rozšířeným významně ve vzduchu, půdě, vodě i potravě (Al-Khashman, 2004; Nagajyoti et al., 2010; Kushwaha et al., 2018). Určení přirozeného obsahu olova v půdě, především v její svrchní vrstvě, je složité, vzhledem k rozsáhlému znečištění, nicméně podle odhadů se pohybuje většinou od 10 do 67 mg/kg s průměrem přibližně 32 mg/kg (Kabata-Pendias a Pendias, 2001).

Olovo se přirozeně v živých organismech nevyskytuje a nemá žádnou známou funkci v biochemii či fyziologii člověka (Duruibe et al., 2007). Pro organismy je toxické a jeho hrozba je, vzhledem k perzistenci tohoto kovu v půdách, dlouhodobá (Wu et al., 2016). Je kumulativním toxinem a do těla vstupuje především prostřednictvím inhalace kontaminovaného vzduchu a ingesce kontaminované potravy a vody (Divrikli et al., 2006; Wu et al., 2016). Chronické nebo akutní vystavení olovu může u člověka způsobit neurologická onemocnění, ztrátu koordinace a koncentrace, hypertenzi, neplodnost, dysfunkci ledvin, snížení

IQ a problémy s dlouhodobou pamětí prostřednictvím poškození mozku (Duruibe et al., 2007; Ray a Ray, 2009; Ali et al., 2013; Wu et al., 2016).

Olovo není pro rostliny esenciálním prvkem (Kabata-Pendias a Pendias, 2001; Sharma a Dubey, 2005), ačkoliv se v nich přirozeně a často vyskytuje (Divrikli et al., 2006). Může být snadno absorbováno a akumulováno, a následně při zvýšených koncentracích působit toxicky (Sharma a Dubey, 2005). V půdě je, nicméně, jeho biodostupnost omezena silnými vazbami na organickou hmotu, která ovlivňuje jeho speciace. Význam mají ale také další půdní charakteristiky, jako například pH nebo textura (Kushwaha et al., 2018). Olovo v rostlinách negativně působí na jejich morfologii, růst, mitózu, absorpci vody a fotosyntetické procesy (Kabata-Pendias a Pendias, 2001; Sharma a Dubey, 2005; Nagajyoti et al., 2010). Symptomy intoxikace se projevují například jako ztmavnutí, vadnutí nebo krnění listů, působí také změny v kořenovém systému (Kabata-Pendias a Pendias, 2001; Sharma a Dubey, 2005).

### 2.4.6 Zinek

Zinek je v životním prostředí běžným prvkem. Je součást minerálů, jako jsou například sfalerit, zinkit (ZnO) nebo smithsonit, jejichž zvětráváním se může dostávat do životního prostředí (Lide, 2005; Salminen et al., 2005). Do něj se dostává také antropogenními cestami, které představuje například doprava, průmysl, pesticidy v zemědělství, těžba nerostných surovin nebo spalování fosilní paliv (Qadir et al., 2005; Kabata-Pendias a Mukherjee, 2007; Wei et al., 2010; Shi et al., 2013; Sarwar et al., 2017). Přirozený obsah zinku v půdách se pohybuje většinou mezi 10 až 300 mg/kg s průměrnou hodnotou přibližně 50 mg/kg (Kabata-Pendias a Mukherjee, 2007; Noulas et al., 2018).

Zinek je pro člověka esenciálním prvkem (Shi et al., 2013) a jedním z důležitých kovů pro normální růst a vývoj (Divrikli et al., 2006; Kabata-Pendias a Mukherjee, 2007). Nicméně, při expozici vyšší, než jsou fyziologické potřeby, může působit toxicky (Faiz et al., 2009; Shi et al., 2013), ačkoliv jeho toxicita není vysoká (Duruibe et al., 2007; Nriagu, 2007). Do těla se dostává především prostřednictvím potravy (Faiz et al., 2009). Projevy intoxikace zinkem jsou podobné jako u olova (Duruibe et al., 2007). Chronické nebo akutní vystavení zinku může u člověka způsobit anémii, selhání jater a ledvin, systémové dysfunkce, které mohou vést k narušení růstu a reprodukce (Duruibe et al., 2007; Nriagu, 2007). Vysoké hladiny zinku mohou také narušit homeostázi jiných esenciálních prvků (Faiz et al., 2009).

Zinek je esenciální prvek v rostlinném metabolismu (Nagajyoti et al., 2010; Shi et al., 2013), kde je významnou složkou řady enzymů (Kabata-Pendias a Pendias, 2001). Díky tomu jsou k jeho vyšším koncentracím rostliny tolerantní, nicméně, při nadměrném obsahu může způsobovat chlorózy, krnění výhonků, deformaci mladých listů, retardaci rostlinného růstu a snížení produkce biomasy (Rout a Das, 2009; Nagajyoti et al., 2010; Obrázek 4). V globálním měřítku je ale problém spíše s deficitem zinku v půdách než s jeho toxicitou (Noulas et al., 2018). V půdách je velmi mobilní, zejména v kyselém prostředí, nicméně, může být také snadno vázán organickou hmotou ve svrchní vrstvě půdy (Kabata-Pendias a Pendias, 2001; Noulas et al., 2018). S ohledem na to je fytotoxicita zinku typická zejména pro kyselé kontaminované půdy (Kabata-Pendias a Mukherjee, 2007).

***Obrázek 4***. *Chloróza listů a růstová retardace výsadeb dubu (Quercus sp.) způsobená nadměrným obsahem zinku v Příbrami, Česká republika (Autor fotografie: Ing. Václav Pecina).*

## 2.5 Fytoremediace

Na silně znečištěných půdách může toxicita těžkých kovů dosáhnout takové úrovně, že neumožňuje růst rostlin v takovém rozsahu, aby vytvořily souvislý půdní pokryv (Mendez a Maier, 2008; Križáni et al., 2009). To zvyšuje hrozbu eroze a odnosu kontaminovaných částic. Znečištění půdy kromě rostlin negativně ovlivňuje také počet, diverzitu a aktivitu půdních organismů (Frey et al., 2006), inhibuje rozklad organické hmoty a také mineralizační procesy v půdě (Wong, 2003), což má vliv na mobilitu těžkých kovů (viz kapitoly 2.2 a 2.4). Synergické působení těchto faktorů, které se vzájemně ovlivňují, může následně přispět k šíření kontaminantů z těžebních oblastí do okolního prostředí. Z tohoto důvodu je nezbytné hledat řešení, která omezí možná rizika pro ekosystémy a člověka.

Toxicita těžkých kovů je druhově specifická (Barbafieri et al., 2011; Gall et al., 2015). Některé organismy jsou adaptovány na vysoký obsah těžkých kovů ve svém těle. Například rostlinné metalofyty dokáží růst v podmínkách s extrémně vysokou zátěží těžkými kovy v půdě díky evolučně dané adaptaci na přirozeně vysoké obsahy kovů v prostředí (Whiting et al., 2004). Některé z nich mají dokonce schopnost hyperakumulace specifických kovů (Mendez a Maier, 2008; Gall et al., 2015; Mahar et al., 2016). Právě tyto rostlinné druhy mohou hrát významnou roli při rekultivaci oblastí postižených těžbou prostřednictvím fytoremediace.

Fytoremediace je ekologický a nákladově efektivní typ managementu krajiny sloužící k řízení problematiky znečištění životního prostředí pomocí rostlin (Sharma et al., 2021). Jiní autoři definují fytoremediaci také jako obnovu ekosystémů nebo jejich jednotlivých složek kontaminovaných těžkými kovy pomocí bylin a dřevin (Gorelova a Frontasyeva, 2017). Při fytoremediaci se využívá potenciálu specifických rostlinných druhů absorbovat a akumulovat těžké kovy z půdy, případně na ně působit tak, aby došlo k minimalizaci jejich toxických rizik pro další živé složky životního prostředí (Ernst, 1996; Wong, 2003; Mendez a Maier, 2008; Barbafieri et al., 2011; Sarwar et al., 2017). Během tohoto procesu rostliny využívají především svůj vlastní metabolismus a doprovází jej interakce mezi kořeny a mikroorganismy (Wei et al., 2021).

Výhody fytoremediace oproti jiným řešením zahrnují například nízkou finanční nákladnost a dobrý estetický projev (Ali et al., 2013; Gall et al., 2015; Sarwar et al., 2017). Zároveň představuje fytoremediace přístup šetrný k životnímu prostředí a možnost udržitelného managementu krajiny (Barbafieri et al., 2011; Sarwar et al., 2017). Výhodou je také to, že se dá využít při řešení znečištění půdy, vody i vzduchu (Wei et al., 2021). Jednou z jejích nevýhod může být časová náročnost potřebná k úplnému obnovení funkčních vlastností půdy (Gorelova a Frontasyeva, 2017). Díky převažujícím pozitivům se fytoremediace jeví jako přijatelná cesta v procesu ozdravování krajiny degradované těžbou nerostných surovin, kterou doprovází znečištění těžkými kovy. Dobrou alternativou technické rekultivace může být především v chudých rozvojových zemích (Mendez a Maier, 2008), kde realizace rekultivačních projektů často nemá dostatečnou finanční a správní podporu.

Fytoremediace zahrnuje řadu technik a aplikací, které se liší způsobem působení rostlin na těžké kovy – ty mohou buď odstraňovat, imobilizovat nebo degradovat (Bolan et al., 2011). Nejčastější formy fytoremediace jsou fytostabilizace nebo fytoextrakce (Mendez a Maier, 2008; Mahar et al., 2016; Korzeniowska a Stanislawska-Glubiak 2019).

### 2.5.1 Fytostabilizace

Fytostabilizace je fytoremediační technika spočívající především ve snížení mobility polutantů v životním prostředí prostřednictvím založení rostlinného krytu na povrchu znečištěného území a následnou interakcí rostlin s těžkými kovy (Bolan et al., 2011). Fytostabilizace se specializuje na vysoce adaptabilní a resistentní druhy rostlin, které mají potenciál omezovat mobilitu nebo biodostupnost těžkého kovu v půdě k omezení jeho vyluhování nebo vstupu do potravinového řetězce, a to především prostřednictvím kořenového systému (Mahar et al., 2016; Korzeniowska a Stanislawska-Glubiak 2019; Shackira and Puthur, 2019). Během fytostabilizace dochází k imobilizaci nebo retenci těžkých kovů akumulací v kořenech rostlin nebo jejich vysrážením v rhizosféře ve formě uhličitanů, fosforečnanů a hydroxidů (Bolan et al., 2011; Gorelova a Frontasyeva, 2017). Proces fytostabilizace zahrnuje snížení rizika vyluhovaní těžkých kovů do podzemní vody, změnu jejich speciace, kontrolu větrné a vodní eroze, vytváření aerobního prostředí v kořenové zóně a zvyšování obsahu organické hmoty v substrátu k vázání polutantů. Dochází také k snížení rizika přímého kontaktu lidí nebo zvířat se znečištěným substrátem. Cílem je, aby se znečištění nešířilo dál mimo zasaženou lokalitu (Bolan et al., 2011; Shackira and Puthur, 2019).

Efektivitu fytostabilizace je možné zvýšit využíváním pomocných organických nebo anorganických látek a materiálů sloužících ke zlepšování kvality půdy, respektive těžebního substrátu. Tato metoda je označována jako asistovaná fytostabilizace (aided phytostabilization). Během ní jsou současně s rostlinami využívány například dolomit, vápenec, kompost, kejda, zeolit, popel nebo biouhel (Martínez-Martínez et al., 2019; Radziemska et al., 2019; Shackira and Puthur, 2019). Díky aplikaci takovýchto aditiv může být zlepšena efektivita snižování biodostupnosti těžkých kovů a půdní úrodnost, snížena eroze a zvýšena diverzita rostlinných i mikrobiálních společenstev v těžebním odpadu (Luo et al., 2019). Například Martínez-Martínez et al. (2019) po realizaci asistované fytostabilizace v důlním odkališti zjistili pokles biodostupnosti Cd, Pb a Zn o 90–99 %. Mimoto došlo díky zlepšení půdní kvality (růst pH, dostupného P, celkového N a C, výměnného K) také k podpoře vývoje rostlinného společenstva a mikrobiální kolonizaci substrátu. Pro zvýšení efektivity imobilizace těžkých kovů v půdě těmito aditivy je nutné pravidelně opakovat jejich aplikaci, aby byl účinek zachován (Bolan et al., 2011). Nicméně, příliš vysoké dávky takto využívaných aditiv už mohou mít i negativní dopad na rostliny (Wei et al., 2021). I přesto je asistovaná fytostabilizace považována za nejslibnější fytoremediační techniku pro sanaci důlní hlušiny, respektive hald těžebního odpadu (Luo et al., 2019).

Fytostabilizace může být považována za pouze dočasné řešení, které vyžaduje neustálý monitoring, protože těžké kovy v půdě zůstávají v imobilní fázi (Bolan et al., 2011; Sarwar et al., 2017).

### 2.5.2 Fytoextrakce

Fytoextrakce je fytoremediační technika spočívající v trvalém odstranění těžkých kovů z půdy prostřednictvím jejich vytěžením rostlinami (Mendez a Maier, 2008; Mahar et al., 2016; Suman et al., 2018; Korzeniowska a Stanislawska-Glubiak 2019). Fytoextrakce představuje vůbec nejvýznamnější fytoremediační přístup pro odstraňování těžkých kovů z půd, vody a sedimentů (Sarwar et al., 2017). Využívají se při ní primárně specializované a vysoce adaptabilní a tolerantní rostlinné hyperakumulátory, které jsou schopny absorbovat, transportovat a akumulovat těžké kovy v biomase orgánů (za současné vysoké produkce biomasy), které se dají snadno sklízet (Suman et al., 2018; Diarra et al., 2021). Jako hyperakumulátory jsou označovány rostliny, které akumulují výjimečné koncentrace těžkých kovů ve svých nadzemních částech bez viditelných příznaků toxicity (Verbruggen et al., 2009). Stejný kolektiv autorů na základě rešeršního shrnutí specifikuje tyto koncentrační kritéria jako 1 % Zn, 0,1 % As, Cr, Cu, Ni a Pb a 0,01 % Cd v sušině listů a řadí mezi hyperakumulátory těžkých kovů přes 450 druhů rostlin.

Významně limitovaná může být fytoextrakce zejména v případě znečištění půdy více těžkými kovy, které se dá v případě těžebních lokalit očekávat. S ohledem na převážně prvkově specifickou schopnost akumulace a tolerance rostlin existuje riziko, že potenciál fytoextrakce jednoho těžkého kovu nebude naplněn s ohledem na toxické působení jiného. To může využití fytoextrakce při fytoremediaci těžebních oblastí významně limitovat. Mezi důležitá kritéria výběru rostliny proto patří i tolerance a bioakumulace širokého spektra těžkých kovů, respektive takovéto druhy jsou pro fytoextrakci upřednostňovány (Diarra et al., 2021).

Zatímco v případě dříve popsané fytostabilizace je záměrem snížit biodostupnost těžkých kovů, v případě fytoextrakce je záměrem stimulovat jejich absorpci rostlinou zvýšením biodostupnosti. Pro zvýšení schopnosti rostlin absorbovat těžké kovy z půdy jsou využívány chelátory (např. kyseliny citrónová, šťavelová a jablečná) nebo inokulace rostliny symbiotrofickými houbami a bakteriemi (Gorelova a Frontasyeva, 2017; Asgari Lajayer et al., 2019; Lu et al., 2020; Diarra et al., 2021). Na efektivitu fytoextrakce ale i přirozeně působí řada faktorů, například pH, obsah organické hmoty, aktivita půdních mikroorganismů a přítomnost sorbentů jako například obsah jílových částic (Gorelova a Frontasyeva, 2017), záměrem tohoto typu managementu je upravit tyto charakteristiky tak, aby byla stimulována absorpce těžkých kovů rostlinami.

# 3. MATERIÁL A METODIKA

Studované těžební oblasti představují města Shariin Gol, Nalaikh a Baganúr v Mongolsku, pro která je charakteristická těžba uhlí (Příloha 1), a lesopark založený na místě bývalého důlně-úpravárenského závodu a těžební haldy v Příbrami v České republice (Příloha 2). Problematika fytoremediace je zpracována ve dvou studiích řešících fytoremediaci kontaminovaných půd v kontrolovaných podmínkách skleníku v přílohách 3 a 4.

S ohledem na specifika metodiky odběru vzorků, jejich zpracování a analýz, které se mezi studiemi značně liší, stejně jako charakteristiky a podmínky experimentů, jsou dílčí materiály a metody podrobně popsány pouze v jednotlivých případových studiích (Přílohy 1–4).

Obecné charakteristiky, jako jsou indikátory znečištění půdy a rostlin a posouzení rizika pro lidské zdraví, jsou rozebrány v následujících podkapitolách. S ohledem na diskuzi věnovanou situaci v Mongolsku je pro pochopení širších souvislostí základní přehled problematiky uveden také v této kapitole.

## 3.1 Indikátory znečištění půdy

Za účelem ochrany životního prostředí a člověka před toxickými dopady těžkých kovů byly stanoveny standardy, které definují limitní hodnoty obsahů či koncentrací těžkých kovů ve vybraných složkách životního prostředí. Tyto limity jsou zakotveny v národní legislativě řady zemí. Některé národní standardy jsou uznávány také na mezinárodní úrovni. Při zaměření na půdu (Tabulka 1) mezi tyto mezinárodně uznávané limity patří například Dutch Soil Guidelines (DSG; VROM, 2013). DSG udávají dvě hodnoty, konkrétně tzv. Target value a Intervention value. Target value reflektuje možné přirozené obsahy daných prvků v půdě, zatímco Intervention values indikuje již vážné ohrožení funkčních vlastností půdy pro člověka a další živočichy, stejně jako pro rostliny, možné ohrožení jejich zdraví, a představuje tedy vážnou kontaminaci.

Jako příklady jsou uvedeny také další standardy. Český standard (Vyhláška 153/2016 Sb.) udává preventivní hodnoty obsahů rizikových prvků v zemědělské půdě. Dlouho užívaný čínský limit (CEPA,1995), konkrétně Chinese Soil Quality Standard (Grade II), taktéž udává preventivní hodnoty obsahů rizikových prvků v půdě za účelem ochrany lidského zdraví a zemědělské produkce. Mongolský standard (MNS 5850:2008) udává maximální povolené limity prvků v půdě.

Z tabulky 1 je zřejmá vysoká variabilita hodnot, která částečně reflektuje přirozené obsahy daných prvků v půdách vybraných zemí vycházejících z rozdílné geologie.

**Tabulka 1**. Limitní hodnoty těžkých kovů v půdě (v mg/kg).

| **Země** | | **As** | **Cd** | **Cu** | **Hg** | **Pb** | **Zn** |
|---|---|---|---|---|---|---|---|
| Holandsko (VROM, 2013) | Target value | 29,0 | 0,8 | 36,0 | 0,3 | 85,0 | 140,0 |
| | Intervention value | 55,0 | 12,0 | 190,0 | 10,0 | 530,0 | 720,0 |
| Česká republika (Vyhláška 153/2016 Sb.) | | 20,0 | 0,5 | 60,0 | 0,3 | 60,0 | 120,0 |
| Čína (CEPA,1995) | | 30,0 | 0,3 | 100,0 | 0,5 | 300,0 | 250,0 |
| Mongolsko (MNS 5850:2008) | | 6,0 | 3,0 | 100,0 | 2,0 | 100,0 | 300,0 |

Kromě legislativních limitů se v odborné literatuře využívají také kontaminační indexy. Tyto indexy jsou počítány zejména na základě znalosti pozaďových/přirozených hodnot těžkých kovů v půdě v místě studie. Jedním z nejhojněji užívaných indexů je Nemerowův index znečištění ($IPI_N$).

**Nemerowův index znečištění (Nemerowův Pollution Index)**

$IPI_N$ je využíván k orientačnímu posouzení stupně znečištění půdy těžkými kovy. Většina podobných indexů vychází z pozaďových (přirozených) hodnot daného těžkého kovu na lokalitě. Takovéto hodnocení ale přináší jen znalost o míře antropogenního nabohacení půdy kovy, tedy o kontaminaci. Nebere ale v potaz možnou hrozbu představovanou těžkými kovy v půdě, respektive o riziku vypovídá velmi slabě. Z tohoto důvodu je v této práci přistoupeno k adaptaci Nemerowa indexu, při kterém byly využity limitní hodnoty z mongolského standardu (MNS 5850:2008) a Target value holandského standardu (VROM, 2013). Rovnice používaná pro výpočet $IPI_N$ je (Cheng et al. 2014; Huang et al., 2018):

$$\mathrm{PI_i} = \frac{\mathrm{C_i}}{\mathrm{T_i}}$$

$$\mathrm{IPI_N} = [(\mathrm{PI^2}_{\mathrm{avg}} + \mathrm{PI^2}_{\mathrm{max}})/2]^{1/2}$$

Kde:

- $PI_i$ představuje pollution index vybraného těžkého kovu
- $C_i$ představuje obsah vybraného těžkého kovu
- $T_i$ představuje limitní hodnotu odpovídajícího těžkého kovu podle MNS 5850:2008 nebo Target value podle VROM (2013)
- $IPI_{avg}$ – představuje průměr hodnot všech $PI_i$ sledovaných kovů

- $PI_{max}$ představuje maximální $PI_i$ hodnotu ze sledovaných kovů.

Výsledky $IPI_N$ se vyhodnocují na základě zařazení do následujícího kategorií: ≤0.7: bezpečné; 0.7–1: možné znečištění; 1–2: mírné znečištění; 2–3: střední znečištění; ≥3: těžké znečištění (Cheng et al. 2014; Brtnický et al., 2019).

### 3.2 Indikátory znečištění rostlin

Zhodnocení vlivu znečištění těžkými kovy na rostliny je možné provádět prostřednictvím bioindikačních metod. Ty vycházejí z přímého hodnocení zdravotního stavu rostliny na základě projevů fytotoxicity, které byly dříve popsány během experimentálních studií. Výhodou této metody je především možnost terénního využití.

Mezi symptomy intoxikace arzenem patří změna barvy listů a kořenů, zvlnění listů a retardace růstu rostliny (Kabata-Pendias a Pendias, 2001). Intoxikace kadmiem způsobuje retardaci růstu a poškození kořenů, chlorózu listů a hnědočervené zbarvení okrajů listů nebo žilnatiny (Kabata-Pendias a Pendias, 2001; Nagajyoti et al., 2010). Symptomy intoxikace mědí jsou tmavě zelené listy přecházející do indukované Fe chlorózy nebo deformace kořenového systému (Kabata-Pendias a Pendias, 2001; Michaud et al., 2008). Intoxikace rtutí se projevuje krněním semenáčků, sazenic a kořenového systému nebo chlorózou listů (Kabata-Pendias a Pendias, 2001). Symptomy intoxikace olovem zahrnují ztmavnutí, vadnutí nebo krnění listů a může způsobovat také změny v kořenovém systému (Kabata-Pendias a Pendias, 2001; Sharma a Dubey, 2005). Nadměrný obsah Zn může způsobovat chlorózy, krnění výhonků, deformaci mladých listů, retardaci rostlinného růstu a snížení produkce biomasy (Rout a Das, 2009; Nagajyoti et al., 2010).

Další možnost hodnocení představují listové analýzy; jejich vyhodnocení je prováděno na základě srovnání s referenčními hodnotami (Tabulka 2).

**Tabulka 2**. Příklady obsahů těžkých kovů (v mg/kg suché váhy) ve zralých listech různých druhů rostlin na základě rešeršního shrnutí, ve kterém nejsou zahrnuty vysoce citlivé, ani vysoce tolerantní druhy (Kabata-Pendias a Pendias, 2001; Kabata-Pendias, 2011).

| | **As** | **Cd** | **Cr** | **Cu** | **Hg** | **Ni** | **Pb** | **Zn** |
|---|---|---|---|---|---|---|---|---|
| Nadměrné až toxické | 5–20 | 5–30 | 5–30 | 20–100 | 1–3 | 10–100 | 30–300 | 100–400 |
| Tolerovatelné v zemědělských plodinách | 0,2 | 0,05–0,5 | 2 | 5–20 | 0,2 | 1–10 | 0,5–10 | 50–100 |

Pro hodnocení vztahu mezi půdním obsahem těžkého kovu a obsahem kovu v rostlině se používá tzv. biokoncentrační faktor (Bioconcentration factor – BCF). BCF představuje jednoduchou metodu, jak kvantitativně charakterizovat transfer biologicky dostupných těžkých kovů z půdy do rostliny (Branzini et al., 2012). Rovnice používaná pro výpočet BCF je (Monterroso et al., 2014; Zhu et al., 2018):

$$\mathrm{BCF} = \frac{C_p}{C_s}$$

Kde:

- $C_p$ představuje obsah těžkého kovu v rostlinné tkáni
- $C_s$ představuje obsah těžkého kovu v půdě.

Hodnota BCF <1 signalizuje akumulaci těžkých kovů v rostlině (Monterroso et al., 2014). Čím vyšší je hodnota BCF, tím vyšší je schopnost rostliny akumulovat těžké kovy (Zhu et al., 2018). Akumulační potenciál rostlin se dá využít při remediacích kontaminovaných půd, pokud tyto rostliny vysoký obsah těžkých kovů ve svém těle dobře snášejí a nepůsobí toxicky.

## 3.3 Posouzení rizika pro lidské zdraví

Riziko pro lidské zdraví je hodnoceno prostřednictvím indexu rizika (Hazard Index – HI) a indexu karcinogenního rizika (Carcinogenic Risk – CR). Tyto indexy jsou založeny na předpokládaném průměrném denním příjmu těžkých kovů (ADD), referenční dávce (RfD) a tzv. slope factor (SF). Expozice člověka těžkým kovům v půdách ($C_{soil}$) může nastat při požití ($ADD_{ing}$), dermálním kontaktu ($ADD_{derm}$) a inhalaci ($ADD_{inh}$). Pro odhady příjmu dávek těmito expozičními cestami se používají následující rovnice (De Miguel et al., 2007; Chabukdhara a Nema, 2013; Urrutia-Goyes et al., 2017):

$$\mathrm{ADD_{ing}} = \frac{\mathrm{C_{soil}} \times \mathrm{IngR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \times 10^{-6}$$

$$\mathrm{ADD_{derm}} = \frac{\mathrm{C_{soil}} \times \mathrm{SA} \times \mathrm{AF} \times \mathrm{ABS} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \times 10^{-6}$$

$$ADD_{inh} = \frac{C_{soil} \times InhR \times EF \times ED}{BW \times AT \times PEF}$$

Nekarcinogenní riziko (HI) je stanoveno výpočtem dílčích rizikových kvocientů (HQ) a jejich následným součtem:

$$HI = \sum HQ_i = \sum \frac{ADDi}{RfDi}$$

Karcinogenní riziko se počítá pro karcinogenní (polo)kovy (např. As, Cd, Cr, Ni a Pb):

$$CR = \sum ADD_i \times SF_i$$

Podrobnosti o obecně užívaných vstupních parametrech rovnic jsou v tabulkách 3 a 4.

**Tabulka 3**. Popis parametrů používaných v rovnicích.

| Faktor | Popis | Jednotka | Dospělý | Dítě | Zdroj |
|---|---|---|---|---|---|
| IngR | Míra požití půdy | mg/den | 100 | 200 | USEPA, 2001 |
| EF | Frekvence expozice | dny/rok | 350 | 350 | Luo et al., 2012 |
| ED | Doba expozice | roky | 24 | 6 | USEPA, 2001 |
| BW | Tělesná hmotnost | kg | 55.9 | 15 | Qing et al., 2015 |
| AT | Průměrná doba | dny | 8760[a] | 2190[a] | Kong et al., 2012 |
| SA | Exponovaný povrch kůže | $cm^2$ | 4350 | 1600 | Qing et al., 2015 |
| AF | Kožní adhezní faktor pro půdu | $mg/cm^2$ | 0.07 | 0.20 | Luo et al., 2012 |
| InhR | Míra inhalovaného vzduchu | $m^3$/den | 12.8 | 7.63 | Qing et al., 2015 |
| PEF | Emisní faktor částic | $m^3/kg$ | $1.36\times10^9$ | $1.36\times10^9$ | USEPA, 2001 |

[a] Pro karcinogenní účinky: LT × 365 (Luo et al., 2012). Například v Mongolsku je LT (průměrná délka života) 70 (World Bank Group, 2020). Pro karcinogenní účinky AT = 25 550.

Hodnoty HI >1 indikují pravděpodobnost nepříznivých účinků obsahu těžkých kovů v půdě na lidské zdraví. Hodnoty CR ≤ $10^{-6}$ představují virtuální bezpečnost a CR ≥$10^{-4}$ indikují potenciálně velké karcinogenní riziko. Rozsah přijatelného celkového rizika pro regulační účely je $10^{-6}$ až $10^{-4}$ (Luo et al., 2012; Chabukdhara a Nema, 2013; Brtnický et al., 2019).

**Tabulka 4**. Hodnoty relativní toxicity (Ferreira-Baptista a De Miguel, 2005; De Miguel et al., 2007; Zheng et al., 2010; Kong et al., 2012; Luo et al., 2012; Chabukdhara a Nema, 2013; Zhang et al., 2015; Cocârţă et al. 2016; Rehman et al., 2018).

| **Prvek** | **$RfD_{ing}$** | **$RfD_{derm}$** | **$RfD_{inh}$** | **ABS**[b] | **$SF_{ing}$** | **$SF_{derm}$** | **$SF_{inh}$** |
|---|---|---|---|---|---|---|---|
| As | 3.00E-04 | 1.23E-04 | 3.00E-04[a] | 0.03 | 1.50E+00 | 3.66E+00 | 1.51E+01 |
| Cd | 1.00E-03 | 1.00E-05 | 1.00E-03[a] | 0.001 | 1.50E+01 | | 6.30E+00 |
| Cr | 3.00E-03 | 6.00E-05 | 2.86E-05 | 0.001 | 5.00E-01 | | 4.20E+01 |
| Cu | 4.00E-02 | 1.20E-02 | 4.00E-02[a] | 0.001 | | | |
| Hg | 3.00E-04 | 2.10E-05 | 8.57E-05 | 0.001 | | | |
| Ni | 2.00E-02 | 5.40E-03 | 2.00E-02[a] | 0.001 | 9.10E-01 | | 8.40E-01 |
| Pb | 3.50E-03 | 5.25E-04 | 3.50E-03[a] | 0.001 | 8.50E-03 | | 8.50E-03 |
| Zn | 3.00E-01 | 6.00E-02 | 3.00E-01[a] | 0.001 | | | |

[a] Vzhledem k tomu, že referenční dávka tohoto kontaminantu dosud nebyla stanovena, byla tato hodnota považována za rovnou hodnotě $RfD_{ing}$, s předpokladem, že po vdechnutí bude mít absorpce toxických látek vázaných na částice za následek podobné účinky na zdraví, jako kdyby byly částice požity (De Miguel et al., 2007; Zheng et al., 2010; Chabukdhara a Nema, 2013).
[b] Dermální absorpční faktor.

## 3.4 Problematika znečištění životního prostředí těžbou v Mongolsku

Těžební průmysl je pro Mongolsko stěžejním sektorem jako hlavní přispěvatel národní ekonomiky (Lkhasuren et al., 2007; Byambaa a Todo, 2011; Regdel et al., 2012). Jeho počátek se datuje do roku 1924, kdy byl ve městě Nalaikh otevřen první důl na uhlí (Lkhasuren et al., 2007). Rapidní rozvoj těžebního průmyslu spojený s intenzivním vydáváním těžebních licencí začal ale až v 90. letech, po přestupu k otevřené ekonomice, a pokračuje dodnes (McIntyre et al., 2016). Mezi hlavní těžené komodity patří Cu, černé a hnědé uhlí, Au, fluorit, Ag, Mo, Pb a Zn (MRPAM, 2020; Surenbaatar et al., 2021).

Problematika znečištění životního prostředí těžbou nerostných surovin v Mongolsku se v posledních letech začíná stávat předmětem rostoucího množství studií (např. Kosheleva et al., 2010; Byambaa a Todo, 2011; Inam et al., 2011; Brumbaugh et al., 2013; Battogtokh et al., 2014; Nadmitov et al., 2015; McIntyre et al., 2016; Timofeev et al., 2016; Kosheleva et al., 2018; Yondonjamts et al., 2019). Vzhledem k velké rozloze země a množství těžebních oblastí je ale možné považovat toho množství stále za nedostatečné.

Kritické riziko, které znečištění představuje, je především znehodnocení lokálních zdrojů povrchové i podpovrchové pitné vody (Byambaa a Todo, 2011; Inam et al., 2011; Regdel et al., 2012; Nadmitov et al., 2015; McIntyre et al., 2016). To může být v semiaridních až aridních podmínkách Mongolska, charakteristických nedostatkem srážek a alternativních zdrojů vody (Regdel et al., 2012), pro místní obyvatelstvo i biotu fatální (McIntyre et al., 2016).

Těžební oblasti se stávají cílem řady Mongolů v rámci procesu urbanizace (Regdel et al., 2012; Thorslund et al., 2012; McIntyre et al., 2016), při které si původně kočující obyvatelé hledají práci nebo nabízejí produkty své zemědělské produkce na trzích. Koncentrace obyvatel do těchto rizikových oblastí může znamenat jejich zdravotní ohrožení skrze celou řadu expozičních cest. Riziko představuje požívání kontaminované vody, obyvatelé jsou vystaveni kontaminovanému prachu, docházet může ke kontaminaci celého potravního řetězce (Gall et al., 2015). Lkhasuren et al. (2007) v jedné z mála studií prakticky se zabývajících touto tematikou v Mongolsku uvádí, že především vysoká prašnost spojená s těžbou uhlí a zlata stojí za vysokým a pořád rostoucím množstvím plicních onemocnění, jako jsou bronchitida a pneumokonióza. Zároveň uvádí, že uhlí představuje hlavní zdroj energie v mongolských městech. Jeho spalování přitom může vést k významnému uvolňování těžkých kovů do životního prostředí (Rout et al., 2015; Pandey et al., 2016; Timofeev et al., 2016; Liang et al., 2017; Tang et al., 2017). I proto je řazeno mezi hlavní zdroje znečištění ovzduší v hlavním městě Mongolska – Ulánbátaru (Batjargal et al., 2010; Chung a Chon, 2014) i v dalších městech (Kosheleva et al., 2010).

Studie na kontaminaci městských půd těžkými kovy v Mongolsku, které se v poslední dekádě konečně začínají pomalu objevovat, jsou zaměřeny bohužel striktně pouze na tři největší města (Tabulka 5), z nichž je s těžebním průmyslem spojen především Erdenet (Cu-Mo těžba). Při srovnání s limity pro Mongolsko je v tomto případě patrné zejména navýšení As a částečně také Cu. Při srovnání s dalšími limity užívanými ve světě (Tabulka 1), lze ale i obsahy As považovat za nízké, se zanedbatelnou mírou rizika. S ohledem na nízké počty odebraných a analyzovaných vzorků v poměru k rozloze měst (Tabulka 5) a jejich převažující nesystematický odběr je, nicméně, nezbytné pokračovat ve sledování situace nejen v nových, ale i stávajících městech.

Problematika znečištění se nemusí týkat pouze bezprostředního okolí těžebních oblastí. Díky transportu větrem a vodní erozi, které patří mezi nejčastější zdroje disperze znečištění v aridních a semiaridních podmínkách (Mendez a Maier, 2008), mohou být těžké kovy zanášeny do značných vzdáleností. Kupříkladu těžba zlata z rozsypů a další zdroje znečištění v Mongolsku jsou spojovány až s kontaminací jezera Bajkal v Ruské federaci (Stubblefield et al., 2005; Thorslund et al., 2012; Brumbaugh et al., 2013; Nadmitov et al., 2015). Pro pochopení rozsahu znečištění a jeho závažnosti je proto nezbytné pokračovat ve výzkumech v této oblasti

(McIntyre et al., 2016). Degradace životního prostředí je v Mongolsku velkým tématem a rekultivaci, která je v těchto podmínkách náročná, vyžaduje celá řada oblastí (Regdel et al., 2012). Podrobnější literární přehled problematiky je uveden v příloze 1.

