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Katedra agroenvironmentální chemie a výživy rostlin

Uplatnění biocharu při remediaci půd kontaminovaných rizikovými prvky

doktorská disertační práce

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Obsah

Poděkování

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1. Úvod

Využívání přírodních zdrojů považujeme za samozřejmou součást všedních aktivit. Přináší to však svá úskalí – kromě odčerpávání surovin také následné znečišťování prostředí jejich zpracováním, kdy významný dopad této kontaminace zaznamenáváme i v případě půdy, která povahou svých vlastností má schopnost akumulovat řadu rizikových sloučenin. V dnešní době si uvědomujeme negativní vliv na přírodu a současným trendem a nutností je nacházet různá alternativní a šetrná řešení dekontaminace prostředí.

V souvislosti s kontaminací půd jsou studovány i možnosti případné remediace daných půd tak, aby se tyto půdy daly využít k zemědělským účelům. Jednou z možností je fytoextrakce s využitím plantáží rychlerostoucích dřevin. Efektivnost takových opatření je v současné době intenzivně pozorována a hodnocena. Problémem na některých lokalitách je extrémně vysoký obsah rizikových prvků, který výrazně negativně ovlivňuje růst sledovaných rostlin. Vzhledem k tomu, že tvorba biomasy je klíčová pro efektivní fytoextrakci, je vhodné navrhnout kombinaci remediačních technologií, a to fytoremediace a imobilizace – aplikace půdních aditiv, která jsou schopna různými mechanismy rizikové prvky v půdě fixovat tak, aby se snížila jejich mobilita v půdě, a tím i jejich toxicita pro pěstované rostliny. V optimálním případě je třeba dosáhnout maximálního odběru prvků rostlinami při minimálním ovlivnění biochemických procesů v těchto rostlinách. Nasnadě je také otázka, jak naložit s kontaminovanou biomasou. Existuje možnost termochemického zpracování biomasy – pyrolýza, kde jsou výsledné produkty olej a plyn dále využitelné pro energetické účely – a zároveň vzniká pevný zbytek označovaný jako koks, běžněji však z angličtiny převzatým termínem *biochar*. Biochar je v posledních letech velmi intenzivně zkoumán jako půdní aditivum zlepšující půdní vlastnosti a byly popsány jeho schopnosti poutat organické i anorganické polutanty. Důvodem jsou zejména jeho fyzikální vlastnosti, kdy bylo potvrzeno, že se jedná o materiál stabilní, alkalický a porézní. Atraktivní je jeho příprava, při níž jsou limitovány emise oxidu uhličitého. Tímto způsobem by se daly velmi efektivně zpracovat určité typy odpadů za vzniku látek, které lze dále využít. Předmětem výzkumu je vhodnost materiálů pro efektivní pyrolýzu a přípravu požadovaných výstupů. Vlastnosti výstupních látek jsou podmíněny vlastnostmi vstupních materiálů. Jestliže předpokládáme, že potřebujeme biochar schopný imobilizovat kontaminanty v půdě, je třeba se zabývat otázkou z čeho a za jakých podmínek vhodný biochar připravit a následně jak a za jakých podmínek ho do půdy aplikovat.

2. Literární přehled

2.1 Kontaminace půd rizikovými prvky a její možná remediace

2.1.1 Rizikové prvky v biosféře

Biosféra je přirozené prostředí živých organismů tvořící komplexní biologickou vrstvu Země. V biosféře se vyvinuly různé typy ekosystémů s vyrovnaným cyklem chemických prvků, sloučenin a toku energie. Ovšem zásahy člověka tyto vyrovnané pochody značně ovlivnily a dále ovlivňují. Stopové prvky jsou přirozené chemické prvky, které se vyskytují v různých množstvích a na rozmanitých místech po celém světě. Většinu chemických prvků důležitých pro život na Zemi poskytuje půda. Stopové prvky jsou přijímány rostlinami a množství přijaté do pletiv rostlin zpravidla koreluje s jejich množstvím v půdě. S tímto jevem vyvstávají problémy pro samotné rostliny, zvířata a následně člověka, kdy organismy mohou trpět přemírou, či naopak nedostatkem stopových prvků, dle jejich obsahů a mobilních podílů v půdě.

Biochemické funkce stopových prvků jsou známy, působí jako kofaktory enzymů, jsou stavebními jednotkami proteinů, jsou odpovědné za přenos elektronů v důležitých chemických procesech. Mnoho stopových prvků je nezbytných pro člověka i rostliny. Existuje ale také mnoho prvků, které nejsou ve vysokých koncentracích toxické pro rostliny, ovšem pro člověka a zvířata ano. Příjmem těchto prvků zvířaty se tak zvyšuje množství těchto prvků v potravním řetězci (Kabata-Pendias a Mukherjee, 2007).

Za rizikové prvky, tedy takové, které mohou ve zvýšených koncentracích způsobovat závažné znečištění biosféry, jsou považovány: As, Ag, B, Ba, Be, Cd, Co, Cr, Cu, F, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Tl, V a Zn. Termín rizikové prvky je také spjat s termínem těžké kovy, který se vztahuje na prvky mající hustotu vyšší než 5 $g.cm^{-3.}$ Označuje kovy a metalloidy, které se všeobecně pojí se znečišťováním prostředí a toxickým působením (Adriano, 2001). Těžké kovy jsou stálé a z prostředí je lze velmi obtížně odstranit. Problém nastává, když je jejich obsah v prostředí vysoký, díky přirozenému podloží nebo antropogenní činnosti (Prasad, 2008).

Půda obsahuje rizikové prvky různých původů: i) litogenní, tzn. původní horninové podloží (například chrom, nikl a kobalt se hojně vyskytují na hadcových půdách, zinek zase na zinkitových (Prasad, 2008)), ii) pedogenní, tedy pocházející z horninového podloží, ale vlivem půdotvorných procesů se mění formy těchto prvků, iii) antropogenní, to znamená spojený s činností člověka (Kabata-Pendias a Mukherjee, 2007).

Se stále rostoucí produkcí a poptávkou po využívání kovů v rozvinutých ale i v rozvojových zemích se zvyšuje pravděpodobnost rozptylování těžkých kovů do prostředí. Tyto prvky mohou být uvolňovány do prostředí od chvíle, kdy jsou těženy, až po dobu, kdy jsou zpracovávány a využívány v průmyslových odvětvích (Adriano, 2001).

Také zemědělská produkce může přispět ke zvýšení obsahu rizikových prvků v půdě, protože s její intenzitou se zvyšuje používání hnojiv, která mohou tyto prvky obsahovat. Mezi riziková hnojiva patří fosforečná nebo používání čistírenských kalů, kde se mohou objevit vyšší obsahy těžkých kovů. Určité množství prvků se mohlo ojediněle vyskytnout i v pesticidech (Gimeno-García a kol., 1996). Další zátěž půdy může souviset i se skladováním odpadů jak komunálních, tak nebezpečných, a s potenciálním únikem prvků z těchto materiálů do půdy. Do ovzduší se pak rizikové prvky dostávají spalováním uhlí. Množství kovů uvolněných pří spalování závisí na technologii a typu uhlí (Adriano, 2001). Tyto prvky se pak šíří v prostředí pomocí větru, vody a nakonec se dostávají do půdy a sedimentů, kde jsou vázány. Biologická přístupnost kovů klesá organismům s mírou jejich sorpce v půdě, kdy je vyšší biologická dostupnost prvků pozorována u antropogenních zdrojů kontaminace, než když se tyto prvky ve vyšších koncentracích nacházejí v podloží (Prasad, 2008).

2.1.2 Možné zdroje kontaminace půd

Kontaminace půdy představuje v současné době jeden z nejdůležitějších problémů ochrany půdy, přičemž takzvaná stará zátěž je významným ohrožením pro lidské zdraví (Němeček a kol., 2010). Jedná se o závažný problém z hlediska dalšího šíření těchto prvků do potravního řetězce nebo jejich vyplavování (Prasad a Hagemeyer, 1999).

Jak uvádějí Panagos a kol. (2013), v Evropě se nachází 1 170 000 potenciálně kontaminovaných lokalit (v 33 zemích), tedy míst, kde je možná kontaminace půdy. Všechny zdroje ale nejsou ověřené, takže na těchto lokalitách musí být ještě proveden detailní výzkum. Lokalit identifikovaných jako kontaminované bylo nalezeno 127 000. Z tohoto počtu je až 46 % remediováno. Kontaminanty půdy jsou především těžké kovy a minerální oleje (graf 1).
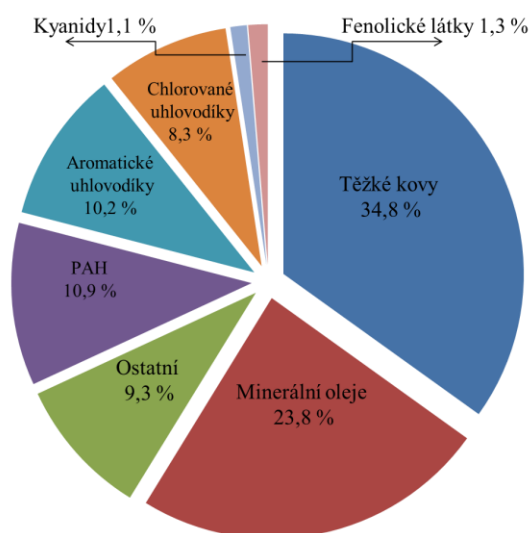
Graf 1. Přehled hlavních kontaminantů v Evropě (Panagos a kol., 2013)

Literatura popisuje možné zdroje kontaminace: skládky, areály s ropným průmyslem, vojenské základny (Panagos a kol., 2013). Další vstupy kontaminantů do půdy uvádí Němeček a kol. (2010). Jedná se o aplikace odpadních látek recyklovatelných v půdě, záplavy v místech výskytu fluvizemí, kde byly zachyceny prvky obsažené ve vodě v důsledku absence či nedostatečné funkce čistíren odpadních vod (ČOV), komposty se zvýšeným obsahem kontaminantů, převrstvení půd stavebními odpady a havárie.

K oblastem s vysokou kontaminací určitě patří místa se staletími trvající metalurgickou a hornickou činností. Hornictví a metalurgie vede ke zvýšení prvků v půdě uvolněním jejich ložisek v mateřské hornině (Vrubel a kol., 1996; Šichorová a kol., 2004). Kromě rozšíření kovů atmosférickou depozicí se lze setkat s nehodami, jako je poničení stěn sedimentačních lagun kovohutí povodní a následným rozšířením rizikových prvků do povodí (Borůvka a Vácha, 2006).

Zdroje možných anorganických kontaminantů lze shrnout následovně:

A / Průmyslové aktivity

- těžební a zpracovatelský průmysl
- stavební průmysl
- cementárny.

B / Výroba energie

- spalování fosilních paliv
- jaderné elektrárny
- spalování komunálního odpadu.

C / Zemědělství

- aplikace čistírenských kalů

- aplikace minerálních hnojiv

- aplikace pesticidů.

D / Doprava

(Kabata-Pendias a Mukherjee, 2007)

2.1.3 Rizikové prvky v půdě

Půda je důležitou součástí terestrických ekosystémů a hraje klíčovou roli v různých kolobězích prvků. Má funkci jako úložiště, filtr a je to místo mnoha přeměn, čímž podporuje vyváženost vztahu biotických a abiotických složek (Kabata-Pendias, 1995). Půda je komplexní heterogenní medium skládající se z pevné fáze (minerály, organická hmota), plynné fáze (půdní vzduch) a fáze kapalné (půdní voda). Fáze na sebe navzájem působí a tvoří ucelený systém, kterým ionty prvků procházejí (Alloway, 1999). Půda je však také místo, kde se mohou kumulovat rizikové prvky, přičemž sorpce na pevnou fázi je hlavním procesem odpovědným za jejich akumulaci (Bradl, 2004). Schopnost půdy zadržovat kovy v pevné fázi půdy (čímž přemisťuje polutanty z půdního roztoku a dochází k interakci mezi těmito rozhraními), je základním mechanismem, kterým půda snižuje možnost rozšíření kontaminantů do dalších složek životního prostředí (Petruzzelli, 1997).

2.1.4 Sorpce prvků v půdě

Sorpci je možno definovat jako akumulaci kovů v půdě a vztahuje se k procesu udržení iontů kovu v ní, což závisí na afinitě kovu k půdním částicím (Petruzzelli, 1997). Sorpce rizikových prvků a jejich distribuce mezi půdou a kapalnou fází je především ovlivňována půdním typem, sloučeninou prvku, jeho koncentrací, půdním pH, poměrem pevné a kapalné fáze půdy, dobou kontaktu, redox potenciálem, výměnnou půdní kapacitou, půdní vrstvou, klimatickými podmínkami a podílem organických částic v půdě (Bradl, 2004; Kabata-Pendias, 1995; Barančíková a Makovníková, 2003). Půdní pH má převládající vliv na sorpci kovů, jako jsou kadmium a zinek. Vliv redox potenciálu je významný především u těch rizikových prvků, které se v půdě mohou vyskytovat ve více než jednom oxidačním stupni (As, Cu, Hg, a Pb). Obsah vody v půdě také souvisí se schopností sorpce, kdy snižující se obsah vody v půdě má za následek vyšší množství navázaných kovů. Přítomnost některých solí může mít také vliv na dostupnost kovů pro rostliny. Například chloridové anionty zvyšují sorpci kovů vznikem iontu nebo neutrální částice (Petruzzelli, 1997).

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Mechanismy sorpce bývají jednoduše popisovány jako: i) specifická sorpce, která je charakterizována jako více selektivní a méně vratná včetně vytváření komplexů na vnitřních površích částic, ii) nespecifická sorpce neboli iontová výměna, která zahrnuje slabé a méně selektivní komplexy vně částic. Specifická sorpce se vyznačuje pevnou a nevratnou vazbou kovu na organickou hmotu či jílový minerál. Nespecifická sorpce je elektrostatický jev, kdy jsou kationty z půdního roztoku vyměněny za kationty poblíž povrchu částice. Kationtová výměna se vyznačuje slabou vazbou mezi kovem a nabitou půdní částicí a je vratná (Bradl, 2004). Existují různé druhy sil vázající kovové ionty k pevným částicím, které je pak drží. Jejich rozsah je od elektrostatických až po kovalentní, z nichž pak vyplývají vazebné energie (Petruzzelli, 1997).

Hlavní složky pevné fáze ovlivňující sorpci jsou přítomnost jílovitých částic, obsah a složení organické hmoty, obsah oxidů železa, manganu a hliníku, fosfátů a karbonátů (Dube a kol., 2001; Bradl, 2004; Sparks, 1995).

Obecně platí, že permanentní náboj na jílovitých částicích reaguje s kovovými ionty prostřednictvím nespecifických elektrostatických sil. Tento náboj jílových minerálů je převážně záporný, což je velmi důležitý faktor ovlivňující sorpční vlastnosti půd (Loughnan, 1969). Jsou možné i další vazby na koncích sloučenin jílovitých minerálů, které jsou schopny reagovat s kovy. Jedná se o konce –SiOH a –AlOH skupin. Je třeba zmínit hodnotu pH, kdy při vysokých hodnotách hydrolytické reakce činí sorpci ireversibilní z důvodu vytvoření hydroxo-polymerových forem na koncích silikátových sloučenin (Petruzzelli, 1997).

Organická složka obsahuje velké množství organických funkčních skupin, které jsou schopny s kovy velmi dobře reagovat. Do těchto funkčních skupin se řadí karboxylové, karbonylové, fenyl-hydroxylové, aminové, imidiazolové, sulfylhydrylové a sulfonové skupiny. Sorpční vlastnosti organických a anorganických částic půdy jsou ovlivněny počtem a typem funkčních skupin, přístupných kovům (Dube a kol., 2001). Tvorba organokovových komplexů je nejvíce závislá na specifickém povrchu půdy a obsahu organického uhlíku (Gao a kol., 1997) a rozpustného organického uhlíku (Al-Wabel a kol., 2002).

Oxidy a hydratované kovové oxidy mají velkou schopnost vázat se s kovy. Tato schopnost souvisí se stupněm krystalizace a morfologie sorpčních povrchů. Na nekrystalizovaných oxidech s –OH skupinou s jediným atomem hliníku se může nestálý náboj vybalancovat pomocí navázání kovu (Dube a kol., 2001).

Dalším faktorem, který ovlivňuje podmínky a intenzitu sorpčních procesů je přítomnost ligandů. Tvoří se pak takové „trojkomplexy" jako ligand – kov – půdní částice. Sorpce kovů se zvyšuje, jestliže má komplex ligand – kov vysokou afinitu k půdním částicím,

nebo jestliže ligand samotný disponuje vysokou afinitou k půdním částicím. Takový ligand pak pomáhá kovu navázat se. Ke snížení sorpce dochází v důsledku slabé afinity k půdním částicím komplexů, ligandů samotných nebo ligandu ke kovu. (Petruzelli, 1997).

Mobilita prvků je dána jejich elektrochemickými vlastnostmi a stabilitou minerálu. V důsledku vysoké dynamiky chemických procesů v půdě jsou přeměny nepřetržité. Obsah mobilních kovů v půdě také velmi ovlivňuje stupeň zvětrávání, ale významnou roli hrají i další faktory: pH, výměnná půdní kapacita a redox potenciál (Kabata-Pendias, 1995).

Litogenní složka je zdrojem spíše imobilních prvků v půdě, může však dojít k jistým přeměnám díky půdním podmínkám, ale také díky činnosti mikroorganismů i kořenovým exudátům (Kabata-Pendias, 1995). Mobilitu kovů a jejich distribuci také ovlivňuje přítomnost oxidů železa a manganu. Je to důležitá skupina tvořící koloidní částice, které za přítomnosti vody vytvářejí hydratované formy schopné velmi silně vázat kovy. Tyto formy nejsou lehce rozpustné, a proto jsou nepřístupné rostlinám (Petruzzelli, 1997). I když mobilita kovů závisí na jejich chemicko-fyzikálních vlastnostech, je možné, že ji ovlivňuje i jejich afinita k určitým půdním částicím. Mobilnější kovy (Cd, Zn) se vyskytují hlavně jako anorganické komplexy, kdežto méně mobilní (Pb) jsou vázány především na silikátových částicích (Kabata-Pendias, 1995).

Pro popisování sorpce rizikových prvků v půdě se využívá empirických či semiempirických modelů, kdy emipirické modely mají za cíl popsat a hodnotit experimentální data, zatímco semiempirické modely udávají popis základních mechanismů sorpce. Empirické modely se obvykle zakládají na jednoduchých matematických vztazích mezi koncentrací prvků v kapalné fázi a pevné fázi po ustanovení rovnováhy za konstantní teploty, kdy je rovnováha dána rovností chemického potenciálu těchto dvou fázi. Tyto vztahy jsou nazývány „izotermy" (Bradl, 2004). Jednovrstevnou sorpci plynu na homogenní planární povrch poprvé matematicky popsal a vysvětlil Irving Langmuir v roce 1916. Freundlichova izoterma je vhodná pro modelování heterogenního povrchu, ovšem ne vždy je správně interpretována, fyzikální význam parametru l/n není ve většině studovaných systémů zcela jasný. Tabulka 1 prezentuje porovnání nejpoužívanějších izoterem (Zhao a kol., 2011). Langmuirova izoterma popisuje monovrstevnou sorpci na homogenní povrchy, přičemž každé vazebné místo zachycuje jednu částici a adsorbované molekuly nemohou po povrchu migrovat a navzájem se ovlivňovat. Existují další rovnice obsahující dva parametry pro modelování dat: Temkinova izoterma, rovnice Redlich-Patersonova, Flory-Hugginsova a Dubinin-Raduskevichova, Halseyho izoterma a Brunauer-Emmer-Tellerova (BET) izoterma. Redlich-Patersonova izoterma je pak kombinací Langmuirovy a Freundlichovy izotermy a jedná se

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o nejpoužívanější model. Při modelování sorpce vysokých koncentrací se přibližuje Freundlichově izotermě, při nižších koncentracích Langmuirově. Tato rovnice je velmi populární pro odhadování sorpce těžkých kovů. Tothova izoterma je podobná Redlich-Patersonově a také kombinuje vlastnosti jak Langmuirovy, tak Freundichovy izotermy. Dobře popisuje sorpci heterogenních systémů (Dursun, 2006). Sipsova izoterma vystihuje model biologické sorpce těžkých kovů s vysokou hodnotou koeficientu korelace (Apirakitul a Pavasant, 2008).

Tabulka 1. Příklady izoterem popisující sorpci (dle Zhao a kol., 2011)

Izoterma dle	Rovnice funkce
Freundlich	$q_e = K_F C_s^{l/n}$
Langmuir	$q_e = q_{max} \dfrac{K_L C_e}{1 + K_L C_e}$
Temkin	$q_e = \dfrac{RT}{b} ln(a C_e)$
Dubinin-Radushkevich	$q_e = q_{max} exp(-(\dfrac{RT ln\left(\frac{C_e}{C_s}\right)}{\beta E_0}))$
Flory-Huggins	$log \dfrac{\theta}{C_0} = log K_{FH} + n_{FH} log(1 - \theta)$
Halsey	$q_e = (\dfrac{K_H}{C_e})^{l/n_H}$
Brunauer, Emmett, Teller (BET)	$q_e = q_{max} \dfrac{BC_e}{q_{max}(C_e - C_s)[1 + (B - 1)\left(\frac{C_e}{C_s}\right)]}$
Sips	$q_e = q_{max} \dfrac{(K_S C_e)^{\gamma}}{1 + (K_S C_e)^{\gamma}}$
Toth	$q_e = q_{max} \dfrac{b_T C_e}{(1 + (b_T C_e)^{n_T})^{l/n_T}}$
Redlich-Paterson	$q_e = \dfrac{K_{RP} C_e}{1 + \alpha_{RP} C_e^{\beta}}$

Vysvětlivky: C_e - koncentrace po ustanovení rovnováhy, C_s - rozpustnost adsorbátu při dané teplotě, E_0 - charakteristika energie vztažená k referenční složce, q_e - nasorbované množství, q_{max} - sorpční kapacita (saturovaná monovrstva). Kinetické rovnice: q_e - množství sorbované v rovnováze, q - množství nasorbované v daném čase „t", C - koncentrace sorbátu v roztoku v čase, C_s - koncentrace sorbátu v sorbentu v čase, parametry a konstanty jednotlivých rovnic.

Sorpce může být popsána čtyřmi obecnými typy izotererm (obrázek 1): i) S – typ indikuje, že za nízkých koncentrací kovů má povrch nízkou afinitu pro jejich sorpci, ta se zvyšuje se zvyšující se koncentrací kovů; ii) L – typ ukazuje vysokou afinitu při nízkých koncentracích kovů, se zvyšují koncentrací se afinita snižuje; iii) C – typ představuje oddělující mechanismus, kdy se ionty kovů nacházejí v mezifázi půdní částice a půdního roztoku rovnoměrně; iv) H – typ vyjadřuje vysokou afinitu a tvorbu komplexů vnitřní sféry povrchů (Sparks a kol., 1995).

Obrázek 1. Základní typy sorpčních izoterem (Sparks a kol., 1995)
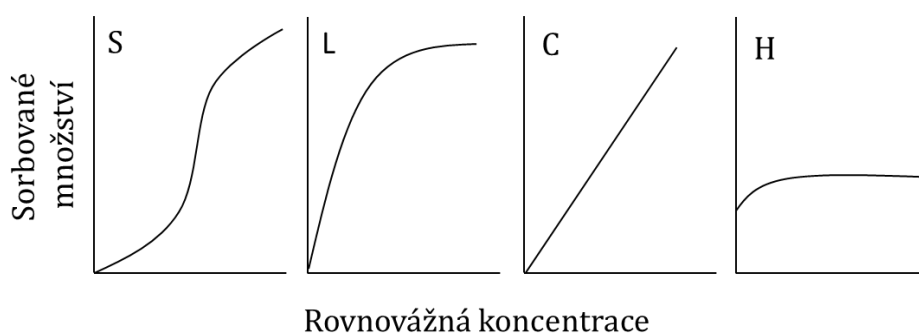
2.1.5 Metody remediace kontaminovaných půd

Z důvodu možné toxicity rizikových prvků vyžaduje tento problém efektivní a dostupné řešení. Byly popsány remediační technologie založené na fyzikálních, chemických nebo biologických procesech. Výběr typu remediační metody závisí na typu kontaminantu, typu půdy a celkové charakteristice remediovaného místa (Alloway, 1999). Mulligan a kol. (2001) shrnují použitelné remediační technologie půd kontaminovaných rizikovými prvky takto: i) zadržování (fyzikální, enkapsulace, vitrifikace); ii) *ex-situ* technologie (fyzikální separace, chemické promývání půd, pyrometalurgie); iii) *in-situ* technologie (reaktivní bariéry, vymývání vodou, elektrokinetickými postupy, fytoremediční technologie). Ačkoliv mohou být některé popsané metody velmi efektivní (Basta a McGowen, 2004), jsou velmi ekonomicky náročné a k půdě nešetrné, až přímo drastické (například vitrifikace: tavení anorganických látek elektrickým proudem na amorfní materiál podobný sklovině). Environmentálně šetrné a ekonomicky výhodné jsou fytoremediační postupy, kdy se k odstraňování, či stabilizaci rizikových prvků používají rostliny. Fytoimobilizace je proces, kdy záměrně pěstované rostliny uvolňují do půdy látky, a tak kovy tvoří komplexy a stávají se imobilní. Při fytoextrakci se využívají rostliny, které kovy z půdy extrahují a kumulují ve svých pletivech (Mulligan a kol., 2001). Existují rostliny, které jsou schopny ve svých pletivech kumulovat extrémně vysoké obsahy rizikových prvků, nazýváme je

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hyperakumulátory. Bylo popsáno až 400 druhů rostlin schopných zvýšené akumulace rizikových prvků. Patří mezi ně rostliny z tropického i mírného pásma, převážně rostoucí na půdách bohatých na těžké kovy a náležících do mnoha různých čeledí (Assunção a kol., 2003). Rychlerostoucí dřeviny, jako jsou vrby a topoly (*Salicaceae*), vykazují velmi dobrou schopnost akumulace rizikových prvků, zejména Cd a Zn, a jejich výhodou je velký nárůst biomasy, což může vést k významnému odběru těchto prvků za vegetační období (Jensen a kol., 2009; Meers a kol., 2007, Robinson a kol., 2000).

Potenciál využívání rychlerostoucích dřevin, které zajišťují dostatečný nárůst biomasy, byl pozorován na extrémně i mírně kontaminovaných, vápenatých i písčitých půdách (Jensen a kol., 2009; Tlustoš a kol., 2007; Meers a kol., 2007). U těchto metod se uvažuje o odstranění (extrakcí) kovů z půdy, z čehož plynou následné otázky, jak skladovat a dál nakládat s kontaminovanou biomasou.

2.2 Stabilizace rizikových prvků pomocí půdních aditiv

Slibnou *in-situ* remediační metodou, kdy bychom uvažovali využívání kontaminované půdy bez extrakce rizikových prvků, je fixace rizikových prvků aditivem aplikovaným do půdy (Guo a kol., 2006). Aplikují se taková aditiva, která jsou schopna sorbovat rizikové prvky, tvořit s nimi komplexy či sraženiny a tím omezit jejich pohyb (Basta a McGowen, 2004; Kumpiene a kol., 2008). Tato metoda vychází z tradičních agrotechnických opatření, jako je aplikace fosfátů, vápna nebo organické hmoty při pěstování rostlin k zvýšení jejich produkce a omezení mobility a přístupnosti rizikových prvků rostlinám (Bolan a kol., 2003). Omezení přístupnosti rizikových prvků rostlinám a omezení jejich vyplavování je dosahováno různými mechanismy sorpce, tedy sorpcí na minerální povrch, tvorbou stabilních komplexů s organickými ligandy, srážením nebo iontovou výměnou. Také srážení ve formě solí a koprecipitace mohou přispívat k redukci mobility kontaminantů. Různé sorpční/desorpční pochody jsou ovlivňovány řadou faktorů: velmi podstatná je změna (zvýšení) pH, dále redox potenciál, typ půdních složek, kationtová výměná kapacita a jen zřídka imobilizaci prvků v půdě ovlivní pouze jeden mechanismus (Kumpiene a kol., 2008).

Jako stabilizační materiál byl popsán například vápenec, fosfát (Basta a McGowen, 2004; Lin a kol., 2005; Xenidis a kol., 2010), oxihumolit, zeolit, humáty (Janoš a kol., 2010; Conesa a kol., 2010), prášek z vaječných skořápek a drůbežích kostí (Lim kol., 2013) nebo nanočástice na bázi zeolitu (Ghaira kol., 2010).

Mezi toto široké množství dostupných půdních stabilizačních materiálů – ať už organických či anorganických – nebo jejich směsí, řadíme také látky uhlíkaté povahy jako

vedlejší produkty spalování uhlí, polétavé popílky vzniklé používáním biopaliv (Clark a kol., 2001) nebo popele vznikající spalováním dřeva (Ochecová a kol., 2014) či lignitu (Uzinger a kol., 2008).

Alkalické materiály jako popílek či červený kal (tvořený především oxidy železa, vzniká jako odpad při výrobě hliníku z bauxitu) snížily vyplavování zinku z půdy téměř ze 100 % (Ciccu a kol., 2003). Mobilita zinku je modifikována přítomností P, Ca, Al, Mn, Fe oxidů a organickou hmotou. Zinek se může srážet s hydroxidy karbonáty, fosfáty, sulfidy, molybdenany a dalšími anionty, stejně jako tvořit komplexy s organickými ligandy. Bylo zjištěno, že významně omezují mobilitu zinku aditiva na bázi fosfátů (Basta a McGowen, 2004). Účinné imobilizace olova bylo dosaženo použitím materiálů na bázi fosfátů, či vápníku (Basta a McGowen, 2004). Všeobecně se udává, že materiály zvyšující pH půdy jsou efektivní pro redukci mobility olova (Kumpiene a kol., 2008). Například Belviso a kol. (2012) popisují schopnost syntetického minerálu (zeolitu) z polétavého popílku vzniklého při spalování uhlí, jehož hodnota pH byla 13, účinně imobilizovat olovo za vzniku nesnadno rozpustných hydroxidů ($[Pb(OH)]_4$). Naopak Vondráčková a kol. (2013) zjistily, že dolomit není vhodným aditivem pro imobilizaci olova na kyselých i neutrálních půdách.

Při sorpci kadmia hraje důležitou roli pH a elektrostatické interakce (Ghair a kol., 2010). Jako vhodné imobilizační medium pro kadmium byly popsány bazické sloučeniny vápníku (Lim a kol., 2013) nebo organické povahy: humáty (Janoš a kol., 2010), či minerály (Ghair a kol., 2010). Efektivní imobilizace těchto prvků po aplikaci vápna (CaO) a vápence byla sledována na kyselých půdách (Vondráčková a kol., 2013) a autoři také vyzdvihují vliv aplikační dávky na účinnou a efektivní fixaci rizikových prvků.

V extrémně konatminovaných půdách je tvorba biomasy extrahujících rostlin významně redukována v důsledku fytotoxického účinku rizikových prvků (Vysloužilová a kol., 2003). Nicméně je účinnost fytoextrakce podmíněna vysokou tvorbou biomasy (Meers a kol., 2007). Ve specifických případech by se tedy dala zvažovat možnost kombinace fytoextrakce se stabilizačními aditivy pro zlepšení růstu rostlin, a tak zvýšit stabilizační a fytoextrakční potenciál pěstovaných rostlin.

2.2.1 Biochar jako půdní aditivum

Foto: K. Pračké

Jednou z nově testovaných možností k remediaci kontaminovaných půd je využití zbytkového materiálu po pyrolýze organické hmoty tzv. biocharu. Biochar je jemnozrnná porézní a na uhlík bohatá substance podobající se dřevěnému uhlí (v angl. *Charcoal,* odtud název *biochar*). Získává se pyrolýzou bioodpadu – termickým rozkladem za různých teplot a bez přístupu médií obsahujících kyslík. V posledních letech se takto získaným materiálem autoři zabývají jako aditivem zlepšujícím půdní vlastnosti. Bylo zjištěno, že biochar nejen ovlivňuje půdní vlastnosti (Amonette a Joseph, 2009), ale také sorbuje rizikové prvky a tím omezuje jejich pohyb v půdě (Chen a kol., 2011).

V důsledku neustálého zvyšování koncentrace CO_2 v atmosféře se novodobě začalo uvažovat, že aplikací materiálu s vysokým obsahem uhlíku, jakým biochar je, se část uhlíku bude dlouhodobě ukládat v půdě. Američtí vědci ve své studii vypočetli, že by bylo možné pyrolýzou biomasy, kde by se získával plyn a olej pro energetické účely a biochar by byl použit pro půdní aplikace, dosáhnout sekvestrace až 10 % ročních emisí z fosilních paliv v USA (Lehmann, 2007a; obrázek 2). Moreira a kol. (2017) shrnují pozitiva nahrazení tradičního aktivního uhlí biocharem především z hlediska využití a zpracování lignin-celulózového odpadu.

Obrázek 2. Sekvestrace uhlíku v podobě pyrolýzního produktu, biocharu
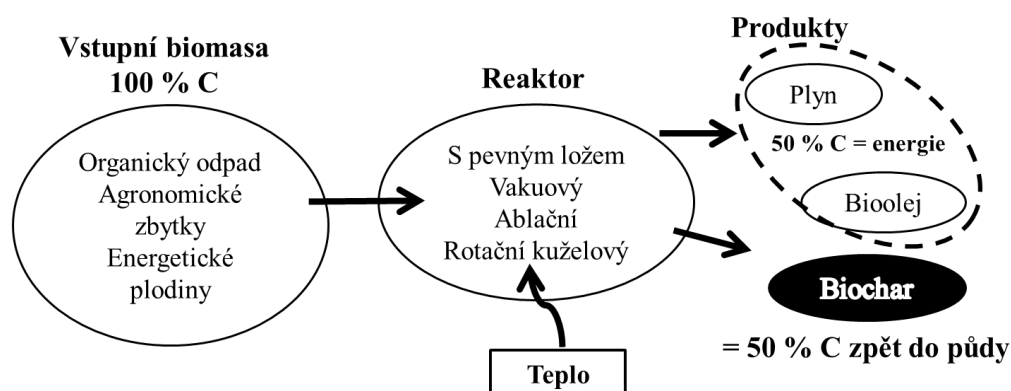
Využívání zuhelnatělé biomasy pro agronomické účely není záležitostí a trendem posledních desetiletí. Již v roce 1929 John Morley zmiňuje v časopise *The National Greenkeeper* pozitiva aplikace hnědého uhlí a popisuje následné zlepšení půdní struktury

(Morley, 1929). V šedesátých letech byla pak popsána území v Amazonii, kde spolupůsobení spálené biomasy a dalších organických materiálů vedlo k vytvoření velmi úrodných půd, které nesou dnes již zažité označení *terra preta* (Sombroek, 1966). Již od starověku používali farmáři hnědé uhlí při pěstování plodin. Příkladem je mýcení a spalování stromů na plochách následně využívaných pro zemědělské účely, které se ještě stále praktikuje v severovýchodní Indii (Jha a kol., 2010). Ve čtyřicátých letech se pak objevuje v disertační práci E. H. Tryona myšlenka, využití dřevěného uhlí k sorpci látek toxických pro rostliny (Tryon, 1948). Vůbec poprvé se termín biochar objevil v publikaci z roku 1999 *An activated carbon product prepared from milo (Sorghum vulgare) grain for use in hazardous waste gasification by ChemChar cocurrent flow gasification* (Bapat a kol., 1999), kdy autoři popisují přípravu tzv. „chemcharu" ze zrn čiroku. Názvem chtěli odlišit uhlí připravené z tohoto materiálu od běžného aktivního uhlí vyráběného z uhlí. Také byl používán termín „charcoal" pro materiál připravovaný pyrolýzou různých zbytků rostlinné biomasy dále využívaný pro energetické účely. Počátkem devadesátých let se objevuje první zmínka spojitosti „charcoal" se zmírněním klimatických změn, avšak autoři neuvádějí možnost aplikace tohoto materiálu do půdy. Až v roce 2005 Lehmann a kolektiv ve své prezentaci *Bio-char sequestration in soil: A new frontier* popisují biochar ve spojitosti sekvestrace uhlíku v půdě dále jako půdní aditivum (Woolf a kol., 2010).

2.2.1.1 Pyrolýza

Pyrolýza je termický rozklad materiálů bez přístupu kyslíku, nebo za významně nižší přítomnosti kyslíku, než vyžaduje dokonalé spalování (Mohan a kol., 2006).

Většina v současné době provozovaných pyrolýzních systémů je založena na termickém rozkladu organického materiálu v rotační peci vytápěné zevně spalinami, které vznikají z následného spalování pyrolýzních plynů v tzv. termoreaktoru. K ohřátí biomasy lze použít přímo i horkého inertního plynu (neobsahujícího kyslík). Příklad schématu reaktoru je na obrázku 3. Reakční mechanismus pyrolýzy rostlinné biomasy je komplexní a je velice těžké ho přesně popsat z důvodu diverzity vstupní biomasy (Mohan a kol., 2006). V závislosti na dosažené teplotě lze při pyrolytickém procesu pozorovat řadu dějů, které je možné pro jednoduchost rozdělit do tří teplotních intervalů. V oblasti teplot do 200°C dochází k sušení a tvorbě vodní páry fyzikálním odštěpením vody. Tyto procesy jsou silně endotermické. V rozmezí teplot 200–500°C následuje oblast tzv. suché destilace. Zde nastává ve značné míře odštěpení bočních řetězců z vysokomolekulárních organických látek a přeměna

makromolekulárních struktur na plynné a kapalné organické produkty a pevný uhlík v důsledku primárních pyrolýzních reakcí.

Dále dochází k výměně mezi plynnými a těkavými podíly a chladnějšími částmi pyrolyzovaného materiálu a částečně ke kondenzaci kapalných podílů. Následuje sekundární kondenzační reakce kondenzovaných kapalných podílů – tvorba dehtu, dále tepelný rozklad (fáze tvorby plynu: 500–1200°C) reformování, rekombinace radikálů v závislosti na teplotě a době zdržení. Přitom jak z pevného uhlíku, tak i z kapalných organických látek vznikají stabilní plyny, jako je H_2, CO, CO_2 a CH_4 (Demirbas a kol., 2004; Mohan a kol., 2006).

Konečnými produkty jsou pyrolytický plyn, pyrolytický olej a pyrolytický koks. Cílem tzv. pomalé pyrolýzy je produkce koksu, naopak při rychlé pyrolýze se získá více pyrolytického oleje (Brown a Stevens, 2011). Správný průběh pyrolýzního procesu je dán extrémně rychlým přívodem tepla do suroviny, udržováním potřebné teploty, krátkou dobou pobytu par v reakční zóně a co nejrychlejším ochlazením vzniklého produktu. Vznikající pyrolytický olej je tmavě hnědá kapalina s hustotou asi 1,2 $kg.dm^{-3}$, výhřevností 16–19 $kJ.kg^{-1}$. Dále vzniká pyrolytický plyn, který má výhřevnost až 20 $kJ.m^{-3}$ (Diebold a Bridgewater, 1997)

Rozeznáváme základní typy pyrolýzy: pomalou (konvenční), rychlou a tzv. „flash" pyrolýzu. Každý typ vyznačuje jinými podmínkami (tabulka 3), což má za následek různé výtěžky produktů (Mohan a kol.; 2006; Jahirul a kol., 2012).

Obrázek 3. Schéma všeobecného procesu rychlé pyrolýzy (podle Frankovské a kol., 2010)

Vysvětlivky:

1 – reaktor
2 – ošetřená biomasa: sušič a drtič
3 – teplo
4 – zkapalněný plyn
5 – zásobník biouhle
6 – cyklon s plynem
7 – zásobník biopaliva/oleje
8 – zhášedlo
9 – plyn
10 – teplo na sušení biomasy
11 – recyklace plynu

Výnos a poměr produktů pyrolýzy biomasy závisí na několika parametrech, zejména na složení a obsahu organických látek v biomase, tedy obsahu (hemi) celulózy, ligninu

a extrahovatelných látek (Gani a Naruse, 2007). Hemicelulóza se rozkládá při teplotách 220–315°C (Yang a kol., 2007), celulóza při teplotách 240–350°C a její pyrolýzní rozklad je rychlý. Lignin se rozkládá při teplotách 280–500°C (Mohan a kol. 2006), pomaleji za vysokých výtěžků pevného produktu pyrolýzy – koksu (Yang a kol., 2007). Minerální složení biomasy, potažmo popelovin velmi ovlivňuje finální produkty pyrolýzy, míru uvolnění těkavých látek a teplotu, kdy se biomasa v reaktoru začne rozkládat, následně ovlivňuje i vlastnosti produktů. Při odstranění popelovin se zvýší únik těkavých látek, počáteční teplota a rychlost rozkladu. Zvýší se produkce oleje a optimalizuje produkce koksu a plynu (Raveendran a kol., 1995), přičemž následné sorpční vlastnosti koksu se po odstranění popelovin u vstupní biomasy zvýší (Raveendran a kol., 1998). V tabulce 2 jsou uvedeny obsahy hlavních organických komponent rostlinné biomasy.

Tabulka 2. Obsahy hlavních organických komponent rostlinné biomasy

Rostlina	Část rostliny	Celulóza	Hemicelulóza	Lignin	Extrahovatelné látky	Citace
Kukuřice	klas	40,3	28,7	16,6	15,4	Raveendran a kol., 1995
	stonek	42,7	23,6	17,5	9,8	
Trávy*		42,7	37,9	19,4	20,5	Vassilev a kol., 2012
Pšenice	sláma	30,5	28,9	16,4	13,4	Raveendran a kol., 1995
Dřevo**		51,2	23,4	25,5	3	Vassilev a kol., 2012
Kůra***		22	47	31	3,3	Vassilev a kol., 2012

*průměrná hodnota z 12 vzorků, **průměrná hodnota z 19 vzorků, ***průměrné hodnoty z 5 vzorků

Tabulka 3 ukazuje, jak poměr výsledných produktů a jejich výtěžek závisí na podmínkách pyrolýzy: na teplotě, době zdržení, rychlosti ohřevu, předúpravě biomasy, množství vstupní biomasy, typu reaktoru a typu ohřevu (Demirbas, 2004). Výnos koksu (biocharu) se snižuje se zvyšující se pyrolýzní teplotou (Uchimiya a kol., 2011a; Keiluweit a kol., 2010; Horne a Williams, 1996).

Tabulka 3. Porovnání různých typů pyrolýzy na výsledné produkty (Demirbas, 2004)

Technologie pyrolýzy	Doba zdržení	Rychlost ohřevu	Finální teplota (°C)	Přibližné zastoupení produktů (%)		
				olej	plyn	koks
Karbonizace	dny	velmi pomalý	400			100
Konvenční	5 –30 minut	pomalý	600	30	35	35
Rychlá	0,5 - 5 vteřin	velmi rychlý	650	50	30	20
"Flash"	< 1 vteřin	rychlý	> 650	75	13	12

V procesech pyrolýzy lze dosáhnout vysoké výtěžnosti produktů za poměrně nízkých energetických ztrát, ovšema předpokladu dostatečně vysušené vstupní biomasy. Vstupní biomasa pro pyrolýzu ale většinou obsahuje velké množství vody. Celý proces tedy vyžaduje vysoušení vstupní biomasy, což znamená další energetický vklad. Z toho důvodu se výzkum zaměřuje i na takzvanou hydrotermální karbonizaci, kdy termochemický rozklad probíhá za přítomnosti vody. Produkty jsou zde také plyn, olej a biochar, který se zde označuje jako hydrochar (Cha a kol., 2016).

2.2.1.2 Pyrolýza kontaminované biomasy

Sas-Nowosielska a kol. (2004) navrhují několik způsobů, jak s kontaminovanou biomasou nakládat. První je lisování čerstvé hmoty pro snížení objemu, kde je nevýhodou riziko zpětného úniku rizikových prvků do prostředí. Další je kompostování, kdy se sníží objem biomasy a zároveň dojde ke stabilizaci rizikových prvků a snížení rizika jejich vyplavování (Šyc a kol., 2012), následně je však nutné s kompostem nakládat jako s nebezpečným odpadem (Sas-Nowosielska a kol., 2004). Dále pak autoři uvádějí tavení biomasy, zpopelňování, extrakci kovů z biomasy kyselinou a také termochemické zpracování biomasy, pyrolýzu. Šyc a kol. (2012) uvádějí jako jeden z nejlepších způsobů zpracování kontaminované biomasy energetické využití, spalování, kde ale může dojít k uvolňování některých relativně těkavých prvků (Cd) do ovzduší za vysokých teplot. Jiné rizikové prvky jako Pb, Zn či Cu se až z 90 % koncentrují v popelu.

Stals a kol. (2010) pyrolyzovali biomasu vrb kontaminovanou rizikovými prvky a zjistili, že při finální teplotě 350 a 450°C lze získat pyrolýzní olej pro energetické účely s přijatelným obsahem rizikových prvků. Při teplotě 350°C se biomasa převážně transformovala do pyrolýzního koksu, tedy biocharu. Uvádějí, že je potřeba dalšího výzkumu následného využití tohoto materiálu buď pro energetické účely, nebo pro účely aplikace do půdy.

Lievens a kol. (2008) pyrolyzovali dřevo břízy kontaminované kadmiem. Zjistili, že při teplotě nad 400°C kadmium těká a doporučují pro retenci kadmia v koksu nižší teploty pyrolýzy (400°C, kdy se 82 % kadmia koncentrovalo v biocharu). Dále uvádějí, že volatilizace kadmia a zinku závisí na vstupní biomase. Jako nosič tepla v reaktoru používali písek, kterému přičítají, že se pak rizikové prvky koncentrovaly v biocharu v podobě silikátů, čímž se staly imobilními.

Jones a Quilliam (2014) testovali aplikaci biocharu (50 t.ha^{-1}) a popela (5 t.ha^{-1}) připravených z biomasy s příměsí kontaminovaného dřeva (kontaminace mědí) na růst pšenice a slunečnice. Zjistili, že měď se ze sledovaných matric do půdy uvolňovala při vyšším procentu zastoupení kontaminovaného dřeva ve vstupní biomase. Zajímavý výsledek je, že negativní účinek aplikace biocharu na rostliny byl sledován, když zastoupení kontaminovaného dřeva ve vstupní biomase činilo 50 %. A při aplikaci popela byl negativní účinek sledován, když obsah kontaminované vstupní biomasy činil 10 %.

2.2.1.3 Vlastnosti biocharu

2.2.1.3.1 Chemické vlastnosti biocharu

Základní vlastnosti biocharu závisí na původní biomase a zvolených podmínkách pyrolýzy (Antal a Groenli, 2003). Jelikož jako vstupní materiál může sloužit bezpočet typů materiálu, nelze tedy jednoznačně vlastnosti biocharu stanovit. Jeho určující vlastností je, že se organický podíl vyznačuje vysokým obsahem uhlíku (Lehmann a Joseph, 2009). Všeobecně lze konstatovat, že je biochar zásaditý materiál: pH ~ 8 – 10 (Gaskin a kol., 2008); pH ~ 8 – 10 (Novak a kol., 2009a); pH~7 – 9 (Zhang a kol., 2013). Celkový obsah uhlíku se pohybuje v rozmezí 40–90 % (Novak a kol., 2009a; Uchymiya a kol., 2011; Park a kol., 2011; Trakal a kol., 2011). Uhlíky jsou zpravidla uspořádány v cyklech o šesti atomech uhlíku. Nepravidelná uspořádání uhlíků mohou dále obsahovat kyslík, vodík nebo – v některých případech – i některé minerály v závislosti na vstupním materiálu (Lehmann and Joseph, 2009). Podmiňující parametry: typ pyrolýzy a vstupní materiál však ovlivňují vlastnosti připraveného biocharu v různé míře. Z uvedených publikací vyplývá, že se vzrůstající pyrolýzní teplotou roste pH i obsah uhlíku. Hodnota pH je ovlivněna podmínkami pyrolýzy, ovšem celkový obsah uhlíku a dalších prvků jsou spíše podmíněny vstupní biomasou (Zhao a kol., 2013). To ostatně dokládá tabulka 4.

Tabulka 4. Obsah vybraných prvků v různých typech biocharu, připraveného při 500°C (Gaskin a kol., 2008)

Vstupní biomasa	C	N	P	K	S	Ca	Mg
	g.kg^{-1}						
Drůbeží trus	392	31	36	59	14	50	13
Arašídové slupky	804	25	2,0	16	0,6	5	3
Štěpka borovice	817	2,2	0,1	1,5	0,1	1,9	0,6

2.2.1.3.1   Fyzikální vlastnosti biocharu

Mezi metody popisující specifický povrch biocharu se řadí například SEM analýza (řádkovací elektronová mikroskopie) nebo FTIR (Fourierova transformační infračervená spektroskopie) pro zjišťování funkčních skupin na povrchu materiálu. Specifický povrch je zjišťován metodou adsorpcí plynů a dále hodnocen dle Brunauer–Emmett-Teller sorpční izotermy a označován jako S_{BET} (Brunauer a kol., 1938). Skeletární hustota je stanovována heliovou pyknometrií a porozita rtuťovou porozimetrií, určování struktury pak pomocí rentgenové difrakční analýzy (XRD) (Dutta kol., 2015; Uchymia a kol., 2011; Mašek a kol., 2013; Jimenez-Cordero a kol., 2013; Keiluwiet a kol., 2010). Uvedenými metodami bylo zjištěno, že se jedná o mikroporézní materiál, kdy například Jimenez-Cordero a kol. (2013) zjistili nejvyšší distribuci mikropórů v biocharu z hroznových zrn při nejvyšších teplotách pyrolýzy, 700–800°C, (0,1 –0,4 nm). Mikropóry hrají důležitou roli v adsorpci molekul na sorpční povrch biocharu. Rozmezí velikosti mikropórů se pohybuje od 750 do 1360 $m^2.g^{-1}$ (Lehmann a Joseph, 2009). Klíčovou roli při vytváření pórů hraje finální teplota pyrolýzy, se zvyšující se teplotou roste i porozita (Dutta a kol., 2015).

Specifický povrch (S_{BET}) se u biocharu zvyšuje se zvyšující se pyrolýzní teplotou. Určitý vliv má i vstupní biomasa, kdy Ronsee a kol. (2013) sledovali, jak vyšší obsah minerálních látek ve vstupní biomase negativně koreluje se S_{BET} u připraveného biocharu, zřejmě z důvodu uzavírání pórů roztavením popela. Příklady specifického povrchu biocharu připravovaných za různých podmínek jsou následující: biochar z travní biomasy (500°C): S_{BET}: 50 $m^2.g^{-1}$, z dřevní biomasy (500°C): S_{BET}: 196 $m^2.g^{-1}$ (Keiluweit a kol. (2010); z kukuřičné slámy (500°C, 600°C): S_{BET}: 245 $m^2.g^{-1}$, 13 $m^2.g^{-1}$ (Zhang a kol., 2011, Chen a kol., 2011); z jehlic borovice (700 °C): S_{BET} 500 $m^2.g^{-1}$ (Chen a kol., 2008).

Existují studie popisující sorpční schopnosti biocharu s různými specifickými povrchy. Uchymia a kol. (2011a) zdůrazňují důležitost funkčních skupin při sorpci těžkých kovů na povrch biocharu a naznačují možnost tzv. aktivace biocharu: úpravu kyselinami, či jinými oxidanty pro zvýšení množství funkčních skupin na površích tohoto materiálu.

Jestliže je vstupním materiálem rostlinná biomasa její složení, tedy konkrétní podíl celulózy, hemicelulózy, ligninu a extrahovatelných sloučenin, je důležitým faktorem ovlivňujícím strukturu biocharu (Brewer a kol., 2009, Downie a kol., 2009). Za vyšších teplot podléhá rostlinná biomasa depolymerizaci a dehydrataci, celulóza a lignin kondenzují a vytváří se grafitická struktura biocharu. To naznačuje, že při zvýšené teplotě se v biocharu vytváří vícevrstvá uhlíková struktura (Chowdhury a kol., 2016). Tento předpoklad je podpořen postupným rozkladem těchto složek během pyrolýzy vedoucí k různým typům biocharu (Demirbas a kol., 2004). Zobecnění a detailní specifikace různých typů biocharu pro přesně dané využití v půdě založené na vlastnostech vstupní biomasy však v literatuře téměř neexistuje.

Fernanda Aller (2016) shrnuje dostupné vědecké údaje o fyzikálních vlastnostech biocharu a dospěla k závěru, že ve srovnání s jinými vlastnostmi biocharu je toto pole stále nedostatečně opublikováno. Její přehledný článek také dokládá, že struktura a vlastnosti, jako je porozita a celková pórovitost, byly popsány s použitím různých technik rentgenové spektrometrie, ale počet článků kvantifikujících uvedené vlastnosti je velmi omezen. Brewer a kol. (2014) naznačuje, že fyzikální charakterizace biocharu je náročná, protože póry tohoto materiálu se mohou měnit v širokém rozmezí velikostí od subnanometrů až po mikrometry a v současné době neexistuje žádná společná standardní metodika pro měření objemu pórů v takovém rozmezí. Tato oblast popisu biocharu je stále výzvou výzkumu.