**Tabulka 5**. Souhrn studií na kontaminaci městských půd Mongolska těžkými kovy (průměrné hodnoty v mg/kg).

| **Město** | **n** | **As** | **Cd** | **Cr** | **Cu** | **Hg** | **Ni** | **Pb** | **Zn** | **Zdroj** |
|---|---|---|---|---|---|---|---|---|---|---|
| Darchan | 25* | | | 704** | 50,8 | 0,06 | 32 | 29,2 | 63,6 | Kosheleva et al. (2010) |
| | 21 | 3,33 | | 31,9 | | | 19,5 | 20,9 | 67,3 | Chonokhuu et al. (2019) |
| | 18[1] | 5,22 | 0,23 | 32,1 | 18,5 | | 15,2 | 29,9 | 82,3 | Timofeev et al. (2019) |
| Erdenet | 18[1] | | | 113 | 450 | 0,10 | 35 | 24,7 | 62,2 | Kosheleva et al. (2010) |
| | 30 | 12,8 | | 65,7 | | | 29,3 | 18,1 | 155 | Chonokhuu et al. (2019) |
| Ulánbátar | 22 | 14 | 0,8 | 20,3 | 35,9 | | 18,7 | 63,9 | 159 | Batjargal et al. (2010) |
| | 23* | | | 72,6 | 56,5 | 0,19 | 30,6 | 59,4 | 105 | Kosheleva et al. (2010) |
| | 90 | 10,8 | 0,25 | 32,7 | 22,4 | | 17,0 | 45,9 | 120 | Kasimov et al. (2011) |
| | 29 | | | | | 0,09 | | | | Chung and Chon (2014) |
| | 10 | | | | | | | 39,1 | | Tserenpil et al. (2016) |
| | x*** | 10,3 | | 685 | | | | 33,0 | 113 | Naidansuren et al. (2017) |
| | 27 | 28,0 | | 16,6 | | | 21,3 | 43,1 | 106 | Chonokhuu et al. (2019) |
| | 42 | 22,9 | 0,2 | 29,0 | 28,9 | | | 34,5 | 136 | Battsengel et al. (2020) |
| | 22 | 9,41 | 0,25 | 30,0 | 20,8 | | | 45,1 | 116 | Bilguun et al. (2020) |
| | 28 | 16,4 | | 1987 | 53,5 | | 13,6 | 33,2 | 111 | Oyunbat et al. (2021) |
| Mongolský standard | 6 | 3 | 150 | 100 | 2 | 150 | 100 | 300 | MNS 5850:2008 | |
| DSG Target value | 29 | 0.8 | 100 | 36 | 0,3 | 35 | 85 | 140 | VROM (2013) | |
| DSG Intervention value | 55 | 12 | 380 | 190 | 10 | 210 | 530 | 720 | VROM (2013) | |

* Hodnoty vybrány z kategorie s nejvíce vzorky

** Pravděpodobně chybná hodnota nekonzistentní s dalším textem studie

*** XRF analýza na 340 bodech

# 4. VÝSLEDKY A DISKUZE

## 4.1 Znečištění půd v městech s těžbou uhlí a související zdravotní rizika

Znečištění půd ve studovaných mongolských městech charakteristických těžbou uhlí je podrobněji rozpracováno v příloze č. 1. Průměrné obsahy Cd, Cu, Pb a Zn nepřekročily mongolský standard (MNS 5850:2008) v žádném ze studovaných měst (Příloha 1). Hodnocení $IPI_N$ klasifikovalo všechna tři města jako bezpečná (Tabulka 6) a také hodnocení prostřednictvím zdravotních indexů nenaznačilo potenciální karcinogenní nebo nekarcinogenní rizika pro místní obyvatele (Příloha 1). Kontrolní měření obsahů As, Cr, Hg a Ni také nenaznačilo hrozbu znečištění. Prostorová analýza distribuce znečištění neukázala přímou závislost znečištění na pozici dolů (Příloha 1). Na základě těchto výsledků je možné vyvodit minimální zasažení půd znečištěním těžbou ve studovaných městech.

Potenciální hrozbu do budoucna může představovat Cd, které překročilo Target value (VROM, 2013) v Baganúru, Nalaikhu i Shariin Golu (Příloha 1). Kontaminace nejen tímto prvkem se může souběžně se zvětráváním těžebního odpadu nadále zvyšovat. Předpokladem pro to jsou vyšší obsahy kovů v substrátu na haldách (Příloha 1).

**Tabulka 6**. Hodnocení Nemerowým indexem znečištění ($IPI_N$). Modifikováno podle přílohy 1.

| | | **Baganúr** | **Nalaikh** | **Shariin Gol** |
|---|---|---|---|---|
| | Průměr | 0,28 | 0,38 | 0,65 |
| | S.D. | 0,10 | 0,09 | 1,10 |
| **$IPI_N$** | Min. | 0,17 | 0,22 | 0,26 |
| | Medián | 0,26 | 0,36 | 0,43 |
| | Max. | 0,80 | 0,74 | 7,49 |

Na základě literární rešerše byly překvapivě nízké obsahy těžkých kovů také v jiných těžebních oblastech Mongolska, a to nejen ve městech poblíž dolů, ale i v samotných těžebních areálech (Příloha 1). Toto zjištění ostře kontrastuje s jinými oblastmi světa, kde těžební průmysl představuje jednu z největších kontaminačních hrozeb (Li et al., 2014). Důvodů pro tuto skutečnost může být hned několik: (I) kontaminační studie se zaměřují na oblasti s nižším rizikem znečištění těžkými kovy, (II) krátká historie těžby, (III) omezené zpracování v místě těžby a (IV) velkoplošná disperze kontaminace větrem.

Ad I) V globálním měřítku se studie na kontaminaci těžbou často zaměřují na rizikovější nerostné suroviny, než je uhlí, které jsou buď přímo těžkými kovy (např. Cu, Pb, Zn; Kapusta et al., 2011; Motyka a Postawa, 2013; Monterroso et al., 2014; Cai et al., 2015), nebo jsou jimi ve větší míře doprovázeny (např. Au, Ag; Kim et al., 2002; Ettler et al., 2006; Drahota et al., 2018). Ačkoliv v Mongolsku rovněž probíhá těžba těchto rizikovějších surovin, jako jsou Ag, Pb a Zn (MRPAM, 2020; Surenbaatar et al., 2021), jejich problematice není v doposud publikovaných studiích v Mongolsku věnována patřičná pozornost. V případě těžby Cu a Au ale výsledky (Příloha 1) ukazují také na působení dalších faktorů, které snižují úroveň kontaminace.

Ad II) Zatímco v jiných zemích s typickým silným znečištěním v těžebních oblastech probíhala těžba a doprovodné zpracovatelské nebo zvětrávací procesy několik století (Ettler et al., 2006; Mihaljevič et al., 2006; Motyka a Postawa, 2013; Stefanowicz et al., 2014), v Mongolsku je těžební průmysl stále mladým sektorem (Lkhasuren et al., 2007). Omezený srážkový úhrn a nízká vlhkost, typické pro aridní Mongolsko (Regdel et al., 2012), spolu se sorpcí prvků na uhelnou hlušinu v případě uhelných dolů, mohou omezit zvětrávání těžebního odpadu a distribuci těžkých kovů do životního prostředí v okolí dolů.

Ad III) Za jednu z největších kontaminačních hrozeb v souvislosti s těžbou nerostných surovin je pokládáno jejich zpracování, jako je například tavení rud (Ettler et al., 2006; Mihaljevič et al., 2006). To ale v Mongolsku probíhá v omezené míře; po těžbě dochází často k přímému exportu vytěžených surovin do zahraničí, zejména do Číny, kde teprve dojde ke zpracování s ohledem na lepší technologické zázemí. To může významně přispívat ke snížení lokální kontaminace.

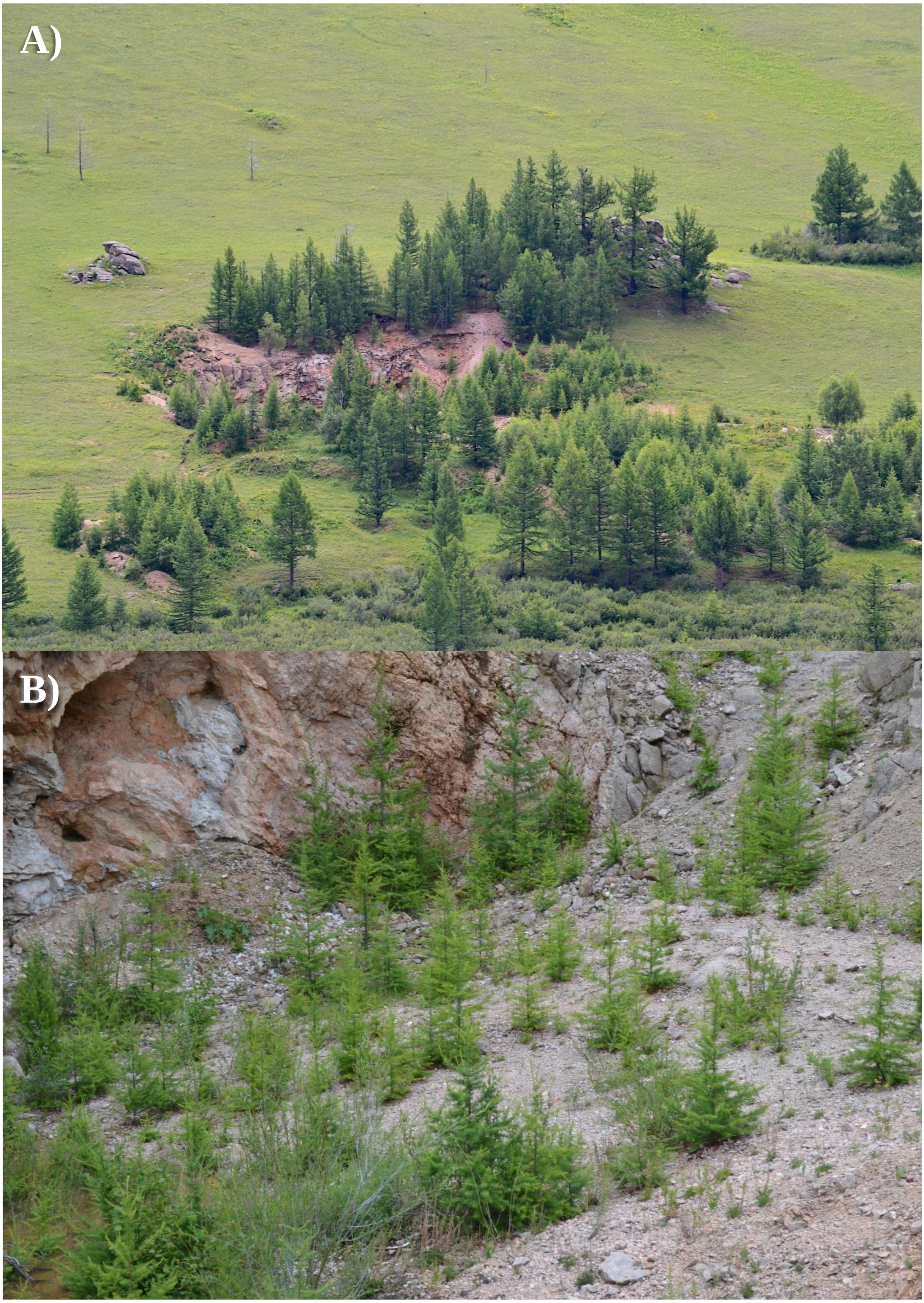
Ad IV) Pro Mongolsko jsou charakteristické silné větry; větrná eroze ovlivňuje do určité míry až 90 % země (Mandakh et al., 2016). V případě těžby uhlí představuje emitovaný prach základní médium pro šíření polutantů v dotčených oblastech (Rout et al., 2013). Transport větrem tedy může významně ovlivnit disperzi kontaminovaných prachových a dalších částic a (a) vést k velkoplošné disperzi kontaminace, a tím k snížení lokální hrozby představované těžkými kovy, nebo (b) k vytváření hotspotů kontaminace v odlehlých oblastech se zvýšeným rizikem zátěže. Velkoplošné znečištění těžkými kovy prostřednictvím atmosférické depozice spojené s těžbou uhlí zmiňuje například také Liang et al., (2017). Vážnou hrozbu představovanou disperzí větrem zmiňují ale i další autoři studující právě znečištění v Mongolsku (Battogtokh et al., 2014; Kosheleva et al., 2018; Nottebaum et al., 2020). Tento scénář a hrozbu lze proto řadit mezi nejvýznamnější. Možným řešením snížení rizika prašnosti a eolického transportu je fytostabilizace těžebních odpadů.

## 4.2 Perspektiva fytoremediace v Mongolsku

Fytostabilizace jako jedna z fytoremediačních technik může představovat vzhledem ke své nízké technické i finanční náročnosti jednu z dostupných cest rehabilitace těžbou degradované krajiny a snížení rizik spojených se znečištěným těžebním odpadem i v rozvojových zemích jako je Mongolsko.

Těžební odpad je specifický extrémními podmínkami pro kolonizaci rostlinami, které zahrnují například převládající kyselé pH, nedostatek živin, nevhodnou strukturu substrátu a nestabilitu (Wong, 2003; Alday et al., 2011). V prostředí semiaridního až aridního klimatu Mongolska s nízkým úhrnem srážek (Regdel et al., 2012) a charakteristickými klimatickými extrémy mohou jako doplňující kritický faktor limitující použití fytostabilizace působit také tyto faktory zahrnující sucho a zasolení (Mendez a Maier, 2008). Při studiu těžebních půd z Baganúru například Park et al. (2020) zjistil desertifikační potenciál. Dokonce i v případě městské zeleně je v Mongolsku typické umělé zavlažování pro zajištění úspěšného pěstování a využíváno je často i při zakládání lesa nebo větrolamů v lesostepních a pouštních oblastech. Tyto skutečnosti významně snižují pravděpodobnost úspěšného založení a udržitelného managementu vegetačního krytu na těžebních odpadech.

Doposud realizované studie na těžební odpady v Mongolsku ale naopak ukázaly vysoký potenciál pro ozelenění. V severním Mongolsku byla popsána úspěšná spontánní sukcese prostřednictvím kolonizace odvalů a lomů po těžbě pegmatitů modřínem sibiřským (*Larix sibirica* Ledeb.), a to i na stanovištích přirozeně pokrytých stepí (Juřička et al., 2016; 2020b). Výsledky naznačují, že růst modřínu sibiřského na těchto extrémních stanovištích na stepi, tedy mimo jeho ekologickou niku, je umožněn zejména kondenzací vzdušné vlhkosti; odvaly a lomy v dané oblasti tedy fungují jako tzv. sběrače rosy. Ačkoliv tato stanoviště umožňují růst a prosperování stromů (Obrázek 5), půdní pokryvnost bylinným patrem je obvykle velmi nízká (Obrázek 5b). Efektivita omezení disperze prachových částic těmito samovolně vzniklými sukcesními nebo případně uměle založenými společenstvy s převahou stromů může proto být značně snížena, a jejich reálný význam v tomto ohledu vyžaduje další výzkum a rozsáhlejší pokusy v terénních podmínkách. Potenciál modřínu může být ale také v jeho využití jako přípravného druhu s ohledem na jeho pionýrskou životní strategii. Pozitivní přínosy ve formě stimulace podmínek pro kolonizaci těžebního substrátu bylinami je možné očekávat už po přibližně pěti letech od založení porostu.




***Obrázek 5**. A) Porosty modřínu sibiřského (Larix sibirica Ledeb.) přirozeně kolonizující bezprostřední okolí lomu ve stepi. B) Nálety modřínu sibiřského dominující na dně lomu s řídce se vyskytujícími bylinami (Autor fotografie: Ing. Václav Pecina).*

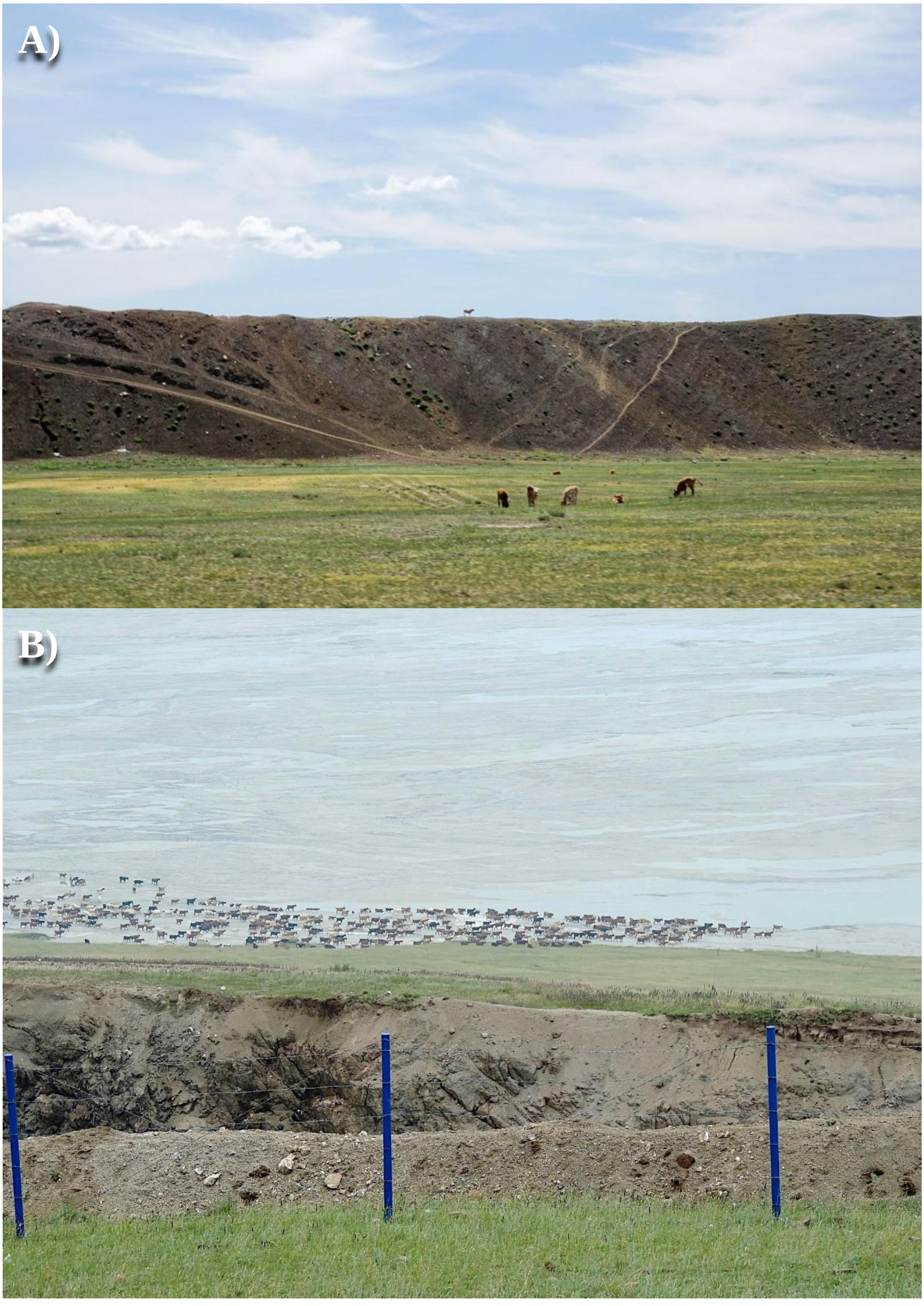
Limitujícím faktorem pro využití modřínu sibiřského v mongolských oblastech s těžbou uhlí nebo rud může být jeho potenciální sensitivita na znečištění. Podle některých přístupů jsou jako preferované rostliny pro fytoremediaci v těžebních oblastech vybírány rychle rostoucí stromy s dlouhými kořeny resistentní vůči toxicitě těžkých kovů a suchu (Wei et al., 2021). Riziko znečištění těžkými kovy při těžbě pegmatitů je zanedbatelné, faktor toxicity substrátu odvalů tedy nebrání modřínům v kolonizaci, a pro vyhodnocení naplnění této podmínky jsou předchozí studie (Juřička et al., 2016; 2020b) nedostatečné. Vztah mezi znečištěním těžkými kovy a modřínem byly ale studovány v jiných oblastech. Afanasyeva a Ayushina (2019) jej označují jako druh citlivý na znečištění, vhodný jako bioindikátor. Gorelova a Frontasyeva (2017) ale zmiňují potenciál modřínu pro fytoremediaci vzhledem ke schopnosti akumulovat Fe a Pb, Saltan a Sviatkovskaya (2020) jej dokonce označují jako druh vysoce resistentní vůči znečištění těžkými kovy s potenciálem snášet zvýšené obsahy Fe, Ni a Cu v jehlicích. Tyto výsledky částečně podporují teorii o potenciálu modřínu pro fytoremediaci znečištěných těžebních oblastí v Mongolsku.

Potenciálním řešením problematiky nízkého pokryvu těžebních opadů bylinným patrem může být aplikace organických materiálů, jako jsou například kompost, dřevní štěpka, biouhel nebo hnůj (Mendez a Maier, 2008; Sarwar et al., 2017), nebo jiných materiálů, které by mimo stimulaci růstu rostlin také snížily exponovanost jemných kontaminovaných prachových částic erozivní činnosti větru. Podobné metody asistované fytostabilizace znečištěných půd již dříve úspěšně testovala například Radziemska et al. (2019) s dolomitem nebo křemelinou. Na základě výsledků v příloze 3 se jako vhodná aditiva pro fytostabilizaci kontaminovaných půd ukázaly také kompost z čistírenských kalů, biouhel z vrbové štěpky nebo popel z drůbežího peří, jejichž aplikace vedla k zvýšení půdního pH a produkci rostlinné biomasy a snížení půdní fytotoxicity. S ohledem na dostupnost by v Mongolsku mohlo být perspektivní využití hnoje nebo štěpky, ať už surových, nebo dále zpracovaných, které mohou být při aktivním managementu snadno získávány ze zemědělské nebo lesní půdy. Aplikace těchto materiálů má potenciál nejen pro snížení prašnosti, ale také pro zlepšení podmínek substrátu pro vývoj rostlinných společenstev, fytostabilizaci a pro absorpci těžkých kovů. Současně nabízí řešení pro využití zbytkové dřevní hmoty z lesní těžby, pro kterou se v Mongolsku aktuálně hledá využití, a trusu hospodářských zvířat akumulovaného v zimovištích, která představují dlouhodobou přehlíženou hrozbu kontaminace vodních zdrojů zejména dusíkem.

Specifická kombinace aditiv aplikovaných v příloze 3 spolu s pěstováním *Lupinus luteus* L., nicméně, nemá perspektivu pro využití v mongolských extrémních podmínkách s ohledem na ekologii a areál druhu, a tak neumožňuje další využití závěrů studie. Odlišná je situace v případě přílohy 4 s ohledem na široký areál *Festuca rubra* L., která splňuje rostlinné fytoremediační kritérium lokálního nebo nativního druhu přizpůsobeného místním podmínkám (Whiting et al., 2004; Asgari Lajayer et al., 2019). Potenciální hrozbu vysoké kontaminace půd chromem, kterou indikují studie realizované v blízkosti koželužen (Příloha 1), je možné snížit kombinací pěstování *F. rubra* spolu s aplikací chalcedonitu (Příloha 4**)**, který je v Mongolsku poměrně běžnou a dostupnou surovinou.

Fytoremediace znečištěných půd v těžebních oblastech nemusí být vždy vhodným typem managementu. Tzv. „zelená řešení“ realizovaná na místech ekologických zátěžích v urbánních oblastech naopak mohou ještě zvýšit úroveň rizika pro člověka, protože vytvořením atraktivního prostředí pro rekreaci na silně znečištěné půdě dochází k navýšení rizika expozice těžkým kovům (Příloha 2). Ačkoliv může být toto riziko významně sníženo volbou vhodných druhů rostlin, doprovodnými inženýrskými řešeními a edukací dotčené společnosti (Wilschut et al., 2013; Rocha et al., 2021), přetrvávajícím rizikovým faktorem je nekontrolovaná pastva hospodářských zvířat, která je v Mongolsku tradičním způsobem obživy (Regdel et al., 2012; McIntyre et al., 2016; Juřička et al., 2020a).

Konflikt mezi pastevectvím a těžbou nerostných surovin je v Mongolsku vnímán především v přístupu k pastvinám a vodním zdrojům (Suzuki, 2013). Urbanizace v tradičně pasteveckém Mongolsku vede v posledních dekádách ke koncentraci pastevců do městských oblastí (McIntyre et al., 2016; Juřička et al., 2019). Vzhledem k běžné vazbě měst na doly, jak je tomu také v případě Baganúru, Nalakhu i Shariin Golu (Příloha 1), je také vyšší pastevní tlak na vegetaci v okolí dolů. Nedostatečný ochranný a kontrolní management těžebních oblastí umožňuje, že se dobytek pase přímo na nezabezpečených těžebních haldách (Obrázek 6a). To vede k hrozbě intoxikace hospodářských zvířat konzumací rostlin volně rostoucích na těžebním odpadu přijímajících a ukládajících těžké kovy ve výhoncích, listech a dalších rostlinných tkáních a doprovodném požívání kontaminovaného prachu nebo substrátu usazeného na rostlinách. Dalším rizikem je pravidelný pohyb hospodářských zvířat na těchto lokalitách a související narušování sukcesně vznikajícího vegetačního pokryvu a doprovodné zvyšování prašnosti narušováním půdního povrchu a resuspenzí.




***Obrázek 6**. A) Pastva dobytka pod haldou těžebního odpadu s viditelnými aktivními stezkami dobytka na haldě poblíž města Shariin Gol. B) Napájení drobného dobytka v odkalovací nádrži za ochranným plotem poblíž města Erdenet s Cu-Mo dolem (Autor fotografie: Ing. Václav Pecina).*

Riziko intoxikace dobytka a neúspěšné fytostabilizace je nezbytné snížit oplocením rizikových oblastí. Nicméně, ani přítomnost plotů nezaručuje v mongolských podmínkách efektivní eliminaci rizik (Obrázek 6b). Proto je v případě řízené fytostabilizace vhodné vybírat druhy s potenciálem akumulovat těžké kovy pouze v podzemní biomase nebo je neakumulovat vůbec. Výběr takovýchto druhů vyžaduje další rozsáhlý výzkum v lokálních podmínkách. S ohledem na výše zmíněná rizika představuje modřín sibiřský potenciálně silně rizikový druh vzhledem k akumulaci těžkých kovů v nadzemní biomase (Gorelova a Frontasyeva, 2017; Afanasyeva a Ayushina, 2019). Jeho využití při fytostabilizaci hald těžebního odpadu by tedy muselo zahrnovat individuální ochranu před okusem. Součástí ochranných opatření musí být nepřetržitý monitoring a doprovodné vzdělávání pastevců.

# 5. ZÁVĚR

Závěry dílčích studií jsou podrobně rozpracovány v přílohách 1-4.

Výsledky dvou případových studií zaměřených na znečištění životního prostředí těžebních oblastí těžkými kovy ukázaly překvapivé výsledky. Výsledky studie realizované v Příbrami (Příloha 2), kde proběhla sanace území bývalého důlně-úpravárenského závodu a haldy a přetvoření na lesopark, poukázaly na silné znečištění půdy těžkými kovy (As, Cd, Pb a Zn), potenciální zdravotní rizika pro návštěvníky parku a toxické působení těžkých kovů (zejména Zn) na sazenice stromů i po realizaci rekultivačního projektu. Závěrem jsou doporučení, jak postupovat v případě podobných projektů na silně znečištěném území. Výsledky terénního výzkumu i rešerše na nedostatečně spravované a zabezpečené těžební oblasti v Mongolsku (Příloha 1) naproti tomu poukázaly na velmi nízkou úroveň až absenci znečištění půdy bez předpokládaných rizik pro obyvatele zasažených oblastí. Obsahy těžkých kovů (zejména Cd) v substrátu na těžebních haldách, nicméně, naznačují možná budoucí rizika a nutnost zásahu.

Výsledky obou fytoremediačních studií (Příloha 3 a 4) potvrdily potenciál studovaných druhů rostlin v kombinaci se specifickými aditivy pro asistovanou fytostabilizaci znečištěných půd (příslušně pro Cd, Cr, Cu, Ni a Zn a Cr(VI)), a tedy i potenciál pro využití při fytoremediaci půd v těžebních oblastech znečištěných těžkými kovy.

V případě areálu parku v Příbrami i těžebních oblastí v Mongolsku je možné využití fytoremediace specificky aplikované s ohledem na lokální podmínky a situaci. Vzhledem k rizikům spojeným s nekontrolovanou pastvou v Mongolsku je nezbytné oplocení těžebních odpadů a pro snížení rizik intoxikace hospodářských zvířat se zaměřit na fytostabilizaci hald. Perspektivním druhem pro fytoremediaci v Mongolsku může být modřín sibiřský, který má potenciál fungovat jako přípravná dřevina. Jeho význam pro formování vegetačního krytu a fungování takto založených rostlinných společenstev, stejně jako fungování na znečištěných půdách, nicméně vyžaduje další výzkum.

Velkou perspektivu v Mongolsku má asistovaná fytostabilizace se zaměřením na využití dostupných organických materiálů s potenciálem zlepšit podmínky těžebního odpadu pro kolonizaci rostlinami a omezit disperzi polutantů. Experimenty s aplikací hnoje, samotného trusu nebo štěpky do svrchní vrstvy hald doprovázené umělou výsadbou a osevem by měly být předmětem dalších výzkumů.

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# 7. SEZNAM OBRÁZKŮ

# 8. SEZNAM TABULEK

# 9. SEZNAM PŘÍLOH

**Příloha č. 1**: Manuskript článku „*Current knowledge on soil pollution with metal(loid)s in resource-rich Mongolia: How serious are the impacts of mining?*“ v podobě, v jaké byl zaslán do redakce Scientific Reports (18.8. 2022), kde je v současné době (13.10. 2022) v recenzi.

**Příloha č. 2**: Manuskript článku „*Polluted brownfield site converted into a public urban park: A place providing ecosystem services or a hidden health threat?*“. Citace: PECINA, Václav, et al. Polluted brownfield site converted into a public urban park: A place providing ecosystem services or a hidden health threat?. Journal of environmental management, 2021, 291: 112669. https://doi.org/10.1016/j.jenvman.2021.112669.

**Příloha č. 3**: Manuskript článku „*Insight into metal immobilization and microbial community structure in soil from a steel disposal dump phytostabilized with composted, pyrolyzed or gasified wastes*“. Citace: RADZIEMSKA, Maja, et al. Insight into metal immobilization and microbial community structure in soil from a steel disposal dump phytostabilized with composted, pyrolyzed or gasified wastes. Chemosphere, 2021, 272: 129576. https://doi.org/10.1016/j.chemosphere.2021.129576.

**Příloha č. 4**: Manuskript článku „*Successful Outcome of Phytostabilization in Cr(VI) Contaminated Soils Amended with Alkalizing Additives*“. Citace: RADZIEMSKA, Maja, et al. Successful Outcome of Phytostabilization in Cr (VI) Contaminated Soils Amended with Alkalizing Additives. International Journal of Environmental Research and Public Health, 2020, 17.17: 6073. https://doi.org/10.3390/ijerph17176073.

# PŘÍLOHA Č. 1

# Current knowledge on soil pollution with metal(loid)s in resource-rich Mongolia: How serious are the impacts of mining?

Václav Pecina [a, *], David Juřička [b], Josef Hedbávný [c], Martin Klimánek [d], Jindřich Kynický [e], Martin Brtnický [a,f], Renata Komendová [a]

[a] *Institute of Chemistry and Technology of Environmental Protection, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, 61200 Brno, Czech Republic*

[b] *Department of Geology and Soil Science, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 3, 613 00 Brno, Czech Republic*

[c] *Department of Chemistry and Biochemistry, Faculty of Agrisciences, Mendel University in Brno, Zemědělská 1, 613 00, Brno, Czech Republic*

[d] *Department of Forest Management and Applied Geoinformatics, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 3, 61300 Brno, Czech Republic*

[e] *BIC Brno, Technology Innovation Transfer Chamber, Purkyňova 125, 612 00, Brno, Czech Republic*

[f] *Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic.*

** Corresponding author, e-mail xcpecina@fch.vut.cz; ORCID 0000-0003-4274-5142*

## Abstract

As Mongolia is considered one of the most resource extraction-dependent countries globally, significant mining-related environmental and human health risks are expected. The aim of this study was to (I) assess the impacts of mining on soil pollution with metals in Mongolia's key coal mining towns (Baganuur, Nalaikh and Sharyn Gol) and (II) review the current knowledge on soil pollution with metal(loid)s in Mongolia. The results showed predominantly low soil contents of Cd, Cu, Pb and Zn and a related absence of severe pollution and potential health risk in the coal mining towns. Urban design, rather than the presence of mines, controlled the pollution distribution. Despite the methodological shortcomings of several studies on soil pollution in Mongolia, their results suggest a similarly low threat in the three largest cities (Ulaanbaatar, Darkhan, Erdenet) and several mining areas. While the generally highlighted risk of As seems like an artificially escalated issue, the content of Cr in urban soil may be a neglected threat. Further pollution research in Mongolia should focus on street dust and drinking water pollution.

**Keywords**: urban soil; coal; health risk assessment; contamination; potentially toxic elements

## 1. Introduction

Mongolia is considered one of the most resource extraction-dependent countries globally[1], and the mining industry has been a major contributor to its economy over recent decades[2,3]. The mining industry's origin is dated to 1924 when the first coal mine was opened in Nalaikh[2]. Its rapid development associated with the intensive release of mining licensing began in the 1990s after the transition to an open economy and is ongoing[4,5]. Simultaneously, the expansion of major official mining operations has been accompanied by artisanal and small-scale mining[6,7]. There are approximately 3,000 deposits and 50 different minerals explored and researched in Mongolia[1], with the most economically important and mined commodities being black and brown coal, fluorite, Au, Cu, Ag, Mo, Pb and Zn[8],[9].

However, mining development and related economic growth have accelerated land degradation[10]. According to UNDP[5], 77% of Mongolian land is classified as degraded or desertified. While mining-related environmental pollution in Mongolia has been intensively studied in recent years, this is still insufficient because of the country's size and the number of mining areas. In the semi-arid to arid conditions of Mongolia[11] with limited water resources[12], water pollution poses a critical risk[4,3,12–16]. Insufficient scientific attention is paid to soil pollution, although mining of most minerals can be associated with soil contamination with metal(loid)s. Furthermore, there is no effective management system to assess the environmental health of mining sites[9].

In recent decades, mining areas in Mongolia have been urbanised by originally nomadic inhabitants[12]. The concentration of people in these areas may be risky due to daily exposure to potentially hazardous substances in the environment released from mining operations and stored mining waste materials. Lkhasuren et al.[2] stated that the high levels of dust associated with coal and gold mining are behind the high and growing number of lung diseases in Mongolia. Suvd et al.[6] found typical chronic Hg intoxication symptoms in artisanal miners from gold mining areas in two sums. Recently, Surenbaatar et al.[9] identified different patterns of mining's impact on children's health in areas of southern Mongolia. However, the results generally indicated the local effect of mining on the content of Pb in blood, As in urine and Hg in blood and hair.

The most resonant issue in urban soil pollution studies in Mongolia is coal combustion. Coal combustion can lead to a significant release of metal(loid)s into the environment and is ranked among the primary sources of contamination in the capital of Ulaanbaatar[17–20], other cities[21] and environments[22]. Other typical and frequently mentioned sources of contamination in Mongolia include transport and various industries[20,23]. However, the studies carried out so far on urban soil pollution with metal(loid)s in Mongolia strictly focus on the three largest cities (Ulaanbaatar, Darkhan, Erdenet), of which only Erdenet is directly linked to mining.

The pollution-related health risk may not only concern the immediate vicinity of mines. Due to aeolian dispersion and water erosion, the most common means of pollutants transport in arid and semi-arid environments[24], metal(loid)s can be deposited over considerable distances. For example, mining activities in Mongolia are associated with the contamination of Lake Baikal in the Russian Federation[13,14]. Another problem may be traditional livestock grazing[4,11]. The accumulation of metal(loid)s in plants growing on polluted soils that livestock graze can endanger the vitality of the animals and the quality of food produced, leading to another human health risk[24,25].

Given that the impacts of mining on soil pollution are serious and pose high health risks to the public in neighbouring, similarly highly mining-dependent China[26], it can be assumed that mining also poses serious environmental and health risks in Mongolia. The absence of relevant studies on this issue points to the need for extensive research. The aims of this study are to: (1) assess the pollution of urban soils with metals in the key coal mining towns of Baganuur, Nalaikh and Sharyn Gol; (2) summarise and evaluate studies on soil pollution with metal(loid)s in Mongolia; and (3) assess the human health risks posed by soil pollution in Mongolia.

## 2. Materials and methods

### 2.1 Study area

Mongolia is characterised by extreme semi-arid to arid climate conditions, with an average annual rainfall reaching about 400 mm. Average annual temperatures range from approximately -50 °C to 40 °C[27]. Strong winds that can easily enhance dust particles dispersion from mining areas significantly influence Mongolian steppe and desert areas. Wind erosion affects up to 90% of the country to some extent. The major soil types are Kastanozem soils, Cambisols and Chernozems[28].

For this study, three key coal mining towns were sampled: Baganuur, Nalaikh and Sharyn Gol (also referred to as Sharyngol or Shariin Gol) (Fig. 1). These towns are or were main coal suppliers to the three largest Mongolian cities: Ulaanbaatar, Erdenet and Darkhan[17,29,30]. While official mining in Nalaikh has already ended[22], small-scale illegal mining continues[7]. The open-pit (Baganuur, Sharyn Gol) or underground (Nalaikh) coal mines are to the south of the towns. The quality of mined coal varies between lignite and sub-bituminous[31,32].

Furthermore, the available studies (up to 2021) were reviewed to evaluate the status of metal(loid) soil pollution in Mongolia.

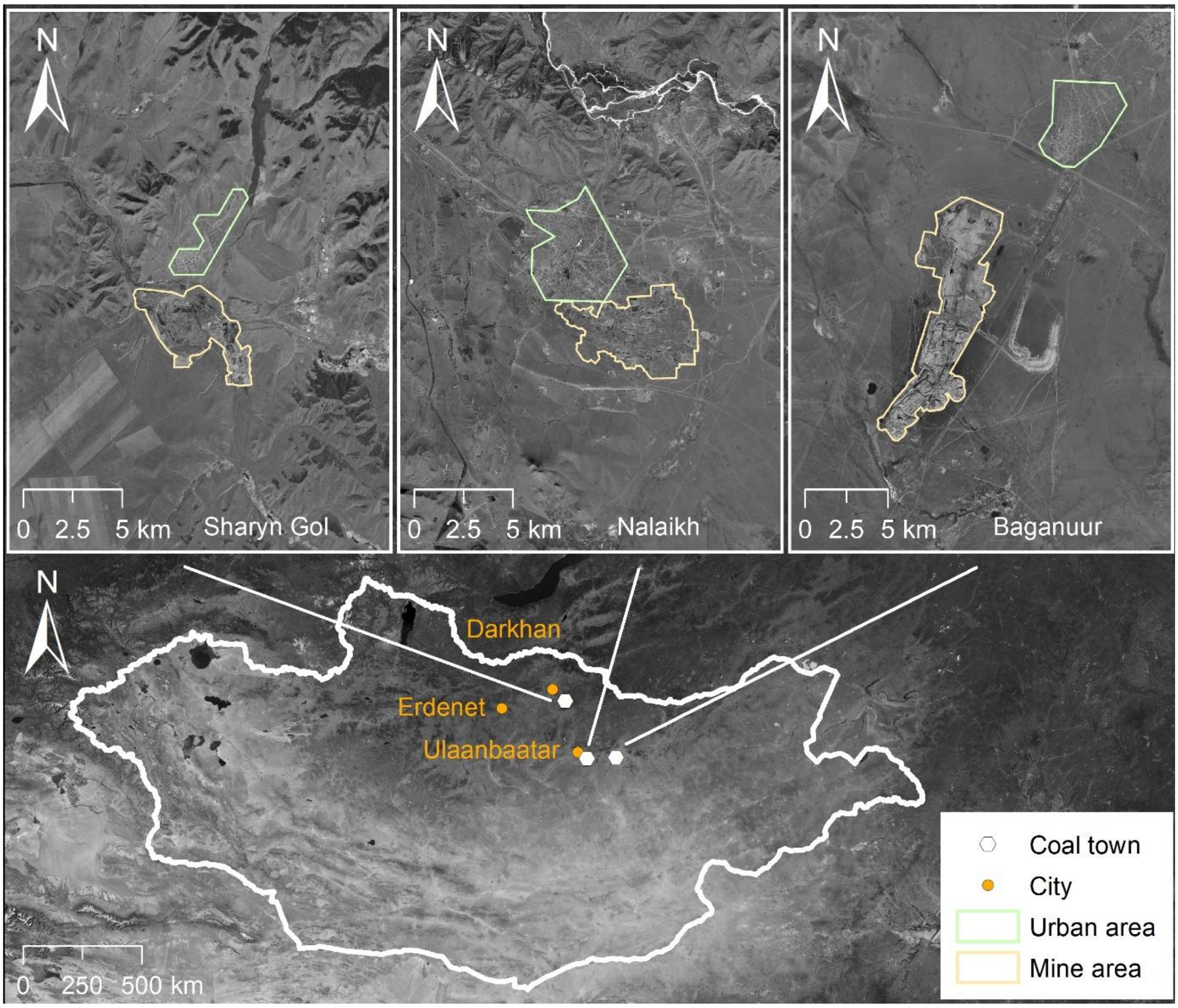


**Fig. 1**. Location of the studied towns and three largest cities in the context of Mongolia.

### 2.2 Soil sampling, preparation and analysis

Topsoil (0–10 cm) samples composited from three sub-samples from approximately 1 $m^2$ were taken in each town according to a regular hexagonal sampling network. Forty-four samples were taken from Sharyn Gol, 50 from Nalaikh and 48 from Baganuur: 142 samples in total. The samples were air-dried at room temperature and sieved through a 2.0 mm nylon sieve.

The decomposition of the samples was performed in two replications according to ČSN EN 16174 by microwave extraction with aqua regia at the microwave extractor ETHOS ONE (Milestone, Italy). 0.5 g of soil sample was weighed to Teflon tubes, and 2 ml of Suprapur nitric acid (65%) and 6 ml of hydrochloric acid (37%) in ACS quality (Sigma Aldrich) were added.

Extraction was performed at 175 °C for 20 min. Total metal (Cd, Cu, Pb and Zn) concentrations in soil samples were determined by flame atomic absorption spectrometry using Varian SpectrAA-30 (Varian, Australia). Air-acetylene flame atomisation (gas flow 13.5 L/min and 2.0 L/min) and an ultrasensitive hollow cathode lamp for Cd, Cu, Pb and Zn (Agilent Technologies, USA) were used. Cd, Cu, Pb and Zn were measured at 228.8 nm, 324.7 nm, 217.0 nm and 213.9 nm, respectively. For calibration, standard solutions of Cd, Cu, Pb and Zn (1 g/L) (Merck) and MilliQ water (Millipore, USA) were used. Certified reference material METRANAL 31 (light sandy soil; Analytika, Czech Republic) was used for quality control.

Based on the analysis of these basic metal pollutants, additional As, Cr, Hg and Ni content measurements were performed in several samples to verify the possibility of severe town-scale pollution with these metal(loid)s. The test samples were selected with regard to their increased content of Cd, Cu, Pb or Zn and diverse spatial distribution; up to fifteen samples were analysed in total. Contents of As, Cr and Ni in the soil samples were analysed using inductively coupled plasma mass spectrometer Agilent 7900 (Agilent, USA) with the SPS 4 autosampler (Agilent, USA) after microwave extraction with aqua regia at the microwave extractor ETHOS ONE (Milestone, Italy). 0.5 g of soil sample was weighed to Teflon tubes, and 3 ml of nitric acid (67–69%) and 9 ml of hydrochloric acid (36%) (both ANALPURE; Analytika, Czech Republic) were added. Extraction was performed at 200 °C for 15 min. For total Hg content analysis, a single-purpose atomic absorption spectrophotometer AMA-254 (Advanced Mercury Analyser; Altec, Czech Republic) was used without the necessity of further sample pre-treatment or pre-concentration. The quality control was the same as in the previous measurements. The median values of As, Cr, Hg and Ni in test samples were 10.9, 31.3, 0.0336 and 17.4 mg/kg, respectively. Due to their prevailing contents being below the limits of the soil pollution standard[33], they were not analysed in the remaining samples with the expectation of a low probability of pollution risk at the town scale.

## 2.3 Soil pollution indicators

The content of the metal(loid)s in soils was assessed in compliance with the maximum allowable limit of the Mongolian standard on soil quality (MNS 5850:2008)[34] and the internationally recognised target and intervention values of the Dutch Soil Guidelines (DSG)[33]. Contamination/pollution indicators (indices) calculate the level of environmental contamination/pollution based mainly on local background values. However, the level of pollution determined in this way only weakly indicates the severity of the threat. Therefore, the adapted Integrated Nemerow Pollution Index ($IPI_N$)[35] with the Mongolian standard values considered was chosen:

$$PI_i = \frac{C_i}{T_i}$$

$$IPI_N = [(PI^2{}_{avg} + PI^2{}_{max})/2]^{1/2}$$

$PI_i$ is the pollution index for a metal(loid), $C_i$ is the content of the metal(loid), $T_i$ is the maximum allowable content of the soil pollutant given by MNS 5850:2008[34], $PI_{avg}$ is the mean value of all the $PI_i$ of the metal(loid)s, and $PI_{max}$ is the maximum $PI_i$ value of the metal(loid)s. $IPI_N$ classes are as follows: ≤0.7: safe; 0.7–1: precaution; 1–2: slight pollution; 2–3: moderate pollution; ≥3: heavy pollution.

## 2.4 Human health risk assessment

The Hazard Index (HI) and Carcinogenic Risk (CR) were calculated to assess the potential health risk posed by metal(loid)s in Mongolian soils. The HI and CR calculations are based on average daily dose (ADD), reference dose (RfD), and slope factor (SF) values of metal(loid)s.

Human exposure to metal(loid)s in soils ($C_{soil}$) arises through ingestion ($ADD_{ing}$), dermal contact ($ADD_{derm}$) and inhalation. However, because ingestion and dermal contact pose the greatest health risks[35], attention was paid only to these two pathways. An explanation of the input parameters and the values used for the calculations are summarised in Tables 1 and 2. To refine the authenticity of the calculation considering different physical features, national values (adult BW, LT) and values used in neighbouring China (children BW, SA) were used instead of the conventional ones. The following equations were used for the intake estimations via each exposure pathway[36]:

$$\mathrm{ADD_{ing}} = \frac{\mathrm{C_{soil}} \times \mathrm{IngR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \times 10^{-6}$$

$$\mathrm{ADD_{derm}} = \frac{\mathrm{C_{soil}} \times \mathrm{SA} \times \mathrm{AF} \times \mathrm{ABS} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \times 10^{-6}$$

Total non-carcinogenic health risk, HI, was determined as a sum of calculated individual hazard quotients (HQ)[30,37]:

$$\mathrm{HI} = \sum \mathrm{HQ}_i = \sum \frac{\mathrm{ADDi}}{\mathrm{RfDi}}$$

HI values >1 indicate the probability of non-carcinogenic adverse health effects.

The carcinogenic risk was calculated for considered carcinogenic metal(loid)s (As, Cd, Cr, Ni and Pb) as follows[35]:

$$\mathrm{CR} = \sum \mathrm{ADD_i} \times \mathrm{SF_i}$$

CR values $\leq 10^{-6}$ indicate virtual safety, whereas values $\geq 10^{-4}$ indicate an unacceptable risk. The acceptable risk range for regulatory purposes is $10^{-6}$ to $10^{-4}$.

**Table 1.** Calculation parameters.

| Factor | Description | Unit | Adult | Children | Reference |
|---|---|---|---|---|---|
| IngR | Ingestion rate of soil | mg/day | 100 | 200 | USEPA[38] |
| EF | Exposure frequency | days/year | 350 | 350 | MEP[39] |
| ED | Exposure duration | years | 24 | 6 | USEPA[38] |
| BW | Bodyweight | kg | 65.0[a] | 15.9[a] | MEP[39] |
| AT | Average time | days | 8,760[b] | 2,190[b] | Pecina et al.[35] |
| SA | Exposed skin surface area | $cm^2$ | 4,350 | 1,600 | Pecina et al.[35] |
| AF | Skin adherence factor for soil | $mg/cm^2$ | 0.07 | 0.20 | Pecina et al.[35] |

[a] BW was calculated as the average of the adult Mongolian weights reported in WHO[40], Otgontuya et al.[41], and WHO[42] studies. The Mongolian children's weight values were not found; therefore, the values from neighbouring China[39] were used.

[b] For carcinogenic effects[43]: LT (lifetime) × 365. In Mongolia[44], LT = 70. For carcinogenic effects, AT = 25,550.

**Table 2.** The relative toxicity values used (based on the literature summary in Pecina et al.[36]).

| Metal | $RfD_{ing}$ | $RfD_{derm}$ | ABS[a] | $SF_{ing}$ |
|---|---|---|---|---|
| Cd | 1.00E-03 | 1.00E-05 | 0.001 | 1.50E+01 |
| Cu | 4.00E-02 | 1.20E-02 | 0.001 | |
| Pb | 3.50E-03 | 5.25E-04 | 0.001 | 8.50E-03 |
| Zn | 3.00E-01 | 6.00E-02 | 0.001 | |

[a] Dermal absorption factor.

### 2.5 Spatial data analysis

Statistica 12® was used for statistical analyses of the dataset. Differences in the soil contents of metals between the towns were tested. Data normality was investigated using the Shapiro–Wilk normality test ($p > 0.01$). One-way ANOVA was used for normal series; if at least one of the series did not pass the normality test, a nonparametric Kruskal–Wallis ANOVA was used. Spatial data of the pre-processing and geostatistical analysis were conducted in SW ESRI ArcGIS Desktop 10.8 using the Geostatistical Analyst extension. All output rasters of the interpolated values were calculated with a spatial resolution of 10 m. The geostatistical kriging method was selected for spatial data interpolation, specifically, Empirical Bayesian Kriging where the mean prediction errors are more accurate for small data sets compared to other kriging methods. Spatial data transformation (with any base function) was not applied. The thin Plate Spline semivariogram model was chosen by testing several different models of semivariogram. Setting the semivariogram has always been simulated for 100 models, and the results for the map outputs were checked by cross-validation. A smooth circular interpolation option with a radius of 500 m and a smoothing factor of 0.5 was selected in search neighbourhood parameters to control the output.

## 3. Results and discussion

### 3.1 Soil pollution in the coal mining towns

The average contents of metals did not exceed the Mongolian standard[34] in any of the studied towns (Fig. 2). The standard was rarely exceeded, once in Cu (Baganuur) and three times in Pb (Sharyn Gol). Cd exceeding the target value[33] in Baganuur, Nalaikh and Sharyn Gol (Fig. 2) indicates contamination with this element. Pb exceeded the intervention value, suggesting severe pollution, only once (Sharyn Gol). $IPI_N$ assessment classified all the towns as safe according to average $IPI_N$ values (Table S1). Therefore, despite the ongoing open-pit mining

(Baganuur, Sharyn Gol) or historical underground mining (Nalaikh) of coal enriched with chalcophile elements[7,17,23,29], the results suggest minor pollution with Cd, Cu, Pb and Zn.

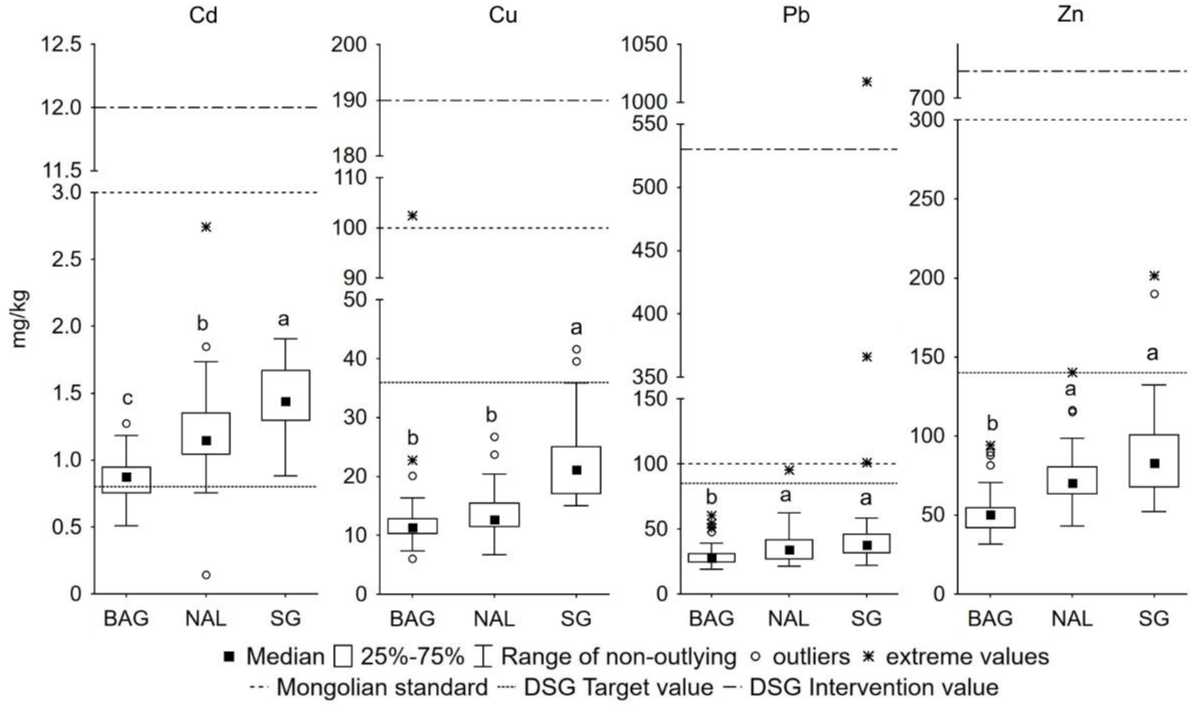


**Fig. 2**. Metal contents (mg/kg) in the urban soils of the coal mining towns.

Dependence of the soil contamination to pollution distribution on the mine's proximity is rather indirect or none (Fig. 3), with predominantly other determining factors. Similarly, Nottebaum et al.[7] did not find an immediate or even dominating impact of mining on soil contamination with As in Nalaikh. Thus, mining does not cause pollution there (Fig. 2, Table S1) and can hardly even be associated with local contamination (Fig. 3).

With Baganuur and Sharyn Gol, the signs of contamination to pollution are mainly in the original town centre with paved roads and multi-storey buildings (Fig. 3). The assumption is that there has been more intensive traffic that is associated with higher emissions of metals. Furthermore, the general coverage of the soil with impermeable surfaces (e.g. buildings, parking lots) could increase the occurrence of pollution hotspots in limited places that have long allowed the retention and accumulation of contaminating particles. However, the situation was the opposite in Nalaikh, with higher $IPI_N$ values in ger areas.

The division of residential areas into areas with multi-storey buildings and gers is such a unique Mongolian phenomenon that it is addressed in other studies. Coal is the primary energy source in ger areas, and burning-generated ash is becoming one of the primary sources of *in situ* soil pollution due to improper disposal[45,46]. Kasimov et al.[47] stated that the soils of the districts with multi-storey buildings are more polluted than the ger districts. Chonokhuu et al.[37] found different pollution patterns in this regard, as in Ulaanbaatar and Erdenet, ger area > apartment area, while in Darkhan, apartment area > ger area; and explain them by different socio-economic features and ageing of cities. However, Bilguun et al.[20] found the opposite pollution order in Ulaanbaatar as downtown > suburban settlement > ger area. These results suggest that there may be considerable variability or inappropriate sampling methodology even within one city. An additional verifying statistical comparison was not applicable for the studied towns due to the limited number of samples from the areas with multi-storey buildings or their borderline characters.

The reason for the different patterns in urban design-moderated pollution distribution in Nalaikh compared to Baganuur and Sharyn Gol may be the utilisation of low-quality coal with a higher content of metals. Due to frequent small-scale illegal mining in Nalaikh[7], such coal without quality control may be often utilised in ger areas, dispersing more contaminated ash and increasing pollution.

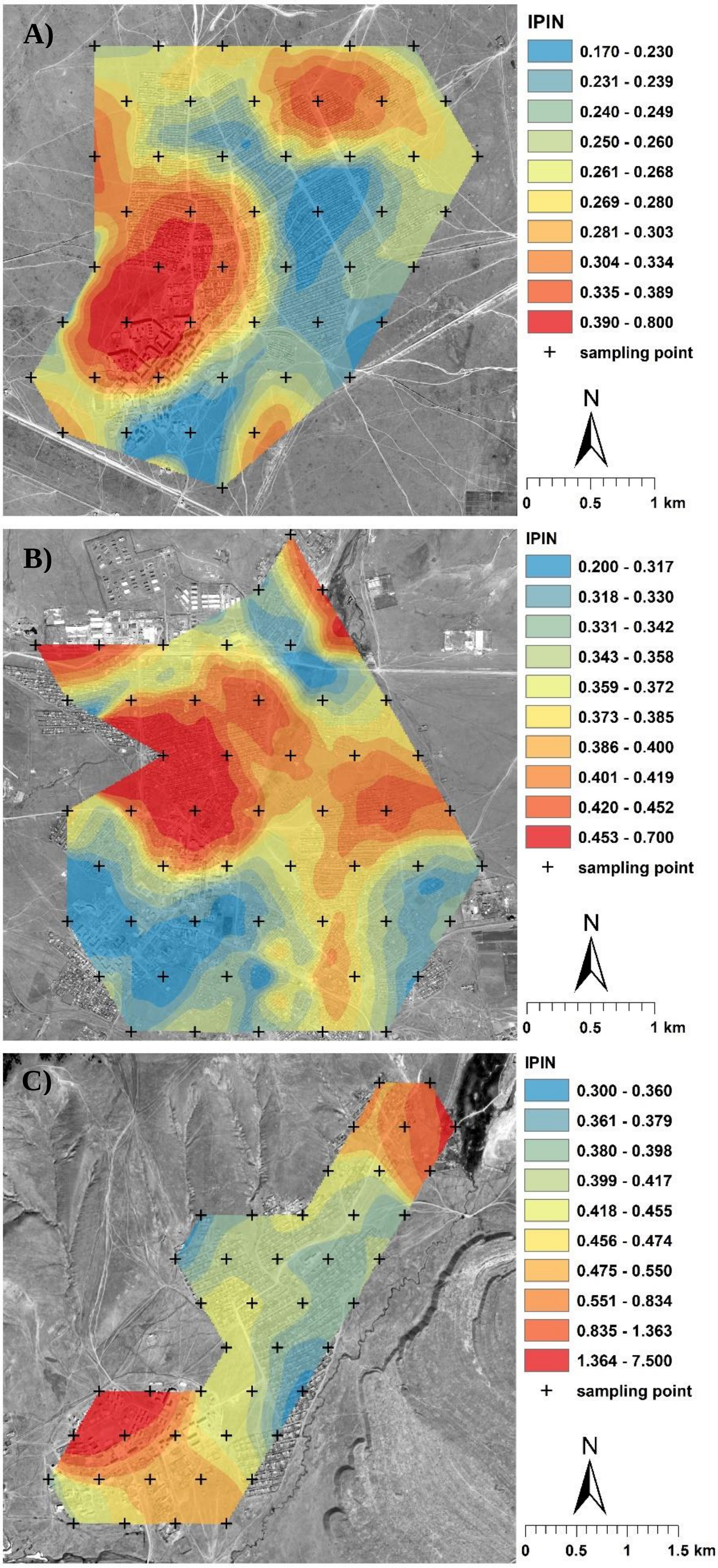


**Fig. 3**. Integrated Nemerow Pollution Index ($IPI_N$) assessment-based pollution distribution in the coal mining towns: A) Baganuur, B) Nalaikh, and C) Sharyn Gol.

Only an increase in $IPI_N$ values in the north-eastern part of Sharyn Gol (Fig. 3C) can be directly linked to mining. Generally, the higher $IPI_N$ values in this town (Table S1) may be related to the mining waste heaps deposited near it in the east. The contents of Cd, Cu, Pb and Zn are mostly increased there, about 1.91, 40.5, 58.4 and 104 mg/kg, respectively (own unpublished data). Due to the absence of vegetation cover or other protection against aeolian transport, dust particles containing metal(loid)s can be easily introduced into the town. Hills form the northern and western borders of the town, and a mine and other heaps form the southern one. The town's location in this valley increases the risk of sedimentation and accumulation of contaminants.

### 3.2 Urban soil pollution in Mongolia

So far, studies performed on urban soil contamination with metal(loid)s in Mongolia have focused strictly on the three largest cities (Table 3). In addition, the limit of these studies is a low number of samples, and the limited sampling design only focused on some parts of the city or a specific phenomenon (e.g. roadside soils, ger areas). Therefore, they do not provide a comprehensive view of the actual pollution situation in the cities. The quality of some may also be questioned due to insufficient or unreliable descriptions of results or methodologies. However, their results suggest that soil pollution in Mongolia is less severe than expected.

**Table 3**. Summary of studies on contamination of Mongolian urban soils with metal(loid)s (average values in mg/kg); n = number of samples; DSG = Dutch Soil Guidelines; studies exceeding all the standards are bold.

| City/Town | n | As | Cd | Cr | Cu | Hg | Ni | Pb | Zn | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Baganuur | 48 | | 0.87 | | 13.6 | | | 29.6 | 51.7 | This study |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Darkhan | 25[*] | | | **704**** | 50.8 | 0.06 | 32 | 29.2 | 63.6 | Kosheleva et al.[21] |
| | 21 | 3.33 | | 31.9 | | | 19.5 | 20.9 | 67.3 | Chonokhuu et al.[37] |
| | 18[1] | 5.22 | 0.23 | 32.1 | 18.5 | | 15.2 | 29.9 | 82.3 | Timofeev et al.[30] |
| Erdenet | 18[1] | | | 113 | **450** | 0.10 | 35 | 24.7 | 62.2 | Kosheleva et al.[21] |
| | 30 | 12.8 | | 65.7 | | | 29.3 | 18.1 | 155 | Chonokhuu et al.[37] |
| Nalaikh | 50 | | 1.21 | | 13.8 | | | 36.4 | 73.7 | This study |
| Sharyn Gol | 44 | | 1.46 | | 22.1 | | | 69.0 | 87.6 | This study |
| Ulaanbaatar | 22 | 14 | 0.8 | 20.3 | 35.9 | | 18.7 | 63.9 | 159 | Batjargal et al.[17] |
| | 23[*] | | | 72.6 | 56.5 | 0.19 | 30.6 | 59.4 | 105 | Kosheleva et al.[21] |
| | 90 | 10.8 | 0.25 | 32.7 | 22.4 | | 17.0 | 45.9 | 120 | Kasimov et al.[47] |
| | 29 | | | | | 0.09 | | | | Chung and Chon[19] |
| | 10 | | | | | | | 39.1 | | Tserenpil et al.[48] |
| | x[***] | 10.3 | | **685** | | | | 33.0 | 113 | Naidansuren et al.[49] |
| | 27 | 28.0 | | 16.6 | | | 21.3 | 43.1 | 106 | Chonokhuu et al.[37] |
| | 42 | 22.9 | 0.2 | 29.0 | 28.9 | | | 34.5 | 136 | Battsengel et al.[45] |
| | 22 | 9.41 | 0.25 | 30.0 | 20.8 | | | 45.1 | 116 | Bilguun et al.[20] |
| | 28 | 16.4 | | **1987** | 53.5 | | 13.6 | 33.2 | 111 | Oyunbat et al.[50] |
| Mongolian standard | | 6 | 3 | 150 | 100 | 2 | 150 | 100 | 300 | MNS 5850:2008[34] |
| DSG target value | | 29 | 0.8 | 100 | 36 | 0.3 | 35 | 85 | 140 | VROM[33] |
| DSG intervention value | | 55 | 12 | 380 | 190 | 10 | 210 | 530 | 720 | VROM[33] |

[*] Data selected from the study's category with the most samples

[**] Possible incorrect value inconsistent with the text of the article

[***] XRF readings on 340 points

Cadmium is one of the most important contaminants in urban soils[35]; however, its contents were atypically low (≤0.25 mg/kg) in most studies or have not been studied (Table 3). The exceptions are the coal mining towns, which exceeded the DSG target value. Arsenic in the urban soils (Table 3), originating mainly from coal combustion[20,47,51], regularly (80%) exceeded the Mongolian limit. However, the value given by this standard can generally be considered extremely low, even compared to background values. In the context of DSG target value, the urban soils content of As is low. In addition, Nottebaum et al.[7], partly studying urban soils of Nalaikh, stated that As does not pose a ubiquitous risk there. Extreme Cr contents (Table 3) exceeding DSG intervention value in Ulaanbaatar (reaching 1987 mg/kg) and possibly in Darkhan (704 mg/kg) are associated with local pollution by the leather processing industry[21,49,50]. Given the importance of the leather processing industry using Cr-based technology in traditionally pastoral Mongolia[52], similarly significant pollution with Cr can be expected elsewhere.

Increased Cu content (450 mg/kg) compared to other cities and standards (Table 3) was found only in Erdenet, where Cu-Mo mining occurs. Timofeev et al.[51] also found soil contamination with Cu in the Erdenet area. The Hg contents (Table 3) commonly associated with coal combustion are surprisingly low[19]. This finding may be misrepresented by the low number of studies addressing this element. However, Chung and Chon[19] explain that Ulaanbaatar's Hg contamination is lower than in other cities with analogous circumstances because of the lower Hg concentration in the coal used and the spatial and temporal trends in coal usage. The contents of Ni and Pb (Table 3) are also low and balanced between cities, which indicates their low anthropogenic emissions or similar contamination patterns without risk expectations. The increased Zn values in Ulaanbaatar compared to other cities (Table 3) may be related to: (I) different natural backgrounds, (II) long-range transport of Zn emissions from the heavy industry area in China potentially affecting Ulaanbaatar[18] or (III) higher local emissions of Zn. However, we can relatively reject hypotheses I and II, given the possible background values (Table 4). Therefore, an important local source of Zn is expected. This may be related to heavy traffic[53] and various industries because these sources of Zn are expected to be important in Ulaanbaatar[20]. However, Zn contents are also low compared to the standards (Table 3).

Due to the different calculation methods, the pollution level mentioned in the summarised studies is not comparable. Despite the oft-cited moderate or high contamination or pollution (the ambiguity is mainly based on incorrectly used terminology) by at least one element (Table 4), comparisons with the standards (Table 3) suggest considerably low contamination and no pollution. A notable exception is the study of Chonokhuu et al.[37], reporting the predominant absence of contamination in the three largest Mongolian cities. However, there is no discussion of other studies' results concerning these cities or a better explanation of the results, reducing the credibility of the conclusions.

**Table 4**. Background values (mg/kg) used in the studies on urban soil pollution in Mongolia together with generalised level of contamination/pollution calculated based on these background values (where clearly indicated); colours signals "contamination/pollution" level found/indicated in the respective study: green = none, yellow = low/minor, orange = moderate, red = high/heavy; $I_{geo}$ = geoaccumulation index; EF = enrichment factor; PI = pollution index.

| City | Background | Index | As | Cd | Cr | Cu | Hg | Ni | Pb | Zn | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Darkhan | Non-affected soil* | $I_{geo}$ | 3.3 | | 38.2 | | | 12.7 | 46.7 | 52.3 | Chonokhuu et al.[37] |
| | Regional background sampling | $I_{geo}$ | 4.1 | 0.20 | 25.0 | 14.0 | | 16.0 | 19.0 | 61.0 | Timofeev et al.[30] |
| Erdenet | Non-affected soil* | $I_{geo}$ | 4.0 | | 60.0 | | | 18.6 | 15.0 | 77.8 | Chonokhuu et al.[37] |
| Ulaanbaatar | Regional background sampling | | 8.6 | 0.14 | 16.2 | 11.7 | | 10.7 | 14.7 | 46.7 | Kasimov et al.[47] |
| | Suburbs of the city | $I_{geo}$ | | | | | 0.02 | | | | Chung and Chon[19] |
| | Side of the Tuul river | PI | 9.0 | | 115 | | | | 29.0 | 97.0 | Naidansuren et al.[49] |
| | Non-affected soil* | $I_{geo}$ | 14.2 | | 13.0 | | | 14.7 | 37.9 | 114 | Chonokhuu et al.[37] |
| | Regional background sampling** | | 12.0 | 1.0 | 45.0 | 25.0 | | | 20.0 | 60.0 | Battsengel et al.[45] |
| | Upper continental crust | EF | 2.0 | 0.10 | 35.0 | 14.3 | | | 17.0 | 52.0 | Bilguun et al.[20] |
| | Unpolluted soil around the city | PI | 9.5 | | 84.5 | 15.5 | | 13.5 | 21.0 | 55.5 | Oyunbat et al.[50] |

* Based on the references (local studies) and the composition of the continental crust

** Cited from the local source studying background concentration of microelements in Ulaanbaatar's regional natural surface soil

An overall weakness in the soil contamination assessment is the lack of knowledge of background values or their usage inconsistency, which can distort the actual situation significantly. Studies assessing local contamination often used general standardised values, such as Kosheleva et al.[21], Chonokhuu et al.[37] and Bilguun et al.[20]. Using local background values is less common, and the values for the same cities vary considerably in different studies (Table 4). As a result, for example, Cr values ranging from 13.0 to 115 mg/kg are used as a background in the Ulaanbaatar. The disagreement emphasises the need for a better methodological basis for sampling. This finding also indicated that the reality of contamination of Mongolian urban soils might differ from what the studies suggest, as there is no credible background that the authors agree with at the local level.

### 3.3 Soil pollution in Mongolian mining areas

Studies on soil pollution related to mining in Mongolia can be divided into two groups: I) accompanying soil assessment in the study of water contamination and II) assessment of mining areas partly including urban units. The studies concern three basic mineral resources: Au (Boroo, Zaamar) Cu (Erdenet) and coal (Baganuur, Nalaikh).

**Au**: The results of Inam et al.[54] suggest that, except for As (3.4–261 mg/kg), soil metal(loid) contents are within the acceptable levels of the Mongolian standard[34] in the vicinity of the Boroo gold mine. However, the authors pointed out that the waste material may be a source of future contamination. Later, Oyuntsetseg et al.[55] found Cu, As and Pb enrichment in soils at the Boroo small-scale mining area that exceeded the standard in As (167 mg/kg) and Cu (109 mg/kg) in the gold washing location. In Zaamar Goldfield, Jarsjö et al.[56] found elevated soil contents of As (13 mg/kg), Ni (28 mg/kg), Cu (25 mg/kg) and Cr (69 mg/kg) compared to non-local backgrounds; however, only As exceeded the standard. Thus, only As can pose a potential threat in limited and extreme cases of gold mining operations.

**Cu**: Most attention has been focused on Cu-Mo mining in Erdenet. Battogtokh et al.[57] stated that the soils in the Erdenet mining area are highly contaminated with Cu. While Timofeev et al.[51] designated the studied soils as urban, we assigned the overall study conclusions to this chapter due to the predominance of out-of-city sampling. The authors found contamination and potential environmental risk related to Cu and As exceeding the Mongolian standard. Yondonjamts et al.[58] achieved the same conclusions. Dust production by grinding and transportation associated with the mine operation and wind erosion of technogenic sands of the tailings were highlighted as important factors for contamination of the mining area[51,57,59].

**Coal**: Park et al.[60] expect the risk of As poisoning due to above-standard As contents (5.57–14.2 mg/kg) in the soil dust from the Baganuur mine; however, they draw conclusions only from five samples and still low As contents in the context of other standards (Table 3). Nottebaum et al.[7] found As contents in Kastanozem topsoil and fluvial-alluvial sediments of the Nalaikh area ranging from 4.6 to 16 mg/kg, and consider them low and not a serious threat to the environmental compartments. Differences in conclusions, despite similar values, highlight the inconsistency in the evaluation of results reported in Mongolian studies.

### 3.4 Risk assessment and future perspectives

Although many of the mentioned studies highlighted the issue of soil pollution with As in particular, its content is predominantly low by other international standards (Table 3). Assumedly, the threat of As in the Mongolian soils is considerably artificially escalated in the context of a low value of the Mongolian standard.

Given that soil contents of metal(loid)s in Mongolia are predominantly low (Table 3, Chapter 3.3), a low health risk may be expected. Chonokhuu et al.[37] confirmed this assumption, not finding serious health threats based on potential health risk assessments in Ulaanbaatar, Darkhan or Erdenet. Conversely, Battsengel et al.[45] emphasised possible health risks in

Ulaanbaatar. However, the description of the results in both studies suggests a partial misunderstanding of the calculation, as the authors present the generally applicable facts arising from the calculation methodology as unique results. In addition, errors can be found in both cases. The conclusions of Battsengel et al.[45] can, therefore, be considered incorrect.

According to a potential human health risk assessment (Table 5), there are no non-carcinogenic or carcinogenic risks in the coal mining towns posed by the soil contents of the metals. Only CR values of Cd indicated a possible threat as they fell into the acceptable total risk category for regulatory purposes.