2.2.2 Biochar v půdě

Jak již bylo uvedeno výše, primární myšlenkou aplikace biocharu do půdy je sekvestrace uhlíku v půdě. Díky základním vlastnostem tohoto materiálu byl pozorován jeho pozitivní vliv na různé půdní funkce či vlastnosti (Verheijen a kol., 2010) a strukturu (Tan a kol., 2017). Oguntunde a kol. (2008) sledovali zvýšení porozity půdy po aplikaci biocharu až o 50 %.

Bylo také zjištěno, že biochar je stabilní, protože aromatický charakter uhlíkatých sloučenin podporuje pomalý rozklad v půdě, protože mikroorganismy využívají tyto sloučeniny pouze s obtížemi (Rosa a kol., 2007).

Molární poměr vodíku, kyslíku a uhlíku u biocharu je užitečná charakteristika pro odhad polarity materiálu a jeho možné interakce s vodou. Se vzrůstající teplotou pyrolýzy se zvyšuje obsah uhlíku, ovšem funkční skupiny (-OH) se během pyrolýzy uvolňují, tudíž polárnější materiál vzniká při nižších teplotách pyrolýzy (Novak a kol., 2009a). Spokas (2010) na základě poměru O /C odhaduje „životnost" biocharu v půdě a to, že O /C < 0,2 předurčuje poločas rozpadu biocharu na 1000 let. Nutno podotknout, že dlouhodobější experimentální pozorování v tomto ohledu chybí.

Laird a kol. (2010a) sledovali v inkubačním experimentu (promývací kolony) vliv biocharu aplikovaného do hlinité půdy. Varianty s biocharem se vyznačovaly vyšší schopností retence vody (potvrzují na jílových půdách i Sun a Lu, 2014 a dále i Downie a kol., 2009), zvýšil se specifický povrch půdy (potvrzují i Chan a kol., 2007; Sun a Lu, 2014 v jílových půdách), o 20 % se zvýšila kationtová výměnná kapacita (KVK) a pH se zvýšilo o jednotku. Vzrostl celkový obsah dusíku, organického uhlíku, extrahovatelného P, K, Mg, Ca (Mehlich, III). Zvýšení pH půdy s dávkou biocharu pozoroval i Lehmann (2007b). Nicméně Yuan a kol. (2011) připisují nárůst retence živin zvýšením pH půdy po aplikaci biocharu. Využití biocharu jako prostředku pro prevenci acidifikace půd se zabývá přehledný článek autorů Dai a kol. (2017). Aplikace biocharu zvyšuje pH půdy díky jeho vlastnostem – alkalickému charakteru i vysoké pufrovací schopnosti. Obvykle se pH půdy zvýší o jednotku (popsáno ve většině případů, kdy je pH biocharu vyšší než 7). Aplikace biocharu zvýší pH půdy, jestliže se jedná o půdu kyselou, v půdě alkalické působí biochar neutrálně, či dokonce dochází ke snížení pH půdy. Yuan a Xu (2011) zjistili, že zvýšení pH půdy více koreluje s obsahem alkalických prvků v biocharu než se samotným pH biocharu. Kationty jako Ca, K, Mg, Na a Si vytváří během pyrolýzy karbonáty a oxidy a ty reagují s vodíkovými ionty a monomerním Al a tím snižují aciditu půdy (Brewer a kol., 2012, Novak a kol., 2009a). Pufrovací schopnost biocharu je zřejmě spojena se schopností biocharu zvýšit KVK půdy a procesy protonace/deprotonace funkčních skupin na povrchu biocharu (Xu a kol., 2012). Bazické kationty obsažené v biocharu jsou uvolněny do půdy a vytěsňují Al^{3+} a H^+ ionty a tím zvýší kationtovou výměnnou kapacitu půdy. KVK stoupá se zvyšující se hustotou náboje v půdě a se zvyšujícím se povrchem tedy místy, kde se kationty sorbují (Liang a kol., 2006).

Novak a kol. (2009b) aplikovali biochar do půdy chudé na živiny, po 67denní inkubaci zjistili zvýšení hodnoty pH a obsahu přístupných živin (P, Ca a K), nezvýšila se však hodnota KVK. Autoři zmiňují možnost oxidace povrchu biocharu pro zvýšení množství funkčních skupin a tím následné zvýšení KVK půdy. Lehman a kol. (2006) uvádějí, že je možné očekávat, že po čase povrch biocharu vlivem kyslíku a vody zoxiduje přímo v půdě.

Mnohé publikace uvádějí velmi pozitivní vliv biocharu na zvýšení přítomnosti živin v půdě a stejně tak mnohé publikace popisují opačný efekt aplikace tohoto materiálu. Je známo, že více živin je obsaženo v biocharu vyrobeném z hnoje, z rostlinných surovin je pak více živin biocharu z bylinné biomasy oproti dřevní (Gaskin a kol., 2008; Novak a kol., 2014). Nejvíce problematickou živinou je zřejmě dusík, který se z důvodu vysokých teplot přípravy v biocharu téměř nenachází. Biochar jako takový ale může ovlivňovat dynamiku dusíku v půdě ať už pozitivně (Pommer a kol., 2014), či negativně (Wang a kol., 2015a). Biochar připravený za vyšších teplot je schopen poutat a tak imobilizovat nitrátový dusík (Zheng a kol., 2013a). Byly zaznamenány případy stimulace nitrifikace po aplikaci biocharu na kyselých půdách (Berglund a kol., 2014) především v souvislosti s aplikací dusíkatých hnojiv (Gul a kol., 2016). Mukherjee a Zimmerman (2013) uvádějí, že množství uvolnitelných živin ze samotného biocharu i ze směsí biocharu a půdy je velmi variabilní v závislosti na vlastnostech půdy, původu biocharu a jeho přípravy. Živiny v biocharu jsou v různých formách a tudíž variabilita jejich možného využití rostlinami v půdě je také vysoká. Gaskin a kol. (2008) ve své studii popisují konzervaci živin v biocharu a uvádějí, že dusík v něm obsažený je rostlinám nepřístupný, ale živiny, jako je vápník, draslík a fosfor, byly slabě kyselými extraktanty uvolnitelné, přičemž Laird a kol. (2010a) i Zheng a kol. (2013b) toto ve své studii potvrzují.

Vliv biocharu na výnos plodin je závislý na podmínkách daného místa. Jestliže byl biochar aplikován na chudou půdu, výnos plodin se zvýšil o 16-35 %, na úrodné půdě byl pozorován mírný pokles výnosu po aplikaci biocharu (Haefele, a kol., 2011). Po aplikaci biocharu a jeho schopnosti snížit toxicitu Al a zvýšit příjem P pozorovali Hong a kol. (2014) zvýšení výnosu kukuřice. Uzoma a kol. (2011) uvádí zvýšení výnosu kukuřice o 150 a 98 % (ve srovnání s kontrolou) při použití biocharu z hovězího hnoje v dávkách 15 a 20 t.ha^{-1} na písčitou půdu. Aplikace biocharu z biomasy prosa způsobila negativní dopady na produkci rostlinné biomasy a změnila zastoupení mikroorganismů v půdě (Kelly a kol., 2015). Když Nelissen a kol. (2014) aplikovali biochar do půdy, v důsledku této aplikace pozorovali výrazné snížení výnosu ředkviček a jarního ječmene. Snížení výnosu bylo způsobeno snížením obsahu nitrátového dusíku na variantách s biocharem.

Další možností využití biocharu se zabývali Ngo a kol. (2013). Ve své studii popisují chování směsi biocharu s kompostem, vermikompostem a hnojem. Zjistili, že přítomnost biocharu v těchto materiálech chrání organickou hmotu před oxidací, a tak mění jejich náchylnost k biodegradaci. Podporuje tedy sekvestraci uhlíku v těchto materiálech.

Při současných poznatcích je třeba uvažovat a předem podrobně charakterizovat biochar, který je do půdy aplikován, vědět z jakého je vstupního materiálu a jak je připraven. Dále chybí poznatky z dlouhodobých experimentů (Lone a kol., 2015).

2.2.3 Využití biocharu v remediačních technologiích

Velký zájem o biochar je díky jeho schopnosti sorbovat nežádoucí kontaminanty organického (Zhang a kol., 2011; Jones a kol., 2011; Cao a kol., 2009; Chen a kol., 2008; Khorram a kol., 2016) a anorganického původu ve vodním prostředí i v půdě (Uchimiya a kol., 2010a, 2011a,b,c ; Beesley a kol., 2011; Trakal a kol, 2011; Qui a kol., 2008; Park a kol., 2011; Jinag a kol., 2012; Rinklebe a kol., 2016). Tabulka 5 uvádí příklady kontaminantů a typů biocharu, schopných tyto kontaminanty poutat.

Tabulka 5. Příklady kontaminantů a typů biocharu schopných omezit jejich mobilitu

Látka		Vstupní biomasa pro biochar	Charakteristiky pokusu	Citace
Organické polutanty	simazine	tvrdé dřevo	S_{BET} (39 m^2.g^{-1}) při 450°C, aplikace 0 –200 t. ha^{-1}	Jones a kol., 2011
	atrazine	hovězí hnůj	S_{BET} (~8 m^2.g^{-1}) při 350°C, aplikace 1 :10 (w /v), vsádkový experiment	Cao a kol., 2009
	naftalen, nitrobenzen	jedlové jehličí	S_{BET} (~112–490 m^2.g^{-1}) při 400–700°C,	Chen a kol., 2008
Rizikové prvky	kadmium	blahovičník	při 550°C, dávky 0,5 a 5 %	Zhang a kol., 2013
	měď, nikl, olovo	bavlník	S_{BET} (~20 m^2.g^{-1}) při 350°C, dávka: 10 %	Uchymia kol., 2011a

Sorpční schopnost biocharu je ovlivněna jeho vlastnostmi stejně jako vlastnostmi sorbovaného prvku. Při sorpci z roztoku bylo zjištěno, že také záleží na množství iontu kovu (Kolodynska a kol., 2012). Uchymia a kol. (2011a) uvádějí, že by se pro konkrétní půdní aplikaci měl vybrat biochar na základě jeho vlastností a požadované funkce a půdních vlastností. K objasnění sorpčních mechanismů rizikových prvků na biochar jsou nejčastěji ve studiích používány adsorpční izotermy Freundlicha a Langmuira (Chen a kol., 2011; Uchymia a kol., 2011b,c).

Možné mechanismy při imobilizaci rizikových prvků jsou: i) srážení: tvorba hydroxidu, karbonátu, fosfátu (především při vysokých hodnotách pH platí pro imobilizaci Pb; Wang

a kol., 2015b; Cao et al., 2009; Cao a Harris, 2010; Xu a kol., 2013), ii) elektrostatická interakce mezi kationty a funkčními skupinami (Wang a kol., 2015b: popsáno jako hlavní mechanismus při sorpci arsenu; Frišták a kol., 2015), iii) povrchová chemisorpce mezi d - elektrony kovů a π-elektrony biocharu (Cao a kol. 2009; Uchimiya a kol., 2010b), iv) fyzikální sorpce řízená specifickým povrchem a porozitou (Uchimiya a kol., 2010b)

Uchimiya a kol. (2011b) uvádějí, že aplikace biocharu způsobuje rovnovážný stav půdního roztoku (hysteréze sorpce a desorpce), a tím se zvyšuje schopnost sorpce dalších kontaminantů vstupujících do půdy. Nepřímo může biochar ovlivnit imobilizace rizikových prvků již zmíněným zvýšením pH; to může dále znásobit negativní náboj povrchu a následně tak zvýšit afinitu půdy ke kationtům rizikových prvků (Jiang a kol., 2012; Kolodynska a kol., 2012; Beesley a Marmiroli, 2010). To potvrzují i Chen a kol. (2015), kteří popisují imobilizaci kadmia biocharem z čistírenských kalů (již při dávce 0,2 g) a jako dva hlavní mechanismy této imobilizace popisují vysrážení kadmia na povrchu biocharu při vyšším pH anebo iontovou výměnu.

V inkubačním experimentu bylo pozorováno významné snížení dostupných forem kadmia a mědi a zvýšení hodnoty pH po aplikaci biocharu během 1 –2 měsíců (Gomez-Eyles a kol., 2011). Ahmad a kol. (2017) popisují imobilizaci Cu, Pb a Zn v kyselé půdě pomocí inkubačního experimentu. Vyzdvihují závěr, že pro efektivní využití biocharu je zapotřebí jak přesné znalosti jeho vlastností, tak i vlastností půdy, kam je aplikován, a to, pro jaké účely má být použit. Snížení mobility prvků v půdě pak vede i ke snížení obsahu rizikových prvků v biomase rostlin. Namgay a kol. (2010) zjistili, že aplikace biocharu (15 g.kg^{-1} půdy) snížila přístupné formy mědi a kadmia. U zinku a arsenu dávka biocharu nezpůsobila snížení jejich přístupných forem. Koncentrace As, Cd, Cu, Pb se v nadzemní biomase kukuřice snížila ve srovnání s kontrolou (bez biocharu) díky vyšší dávce biocharu (15 g.kg^{-1}). Významné snížení obsahu Cd, Cu, a Pb bylo v předešlých studiích sledováno v kořenech brukve sítinovité při aplikaci biocharu ze zeleného odpadu a drůbežího hnoje v aplikační dávce nepřevyšující 1 % do slabě kyselé půdy (Park a kol., 2011). Autoři také sledovali významný nárůst biomasy oproti kontrole v důsledku zvýšení přístupnosti fosforu a draslíku v půdě. Cui a kol. (2011) aplikovali 10–40 t.ha^{-1} biocharu v polním experimentu a při dvouletém sledování zjistili významné snížení příjmu kadmia rostlinami rýže již při nejnižší dávce. Nezjistili však vyšší výnos na variantách s biocharem oproti kontrole. V dávkách 1,5 %, 3 % a 5 % na hlinitopísčitou půdu kontaminovanou kadmiem bylo dosaženo zvýšení výnosu nadzemní biomasy i kořenů pšenice ve studii Abbas a kol. (2017).

Otázkou také zůstává vhodně zvolená aplikační dávka biocharu. Uchymia a kol. (2011a) uvádějí, že vhodná dávka pro snížení přístupných forem rizikových prvků na silně kontaminované půdě je 10–20 % biocharu. Jak je ale vidět v tabulce 5, sorpční či imobilizační schopnost biocharu byla sledována i při mnohem nižších dávkách.

Zavedení biocharu do praxe naráží na úskalí, která shrnují O'Connor a kol. (2018). Zmiňují například interakci aplikovaného biocharu s tradičními hnojivy, kdy se efekt biocharu sníží s nadměrným používáním hnojiv. Další faktory, které ovlivňují působení biocharu ve smyslu omezení vstupu rizikových prvků do pěstovaných plodin, jsou: klimatické podmínky, dávka a hloubka zapravení biocharu, půdní vlastnosti, působení mikroorganismů, vlastnosti biocharu i biomasy, z které je připraven. Jak uvádějí Houben a Sonnet (2015) lze považovat i vlastnosti rhizosféry různých typů rostlin jako faktor ovlivňující efektivnost biocharu a shrnují, že při aplikaci biocharu je třeba volit správnou formu hnojení dusíkem (upřednostňovat nitrátovou před amonnou) či vybírat rostliny s nižší schopností acidifikace rhizosféry. Rinklebe a kol. (2016) uvádějí, že redox potenciál půdy má jen malý efekt na působení biocharu na uvolňování těžkých kovů (Cd, Cu, Ni, Zn) z kontaminované nivní půdy.

Pohyb prvků půdním profilem se nejčastěji sleduje v různě upravených nádobách, ve kterých se zachycuje průsaková voda neboli perkolát, tedy tzv. lyzimetrech (Jordan, 1968). Autoři používají válcové nádoby, které se následně umísťují buď do laboratorních, skleníkových nebo přirozených podmínek (Trakal a kol., 2011; Laird a kol., 2010a,b ; Puga a kol., 2016). Průtok vody je simulován zavlažovacími systémy, kdy je používána dešťová (Uchimiya a kol., 2011a) nebo demineralizovaná voda (Trakal a kol., 2011). Laird a kol. (2010a,b) při inkubačním experimentu používali každý týden k promývání místo vody vyluhovadlo 0,001 mol.l $^{-1}$ $CaCl_2$. Zjistili, že se rostoucí dávkou (5, 10 a 20 g.kg^{-1}) biocharu zamezilo vyplavování živin ze zemědělské půdy Při dávce biocharu 20 g.kg^{-1} se v roztoku snížil obsah celkového dusíku o 11 % a rozpustného fosforu o 69 %. Puga a kol. (2016) pozorovali redukci kadmia (57–73 %), olova (45–55 %) a zinku (46 %) ve výluhu při aplikaci 3 % biocharu do slabě kyselé půdy.

Chen a kol. (2011) uvádějí, že využívání biocharu jako sorbentu má velký potenciál, avšak mohou nastat komplikace při remediaci vícečetné kontaminace a vyzdvihují potřebu individuálního přístupu ke specifickým podmínkám kontaminované oblasti.

3. Hypotézy a cíle práce

1) První hypotézou práce je, že vstupní biomasa a pyrolýzní podmínky ovlivní vlastnosti biocharu.

Cílem bylo připravit biochar z různých druhů kontaminované i nekontaminované vstupní rostlinné biomasy. Biomasa rostlin byla pěstována na kontaminované půdě, následně byla upravována termochemickou konverzí – pyrolýzou – za různých finálních teplot. Tak byl připraven biochar a budou popsány jeho fyzikálně-chemické vlastnosti.

2) Druhou hypotézou je, že aplikace biocharu ovlivní sorpční vlastnosti půdy, mobilitu rizikových prvků v půdě a zároveň kontaminovaná vstupní biomasa neovlivní sorpční vlastnosti připraveného biocharu.

Cílem bylo sledovat sorpční vlastnosti biocharu pomocí "batch" sorpčního a desorpčního experimentu. Byla aplikována dávka biocharu do kontaminované půdy a sledována sorpční schopnost vzniklé matrice poutat rizikové prvky, přičemž byla porovnána se sorpční schopností neošetřené půdy. Dále bylo hodnoceno uvolňování (desorpce) zmíněných prvků z biocharu, připraveného z kontaminované biomasy, a zároveň uvolňování rizikových prvků z připravené matrice, půda + biochar.

3) Třetí hypotézou je, že biochar aplikovaný do kontaminované půdy ovlivní růst rostlin a jejich schopnost akumulace rizikových prvků v biomase.

Cílem práce bylo sledovat vliv aplikované dávky biocharu do kontaminované půdy na růst rostlin, příjem rizikových prvků rostlinami a vyplavování těchto prvků v modelovém lyzimetrickém experimentu ve skleníkových podmínkách. Různé dávky biocharu byly aplikovány na půdu extrémně kontaminovanou kadmiem, olovem a zinkem. Ve variantách byly pěstovány vrby (*Salix* spp.) a byl odebírán perkolát (průsaková voda) z lyzimetrických nádob.

4. Publikované články

4.1

Břendová, K., Száková, J., Lhotka, M., Krulikovská, T., Punčochář, M., Tlustoš, P. 2017. **Biochar physicochemical parameters as a result of feedstock material and pyrolysis temperature: predictable for the fate of biochar in soil?** Environmental Geochemistry and Health, 39 (6), 1381–1395.

4.2

Břendová, K., Tlustoš, P., Száková, J. 2015. **Can Biochar From Contaminated Biomass Be Applied Into Soil For Remediation Purposes?** Water, Soil and Air Pollution, 226 (6), 1.

4.3

Břendová, K., Tlustoš, P., Száková, J. 2015. **Biochar immobilizes cadmium and zinc and improves phytoextraction potential of willow plants on extremely contaminated soil.** Plant, Soil and Environment, 61 (7), 303-308.

4.4

Břendová, K., Tlustoš, P., Száková, J. 2018. **Biochar applications enhance the phytoextraction potential of *Salix smithiana* [Willd.](willow) in heavily contaminated soil – potential for a sustainable remediation method?** Journal of Environmetnal Managment. under review.

4.5

Břendová, K., Zemanová, V., Pavlíková, D., Tlustoš, P. 2016. **Utilization of biochar and activated carbon to reduce Cd, Pb and Zn phytoavailability and phytotoxicity for plants.** Journal of Environmental Management, 181, 637-645.

4.6

Zemanová, V., Břendová, K., Pavlíková, D., Kubátová, P., Tlustoš, P. 2017. **Effect of biochar application on the content of nutrients (Ca, Fe, K, Mg, Na, P) and amino acids in subsequently growing spinach and mustard.** Plant, Soil and Environment, 7, 322-327.

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ORIGINAL PAPER

Biochar physicochemical parameters as a result of feedstock material and pyrolysis temperature: predictable for the fate of biochar in soil?

Kateřina Břendová · Jiřina Száková · Miloslav Lhotka · Tereza Krulikovská · Miroslav Punčochář · Pavel Tlustoš

Abstract Biochar application is a widely investigated topic nowadays, and precisely described biochar parameters are key information for the understanding of its behaviour in soil and other media. Pore structure and surface properties determine biochar fate. However, there is lack of complex, investigative studies describing the influence of biomass properties and pyrolysis conditions on the pore structure of biochars. The aim of our study was to evaluate a wide range of gathered agriculture residues and elevated pyrolysis temperature on the biochar surface properties and pore composition, predicting biochar behaviour in the soil. The biomass of herbaceous and wood plants was treated by slow pyrolysis, with the final temperature ranging from 400 to 600 °C. Specific surface ranged from 124 to 511 cm^2 g^{-1} at wood biochar and from 3.19 to 192 cm^2 g^{-1} at herbaceous biochar. The main properties influencing biochar pore composition were increasing pyrolysis temperatures and lignin (logarithmically) and ash contents (linearly) of biomass. Increasing lignin contents and pyrolysis temperatures caused the highest biochar micropore volume. The total biochar pore volume was higher of wood biomass (0.08–0.3 cm^{-3} g^{-1}). Biochars of wood origin were characterised by skeletal density ranging from 1.479 to 2.015 cm^3 g^{-1} and herbaceous ones 1.506–1.943 cm^3 g^{-1}, and the envelope density reached 0.982 cm^3 g^{-1} at biochar of wheat grain origin and was generally higher at biochars of herbaceous origin. Density was not pyrolysis temperature dependent.

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Graphical Abstract

PYROLYSIS

Defined properties

Choice of proper biochar

EFECTIVE SOIL APPLICATION

Keywords Specific surface area · Sorption · Pore volume · Wood biomass · Herbaceous biomass

Introduction

Biochar is a highly porous, fine, carbon-rich material produced by pyrolysis from different feedstock materials of organic origin. The difference between biochar and char is in their subsequent use: biochar is designed for soil application and char mainly for combustion. Biochar is prepared in a limited or no oxygen atmosphere (Lehmann and Joseph 2009). Moreover, its soil application has been widely investigated over the last decade, due to its many positive impacts on soil properties. This material is able to sorb organic and inorganic contaminants in soil (Břendová et al. 2015; Uchimiya et al. 2011; Oleszczuk et al. 2014). Biochar application leads to an increase in soil properties determining soil fertility, e.g. cation exchange capacity, water retention, pH adjustment, nutrient availability, increase in microbial habitat (Tang et al. 2013; Van Zwieten et al. 2010). However, over time, the published results have highlighted not only the positive effects of biochar application, but also negative or zero effects. Determination of the wide range of biochar properties can substantially help in predicting biochar effectiveness on soils with different properties.

The enormous diversity of feedstock biomass, as well as the various technologies of thermochemical decomposition of feedstock (high-/low-temperature pyrolysis, carbonisation, gasification, etc.), influences the quality of final biochar and leads to highly diverse chemical (carbon content, composition and content of other elements, content of functional groups) and physical properties (e.g. specific surface area, structure, pore geometry). Therefore, the fate of biochar in contact with soil environment (i.e. hydrophobicity, sorption of nutrients and contaminants) is very different, as well (Schnee et al. 2016; Keiluweit et al. 2010; Ahmad et al. 2014). Both feedstock properties and production conditions are important for the final yield and properties of biochar, but their effect differs with the individual property of interest (Zhao et al. 2013). The feedstock materials mainly influence the element composition, content of fixed carbon, ash content (Gaskin et al. 2008; Lee et al. 2013; Zhao et al. 2013). The pyrolysis temperature positively correlates with pH, Brunauer–Emmett–Teller (BET) surface area, aromaticity and recalcitrance of the biochar (Jindo et al. 2014; Novak et al. 2009; Keiluweit et al. 2010; Ronsee et al. 2013; Ahmad et al. 2012; Zhao et al. 2013). The mutual effect of both conditions of the pyrolysis process and feedstock material demonstrates cation exchange capacity of biochar. Some authors have observed decreased value of cation exchange capacity (CEC) with increasing peak temperature of pyrolysis (Novak et al. 2009). Zhao et al. (2013) further specified that this property is more related to feedstock material, because CEC is related to cations (e.g. K, Ca, Mg) present in biochar. Biomass composition, which is defined by the proportion of cellulose, hemicelluloses, lignin and extractive compounds, is an important factor affecting biochar structure; it is suggested that the original plant cellular structure is printed in biochar one (Brewer et al. 2009; Downie et al. 2009). This assumption is supported by the gradual decomposition of these components during pyrolysis leading to various types of biochar (Demirbas 2004). However, the generalisation and verification of specified types of biochar for effective soil utilisation based on these properties of feedstock material are hardly present in the literature.

Knowledge related to the physical biochar properties is necessary, due to their direct or indirect relation to the ways in which biochars interact with and affect soil systems (Downie et al. 2009). Density and porosity are fundamental physical characteristics that play a key role in determining residence time of

biochar in soil (Masiello et al. 2012). Faur-Brasquet et al. (2002) showed the dependence of adsorption capacities on adsorbent porosity and other properties, e.g. chemical properties of individual ions (molecular weight, ionic radius and electronegativity), etc. Similarly, Ahmad et al. (2012) observed linear relationships between sorption parameters and surface area, as well as molar elemental ratios of biochar. However, Schnee et al. (2016) determined that physical description of biochars alone is insufficient for the reliable prediction of microbial habitat quality and they recommended that physical and surface chemical data should be linked for this purpose; the biochar structure is an important property in predetermining biochar as a suitable niche for soil microorganisms.

Aller (2016) reviewed the available scientific data of the physical properties of biochar and concluded that compared with other biochar properties there was still an overwhelming lack of these published data. The review also showed that structure and properties such as porosity and total porosity were described using various techniques of X-ray spectrometry, but the number of papers quantifying these properties is very limited. Brewer et al. (2014) indicated that the physical characterisation of biochar is challenging, because biochar pores can change over an extensive range of sizes from sub-nanometre to micrometre size and currently there is no common standard methodology to measure pore volume precisely across these scales. As a result, this area is not fully understood.

The aim of our study was to prepare biochar from various organic waste materials originating mostly from the crop production. We observed the suitable pyrolysis temperature to gain the sufficient yield of biochar. The biochar physical properties based on non-destructive and solid sample-based analytical techniques were determined. The results were compared with the literature, and their applicability was considered for evaluation of biochar expediency as soil amendment.

Materials and methods

Feedstock biomass

The plant biomass for pyrolysis was harvested in a relatively small area close to Příbram region (Czech Republic, 60 km from Prague; 49°70′63″N,

13°97′57″E). The sampled plant biomass samples were: (i) plants from the *Salicaceae* family (various clones of *Populus* and *Salix* spp.) representing the bark wood biomass; (ii) for comparison, soft deciduous bark-free poplar wood, purchased from J. Rettenmaier & Söhne GmbH, this was used to determine the influence of the presence of bark on the properties of the final biochar; (iii) whole above-ground biomass of maize (*Zea mays*); (iv) winter wheat in full maturity, where the grains and straw were pyrolysed separately; and (v) meadow grass harvested near the plantation growing the wood biomass.

The plant material was first air-dried to the optimum moisture content of 12–15%. The biomass was ground and homogenised. It was subsequently processed into pellets with a diameter of 6 mm. The wheat grain biomass was pyrolysed as whole grains.

Pyrolysis process

The pyrolysis process was carried out on a fixed bed, under oxygen-limited conditions in a muffle furnace under the flow of nitrogen (inert gas) 1 m^3 h^{-1}, at atmospheric pressure and with a retention time of 30 min for the individually designed temperatures, and the heating rate was 7 °C min^{-1}. Pyrolysis was conducted at five different temperatures: 400, 450, 500, 550 and 600 °C for all materials.

Analytical methods

The yield of biochar (Y; in %) was calculated using the following equation (Eq. 1):

$$\left(Y = \frac{w_B}{w_F} \cdot 100 \right), \tag{1}$$

where w_B is the weight of biochar and w_F is the weight of dry feedstock.

Water content (W) in the original biomass sample was determined according to the Czech/European standard (CSN EN 15414-3), i.e. by sample drying in an analytical drying oven at (105 ± 2) °C until a constant weight rounded to two decimal places was reached.

Ash content (A) in the biomass sample was determined according to the Czech/European standard (CSN EN 15403), i.e. by low-temperature incineration of the sample at (550 ± 10) °C until a constant weight rounded to two decimal places was reached.

Volatiles content (V) in the biomass sample was determined according to the Czech/European standard (CSN EN 15402), i.e. by sample devolatilisation in a closed crucible in a muffle furnace at (900 ± 10) °C for 7 min.

The content of C, H, N, O and S was determined by using the Flash EA 1112 apparatus in the CHNS/O configuration (Thermo Fisher Scientific, USA), where the sample is incinerated in an oxygen stream at high temperature, and the gaseous incineration products (nitrogen, carbon dioxide, sulphur dioxide and water) are separated on a packed chromatographic column and detected by a thermal conductivity detector. In this method, the oxygen content is then calculated by the difference. These results were used to calculate the atomic H/C, O/C ratios. Organic compounds of feedstock biomass were determined by TAPPI 264 cm^{-97} standard based on determining the amount of solvent-soluble, non-volatile material in wood and pulp.

The pH value of biochar was determined in H_2O in 1:10 (w/v) ratio according to Gaskin et al. (2008) and Bachmann et al. (2016).

The specific surface area, micropore analysis and distributions of volume mesopores were measured on an ASAP 2020 (accelerated surface area and porosimetry) analyser (Micromeritics, Norcross, GA, USA) using the gas sorption technique Brewer et al. 2014). The adsorption isotherms were fitted by using the Brunauer–Emmett–Teller (BET) method for specific surface area (Brunauer et al. 1938), the micropore volume by the *t*-plot method (Webb and Orr 1997) and the pore size distribution by the Barrett–Joyner–Halenda (BJH) method (Barrett et al. 1951).

Skeletal density was measured on a He-pycnometry 1305 analyser (Micromeritics, Norcross, GA, USA).

Mercury porosimetry measurements were made using an AutoPore IV 9500 porosimeter (Micromeritics, Norcross, GA, USA). The measurements comprised of two parts. There are two low pressure ports on the top, where the evacuation of sample and low pressure from 0.01 to 0.25 MPa takes place. This means that pore radii from 100 to 3 μm approximately are determined. The high-pressure chamber is used for high-pressure analysis from 0.25 to 400 MPa. It covers the pore radius range from 3 to 1.5 nm.

In addition to the basic analytical methods, each type of feedstock biomass was analysed by thermal analysis using the Setsys Evolution unit (Setaram, France). The mass of the sample for the thermal analysis was approximately 5 mg of the explored types of biomass. Nitrogen was used as a carrier gas with the flow rate of 100 ml min^{-1}. The heating rate for the analysis described and discussed was 10 °C min^{-1}.

Porosity (*p*) was calculated by equation $p = 1 -$ envelope density/skeletal density.

Testing of the mechanical resistance of selected pyrolysed pellets was carried out according to the Czech/European standard (CSN EN 15210). The selected pellets were tested in a rotary drum at 50 rpm for 10 min.

The morphology of the biochar was evaluated by scanning electron microscopy (SEM) using a Hitachi S-450 with an EDS analyser Kevex Delta 5. Images of the bark wood biochar and wheat grains were taken under a stereomicroscope Nikon SMZ800N (Nikon, Japan) equipped with a Canon PowerShot A620 digital camera and illuminating system Intralux 6000^{-1} (Volpi, Switzerland). Images were evaluated by image analysis software NIS-Elements 3.1 (Laboratory Imaging Ltd, Czech Republic).

Results and discussion

Feedstock biomass characteristics

Ultimate and proximate analysis, organic compounds content

The results of the main characteristics of the feedstock biomass are summarised in Table 1. The content of volatile matter is a good precursor for the development of porous material. As it was reported before, the high content of volatile matter presented in the biomass is suitable for the production of highly porous structure of activated carbon (Lua et al. 2006). During pyrolysis, the evolution of volatiles from the feedstock results in enhanced pore development in the chars (Lua et al. 2004). As the pyrolysis temperature increased, the evolution of volatile products increased. This will lead to an increase in the formation of bubbles and pores in the melt and the surface area of the char (Sharma et al. 2004). Fixed carbon is a parameter that can help to predict the combustion behaviour of fuels (Demirbaş 1997). In comparison to coal, biomass is characterised by a lower content of fixed carbon, by approx. 20%

(Demirbas 2004). Table 1 also documents differences in the contents of organic compounds in feedstock biomass. The highest lignin was determined in wood, whereas the lowest content of lignin was found in grains of wheat.

Thermogravimetric analysis

The results of thermogravimetry (TG) and differential thermal analysis (DTA), carried out in an inert nitrogen atmosphere, are presented in Fig. 1, where Fig. 1a shows that the stage of losing moisture was observed up to 200 °C in all types of biomass. Devolatilisation of the feedstock biomass was observed between 200 and 400 °C, where the cellulose, hemicelluloses and part of the lignin are decomposed. The highest mass loss was observed in poplar (70%), whereas only 40% of the mass loss was determined for wheat straw. Wheat straw biomass showed the highest amount of residues at the end of the experiment, which could be explained by the highest content of ash. Abdullah et al. (2010) observed the lowest mass loss in pure lignin, followed by rice straw in their study, and the residues were comparable to the amount of wheat residues in our experiment. From 400 °C, the slope of mass loss was very slight due to the slow decomposition of lignin. For maize (Fig. 1b), a double peak in the curve was observed. The first peak (290 °C) and the second peak (300 °C) correspond to hemicelluloses and cellulose, respectively. Wheat grain is represented by the sharpest peak at 310 °C on the DTA curve, and this reflects cellulose decomposition. This confirmed the very low lignin content in this material, as is already documented in Table 1.

The curve with very low intensity at temperatures exceeding 500 °C indicates the pyrolysis of lignin in all the observed biomass.

Biochars characteristics

Element, ash content and pH of prepared biochars

Table 2 shows the basic element composition, ash content and pH of the prepared biochars. The molar ratios were also calculated.

The carbon conversion (carbon content in biochar/carbon content in feedstock × 100%) was highest for woody biomass: bark free up to 162%, wood biomass with bark 149%. Among the herbaceous feedstocks, relatively high carbon conversation reached up to 161%. Lower conversation representing 129, 133 and 139% was determined for meadow grass, maize and wheat grain, respectively. Thus, the wood feedstock biomass can be considered as better material after pyrolysis for potential carbon sequestration in soil. This statement is supported by the results of the microbial activity in the biochar-treated soil. Biochar from straw improved the number of microorganisms in the soil, but biochar from woody biomass did not, suggesting a higher stability of woody biochar rather for carbon sequestration purposes (Schnee et al. 2016). On the other hand, herbaceous biomass oxygen content indicated the presence of functional groups, which could serve for higher nutrient sorption in soil.

The effect of pyrolysis temperature on element content in the biochar was also evident. The carbon content increased with the temperature (woody biomass), remained unchanged (wheat biomass) or even slightly decreased (maize and meadow grass).

Zhao et al. (2013) assessed the effect of feedstock and pyrolysis temperature effect on biochar pH. They found that pH was mainly temperature-affected. In our study, we found a link between the pH of biochars and ash content in feedstock materials. The pH linearly correlated with ash content in biochars, which is conditioned by ash-based element content in feedstock biomass (for wood biochars: $R^2 = 0.74$–0.98; for maize, meadow grass and wheat straw biochar: $R^2 = 0.94$, 0.6 and 0.57, respectively).

The ratio of H/C and O/C (Table 2) can be helpful for the estimation of the biochar polarity and its potential interaction with water and provides the level of oxidative alteration of biochar in the soil (Sohi et al. 2010). With increasing temperature, the carbon content increased, while the biomass surface –OH groups were released during the pyrolysis process. Thus, higher polarity is observed at lower temperature (Novak et al. 2009). At higher temperatures, plant-based biomass undergoes dehydration and depolymerisation reactions to form volatile lignin and cellulose which condensate subsequently to yield graphitic structures inside the biochar matrix. These results indicate that at elevated temperature more recalcitrant carbon structure was formed inside biochar matrix (Chowdhury et al. 2016). If biochar is applied into soil, it is generally said that molar ratios of O/C lower than 0.2 appear to provide, at minimum, a

Table 1 Element content and characteristics of feedstock biomass

Parameters % (w/w)	Feedstock biomass					
	Bark-free wood	Bark wood	Maize	Meadow grass	Winter wheat straw	Winter wheat grain
C	47 ± 5.2	49 ± 3.5	45 ± 3.1	46 ± 4.0	45 ± 2.3	45 ± 3.4
H	6.1 ± 0.2	5.9 ± 0.1	5.9 ± 2.0	5.8 ± 1.2	6.3 ± 0.3	6.6 ± 0.4
O[a]	46 ± 4.3	41 ± 2.7	41 ± 5.6	41 ± 2.5	40 ± 1.6	43 ± 5.8
N	0.1 ± 0.02	0.7 ± 0.01	1.5 ± 0.6	1.2 ± 0.02	0.9 ± 0.1	2.6 ± 0.4
Ash[b]	0.8 ± 0.03	3.7 ± 0.2	6.8 ± 1.3	6.5 ± 0.6	8.2 ± 0.9	2.2 ± 0.6
Volatile matter[b]	86 ± 18	77 ± 5.8	77 ± 11.2	76 ± 2.3	75 ± 6.3	81 ± 7.8
Fixed carbon[b]	13 ± 2.1	20 ± 2.1	16 ± 3.8	18 ± 1.2	17 ± 1.7	17 ± 2.5
Holocellulose[b]	79 ± 10	63 ± 5.7	60 ± 3.7	63 ± 2.7	65 ± 9.5	54 ± 9.8
Lignin[b]	17 ± 2.8	18 ± 2.1	5.0 ± 2.3	9.6 ± 1.6	9.4 ± 1.1	0.3 ± 0.06
Resins[b]	4.5 ± 1.2	6.5 ± 0.4	26 ± 1.1	18 ± 1.8	19 ± 2.7	36 ± 7.4
Tannins[b]	0.01 ± 0.00	12 ± 0.2	8.9 ± 1.2	10 ± 0.5	6.0 ± 0.02	10 ± 1.2

[a] Oxygen content was calculated by difference; data are mean of 2 replications ± standard error of the mean

[b] Data given in dry basis

Fig. 1 Results of thermogravimetric analysis: **a** thermogravimetric analysis of feedstock biomass; **b** differential thermal analysis of feedstock biomass
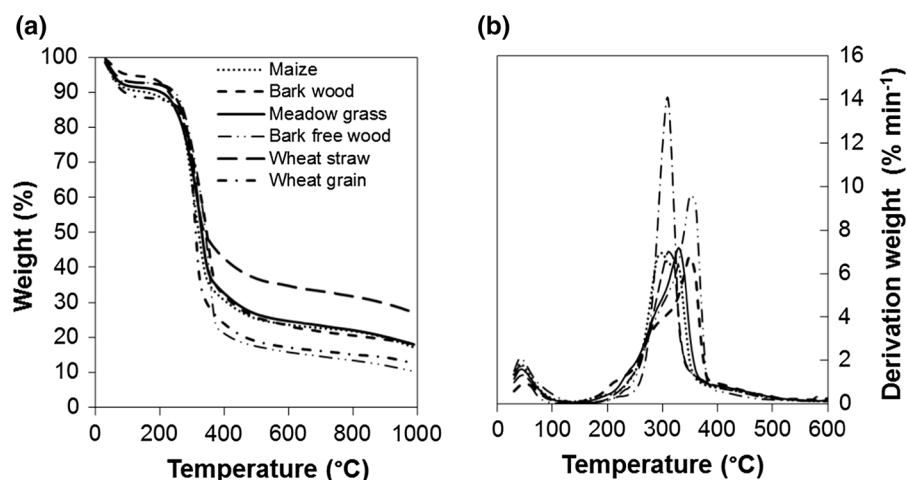
1000-year biochar half-life (Spokas 2010). Therefore, the biochars prepared at 500–600 °C indicated high stability in soil. Comparing the atomic ratio of biochar with carbon-rich soil organic compounds, humic and fulvic acids, the common O/C ratio is 0.5 and 0.7 for humic and fulvic acids, respectively, and the H/C ratio of both soil organic compounds approaches 1.0 (Stevenson 1994). The atomic ratios of our biochars were lower.

The differences between wood and herbaceous feedstock and their influence on the biochar yield were defined and strong linear dependence was determined between feedstock and final temperature of pyrolysis (a) wood feedstock biomass ($R^2_{\text{bark free wood}} = 0.97$, $R^2_{\text{bark wood}} = 0.98$); (b) herbaceous feedstock biomass ($R^2_{\text{all materials}} = 0.98$).

According to our results, the yield of biochar decreased with increasing temperature, as confirmed by Uchimiya et al. (2011), Keiluweit et al. (2010) and Horne and Williams (1996). The highest yields were achieved for wheat and grass biomass. This seemed to be due to the high content of ash, in these types of biomass (Vassilev et al. 2010), and subsequently in biochars. The yield dropped by 63, 50, 58, 26 and 27% for maize, wood chips, poplar and wheat straw and grain, respectively, between pyrolysis temperatures of

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Table 2 Yield and characteristics of prepared biochars

Type of biochar	Temperature (°C)	Yield (%)	C (%)	H (%)	N (%)	O[a] (%)	C/N	O/C	H/C	pH	Ash (%)
Bark-free wood	400	23	73.7	2.93	0.15	20.9	491	0.28	0.04	7.31	2.37
	450	19	73.1	2.48	0.17	16.5	430	0.23	0.03	7.52	7.81
	500	18	74.5	2.54	0.17	12.9	438	0.17	0.03	8.66	9.92
	550	13	75.5	2.41	0.23	11.7	328	0.16	0.03	9.37	10.2
	600	10	76.8	2.06	0.17	9.39	452	0.12	0.03	9.67	11.5
Meadow grass	450	28	59.4	2.44	1.78	16.7	33.4	0.28	0.04	6.23	19.7
	500	25	57.9	2.21	1.72	17.3	33.7	0.30	0.04	6.54	20.8
	550	21	55.2	1.83	1.66	17.6	33.3	0.32	0.03	6.90	23.6
Maize	400	26	59.8	2.80	2.08	13.7	28.7	0.23	0.05	7.61	21.7
	450	24	58.8	2.15	1.88	14.2	31.3	0.24	0.04	8.00	23.0
	500	19	54.9	2.05	1.95	13.8	28.2	0.25	0.04	9.82	27.3
	550	14	44.2	1.56	1.79	16.7	24.7	1.06	0.04	9.96	35.7
	600	10	29.0	1.07	1.22	23.9	23.8	0.82	0.04	10.2	44.9
Bark wood	400	29	62.5	2.65	1.12	21.8	55.8	0.35	0.04	10.5	12.0
	450	25	63.6	2.35	1.17	20.2	54.3	0.32	0.04	10.0	12.7
	500	21	63.8	2.21	1.11	19.1	57.5	0.30	0.04	9.65	13.8
	550	16	62.8	1.97	1.01	19.3	62.2	0.31	0.03	9.90	14.9
	600	15	68.3	1.78	0.99	13.2	69.0	0.19	0.03	9.91	15.8
Wheat straw	400	31	70.6	3.50	4.46	15.8	15.8	0.22	0.05	10.1	5.69
	450	29	69.2	3.00	4.12	17.6	16.8	0.25	0.04	10.4	6.17
	500	28	71.5	2.35	4.54	14.6	15.7	0.20	0.03	10.3	7.01
	550	26	70.8	2.10	4.61	13.7	15.4	0.19	0.03	10.4	8.71
	600	23	73.4	1.85	4.62	11.7	15.9	0.16	0.03	10.5	8.31
Wheat grain	400	27	61.1	2.84	1.30	12.0	47.0	0.20	0.05	6.93	22.6
	450	26	62.6	2.45	1.25	9.64	50.1	0.15	0.04	6.91	23.9
	500	24	61.9	2.20	1.22	10.3	50.8	0.17	0.04	7.02	24.4
	550	22	61.5	1.92	1.20	9.48	51.2	0.15	0.03	6.93	25.9
	600	20	57.5	1.62	1.09	8.23	52.8	0.14	0.03	6.94	31.5

[a] Calculated by difference

400 and 600 °C. The lower biochar yield from woody biomass can also be caused by higher content of extractives in feedstock material. The higher yield at the lower temperature can be seen as a consequence of incomplete charring, where at low temperatures of 200–300 °C, a gas portion escapes from the biomass primarily and up to 90% of the solids remain (Bergman and Kiel 2005).

Biochar surface physical properties

The specific surface area, envelope density, skeletal density, porosity and pore distribution of biochars were assigned. The physical structure of biochars, such as surface area, pore volume and average pore size, is typically related to its sorption and water holding capacity which, in turn, relates to its effect on soil structure, contaminant mobility and microbial interactions (Zhao et al. 2013). The necessary conditions for the production of biochars for water holding applications are: (a) to create sufficient porosity through feedstock selection and (b) to determine suitable production temperature that reduces hydrophobicity to an acceptable level (Gray et al. 2014).

We found that the biochar from bark-less wood (which has ash content of 0.79%, Table 3) achieved the highest specific surface area: 511 m² g⁻¹ at the

highest temperature (600 °C). The biochar from bark wood reached the highest S_{BET} at 600 °C: 428 m^2 g^{-1}. The smallest surface area of this material corresponded to the highest ash content in the biochar (Table 2). The reduction in the surface area in the temperature range 550–650 °C can be explained by the loss of the secondary volatiles in the intermediate thermoplastic phase during secondary devolatilisation (Lu et al. 1995). The highest S_{BET} among the herbaceous materials was observed for wheat grain and straw (143 and 192 m^2 g^{-1}, respectively). Fu et al. (2009) found the highest specific surface area of maize stalk biochar (81.6 m^2 g^{-1}) from fast pyrolysis at 900 °C. Keiluweit et al. (2010) reached the S_{BET} value of 50 m^2 g^{-1} for grass at 500 °C. Our results showed higher values of the S_{BET}, where the biochar from meadow grass and maize reached 81 and 64 m^2 g^{-1} at 500 °C, respectively. These values are lower than observed by Zhang et al. (2011), where the S_{BET} value from maize straw pyrolysis was 245 m^2 g^{-1}, but higher than Chen et al. (2011), who obtained biochar of corn straw with S_{BET} of 13 m^2 g^{-1}. Ronsee et al. (2013) observed that the highest content of minerals negatively correlated with specific surface area, most probably due to the fusion of molten ash filling up pores in the biochar.

The S_{BET} values increased logarithmically (bark-free wood except value of S_{BET} at 600 °C: $R^2 = 0.98$; bark wood: $R^2 = 0.98$; maize: $R^2 = 0.89$; meadow grass: $R^2 = 0.99$; winter wheat straw: $R^2 = 0.94$, winter wheat grain: $R^2 = 0.55$) with the temperature of pyrolysis, e.g. for bark wood the values increased from 124.4 to 428.1 m^2 g^{-1}, for maize from 4.75 to 105 m^2 g^{-1} (Table 3). The general trend of increasing biochar surface area along with pyrolysis temperature was likely due to the release of volatile organic compounds which contributed to the creation of voids or pores within the biochar matrix (Downie et al. 2009).

Bachmann et al. (2016) mentioned that the BET model is pore size and distribution dependent and may lead to over- or underestimation of the total specific surface area, depending on the partial pressure ratio of the measurement and/or the type of pores dominating in the sample. Moreover, most biochar literature reports surface area values in terms of the BET method, but more work is needed to demonstrate how this or other measurements relate to the quantity of the reactive surface sites (Brewer et al. 2009). Thus, it could be suggested that not only the BET model, but next surface properties, should be determined for better biochar description.

To get a fuller picture of biochar, the total pore volume and pore distribution were determined. The next analysis revealed that biochars were microporous materials.

Total pore volume (g cm^{-3}) revealed that biochars were microporous materials (Table 3), but increased with increasing temperature for all the investigated feedstock materials. The influence of feedstock was also determined. Herbaceous materials provided approximately 100-fold lower total pore volume and the highest values were determined at wheat grains at 550–600 °C: 0.094 and 0.110 cm^3 g^{-1}. At woody biomass, the values were 0.216 and 0.291 cm^3 g^{-1} at 550 °C, at bark-free wood and bark wood, respectively. Pore closing was observed at 600 °C, at bark-free wood, total pore volume increased to 0.149 cm^3 g^{-1}.

When we expect biochar to be applied to soil as a porous material, we can compare this amendment with the commonly occurring porous clay minerals in soil. The pore volume of biochars prepared from wood biomass is comparable to natural zeolite (~ 0.3 cm^{-3} g^{-1}; Herron and Corbin 1995) or montmorillonite (~ 0.21 cm^{-3} g^{-1}; Diamond 1970), whereas the pore volume of biochar from herbaceous biomass is lower.

The relation of ash content, pyrolysis temperature, micropore and total pore volume is shown in Fig. 2. Ash content in feedstock and pyrolysis temperature correlated with the described surface properties of biochars linearly.

Pyrolysis of pure lignin was described in detail, e.g. in Sharma et al. (2004). The lignin content in the feedstock material was tested as the potential fingerprint of feedstock biomass in biochar for estimation of the interrelationships between pyrolysis temperature and final pore volume of biochars. Lignins are highly branched, substituted mononuclear aromatic polymers in the cell walls of certain biomass, especially woody species, and are often bound to adjacent cellulose fibres to form a lignocellulosic complex (Klass 1998). The influence of lignin content together with pyrolysis temperature on the pore and micropore volume is presented in Fig. 3. A paraboloidal equation preferably describes these relations. Both the micropore and total pore volume increased with higher lignin content

Table 3 Specific surface area and pore volume of the investigated biochars

Feedstock material	Temperature (°C)	Specific surface area (S_{BET}) (m^2 g^{-1})	Specific surface area ($S_{t\text{-plot}}$) (m^2 g^{-1})	Total pore volume (V_{total}) (cm^3 g^{-1})	Micropore volume ($V_{t\text{-plot}}$) (cm^3 g^{-1})
Bark-free wood	400	164	35.9	0.09	0.06
	450	327	87.9	0.18	0.13
	500	376	100	0.21	0.14
	550	511	181	0.29	0.16
	600	214	104	0.15	0.06
Bark wood	400	124	32.6	0.08	0.04
	450	215	56.3	0.14	0.07
	500	323	91.7	0.19	0.11
	550	382	117	0.22	0.12
	600	428	133	0.24	0.14
Maize	400	4.75	0.44	0.02	0.00
	450	40.1	12.7	0.04	0.01
	500	64.3	21.2	0.06	0.02
	550	105	37.5	0.09	0.03
Meadow grass	450	13.4	7.39	0.02	0.00
	500	57.0	16.7	0.04	0.02
	550	77.2	21.9	0.06	0.03
Wheat straw	400	4.47	4.40	0.03	0.00
	450	4.00	2.14	0.03	0.00
	500	11.5	7.21	0.03	0.00
	550	52.9	17.4	0.05	0.02
	600	144	35.6	0.11	0.06
Wheat grain	400	3.19	3.20	0.01	0.00
	450	29.4	7.66	0.02	0.01
	500	87.5	18.6	0.05	0.04
	550	160	32.8	0.09	0.07
	600	192	35.3	0.11	0.08

in the feedstock biomass and the highest pyrolysis temperature. A slight increase in both characteristics was observed in the range of 5–10% of lignin content (it meant maize: 5%, meadow grass: 9.6% and wheat straw: 9.4%).

The sorption ability of biochar of different S_{BET} was already intensively investigated. Relatively high pyrolysis temperatures generally produce biochars that are effective in the sorption of organic contaminants by increasing surface area, microporosity and hydrophobicity, whereas the biochars obtained at low temperatures are more suitable for removing inorganic/polar organic contaminants by oxygen-containing functional groups, electrostatic attraction and precipitation (Ahmad et al. 2014). Biochars with high surface area may cause nutrient retention in soil

(Wang et al. 2015). The sorption is effective due to the hydrophobic effect, charge transfer, interaction and the pore-filling mechanism (Zhang et al. 2011). Uchimiya et al. (2011) highlighted the importance of the functional groups during heavy metal sorption and suggested further biochar treatment by acids and other oxidants to increase the amount of oxygen-containing surface functional groups.