**Table 5**. Average values of human health risk assessment via potential non-carcinogenic (HI) and carcinogenic risk (CR) following different exposure pathways in the coal mining towns.

| | | **Cd** | | | | **Cu** | | |
|---|---|---|---|---|---|---|---|---|
| | | **HQingest** | **HQdermal** | **HI** | **CR** | **HQingest** | **HQdermal** | **HI** |
| **Baganuur** | **Adult** | 1.28E-03 | 3.90E-04 | 1.67E-03 | 6.59E-06 | 5.03E-04 | 5.11E-06 | 5.09E-04 |
| | **Children** | 1.05E-02 | 1.68E-03 | 1.21E-02 | 1.35E-05 | 4.12E-03 | 2.20E-05 | 4.14E-03 |
| **Nalaikh** | **Adult** | 1.79E-03 | 5.44E-04 | 2.33E-03 | 9.19E-06 | 5.09E-04 | 5.16E-06 | 5.14E-04 |
| | **Children** | 1.46E-02 | 2.34E-03 | 1.69E-02 | 1.88E-05 | 4.16E-03 | 2.22E-05 | 4.18E-03 |
| **Sharyn Gol** | **Adult** | 2.15E-03 | 6.55E-04 | 2.81E-03 | 1.11E-05 | 8.16E-04 | 8.28E-06 | 8.24E-04 |
| | **Children** | 1.76E-02 | 2.82E-03 | 2.04E-02 | 2.26E-05 | 6.67E-03 | 3.56E-05 | 6.71E-03 |
| | | **Pb** | | | | **Zn** | | |
| | | **HQingest** | **HQdermal** | **HI** | **CR** | **HQingest** | **HQdermal** | **HI** |
| **Baganuur** | **Adult** | 1.25E-02 | 2.53E-04 | 1.27E-02 | 1.27E-07 | 2.54E-04 | 3.87E-06 | 2.58E-04 |
| | **Children** | 1.02E-01 | 1.09E-03 | 1.03E-01 | 2.60E-07 | 2.08E-03 | 1.66E-05 | 2.10E-03 |
| **Nalaikh** | **Adult** | 1.53E-02 | 3.12E-04 | 1.57E-02 | 1.57E-07 | 3.62E-04 | 5.52E-06 | 3.68E-04 |
| | **Children** | 1.25E-01 | 1.34E-03 | 1.27E-01 | 3.20E-07 | 2.96E-03 | 2.37E-05 | 2.99E-03 |
| **Sharyn Gol** | **Adult** | 2.91E-02 | 5.91E-04 | 2.97E-02 | 2.97E-07 | 4.31E-04 | 6.56E-06 | 4.37E-04 |
| | **Children** | 2.38E-01 | 2.54E-03 | 2.40E-01 | 6.07E-07 | 3.52E-03 | 2.82E-05 | 3.55E-03 |

Low metal contents in Mongolian mining areas (Fig. 2, Table 3, Chapter 3.3) and the associated low health risks contrast sharply with other mining areas of the world[26,36]. The reason may be a short history of mining. While intensive mining and mineral processing has been taking place for several centuries in some countries, the mining industry is still a young sector in Mongolia[2]. Limited precipitation and low humidity typical for Mongolia and the sorption stability of elements in lignite and sub-bituminous coal likely reduce weathering of mining waste and release of metal(loid)s into the environment within such a short time. Furthermore, limited local ore processing followed by direct export could also contribute to the current favourable situation. The last important factor is probably the wind erosion and wind transport of dust particles far from the deposits. However, the positives of this phenomenon can be ambiguous as particle accumulation must occur somewhere, and the creation of pollution hotspots can be expected.

A significant threat posed by the aeolian dispersion of contaminated dust particles from mining waste heaps is indicated in Sharyn Gol (Fig. 3C) and mentioned as risky in other related studies[7,57,59]. Park et al.[60] even stated that all waste soil samples from Baganuur appeared to have desertification potential. A possible solution for the dust risk decrease is phytostabilisation. Extreme conditions of mining waste, such as acidic pH, lack of nutrients, unsuitable substrate structure, and instability[61,62], can be mitigated using available organic materials, such as wood chips or manure[24]. This topic should be addressed by the relevant authorities and further research.

Poor management of mining sites often leads to livestock grazing on sparse vegetation of heaps and drinking from tailings dams in Mongolia (own observation). The risk of livestock intoxication and unsuccessful phytostabilisation must be reduced by fencing and permanent monitoring.

## 4. Conclusions

The results showed that the contents of Cd, Cu, Pb and Zn are low without significant pollution and health risk in the coal mining towns of Baganuur, Nalaikh and Sharyn Gol. Based on the available studies, the same conclusions can be drawn for other Mongolian cities. Only the Cr content associated with the leather industry can likely pose a significant threat and, therefore, warrants increased attention. The generally highlighted risk of As contamination, on the contrary, acts more like a virtual problem. Many soil contamination studies focused on Mongolia are based on the inappropriate methodology or an insufficient number of samples, reducing their results' quality and conclusions' relevance. Due to the potential for the long-term growth of the Mongolian mining sector, a precise metal(loid)s contamination assessment is highly needed. Given the results, future research on pollution in Mongolia should focus more on drinking water and street and mining dust pollution.

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### Author Contributions

V. P.: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Methodology, Writing - original draft. D. J.: Investigation, Methodology, Software, Visualization, Writing - review & editing. J. H.: Validation, Writing - review & editing. M. K.: Visualization, Software. J. K.: Funding acquisition, Resources, Supervision, Writing - review & editing. M. B.: Conceptualization, Funding acquisition, Resources, Project administration. R. K.: Supervision, Project administration, Writing - review & editing.

### Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

# PŘÍLOHA Č. 2

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Research article

# Polluted brownfield site converted into a public urban park: A place providing ecosystem services or a hidden health threat?

Václav Pecina[a,b], David Juřička[c], Michaela Vašinová Galiová[b,*], Jindřich Kynický[d], Ludmila Baláková[c], Martin Brtnický[a,b]

[a] Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00, Brno, Czech Republic
[b] Institute of Chemistry and Technology of Environmental Protection, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, 61200, Brno, Czech Republic
[c] Department of Geology and Soil Science, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 1, 613 00, Brno, Czech Republic
[d] BIC Brno, Purkyňova 125, 612 00, Brno, Czech Republic

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ABSTRACT

The conversion of old brownfield sites into places once again serving society is becoming an upward global trend, especially in urban areas. Due to the increasingly growing pressure on the expansion of urban green spaces, such sites can become, for instance, urban parks. The aim of the study was to assess whether the solution is appropriate and if it does not pose a potential health risk. Heavy pollution of soils was found out by means of the example of the urban park newly established in a reclaimed area of a historic mining town. The high average values in the topsoil were found out mainly in As (132 mg/kg), Cd (6.8 mg/kg), Pb (535 mg/kg) and Zn (1604 mg/kg). The assessment of the non-carcinogenic health risk has revealed possible As-related adverse health effects in children even at irregular park visits. According to the carcinogenic risk assessment, As, Cd, Cr and Ni can be ranked in the category of an acceptable total risk for regulatory purposes. The health status of park vegetation as a significant component of the urban ecosystem was also assessed. Soil phytotoxicity brought about severe damage to the seedlings, with a mortality rate of up to 84% locally. The results indicate that heavily polluted brownfield sites with historic mining-related activities are not suitable for establishing urban parks even after reclamation and nature-based solutions may not be invariably appropriate. Based on the findings, the management steps that ought to be implemented in the process of brownfield redevelopment into the urban park even after its establishment have been highlighted in order to minimize the health risk to park visitors while providing the required ecosystem services by vegetation.

## 1. Introduction

Historical environmental burdens (brownfield sites) pose a serious problem as potential sources of toxic substances (Križáni et al., 2009; Stefanowicz et al., 2014). Their risk arises mainly in urbanized areas where many people are exposed to their potentially negative impacts (Peña-Fernández et al., 2014). Based on the increasing knowledge of this issue, the current society has been trying to eliminate these burdens in recent years (Loures and Panagopoulos, 2007; Shackelford et al., 2018). Abandoned factories, landfills, heaps, and so forth, are gradually being redeveloped into sites that can serve society again, such as recreation sites (Loures and Panagopoulos, 2007; Janků et al., 2014; Song et al., 2019). One of the realized ways is the redevelopment of brownfields into urban parks (Dagenhart et al., 2006; Carew et al., 2015; Klenosky et al., 2017). Converting brownfield sites into green spaces brings social, economic, and environmental benefits (Song et al., 2019). In the case of redevelopment of a site with a historic deposition of potentially toxic elements (PTEs) into an urban park, reclamation, however, might conceal the threat of remaining pollution (Erdem and Nassauer, 2013; Urrutia-Goyes et al., 2017).

Urban parks and open green spaces have a strategic importance for the quality of life in cities (Rodríguez-Seijo et al., 2017; Brown et al., 2018). They provide many environmental and ecological services and important social and psychological benefits to people (Chiesura, 2004;

* Corresponding author.
*E-mail addresses:* vaclav.pecina@mendelu.cz (V. Pecina), david.juricka@mendelu.cz (D. Juřička), galiova@fch.vut.cz (M. Vašinová Galiová), jindrak@email.cz (J. Kynický), ludmila.balakova@mendelu.cz (L. Baláková), martin.brtnicky@mendelu.cz (M. Brtnický).

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Shanahan et al., 2015; Song et al., 2019). Urban society spends much of its free time in urban parks during recreation, particularly mothers with children, athletes, and elderly people (Brown et al., 2018). These park users, especially children, may easily get into direct contact with the soil (Gu et al., 2016). In the case of soil pollution, contaminants pose serious human health risks through dermal contact, oral ingestion, and particle inhalation (Wuana and Okieimen, 2011; Luo et al., 2012). Moreover, soil pollution presents risks not only for park visitors, but also for park vegetation (Brtnický et al., 2019), which is an essential component of the urban ecosystem (Rodríguez-Seijo et al., 2017).

PTEs can easily enter living organisms and pose a constant risk to the ecosystem and humans due to their nature and behaviour (Wuana and Okieimen, 2011; Peña-Fernández et al., 2014). Some PTEs, such as Cr, Cu, Mn, Ni and Zn, are essential for living organisms. However, these metals and metal(loid)s that are considered to be nonessential, such as As, Cd and Pb, can pose risks when their content and bioavailability are high in soil (Nagajyoti et al., 2010; Kabata-Pendias, 2011). The risk is great especially in the case of a chronic high intake of PTEs, which results in adverse effects on blood formation, nervous system, cardiovascular system, renal and reproductive system in humans (Christoforidis and Stamis, 2009). Some metal(loid)s, such as As, Cd, Cr, Ni and Pb, may even cause various types of cancer (Koedrith et al., 2013). PTEs toxicity also results in negative effects on plants, such as root damage, inhibition of photosynthesis, damage to photosynthesis apparatus, growth retardation and may eventually cause death (Nagajyoti et al., 2010; Kabata-Pendias, 2011). In heavily polluted soils, the soil toxicity can be so high that it does not allow the growth of any plants (Križáni et al., 2009).

The Příbram region is one of the most polluted areas in the Czech Republic (Šichorová et al., 2004), as evidenced by a number of studies on environmental contamination by PTEs as a consequence of polymetallic ore mining and smelting (e.g., Rieuwerts et al., 1999; Vysloužilová et al., 2003; Šichorová et al., 2004; Ettler et al., 2006). Mining had been active there since the Middle Ages when primary silver was extracted (Ettler et al., 2009). During the 20th century mining began to be unprofitable. Therefore, it was gradually limited until it was finally terminated in 1979 (Ettler et al., 2006). The town of Příbram actively and gradually tries to return the landscape degraded by mining to the possibility of recreational use (Janků et al., 2014). An example of this effort is the newly established forest park Březové Hory.

Human health risk assessments of PTEs in urban soils (Peña-Fernández et al., 2014; Gu et al., 2016) as well as assessment of their phytotoxicity in contaminated soils (Vysloužilová et al., 2003; Brtnický et al., 2019) have been frequently discussed topics over recent years. Nevertheless, their combination on the mining related site redeveloped into an urban park is a unique research topic, as the elimination of environmental threats after technical and biological reclamation is expected. Moreover, scientific evidence regarding the suitability of nature-based solutions for brownfield redevelopment in cities is demanded (Song et al., 2019).

The hypothesis of this study was that public green spaces redeveloped from brownfield sites may still pose a serious and persistent risk for living organisms. This hypothesis was verified on the example of the forest park Březové Hory. The aims of this study were: (1) to determine PTEs (As, Cd, Cr, Cu, Mn, Ni, Pb and Zn) contents in the urban park soils and trees; (2) to calculate indices assessing the level of pollution and health risks; (3) to assess the soil pollution impact on the park trees; (4) to evaluate redevelopment of brownfield sites associated with mining into public spaces; and (5) to highlight the management steps necessary to ensure safe redevelopment of brownfield sites into urban parks and subsequent park maintenance.

## 2. Materials and methods

### *2.1. Study area*

The area of the forest park Březové Hory originally included the mining-processing plant and a heap. Flotation in the ore processing plant was finished in 1991. Although the risk analysis carried out in 2009 demonstrated no human health risks, a hazard to the Litavka River ecosystem was found (Řehoř, 2014; DIAMO, 2017). Based on that, a reclamation project was developed. Firstly, in the period 2011–2012, the plant was demolished, and its area was reclaimed. Subsequently, between 2012 and 2013, the surface of the heap was remodelled, to ensure precipitation-runoff through the drainage ditch system, and to modify the surface with materials to reduce groundwater contamination by rainwater, which would leach deeper deposited waste material. Landscape reclamation consisted of biological reclamation with predominantly forest-technical reclamation combined with grassed areas (DIAMO, 2017). Based on the type of planned vegetation, the depth of the soil cap (the layer of uncontaminated soil) was realized. In the afforestation sections, a 15 cm deep topsoil layer was created (Řehoř, 2014). The park has already been used for recreation by the residents.

### *2.2. Soil and vegetation sampling*

The samples were collected in the autumn of 2017. Ten randomly selected representative sampling plots (10 × 10 m) were established on the reclaimed area (RA) following a random distribution of the afforestation (Fig. 1). Moreover, two comparative plots in the park area with spontaneously developed tree vegetation (succession area) on the original material and two comparative plots on the nearby heap (heap area) with spontaneously developed vegetation were established.

Soil sampling on the RA was carried out at two depths (0–10 and 10–20 cm) of the upper rooting zone. The division into the two depths was chosen for the sake of more precise potential risk demonstration posed to humans and plants. The depth of 10–20 cm already represented a mixture of the original contaminated and new uncontaminated soil. Five soil samples (4 in corners and 1 central) were collected on each sampling plot and mixed into one composite sample for each depth. The final composite sample consisted of 500–1000 g of soil. The same methodology was followed for the succession area. In the case of the heap, only the topsoil (0–10 cm) was sampled due to the character of the soil-forming substrate. In total, 26 soil samples were taken.

Tree leaves were studied to assess the PTE contents in the vegetation, their sampling was carried out in parallel with the soil sampling. Considering the aims of the study, only a composite sample of present tree species was made. The leaves were taken with petioles from the representative trees. In the case of the plot Succession 1, needles (older) were taken due to the absence of mature broadleaf species. In total, 14 samples (13x leaves and 1x needles) were collected.

### *2.3. Dendrological-dendrometric mapping*

Tree species composition and their numbers were surveyed on all the plots. At the same time, the number of living and dead individuals was counted, and their visual inspection was performed evaluating leaf browning, deformation of young leaves, chlorosis of the remaining leaves, defoliation, and crown dieback.

Several tree species were used for afforestation, namely dominating common oak (*Quercus robur* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.), as well as small-leaved lime (*Tilia cordata* Mill.), Norway maple (*Acer platanoides* L.), sycamore (*Acer pseudoplatanus* L.) and common hornbeam (*Carpinus betulus* L.). In spontaneously developed park vegetation mature individuals of scots pine (*Pinus sylvestris* L.) dominated, accompanied by silver birch (*Betula pendula* Roth). In the undergrowth, there was a natural regeneration of other tree species such as *Acer* sp. and *Quercus* sp. The composition of the stands growing on the heap consisted of sycamore, Norway maple, silver birch, Norway spruce (*Picea abies* (L.) H. Karst), ash (*Fraxinus excelsior* L.) and goat willow (*Salix caprea* L.).

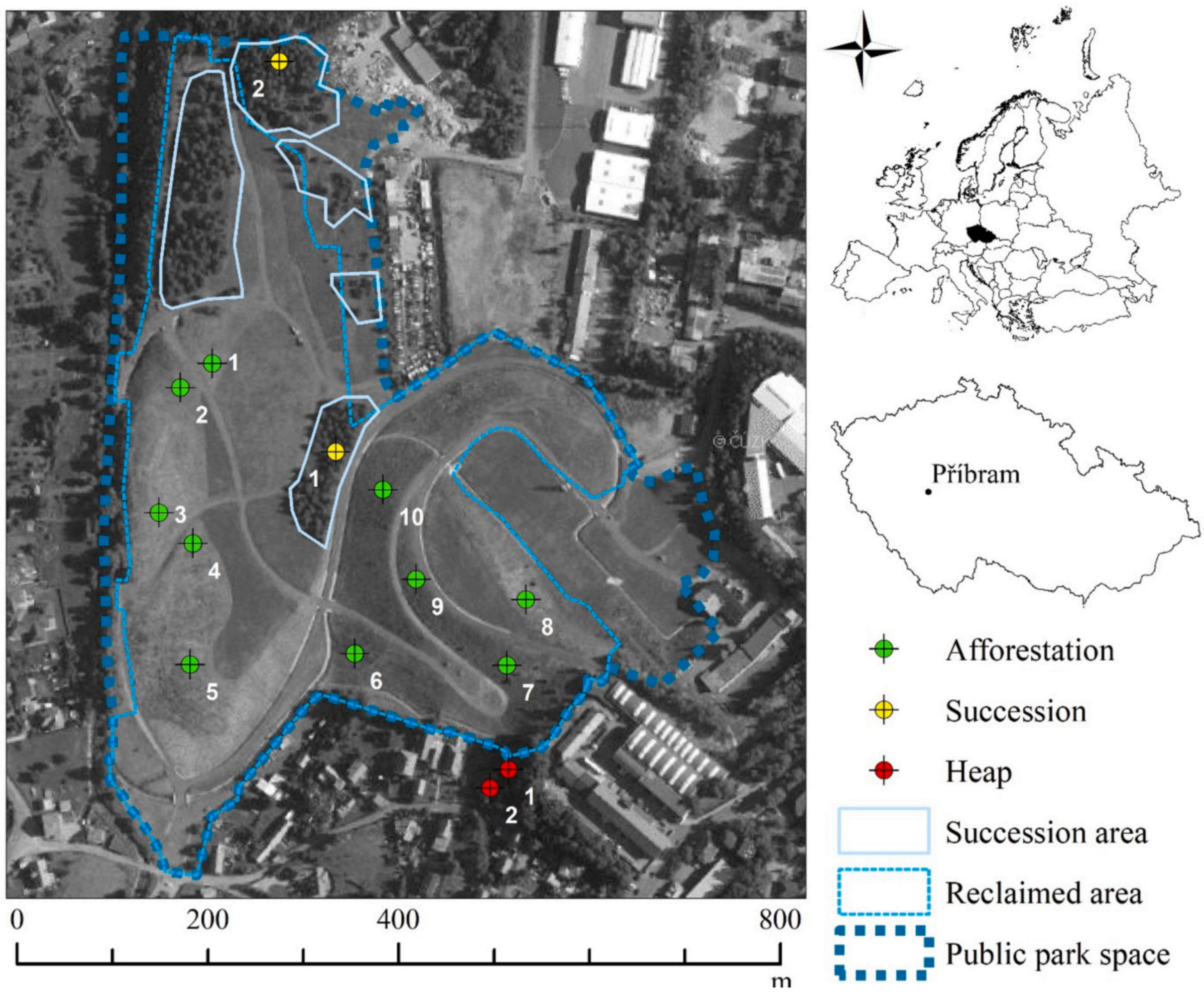


**Fig. 1.** Study area.

*2.4. Sample analysis*

The soil samples were dried at room temperature, gently disaggregated, sieved through a nylon sieve (2.0 mm mesh), and milled for analysis of the PTEs. The leaf samples were washed with running distilled water to remove dust particles, dried at room temperature in dust-free environment and ground into fine powder.

To determine the soil organic matter content, a loss of the ignition method was chosen (Zbíral et al., 1997), soil texture was determined using the sedimentation method (ISO 11277:2009), and soil pH was measured in 1M KCl (ISO10390:2005).

The PTEs from the soil samples were extracted with the *aqua regia* (Zbíral, 2011). Three grams of the sample were left in the acid mixture (21 ml HCl and 7 ml $HNO_3$) for 16 h to self-interact and then were subsequently heated for 2 h under the reflux condenser. The PTEs were measured in the filtered extract with volume of 100 ml.

Two types of decomposition were performed in case of plant material (ÚKZÚZ, 2014). As, Cd and Pb were determined in the solution after decomposition of 0.5 g of the dried sample in 2 ml of $HNO_3$ and 2 ml of $H_2O_2$ in a microwave device MILESTONE MLS-1200MEGA. After decomposition, the solutions were quantitatively transferred to a volume of 50 ml. Other elements (Cr, Cu, Mn, Ni and Zn) were determined in the solution after dry mineralization, where 5 g of the sample were burned in a muffle furnace at 450 °C for 12 h. The ash was then dissolved by boiling in 3 mol/l HCl, transferred to 50 ml and filtered.

Determination of As, Cd in all the samples and Pb in the case of plant material was performed using GBS AVANTA PM spectrometer (GF-AAS) with temperature programmes given in Table S1. The hollow cathode lamps emitting the wavelengths of As 188.6 nm, Cd 228.8 nm and Pb 283.3 nm were applied as the radiation source. The 20 µl of sample with 2 µl of Ni (1 g/l) as a modifier for As and 10 µl + 2 µl of 1% $NH_4H_2PO_4$ for the determination of Cd and Pb were dosed and argon was used as inert gas. The relative standard deviation was, in all the cases, below 8%.

The contents of Cr, Cu, Mn, Ni, Zn (for all samples) and Pb (for soil samples only) were measured by GBS SavantAA (F-AAS) in the flame $N_2O$-acetylene (Cr) and acetylene-air (Cu, Mn, Ni, Zn and Pb). The following wavelengths were used: Cr 357.9 nm, Cu 324.8 nm, Mn 280.1 nm, Ni 232 nm, Zn 213.9 nm and Pb 217 nm. LOQ of the entire methodology including decomposition, solution preparation and measurement for soil samples was 2.5 mg/kg for all the elements. In case of plant tissue, LOQ equal to 0.1 mg/kg for Cr, Cu, Ni, and Zn and 0.2 mg/kg Mn has been established. The relative standard deviation was, in all the cases, below 3%.

The background correction with a deuterium lamp was applied for all the elements and the certified reference materials Astasol (Analytika, Czech Republic) were used for the preparation of the calibration solutions and quantification.

Verification of decomposition as well as solution analysis have been accomplished using CRM Metranal 3 (strawberry leaves) and AN-ZP01 (heavy loam soil). They allow LOQ to be 0.5 mg/kg As, 0.1 mg/kg Cd for soil samples and 0.05 mg/kg As, 0.01 mg/kg Cd and 0.01 mg/kg Pb for plant material. The soil recovery for the PTEs reached the following values: As 96%; Cd 100%; Cr 98%; Cu 103%; Mn, Ni and Zn 102%; and Pb 97%. For plants, the values were: As 110%; Cd and Ni 103%; Cr 89%;

Cu 97%; Mn 94%; Pb 91%; and Zn 99%.

### 2.5. Soil and plant pollution indicators

The content of the PTEs in soil was assessed in compliance with the Czech legislation, Decree No. 153/2016 Coll., on the quality of soil and Dutch Soil Guidelines (VROM, 2013). The Dutch standard is widely applied in research studies throughout the world (Chabukdhara a Nema, 2013; Cheng et al., 2014; Brtnický et al., 2019).

Pollution indicators (indices) calculate the level of environmental contamination, especially in relation to background values. However, under reclamation conditions with non-originating imported substrate, the background values are difficult to be determined. In this case, the situation is made even more demanding by the fact that the area is in the mining region with large-scale and long-term anthropogenic deposition of PTEs. Therefore, for the purposes of assessing the level of environmental pollution, the Integrated Nemerow Pollution Index ($IPI_N$) using standard values was chosen. $IPI_N$ classes are: ≤0.7: safe; 0.7–1: precaution; 1–2: slight pollution; 2–3: moderate pollution; ≥3: heavy pollution. $IPI_N$ was calculated as follows (Cheng et al., 2014):

$$PI_i = \frac{C_i}{T_i}$$

$$IPI_N = [(PI^2{}_{avg} + PI^2{}_{max})/2]^{1/2}$$

Where:

$PI_i$ means single pollution index of individual PTE.

$C_i$ means content of the PTE.

$T_i$ means target value of the PTE by VROM (2013).

$PI_{avg}$ means average value of all $PI_i$ of the PTEs.

$PI_{max}$ means maximum $PI_i$ value of the PTEs.

To evaluate the relationship between PTEs in soils and plants, bioconcentration factor (BCF) was used. It provides a simple method to characterize quantitatively the transfer of available PTEs from soil to a plant (Branzini et al., 2012). BCF was calculated as the ratio of the PTE content in the leaves to the total PTE content in the soil (Monterroso et al., 2014; Zhu et al., 2018). Plants with a BCF value of <1 correspond to PTE excluders, and a BCF value of >1 to accumulators (Monterroso et al., 2014). Possible interpretation of a BCF is also that its greater value indicates a stronger accumulation ability of PTEs (Zhu et al., 2018).

### 2.6. Human health risk assessment

Potential human health risk assessment was represented by Hazard Index (HI) and Carcinogenic Risk (CR) in this study. The HI and CR for PTEs with potentially non-carcinogenic and carcinogenic effects, were calculated, respectively, based on their corresponding average daily dose (ADD), reference dose (RfD), and slope factor (SF) values. Human exposure to PTE in soils ($C_{soil}$) can occur via ingestion ($ADD_{ing}$), dermal contact ($ADD_{derm}$) and inhalation ($ADD_{inh}$). HI values of <1 indicate no adverse health effects, whereas HI values > 1 indicate probability of adverse health effects. CR values $\leq 10^{-6}$ represent virtual safety, and CR values $\geq 10^{-4}$ indicate a potentially great risk. The range of acceptable total risk for regulatory purposes is $10^{-6}$ to $10^{-4}$ (Luo et al., 2012; Chabukdhara and Nema, 2013; Brtnický et al., 2019).

The following equations were used for the intake estimations via each exposure pathways (De Miguel et al., 2007; Chabukdhara and Nema, 2013; Urrutia-Goyes et al., 2017):

$$ADD_{ing} = \frac{C_{soil} \times IngR \times EF \times ED}{BW \times AT} \times 10^{-6}$$

$$ADD_{derm} = \frac{C_{soil} \times SA \times AF \times ABS \times EF \times ED}{BW \times AT} \times 10^{-6}$$

$$ADD_{inh} = \frac{C_{soil} \times InhR \times EF \times ED}{BW \times AT \times PEF}$$

Non-carcinogenic toxic risk was determined by calculating the Hazard Quotients (HQ) and HI as follows:

$$HI = \sum HQ_i = \sum \frac{ADDi}{RfDi}$$

Carcinogenic risk was calculated for potentially carcinogenic PTEs (As, Cd, Cr, Ni and Pb) as follows:

$$CR = \sum ADD_i \times SF_i$$

The details of the input parameters and the used values are given in Tables S2 and S3. The values were selected based on their frequency in research studies, which are widely accepted at the international level. Studies with a similar topic (especially urban parks, urban soils) were preferred. At the same time, there was the effort to use local (national) values (weight, age) for more accurate results.

### 2.7. Statistical data treatment

Basic raw data processing was performed using Microsoft Excel®. Statistica 12® was used for statistical analyses. Differences in the soil PTE contents, pH and organic matter between the depths were tested. Data normality was investigated using Shapiro–Wilk normality test ($p > 0.01$). One-way ANOVA was used for both normal series; in the case that at least one of the series did not pass the normality test, a nonparametric Kruskal-Wallis ANOVA was used. The Spearman's Rank-Order Correlation analysis was applied to investigate the relations between the leaves and soils PTE contents.

## 3. Results and discussion

### 3.1. Soil pollution

The basic soil properties were balanced at both depths of the RA soils (Fig. S1). The range of pH values 7.45–7.74 indicated the alkaline type of reaction. The average organic matter contents pointed to slightly humic horizon with values of 1.81% (0–10 cm) and 1.84% (10–20 cm). The RA soils were classified as sandy loam.

The PTE contents in the RA soils (Fig. 2, Table S4) showed considerable variance even in the topsoil (0–10 cm). Such a high level of heterogeneity is typical for many mine tailings (Monterroso et al., 2014), but is striking for the RA with the soil cap (0–15 cm) using the uncontaminated material. This finding indicates mixing with the original contaminated material, thus a poor implementation of the technical part of the reclamation. This assumption was confirmed by the absence of a significant difference in PTE contents between the depths (Fig. 2).

In comparison with the original soils (succession and heap areas) (Table S4), it is evident that the reclamation resulted in a decrease in the contents of As, Cd, Cu, Mn, Ni, Pb and Zn. Despite this, the average values are above the standards (Fig. 2, Table S4). The Czech Decree No. 153/2016 Coll. was exceeded several times in the case of As, Cd, Pb and Zn in the RA soils. The Intervention Value was exceeded by As, Pb and Zn in the same category; the exceedance of this value indicates that functional properties of the soil for human, plant and animal life, are seriously impaired or threatened (VROM, 2013). A low and balanced Cr content indicates that there is no seriouscontamination by this element. The Ni content exceeded the Target Value in the case of non-reclaimed areas, but the reclamation led to its reduction below the values of the standards. There are no limits for Mn in the given standards.

The pollution assessment has shown that the park topsoil can be classified as heavily polluted ($IPI_N = 9.12$ at 0–10 cm depth) even after reclamation. The mixed soil (10–20 cm) can also be classified as heavily polluted ($IPI_N = 11.3$).

When comparing the average values found in this urban park with

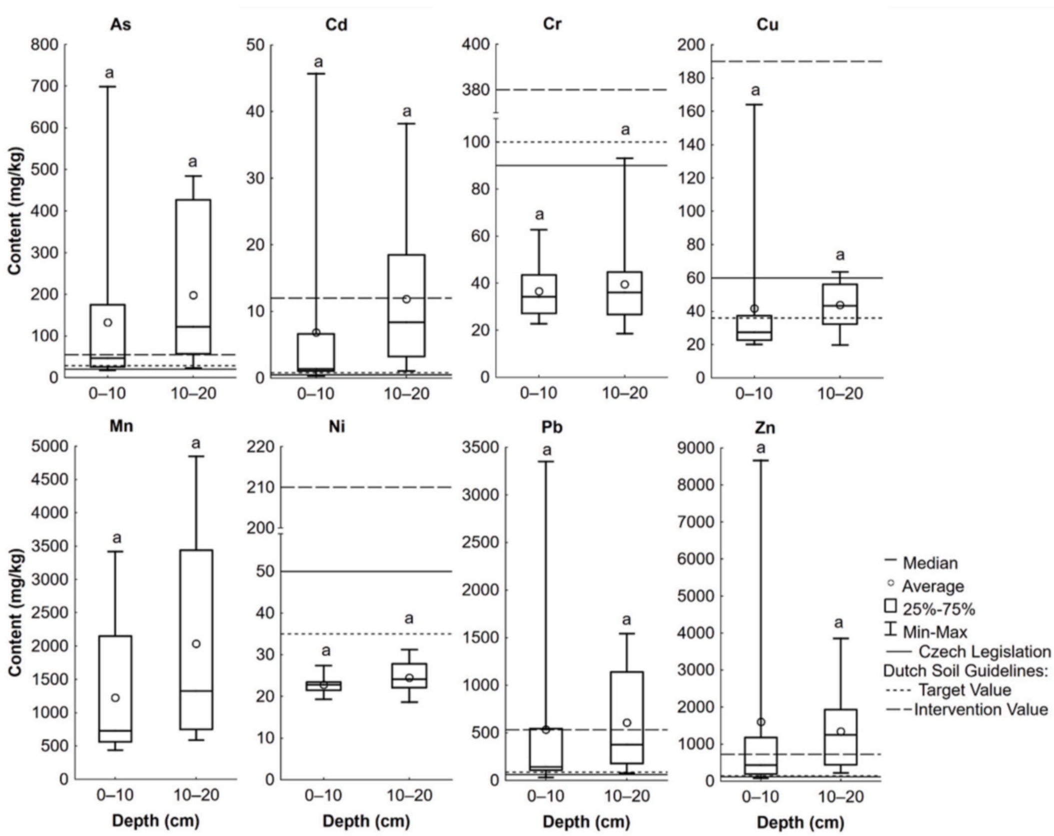

**Fig. 2.** PTE contents (mg/kg) in soils of the RA forest park Březové Hory; available soil pollution standards for As, Cd, Cr, Cu, Ni, Pb and Zn from the Czech Legislation (Decree No. 153/2016 Coll.) and Dutch Soil Guidelines (VROM, 2013); different letters indicate statistically significant differences at $p < 0.05$ (Kruskal-Wallis ANOVA).

other parks (Luo et al., 2012; Peña-Fernández et al., 2014; Gu et al., 2016; Urrutia-Goyes et al., 2017; Brtnický et al., 2019), it is particularly evident that the high average contents of As, Cd and Zn are well above the maximum values found out in the previously mentioned studies. The content of PTEs in the soils after reclamation is closer to the content in garden soils polluted by a secondary lead smelter located nearby the study area, where, for instance, As, Cd and Zn reached 153, 10.6 and 1180 mg/kg (Rieuwerts et al., 1999).

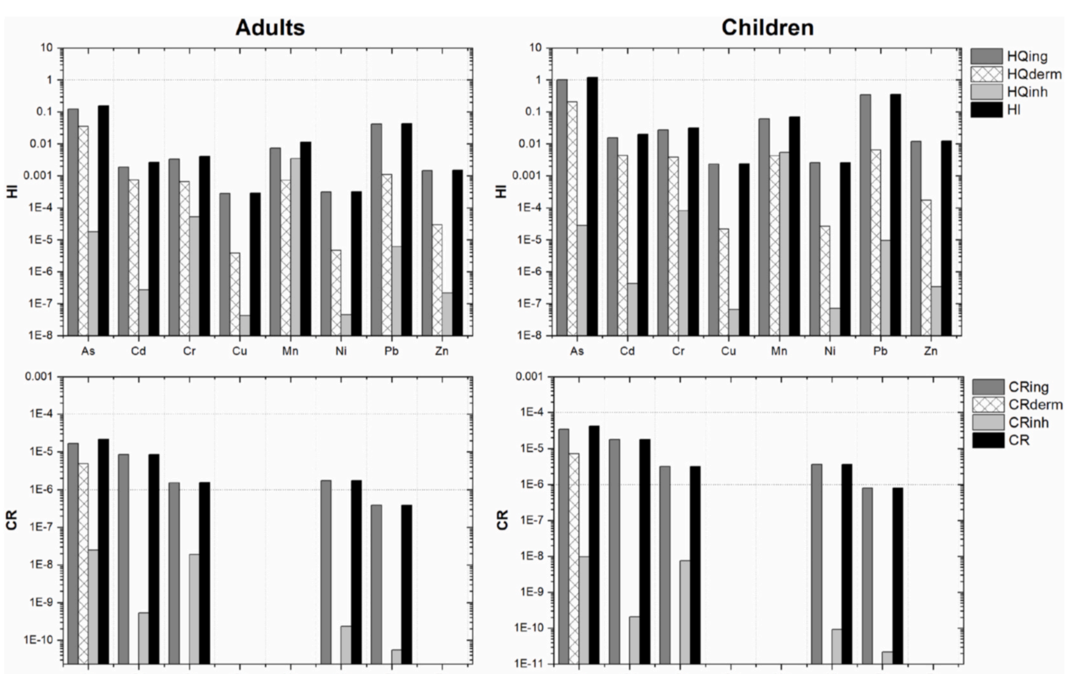

**Fig. 3.** HI and CR for each element and exposure pathway for adults and children.

### 3.2. Human health risk assessments of PTEs in urban park soils

The results of potential health risk assessment indices, both non-carcinogenic (HI) and carcinogenic (CR), for the RA topsoil (0–10 cm), are shown in Fig. 3. The average HI did not exceed the limit value for any of the elements in the case of adults. However, in children, the possible adverse health effect is posed by As, which reached the average HI 1.2. In the case of CR, no element exceeded the limit value ($1 \times 10^{-4}$). Nonetheless, As, Cd, Cr and Ni can be ranked to the category of the acceptable total risk for regulatory purposes.

The low values of the indices contrasting with the high PTE contents are due to other inserted variables used for the calculation, especially the exposure frequency factor, which represents the number of visits per year. The exposure frequency for recreation is lower than that of regular residential use (Luo et al., 2012; Urrutia-Goyes et al., 2017), and therefore the value of 75 was chosen (Luo et al., 2012). However, in the case of urban parks it is necessary to consider, for instance, that dog owners from the surrounding built up area can visit them every day, and the potential risk they face is thus higher. Similarly, mothers with children visit parks regularly. At the same time, these groups can easily come in direct contact with soil.

In case of an increase in the frequency of visits of these groups, the value of 350, applied in similar studies (Gu et al., 2016; Beroigui et al., 2020), might result in a local HI increase of up to 3.8 with an average value of 0.7 for adults. In children, then, HI of As would increase up to 5.6 while Pb, with an average value of 1.6, would also pose a risk. It would also signify the exceedance of the CR limit and thus the potentially great threat of the carcinogenic risk due to As for adults ($1.00 \times 10^{-4}$) and children ($1.93 \times 10^{-4}$). This fact should generally be considered when drawing conclusions about the acute need to address the current state and management steps leading to risk reduction.