Pore size of observed biochars

Pore structure of soils affects many physical, chemical and biological properties, such as gas diffusivity, water transmission and storage, mechanical resistance, carbon dynamics, microbial habitat and root penetration (Zaffar and Lu 2015). The pore size distribution is
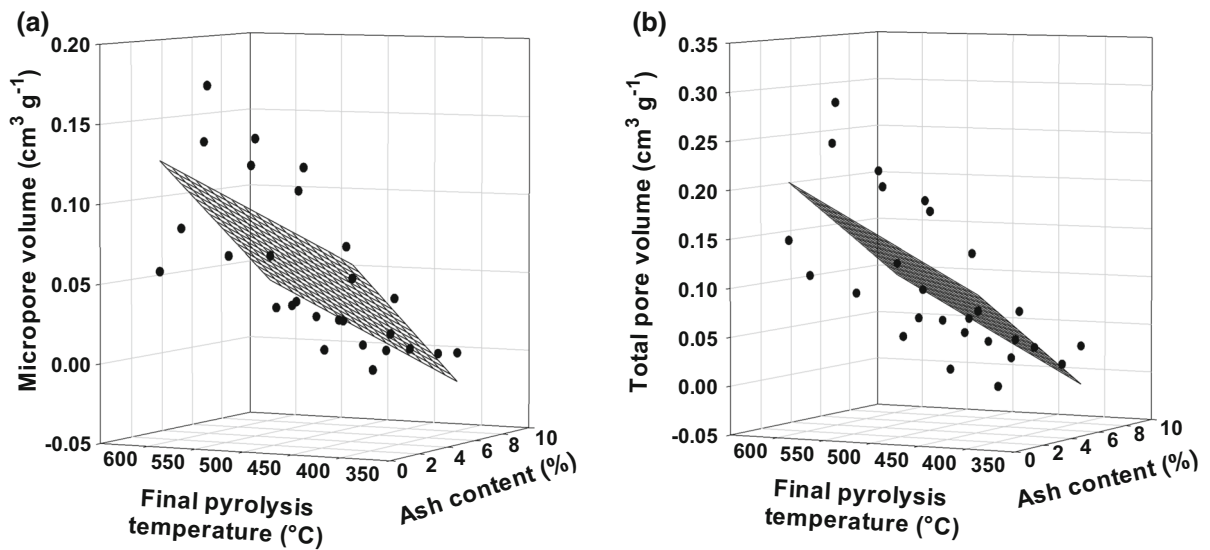
Fig. 2 Influence of ash content and pyrolysis temperature on **a** micropore volume ($p < 0.0001$; $R^2 = 0.9822$) and **b** total pore volume ($p < 0.0001$; $F = 14$; $R^2 = 0.9915$)
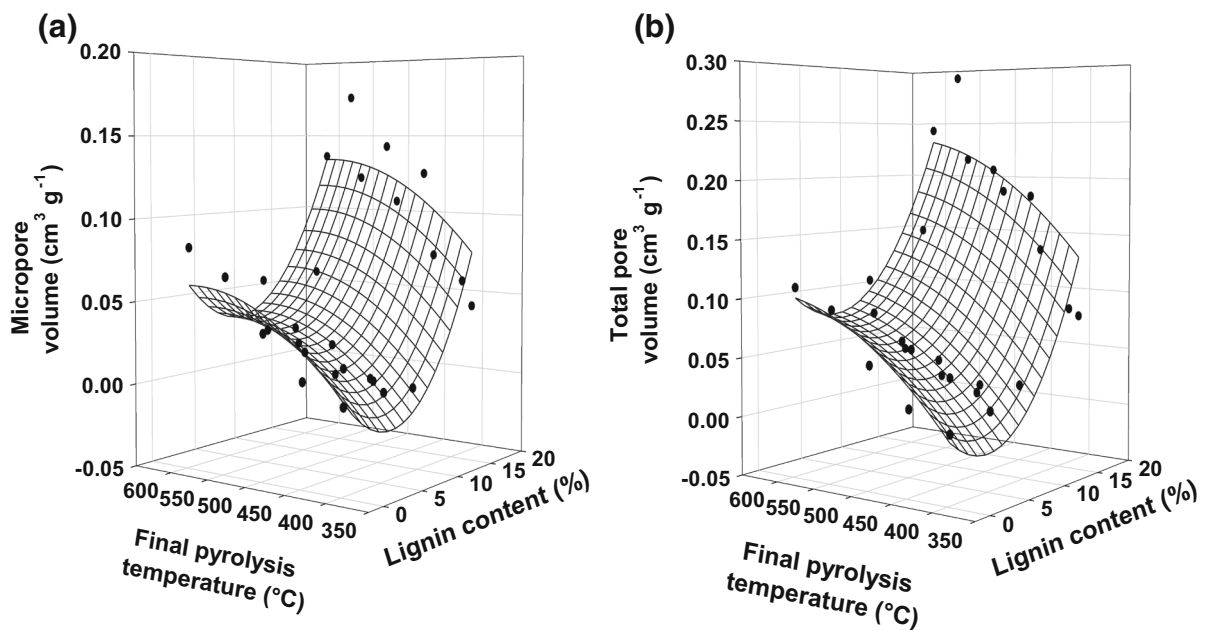

Fig. 3 Influence of lignin content and pyrolysis temperature on **a** micropore volume ($p < 0.0001$; $F = 16$; $R^2 = 0.7446$) and **b** total pore volume ($p < 0.0001$; $F = 14$; $R^2 = 0.8179$)

a key element in the characterisation of porous activated carbons (Jimenez-Cordero et al. 2013).

The shapes of the adsorption isotherms (Fig. 4) correspond to type I and type III according to the Thommes et al. (IUPAC, 2015) definition. The isotherm profiles indicate that there is a large portion of micropores in the biochar samples, the highest of which occurs in wood biochar (Fig. 4a) in comparison to herbaceous materials (Fig. 4b). Similarly, the predominance of micropores was shown also by Jimenez-Cordero et al. (2013) for the biochar based on the grape seeds as a feedstock. Cetin et al. (2005)

Fig. 4 N$_2$ adsorption and desorption isotherms of **a** bark wood biochar and **b** maize biochar at different temperatures: *filled diamond* 400 °C; *filled square* 450 °C; *filled triangle* 500 °C; *times* 550 °C; *asterisks* 600 °C
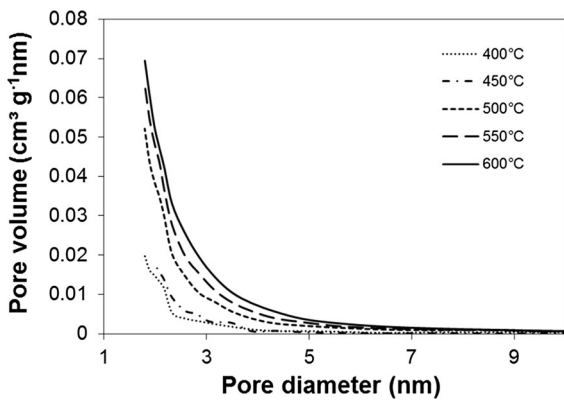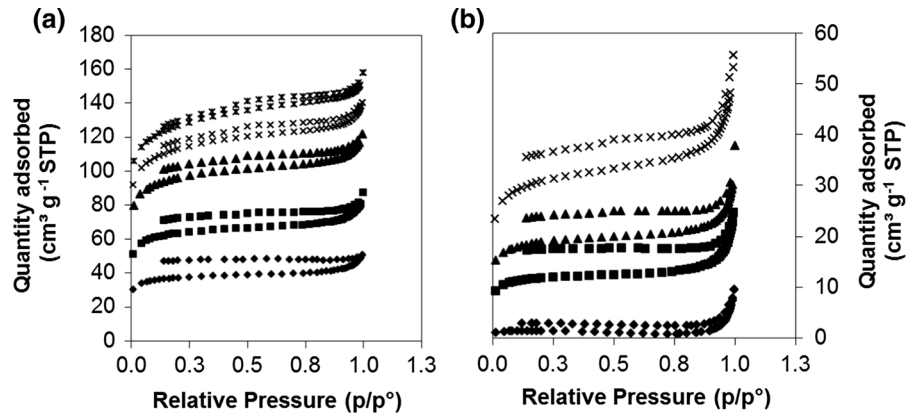
Fig. 5 Distribution of mesopores volume of bark wood biochar at different pyrolysis temperatures
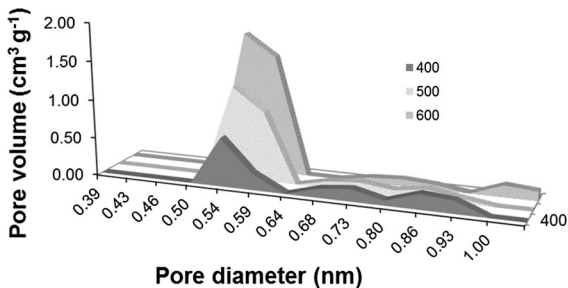
Fig. 6 Distribution of micropores volume of bark wood biochar at different pyrolysis temperatures determined by using the BJH method

Fig. 7 Distribution of macropores volume of bark wood biochar at different pyrolysis temperatures determined by the mercury porosimetry

Data processing by using the *t*-plot method showed that the specific surface area of the mesopores ($S_{t\text{-plot}}$) is significantly lower than the specific surface area determined by the BET method (Table 3). This confirms the presence of micropores in the samples, e.g. for bark wood at 500 °C S_{BET} 323.7 m^2 g^{-1}, $S_{t\text{-plot}}$ 91.7 m^2 g^{-1}.

The overall pore volume (micro and mesopores) was determined from the adsorption isotherms at a relative pressure of 0.995 p/p°. This value gradually increases with the pyrolysis temperature. The same trend has been found for the micropore volume as determined by the *t*-plot method (Table 3). A comparison of these results leads to the conclusion that the largest portions of pores are micropores, e.g. for bark wood pyrolysis at 600 °C, 58% of the adsorbed nitrogen is present in micropores. The microspores/mesopores ratio increases with the temperature of pyrolysis.

showed that at low pyrolysis temperature and atmospheric pressure, the final char (they described char from coal) is characterised by micropores, whereas at high temperature the char is characterised by macropores as a result of the melting. Similar pattern occurs in biochar from bark-less wood.

A comparison of the pore volume distribution curves determined according to the BJH method (Fig. 5) indicates that the final character of the biochar samples is very similar. The mesopore volume is very low, and the significant increase in the number of pores with diameter 3–5 nm shows the presence of micropores and the smallest mesopores.

The distributions of the micropores volume are shown in Fig. 6. In the biochar prepared from bark wood, the volume of micropores increased with the pyrolysis temperature, reaching a maximum at a temperature of 600 °C with a diameter of 0.54 nm. This result confirmed that biochar is a microporous material and pore melting can occur at pyrolysis temperature above 600 °C.

Mercury porosimetry results have confirmed the nitrogen adsorption results. There are hardly mesopores in the system; macropores can be found only in the pore radius interval of 0.2–7 µm (Fig. 7). The SEM analysis confirmed the previous determination and showed a significant amount of macropores in the biochar, particularly in the range of radius from 2 to 10 µm (supplementary material I.). Maize biochar contains fewer pores than bark wood biochar, and the volumes of mesopores and micropores are four times lower. On the other hand, the overall macropores volume is about 10% higher. Meadow grass biochar has similar characteristics to maize biochar, while bark wood biochar has comparable properties to bark-less wood.

Table 4 Density and porosity of prepared biochars

Feedstock material	Temperature (°C)	Skeletal density (g cm^{-3})	Envelope density (g cm^{-3})	Porosity (−)
Bark-free wood	400	1.479	0.799	0.460
	450	1.611	0.830	0.485
	500	1.582	0.775	0.510
	550	1.571	0.713	0.546
	600	2.015	0.875	0.566
Bark wood	400	1.545	0.752	0.514
	450	1.561	0.751	0.519
	500	1.607	0.716	0.555
	550	1.678	0.690	0.589
	600	1.673	0.705	0.578
Maize	400	1.674	0.686	0.591
	450	1.680	0.727	0.567
	500	1.695	0.755	0.554
	550	1.794	0.702	0.609
	600			
Meadow grass	450	1.638	0.902	0.450
	500	1.732	0.893	0.484
	550	1.705	0.875	0.487
Wheat straw	400	1.705	0.820	0.519
	450	1.619	0.820	0.493
	500	1.699	0.832	0.510
	550	1.721	0.859	0.501
	600	1.943	0.839	0.568
Wheat grain	400	1.506	0.858	0.430
	450	1.590	0.887	0.442
	500	1.578	0.879	0.443
	550	1.636	0.952	0.418
	600	1.620	0.982	0.394

Skeletal and envelope density and porosity of biochars

The amounts of biochar obtained, with the appropriate density and porosity of each type of biochar, are shown in Tables 2 and 4.

Density and porosity are fundamental physical characteristics that play a key role in determining the soil residence time of charcoal (Masiello et al. 2012). The skeletal densities of the biochar increased with the pyrolysis temperature and the envelope densities of the biochar slightly decreased or showed no effect of the pyrolysis temperature (Table 4).

Porosity means a pore volume per volume unit of porous particles (including pores). The porosity of the biochar is feedstock dependent, but it is not affected by the pyrolysis temperature, e.g. the bark wood porosity varies in the interval from 54 to 58% across all temperatures. Porosity was considered as the main property allowing water retention. Low-temperature biochars took up less water than high temperature biochars but the same amount of ethanol, suggesting that differences in water uptake based on production temperature reflect differences in surface hydrophobicity, not porosity; conversely, greater porosity of biochar provided more water uptake (Gray et al. 2014).

Similarly, Brewer et al. (2014) described the results of measurements of the skeletal and envelope density of biochar, where the skeletal density ranged from 1.34 to 1.96 g cm^{-3}; these results are comparable to our study. However, they determined lower envelope density, ranging from 0.25 to 0.60 g cm^{-3}, and found that this parameter was higher for wood biochars than grass biochars. The envelope density was comparable or slightly higher in the case of grass biomass, as confirmed also by our results. From these characteristics, they concluded the possible further behaviour of biochars in soils: (i) the high porosity of the biochars in this study explains why many biochars will initially float when exposed to water even though their skeletal densities are greater than that of water, or (ii) biochars with a low envelope density are more likely to float and be susceptible to preferential erosion during surface run-off.

Conclusion

For our study, different sources of types of biomass, different common agriculture residues were collected, gathered and then treated by pyrolysis at elevated temperatures (400–600 °C). The relations of biomass properties, and pyrolysis conditions and properties of biochars structures were observed. Strong relations were determined between lignin and ash contents of feedstock biomass and pore biochar size and volume. Microporosity of biochars was mainly affected by growing lignin contents and increasing pyrolysis temperatures. The total pore biochar volume was higher for wood biomass (0.08–0.3 cm^{-3} g^{-1}), than for the herbaceous one (0.01–0.11 cm^{-3} g^{-1}).

Wood biochars were characterised by higher skeletal density ranging from 1.479 to 2.015 cm^3 g^{-1}, and herbaceous biochars from 1.506 to 1.943 cm^3 g^{-1}, and the envelope density reached 0.982 cm^3 g^{-1} at wheat grain biochar and generally was higher with herbaceous biochars. The skeletal and enveloped densities were not pyrolysis temperature dependent. Our findings are important in providing the possibility for a choice of feedstock material and pyrolysis temperature to make biochar with specific properties, tailored to the exact required effect of biochar in soil.

References

Abdullah, S. S., Yusup, S., Ahmad, M. M., Ramli, A., & Ismail, L. (2010). Thermogravimetry study on pyrolysis of various lignocellulosic biomass for potential hydrogen production. *International Journal of Chemical and Biological Engineering, 3*(3), 137–141.

Ahmad, M., Lee, S. S., Dou, X., Mohan, D., Sung, J. K., Yang, J. E., et al. (2012). Effects of pyrolysis temperature on soybean stover and peanut shell-derived biochar properties and TCE adsorption in water. *Bioresource Technology, 118,* 536–544.

Ahmad, M., Rajapaksha, A. U., Lim, J. E., Zhang, M., Bolan, N., Mohan, D., et al. (2014). Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere, 99,* 19–33.

Aller, M. F. (2016). Biochar properties: Transport, fate, and impact. *Critical Reviews in Environmental Science and Technology, 46*(14–15), 1183–1296.

Bachmann, H. J., Bucheli, T. D., Dieguez-Alonso, A., Fabbri, D., Knicker, H., Schmidt, H. P., et al. (2016). Toward the standardization of biochar analysis: The COST action TD1107 interlaboratory comparison. *Journal of Agricultural and Food Chemistry, 64*(2), 513–527.

Barrett, E. P., Joyer, L. G., & Halenda, P. P. (1951). The determination of pore volume and area distributions in

porous substances. I. Computations from nitrogen isotherms. *Journal of the American Chemical Society, 73*(1), 373–380.

Bergman, P. C. A., & Kiel, J. H. A. (2005). *Torrefaction for biomass upgrading*. Paris: 14th European Biomass Conference & Exhibition.

Břendová, K., Tlustoš, P., & Száková, J. (2015). Can biochar from contaminated biomass be applied into soil for remediation purposes? *Water, Air, and Soil pollution, 226*(6), 1–12.

Brewer, C. E., Chuang, V. J., Masiello, C. A., Gonnermann, H., Gao, X., Dugan, B., et al. (2014). New approaches to measuring biochar density and porosity. *Biomass and Bioenergy, 66,* 176–185.

Brewer, C. E., Schmidt-Rohr, K., Satrio, J. A., & Brown, R. C. (2009). Characterization of biochar from fast pyrolysis and gasification systems. *Environmental Progress & Sustainable Energy, 28*(3), 386–396.

Brunauer, S., Emmett, P. H., & Teller, E. (1938). Adsorption of Gases in Multimolecular Layers. *Journal of the American Chemical Society, 60*(2), 309–319.

Cetin, E., Gupta, R., & Moghtaderi, B. (2005). Effect of pyrolysis pressure and heating rate on radiata pine char structure and apparent gasification reactivity. *Fuel, 84*(10), 1328–1334.

Chen, X., Chen, G., Chen, L., Chen, Y., Lehmann, J., McBride, M. B., et al. (2011). Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. *Bioresource Technology, 102,* 8877–8884.

Chowdhury, Z. Z., Karim, M. Z., Ashraf, M. A., & Khalid, K. (2016). Influence of carbonization temperature on physicochemical properties of biochar derived from slow pyrolysis of durian wood (*Durio zibethinus*) Sawdust. *BioResources, 11*(2), 3356–3372.

CSN EN 15402. (2011). Alternative fuels—Standards for volatile content determining. Stanovení obsahu prchavé hořlaviny. Czech Office For Standards, Metrology and Testing.

CSN EN 15403. (2011). Alternative fuels—Standards for ash determination. Czech Office For Standards, Metrology and Testing.

CSN EN 15414-3. (2011) Alternative fuels—Standards for water content determining by drying—Water content in analytical sample. Czech Office For Standards, Metrology and Testing.

Demirbas, A. (2004). Effects of temperature and particle size on bio-char yield from pyrolysis of agricultural residues. *Journal of Analytical and Applied Pyrolysis, 72*(2), 243–248.

Demirbaş, A. (1997). Calculation of higher heating values of biomass fuels. *Fuel, 76*(5), 431–434.

Diamond, S. (1970). Pore size distributions in clays. *Clays and Clay Minerals, 18*(1), 7–23.

Downie, A., Crosky, A., & Munroe, P. (2009). Physical properties of biochar. Biochar for environmental management: Science and technology. In J. Lehmann & J. Joseph (Eds.), *Biochar for environmental management: Science and technology* (pp. 13–32). London: Earthscan.

Faur-Brasquet, C., Kadirvelu, K., & Le Cloirec, P. (2002). Removal of metal ions from aqueous solution by adsorption onto activated carbon cloths: Adsorption competition with organic matter. *Carbon, 40*(13), 2387–2392.

Fu, P., Hu, S., Xiang, J., Sun, L., Li, P., Zhang, J., et al. (2009). Pyrolysis of maize stalk on the characterization of chars formed under different devolatilization conditions. *Energy & Fuels, 23*(9), 4605–4611.

Gaskin, J. W., Steiner, C., Harris, K., Das, K. C., & Bibens, B. (2008). Effect of low-temperature pyrolysis conditions for agriculture use. *Transactions of the ASABE, 51*(6), 2061–2069.

Gray, M., Johnson, M. G., Dragila, M. I., & Kleber, M. (2014). Water uptake in biochars: The roles of porosity and hydrophobicity. *Biomass and Bioenergy, 61,* 196–205.

Herron, N., & Corbin, D. R. (1995). *Inclusion chemistry with zeolites: Nanoscale materials by design*. Dordrech: Springer.

Horne, P. A., & Williams, P. T. (1996). Influence of temperature on the products from the flash pyrolysis of biomass. *Fuel, 75*(9), 1051–1059.

Jimenez-Cordero, D., Heras, F., Alonso-Morales, N., Gilarranz, M. A., & Rodriguez, J. J. (2013). Porous structure and morphology of granular chars from flash and conventional pyrolysis of grape seeds. *Biomass and Bioenergy, 54,* 123–132.

Jindo, K., Mizumoto, H., Sawada, Y., & Sonoki, T. (2014). Physical and chemical characterization of biochars derived from different agricultural residues. *Biogeosciences, 11*(23), 6613.

Keiluweit, M., Nico, P. S., Johnson, M. G., & Kleber, M. (2010). Dynamic molecular structure of plant biomass-derived black carbon (biochar). *Environmental Science and Technology, 44*(4), 1247–1253.

Klass, D. L. (1998). *Biomass for renewable energy, fuels, and chemicals*. California: Academia Press.

Lee, Y., Park, J., Ryu, Ch., Gang, K. S., Yang, W., Park, Y. K., et al. (2013). Comparison of biochar properties from biomass residues produced by slow pyrolysis at 500 °C. *Bioresource Technology, 148,* 196–201.

Lehmann, J., & Joseph, J. (2009). *Biochar for environmental management: Science and technology*. London: Earthscan.

Lu, G. Q., Low, J. C. F., Liu, C. Y., & Lua, A. C. (1995). Surface area development of sewage sludge during pyrolysis. *Fuel, 74*(3), 344–348.

Lua, A. C., Lau, F. Y., & Guo, J. (2006). Influence of pyrolysis conditions on pore development of oil-palm-shell activated carbons. *Journal of Analytical and Applied Pyrolysis, 76*(1), 96–102.

Lua, A. C., Yang, T., & Guo, J. (2004). Effects of pyrolysis conditions on the properties of activated carbons prepared from pistachio-nut shells. *Journal of Analytical and Applied Pyrolysis, 72*(2), 279–287.

Masiello, C. A., Liu, Z., Ziegelgruber, K. L., Dugan, B., Gonnermann, H., Chuang, V. J., et al. (2012). Density and porosity as controls on charcoal storage in soils. *EGU General Assembly Conference Abstracts, 14,* 830.

Novak, J. M., Busschei, W. J., Laird, L. D., Ahmedna, M., Watts, D. W., & Niandou, M. A. S. (2009). Impact of biochar amendment on fertility of a south eastern coastal plain soil. *Soil Science, 174*(2), 105–112.

Oleszczuk, P., Jośko, I., Kuśmierz, M., Futa, B., Wielgosz, E., Ligęza, S., et al. (2014). Microbiological, biochemical and

ecotoxicological evaluation of soils in the area of biochar production in relation to polycyclic aromatic hydrocarbon content. *Geoderma, 213,* 502–511.

Ronsee, F., Hecke, S. V., Dickinson, D., & Prins, W. (2013). Production and characterization of slow pyrolysis biochar: Influence of feedstock type and pyrolysis conditions. *Global Change Biology Bioenergy, 5*(2), 104–115.

Schnee, L. S., Knauth, S., Hapca, S., Otten, W., & Eickhorst, T. (2016). Analysis of physical pore space characteristics of two pyrolytic biochars and potential as microhabitat. *Plant and Soil, 408*(1–2), 357–368.

Sharma, R. K., Wooten, J. B., Baliga, V. L., Lin, X., Chan, W. G., & Hajaligol, M. R. (2004). Characterization of chars from pyrolysis of lignin. *Fuel, 83*(11), 1469–1482.

Sohi, S. P., Krull, E., Lopez-Capel, E., & Bol, R. (2010). A review of biochar and its use and function in soil. *Advances in Agronomy, 105,* 47–82.

Spokas, K. A. (2010). Review of the stability of biochar in soils: Predictability of O:C molar ratios. *Carbon Management, 1*(2), 289–303.

Stevenson, F. J. (1994). *Humus chemistry: Genesis, composition, reactions.* New York: Wiley.

Tang, J., Zhu, W., Kookana, R., & Katayama, A. (2013). Characteristics of biochar and its application in remediation of contaminated soil. *Journal of Bioscience and Bioengineering, 116*(6), 653–659.

Thommes, M., Kaneko, K., Neimark, A. V., Olivier, J. P., Reinoso, F. R., Rouquerol, J., et al. (2015). Physisorption of gases, with special reference to the evaluation of surface area and pore size distribution (IUPAC technical report). *Pure and Applied Chemistry.* doi:10.1515/pac-2014-1117.

Uchimiya, M., Wartelle, L. H., Klasson, K. T., Fortier, C. A., & Lima, I. M. (2011). Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. *Journal of Agricultural and Food Chemistry, 59*(6), 2501–2510.

Van Zwieten, L., Kimber, S., Morris, S., Chan, K. Y., Downie, A., Rust, J., et al. (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and Soil, 327*(1–2), 235–246.

Vassilev, S., Baxter, D., Andersen, L. K., & Vassileva, C. G. (2010). An overview of the chemical composition of biomass. *Fuel, 89*(5), 913–933.

Wang, X., Zhou, W., Liang, G., Song, D., & Zhang, X. (2015). Characteristics of maize biochar with different pyrolysis temperatures and its effects on organic carbon, nitrogen and enzymatic activities after addition to fluvo-aquic soil. *Science of the Total Environment, 538,* 137–144.

Webb, P. A., & Orr, C. (1997). *Analytical methods in fine particle Technology.* Norcross, GA: Micromeritics Instrument Corporation.

Zaffar, M., & Lu, S. G. (2015). Pore size distribution of clayey soils and its correlation with soil organic matter. *Pedosphere, 25*(2), 240–249.

Zhang, G., Zhang, Q., Sun, K., Liu, X., Zheng, W., & Zhao, Y. (2011). Sorption of simazine to corn straw biochars prepared at different pyrolytic temperatures. *Environmental Pollution, 159*(10), 2594–2601.

Zhao, L., Cao, X., Mašek, O., & Zimmerman, A. (2013). Heterogeneity of biochar properties as a function of feedstock sources and production temperatures. *Journal of Hazardous Materials, 256,* 1–9.

Can Biochar From Contaminated Biomass Be Applied Into Soil for Remediation Purposes?