The potential health risk is further increased by the areas of remaining successional vegetation on original mining waste that are included in the park complex. If the planned technical part of the reclamation was correctly implemented, these areas would constitute individual hotspots of persistent soil contamination (Stefanowicz et al., 2014) representing the main source of health risk for visitors. In these areas the HI limit was exceeded for As (5.1) and Pb (1.2) in the case of children. The finding points out that it is not safe to use the original spontaneous succession areas on heavily polluted soils in such projects.

### 3.3. Vegetation assessment

Non-significant correlation (Spearman's Rank-Order Correlation) was found between the PTE contents in the leaves (Table 1) and in the soil. The only exception was Cd with a weak correlation ($\rho = 0.70$) for a depth of 0–10 cm. This finding shows that the total soil PTE contents are not the only factor significant for their uptake and deposition in plants, but other physical, chemical, and biological factors in the soil are also significant (Ernst, 1996; Hartley et al., 2009; Zhu et al., 2018). Soil alkalinity (pH 7.45–7.74 in this study) can be especially important, which may significantly reduce PTEs availability in the soil, thus reduce PTEs uptake by plants (Bravo et al., 2017).

The average PTE contents in the leaves of the seedlings at the RA (Fig. S2) did not exceed the excessive or toxic limits (Table 1), however, variable health status of the seedlings was found (Table S5). The possible toxicity limit was exceeded merely in Zn, on the plots RA 1 (106 mg/kg) and 8 (169 mg/kg). These plots were characterized by impaired vitality of the seedlings with mortality reaching 84% and the growth retardation and leaf chlorosis up to 100% (Fig. 4), respectively. This damage can be associated with excessive Zn content in the plant, as reported by Rout and Das (2009) and Kabata-Pendias (2011). Furthermore, on the plot RA 8, the highest values of As, Cd, Cr, Mn, Ni and Zn, in comparison with other RA plots, were found. This plot shows a rather lower PTE contents in the soil compared to other plots, which may indicate an increased PTEs mobility there, though. The importance of PTEs accumulation in the plant tissue there is also demonstrated by exceeding the BCF limit by Cd (1.1). This may reflect stronger accumulation ability of PTEs (Zhu et al., 2018), which could be influenced by the plant species and the bioavailability of the metal(loid)s themselves depending on the soil physico-chemical properties (Ernst, 1996; Zhu et al., 2018). However, present *Q. robur* also dominated in other plots and studied soil physico-chemical properties of the plot were not significantly different from other plots. Therefore, it can be assumed that there is another factor which, as the subject of further research, must be revealed.

All the plots with mortality >10% (Table S5) were also characterized by extensive leaf browning, deformation of young leaves, chlorosis of the remaining leaves, defoliation, and crown dieback of the seedlings. These symptoms may be associated with soil toxicity, such as typical manifestations of PTEs toxicity (Nagajyoti et al., 2010; Kabata-Pendias, 2011). Similar PTEs bound phytotoxic effects, specifically chlorosis, partial defoliation, and significant yield reduction of aboveground biomass, were found by Vysloužilová et al. (2003) on the contaminated soils in the vicinity of Příbram. Extensive crown dieback of the *T. cordata* at the plot RA 10 and browning of the *C. betulus* leaves at the plot RA 6 contrasting with good health of other species there can be explained by species-dependent PTEs toxicity variation (Nagajyoti et al., 2010).

The low PTE levels in the leaves that do not exceed toxic limits indicate that PTEs may not be the only stressor affecting vegetation. The limit of mined degraded soils for vegetation is not only their toxicity but also other accompanying adverse factors such as drought, erosion, compaction, wide temperature fluctuations, absence of soil-forming fine materials and the shortage of essential nutrients (Wong, 2003; Burger et al., 2017). Resulting poor soil quality may be among the most significant factors limiting the optimal tree growth and its survival (Adamo et al., 2015; Layman et al., 2016). In particular, the insufficient depth of

**Table 1**
The PTE contents (mg/kg) in leaves.

| | | As | Cd | Cr | Cu | Mn | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|
| Reclaimed area | Average | 1.07 | 0.40 | 1.21 | 5.25 | 116 | 1.19 | 6.86 | 66.1 |
| | Min. | 0.75 | 0.07 | 0.50 | 4.06 | 46.4 | 0.54 | 4.96 | 29.1 |
| | Max. | 1.67 | 2.25 | 3.58 | 7.71 | 303 | 2.89 | 10.4 | 169 |
| | S.D. | 0.30 | 0.63 | 1.03 | 1.01 | 74.3 | 0.65 | 1.60 | 42.7 |
| Succession area | Average | 1.02 | 3.24 | 0.98 | 4.49 | 167 | 1.58 | 10.2 | 463 |
| | Min. | 0.91 | 0.41 | 0.88 | 3.50 | 108 | 0.74 | 6.78 | 194 |
| | Max. | 1.13 | 6.06 | 1.08 | 5.48 | 226 | 2.41 | 13.6 | 732 |
| | S.D. | 0.11 | 2.83 | 0.10 | 0.99 | 59.0 | 0.84 | 3.41 | 269 |
| Heap area | Average | 1.66 | 10.4 | n.d. | 5.20 | 67.2 | 1.15 | 7.02 | 403 |
| | Min. | 1.48 | 0.22 | n.d. | 4.59 | 30.3 | 0.67 | 3.53 | 102 |
| | Max. | 1.83 | 20.5 | n.d. | 5.80 | 104 | 1.63 | 10.5 | 703 |
| | S.D. | 0.18 | 10.1 | n.d. | 0.60 | 36.9 | 0.48 | 3.49 | 301 |
| Limit[a] | | 5–20 | 5–30 | 5–30 | 20–100 | 400–1000 | 10–100 | 30–300 | 100–400 |

[a] Kabata-Pendias (2011). The values are based on a review of the range of plant sensitivity values that are excessive or toxic and does not include highly sensitive or highly tolerant species.

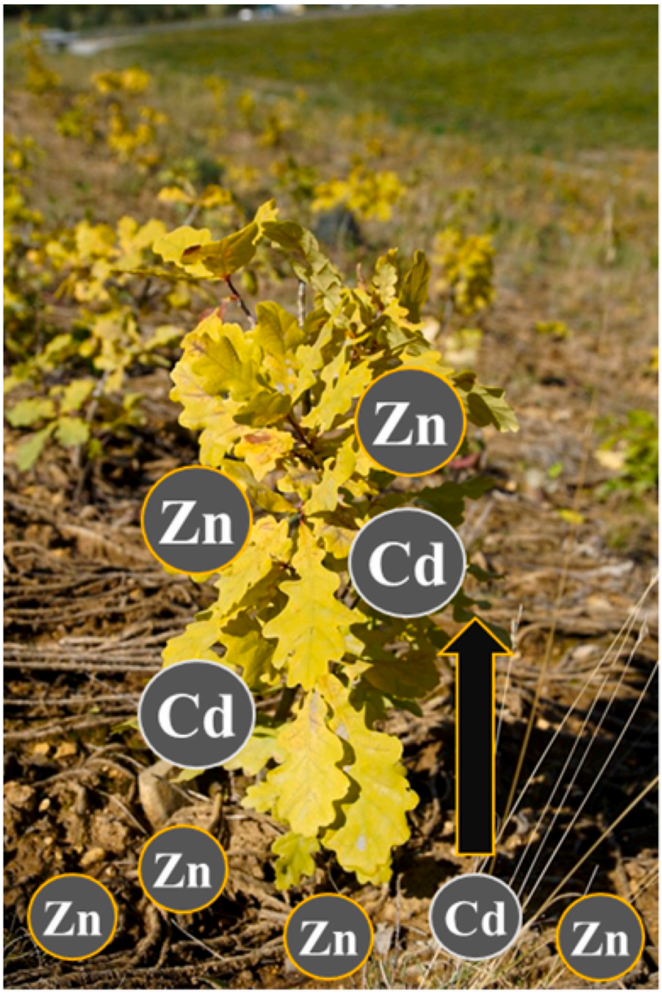


**Fig. 4.** Leaf chlorosis on the plot RA 8.

the soil cap may be a problem (only 15 cm). Carew et al. (2015) state that in the case of brownfield redevelopment, it is preferable to raise soft soil cap grade above the polluted soil to accommodate the roots of trees. In this case, on the contrary, it was reduced (from 20 cm). New plantings are, therefore, exposed to synergistic impacts of several stressors. It can be assumed that their long-term negative effects can lead to the growth of seedling mortality.

This worrying situation was found despite the implementation of the management plan, which included fertilization, replanting new seedlings instead of the dead ones, the protective coating against game, and the weed control (DIAMO, 2017). Artificial afforestation for the purpose of valuable park vegetation establishing in such heavily polluted areas may therefore be unsuccessful despite active and money-consuming care. In such cases, the process of revegetation could risk being ineffective to prevent PTEs dispersion (Adamo et al., 2015). And although the soil is covered at least by grass and herb vegetation on most of the area, a number of bare places remain (Figs. 1 and 4).

When comparing the PTE contents in artificial afforestation and natural spontaneous succession (Table 1), it is evident that the above-limit values were especially in the leaves of the trees growing on the original mining waste (succession and heap areas); however, the trees showed no signs of intoxication. The PTE contents in their leaves were high especially in the case of Cd and Zn, and at the heap area they were probably influenced by the presence of goat willow which can effectively accumulate PTEs in their leaves (Unterbrunner et al., 2007).

The good health of spontaneously developed vegetation may reflect its better adaptation as a result of natural development and may indicate that spontaneous succession is the appropriate process of restoration of such sites. This assumption was also supported by the finding that in the case, that pioneer species formed closed canopy, they created good conditions for natural regeneration of the species, which have been deliberately used for afforestation there. Nevertheless, in contrast to financially very expensive (Wuana and Okieimen, 2011; Song et al., 2019) technical reclamation, this economically more realistic and cost-effective option (Adamo et al., 2015) is slow (Tropek et al., 2010) and under extreme conditions of high contamination inappropriate (Prach and Hobbs, 2008), especially in an urbanized area, where contamination represents immediate health risk.

Planting of park-valuable K-strategist species (e.g., dominating *Quercus* sp.) has shown to be unsuitable (Table S5, Fig. 4) in such extreme conditions. The successful tree growth and fulfilment of the required ecosystem services of such heavily stressed vegetation can be seriously disrupted (Layman et al., 2016). The selection of appropriate plant species which can establish, grow, and colonize PTEs-contaminated soils is important for successful reclamation (Wong, 2003). For this reason, it is necessary to use resistant R or S strategist species, such as *B. pendula*, *P. sylvestris* or *S. caprea* which naturally grow on the mining waste. Based on the results, these species can serve as suitable preparatory species, under which protection species, namely *Quercus* sp. or *Acer* sp. can naturally regenerate despite the pollution. Faster vegetation cover establishment will lead to faster return on investment for municipalities, using trees for ecosystem services (Layman et al., 2016).

### 3.4. Future perspectives and possible solutions

The problem which remains is the park soil heavy pollution, which can still grow in the future because urban park soils have the potential to accumulate and store PTEs (Setälä et al., 2017). One of the possible solutions is phytoremediation (Wong, 2003; Unterbrunner et al., 2007; Wilschut et al., 2013; Mahar et al., 2016; Radziemska et al., 2020). This kind of management would involve the removal of organic matter, for example by mowing local lawns and tree leaves harvesting. Species such as willows (Vysloužilová et al., 2003; Unterbrunner et al., 2007) can contribute to the phytoextraction of PTEs and reduce soil pollution. The abovementioned goat willow may be particularly useful.

However, there is a general lack of research outputs dealing with the relation between effectiveness of full-scale phytoremediation and field environmental factors (Song et al., 2019). Given the current situation, other landscape architectural-engineering solutions are also feasible, based on the detailed field research to distinguish safe zones from hazardous ones, and building elements, such as raised pathways to prevent a direct contact with the contaminated soil (Wilschut et al., 2013). Nevertheless, this entails high additional costs. To summarize, the high level of residual pollution found there will pose long-term uncertainty and the risk will be difficult to control by means of post-remediation long-term management. Therefore, sustainability is improbable even at high maintenance costs.

### 3.5. Management steps ensuring safe redevelopment and following park maintenance

In the case of a brownfield conversion into a public place, it is essential to remove all the possible risks posed by the original source of pollution. Parks constructed at former brownfield sites adhere to strict public safety regulations and are designed to be safe for public use (Klenosky et al., 2017). However, these sites still contain residual pollution, even though with acceptable risks for human health based on safety standards which are varying across the world and changing with time. This may result in a different assessment of the same situation and thus a disregarding of the real threat (Pediaditi et al., 2005; Erdem and Nassauer, 2013).

The correctness of the conversion of polluted sites into urban parks may be the subject of discussions, particularly in the case of such serious original pollution associated with mining. The urban park has the potential to concentrate a greater number of people, therefore, the impact of health risk is increasing. Even in the case of low PTEs mobility, the risk of long-term and recurrent human exposure through potential inhalation and unwashed hand-to-mouth transfer must be considered (Hartley et al., 2009). Nevertheless, given the importance of greenery in the urban environment (Rodríguez-Seijo et al., 2017; Brown et al., 2018), it is advisable to expand green areas, and brownfield sites appear to be suitable for such an expansion (Dagenhart et al., 2006; Loures and Panagopoulos, 2007), with a potential to bring many benefits in case of successful redevelopment (Klenosky et al., 2017; Song et al., 2019). This is also confirmed by the finding that citizens using RAs serving as parks recognize that landscape reclamation has a significant positive effect on the urban environment (Janků et al., 2014) and similar projects are demanded, although city dwellers have health risk concerns (Klenosky et al., 2017).

However, it has historically been shown that these concerns may be justified (Erdem and Nassauer, 2013) and this study demonstrates that health threats can also arise despite the usage of modern reclamation practices. It ought to be taken into consideration whether the serious potential health risks associated with redevelopment of heavily polluted brownfields into public urban parks should not be prioritized in the decision-making process on the future use of brownfield sites over other undisputed parks benefits for cities and whether other redevelopment outputs are more appropriate. Even high financial burden associated with the remediation of the polluted soil, as well as the construction and maintenance of green spaces (Song et al., 2019), do not have to guarantee that the project will be sustainable and safe.

For future projects related to this issue, it is essential to highlight necessary steps to minimize the risks. Based on the outcomes of this study and other similar studies (Erdem and Nassauer, 2013; Carew et al., 2015; Klenosky et al., 2017; Song et al., 2019), several management steps that should be followed when setting up an urban park on a brownfield site were defined (Table 2).

**Table 2**
Management steps that should be followed when setting up an urban park on a brownfield site.

| Management step | Comment |
|---|---|
| Risk management | Consideration of the environmental pollution severity and evaluation whether redevelopment into a public park is a safe solution. If the risk is assessed to be extremely high, a green area with restricted access can be created. In this model, vegetation will have a protective function (phytostabilization) to minimize the PTEs uptake, it will provide important ecological services in the urban ecosystem and simultaneously there will be no human health threat through exposure to the polluted soil. |
| Elimination of other sources of pollution | Given the increasing number of visitors to these places and the time spent at them, there is also a higher risk of PTEs intake from the immediate surroundings. Therefore, it is necessary to continue in reclamation activities in the surrounding area and removal of other possible sources of pollution (e.g., heaps), and the associated health risks. |
| Precise technological solution | A precise solution is essential to avoid mixing the original polluted material and the soil cap in the technical part of the reclamation. It is necessary to reclaim the entire area, to cover all the original material with uncontaminated soil and not to leave local pollution hotspots. A larger thickness of the soil cap, of at least 50 cm, is also suitable. |
| Selection of suitable tree species | When selecting trees, it is advisable to use preparatory species, especially early successional species (S strategists), which can cope with extreme conditions and prepare the environment for the subsequent cultivation of more valuable park trees. Native species that naturally colonize polluted soils in a given locality are suitable. In the case of the soil cap construction, deep rooting trees that would be able to penetrate the soil cap should be excluded. The selection of species suitable for phytoextraction or phytostabilization depends on the situation. |
| Intensive management | Management ought to be directed primarily towards greenery care. It is necessary to remove fallen tree leaves, and plant debris regularly after mowing lawns, which can contaminate topsoil via decomposition by means of PTEs transported from the deeper parts of the soil under the soil cap. |
| Permanent monitoring | Monitoring of the PTEs in the environment is an important basis for all management decisions. It should also be carried out during reclamation (including air monitoring) to eliminate potential risks at the initial stage. After the park establishment, monitoring must be held in regular intervals at least once every six months during the first five years of the park establishment, once a year after that. The methodology presented in this study can be used as a methodological basis for monitoring. It is also advisable to perform an investigation and assessment of the situation by an independent entity to prevent unilateral misleading assessment. Health risks monitoring should be transparent, and its outcomes ought to be accessible to the public. |

## 4. Conclusions

Reclamation has resulted in the reduction of possible exposure and potential intake of PTEs from the environment. Notwithstanding that, park visits may pose a health risk to visitors in a long-term horizon. A potential non-carcinogenic health risk for children may remain even at a low frequency of park visits (75 per year). When visiting the park on a regular basis, the risk may entail not only an increase in already proven non-carcinogenic risk but also potentially great carcinogenic risk for adults and children. In general, this fact should be considered when drawing conclusions about the urgent need to address the current state and management steps leading to the risk reduction.

In the case of reclamation of areas with such heavy pollution, it is essential to carry out a complete remediation of the area and not to leave fragments of the original spontaneously developed ecosystems, as they may pose a serioust health risk to visitors in the form of easily accessible pollution hotspots. In some instances, architectural elements can also be used to prevent direct contact with polluted soil.

The RA proved to be an unsuitable environment for the planted park-valuable K-strategist tree species, which were negatively affected by soil toxicity. Artificial afforestation for the purpose of establishing of valuable park vegetation is complicated in such heavily polluted areas despite active and money-consuming care. For successful reclamation with afforestation, it is more suitable to plant resistant R or S strategist species, which can naturally colonize extreme sites and can serve as good preparatory species. The protection they provide enable more park-valuable species to naturally grow there despite the high soil PTE contents.

It should be considered whether the serious potential health risks associated with redevelopment of heavily polluted brownfields into public urban parks ought not to be prioritized in the decision-making process on the future use of brownfield sites. Based on the findings, it is obvious that the establishment of public urban parks on such heavily polluted soils is inappropriate.

## Author statement

**Václav Pecina**: Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Visualization; Roles/ Writing - original draft. **David Juřička**: Data curation; Software; Validation; Visualization; Writing - review & editing. **Michaela Vašinová Galiová**: Formal analysis; Project administration; Supervision; Validation; Writing - review & editing. **Jindřich Kynický**: Investigation; Methodology; Project administration. **Ludmila Baláková**: Formal analysis; Visualization; Writing - review & editing. **Martin Brtnický**: Conceptualization; Funding acquisition; Resources; Supervision; Writing - review & editing.

## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.112669.

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**PŘÍLOHA Č. 3**

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# Insight into metal immobilization and microbial community structure in soil from a steel disposal dump phytostabilized with composted, pyrolyzed or gasified wastes

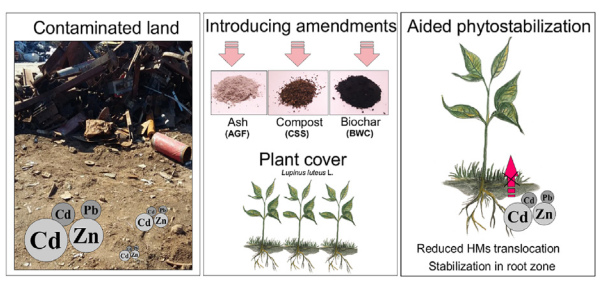
Maja Radziemska [a], Zygmunt M. Gusiatin [b,*], Agnieszka Cydzik-Kwiatkowska [b], Artemi Cerdà [c], Vaclav Pecina [d,e], Agnieszka Bęś [f], Rahul Datta [d], Grzegorz Majewski [a], Zbigniew Mazur [f], Justyna Dzięcioł [g], Subhan Danish [h], Martin Brtnický [d,e]

[a] Institute of Environmental Engineering, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776, Warsaw, Poland
[b] Faculty of Geoengineering, University of Warmia and Mazury in Olsztyn, Słoneczna St. 45G, 10-719, Olsztyn, Poland
[c] Soil Erosion and Degradation Research Group, Department of Geography, University of Valencia, Blasco Ibañez 28, Valencia, 46 010, Spain
[d] Faculty of AgriSciences, Mendel University in Brno, Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, 61300, Brno, Czech Republic
[e] Institute of Chemistry and Technology of Environmental Protection, Faculty of Chemistry, Brno University of Technology, 61200, Brno, Czech Republic
[f] Faculty of Environmental Management and Agriculture, University of Warmia and Mazury in Olsztyn, Pl. Łódzki 4, 10-727, Olsztyn, Poland
[g] Water Centre Laboratory, Faculty of Civil and Environmental Engineering, Warsaw University of Life Sciences, 02-787, Warsaw, Poland
[h] Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Punjab, Pakistan

## HIGHLIGHTS

- Compost (CSS), ash (AGF) and biochar (BWC) were used in aided phytostabilization.
- CSS, AGF and BWC improved accumulation of Cd, Cr, Cu, Ni, Zn by *L. luteus* roots.
- Ni and Cr showed the highest stability in phytostabilized soil.
- *Arenimonas*, *Brevundimonas*, *Gemmatimonas*, *Variovorax* may facilitate soil restoration.
- *L. luteus* with AGF or BWC are good for phytomanagement of steel disposal dump areas.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The soil system is a key component of the environment that can serve as a sink of pollutants. Using processed waste for aided phytostabilization of metals (HMs) in contaminated soils is an attractive phytoremediation technique that integrates waste utilization and recycling. In this study, we evaluated the effect of biologically and thermally processed wastes, i.e. sewage sludge compost (CSS), poultry feather ash (AGF) and willow chip biochar (BWC), on phytostabilization of contaminated soil from a steel disposal dump. Greenhouse experiments with *Lupinus luteus* L. and amendments (dosage: 3.0%, w/w) were conducted for 58 days. Soil toxicity was evaluated with Ostracodtoxkit and Phytotoxkit tests. At the end of the experiment, soil pH, plant biomass yield, and HM accumulation in plant tissues were determined. HM distribution, HM stability (reduced partition index) and potential environmental risk (mRI index) in the soil were assessed. During phytostabilization, changes in the diversity of the rhizospheric bacterial community were monitored. All amendments significantly increased soil pH and biomass yield

* Corresponding author.
*E-mail address:* mariusz.gusiatin@uwm.edu.pl (Z.M. Gusiatin).

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Immobilization
Soil risk minimization

and decreased soil phytotoxicity. AGF and BWC increased accumulation of individual HMs by *L. luteus* roots better than CSS (Cu and Cr, and Ni and Zn, respectively). The soil amendments did not improve Pb accumulation by the roots. Improvements in HM stability depended on amendment type: Ni and Pb stability were improved by all amendments; Zn stability, by AGF, and BWC; Cd stability, by AGF; and Cr stability, by BWC. AGF reduced the mRI most effectively. Microbial diversity in amended soils increased with time of phytostabilization and was up to 9% higher in CSS amended soil than in control soil. AGF application favored the abundance of the genera *Arenimonas, Brevundimonas, Gemmatimonas* and *Variovorax*, whose metabolic potential could have contributed to the better plant growth and lower mRI in that soil. In conclusion, AGF and BWC have great potential for restoring steel disposal dump areas, and the strategies researched here can contribute to achieving targets for sustainable development.


## 1. Introduction

The increase in the level of heavy metal (HM) concentration in soils is one of the most troublesome issues in environmental contamination (Mandal et al., 2020). HM contamination is found in different types of environments such as road dusts, agricultural soils, military areas and river sediments (Trujillo-González et al., 2016; Rodrigo-Comino et al., 2020; Kumar et al., 2020; Radziemska et al., 2019a). The impact of HMs on ecological risk is high due to the damage caused to biota (Kumar et al., 2019; Li et al., 2019) and the widespread distribution of HMs that are transported by irrigation, and surface and subsurface flows (Trujillo-González et al., 2017; Atifah and Harahap, 2019).

Areas where steel wastes have been stored deserve special attention due to the high level of land contamination (Radziemska et al., 2019b). The deposition of metal wastes from factories, houses, bridges, road infrastructure, weapons or vehicles poses an environmental problem (Palansooriya et al., 2020). For decades, metal wastes have been deposited in the soil, due to the lack of laws regarding waste disposal and management. As a result, it has become evident that there is a need to develop new and efficient methods of preventing the spread of contaminants between individual components of the natural environment.

In recent years, methods of using the plant potential in cleaning the soil-water environment have been gaining much interest because they are environmentally friendly, improve the landscape, and are thus supported by society (Ghazaryan et al., 2019; Saran et al., 2020). Phytostabilization can immobilize HMs in the root layer and can recreate the vegetation cover in areas where it disappeared as a result of high concentrations of HMs in the soil (Radziemska, 2018; Clemente et al., 2019; Oleńska et al., 2020; Xie and van Zyl, 2020). Wastes can be biologically and thermally processed and utilized. Composting is a process of humification and stabilization of organic wastes in which microbial and enzymatic activities accelerate organic waste degradation (Smith, 2009; Palansooriya et al., 2020). Composts are recognized as potentially important sources of humic substances, mineral ions and microorganisms that have a high affinity for binding HMs and reducing ecological and environmental risks associated with HMs (Udovic and McBride, 2012). Compost can, however, have varying effects on HM immobilization, depending on the type and composition of composted wastes as well as the soil properties. Until now, plant-derived composted organic amendments/green compost have commonly been used to immobilize HMs in contaminated soils (Palansooriya et al., 2020). On the other hand, due to the increasing volume of sewage sludge in wastewater treatment plants, it is expected that utilization of composts produced from this waste will increase.

Biochar is formed during the thermochemical decomposition (i.e., pyrolysis) of biomass under oxygen-limited conditions. It has been widely studied and applied as a soil conditioner and remediation material. Due to its porous structure, its large surface area and the presence of various functional groups, biochar has a high affinity for HM sorption (Kwak et al., 2019). Until now, biochars made from crop residues and livestock manure were mostly used for remediating HM-contaminated soils (Hamid et al., 2020). Biochar from wood-based biomass is more recalcitrant than biochar from manure and crop residues (Shaaban et al., 2018) and can help in long-term immobilization of HM in the soil. Biochars from wood also often have a higher surface area and lower ash content than biochars from grass biomass. Hardwood biochar exhibits much higher microporosity, alkalinity and electrostatic capacity than softwood biochar (Jiang et al., 2017), which can be crucial for HM sorption. Willow (*Salix* spp.) is a lightweight hardwood and is a leading bioenergy feedstock because, among other reasons, it is among the fastest-growing trees, producing large amounts of biomass in a short period. Thus, it is one of the most readily available forms of wood biomass and waste material in the world. To date, research on pyrolysis of willow feedstock has focused primarily on evaluating the influence of variable process parameters on relative product yields and characteristics (Greenhalf et al., 2013). Although willow biochar is used in agricultural applications (Fletcher et al., 2014; Ścisłowska et al., 2015), little work (Visconti et al., 2020) has been done to investigate the effect of willow biochar on HM immobilization.

The expansion of poultry production around the world has increased the generation of by-products, including viscera, feet, head, bones, blood and feathers. Feathers are one of the most resistant slaughterhouse by-products and constitute up to 10% of all waste generated by the poultry industry (Kwiatkowski et al., 2013). Although feathers are not used for industrial applications on a large scale, there are examples of effective management of this poultry by-product, mainly as a sorbent for water and wastewater pollutants (Chen et al., 2016; Zhang et al., 2019). Considering the growing interest in thermal waste treatment technologies, converting feathers or poultry litter into energy by thermochemical processes is also an attractive method of managing this type of waste (Kwiatkowski et al., 2013; Dalólio et al., 2017; Gusiatin et al., 2020). Because the thermochemical conversion of feathers produces ash as a by-product, great attention should be given to proper disposal and use of the ash. A potentially attractive form of managing this by-product is using it as a soil amendment. However, no research has been carried out on the use of ash from feather gasification in soil remediation.

Most of the studies on aided phytostabilization of contaminated soils have focused on the effect of different amendments on metal bioavailability and plant growth (Kabiri et al., 2019; Lan et al., 2020). Since soil amendments and plant growth may induce shifts in the soil microbial community, the structure of soil microorganisms should also be considered when examining the phytostabilization process. Soil microbes aid in many metabolic reactions associated with biogeochemical cycling of nutrients, enhance detoxification of pollutants and maintain soil microbial structure (Khan et al., 2010). The composition of soil bacterial communities is mainly driven by geochemical factors, including content of HMs (Liu et al., 2018). Microorganisms in the rhizosphere may increase the availability of nutrients and limit the negative effects of HMs on plants. Bacteria resist HM toxicity via HM complexation, volatilization, sequestration in extracellular polymeric substances, and enzymatic detoxification (Ashraf et al., 2017). Bacteria may also contain plasmids with genes that ensure resistance against some metalloids and HMs (Endo et al., 1995). Microorganisms are a determinant of ecosystem resilience and functional efficiency, and can assist restoration ecology (Basanta et al., 2017). Little is known about the effects of phytostabilization on microbial community composition in contaminated calcareous soils, particularly soil from steel disposal dumps. Since the microbiota in HM-polluted soils is a crucial factor in the reclamation process, understanding the effect of a soil amendment on changes in the bacterial community during phytoremediation is of the utmost importance.

Up to now, aided phytostabilization has been performed with mining and acidic soils (Basanta et al., 2017; Yang et al., 2017, 2019). Restoration of soil from steel disposal dumps is still overlooked. In many projects, the effectiveness of soil phytostabilization with different amendments was assessed only on the basis of metal bioavailability and plant growth. In those projects, other important aspects of soil phytostabilization were not assessed, such as changes in soil phytotoxicity, metal stability and succession of rhizosphere microorganisms. Although biochars and composts have been tested as soil amendments, ash from gasification of poultry feathers is proposed here for the first time as a novel amendment for aided phytostabilization.

The overall aim of this study was to investigate the effect of three amendments produced from wastes by composting (compost from sewage sludge, CSS), pyrolysis (biochar from willow chips, BWC) or gasification (ash from gasification of poultry feathers, AGF) on aided phytostabilization of HM-contaminated soil collected from a steel disposal dump. It was hypothesized that the manner of processing the wastes (biological or thermal) and their use as soil amendments are important for restoration of soil from a steel disposal dump. The experiments were conducted with *Lupinus luteus* L., a plant known to be a good phytostabilizator of HMs in contaminated soil. The specific objectives of this study were to characterize the soil and its phytotoxicity before and after phytostabilization, to determine the effect of soil amendments on plant growth and HM accumulation in above- and below-ground plant parts, to investigate HM transformations and immobilization in soil, and to determine HM stability ($I_R$ index) and environmental risk (mRI index). In addition, the effects of the different amendments used for aided phytostabilization on the microbial community structure and diversity were investigated.

## 2. Materials and methods

### 2.1. Sampling area and soil sampling procedure

Soil for the experiment was taken from an area of steel disposal dumps (north-eastern Poland, 53°47′3.48″N; 20°30′52.56″E) that have been operating since 1946. In the studied area, wastes containing ferrous and non-ferrous metals are stored. During the storage, cutting and sorting of steel scrap metal, and the removal of various organic and non-organic contaminants, HMs can make their way into the soil, and later, into groundwaters (Sofilić et al., 2013; Xuan and Yue, 2016; Radziemska et al., 2020). These can be alloy components of steel, such as Zn, Fe, Ni, Cr, Cu, Co or Cd (Ogunkunle et al., 2016), originating from external anticorrosive metallic layers covering the steel elements (Adereti et al., 2017).

Topsoil (0–25 cm) samples were taken from 20 sampling sites in a 200 $m^2$ area in the oldest part of the scrap yard. The sampling sites were selected for equal coverage of the area. At each sampling site, 5 kg of soil was collected with a stainless-steel shovel. All the samples were carefully transferred to clean polyethylene bags before being transported to the laboratory. The soil samples from all sampling sites were thoroughly mixed to obtain a composite soil sample (100 kg). Then, the soil was air-dried at room temperature, sieved (2-mm mesh) and stored in a 4 °C refrigerator for phytostabilization experiments.

### 2.2. Processed wastes for use as soil amendments

Three types of wastes, municipal sewage sludge, willow chips and poultry feathers, were each processed via composting, pyrolysis and gasification, to produce compost, biochar and ash, respectively. The compost was produced at the Department of Environmental Biotechnology, at the University of Warmia and Mazury in Olsztyn (Poland). It was produced from municipal sewage sludge (60% w/w) mixed with wood chips (15% w/w), rape straw (22% w/w) and mature compost as the inoculant (3% w/w) in an aerated 1 $m^3$ bioreactor. The compost was matured for 12 months in a periodically turned 1 $m^3$ windrow (Kulikowska and Klimiuk, 2011). The biochar was supplied by Fluid S.A. (Sędziszów, Poland) and was made from willow chips pyrolyzed at 650 °C for 15 min at a heating rate of about 3 °C/s. The ash came from a full-scale industry plant located in the voivodeship of Warmia and Mazury (Poland). It was produced from poultry feathers gasified in a fixed bed gasifier at 1000–1200 °C with a small quantity of wood pellets used as fuel. Before the following experiments, the amendments were milled in a RETSCH SM-100 cutting mill to obtain a particle size less than or equal to 0.5 mm and then characterized (Table 1).

### 2.3. Experimental set-up

The greenhouse experiment was implemented under natural day/night conditions; the air temperature was 26 ± 3 °C during the day (14 h) and 16 ± 2 °C at night (10 h), with a relative humidity of 75 ± 5%. Twenty 5.0 kg polyethylene pots were used for three different treatments and a control (non-amended soil) in five replicates each. Soil amendments were added to the soil at a dose of 3.0% (w/w). The soil was thoroughly mixed with different amendments, and pots were placed in a dark room for over two weeks to equilibrate the soil mixture. In the plant growth assays, no fertilizer was applied to avoid potential fertilizer-amendment interactions. Yellow lupin (*Lupinus luteus* L.) cv. Perkoz was purchased form the Seed Production Centre in Olsztyn (Poland). Five seeds were planted per pot. The plants were watered every other day with distilled water to 60% of the maximum water holding capacity of the soil. The soil moisture content for each pot was monitored at field capacity every three days. Soil samples for molecular analyses were taken from each experimental replicate after 0, 10, 30 and 58 days of the experiment, and, for example, soil-AGF10 refers to the soil amended with AGF after ten days of the experiment. Soil samples were stored at 4 °C prior to analysis. The plants were harvested 58 days after planting, then weighed and separated into above-ground parts and roots, which were carefully washed with deionized water.

**Table 1**
Selected physical and chemical characteristics of soil amendments (n = 3 ± standard deviation).

| Characteristic | Unit | CSS | AGF | BWC |
|---|---|---|---|---|
| Surface area BET | $m^2\ g^{-1}$ | 12.5 | 1.2 | 313.7 |
| Total area in pores | $m^2\ g^{-1}$ | 1.1 | 0.6 | 92.9 |
| Total volume in pores | $cm^3\ g^{-1}$ | 0.011 | 0.002 | 0.113 |
| pH | – | 7.1 ± 0.2 | 12.9 ± 0.4 | 10.4 ± 0.3 |
| Electrical conductivity | mS $cm^{-1}$ | 12.2 ± 0.4 | 34.0 ± 1.4 | 2.9 ± 0.1 |
| Volatile matter | % | 34.7 ± 0.7 | 1.0 ± 0.1 | 16.5 ± 0.5 |
| Cation exchange capacity | cmol $kg^{-1}$ | 49.5 ± 1.4 | n.a. | 48.6 ± 2.3 |
| Cd | mg $kg^{-1}$ | 0.8 ± 0.2 | 0.0 | 0.2 ± 0.1 |
| Cr | mg $kg^{-1}$ | 55.2 ± 2.8 | 1.8 ± 0.5 | 9.6 ± 0.7 |
| Cu | mg $kg^{-1}$ | 57.6 ± 7.6 | 2.4 ± 0.1 | 13.9 ± 6.3 |
| Ni | mg $kg^{-1}$ | 23.4 ± 3.6 | 0.0 | 10.2 ± 0.1 |
| Pb | mg $kg^{-1}$ | 8.2 ± 0.8 | 0.0 | 1.1 ± 1.6 |
| Zn | mg $kg^{-1}$ | 253.7 ± 18.2 | 0.3 ± 0.1 | 200.2 ± 10.7 |
| **Chemical composition of amendments** | | | | |
| $P_2O_5$ | wt% | 2.6 | 39.9 | 6.0 |
| CaO | wt% | 1.0 | 35.5 | 29.8 |
| $SiO_2$ | wt% | 4.6 | 10.9 | 19.7 |
| $Na_2O$ | wt% | 0.9 | 3.5 | 1.1 |
| MgO | wt% | 1.4 | 2.2 | 3.7 |
| $K_2O$ | wt% | 0.6 | 2.2 | 14.4 |
| $Al_2O_3$ | wt% | 2.2 | 1.5 | 2.5 |
| $Fe_2O_3$ | wt% | 3.2 | 0.7 | 3.7 |

n.a. – not applicable.