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Abstract The carbon rich material obtained from pyrolysis process, i.e. biochar, has been widely discussed during the last decade due to its utilisation as a soil amendment. Furthermore, there is an unsolved question of biomass disposal from phytoremediation technologies. The idea of contaminated biomass pyrolysis has appeared, but there is lack of information about possible biochar utilisation obtained by this process. The aim of our study was to observe sorption properties of biochar prepared from contaminated biomass and release of contaminants from biochar back into the environment. The biomass of fast growing trees and maize was harvested on a site significantly damaged by risk element contamination (Cd, Pb and Zn). Plant biomass was pyrolysed and then the batch (de)sorption experiments were settled. The results confirmed no significant differences in metal sorption ability between biochars prepared from contaminated and uncontaminated biomass under the same conditions. The trend of maximum sorption capacity of observed matrices followed the order: wood biochar + soil (WB + soil) > wood uncontaminated biochar + soil (WUB + soil) > maize biochar + soil (MB + soil) > soil for cadmium, WB + soil > WUB + soil > soil for lead and MB + soil > WUB + soil > WB + soil > soil for zinc. Despite of increase of Zn desorption from wood biochars, maximum sorption capacity of the final WB + soil system was comparable to the WUB+soil sample. Our laboratory experiments showed high potential of biochar from contaminated plants as a soil amendment with sorption abilities and minimal risk of metal release.

1 Introduction

The soil contamination is presented as the most important problem of soil protection nowadays, while the so-called old loads are the most significant health threat (Němeček et al. 2010). For the soft remediation technologies, mainly for phytoextraction, the fast growing trees—willows and poplars—were introduced in pot experiments (Vysloužilová et al. 2003) and also in field conditions on medium contaminated soils (Pulford and Watson 2002).

The important task is management of the contaminated biomass obtained from the phytoremediation technologies. Šyc et al. (2012) evaluated the thermal process (fluidized bed incineration) as a potentially suitable disposal method of this biomass. Most of observed elements (Cu, Pb, Zn) were deposited into ashes, only cadmium left incineration system in flue gas either deposited on submicron particles or in volatilized form. In this study, it was concluded that the described basic character of ash and the insufficient heavy metal content excluded heavy metal recovery.

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Sas-Nowosielska et al. (2004) proposed pyrolysis as suitable way for disposal of contaminated biomass.

Stals et al. (2010) pyrolyzed willow wood of the phytoextraction origin using hot-gas filter and prepared bio-oil of good quality did not contain considerable amount of heavy metals. Fletcher et al. (2014) focused their study on factors affecting the quality of pyrolysis process producing biochar for agriculture application and concluded that future work has to evaluate the risk elements release during the pyrolysis process. However, the potential risk elements release from applied biochar back into soil was not yet systematically discussed.

The utilisation of charred biomass for agriculture purposes is definitely not a new idea. In 1929, John Morley noted the positive effect of charcoal application into soil and described the improvement of soil porosity in *The National Greenkeeper* (Morley 1929). Similarly, the burnt biomass and other organic matter application led to formation of very fertile soil in Amazonia during thousands of years (Sombroek 1966). In ancient times, farmers had used it to enhance the production of agricultural crops. Several decades ago, it was noticed that substances toxic to plants may be absorbed by charcoal (Tryon 1948), and in recent years, the sorption properties of biochar are widely discussed and described. The biochar sorption ability of organic pollutants was observed (Cao et al. 2009), and the sorption ability of heavy metals described (Uchimiya et al. 2011a, b; Trakal et al. 2012; Qui et al. 2008).

There is lack of knowledge concerning utilisation and properties of biochar derived from contaminated biomass. Several questions indicating the main objectives of our study arise as follows: (i) Gaskin et al. (2008) mentioned the nutrient-rich feedstock could be pyrolyzed into nutrient-rich biochar, though it is not clear if the nutrients are available to plant after biochar application into the soil; similarly, to this question of nutrients release from biochar, we will consider the mobility of risk elements retained into biochar and their potential release into the soil: (ii) can this specific biochar increase sorption ability of soil similarly as the biochar from uncontaminated biomass; and (iii) can we provide sustainable agent in contaminated soil? To answer these questions, a set of sorption/desorption experiments was provided and evaluated.

2 Materials and Methods

2.1 Soil Samples

For batch sorption and desorption experiments, the medium contaminated soil was used. This soil originated from Příbram locality (49°42′N, 13°59′E) (Czech Republic). The soil was taken from arable layer (0–20 cm). The pH (leachate of 0.01 M $CaCl_2$, 1:5 w/v) and cation exchange capacity (CEC) (ISO 11260, 1994) of soil were determined. The type of soil was Cambisol. Particle size distribution (%) was follows: sand 42.8 %, silt 41.8 %, clay 15.4 % and soil texture is loamy. Total organic carbon in soil was 1.7 %.

For determination of the total content of risk elements in soils, 0.5 g of soil sample was decomposed in a closed system with microwave heating in the device Ethos 1 (MLS GmbH, Germany) in a mixture of 8 ml HNO_3, 5 ml HCl and 2 ml HF. For determination of the plant-available risk elements, the soil samples were extracted by 0.11 M CH_3COOH in ratio 1:40 (w/v) (Ure et al. 1993). The element contents in the soil digests and extracts were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Varian Vista Pro, Varian, Australia).

The phosphate and sulphate anions were detected in 0.01 M KNO_3 extractant and measured by ion-exchange chromatography with suppressed conductivity. The ion chromatograph ICS 1600 (Dionex, USA) equipped with IonPac AS11-HC (Dionex, USA) guard and analytical columns was used. The eluent composition was 1–37.5 mM KOH with gradient 1–50 min, and flow rate was set to 1 mL min^{-1}. To suppress eluent conductivity, the ASRS 300 4-mm suppressor (Dionex, USA) and the Carbonate Removal Device 200 (Dionex, USA) were used. Samples were introduced by the autosampler AS-DV (Dionex, USA). Chromatograms were processed and evaluated using the software Chromeleon 6.80 (Dionex, USA).

2.2 Biochar

Biochar was derived by pyrolysis from biomass of willows and poplars representing wood biomass and maize representing herbaceous biomass. Willow and maize were harvested on medium contaminated site of old smelter area at Příbram locality (central Bohemia). As a comparative material, the bark-free wood of poplar with low content of risk elements, purchased from J.

Rettenmaier & Söhne GmbH was used. The process was conducted in a muffle furnace in the inert atmosphere of nitrogen (nitrogen flow 1 m^3 h^{-1}), at atmospheric pressure and retention time of 30 min. The process followed final temperature of 600 °C. The wood biochar (WB), maize biochar (MB) and wood uncontaminated biochar (WUB) were prepared at identical conditions.

Surface areas were measured by nitrogen adsorption isotherms at 77 K using ASAP 2050 (Micrometrics Instrument Corporation, USA) surface area analyser. Specific surface areas (SSA) were detected by layered adsorption isotherm Brunauer-Emmett-Teller (BET) model (Brunauer et al. 1938).

Content of C, H, N, O and S was determined by using the apparatus Flash EA 1112 in the CHNS/O configuration (Thermo Fisher Scientific, USA). In this method, the oxygen content is then determined by difference. These results were used to calculate the atomic H/C and O/C ratios. Ash content in the biomass sample was determined according to the CSN EN 15403 (2011) standard. The risk element content both in feedstock material and biochar was determined by neutron activation analysis according to Kubešová and Kučera (2010).

The infrared spectra of the biochars were scanned over the region of 4000–400 cm^{-1} in the transmission mode on a NICOLET 740 FT-IR spectrometer (DTGS detector, KBr beam splitter); 512 spectral accumulations, resolution 2 cm^{-1}, Happ-Genzel apodization. The spectra were processed mathematically by using OMNIC 3.1 software (Nicolet Instruments Co., USA). RAMS 32 software (Galactic Co., USA) was applied to the spectral band separation within the 1500–1800 cm^{-1} range. All absorbance values were converted to 50 μm foil thickness.

2.3 Batch Sorption Experiment

The metal adsorption experiments were performed using a batch equilibration technique. Stock solutions of different concentration of Cd(II), Zn(II), Pb(II) were prepared by dissolving nitrate salts $Cd(NO_3)_2$ $3H_2O$ (Sigma–Aldrich), $Zn(NO_3)_2$ $6H_2O$ and $Pb(NO_3)_2$ (Lachner) in background electrolyte 0.01 M KNO_3. Each stock solution was added separately to observe a single-metal sorption. The concentration series of Pb was 0.12; 0.27; 0.75; 1.6; 2.7; 15.8 and 30.4 mM; for Cd: 0.04; 0.06; 0.12; 0.25; 0.5; 1.8 and 3.7 mM; for Zn: 0.09; 0.1; 0.23; 0.44; 0.78; 3.61 and 6.28 mM. The experiment was conducted in 50 ml centrifuge tubes,

where the volume of 20 ml of single-metal solution (Trakal et al. 2012) was added to 1 g of soil with 20 % (w/w) of different type of biochar, thus the following mixtures were prepared: (i) 20 % maize biochar+80 % soil (MB + soil), (ii) 20 % wood biochar+80 % soil (WB + soil), (iii) 20 % uncontaminated wood biochar+ 80 % soil (WUB + soil) and finally (iv) 100 % soil. Each mixture was then equilibrated on a reciprocating shaker for 24 h (Uchymia et al. 2011b). Samples were centrifuged and the supernatant was measured by ICP-OES (Varian Vista Pro, Varian, Australia).

The metal uptake was calculated (Eq. (1)) as follows:

$$C_{sorb} = \left(C_0 - C_{eq}\right)\frac{V}{m} \qquad (1)$$

where C_{sorb} is the uptake (μmol g^{-1}), C_0 and C_{eq} are the initial and equilibrium liquid-phase concentrations of metal (μmol L^{-1}), respectively, V is the volume (L), and m is the amount of dried biosorbent (g).

The obtained data were then fitted using a Langmuir isotherm; the Langmuir isotherm equation is defined as follows (Eq. (2)):

$$C_{sorb} = \frac{S_{max}K_L C_{eq}}{1 + K_L C_{eq}} \qquad (2)$$

where K_L characterise the bonding energy associated with an equilibrium constant, and S_{max} represents the maximum sorption capacity determined by the number of reactive surface sorption sites in an ideal monolayer system (Trakal et al. 2011), C_{sorb} is the uptake (μmol g^{-1}), and C_{eq} is the initial and equilibrium liquid-phase concentrations of metal (μmol L^{-1}). The model of Bolster and Hornberger (2007) was used to evaluate non-linear isotherm parameters.

The estimation of metal species of investigated elements was modelled by using Visual MINTEQ ver. 3.1, Royal Institute of Technology (2014).

2.4 Batch Desorption Experiment

The experiment was conducted in 50 ml centrifuge tubes, where the volume of 20 ml 0.01 M KNO_3 (Trakal et al. 2012) was added as a background electrolyte to 0.5 g of individual biochar samples or soil and consequently of the mixtures of biochar and soil (MB + soil, WB + soil and WUB + soil). The reaction mixture was then agitated on a reciprocating shaker for 24 h (Uchymia et al. 2011b). Alternatively, mild acidic

solution (pH ~3) of 0.11 M CH_3COOH in ratio 1:40 (*w/v*) was applied. The reaction mixtures were agitated on a reciprocating shaker for 24 h, as well.

Samples were centrifuged at 4000 rpm and the supernatant was measured by ICP-OES (Varian Vista Pro, Varian, Australia). Desorbed concentrations of risk elements were calculated as desorbed concentrations in 1 kg of the entry matrix.

3 Results and Discussion

Table 1 shows the basic biochar and feedstock characteristics. The risk elements were retained and concentrated into biochar. Zinc content was four times higher in wood biochar, ten times in maize biochar and six times in uncontaminated wood biochar in comparison to the original feedstock biomass. The cadmium content is lower in prepared biochar than in feedstock biomass, confirming cadmium volatility at high temperatures of pyrolysis as mentioned by Stals et al. (2010), and Cd recovery can remain for further research.

The carbon content followed the order MB < WB < WUB. The absence of bark in uncontaminated wood resulted in the lowest ash content in WUB.

The *C:N* ratio is 69, 24 and 452 for biochar derived from wood, maize and uncontaminated wood, respectively. Lehmann (2007) stated that the wide *C:N* ratio is associated with biochar aromaticity, and will cause slow biochar decomposition. Thus, it seems that biochar prepared from uncontaminated wood should

have the longest stability in soil. The biochar is alkaline material. The cation exchange capacities (CEC) of biochars are multiply higher than of the soil and the biochar application led to the increase of the soil CEC level (Jiang et al. 2012). The highest value was observed for MB. Similarly, Yuan et al. (2011) determined the CEC values of different straw-based biochars as 10–20 times higher than that of soil CEC.

In the opposite, the lowest specific surface area (SSA) was determined for MB, whereas the WUB is characterised by the highest SSA level 556 m^2 g^{-1}. Chun et al. (2004) compared different pyrolyzing temperatures (300–700 °C) of wheat biomass residues where higher temperatures resulted in relatively high surface area (>300 m^2 g^{-1}) and low oxygen content (≤10 %). These observations were confirmed for both wood-derived biochars, but different pattern was reported for MB (Table 1) confirming the important role of both biomasses composition and pyrolysis conditions for final properties of biochar (Chen et al. 2011).

The soil pH is acidic (Table 2). The cation exchange capacity of soil is lower compared to the applied biochars. The maximum permissible limits of elements in soils of the Czech Republic is given by public notice (Anonymous 1994); according to this notice, the total element concentrations are set as 1.0, 140 and 200 mg kg^{-1} for Cd, Pb and Zn, respectively. Therefore, in our experiment, the risk element contents showed that the soil is medium contaminated by these three elements.

Table 1 Feedstock and biochar characteristics

Element content		Wood chips	WB	Maize	MB	Wood uncon.[b]	WUB
Pb	mg kg^{-1}	3.4	285	1.6	268	<0.025	17
Cd		17.8	5.1	1.5	3.5	<0.90	2
Zn		220	1590	162	1420	11.8	132
Si			9947		68,800		501
C	(% *w/w*)	50.4	68.3	48.2	29.1	47.3	76.8
O[a]		42.7	13.2	43.8	23.9	45.7	9.4
H		6.2	1.8	6.4	1.07	6.2	2.1
N		0.7	1	1.6	1.22	0.9	0.2
Ash		3.7	15.8	6.7	44.9	0.6	11.5
pH			11		10		8
CEC	mmol$_+$kg^{-1}		646		2062		719
SSA[c]	m^2 g^{-1}		414		137		556

[a]Oxygen content was calculated by difference

[b]Wood uncontaminated

[c]Specific surface area

Table 2 Soil characteristics, data are expressed as averages ± standard deviation

	Cd mg kg^{-1}	Pb mg kg^{-1}	Zn mg kg^{-1}	P g kg^{-1}	K g kg^{-1}	Ca g kg^{-1}	Mg g kg^{-1}	pH	CEC mmol$_+$kg^{-1}
Total[a]	5.1±0.4	805±38	294±1	94.4±3 mg kg^{-1}	11.8±1.3 mg kg^{-1}	31.7±0.1 mg kg^{-1}	32.6±1.1 mg kg^{-1}	5.7	175.5
Mobile[b]	1.4±0.0	15.4±0.7	46.8±3.4	48.3±2.5	165.1±7.8	1.8±0.0	124.8±0.9		

[a] Total element concentration

[b] 0.11 M CH$_3$COOH extractable element fraction

The FT-IR (Fourier transform infrared spectroscopy) analysis (Fig. 1) showed the similarity between biochar spectra derived from contaminated and uncontaminated wood. The MB can be characterised as a material with high content of SiO$_2$. For WB, the COO$^-$ was detected at stretching vibration of 1685 and 1558 cm^{-1}. The peak of 1410 and 710 cm^{-1} detected the CO$_3^{2-}$ and the next one (1036 cm^{-1}) determined silicates. The functional group of –COOH was found for WUB (1701 and 1242 cm^{-1}), and the carboxylates are peaked at 1568 and 1448 cm^{-1}. This biochar is characterised by the lowest abundance of SiO$_2$. The MB is characterised by carboxylate (1565 and 1482 cm^{-1}) and carbonate (1403 cm^{-1}) functional groups. Generally, it has the strongest signal response on SiO$_2$. Novak et al. (2009) stated that increase of the soil CEC, the surfaces of the biochar must be oxidised to produce negatively charged carboxyl groups; our prepared biochars have thus the real potential to increase soil CEC at the very beginning of the experiment.

Desorption experiment shows that the released zinc concentrations are significantly higher (Fig. 2), most probably because of its highest concentrations in comparison with other elements in prepared biochars (Table 1). Extracted Zn was significantly higher at WB and MB in comparison with WUB and soil, especially in acidic extract of diluted CH$_3$COOH. The WUB was characterised by high leachable amount of zinc (25 % of total zinc content was extracted by CH$_3$COOH). Considering the zinc is not only a toxic element, but predominantly also a micronutrient, the slow release would not cause recontamination; conversely the biochars would be used as a Zn slow release fertiliser. The lead was desorbed from soil significantly more in comparison with biochar. The acetic acid leachable Pb was 8 % from total content in soil. This behaviour can be explained by different pH of each matrix: soil was characterised as acidic, while biochars are characterised by alkaline reaction (Table 1). For cadmium, slight differences occurred when MB, WB and WUB were leached by acetic acid, thus significantly higher concentration of Cd was extracted from MB and WB. From all three observed biochars, lead and cadmium concentrations were just at the limit of detection in KNO$_3$ extraction.

Figure 3 shows desorption of Cd, Pb and Zn from mixtures of soil and 20 % of different types of biochars. The influence of extraction agent was clearly visible, acid mostly released higher amount of metals. The highest desorbed amount was observed at Zn, while more acidic environment caused higher desorption of this element, as well the highest

Fig. 1 The FT-IR analysis of biochar derived from wood chips, maize and uncontaminated wood
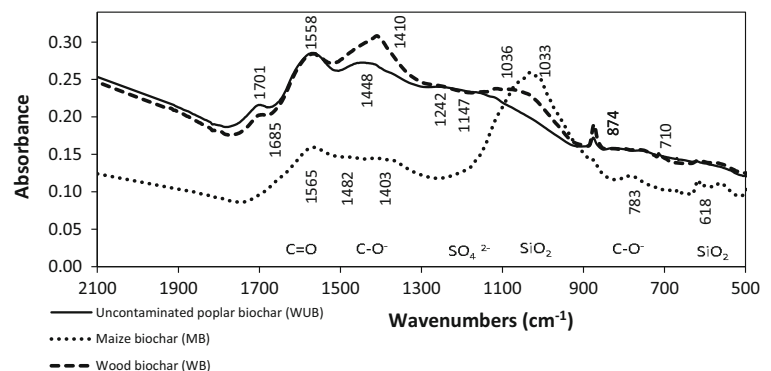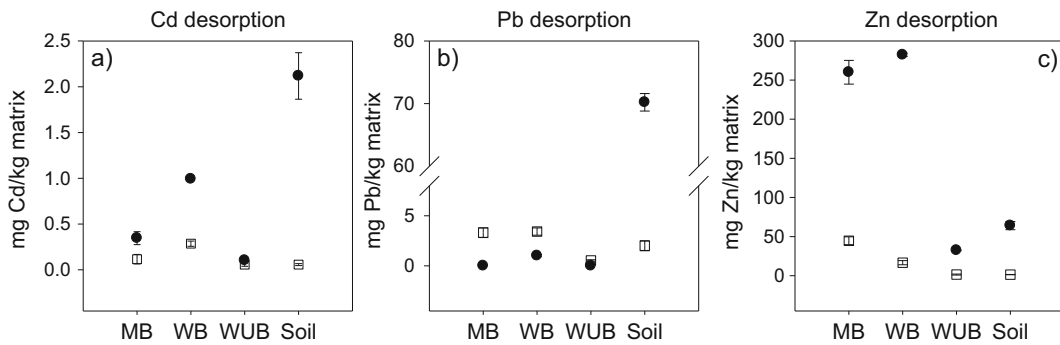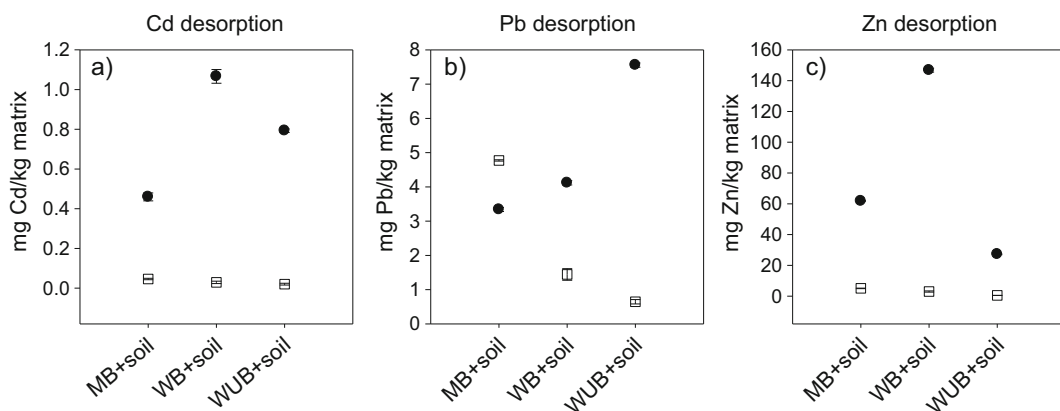
Fig. 2 Cadmium (**a**), lead (**b**) and zinc (**c**) desorption from different types of biochar and soil. *MB* maize biochar, *WB* wood biochar, *WUB* wood (uncontaminated) biochar; soil; (*black circle*) CH$_3$COOH extraction; (*white square*) KNO$_3$ extraction; data are means of 4 replicates; *error bars* represent standard error of the means (SE)

concentration of Zn in WB caused the highest desorption. Similarly, Cd desorption from this matrix showed comparable behaviour of Cd-rich WB. This hypothetical influence of risk element content in prepared biochar on their increased desorption from biochar + soil mixture does not correspond with behaviour of third biochar, WUB, characterised by lowest risk element content. Desorption of Pb by acidic extraction was highest at WUB + soil mixture in comparison to other mixtures and also desorption of Cd was high; whereas the Cd desorption from WUB (Fig. 2a) was very low. Considering the pH of biochars (Table 1), this trend can be explained by lower pH of WUB, hence WUB + soil mixture, in comparison with other ones, and the application of acidic extractant could decrease pH of whole system caused the higher desorption of Cd and Pb (Zn was

not desorbed in such extent, because its content in WUB as well as in soil is lowest).

Comparing the adsorbed and desorbed mutual concentrations of Cd, Zn and Pb (Fig. 4) of observed biochars and soil mixtures, it was found that with increasing adsorbed concentrations of Cd and Zn, the concentration of desorbed Pb decreased. Thus, the lead was bound stronger and was not replaced. While the Pb adsorbed concentrations increased, the desorbed concentrations of Zn and Cd slightly increased from WUB + soil and soil and extremely from WB + soil. From MB + soil, cadmium and zinc were not desorbed in such extent with increasing Pb concentrations comparing other matrices, and it can be explained by high amount of silicates in maize biochar, which could lead to high sorption of this matrices or higher value of pH

Fig. 3 Cadmium (**a**), lead (**b**) and zinc (**c**) desorption from mixtures of 20 % maize biochar+80 % soil; 20 % wood biochar+80 % soil; 20 % wood (uncontaminated) biochar+80 % soil. MB + soil=20 % maize biochar+80 % soil; WB + soil=20 % wood biochar+80 % soil; WUB + soil=20 % wood (uncontaminated) biochar+80 % soil; (*black circle*) CH$_3$COOH extraction; (*white square*) KNO$_3$ extraction; data are means of 4 replicates; *error bars* represent standard error of the means (SE)
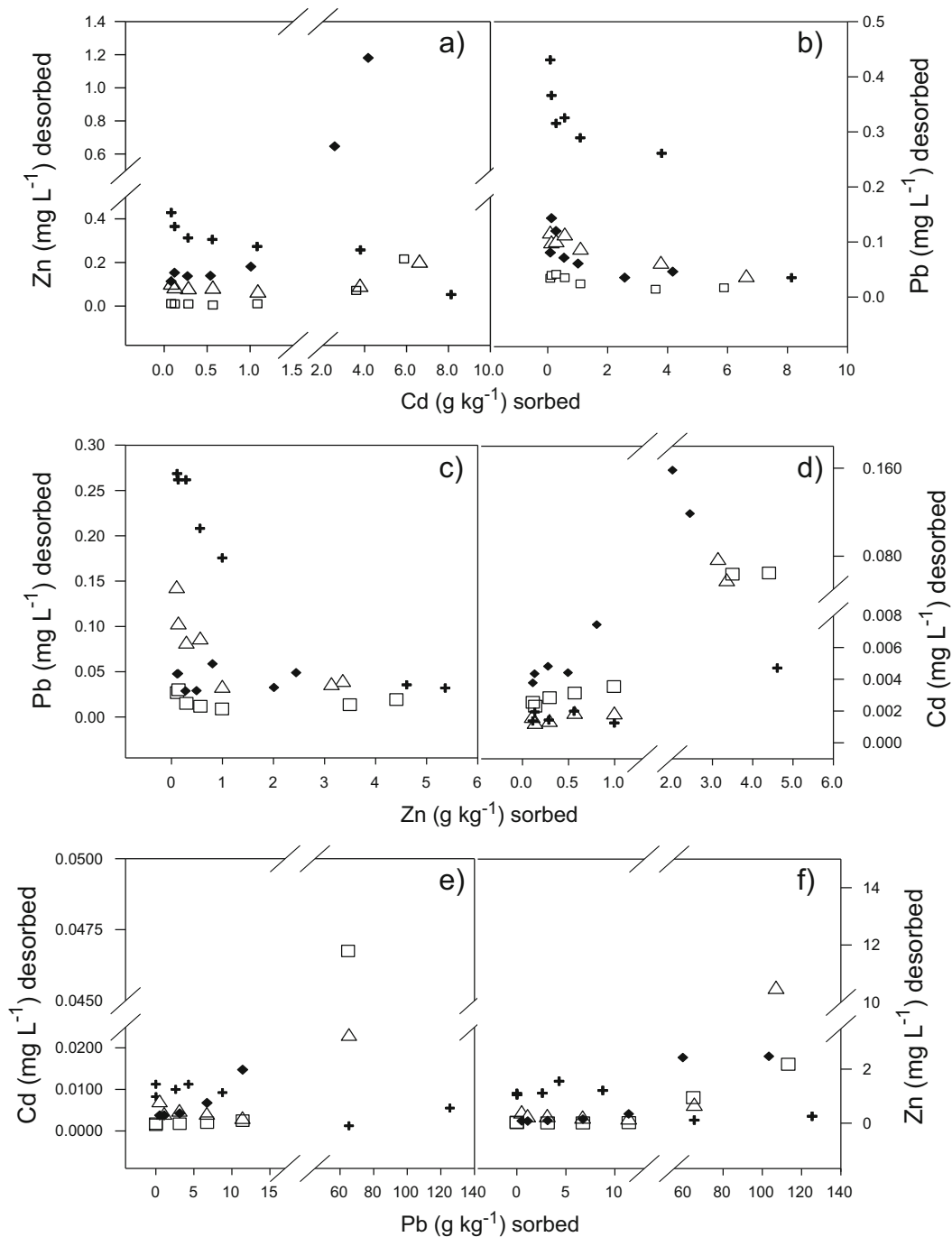
Fig. 4 Relationships between sorption and desorption of the investigated risk elements. + 20 % maize biochar+80 % soil (MB + soil); (*white up-pointing triangle*) 20 % wood biochar+ 80 % soil (WB + soil); (*white square*) 20 % wood (uncontaminated) biochar+80 % soil (WUB + soil); (*black diamond*) 100 % soil; data are means of 4 replicates; *error bars* represent standard error of the means (SE)
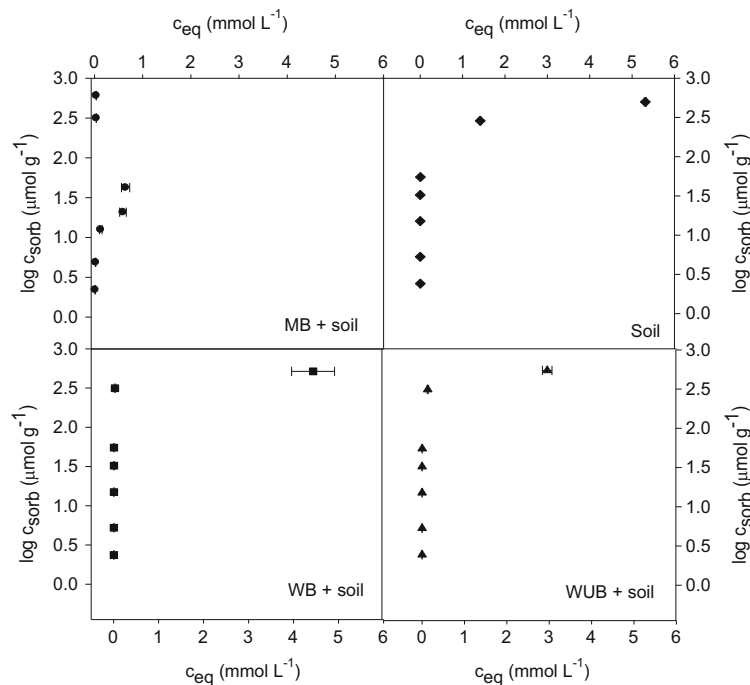
at equilibrium at this treatment in comparison to other treatments. With increasing adsorbed Cd and Zn concentrations, the Zn and Cd desorption is

significantly higher from soil in comparison to biochars indicating the potential of biochars to decrease bioavailability of the metals in soil.