### *2.4. Physical and chemical analyses of soil, amendments and plants*

The soil was air dried at room temperature for two weeks. The pH of the soil, amendments and amended soil was measured in distilled water extracts (1:2.5 w/v) using a pH-meter (HI 221, USA). The electrical conductivity (EC) in the soil and amendments was measured in distilled water extracts (1:2.5 w/v) using a conductivity meter (HI 8733, USA). The cation exchange capacity (CEC) of the soil and amendments was calculated as the sum of hydrolytic acidity (in 1 M Ca $(CH_3COO)_2$) and exchangeable bases (in 0.1 M HCl) (Sulieman et al., 2018). The particle size of the soil was determined using a Mastersizer 2000 particle size analyzer. The Brunauer-Emmet-Teller (BET) specific surface area of the amendments was determined by fitting the BET equation to the linear portion of the BET plot; the pore size distribution was calculated on the basis of the desorption plot of the $N_2$ adsorption-desorption isotherm using the Barret-Joyner-Halend method (Micrometrics ASAP 2000, USA). Organic matter in soil and volatile matter in compost were determined by sample ignition in a muffle furnace at 550 °C for 4 h (Jeong and Kim, 2001). Volatile matter in biochar and ash was determined in a muffle furnace at 950 °C for 6 min (Zhang et al., 2014).

To determine the total content of HMs in the soil, amendments and amended soil, the samples were mineralized in a mixture of concentrated HCl, $HNO_3$, and $H_2O_2$ in a microwave oven (MARSXpress, CEM USA). HM contents (Cu, Ni, Cd, Pb, Zn, Cr) were measured by an atomic absorption spectrometer (AAS) (Varian, AA28OFS). The quality of the analyses was assessed using reference material (CRM 142 R) and the obtained recoveries ranged from 95% to 101%. The distribution of HMs in soil before and after phytostabilization was determined according to the modified Community Bureau of Reference procedure (Gusiatin and Kulikowska, 2016a). HMs were fractionated into four fractions of differing in mobility using the following reagents: exchangeable and acid-soluble fraction with 0.11 M $CH_3COOH$ (mobile, fraction F1), reducible fraction with 0.5 M $NH_2OH \cdot HCl$ (potentially mobile, fraction F2), oxidizable fraction with 1 M $CH_3COONH_4$ (potentially mobile, fraction F3), and residual fraction with HCl, $HNO_3$ and $H_2O_2$ (immobile, fraction F4).

The elemental composition of the soil amendments was analyzed using a Quantax 200 energy dispersive X-ray spectrometer (EDX) with an XFlash 4010 detector coupled with a Zeiss Leo 1430 VP scanning electron microscope (SEM).

Upon completion of the experiment, the above-ground parts and roots of the test plants were washed in tap water and then in deionized water. Next, they were air-dried at room temperature and finally oven-dried at 55 °C to a stable weight, and the dry mass of each was recorded. Before the analysis, the plants were powdered using an analytical mill (Retsch, ZM300). The plant material (the above-ground parts and the roots) were mineralized in concentrated $HNO_3$ and 30% $H_2O_2$ using a microwave oven (MARSXpress, CEM USA). After filtration, the volume of the digested samples was adjusted to 100 mL with ultrapure water (Milli-Q System, USA). Total HM concentrations in extracts were determined by the AAS method (Varian, AA28OFS spectrometer). The detection limits were 0.01, 0.1, 0.05, 0.48, 0.24 and 0.20 μg/g for Cd, Cr, Cu, Ni, Pb and Zn, respectively. All analyses in soil, amendments and plants were performed in triplicate.

### *2.5. Soil toxicity assessment*

Soil toxicity was evaluated with two test kits: an Ostracodtoxkit and a Phytotoxkit. A 6-day toxicity test with *Heterocypris incongruens* using an Ostracodtoxkit F was employed for assessing the quantitative toxicity of amended and non-amended contaminated soil. *H. incogruens* were chosen due to their high sensitivity to various toxic substances. The test was carried out in darkness in a phytotron chamber at a controlled temperature (25 °C). The soil was analyzed twice with the Ostrakodtoxkit test: prior to sowing the lupin and upon harvesting the plant. The mortality of crustaceans was calculated, as well as the average plant growth inhibition in μm and in %.

The growth inhibition of *H. incongruens* (GI) was calculated as:

$$GI = 100 - \frac{A}{B} \times 100 \tag{1}$$

where A is the mean growth of the ostracods in the reference (control) soil and B is the mean growth of the ostracods in the contaminated soil (Oleszczuk and Hollert, 2011).

For the phytotoxicity test, a Phytotoxkit test was used, employing *Sorghum saccharatum* seeds, which germinate quickly and are characterized by fast growth of roots and sprouts (Phytotoxkit, Microbiotests Inc., Belgium). The test was carried out under controlled temperature and humidity in special see-through tiles, which made direct observation and measurement of root length possible. For the phytotoxicity tests, OECD soil (reference), the soil from the steel disposal dumps and the soil with amendments were used. The observations were carried out for 7 days, and digital images were taken daily. After this period, the percentage of germinating roots and their growth in length were recorded. To measure the length of the roots, the digital images were analyzed using Image Tool 3.0 (UTHSCSA, USA).

Seed germination inhibition (SG) and root growth inhibition (RI) percentages for the plants were calculated as percentages using the following formula:

$$SG \text{ or } RI = \frac{A - B}{A} \times 100 \tag{2}$$

where A is either the average number of seeds that germinated or the mean root length in the control, and B is the corresponding value in the amended soil (Czerniawska-Kusza et al., 2006).

### 2.6. Assessment of HM phytostabilization in soil

The assessment of the HM phytostabilization was based on:

- HM accumulation in plant shoots and roots,
- changes in HM distribution patterns in amended and non-amended soil after phytostabilization,
- HM stability in amended and non-amended soil after phytostabilization, assessed with the reduced partition index ($I_R$) (Gusiatin and Kulikowska, 2016a):

$$I_R = \frac{\sum_{i=1}^{k} i^2 \times F_i}{k^2} \tag{3}$$

- potential environmental risk of HMs in amended and non-amended soil after phytostabilization, assessed with the modified potential environmental risk index (mRI), including the share of the metal in the mobile fraction and the toxicity of the HM (Håkanson, 1980 with a modification to $C_f^i$ proposed by the Authors):

$$mRI = \sum_{i=1}^{m} E_r^i = \sum_{i=1}^{m} \frac{C_f^i}{C_t^i} \times T_r^i \tag{4}$$

where $i$ is the index number of the extraction step (from 1 for F1 fraction to 4 for F4 fraction in the BCR procedure), $F_i$ is the percentage of the HM in fraction $i$, $k$ is the total number of HM fractions in the BCR procedure, $E_r^i$ is the potential risk from an individual HM; $T_r^i$ is the toxic-response factor for a given HM (Zn = 1; Cr = 2; Cu, Ni and Pb = 5; Cd = 30) (Håkanson, 1980); $C_f^i$ is the HM concentration in the F1 fraction (mg $kg^{-1}$), $C_t^i$ is the total HM concentration in soil (mg $kg^{-1}$). The potential environmental risk (mRI) for soils was evaluated using the following scale: <1 (no risk), 1–10 (low risk), 11–30 (medium risk), 31–50 (high risk) and >50 (very high risk).

### 2.7. Assessment of microbial community based on 16S rRNA gene amplicon sequencing

Total genomic DNA was extracted from 500 μL of soil using a Fast DNA Spin Kit for Soil (Q-BioGene) according to the manufacturer's protocol. The DNA obtained from each experimental replicate was mixed and the purity and concentration of the isolated DNA was measured using a NanoDrop solid spectrometer (Thermo Scientific). The V4 hypervariable region of the 16S rRNA gene was amplified using the 926wF/1392R (5′-AAACTYAAAKGAATTGRCGG-3′/5′-ACGGGCGGTGTGTRC-3′) universal primer set (Rinke et al., 2014) targeting bacterial, archaeal and eukaryotic 16S rDNA genes. The amplicons were sequenced in the same run using the Illumina MiSeq platform at Research and Testing Laboratory (USA). Detection and removal of chimeras from the raw reads was performed by executing UCHIME (Edgar et al., 2011) in *de novo* mode on the clustered, denoised data. Then, the reads were condensed into FASTA format. Sequences with low-quality tags and those with a length less than half of the expected length were removed. The remaining sequences were clustered into operational taxonomic units (OTUs) using the UPARSE (Edgar, 2013) algorithm. The centroid sequence from each cluster was then run against a database of high-quality sequences derived from the NCBI database using the USEARCH global alignment algorithm (Bokulich et al., 2015), and taxonomic information was assigned. In complex consortia, bacteria with a low abundance may be of great importance for system operation. The number of reads was, therefore, not normalized before calculation of diversity indices to maintain the highest possible precision for each sample (McMurdie and Holmes, 2014). The sequences have been deposited in the NCBI Sequence Read Archive (SRA) as BioProject ID PRJNA659392.

### 2.8. Statistical data analysis

Statistical analysis of the soil properties, HM accumulation in plant biomass, HM distribution in soil was conducted using Statistica 13.3 software. The data was analyzed using one-way analysis of variance (ANOVA) or (when the ANOVA assumptions were not met) the Kruskal-Wallis test. For data with significant differences identified by ANOVA, further analyses were conducted using Tukey's HSD test. For statistical analyses and visualizations of molecular results (heatmap, principal component analysis, i.e. PCA, and diversity indices), the ampvis2 package (Andersen et al., 2018) for R version 3.6.2 (R Core Team, 2018) was used in the RStudio environment, version 0.99 (RStudio Team, 2015).

## 3. Results and discussion

### 3.1. Characteristics of the soil before phytostabilization

The soil from the steel disposal dumps was characterized by an alkaline pH (8.4 ± 0.13), low organic matter content (1.08 ± 0.09%) and a relatively high CEC (56.8 ± 0.11 cmol $kg^{-1}$). Based on particle size analysis, the soil was classified as loamy sand (72.2% sand, 26.4% silt, 1.4% clay). Concentrations of HMs (in mg $kg^{-1}$) in the soil were high (13.6 ± 1.4 Cd, 142.8 ± 5.3 Cr; 396.8 ± 10.3 Cu; 10,115.4 ± 65.1 Pb; 107.2 ± 6.3 Ni; 3,620.1 ± 40.2 Zn), and Pb and Zn contents exceeded the acceptable values specified in the regulations of the Polish Ministry of the Environment, Poland (2016), by approximately 17- and 4-fold, respectively.

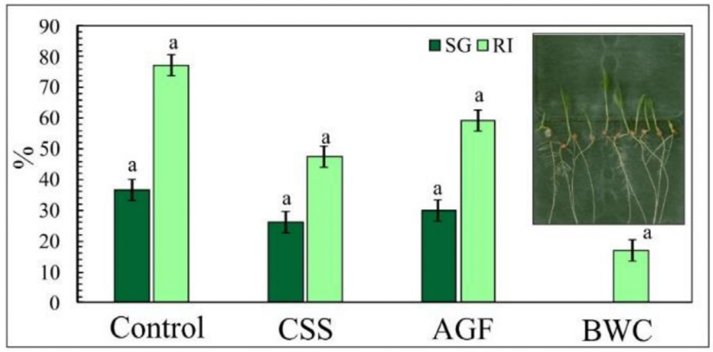
Regarding soil phytotoxicity, the amendments had a significant influence on the germination and root length of *S. saccharatum* (Fig. 1a). A similar effect was reported in an earlier work by Radziemska et al. (2020), in which the toxicity of soil from post-military areas that were contaminated with HMs was assessed using a phytotoxicity test. This is most likely caused by high accumulation of contaminants in the early growth stages of plants (Molnárová and Fargašová, 2012).

In soil amended with BWC, all plants germinated, and they had the longest roots of all variants. The addition of CSS improved the quality of the contaminated soil, as shown by the fact that 83.3% of sorghum seeds germinated in soil with this additive. Measurements of the length of roots after seven days revealed that CSS positively affected the germination of seeds, but the length of the roots was shorter by 40% than that in the soil amended with BWC (Figure S1). In soil amended with AGF, on the other hand, 70% of seeds germinated, with the root length being 50% shorter than that in the soil amended with BWC. The AGF serves mainly as a source of P and, due to its high pH (Table 1), it could decrease extractable nutrients for the plants. The differences in the length of the plant roots between the amendments were not significant. The highest SG was recorded for soils with AGF addition. The RI was also dependent on the additive used. The lowest RI was recorded for soil with BWC (Fig. 1a).

Soil toxicity testing using the Ostrakodtoxkit revealed high mortality of the crustaceans (30–40%) (Fig. 1b). Clear inhibition of the growth of the crustaceans was also observed. However, no significant differences between the variants were noted.

### 3.2. Characteristics of the soil after phytostabilization

Lowering the soil pH decreases the strength of bonds and breaks a)




b)

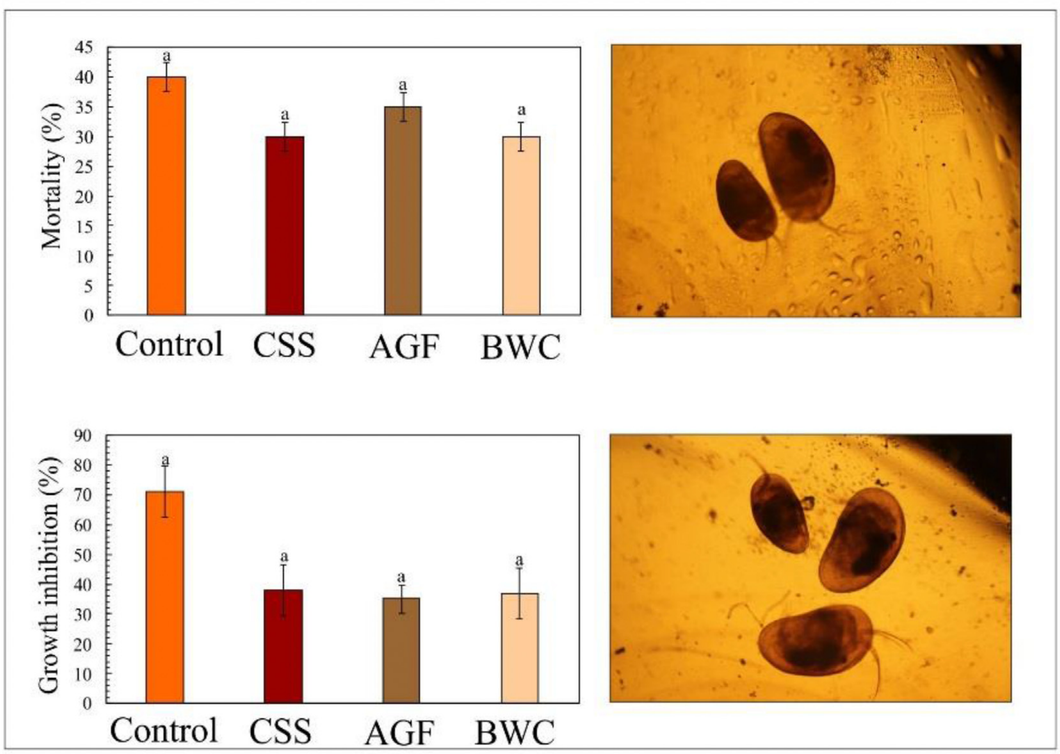



**Fig. 1.** Seed germination inhibition (SG) and root growth inhibition (RI) of *S. saccharatum* (a) and mortality and growth inhibition of *H. incogruens* (b) in soil. Error bars represent standard deviation error (SD, n = 3 determinations). The values followed by different letters differ significantly between sampling sites (ANOVA followed by Tukey's HSD test, $p < 0.05$). CSS is sewage sludge compost, AGF is poultry feathers ash and BWC is willow chips biochar.

down the microcrystalline structure of soil minerals, which leads to breakdown of the soil sorption complex, as well as leaching of alkaline cations to deeper layers of the soil profile (Cui et al., 2018). The main approach to immobilization of HMs in soil is increasing the pH, and thus decreasing the HMs' availability (Madejón et al., 2018). Addition of the amendments increased the pH significantly (Fig. 2a). Upon completing phytostabilization, the pH in non-amended soil was 7.06. The largest increase in the soil pH (up to 8.98) as compared to the non-amended soil resulted from the application of CSS. The increase in pH in soil amended with compost may have been caused by the mineralization of carbon and the subsequent production of $OH^-$ ions by ligand exchange and an increase of the content of basic cations (Mkhabela and Warman, 2005; Gusiatin and Kulikowska, 2016b). Compost with a high pH and organic matter value may increase soil surface ligands, strengthening their ability to bind and immobilize HMs (Chen et al., 2018).

In soil after plant harvesting, the death rate of *H. incongruens* ranged from 6.7 to 33.3%. In the amended soil, the death rate of organisms was found to average 11.1%. The addition of BWC had a

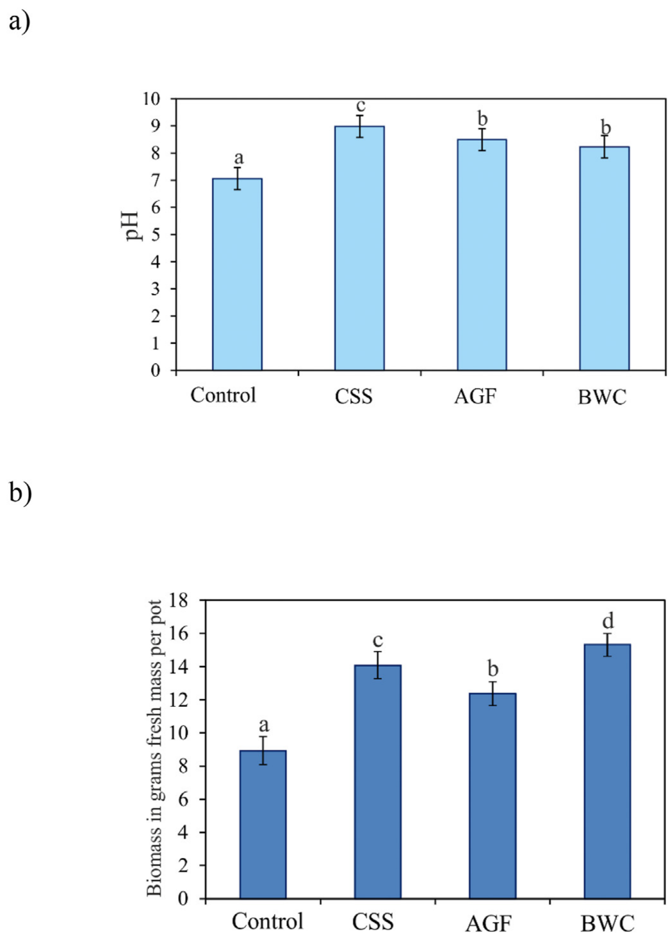



**Fig. 2.** The effect of aided phytostabilization on: a) soil pH, b) *L. luteus* biomass. Error bars represent ± standard deviation (n = 3). The pH or *L. luteus* biomass values followed by different letters differ significantly between sampling sites (ANOVA followed by Tukey's HSD test, $p < 0.05$). CSS is sewage sludge compost, AGF is poultry feathers ash and BWC is willow chips biochar.

positive effect on the development of organisms, and the death rate was at a low level of 10%. When BWC was applied, the crustaceans were characterized by higher growth than with the remaining amendments (Figure S2).

### 3.3. Plant growth

The effects of CSS, BWC and AGF amendments on above-ground biomass production of *L. luteus* grown in HM-contaminated soil are shown in Fig. 2b. *L. luteus* yield in non-amended soil was influenced by a high sensitivity to soil HM contamination. The crop yield in non-amended soil was lower than of that in which amendments had been applied by 36% on average. The addition of CSS, BWC or AGF amendments significantly improved plant growth. Compared to non-amended soil, the total above-ground plant biomass in soil amended with CSS, AGF and BWC increased by 37%, 28% and 42%, respectively. The results obtained here suggest that the addition of BWC could significantly improve *L. luteus* growth. Biochar may protect plants against infection, improve soil fertility and productivity and possess the capacity to retain and exchange nutrients, which would result in better availability of nutrients to plants (Saletnik et al., 2019). These results were consistent with those of Gonzaga et al. (2019) for *Brassica juncea* L. with coconut husk and orange shell biochars and Mandal et al. (2019) for *Triticum aestivum* L. cv. Calingiri. The additive which increased the tested crop yield to a lesser extent was CSS. This observation was similar to that reported by Radziemska et al. (2019c), where the positive impact of compost from food waste on *Lolium perenne* L. growth was observed.

### 3.4. Concentrations of HMs in lupine shoots and roots

HM mobility in the soil-plant system depends, above all, on the soil conditions, including the contamination level, the plant species and the environmental factors (Radziemska et al., 2014). The degree of mobility is proportional to the soil HM concentration. The accumulation of HMs was considerably higher in the roots than in the above-ground parts of *L. luteus* (Fig. 3) and depended on the type of amendment. Compared to non-amended soil, the greatest accumulation of Cu and Cr in the roots was observed in soil amended with AGF, whereas Ni and Zn accumulation was greatest in soil amended with BWC, and that of Cd in soil amended with CSS (Fig. 3). Incorporation of soil amendments usually reduces HM metal uptake by the plant, and facilitates concentration of HMs in the roots rather than in the shoots. Amendments increase the absorption and accumulation of HMs by roots, but the effect of an amendment on HM uptake varies with the type of HM (Mu et al., 2020). Wang et al. (2019) demonstrated that biochar can immobilize HMs in contaminated soils while improving soil quality, which can significantly reduce HM uptake by crops. For example, Cu and Ni concentrations in roots are considerably higher than the concentrations in the aerial organs of plants, which might be due to the complexation and sequestration of these HMs in the vacuoles of the root cells, making them unavailable for translocation to the shoots (Lasat et al., 2000). In the present study, amendments, especially BWC, facilitated root growth and development (data not shown). Some HMs could be preferably adsorbed onto the root surfaces as a first-line barrier, followed by binding to polysaccharides of the rhizodermal cell surface (Xie et al., 2020). In addition, root exudates (e.g. carboxylic acid and amino acids) form complexes with HMs promoting their stabilization within roots (Luo et al., 2014). BWC amendment may also influence iron plaque formation on root surfaces; this plaque exhibits a high capacity to sequestrate certain HMs (Zheng et al., 2012). Sequestration of Zn in iron plaque on *Typha latifolia* L. roots was reported by Ye et al. (1998), and it was positively correlated with Fe concentrations on the root surface. This might be a reason for the increased Zn accumulation in roots in the BWC-amended soil.

### 3.5. The effect of aided phytostabilization with processed waste on HM transformation and immobilization in soil

Apart from HM accumulation in *L. luteus* tissues, changes in their distribution patterns in soil were also determined. It was shown that phytostabilization with processed waste amendments led to HM redistribution, but its extent depended on the type of HM and the type of amendment. The distribution patterns of Cd, Cr, Cu, Ni, Pb and Zn in soil after phytostabilization are shown in Fig. 4, and their concentrations in individual fractions are summarized in Table S2. Both mobile (F1) and recalcitrant (F2, F3, F4) fractions can be used to evaluate HM immobilization in soil (He et al., 2019). In this study, HM immobilization was also assessed with the $I_R$ index (Fig. 4). The $I_R$ includes HM transformations in all these fractions. The higher the value of the $I_R$ (from 0.06 to 1.0 for the BCR procedure), the greater the stability and immobilization of a given HM (Gusiatin and Kulikowska, 2016b). In addition, the mRI (Fig. 5) was used to evaluate HM toxicity and environmental risk posed by the mobile HM fraction.

In non-amended soils, HMs considerably differed in distribution

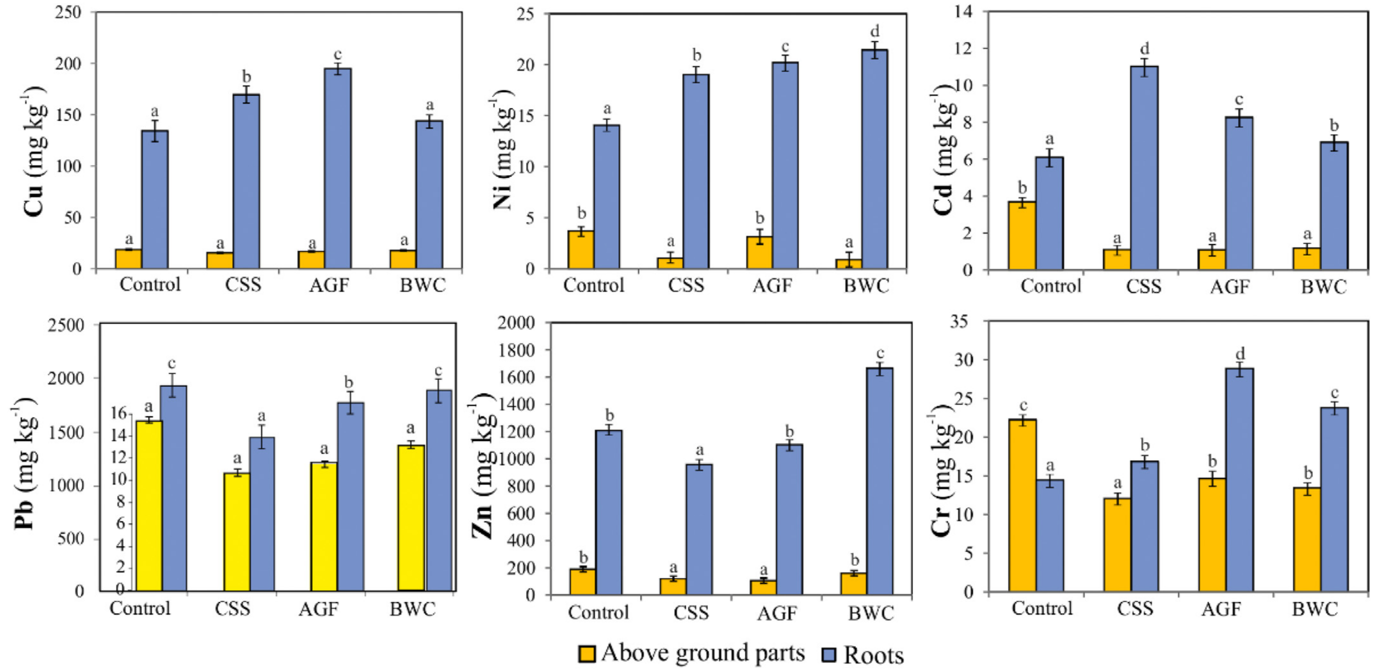



**Fig. 3.** The effect of processed waste amendments on HMs content in above-ground parts and root tissues of *L. luteus*. Error bars represent ± standard deviation (n = 3). For individual HMs, their concentrations followed by different letters differ significantly between treatments (ANOVA followed by Tukey's HSD test, $p < 0.05$). CSS is sewage sludge compost, AGF is poultry feathers ash and BWC is willow chips biochar.

patterns. The largest shares of Zn and Cd were in the exchangeable F1 fraction; that of Pb, in the reducible fraction (F2); those of Cr and Cu, in the oxidizable fraction (F3); and that of Ni, in the residual (F4) fraction (Fig. 4). In scrapyard soil with a pH of 7.73 and lower total concentrations of Cd, Cu, Pb and Zn than those in the present study, Jaradat et al. (2006) found Cd to be the most mobile. In addition, the F2 fraction was shown to be the most important for HM retention (28–53%) (Jaradat et al., 2006). This is because HMs in soil either co-precipitate with metal oxides or are adsorbed on the surface of metal oxides at pH > 7.

The effect of soil amendments on HM transformations varied. With regard to Cu, BWC caused the largest increase in its share in the F2 and F3 fractions, and the share of Cu in the F4 fraction was the lowest among the treatments. Because Cu was mostly redistributed into the F2 fraction and its share in the most stable F4 fraction decreased, its stability (in terms of the $I_R$) was lower in amended soil than in non-amended soil (Fig. 4a).

All amendments had a positive effect on Ni immobilization by decreasing its share in the F1 fraction, and by increasing its share in the most stable F3 and F4 fractions (Fig. 4b). As a result, the stability of Ni (as $I_R$) in amended soil (especially in soil with CSS) was higher ($p < 0.05$) than in non-amended soil.

Similar to what was observed with Ni, the application of the three amendments improved the immobilization of Pb in soil after phytostabilization. This improvement in HM stability was caused by decreasing its share in the F1 fraction, and increasing its share in the F2 and F4 fractions (Fig. 4c). With regard to Pb immobilization and stability (in terms of $I_R$), AGF was more useful than CSS or BWC.

AGF had the best effect on Cd immobilization of the processed wastes that were investigated. Its application caused the largest decrease in Cd content in the F1 fraction, and a concurrent increase in the F2 and F3 fractions (Fig. 4d). The distribution pattern of Cd in soil amended with AGF was reflected by the highest $I_R$ value (0.34). Our previous research has revealed that the AGF is an effective adsorbent with high maximum adsorption capacity for Cd (Gusiatin et al., 2020). In contrast, application of BWC decreased the Cd share in the F4 fraction.

As for Zn, AGF and BWC increased its immobilization to a greater degree than CSS. AGF and BWC were the most effective amendments for decreasing the share of Zn in the F1 fraction and increasing its share in the F2, F3 and F4 fractions (Fig. 4e). As a result, the $I_R$ values for Zn in AGF and BWC amended soils were higher ($p < 0.05$) than the $I_R$ values for Zn in non-amended and CSS-amended soils.

In contrast to the other HMs, Cr did not occur in the F1 fraction in both non-amended and amended soils. In BWC amended soil, there was a visible increase of the Cr share in the residual (F4) fraction as compared to non-amended soil (Fig. 4f). As a result, Cr stability increased, from $I_R = 0.48$ (non-amended) to $I_R = 0.57$ (BWC amended soil).

The HM transformations in soils with aided phytostabilization might be ascribed to the changes in the chemical properties of the soil caused by the addition of the amendments (i.e. an increase in pH and organic matter content, and changes in the mineral composition of the soil) (Grobelak and Napora, 2015). In this study, BWC decreased the shares of HMs in the most mobile fraction, especially those of Zn, Ni and Pb. The primary mechanisms of HM immobilization by biochar in soils are generally an increase in soil pH, ion exchange, adsorption and precipitation processes (He et al., 2019). Ali et al. (2019) found that phytostabilization of Zn, Pb, Cd, and Cu in alkaline soils amended with wood biochar resulted from ion exchange, precipitation of HM-carbonates and HM-phosphates, and chemisorption on the biochar surface. Ahmad et al. (2017) found that biochars from agricultural waste were effective in reducing Cu and Pb mobility in alkaline shooting range soil, as a result of surface metal complexes with Fe-/Al-minerals in biochars, metal-phosphate precipitation, and metal-hydroxide precipitation due to a biochar-induced pH increase. Biochar may facilitate the formation of stable inner sphere complexes between HMs and functional surface groups on the biochar, as well as fixation via

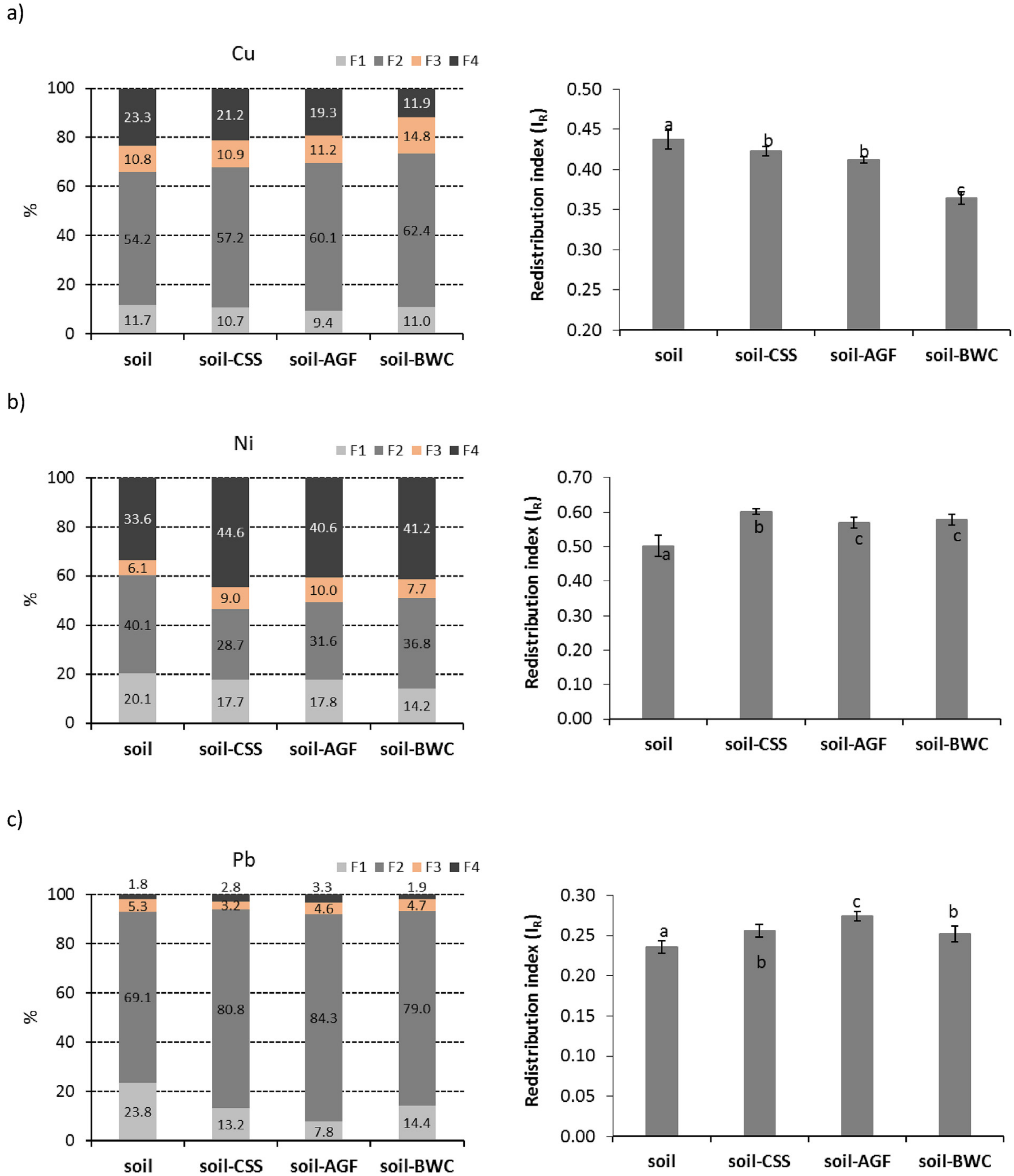



**Fig. 4.** Distribution patterns and redistribution indices ($I_R$) for different HMs in soil after enhanced phytostabilization with compost (CSS), ash (AGF) and biochar (BWC): d) Cd, e) Zn, f) Cr. Error bars represent standard deviation error (SD, n = 3 determinations). For the $I_R$, indices followed by different letters differ significantly between soil amendments (ANOVA followed by Tukey's HSD test, $p < 0.05$).

intraparticle diffusion in meso- and micropores in the biochar (Fang et al., 2016; Qiao et al., 2018). Shaheen et al. (2019) and Xia et al. (2019) demonstrated that Cr retention in biochar can occur through adsorption via π−π electron donor−acceptor interactions and pore diffusion. All of the above-mentioned mechanisms may be related to the increase in the share of some HMs in the residual fraction that was observed in the study reported here.