Figures 5, 6 and 7 give the sorption isotherms of lead, zinc and cadmium on different matrices. In each figure, the matrix of different types of biochar and soil is compared with soil without biochar. It was observed that all the types of biochar increased the sorption ability of the soil for all three contaminants. The sorption ability of prepared biochars strongly depends on the type of adsorbed metal ion (Uchymia et al. 2011a); our results showed the following trend: $Cd^{2+} < Zn^{2+} < <Pb^{2+}$. Although the different biochar (based on dairy manure) prepared under different conditions (350 °C), the order of maximum sorption capacity was $Zn^{2+} < Cd^{2+} < <Pb^{2+}$, respectively (Xu et al. 2013; Cao et al. 2009), confirming strong affinity of Pb to the biochar matrix compared to other investigated metals (Fig. 5). High efficiency to adsorb Pb compared to Cd was observed also by Mohan et al. (2007). However, Figs. 5, 6 and 7 show the highest sorption ability of MB + soil; the data can be weakly fitted by Langmuir model. The sorption isotherms of WB + soil and WUB + soil are comparable. Xu et al. (2013) observed that more than 75 % of the metals retention was attributed to precipitation, especially as metal carbonates. Thus, the differences in the abundance of the carbonate functional groups (Fig. 1) could be also related to the differences among the different biochars.

Biochar application into soil increases the pH of the matrix (Lehmann 2007). The increase in pH can increase the negative surface charge, and consequently, the affinity of soil and biochar surface for cations is expected to increase (Jiang et al. 2012). The ameliorative effect of biochar application resulting in increased soil pH and/or retention of nutrients was proven by Yuan et al. (2011) in acidic soil. We observed the high affinity of metals onto the matrices at lower concentrations (Figs. 5, 6 and 7) at high pH, and consequently, we observed similarly to Mustafa et al. (2002) that the sorption of metal cations was accompanied by the release of H^+ ions into background electrolyte that caused the decrease of pH (Fig. 8) with increasing cation adsorption. The ability of MB to increase pH of the system to nine at Cd or Zn sorption led to precipitation of these elements (up to 80 % of Cd^{2+} and Zn^{2+}) according to modelled by data by Visual MINTEQ, where the amount of anions from soil are neglected. Taking into account, the content of anions in soil (SO_4^{2-} 3.2 mmol L^{-1} and PO_4^{3-} 1.6 mmol L^{-1}; extracted with KNO_3), it was found that up to 98 % of Cd was probably precipitated as a complex of phosphateat the whole concentration range of added Cd^{2+}. Zn could be also precipitated as phosphate on WB + soil and WUB + soil matrixes at lowest concentrations of these elements (high pH; Fig. 8c). Thus, it can be concluded that the
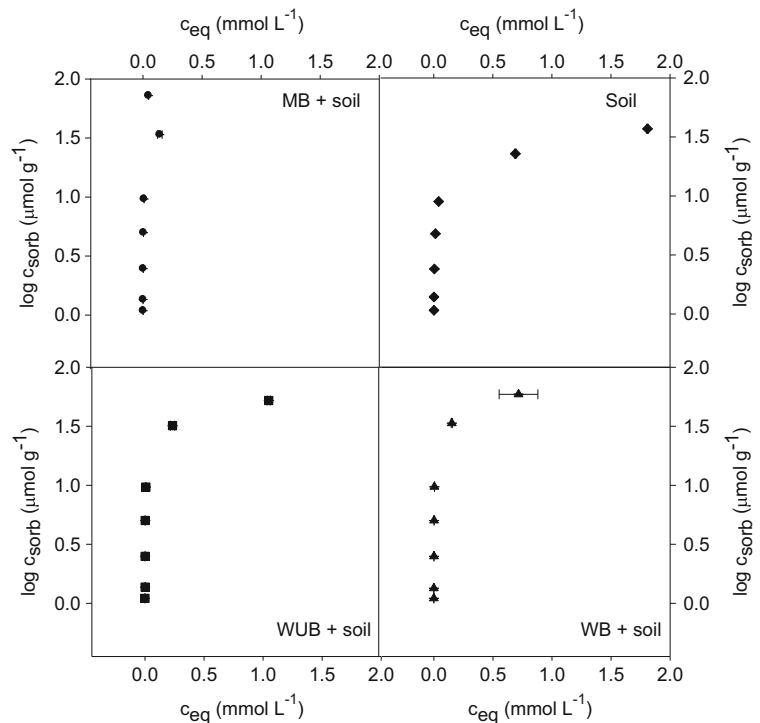

Fig. 5 Sorption isotherms of lead on different types of matrix. (*Black circle*) 20 % maize biochar+80 % soil (MB + soil); (*black square*) 20 % wood biochar+80 % soil (WB + soil); (*black up-pointing triangle*) 20 % wood (uncontaminated) biochar+ 80 % soil (WUB + soil); (*black diamond*) 100 % soil; data are means of 4 replicates; *error bars* represent standard error of the means (SE)
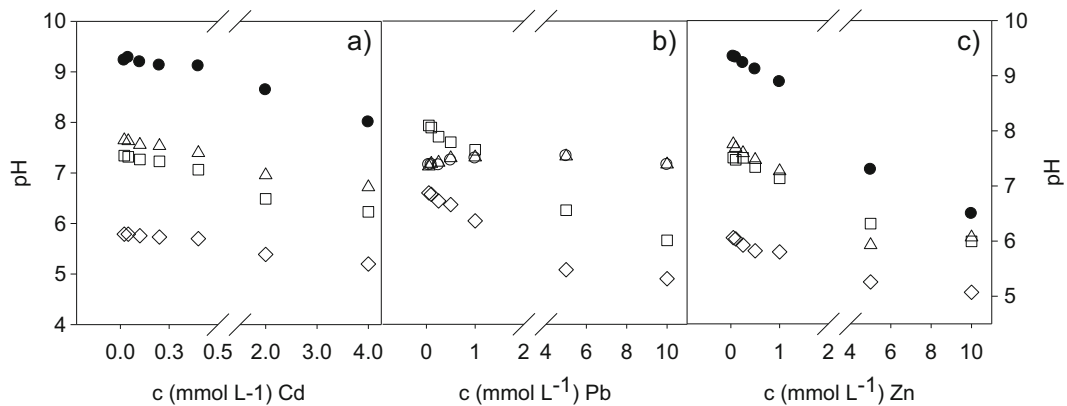
Fig. 6 Sorption isotherms of zinc on different types of matrix. (*Black circle*) 20 % maize biochar+80 % soil (MB + soil); (*black square*) 20 % wood biochar+80 % soil (WB + soil); (*black up-pointing triangle*) 20 % wood (uncontaminated) biochar+ 80 % soil (WUB + soil); (*black diamond*) 100 % soil; data are means of 4 replicates; *error bars* represent standard error of the means (SE)
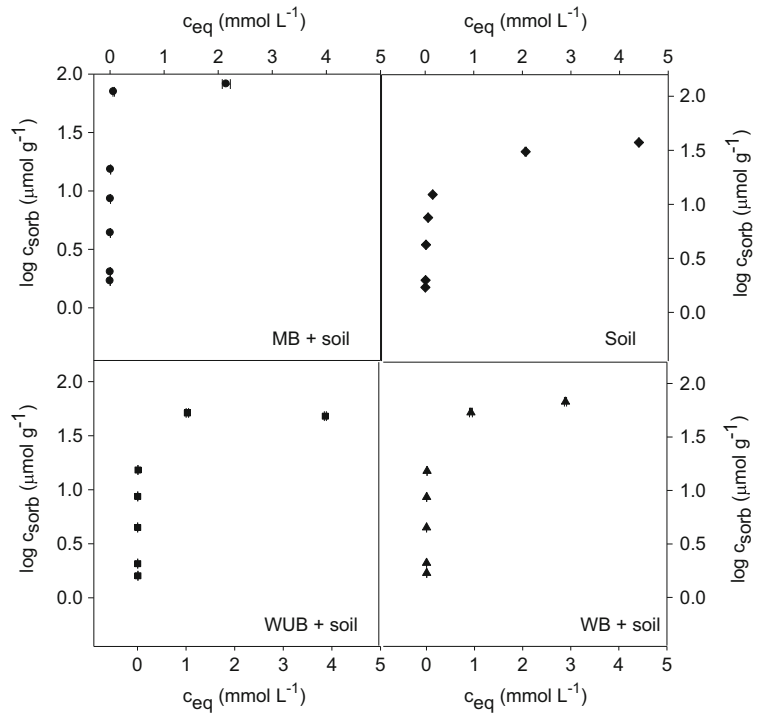
biochar indirectly but highly influence the risk element sorption onto soil. This occurred when the DOC of soil was 212 mg kg^{-1} of dry matter.

The opposite pattern was observed for Pb adsorption on MB + soil and WB + soil, where the pH slightly increased while the higher concentrations of this

Fig. 7 Sorption isotherms of cadmium on different types of matrix. (*Black circle*) 20 % maize biochar+80 % soil (MB + soil); (*black square*) 20 % wood biochar+80 % soil (WB + soil); (*black up-pointing triangle*) 20 % wood (uncontaminated) biochar+ 80 % soil (WUB + soil); (*black diamond*) 100 % soil; data are means of 4 replicates; *error bars* represent standard error of the means (SE)

Fig. 8 pH at equilibrium at observed sorption isotherms. (*Black circle*) 20 % maize biochar+80 % soil (MB + soil); (*white square*) 20 % wood biochar+80 % soil (WB+ soil); (*white up-pointing triangle*) 20 % wood (uncontaminated) biochar+80 % soil (WUB + soil); (*white diamond*) 100 % soil; data are means of 4 replicates

element were adsorbed. This can be caused by presence of SiO_2 in both matrices on which Pb^{2+} can be highly adsorbed as Hao et al. (2012) described the SiO_2/ graphene composite with high adsorption efficiency and fast adsorption equilibrium as a practical adsorbent for Pb^{2+} ion. Lu et al. (2012) observed a new precipitate on Pb-loaded sludge-derived biochar as lead phosphate silicate confirming the important role of silicates in lead sorption process. The Pb^{2+} on WUB + soil is most probably precipitated as $Pb(SO_4)$, and thus the pH decreased while H^+ are released with higher concentrations of cations are precipitated.

The data gained from batch sorption experiment were fitted by Langmuir isotherms (Table 3). According to Nash-Sutcliff's coefficient of model efficiency (E) (Nash and Sutcliffe 1970), Langmuir isotherms are more suitable for description of our observed matrix and their sorption ability. Similar statements were published by Mohan et al. (2007) for biochars based on wood/bark pyrolysis at 450 °C. Thus, the surface of the matrices is homogenised, the adsorption of metal ions is mono layer and the metal ions adsorbed on the surface cannot affect each other (Langmuir 1916). The trend of maximum sorption capacity of observed matrices were WB + soil > WUB + soil > soil for lead, WB + soil > WUB + soil > MB + soil > soil for cadmium and MB + soil > WUB + soil > WB + soil > soil for zinc.

Table 3 Fitted data by Langmuir isotherms

Element	Treatment	E[b]	Langmuir parameters	
			K	S_{max} (mmol$_+$kg^{-1})
Pb	Soil	0.9	0.004	502
	MB + soil	n.a.[a]	n.a.[a]	n.a.[a]
	WB + soil	0.7	0.003	553
	WUB + soil	0.94	0.03	552
Cd	Soil	0.93	0.01	37.3
	MB + soil	0.52	0.03	44.9
	WB + soil	0.92	0.03	60.5
	WUB + soil	0.93	0.02	53.7
Zn	Soil	0.97	0.005	37.9
	MB + soil	0.76	0.01	87.0
	WB + soil	0.94	0.01	49.2
	WUB + soil	0.96	0.01	68.1

MB + soil=20 % maize biochar+80 % soil; WB + soil=20 % wood biochar+80 % soil; WUB + soil=20 % wood (uncontaminated) biochar+80 % soil; soil=100 % soil

[a] Results not available

[b] Model efficiency

4 Conclusion

The results confirmed no significant differences in metal sorption ability between biochars prepared from contaminated and uncontaminated biomass for Pb and Cd and in the case of MB + soil also for Zn. However, the extremely high Zn content together with lower CEC of the wood-derived biochar resulted in increase of Zn desorption and lower maximum sorption capacity of the final WB + soil system compared to the WUB + soil sample. Therefore, the results suggest good and promising potential of the biochar originated from risk element contaminated biomass to suppress the bioavailable contents of these elements in the contaminated

soils. The potential applicability of these materials needs detailed specification of the sorption parameters of the different biochars as well as to determine the maximum risk element contents in the contaminated biomass regarding (i) potential desorption of elements and (ii) sufficient effectivity of the element sorption.

References

Anonymous, (1994). Public Notice No. 13/1994, regulating some details concerning the preservation of agricultural lands available. Czech Ministry of the Environment, Prague.

Bolster, C. H., & Hornberger, G. M. (2007). On the use of linearized Langmuir equations. *Soil Science Society of America Journal, 71*(6), 1796–1806.

Brunauer, S., Emmett, P. H., & Teller, E. (1938). Adsorption of gases in multimolecular layers. *Journal of the American Chemical Society, 60*(2), 309–319.

Cao, X., Ma, L., Gao, B., & Harris, W. (2009). Dairy-manure derived biochar effectively sorbs lead and atrazine. *Environmental Science & Technology, 43*(9), 3285–3291.

Chen, X., Chen, G., Chen, L., Chen, Y., Lehmann, J., McBride, M. B., & Hay, A. G. (2011). Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. *Bioresource Technology, 102*(19), 8877–8884.

Chun, Y., Sheng, G., Chiou, C. T., & Xing, B. (2004). Compositions and sorptive properties of crop residue-derived chars. *Environmental Science & Technology, 38*(17), 4649–4655.

CSN EN 15403 (2011). Solid alternative fuels—determination of ash. Prague, Czech Office for Standards, Metrology and Testing. (In Czech).

Fletcher, A. J., Smith, M. A., Heinemeyer, A., Lord, R., Ennism, C. J., Hodgsonm, E. M., & Farrar, K. (2014). Production factors controlling the physical characteristics of biochar derived from phytoremediation willow for agricultural applications. *Bioenergy Resources, 7*(1), 371–380.

Gaskin, J. W., Steiner, C., Harris, K., Das, K. C., & Bibens, B. (2008). Effect of low-temperature pyrolysis conditions on biochar for agricultural use. *Transaction of American Society of Agricultural and Biological Engineers, 51*(6), 2061–2069.

Hao, L., Song, H., Zhang, L., Wan, X., Tang, Y., & Lv, Y. (2012). SiO2/graphene composite for selective adsorption of Pb(II) ion. *Journal of Colloid and Interface Science, 369*(1), 381–387.

ISO 11260 (1994). Soil quality—determination of effective cation exchange capacity and base saturation level using barium chloride solution. American National Standards Institute.

Jiang, J., Xu, R. K., Jiang, T. Y., & Li, Z. (2012). Immobilization of Cu(II), Pb(II) and Cd(II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. *Journal of Hazardous Materials, 229*, 145–150.

Kubešová, M., & Kučera, J. (2010). Validation of k0 standardization method in neutron activation analysis—the use of Kayzero for Windows programme at the nuclear physics institute, Řež. *Nuclear Instruments and Methods in Physics Research A, 622*, 403–406.

Langmuir, I. (1916). The constitution and fundamental properties of solids and liquids. Part I. Solids. *Journal of the American Chemical Society, 38*(11), 2221–2295.

Lehmann, J. (2007). Bio-energy in the black. *Frontiers in Ecology and the Environment, 5*(7), 381–387.

Lu, H., Zhang, W., Yang, Y., Huang, X., Wang, S., & Qiu, R. (2012). Relative distribution of Pb^{2+} sorption mechanisms by sludge-derived biochar. *Water Research, 46*(3), 854–862.

Mohan, D., Pittman, C. U., Jr., Bricka, M., Smith, F., Yancey, B., Mohammad, J., Steele, P. H., Alexandre-Franco, M. F., Gómez-Serrano, V., & Gong, H. (2007). Sorption of arsenic, cadmium, and lead by chars produced from fast pyrolysis of wood and bark during bio-oil production. *Journal of Colloid and Interface Science, 310*(1), 57–73.

Morley, J. (1929). Compost and charcoal. *The National Greenkeeper, 3*(9), 8–26.

Mustafa, S., Dilara, B., Naeem, A., Rahana, N., & Shahida, P. (2002). Sorption of metal ions on a mixed oxide [0.5 M SiO2:0.5 M Fe(OH)3]. *Adsorption Science & Technology, 20*(3), 215–230.

Nash, J. E., & Sutcliffe, J. V. (1970). River flow forecasting through conceptual models part I—a discussion of principles. *Journal of Hydrology, 10*(3), 282–290.

Němeček, J., Vácha, R., Podlešáková, E. (2010). Assessment of soil contamination in the Czech Republic. Research Institute for Soil and Water Conservation, Prague. (In Czech).

Novak, J. M., Busscher, W. J., David, L., Laird, D. L., Ahmedna, M., Watts, D. W., & Niandou, M. A. S. (2009). Biochar amendment on fertility of a southeaster coastal plain soil. *Soil Science, 174*(2), 105–112.

Pulford, I. D., & Watson, C. (2002). Phytoremediation of heavy metal-contaminated land by trees—a review. *Environment International, 29*(4), 529–540.

Qui, Y., Cheng, H., Xua, C., & Sheng, G. D. (2008). Surface characteristics of crop-residue-derived black carbon and lead (II) adsorption. *Water Research, 42*(3), 567–574.

Sas-Nowosielska, A., Kucharski, R., Malkowski, E., Pogrzeba, M., Kuperberg, J. M., & Krynski, K. (2004). Phytoextraction crop disposal—an unsolved problem. *Environmental Pollution, 128*(3), 373–379.

Sombroek, W. G. (1966). Amazon soils (Doctoral dissertation, Centre for agricultural publications and documentation).

Stals, M., Carleer, R., Reggers, G., Schreurs, S., & Yperman, J. (2010). Flash pyrolysis of heavy metal contaminated hardwoods from phytoremediation: characterisation of biomass, pyrolysis oil and char/ash fraction. *Journal of Analytical and Applied Pyrolysis, 89*(1), 22–29.

Šyc, M., Pohořelý, M., Kameníková, P., Habart, J., Svoboda, K., & Punčochář, M. (2012). Willow trees from heavy metals phytoextraction as energy crops. *Biomass and Bioenergy, 37*, 106–113.

Trakal, L., Komárek, M., Száková, J., Zemanová, V., & Tlustoš, P. (2011). Biochar application to metal-contaminated soil: evaluating of Cd, Cu, Pb and Zn sorption behavior using single-

and multi-element sorption experiment. *Plant, Soil and Environment, 57*(8), 372–380.

Trakal, L., Komárek, M., Száková, J., Tlustoš, P., Tejnecký, V., & Drábek, O. (2012). Sorption behavior of Cd, Cu, Pb, and Zn and their interactions in phytoremediated soil. *International Journal of Phytoremediation, 14*(8), 372–380.

Tryon, E. H. (1948). Effect of charcoal on certain physical, chemical and biological properties of forest soils. *Ecological Monographs, 18*, 81–115.

Uchimiya, M., Wartelle, L. H., Klasson, K. T., Fortier, C. A., & Lima, I. M. (2011). Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. *Journal of Agriculture and Food Chemistry, 59*(6), 2501–2510.

Uchimyia, M., Klasson, K. T., Wartelle, L. H., & Lima, I. M. (2011). Influence of soil properties on heavy metal sequestration by biochar amendment: 1. Copper sorption isotherms and the release of cation. *Chemospere, 82*(10), 1431–1437.

Ure, A. M., Quevauviller, P., Muntau, H., & Griepink, B. (1993). Speciation of heavy metal in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the commission of the European Communities. *International Journal of Environmental Analytical Chemistry, 51*(1–4), 135–151.

Visual MINTEQ ver. 3.1 (2014). Royal Institute of Technology, Div. of Land and Water Resources Engineering, Stockholm, Sweden. Available at: http://www2.lwr.kth.se/English/OurSoftware/vminteq/download.html.

Vysloužilová, M., Tlustoš, P., Száková, J., & Pavlíková, D. (2003). As, Cd, Pb and Zn uptake by *Salix* spp. clones grown in soils enriched by high loads of these elements. *Plant, Soil and Environment, 9*(5), 191–196.

Xu, X., Cao, X., Zhao, L., Wang, H., Yu, H., & Gao, B. (2013). Removal of Cu, Zn, and Cd from aqueous solutions by the dairy manure-derived biochar. *Environmental Science and Pollution Research, 20*(1), 358–368.

Yuan, J. H., Xu, R. K., Qian, W., & Wang, R. H. (2011). Comparison of the ameliorating effects on an acidic Ultisol between four crop straws and their biochars. *Journal of Soils and Sediments, 11*(5), 741–750.

Biochar immobilizes cadmium and zinc and improves phytoextraction potential of willow plants on extremely contaminated soil

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ABSTRACT

The availability of risk elements in soil can be possibly reduced by various soil additives. Among them, the attention has been recently focused on the research of unconventional soil additive – biochar. The aim of this study was (i) to observe the effect of biochar application on risk elements transport through the soil profile and (ii) to assess the availability of risk elements in biochar amended soil to willow growth. The experiment was established at greenhouse conditions and extremely contaminated soil, reaching 43 mg/kg cadmium (Cd) and 4340 mg/kg zinc (Zn), was used. To observe risk element content in leachate, the lysimeter cylinders were tested. The rates of biochar were 0 (control); 5, 10, and 15% per mass of soil. The results showed that biochar significantly increased biomass production whereas the plant Cd and Zn contents remained unchanged in most cases. In leachate, Cd and Zn content decreased by 99% at all the biochar treatments. We can summarize that biochar appears to be a very effective regulator of availability of observed risk elements and improver agent for biomass production of plants and remediation efficiency.

Keywords: heavy metals; soil contamination; *Salix × smithiana*; phytoremediation; stabilization

Under the European Union (EU) Thematic Strategy for Soil Protection, the European Commission identified soil contamination; occurrence of 342 000 polluted sites was reported, most commonly polluted with heavy metals and mineral oil (Panagos et al. 2013). The soil contamination is presented as the most important problem of soil protection nowadays, while the so-called old load is the most significant health threat (Němeček et al. 2010). Risk elements soil contamination in the region of Příbram in the central part of the Czech Republic was described and pollution by cadmium (Cd), zinc (Zn) was determined as extreme at specific parts of this location (Vondráčková et al. 2013).

For metal-polluted soil, phytoremediation appears to be an economically and aesthetically attractive *in situ* technology (Pulford and Watson 2003). Willow potential for phytoextraction technologies was observed on heavily and moderate polluted calcareous, sandy soils or Cambisol (Meers et al. 2007, Tlustoš et al. 2007, Jensen et al. 2009).

The phytoextraction was introduced in Příbram Fluvisol in pot experiments and the willow biomass reduction due to extremely high content of zinc was observed (Vysloužilová et al. 2003). However, for reasonable efficiency of phytoextraction the biomass production in field conditions will mainly determine metal removal (Meers et al. 2007). Thus, in specific cases it should be considered to combine phytoextraction and stabilization technologies to improve plant growth and support the phytoextraction potential.

Among a wide scale of available soil stabilization materials, inorganic and organic substances based on coal-like materials or combustion by-products have been investigated, e.g. coal or bio-fuel fly

ashes (Clark et al. 2001), wood fly ash (Ochecová et al. 2014) or lignite (Uzinger and Anton 2008).

In recent years the investigation was focused on biochar, stable carbon-rich charred biomass and its utilization as a soil additive (Qayyum et al. 2014). The biochar sorption ability of organic pollutants (Zhang et al. 2011) and heavy metals (Beesley et al. 2010) was observed. The specificity of heavy metal has a high impact on biochar sorption ability of these contaminants. While biochar application increases soil pH, the mobility of arsenic (As) increased and due to increased dissolved carbon also copper (Cu) mobility increased; opposite pattern was observed for Cd and Zn (Beesley et al. 2010, Jiang et al. 2012).

Element transport through the soil profile can be observed with lysimeter (Jordan 1968). The utilization of cylinder pots placed in laboratory, greenhouse or into field conditions were described as a suitable way for this type of investigation (Trakal et al. 2011).

As a general conclusion from the review paper, the potential of combination of biochar amendment and phytoremediation technologies have been suggested (Paz-Ferreiro et al. 2014). However, relevant study supporting this statement with experimental data is still missing.