The capacity of biochar to redistribute HMs in soil may also be due to its high specific surface area (Qiao et al., 2018). As BWC had the highest surface area among the amendments (Table 1), it could have facilitated HM redistribution (i.e. Cr, Ni and Zn) into the residual fractions. Some researchers, using synchrotron-based X-ray absorption spectroscopy, confirmed that biochar stimulated Cd and Cu to form (oxy)hydroxide, carbonate and organically bound

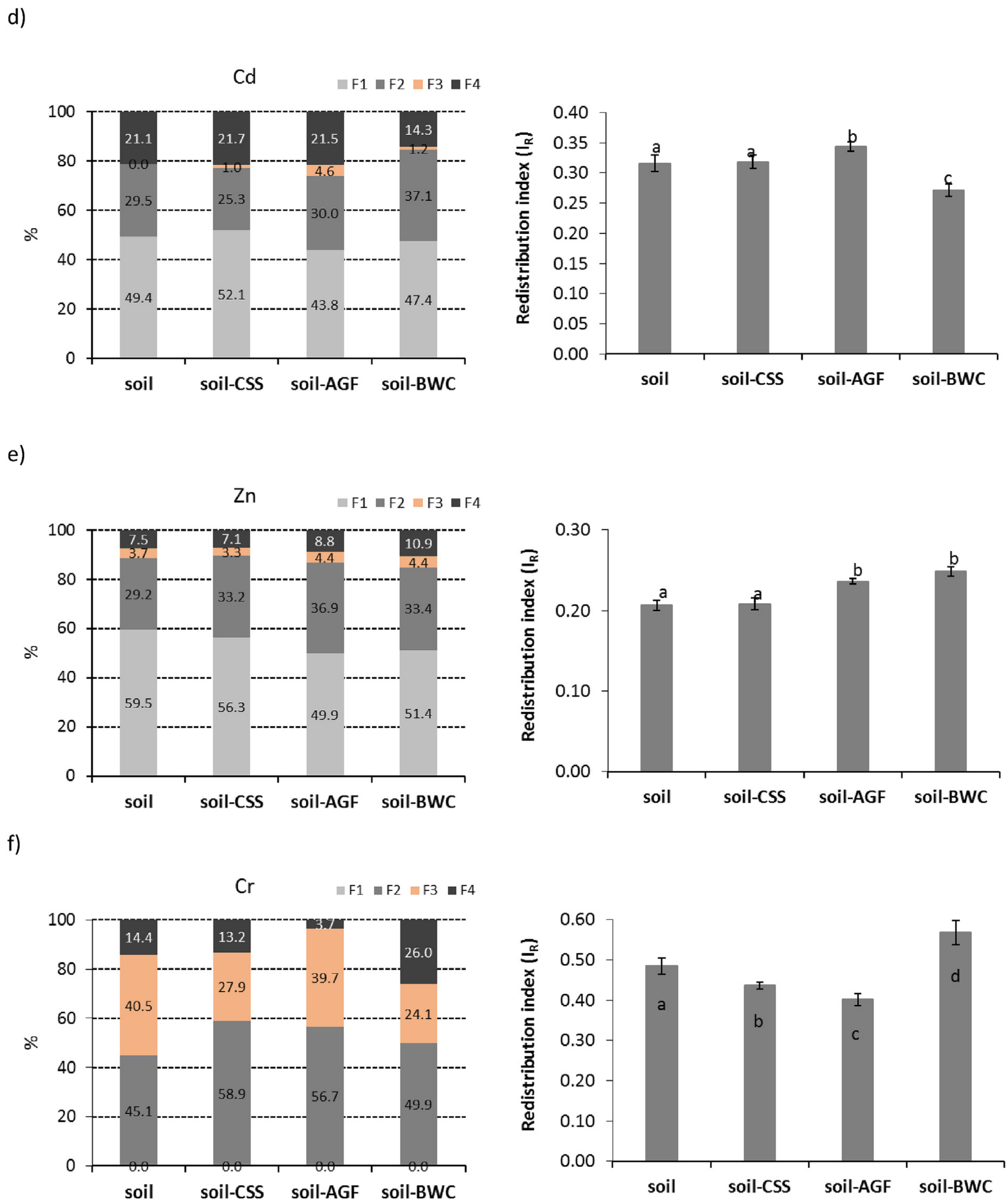



**Fig. 4.** (*continued*).

fractions (Ippolito et al., 2012; Cui et al., 2019). In this study, the share of Cd and Cu in the reducible (F2) and oxidizable (F3) fractions was higher than that in non-amended soil. In contrast, application of BWC decreased the share of Cu and Cd in the residual (F4) fraction. This was associated with HM redistribution into the reducible (F2) fraction in the case of Cd, and into the reducible (F2) and oxidizable (F3) fractions in the case of Cu. Cui et al. (2019) have also demonstrated decreasing Cd content in the F4 fraction in biochar-amended soil, which was derived from Cd transformations to the exchangeable fraction and promotion of the formation of Cd bound to iron/manganese oxyhydroxides. Another probable explanation for the decrease in the Cd and Cu share in the F4 fraction is that metal-(hydr)oxide precipitates might block active sites on biochar surfaces (Bogusz et al., 2017), reducing effective redistribution of Cd and Cu into the residual fraction.

The superiority of AGF and BWC over the CSS may result from the strong ability of these amendments to increase soil pH. AGF was more alkaline than BWC, whereas CSS had a neutral pH. The liming effect of the amendments may have promoted HM precipitation, thus contributing to a reduction in pools of labile HMs and an

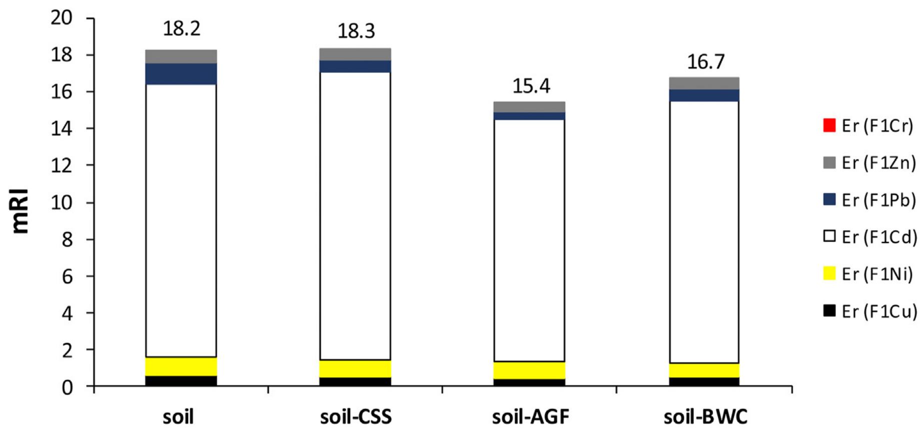



**Fig. 5.** The modified risk indices (mRI) in soils after aided phytostabilization. Each mRI value is composed of potential risk from the F1 fraction for individual HMs, i.e. $E_r$(F1). CSS is sewage sludge compost, AGF is poultry feathers ash and BWC is willow chips biochar.

increase in their content in the residual fraction (Abou Jaoude et al., 2020). The immobilization of HMs in this study may also have resulted from their precipitation as insoluble salts in the form of phosphates. It is possible that this is the predominant mechanism of action for AGF, which contained a high concentration of P (17.4%). Phosphate-derived materials are known to reduce HM bioavailability and mobility in soil (Clemente et al., 2006).

The higher efficiency of HM immobilization by AGF and BWC than by CSS during aided phytostabilization could be related to the high content of silica in both amendments, which have a negative charge at a high pH, favoring HM bonding.

To reduce the harmful effects of HMs, it is necessary to monitor the soluble and exchangeable fractions of HMs in soils (Eissa, 2019). For that purpose, the mRI was used, which is a modified version of the Ecological Risk Index (Håkanson, 1980) and a simplified version of the modified risk index (Zhu et al., 2012). The mRI takes into account the share of mobile HMs in combination with the toxicity of the HMs. Although, regardless of the treatment used, the soil displayed medium risk after phytostabilization, (Fig. 5), AGF decreased environmental risk to the largest extent, followed by BWC and CSS. Among all HMs, Cd had the highest potential risk.

In general, CSS facilitated HM redistribution and decreased the potential environmental risk of HMs in calcareous soil less effectively than AGF and BWC. The results therefore suggest that more efficient CSS performance in terms of HM redistribution may well be observed in acidic soil.

### 3.6. Changes in microbial community structure with different soil treatments during aided phytostabilization

The soil microbial community was characterized using 16S rRNA gene amplicon sequencing. About 400 thousand high-quality reads were obtained with an average of about 30 thousand reads per soil sample (Tab. S3). The sequencing depth was sufficient, as indicated by rarefaction curves (not shown) and the similarity of the OTU number and the Chao1 index for each sample, which were within 10% of each other. The lowest number of operational taxonomic unit (OTUs) in all analyzed samples was noted in samples that were used as inoculum (soil0 and soil-CSS0). In all amended soils, the number of OTUs gradually increased over time, whereas in the non-amended soil, the number first increased, then decreased to 735 OTUs at the end of the experiment (Tab. S3).

HM contamination can negatively influence the microbial diversity and biological activities of soil microbes (Xie et al., 2016). In this study, all amendments improved microbial community diversity, expressed by the Shannon index (H′), compared to that of non-amended soil. In non-amended soil, H′ increased from 4.27 to 5.44 at the end of the experiment (Tab. S3). In amended soils, H′ at the end of the experiment was about 9, 4 and 5% higher than in non-amended soil for CSS, AGF and BWC, respectively. High microbial diversity is advantageous because it translates into a more diverse metabolic potential of the community, which allows it to better cope with unfavorable environmental conditions. The higher microbial diversity in amended soil can also explain its higher *L. luteus* biomass yield compared to non-amended soil. The presence of a diverse microbial community can protect plants grown in HM-contaminated soils by enhancing HM retention in roots and helping plants acquire sufficient nutrients and recycle the organic matter (Xie et al., 2016). Changes in microbial composition due to soil amendment with organic/inorganic additives can be attributed to increased nutrient availability, pH changes, and the soil's capacity to retain water and chelate HMs (Basanta et al., 2017). Zhou et al. (2019) have found that a calcareous soil amended with biochar and compost had higher soil nutrient content, higher bacterial diversity and higher microbial biomass compared to non-amended soil. A high biochar porosity provides a habitat for microorganisms and protects them from predators, supporting microbial diversity (Lehmann et al., 2011).

PCA showed that the soil microbial community changed significantly over time ($r^2 = 0.92$, $p = 0.001$). The ten most loaded taxonomic groups in the PCA model are presented in Fig. 6. *Acidovorax* sp., *Acinetobacter* sp., *Dietzia* sp., and *Brevundimonas* sp. predominated in all variants at the beginning of the experiment (Fig. 7). *Acidovorax* sp. was abundant in both soil (soil0, 24.8%) and compost (soil-CSS0, 17.5%), but its abundance decreased drastically to less than 2% in all samples at the end of the study (Fig. 6). The high abundance of *Acidovorax* sp. in soil taken for the experiment confirms the fact that this genus is often identified in the rhizosphere microbiome during phytoremediation. It was one of the most common genera, next to *Pseudomonas* sp., *Flavobacterium* sp. and *Variovorax* sp., which also appeared in our experiments and in other studies on phytoremediation (Brown et al., 2012). The high percentages of *Acinetobacter* sp. and *Dietzia* in the inoculums (Fig. 6) decreased after ten days of the experiment, and after 30 days they comprised no more than 0.5% of the bacterial biomass in all samples.

*Parasegetibacter* sp. and *Stentrophomonas* sp. predominated in all variants in the transition period of adaptation of microorganisms to environmental conditions during the experiment. *Parasegetibacter* sp. were most numerous after ten days of the

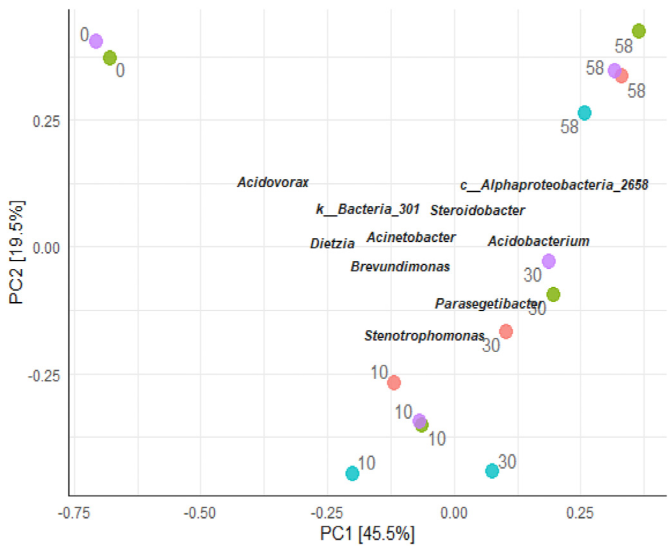



**Fig. 6.** Principal component analysis (PCA) of the microbial community profiles of soil samples with the 10 most loaded OTUs with their highest possible taxonomic assignment; soil-BWC (red), soil-CSS (green), soil-AGF (blue), soil (purple); numbers next to points refer to the day of the experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

experiment in CSS- and BWC-amended soil, and after 30 days in AGF amended soil (Fig. 7). *Stentrophomonas* sp. were most abundant after ten days of incubation constituting from 4.2 to 7.6% of all obtained sequences in AGF amended soil and non-amended soil, respectively. The presence of *Stentrophomonas* sp. in the transition phase was favorable because species belonging to this genus possess different mechanisms to decrease HM toxicity in the soil, which favors plant growth. *Stenotrophomonas maltophilia* tolerates high levels (0.1–50 mM) of such toxic HMs as Cd, Pb, Co, Zn or Hg and can accumulate Cd up to 4% of its biomass. *S. maltophilia* Sm777 overcomes HM toxicity by reduction of oxyanions to non-toxic elemental ions and detoxification of Cd into CdS (Pages et al., 2008).

In the soils sampled at the end of the experiment, the abundance of *Steroidobacter* sp., *Acidobacterium* sp. and microorganisms from class Alphaproteobacteria had increased the most from the beginning of the experiment (Fig. 7). The presence of *Acidobacterium* sp. was highest in non-amended soil (soil58, 9.8%), which indicated a better growth of *Acidobacterium* sp. in the absence of soil amendments. This may be due to the fact that all amendments caused an increase in pH, and *Acidobacterium* sp. was identified mainly in HM-contaminated environments with low pH such as pyrite acidic mine waters (Santofimia et al., 2013) or acid mine drainage (García-Moyano et al., 2012). The percent share of *Steroidobacter* sp., which is commonly found in different soils (Sakai et al., 2014; Gong et al., 2016), varied from 1.4% in AGF amended soil to 2.8% and 2.7% in BWC and CSS amended soil, respectively.

At the end of the experiment, *Flavobacterium* sp. constituted from 1 to 3.2% of all bacteria in the experimental soils while abundance of *Pseudomonas* sp., although high at the beginning of the study (2–3%), decreased to below 1% at the end of the experiment. Microorganisms from both genera may improve plant growth via e.g. phytohormone production, biocontrol through synthesis of siderophores or low molecular weight organic acids that increase the solubility and mobility of toxic HMs (Ullah et al., 2015). Siderophore production by *P. aeruginosa* increased bioavailable concentration of Cr and Pb, and as a result maize plants exhibited enhanced uptake of these toxic HMs (Braud et al., 2009). Generally, in soils, $Fe^{3+}$, occurs mostly in the form of insoluble oxyhydroxides and insoluble hydroxides, which are not available to microorganisms and plants. Siderophores chelate $Fe^{3+}$, and such a form of iron is subsequently taken up by plants as iron nutrient. Reducing the percentage of *Pseudomonas* sp. both in the non-amended and in the amended soil may indicate that the type of plant used for phytoremediation does not support its growth.

Application of AGF as an amendment resulted in the highest lowering of the potential environmental risk of soil. Moreover, the highest soil stability was obtained for Pb and Zn, with over-normative concentrations in the investigated soil, and for the most toxic Cd. AGF contained more P than other amendments which favored microbial growth as P is often a limiting factor for microbial growth in soil (Demoling et al., 2007). The microbiological composition indicated that the addition of AGF to the soil resulted in an increase in the share of microorganisms from the genera *Arenimonas*, *Brevundimonas*, *Gemmatimonas* and *Variovorax*, which were identified in HM-contaminated areas (He et al., 2007; Liu et al., 2018) and whose presence and the metabolic potential could have contributed to the better plant growth.

The highest percentage share of *Arenimonas* sp. in AGF amended soil was observed after ten days of the experiment and at the end of the experiment, it was more than two times higher than in the case of the other amendments and in non-amended soil. Within the genus *Arenimonas*, there are species with a high tolerance to the concentration of HMs. For example, activity of aerobic *Arenimonas metalli* sp. nov. isolated from an iron mine is not inhibited by concentrations of up to 0.3, 0.3, 0.4, 0.5 and 0.5 mM for $Zn^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Cr^{6+}$ and $Fe^{3+}$, respectively (Chen et al., 2012). *Arenimonas* sp. carry genes for alkaline phosphatase, a metalloenzyme with Cd, Zn, and Mg, and a sulfate binding site (Stec et al., 2000). Alkaline phosphatase enables this genus to solidify or mineralize metal(loid)s by biosorption.

At the end of the experiment, abundance of *Brevundimonas* sp. was 4–5 times higher in AGF amended soil than in the soil amended with other amendments and over three times higher than in non-amended soil. This genus was observed in both HM- and oil-polluted soils (He et al., 2007; Chaudhary and Kim, 2018). *Brevundimonas* sp. can tolerate high HM concentrations in soils and their presence significantly affect the growth of plants in polluted sites. The growth of *Brevundimonas* sp. strain X08 isolated from soils co-contaminated by Cd and polycyclic aromatic hydrocarbons was prohibited at as high concentration of Cd as 0.5 mM (Jiajun et al., 2010). *B. diminuta* strain NBRI012 improved phytostabilization of As in rice plants. The plant growth-promoting traits of this bacterial strains revealed the inherent ability of siderophores, phosphate solubilization, indole acetic acid, 1-aminocyclopropane-1-carboxylic acid deaminase production which may be associated with increased biomass and chlorophyll content of rice. The strain was also able to accumulate As and its presence in soil restored the hampered root epidermal and cortical cell growth of rice plant (Singh et al., 2016).

Abundance of *Gemmatimonas* sp. in AGF amended soil was 5, 3 and 2 times higher, than in BWC, CSS amended soil and in non-amended soil, respectively. Gemmatimonadetes has been identified as one of the top phyla found in soils and members of *Gemmatimonas* sp. co-predominated in bacterial communities associated with the rhizosphere of pioneer plants such as *Bahia xylopoda* and *Viguiera linearis* growing on HM-contaminated soils (Navarro-Noya et al., 2010). Analysis of abundance of *Gemmatimonas* sp. in soils indicates that these bacteria prefer low soil moisture (DeBruyn et al., 2011). This genus was identified in systems with enhanced biological P removal (Zhang et al., 2003) which may explain its high percentage in the AGF amended soil characterized by the highest P content.

The percentage of *Variovorax* sp. sequences in AGF amended soil

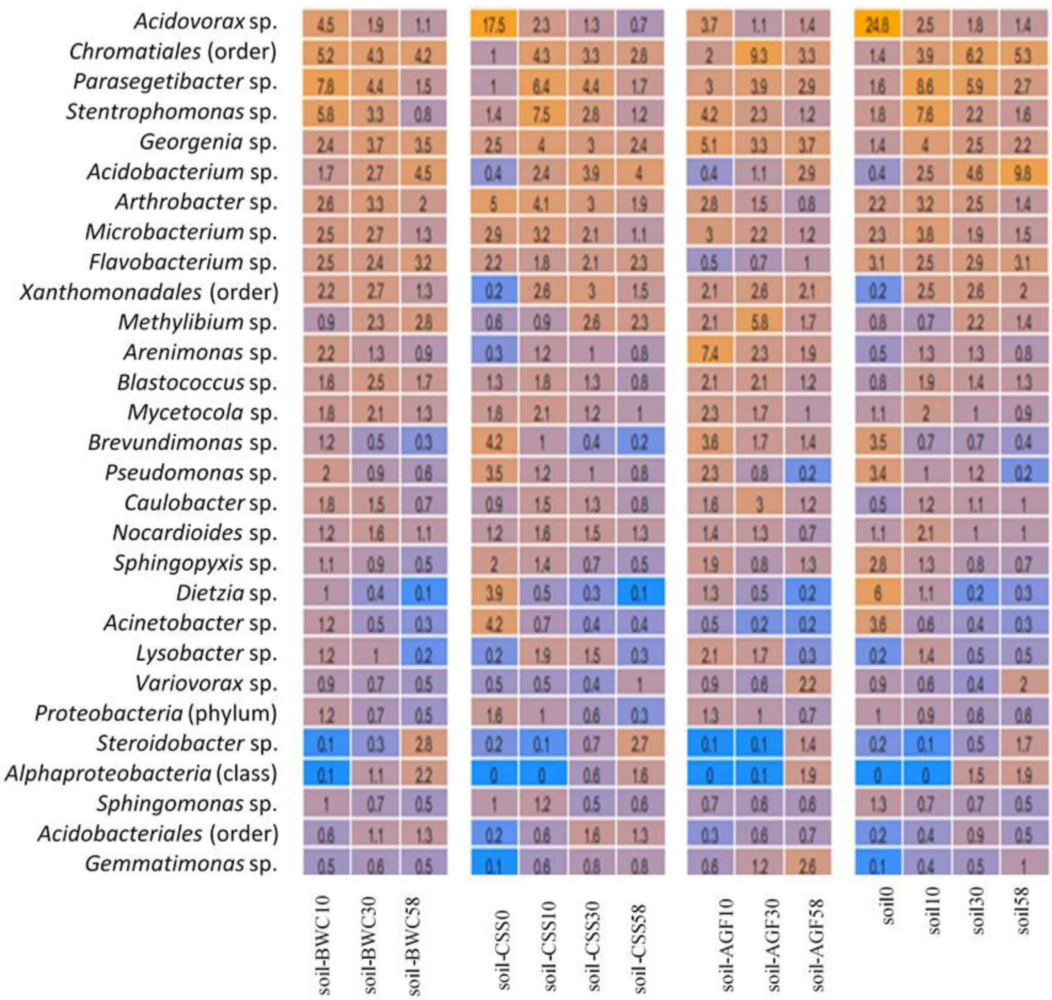



**Fig. 7.** Heatmap presenting changes in the percent share of sequences of the most abundant microbial taxa in control soil and soil with amendments; BWC is biochar, CSS is compost, AGF is ash, soil (non-amended); the numbers in sample name indicate the day of sampling.

was slightly higher than in non-amended soil and it was much higher than in the soil amended with other amendments. The high proportion of this group of microorganisms in the rhizosphere is beneficial because ureolytic bacteria belonging to *Variovorax* sp. can bioprecipitate HMs. *V. boronicumulans* removed 95.9% of Pb, 73.5% of Cd, and 73.8% of Zn from solutions containing these metals at concentrations of 100, 300, and 500 mM, respectively (Jalilvand et al., 2020). In some *Variovorax* sp. the presence of HMs can also increase the activity of catechol 2,3-dioxygenase involved in the degradation of aromatic hydrocarbons. Since environmental contamination with aromatic compounds is often accompanied by the presence of HMs such a feature of *Variovorax* sp. is indicated as a powerful tool in soil bioremediation (Hupert-Kocurek et al., 2013).

Some OTUs were shared between all experimental variants. In this study, such OTUs that constituted at least 0.5% of the sequences in the soil, independent of the time of the experiment and the type of amendment that was used were defined as core genera. Among core genera, the most numerous were *Acidovorax* sp., *Parasegetibacter* sp. and *Stentrophomonas* sp., and microorganisms belonging to order Chromatiales and *Georgenia* sp. Chromatiales constituted 4–5% in BWC amended soil, while in other experimental variants their abundance increased from the 10th day of the experiment, reaching 9.3% in soil-AGF30. Less numerous core genera with abundances of approximately 1–3% were *Microbacterium* sp., *Arthrobacter* sp., *Mycetocola* sp. and *Blastococcus* sp. (Fig. 7).

### 3.7. Implications of aided phytostabilization as a nature-based solution for achieving the sustainable development goals of the United Nations

Phytostabilization is a nature-based solution to the problems caused by pollution with HMs. Nature-based solutions are easy to apply and efficient as they imitate nature (Keesstra et al., 2018a). The use of plants to restore ecosystems is widespread today, and it can be done with the objective of removing HMs (Guo et al., 2019; Wu et al., 2020). Plants have been used also to protect the soil from the soil erosion, as is done with catch crops (Cerdà et al., 2020; López Vicente et al., 2020). This research contributes with information about restoring soils affected by pollution. This will contribute to achieving a more sustainable planet, as soil is a key component of the Earth and determines the distribution of plants and water resources (Rodrigo-Comino et al., 2018). The United Nations has developed the Sustainable Goals for Development for 2030, and the soil system is a relevant objective for reaching targets such as the Land Degradation Neutrality challenge (Keesstra et al., 2016; 2018a,b, Visser et al., 2019).

The research presented here shows that phytostabilization can contribute to reducing the human impact on the soil and Earth system and to achieving sustainability. A reduction in the level of toxics in the soil is a key change for achieving sustainability, and phytostabilization is contributing to this as an efficient technique of soil restoration.

## 4. Conclusions

Amending soil with processed wastes, i.e. compost (CSS), ash (AGF) and biochar (BWC), considerably decreased soil phytotoxicity and improved *L. luteus* biomass growth in soil from a steel disposal dump. The efficiency of aided phytostabilization by *L. luteus* depended on the type of metal and the specific amendment used. CSS and AGF facilitated the accumulation of Ni, Cd and Cu in the roots. AGF facilitated further accumulation of Cr, and BWC, accumulation of Zn. In the presence of the tested amendments, the metals showed different redistribution patterns in soil and were mostly redistributed into the reducible fraction, and/or partially into the oxidizable and residual fractions. AGF and BWC were more effective in increasing metal stability and decreasing potential environmental risk than CSS. All amendments facilitated microbial diversity and stimulated the growth of specific microbial species as the phytostabilization process progressed. For aided phytostabilization, amendment with pyrolyzed poultry feathers or with pyrolyzed willow chips seem to be good options for the phytomanagement of multi-metal contaminated soils from steel disposal dumps. These amendments simultaneously facilitate the improvement of the vegetation cover, decrease metal uptake and increase metal stability in soil, as well as inducing positive changes in the microbial community.

## Credit author statement

**Maja Radziemska**: Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing, Visualization. **Zygmunt M. Gusiatin**: Conceptualization, Methodology, Investigations, Resources, Formal analysis (statistical analysis), Writing - Original Draft, Writing - Review & Editing, Visualization. **Agnieszka Cydzik-Kwiatkowska**: Investigations, Resources, Writing - Original Draft, Visualization. **Artemi Cerdà**: Validation, Writing - Review & Editing, Visualization. **Vaclav Pecina**: Validation, Writing - Review & Editing. **Agnieszka Bęś**: Investigations (conducting pot experiments). **Rahul Datta**: Formal analysis (statistical analysis). **Grzegorz Majewski**: Investigations. **Zbigniew Mazur**: Investigations (conducting pot experiments). **Justyna Dzięcioł**: Investigation. **Subhan Danish**: Validation. **Martin Brtnicky**: Validation.

## 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.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.129576.

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**PŘÍLOHA Č. 4**

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# Successful Outcome of Phytostabilization in Cr(VI) Contaminated Soils Amended with Alkalizing Additives

**Maja Radziemska [1,*], Agnieszka Bęś [2], Zygmunt M. Gusiatin [3], Łukasz Sikorski [2], Martin Brtnicky [4,5,6], Grzegorz Majewski [1], Ernesta Liniauskienė [7], Václav Pecina [4,5], Rahul Datta [4], Ayla Bilgin [8] and Zbigniew Mazur [2]**

1 Institute of Environmental Engineering, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland; grzegorz_majewski@sggw.edu.pl
2 Faculty of Environmental Management and Agriculture, University of Warmia and Mazury in Olsztyn, Pl. Łódzki 4, 10-727 Olsztyn, Poland; agnieszka.bes@uwm.edu.pl (A.B.); lukasz.sikorski@uwm.edu.pl (Ł.S.); zbigniew.mazur@uwm.edu.pl (Z.M.)
3 Faculty of Geoengineering, University of Warmia and Mazury in Olsztyn, Słoneczna St. 45G, 10 719 Olsztyn, Poland; mariusz.gusiatin@uwm.edu.pl
4 Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemedelska1, 61300 Brno, Czech Republic; martin.brtnicky@seznam.cz (M.B.); vaclav.pecina@mendelu.cz (V.P.); rahul.datta@mendelu.cz (R.D.)
5 Institute of Chemistry and Technology of Environmental Protection, Brno University of Technology, Faculty of Chemistry, Purkynova 118, 62100 Brno, Czech Republic
6 Department of Geology and Pedology, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemedelska 3, 613 00 Brno, Czech Republic
7 Kaunas Forestry and Environmental Engineering, University of Applied Sciences, Liepu str. 1, Girionys, LT-53101 Kaunas reg., Lithuania; e.liniauskiene@kmaik.lm.lt
8 Faculty of Engineering, Artvin Coruh University, Seyitler Campus, 08000 Artvin, Turkey; ayla.bilgin@artvin.edu.tr
* Correspondence: maja_radziemska@sggw.edu.pl; Tel.: +48-2259-353-70

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**Abstract:** This study analysed the effect of three alkalizing soil amendments (limestone, dolomite chalcedonite) on aided phytostabilization with *Festuca rubra* L. depending on the hexavalent chromium (Cr(VI)) level in contaminated soil. Four different levels of Cr(VI) were added to the soil (0, 50, 100 and 150 mg/kg). The Cr contents in the plant roots and above-ground parts and the soil (total and extracted Cr by 0.01 M $CaCl_2$) were determined with flame atomic absorption spectrometry. The phytotoxicity of the soil was also determined. Soil amended with chalcedonite significantly increased *F. rubra* biomass. Chalcedonite and limestone favored a considerable accumulation of Cr in the roots. The application of dolomite and limestone to soil contaminated with Cr(VI) contributed to a significant increase in pH values and was found to be the most effective in reducing total Cr and $CaCl_2$-extracted Cr contents from the soil. *F. rubra* in combination with a chalcedonite amendment appears to be a promising solution for phytostabilization of Cr(VI)-contaminated areas. The use of this model can contribute to reducing human exposure to Cr(VI) and its associated health risks.

**Keywords:** soil contamination; soil remediation; immobilizing amendments; risk minimization; *Festuca rubra* L.

## 1. Introduction

The issue of soil contamination with chromium (Cr) compounds afflicts various regions all over the world [1]. No risk of the global natural environment contamination with Cr compounds has yet been observed. However, when emitted to the atmosphere, soil and water locally, it can be excessively present in biogeochemical circulation [2]. Cr compounds in the environment naturally originate from rock and soil erosion, aerosol deposition and volcano eruptions [3]. Cr is a component of mineral deposits, with chromite ore ($FeCr_2O_4$) being the only one of industrial importance. Among the other Cr compounds, only two occur in small amounts as minerals in the natural environment: lead chromate ($PbCrO_4$—crocoite) and sodium dichromate ($Na_2Cr_2O_7$) [4]. Much larger amounts of Cr are released to the soil from anthropogenic sources as this element is used in many branches of industry [5].

Cr compounds are applied in a variety of industries, e.g., galvanizing processes for protective and ornamental chrome plating of steel and brass items [6] and as an additive to construction steel. Chromates and dichromates are applied as pigments (e.g., chrome yellow PbCr) in manufacturing mineral paints used in ceramic, textile, plastic and paper industries and as paints [7]. Cr compounds are also used as corrosion inhibitors, as polymerization catalysts, as oxidizers in organic synthesis, as raw materials in perfume production and in making inks, as wood preservatives and as light-sensitive materials in photography [8]. Chromite ($FeCr_2$) is applied mainly in the production of fireproof materials (fireproof bricks and cement) [9]. Ferrochrome and other compounds, mainly chromates and dichromates are produced from $FeCr_2$. Cr(VI) is present during ore processing and the production of sodium and potassium chromate and dichromate, ammonium dichromate, Cr oxide, pigment production (lead and zinc chromate), dyeing textiles (Cr oxide, Cr sulfate) stainless steel and in tanning ($Cr_2[SO_4]_3$), glass production (Cr oxides) and galvanization ($CrO_3$) [10].

However, all Cr(VI) compounds are classified as carcinogenic [11,12]. People are exposed to Cr(VI) compounds present in contaminated potable water, air and soil. Factors that can affect the toxicity of Cr(VI) compounds include bioavailability, oxidative properties and solubility [12]. Even small amounts of Cr(VI) compounds can be harmful to human health [13]. Contact with Cr-containing materials often results in allergic reactions [14].

Soil is an important part of the natural environment and, as such, it should be covered by special protection [15]. Deterioration of the soil quality is largely caused by the ambient concentration of industrial pollutants containing toxic substances, including Cr(VI) compounds [16]. Soil Cr concentration can vary widely, from 1 up to 3000 mg/kg [17]. In soil, highly toxic Cr(VI) is more soluble and more mobile than essential Cr(III). Thus, soil contamination with Cr is dangerous because of the risk of it being taken up by crops grown on such soils, groundwater infiltration and the contamination of potable water intakes [18]. Of all the natural environment components, contamination with Cr is the most persistent in soil. It is associated with metal adsorption on humic colloids and silty minerals [19].

According to current knowledge, phytostabilization is one of the biological techniques of decreasing the health risks of contaminated soil [20,21]. Owing to its high effectiveness and non-invasiveness, it is increasingly often chosen as an effective method for the immobilization of contaminants in the near-surface soil layers [22]. Vegetation (especially the root zone) plays an important role in the stabilization of degraded land. It stabilizes contaminants, including heavy metals [23]. Numerous studies have been performed in terms of tolerance, uptake and accumulation of Cr by several plant species to evaluate the potential of these species for Cr phytoextraction [24,25]. Research on aided phytostabilization of Cr in contaminated soil, in contrast to conventional heavy metals such as Cd, Cu, Zn or Pb, is not so common. Plants used for phytostabilization should immobilize heavy metals in roots (or soil) rather than transport them up to the above-ground parts as they can be further mobilized in the food chain [26]. Until now, plants used for Cr phytostabilization in soil include: plant rose [27], Indian mustard (*Brassica juncea* L.) [28], *Cynodon dactylon*, *Chloris virgata* and *Desmostachya bipinnata* [29]. Among the different plant species, *Festuca rubra* L. has great potential for the immobilization of conventional heavy metals or their fixing in the rhizosphere to reduce metal transport to above-ground parts [30,31]. Since the role of *F. rubra* in phytostabilization of Cr in soil is poorly recognized, it is reasonable to examine the effect of *F. rubra* on the phytostabilization of Cr, especially when it occurs in the soil at different concentrations.

In a relatively new approach to the phytostabilization process, various process additives have been used to enhance phytostabilization (so-called "aided phytostabilization") [32]. This approach benefits from available natural immobilizing materials (both mineral and organic) and the effects of soil purification. In the present study, natural alkalizing amendments, such as limestone, dolomite and chalcedonite were used as additives aiding the Cr(VI) immobilization processes in acidic soil. The availability of mobile heavy metal species in the soil solution is increased in highly acidic and acidic soils [33]. This is associated with an increase in the solubility of these element compounds and a decrease in their adsorption on soil colloids when the soil pH is low [34]. Therefore, the additives proposed by the authors can be used in the remediation of soil in which the acceptable levels of heavy metal pollution are exceeded because of the alkalizing properties. The novelty of the experiment lies in using new soil amendments, which have not been analysed for an effect of different Cr concentrations on its phytostabilization.

The main objectives of this study were to compare the effectiveness of natural alkalizing amendments in the phytostabilization of Cr with *Festuca rubra* L. and to assess the effect of different Cr concentrations on its phytostabilization. For this purpose, the above-ground biomass of the test plant was determined, and the Cr content was examined in its various organs (above-ground parts and roots). The degree of Cr immobilization in the soil was calculated by determining the total concentration of Cr and the $CaCl_2$-extractable Cr. Moreover, an assessment of the phytotoxicity of soil exposed to Cr(VI) compounds was made with the use of *Sinapsis alba* L. and effective concentrations ($EC_{10}$, $EC_{20}$, and $EC_{50}$) were determined.

## 2. Materials and Methods

### *2.1. Plant Experiment*

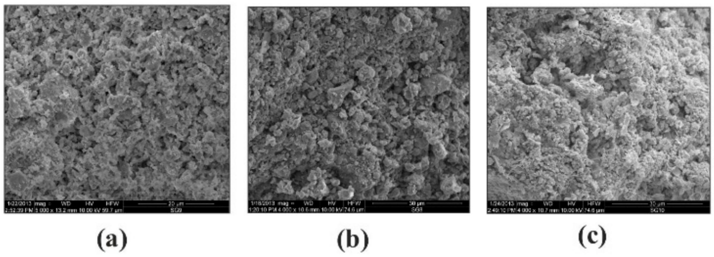
The impact of Cr(VI) at various levels of concentration and amendment of limestone, dolomite and chalcedonite on aided phytostabilization was examined. Pot experiments were performed in a greenhouse (natural daylight, 20–25 °C, 60–70% of humidity). The mass of soil in each polyethylene pot was 3.0 kg. For phytostabilization experiments, the surface soil samples (0–30 cm) from a non-contaminated, agricultural area (northern Poland) were collected. The soil was sandy in texture (86.6% sand, 11.2% silt, 2.2% clay) with acidic pH (5.81). The cation exchange capacity was 94.2 mmol/kg, whereas organic carbon amounted to 0.64%. The nutrient contents in the soil were as follows: total N 0.1%, extractable P 43.2 mg/kg, extractable K 8.72 mg/kg, extractable Mg 31.2 mg/kg.

The soil after air-drying was crushed and sieved to obtain a particle size of ≤ 2 mm. It was then spiked with aqueous solutions of $K_2Cr_2O_7$ as a source of Cr(VI) to obtain four different metal concentration, i.e., 0 (control), 50, 100 and 150 mg/kg. Before phytostabilization, the soil was fertilized with minerals (N-26%, $K_2O$-26%, Cu-0.025%, Mo-0.20%, B-0.013%, Mn-0.25% and Fe-0.05%).

Natural alkalizing amendments (limestone, dolomite, and chalcedonite) were mixed with the spiked and fertilized soil at a dosage of 3.0% (each one). Each treatment was replicated three times. The soil samples were carefully mixed and left for three weeks for stabilization under natural conditions. Seeds of *Festuca rubra* L. cv. Dark were then sown (5 g per each pot) and they germinated six days after sowing. Demineralized water was used for plant watering every other day. The amount of added water corresponded to 60% of the maximum water holding capacity of the soil. The experiment was finished after ca. 47 days after seed sowing. The harvested plants were weighed and then separated into above-ground parts and roots.

### *2.2. Soil Amendments*

Readily available alkalizing amendments were used in the experiment: limestone, dolomite, and chalcedonite. Figure 1 presents the amendments used (as scanning electron microscope (SEM) images) and their physico-chemical characteristics. Limestone was obtained from the PG Silesia company (Czechowice-Dziedzice, Poland); dolomite from the Dolomite Mine company (Sandomierz, Poland); and chalcedonite from the Chalcedon Poland company in Inowłódz.




| Parameter | Chemical Composition in Oxide (wt. %) | Specific Surface Area ($m^2/g$) | pH* |
|---|---|---|---|
| Limestone | CaO-87.47; $Fe_2O_3$-7.80; $C_2O$-1.50; $SiO_2$-1.34; $N_2O_5$-0.78; $Al_2O_5$-0.49; $K_2O$-0.48; MgO-0.05 | 0.91 | 10.02 |
| Dolomite | CaO-38.12; $N_2O_2$-22.30; $C_2O$-20.18; $SiO_2$-6.91; $Al_2O_5$-4.51; $Fe_2O_3$-4.41; CuO-2.28; MgO-1.29 | 1.22 | 7.51 |
| Chalcedonite | $SiO_2$-84.77; $Al_2O_5$-9.33; $C_2O$-4.29; $K_2O$-1.21; MgO-0.40 | 7.44 | 7.12 |

*pH in $H_2O$, 1:5 w/v

**Figure 1.** Scanning electron microscope (SEM) images of limestone (**a**), dolomite (**b**), chalcedonite (**c**).

### 2.3. Soil Analytical Methods

Soil samples were characterized for: pH (1:5 *w*/*v* suspension in distilled water using a pH meter EA940, Orion, IL, USA), total N (Kjeldahl method [35]), total organic carbon (TOC) (dichromate oxidation of samples followed by titration with ferrous ammonium sulfate [36]), available P (colorimetrically with vanadium-molybdenum method [37]), available K (atomic emission spectrometry) and available Mg (flame atomic absorption spectrometry, FAAS [38]). Total Cr was determined with FAAS using a SpectrAA 280FS spectrometer (VARIAN, Mulgrave, Australia). Before FAAS analyses, soil samples were digested in a mixture of concentrated HCl and $HNO_3$ (at 3:1 ratio) in a microwave oven (MARSXpress, CEM Corporation, Matthews, NC, USA) [39]. The samples were then filtered and analyzed for Cr. The accuracy of Cr analysis by FAAS was validated with the CRM 142R reference material.

### 2.4. Plant Chemical Analyses

After the experiment was completed, the plants were removed from the pots to avoid damaging their above-ground and root parts. Harvested *F. rubra* biomass was washed several times with de-ionized water to remove any soil particles and then dried for two weeks at room temperature. Finally, the biomass was homogenized to a fine powder using an analytical mill (Retsch type ZM300, Hann, Germany) and kept at room temperature in closed containers. The dry biomass of roots and shoots was recorded by their drying at 55 °C to a stable weight. The samples of dried and shredded roots and shoots were mineralized in nitric acid (65% *w*/*w*, Chempur, Piekary Śląskie, Poland) and hydrogen peroxide (30% *w*/*w*, Merck, Darmstadt, Germany) in a microwave oven (MARSXpress, CEM Corporation, Matthews, NC, USA). The filtered samples were analyzed for Cr with FAAS using a SpectrAA 280FS spectrometer (VARIAN, Mulgrave, Australia).

### 2.5. Phytotoxicity Analysis

The Cr(VI) toxicity assessment was performed with the germination and early plant growth test (Phytotoxkit FTM) using white mustard (*Sinapsis alba* L.) seeds. The phytotoxicity of Cr(VI) was expressed with the seed germination index, commonly applied in a phytotoxicity assessment. The tests were performed on soils contaminated with Cr (VI) at the following rates: 0, 50, 100 and 150 mg/kg of soil. Soil with no additives and soil with additives (limestone, dolomite, chalcedonite) were tested.

A standard OECD soil was used as a control. The test was conducted before *F. rubra* was sown and after the pot experiment was completed. All of the tests were conducted in three replicates. Ten seeds of *S. alba* germinated in special plastic plates on soil covered with filter paper. The plants under study were exposed to the contaminants for 72 h. The plates were incubated in darkness in a thermostatic cabinet (temperature 25 °C). After the set time, the germinated seeds were counted and the root length was measured (Image Tool 3.0 for Windows; UTHSCSA, San Antonio, TX, USA). The germination inhibition (GI) and root growth inhibition (RI) were calculated from the following formula:

$$\mathrm{RI\ (GI)} = \frac{\mathrm{A} - \mathrm{B}}{\mathrm{A}} \times 100 \tag{1}$$

where:

A—seed germination and root length in control (OECD soil),

B—seed germination and root length in a soil sample under examination (soil contaminated with Cr(VI) with and without additives).

Effective concentration (ECx) data were analyzed with a selected regression model to calculate the concentrations at 10, 20 and 50 response levels.

### 2.6. Accumulation Evaluation

The bioaccumulation coefficient (BCF) and translocation factor (TF) were used to analyze the Cr accumulation in the roots of *F. rubra* and the Cr translocation to above-ground parts of the plant and was calculated as follows: BCF = Cr concentration in roots/Cr concentration in soil, TF = Cr concentration in above-ground parts/Cr concentration in roots.

### 2.7. Statistical Analysis

Statistica 13.3 software (San Diego, CA, USA) was used for processing the experimental data. To test the normality of data distribution, the Shapiro–Wilk test was used, and Levene's test was used to test the homogeneity of variance. A one-way ANOVA and Tukey's test were applied to determine significant differences ($p < 0.05$) between phytostabilization treatments. The results were also subjected to Principal Component Analysis (PCA) in the XLStat program (Addinsoft, Paris, France).

## 3. Results and Discussion

### 3.1. Effect of Cr(VI) and Soil Amendments on the Biomass of F. rubra

Plant response to Cr depends on its concentration and the species in which it is present in the soil [40]. Symptoms of Cr toxicity in plants mainly include water balance disorders, root damage and biomass reduction [41]. Moreover, the visible effects of high contents of Cr include chlorosis and accelerated plant wilting. Plants that are highly sensitive to Cr show these symptoms at concentrations as low as 2 mg/kg [42]. The effects of soil amendments on the biomass of *F. rubra* grown in Cr(VI)-contaminated soils are shown in Figure 2. In pots with no soil amendments, the above-ground parts of *F. rubra* were affected by the highest Cr(VI) concentration, which is demonstrated by a significantly lower yield compared to pots with the amendments. This relationship was corroborated by Seleiman et al. [43] who found that Cr negatively affected biomass and yield of *Triticum aestivum* L., and Shankera et al. [44] and Golovatyj et al. [45], who reported that the above-ground biomass yield of *Zea mayze* L. and *Hordeum vulgare* L. decreased significantly in the presence of Cr compounds in soil. Among the soil amendments applied in this experiment, limestone increased the mean yield (for all the variants Cr(VI) level) of the above-ground parts of *F. rubra* by 48% and chalcedonite by 45% as compared to the control. The lowest above-ground biomass was found for the experiment with dolomite. Wyszkowski and Radziemska [3,46] reported that the application of alkaline soil amendment increased the mean yield of plants (*Avena sativa* L., *Hordeum vulgare* L. and *Zea mayze* L.) grown on Cr(VI)-polluted soil.

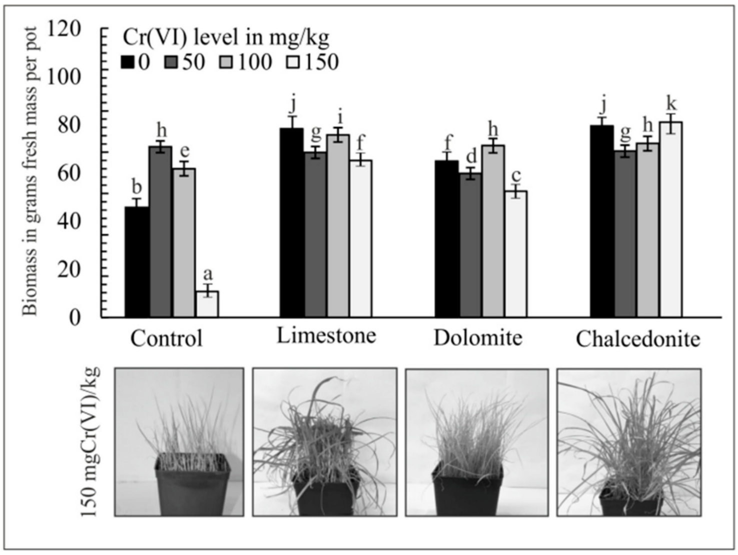



**Figure 2.** Effect of Cr(VI) and soil amendments (3% *w*/*w*) on the above-ground biomass of *F. rubra*. Error bars are ± standard error ($n = 3$). For a given Cr(VI) level, different letters indicate significant differences between treatments ($p < 0.05$).

## 3.2. Cr Accumulation in F. rubra

Physicochemical soil properties, such as pH, texture and humus content, considerably affect the degree of oxidation of Cr compounds [47] which, in consequence, affects their toxic effect on plants. Cr(VI) is very harmful to plants—the $Cr_2O_7^{2-}$ compound is highly toxic, while $Cr^{3+}$ contamination at the same concentration causes no damage [48]. The concentration of Cr in the roots and above-ground parts of *F. rubra* correlated with the Cr(VI) concentration and all amendments added to the soil (Figure 3). The Cr content was higher in the roots than in the above-ground parts of the test plant. This relationship is corroborated by the findings of a study by Ram et al. [49], in which the Cr concentration and accumulation in roots of hybrid Napier grass was significantly higher than in the above-ground parts. Soil contamination with Cr(VI) at 150 mg/kg resulted in the highest Cr accumulation in the analyzed plant. The alkalizing mineral amendments, which can increase soil sorptive capacity, can also reduce the content of heavy metals in the soil available to plants [48,50].

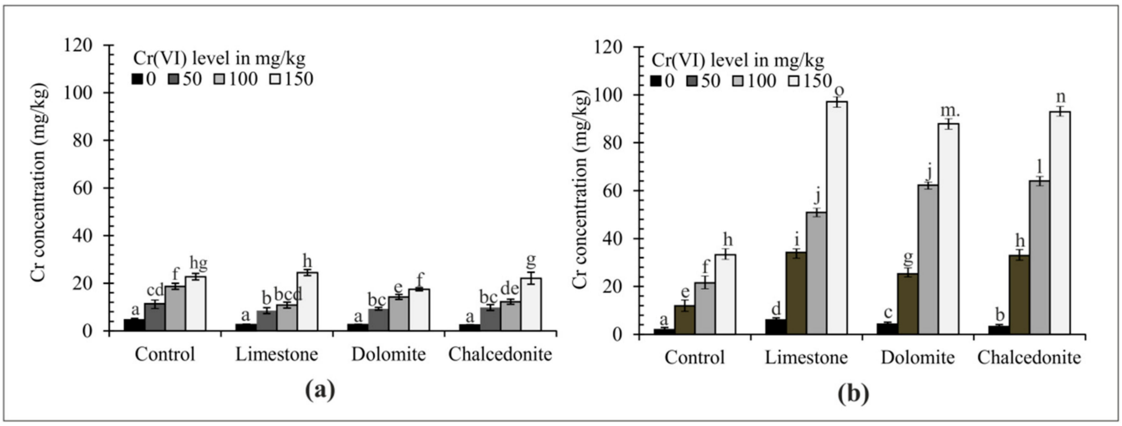



**Figure 3.** The effect of soil amendments (3% *w*/*w*) on Cr accumulation in the above-ground part (**a**) and roots (**b**) of *F. rubra*. Error bars are ± standard error ($n = 3$). For a given Cr(VI) level, different letters indicate significant differences between treatments ($p < 0.05$).

For parameters such as BCF and TF, which determine the plant effectiveness for phytostabilization, according to Mendez and Maier [51], BCF should be higher than 1 and TF should be lower than 1. The species in which heavy metals are present in soil are also important soil parameters, affecting the translocation process [52]. The highest mean BCF for *F. rubra* was observed under the chalcedonite treatment, whereas the lowest TF was observed under the limestone treatment. The highest BCF values were observed following limestone, dolomite, and chalcedonite application, when the soil was contaminated with Cr(VI) at 50 mg/kg.

### 3.3. Phytotoxicity

The phytotoxicity test, performed on the soil before the phytostabilization experiment was set up, showed increasingly high GI in soil with no additives and increasing Cr(VI) concentrations (Figure 4a). The analysis of root growth produced similar observations (Figure 5a). The strongest inhibitory effect on the germination potential and the root length in *S. alba* was observed at the Cr(VI) rate of 150 mg/kg of soil. The lowest RI index was observed in soil contaminated with Cr(VI) at 0 and 50 mg/kg soil—6.18% and 16.4%, respectively. A similar effect was observed in the soil with the additives. The highest indices: of GI and RI—37.8% and 40.5%, respectively—were noted for the Cr(VI) rate of 150 mg/kg of soil, regardless of the additive applied. However, these values were lower than in the soil with no additives, which indicates that alkalizing additives alleviate the toxic effect of Cr. These data indicate a positive response of the test plants to the applied additives, which manifests itself by an increase in the produced biomass [53]. Growth and germination inhibition were observed at the rates of 50 and 100 mg/kg of soil. The differences were small, but statistically significant. A comparison of the findings with those for soil with no additives demonstrated that the indices at the doses of 50 and 100 mg/kg of soil were much lower (Figures 4a and 5a). The dose of 50 mg/kg of soil proved to be the least toxic to plants, which was similar to soil with no additives. A comparison of the additives showed that chalcedonite had the greatest stimulating effect on germination and root growth, followed by limestone and dolomite, regardless of the contamination level.

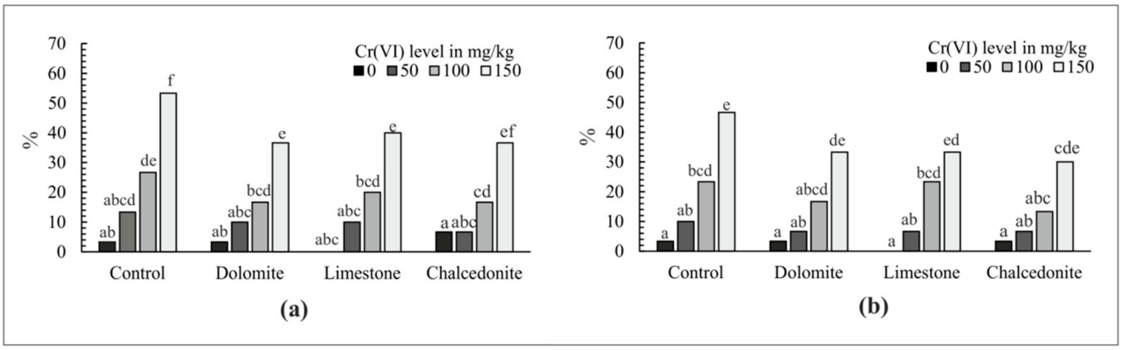



**Figure 4.** Germination inhibition index for *S. alba*—GI (%); (**a**) before phytostabilization started; (**b**) after phytostabilization was completed. For a given Cr(VI) level, different letters above the columns indicated a significant difference at $p < 0.05$. Error bars are ± standard error ($n = 3$).

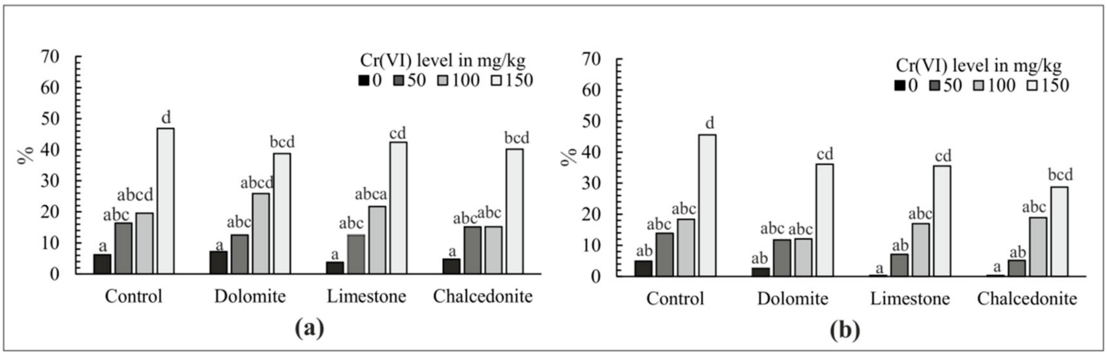



**Figure 5.** Root growth inhibition index for *S. alba*—RI (%); (**a**) before phytostabilization started; (**b**) after phytostabilization was completed. For a given Cr(VI) level, different letters above the columns indicated a significant difference at $p < 0.05$. Error bars are ± standard error ($n = 3$).

The phytotoxicity test performed on soil samples after the phytostabilization indicated that soil contaminated with Cr(VI) with no additives was still highly toxic to *S. alba*. However, the application of additives decreased the GI index and the RI index considerably. Chalcedonite proved to be the most effective additive, as in the first test (Figures 4b and 5b).

The lowest observed effective concentration is the lowest concentration of a contaminant which reduces the measured response by more than 20% [54]. It was found that the 20% of GI in *S. alba* in the control soil was caused by its contamination with Cr(VI) at 77.3 mg/kg. Mixing the same soil with dolomite, limestone and chalcedonite decreased the phytotoxicity of Cr(VI). This effect was the most clearly visible in soil with chalcedonite, where $EC_{20}$ = 114 mg/kg. The response of *S. alba* in the control soil as 50% GI ($EC_{50}$) was observed at a Cr(VI) concentration of 146 mg/kg. However, in the experiment variants using mixtures with dolomite, limestone and chalcedonite, the germination was inhibited by 37.4, 21.4 and 28.6 mg/kg at a higher concentration of Cr (Table 1, Figure 6).

**Table 1.** The effect of Cr(VI) on inhibition in germination (GI) and growth inhibition of roots (RI) of *S. alba* growing in control soil and soil supplemented with dolomite, limestone and chalcedonite. $EC_{10}$, $EC_{20}$ and $EC_{50}$ values are expressed in mg/kg.

| Effective Concentration mg/kg | Control | Dolomite | Limestone | Chalcedonite |
|---|---|---|---|---|
| | Inhibition in germination (GI) before remediation | | | |
| $EC_{10}$ | 38.0 | 58.6 | 55.3 | 63.3 |
| $EC_{20}$ | 77.3 | 110 | 96.2 | 114 |
| $EC_{50}$ | 146 | 183 | 167 | 174 |
| Mean Effective Concentration | 87.0 | 117 | 106 | 117 |
| | Inhibition in germination (GI) after remediation | | | |
| $EC_{10}$ | 47.6 | 91.9 | 53.8 | 68.2 |
| $EC_{20}$ | 89.6 | 141 | 96.9 | 121 |
| $EC_{50}$ | 152 | 173 | 203 | 179 |
| Mean Effective Concentration | 96.5 | 136 | 118 | 123 |
| | Growth inhibition of roots (RI) before remediation | | | |
| $EC_{10}$ | 28.1 | 25.0 | 42.3 | 45.5 |
| $EC_{20}$ | 86.5 | 79.6 | 89.7 | 99.0 |
| $EC_{50}$ | 154 | 172 | 161 | 172 |
| Mean Effective Concentration | 89.4 | 92.2 | 97.5 | 106 |
| | Growth inhibition of roots (RI) after remediation | | | |
| $EC_{10}$ | 44.2 | 65.5 | 67.1 | 59.4 |
| $EC_{20}$ | 95.8 | 115 | 110 | 107 |
| $EC_{50}$ | 153 | 182 | 181 | 202 |
| Mean Effective Concentration | 97.6 | 121 | 120 | 123 |

After six weeks of growing *F. rubra*, a three-day Phytotoxkit test was performed on each soil under test. Germination inhibition in *S. alba* caused by Cr(VI) was lower both in the control soil and in soil with amendments than before sowing *F. rubra*. Cr was the most toxic in the control soil (mean EC = 96.5 mg/kg Cr(VI), and the least toxic in soil with dolomite (mean EC = 136 mg/kg of Cr) (Table 1, Figure 7).

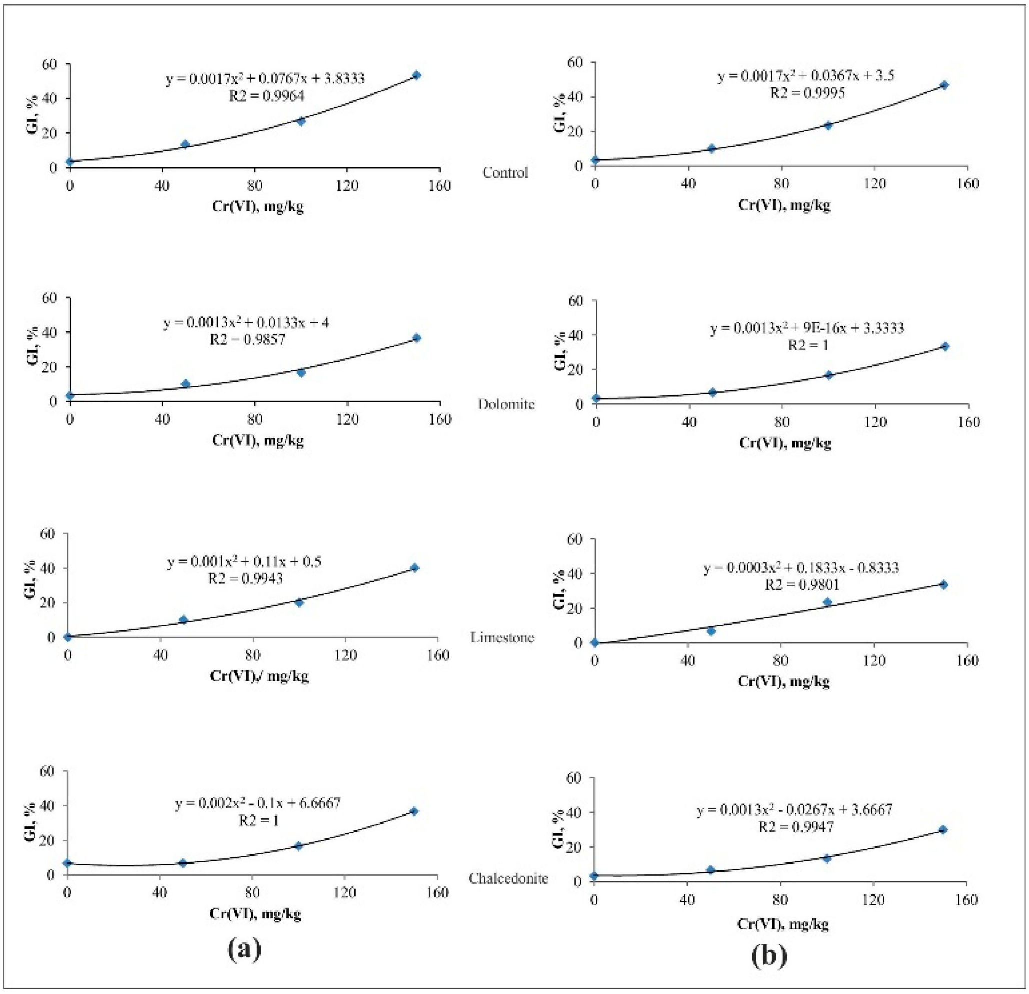



**Figure 6.** The effect of Cr(VI) on inhibition in germination (GI) of *S. alba* (**a**) before phytostabilization, (**b**) after phytostabilization.

Increasing Cr(VI) concentrations not only inhibited *S. alba* germination but also the growth of its roots (RI). The effective concentration of Cr(VI) in the control soil before growing *F. rubra*: $EC_{10}$, $EC_{20}$ and $EC_{50}$ inhibiting the growth of *S. alba* roots were 28.1, 86.5, 154 mg/kg, respectively. However, the effective concentrations in the least toxic soil (with an addition of chalcedonite) were, respectively: $EC_{10}$ = 45.5, $EC_{20}$ = 99.0 and $EC_{50}$ = 172.3 mg/kg of Cr. Growing *F. rubra* for six weeks improved the quality of all the soils with additives. In each soil contaminated with the same Cr(VI) doses, the roots of *S. alba* grew more intensively than before sowing *F. rubra*. The highest growth of *S. alba* roots was observed in soil with chalcedonite (mean EC = 123 mg/kg Cr). The highest RI was observed in the control soil (mean EC = 97.6 mg/kg of Cr) (Table 1, Figure 8).

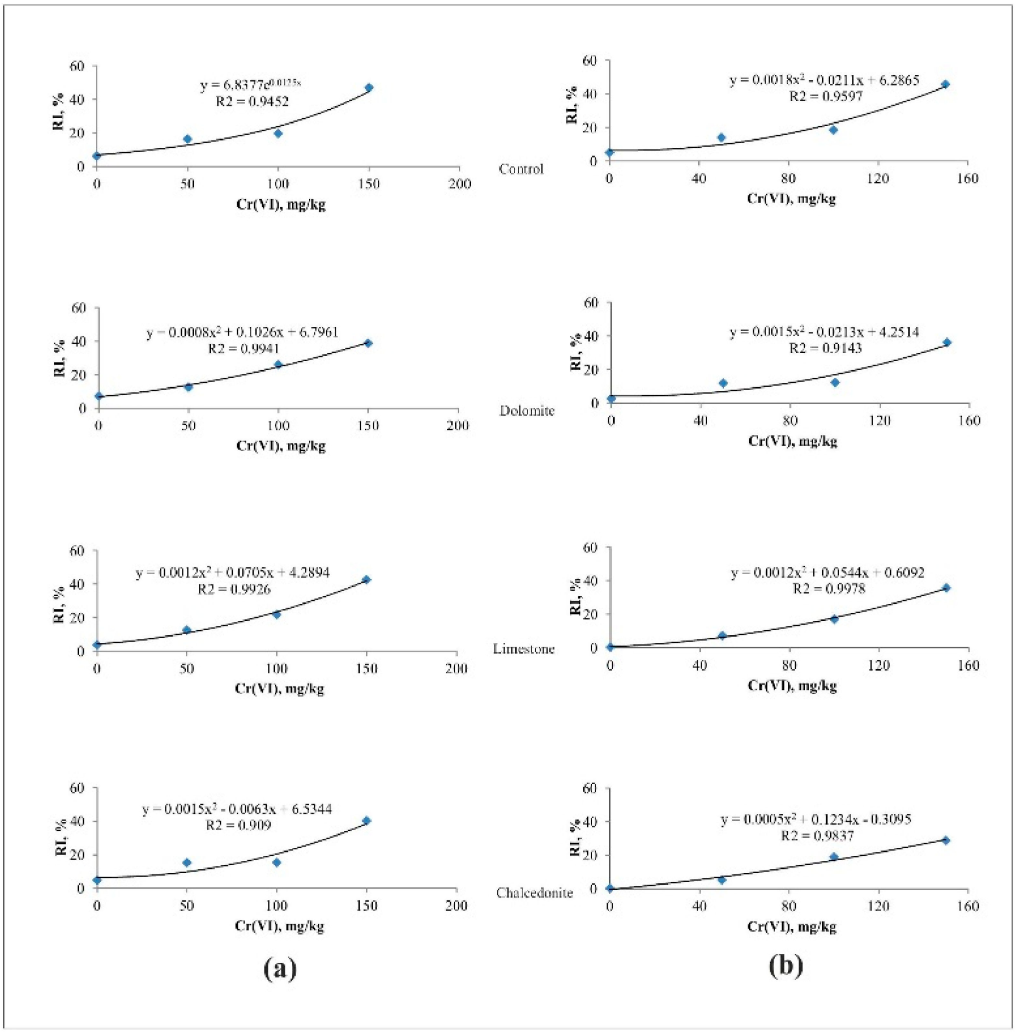



**Figure 7.** The effect of Cr(VI) on growth inhibition of roots (RI) of *S. alba* (**a**) before phytostabilization, (**b**) after phytostabilization.

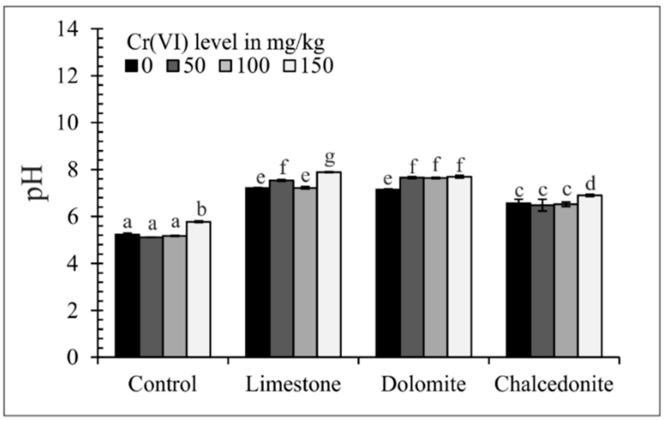



**Figure 8.** Soil pH. Error bars ± standard error ($n$ = 3). For a given Cr(VI) level, different letters indicate significant differences between treatments (ANOVA# followed by Tukey's HSD test, $p < 0.05$).

### 3.4. Soil Chemical Properties

The occurrence of Cr compounds in soil depends on the pH and redox potential [55]. The sorption of Cr(III) increases and that of Cr(VI) decreases with increasing soil acidity. An increase in soil acidity accelerates many processes in which such harmful elements as Al and Mn are released from the sorption complex to the soil solution [56]. Therefore, it seems justified to use soil additives which increase the soil pH significantly. The soil pH following the phytostabilization experiment is shown in Figure 8. The greatest increases were 2.15 and 2.22 pH units, with limestone and dolomite added into soil. This was corroborated by Ye et al. [57], who found that an addition of an alkalizing amendment (diatomite) was found to increase the soil pH.

The mobility and availability of heavy metals in soil depend on their total concentration and that of exchangeable forms [58]. In the natural environment Cr(VI) compounds are easily reduced by organic matter to Cr(III) compounds. [59]. The total (a) and $CaCl_2$-extractable (b) Cr content is shown in Figure 9. The Cr concentration after the phytostabilization experiment was the lowest following the application of limestone and chalcedonite compared to the control pots. $CaCl_2$ extraction is a measure of metal availability [30]. The application of alkalizing amendments reduced $CaCl_2$-extractable content of Cr in soil, and the most significant reduction was observed under dolomite and chalcedonite treatment.

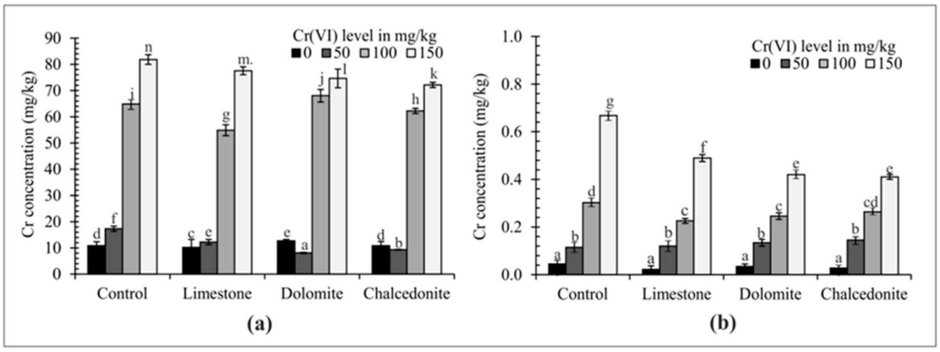



**Figure 9.** Total (**a**) and $CaCl_2$-extractable (**b**) Cr concentrations in the soil after the application of soil amendments (3% *w*/*w*). Error bars are ± standard error ($n$ = 3). For a given Cr(VI) level, different letters indicate significant differences between treatments ($p < 0.05$).

### 3.5. Statistical Analysis

The correlation matrix of data obtained from PCA is shown in Table 2 and Figure 10. There was a significant positive relationship between Cr content in the shoots and in the roots ($r$ = 0.757), Cr total content in the soil ($r$ = 0.876) and Cr in the soil in an available form ($r$ = 0.936). There was also a significant positive correlation between Cr in the root and the soil available forms ($r$ = 0.695) and soil pH ($r$ = 0.505), respectively. A significant positive correlation was also found between total Cr in the soil and in an available form ($r$ = 0.895). There was also a significant negative correlation between *F. rubra* biomass and Cr content in the soil in an available form ($r$ = –0.538). The results indicate that the accumulation of Cr in plant tissues depended on total Cr concentration, its available form and soil pH.

**Table 2.** Correlation matrix between soil physico-chemical properties and plant-related parameters.

| | Biomass | Cr Shoot | Cr Root | Cr Soil Total | Cr Soil Available | pH |
|---|---|---|---|---|---|---|
| Biomass | 1 | | | | | |
| Cr shoot | –0.338 | 1 | | | | |
| Cr root | 0.086 | 0.757 | 1 | | | |
| Cr soil total | –0.278 | 0.876 | 0.776 | 1 | | |
| Cr soil available | –0.538 | 0.936 | 0.695 | 0.895 | 1 | |
| pH | 0.317 | 0.023 | 0.505 | 0.106 | 0.055 | 1 |

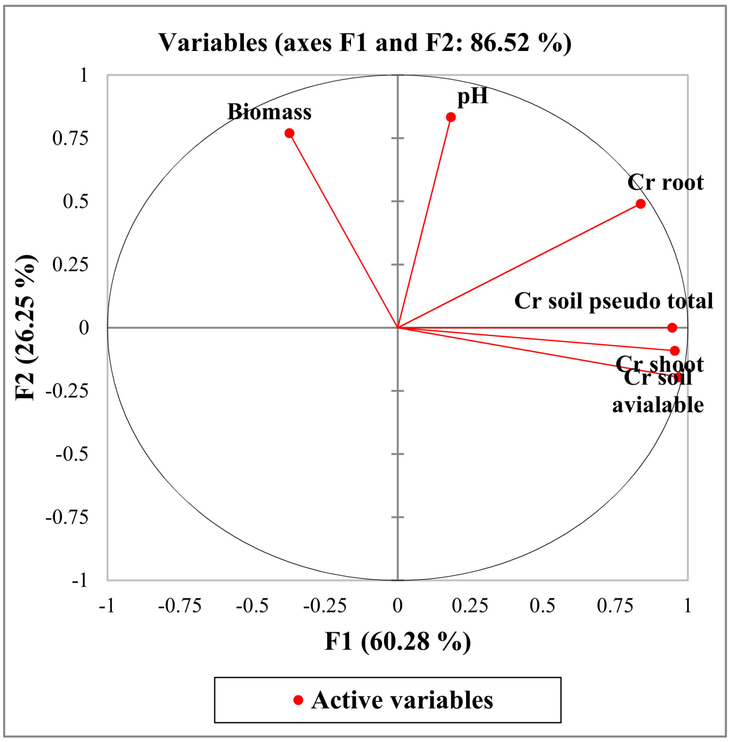



**Figure 10.** Plot of principal component scores (PCA).

Suitability for a data factor analysis was tested by Kaiser–Meyer–Olkin (KMO) and Barlett Tests. The value of KMO should be greater than 0.5. The data are suitable for the factor analysis as the KMO value gets closer to 1 between 0 and 1 [60,61]. According to the analysis results, the value of KMO = 0.621 is suitable to be used in this analysis. As a result of the factor analysis, two factors were identified with eigenvalues greater than 1. These three factors explain 86.5% of the total variance. The first factor explains 60.3% of the total variance and Cr in the shoot and root, total content and available form in the soil have strong positive load values, respectively. The second factor explains 26.3% of the total variance and soil pH and plant biomass have strong positive load values, respectively.

## 4. Conclusions

Based on the present study, it can be concluded that the application of alkalizing amendments such as limestone, dolomite and chalcedonite has a beneficial effect on aided phytostabilization of soil contaminated with Cr(VI) at various doses. This effect is accompanied by increased soil pH, which is the highest following the application of limestone and dolomite. The current results show that an addition of chalcedonite brings about the highest yield of *F. rubra*, the highest concentration of Cr in roots and the highest mean bioconcentration factor. The phytotoxicity test results show that chalcedonite has the greatest stimulating effect on *S. alba* germination and root growth, followed by limestone and dolomite, regardless of the Cr contamination level. The findings present great potential for practical application because of the availability of the amendments under study, their environmental safety and high effectiveness in chromium immobilization. Moreover, they help to recreate vegetation in degraded areas. Using this kind of phytostabilization may contribute to the reduction of Cr(VI) exposure and thus to the reduction of human health risk in Cr-contaminated areas.

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