The aim of our study was (i) to evaluate the potential effect of elevated rates of biochar application on risk elements transport through the soil profile, and (ii) to assess the effect of biochar amendment on plant growth as well contaminant accumulation in willow tissues was evaluated.

MATERIAL AND METHODS

Biochar and soil characterization. Biochar was purchased from Erspol., Ltd. (Czech Republic). Biochar derived from coconut shells was characterized by ash content: 12%, pH_{CaCl_2}: 8.9, cation exchange capacity (CEC): 73 $mmol_+/kg$, specific surface area (SSA_{BET}): 486 m^2/g (activated by water steam), particle fraction: $4 \times 2 \times 2$ mm. Soil was sampled from top layer (0–30 cm) of grassland in Trhové Dušníky (Czech Republic) 49°71'8.8742N, 14°0'12.8814E. The type of soil was Fluvisol, pH_{CaCl_2}: 6.6, CEC: 157 $mmol_+/kg$. Soil was air-dried and homogenized.

For determination of the total content of risk elements in soils 0.5 g of soil sample was decomposed in a closed system with microwave heating in the device Ethos 1 (MLS GmbH, Leutkirch, Germany) in a mixture of 8 mL HNO_3, 5 mL HCl and 2 mL HF. Plant-available risk elements in soil were determined according to Ure et al. (1993). The element contents in the soil digests and extracts were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., Santa Clara, USA).

The risk element content in biochar was determined by the neutron activation analysis (INAA) (Kubešová and Kučera 2010).

Experimental design. Each pot was filled with 8 kg of contaminated soil. *Salix × smithiana* was chosen as an experimental plant. At each treatment two willow cuttings were planted. The experiment consists of 4 treatments: control (no applied biochar), and rates of 5, 10, and 15% of biochar from total mass of soil. Pots were uniquely fertilized with 0.1 g N; 0.16 g P; 0.4 g K per 1 kg of soil. Trees were harvested twice, firstly in July, secondly in early October to test the maximum accumulation potential of plants. The twigs and leaves were analysed separately. Total element contents in plant biomass were determined in the digests obtained by dry ashing decomposition (Street et al. 2006) and Cd and Zn contents were determined by ICP-OES. The experiment was established at greenhouse controlled conditions. To observe risk element content in leachate, the lysimeter cylinders were used. The pots were 40 cm tall. At the bottom end each pot was drained with gravel and placed onto funnel. The leachate was collected into polyethylene laboratory bottle pitched on the funnel and analysed (ICP-OES) each 5 weeks during vegetation.

Statistics. All statistical analyses were performed using the Statistica12 software (Tulsa, USA).

RESULTS AND DISCUSSION

Soil and biochar element content. Nutrient and risk element content of experimental biochar and soil are given in Table 1. There are no legislative limits of risk elements for biochar use in the Czech Republic. If the content of risk elements in used biochar is compared to limits for field application of ash (according to public notice No. 131/2014), the content of Cd is under limit (the limits are: Cd: 5 mg/kg, for Zn no limit was established). Within Europe, plant-available Cd and Zn concentrations in ordinary uncontaminated arable land are up

Table 1. Element content of biochar and soil

		K	Ca	Mg	Fe	Cd	Zn	C (% w/w)
		(g/kg)				(mg/kg)		
Biochar	total*	0.5	2.9	2.2	4.1	< 0.1	8.3	C$_{total}$: 93
Soil	total**	10 ± 2	2 ± 0.07	1.9 ± 1.6	6.3 ± 0.8	42.7 ± 0.4	4341 ± 1	C$_{org}$: 3.6 ± 0.01
	available***	0.1 ± 10^{-3}	1.6 ± 0.06	0.089 ± 6.10^{-3}	0.005 ± 10^{-3}	24.74 ± 1.4	2236 ± 187	

*determined by INAA; **Aqua regia* and hydrofluoric acid decomposition, *** 0.11 mol/L CH$_3$COOH extraction

to 0.05 and 0.2 mg/kg, respectively (Uprety et al. 2009). In our experimental soil, these forms are three and four orders higher, respectively. The high content of risk elements in light soil can indicate a risk of groundwater contamination, moreover the contamination can be spread by near river.

Biomass yield. Figure 1a compares the willow biomass yield of leaves and twigs separately and evidently, the aboveground biomass yield was significantly higher at summer harvest compared to the autumn one. However, differences among individual treatments were more balanced at autumn harvest. The yield of leaves was higher than the yield of twigs at all observed treatments.

Significantly lower biomass yield of aboveground biomass was observed at control at both harvests in comparison to other treatments. With elevating rates of biochar the yield of aboveground biomass increased. The strong phytotoxic symptoms were observed at control. Yellow leaves indicated Fe deficiency due to competition between Zn and Fe uptake. Amended treatments generally did not show phytotoxic symptoms. Meers et al. (2007) planted willows on contaminated soil in pot experiment (5 mg/kg Cd, 276 mg/kg Zn; *aqua regia* extraction) and achieved yield (leaves and twigs) of 4 g per one plant. Soil in our experiment was more contaminated and at amendments treat-
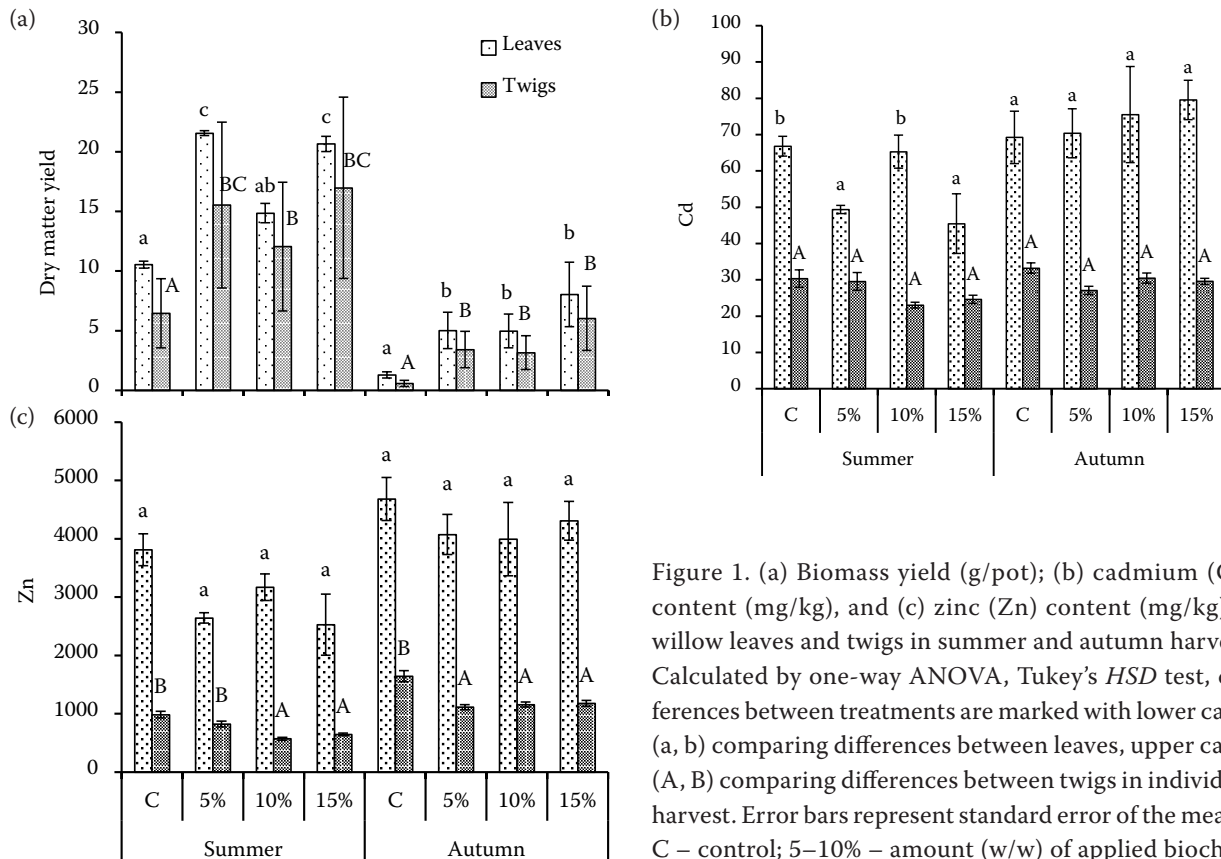

Figure 1. (a) Biomass yield (g/pot); (b) cadmium (Cd) content (mg/kg), and (c) zinc (Zn) content (mg/kg) in willow leaves and twigs in summer and autumn harvest. Calculated by one-way ANOVA, Tukey's *HSD* test, differences between treatments are marked with lower cases (a, b) comparing differences between leaves, upper cases (A, B) comparing differences between twigs in individual harvest. Error bars represent standard error of the means. C – control; 5–10% – amount (w/w) of applied biochar

ment with 5% of biochar gave almost 11 g/plant in summer. Thus, biochar application was able to overcome the phytotoxicity of the extremely contaminated soil.

Risk elements content in aboveground biomass. The content of Cd was significantly higher in willows leaves in comparison to twigs at summer and also at autumn harvest (Figure 1b). At summer harvest significantly higher Cd content was determined at control and at 10% treatment. There were no differences in Cd content in twigs at all treatments both in summer and autumn. Similarly to Vysloužilová et al. (2003) and Meers et al. (2007) Cd and Zn were transferred from roots to aboveground tissues and all treatments confirmed higher Cd and Zn accumulation in leaves than in twigs. Higher concentration of risk elements was observed both in leaves and twigs in the autumn, in the end of the vegetation of willows. This was confirmed by Lettens et al. (2011) reporting increased foliar concentrations of Cd, Zn, Cu and Mn towards the end of the season.

Zinc content in plant tissues was hundredfold higher in comparison to the content of Cd (Figure 1c). This trend was also observed in the study of Tlustoš et al. (2007). The content of Zn was higher at autumn harvest. There was a tendency for higher concentration in the control treatment. Differences between elevating rate of biochar were not significant. If we compare mean values of foliar Zn content, the higher Zn concentrations were determined at autumn harvest. In twigs, significantly higher Zn content was at control and 5% treatment at summer harvest and only at control at autumn harvest.

The Zn phytotoxicity threshold was determined at 400 mg/kg (Kabata Pendias and Pendias 2001). Despite of exceeding these values in aboveground biomass of willows, plants at amendment treatments did not have phytotoxic symptoms. Beesley et al. (2010) documented the decrease of phytotoxicity of Zn by biochar amendment into soil by using seed phytotoxicity test. With our results, we can confirm and expand this statement on high plants. High concentration of both Cd and Zn in plant leaves require annual harvesting both to avoid the risk element return by leaf-bound in autumn (Robinson et al. 2000).

Figure 2 shows the Cd and Zn concentration in leachate collected during the whole willow
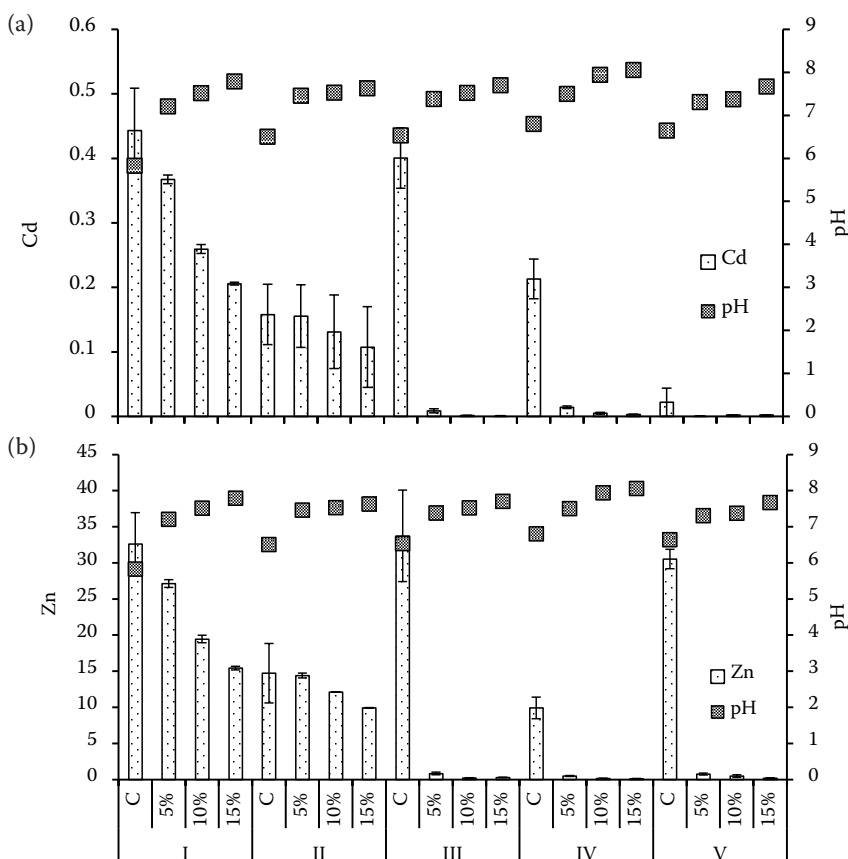

Figure 2. (a) Cadmium (Cd) and (b) zinc (Zn) content in leachate collected during willow vegetation (mg/L). Error bars represent standard error of the means. C – control; 5–10% – amount (w/w) of applied biochar; I, II, III, IV, V – samplings

vegetation. First sampling was after 1 month of growth. There was a visible decrease of Cd content in leachate with elevating rate of biochar at first and second sampling. After three months the reduction of Cd at biochar amendment treatments was by 97, 99 and 99% at 5, 10 and 15% of biochar amended treatments, respectively in comparison to concentrations determined after one month of willow growth. Similarly Jiang et al. (2012) observed, the acid-soluble Cd decrease by 86% after addition of 3% biochar with no significant difference between 3% and 5% of biochar addition. It can be concluded that lower dose of biochar is a sufficient stabilization agent.

Questionable and worthy to further observation is the Cd and Zn significant reduction in leachate at all biochar treatments, while the concentration of these elements in aboveground biomass changed little. This phenomenon can be caused by the plant rhizosphere ability to release weakly bound Cd and Zn for plants. The higher concentrations of elements at autumn harvest compared to summer one, can be explained by low autumn biomass yield, which caused lower dilution effect (elements content in the amount of biomass), as Vaněk et al. (2012) described for nutrients.

The pH value of leachate increased (in average by 1 unit) with elevating rate of biochar (Figure 2). Leachate from the control treatment was slightly acidic and the highest pH was 8 at 15% treatment. This effect in our study is comparable with Laird et al. (2010) who observed that the application of biochar (0.5, 1, 2% of biochar was applied in their study) resulted in higher pH values (up to 1 pH unit) relative to the non-amended control.

As was mentioned above, trace elements behaviour is primarily related to soil pH. So the biochar ability to increase pH value is probably one of the crucial factors of reduction of the observed element leachability.

Alkaline materials like coal fly ash and red mud also decreased Zn leaching by 99.7% and 99.6%, respectively (Ciccu et al. 2003); comparatively, our slightly alkaline biochar provides reduction of Zn leaching. The decrease of leached Zn was 97, 99 and 98% at 5, 10 and 15% treatment between first and third sampling, respectively.

Balance of Cd and Zn removal and leachability. An evaluation of the total Cd and Zn removal by the willow plants was calculated by using remediation factor (Rf) derived as a ratio of element removed by

harvested biomass to the total content of elements in soil (Figure 3). A percentage removal of Cd by willow plants was higher than Zn removal. The lowest Rf both of Cd and Zn was observed at control: 1 and 0.6%, respectively, of the total soil content. The highest removal of both elements was detected at 15% treatment: 2.7 for Cd and 1.3 for Zn. Tlustoš et al. (2007) investigated willow phytoextraction potential on highly contaminated Fluvisol, resulting in phytoextraction efficiency not exceeding 1% for both Cd and Zn, respectively. Jensen et al. (2009) concluded that phytoextraction by willows on heavily polluted soils is unsuited, because of the poor growth. On moderately contaminated Cambisol, they achieved the extraction of total Cd by 0.13% and of total Zn by 0.29%. Total leaching of Cd and Zn was calculated as a ratio of total element in soil to the amount of leached water during the whole willows vegetation period. The restriction of the risk element transport through soil profile in comparison to control was remarkable. Beesley et al. (2010) observed reduction of water-soluble Zn and Cd in incubation experiment, where they applied 50% of biochar. In our experiment 5% of biochar provided a significant reduction of Cd and Zn leaching. Thus, biochar application was proved as the effective measure for improvement of phytoremediation efficiency at the extremely contaminated soil.

Finally, we can summarize that biochar appears to be a very effective regulator of availability of observed risk elements and it improves biomass growth and thus increases total uptake of Cd and Zn and decreases leaching of these elements.
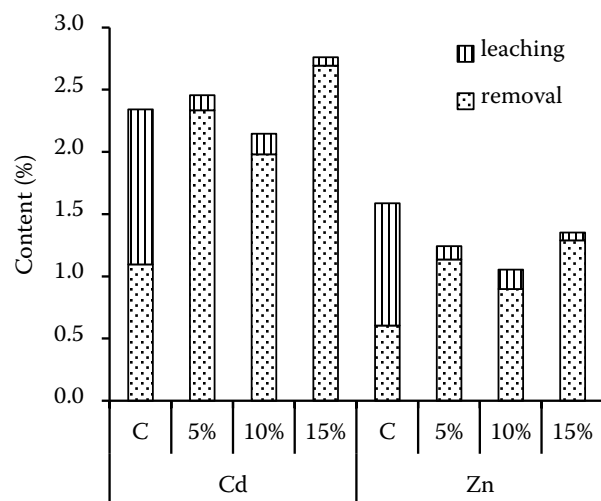

Figure 3. Relative comparison of zinc (Zn) and cadmium (Cd) removal and leaching from contaminated soil (%). C – control; 5–10% – amount (w/w) of applied biochar

REFERENCES

Beesley L., Moreno-Jiménez E., Gomez-Eyles J.L. (2010): Effects of biochar and greenwaste compost amendments on mobility, bio-availability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental Pollution, 158: 2282–2287.

Ciccu R., Ghiani M., Serci A., Fadda S., Peretti R., Zucca A. (2003): Heavy metal immobilization in the mining-contaminated soils using various industrial wastes. Minerals Engineering, 16: 187–192.

Clark R.B., Ritchey K.D., Baligar V.C. (2001): Benefits and constrains for use of FGD products on agricultural land. Fuel, 80: 821–828.

Jensen J.K., Holm P.E., Nejrup J., Larsen M.B., Borggaard O.K. (2009): The potential of willow for remediation of heavy metal polluted calcareous urban soils. Environmental Pollution, 157: 931–937.

Jiang J., Xu R.K., Jiang T.Y., Li Z. (2012): Immobilization of Cu(II), Pb(II) and Cd(II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. Journal of Hazardous Materials, 229–230: 145–150.

Jordan C.F. (1968): A simple, tension-free lysimeter. Soil Science, 105: 81–86.

Kabata-Pendias A., Pendias H. (2001): Trace Elements in Soils and Plants. 3rd Ed. Boca Raton, CRC Press.

Kubešová M., Kučera J. (2010): Validation of k0 standardization method in neutron activation analysis – The use of Kayzero for Windows programme at the nuclear physics institute, Řež. Nuclear Instruments and Methods in Physics Research, Section A: Accelerators, Spetrometers, Detectors and Associated Equipment, 622, 403–406.

Laird D., Fleming P., Wang B., Horton R., Karlen D. (2010): Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma, 158: 436–442.

Lettens S., Vandecasteele B., De Vos B., Vansteenkiste D., Verschelde P. (2011): Intra- and inter-annual variation of Cd, Zn, Mn and Cu in foliage of poplars on contaminated soil. Science of the Total Environment, 409: 2306–2316.

Meers E., Vandecasteele B., Ruttens A., Vangronsveld J., Tack F.M.G. (2007): Potential of five willow species (*Salix* spp.) for phyto-extraction of heavy metals. Environmental and Experimental Botany, 60: 57–68.

Němeček J., Vácha R., Podlešáková E. (2010): Assessment of Soil Contamination in the Czech Republic. Prague, Research Institute for Soil and Water Conservation. (In Czech)

Ochecová P., Tlustoš P., Száková J. (2014): Wheat and soil response to wood fly ash application in contaminated soils. Agronomy Journal, 106: 995–1002.

Panagos P., Van Liedekerke M., Yigini Y., Montanarella L. (2013): Contaminated sites in Europe: Review of the current situation based on data collected through a European network. Journal of Environmental and Public Health, 2013. doi:10.1155/2013/158764

Paz-Ferreiro J., Lu H., Fu S., Méndez A., Gascó G. (2014): Use of phytoremediation and biochar to remediate heavy metal polluted soils: A review. Solid Earth, 5: 65–75.

Pulford I.D., Watson C. (2003): Phytoremediation of heavy metal-contaminated land by trees – A review. Environment International, 29: 529–540.

Qayyum M.F., Steffens D., Reisenauer H.P., Schubert S. (2014): Bio-chars influence differential distribution and chemical composition of soil organic matter. Plant, Soil and Environment, 60: 337–343.

Robinson B.H., Mills T.M., Petit D., Fung L.E., Green S.R., Clothier B.E. (2000): Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation. Plant and Soil, 227: 301–306.

Street R., Száková J., Drábek O., Mládková L. (2006): The status of micronutrients (Cu, Fe, Mn, Zn) in tea and Te infusions in selected samples imported to the Czech Republic. Czech Journal of Food Science, 24: 62–71.

Tlustoš P., Száková J., Vysloužilová M., Pavlíková D., Weger J., Javorská H. (2007): Variation in the uptake of arsenic, cadmium, lead, and zinc by different species of willows *Salix* spp. grown in contaminated soils. Central European Journal of Biology, 2: 254–275.

Trakal L., Neuberg M., Tlustoš P., Száková J., Tejnecký V., Drábek O. (2011): Dolomite limestone application as a chemical immobilization of metal-contaminated soil. Plant, Soil and Environment, 57: 173–179.

Uprety D., Hejcman M., Száková J., Kunzová E., Tlustoš P. (2009): Concentration of trace elements in arable soil after long-term application of organic and inorganic fertilizers. Nutrient Cycling in Agroecosystems, 85: 241–252.

Ure A.M., Quevauviller Ph., Muntau H., Griepink B. (1993): Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the commission of the European Communities. International Journal of Environmental Analytical Chemistry, 51: 135–151.

Uzinger N., Anton A. (2008): Chemical stabilization of heavy metals on contaminated soils by lignite. Cereal Research Communications, 36: 1911–1914.

Vondráčková S., Hejcman M., Tlustoš P., Száková J. (2013): Effect of quick lime and dolomite application on mobility of elements (Cd, Zn, Pb, As, Fe, and Mn) in contaminated soils. Polish Journal of Environmental Studies, 22: 577–589.

Vysloužilová M., Tlustoš P., Száková J., Pavlíková D. (2003): As, Cd, Pb and Zn uptake by *Salix* spp. clones grown in soils enriched by high loads of these elements. Plant, Soil and Environment, 49: 191–196.

Zhang G., Zhang Q., Sun K., Liu X., Zheng W., Zhao Y. (2011): Sorption of simazine to corn straw biochars prepared at different pyrolytic temperatures. Environmental Pollution, 159: 2594–2601.

Vaněk V., Balík J., Černý J., Pavlík M., Pavlíková D., Tlustoš P., Valtera J. (2012): Nutrition of Horticulture Crops. Praha, Academia. (In Czech)

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1 **Biochar applications enhance the phytoextraction potential of Salix**

2 **smithiana [Willd.](willow) in heavily contaminated soil – potential**

3 **for a sustainable remediation method?**

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11

12 **Abstract**

13 The combination of chemostabilization and phytoextraction provide an affordable and

14 environmentally effective remediation technology for the heavy metals in contaminated soils.

15 This study investigated how biochar applications in heavily contaminated alluvium soils

16 reduced the toxic effects of heavy metal (Cd, Pb, Zn) and their transport through the soil

17 profile. It also enhanced the phytoextraction potential of willow plants in the first and second

18 year of a pot experiment. High biochar applications (5, 10 and 15% w / w) reduced the

19 phytotoxicity of metals in soil solution and improved biomass growth in *Salix smithiana* and

20 enhanced heavy metal uptake by plants in the amendment treatments. In the 3rd year after

21 planting, the pH of both the soil and leachate decreased by 1 unit. The pH of the system was

22 probably the most crucial factor of Cd and Zn immobilization. Thus, the decrease of pH led to

23 the increase of Cd and Zn in the leachate and/or limited the growth of plants. Although the

24 changes in heavy metals transferring from the soil exchangeable to the reducible fraction after

25 biochar application were recorded, the change was not sufficient for any reduction of heavy

26 metal mobility in the contaminated soils. Further research is necessary to evaluate the

27 effectiveness of biochar utilization for remediation of extremely contaminated alluvial soils.

28

29 **Key words:** cadmium, zinc, carbon-based soil amendment, short rotation coppice,

30 phytoremediation, immobilization

31 **1   Introduction**

32 Soil contamination in 2011 was estimated at 2.5 million potentially contaminated sites in

33 European countries of which about 45 % have been identified to date (EEA, 2015). In the

34 Czech Republic approximately 7,000 potentially contaminated sites are registered, mostly of

35 which originated from historical mining and smelting process and are referred to as old loads

36 (SEKM, ČR). Soil contamination is the most important problem in soil protection nowadays,

37 while the contaminated site, old loads persist as significant health threats to humans (Němeček

38 et al., 2010). Schwartz et al. (2006) reviewed how the contamination of Fluvisols resulted from

39 solid contaminant deposition in regions of slack water from Industry or old loads and in

40 floodplains adjacent to these depositions in Central Europe. The old mining and smelting area,

41 Příbram, is one of the most polluted sites in the Czech Republic. Alluvial soils of the Litavka

42 river, flowing through this site, have been previously studied by Vaněk et al. (2005) and they

43 found that in the areas which flood, the mean contents of Cd, Pb, and Zn in topsoil were 34;

44 2979; and 3363 mg kg^{-1}, respectively. The walls of waste sedimentation ponds of smelters,

45 located close to the river, were broken due to river floods several times in history. The content

46 of ponds contained high amounts of heavy metals and they were spread with floods into the

47 alluvium of the Litavka River (Borůvka and Vácha, 2006). In addition, metal mobility through

48 the soil profile was higher than in areas influenced by atmospheric deposition (Borůvka et al.,

49 1996).

2

50   According to the European Environment Agency, the remediation of contaminated soil is

51   commonly managed using "traditional" techniques, e.g. excavation and off-site disposal, which

52   accounts for about one third of remediation management practices. In-situ and ex-situ

53   remediation techniques for contaminated soil are applied more or less equally (EEA, 2015).

54   For metal polluted soils, phytoremediation appears to be an economically, esthetical method

55   with low side effects and an attractive *in situ* technology (Pulford and Watson, 2003; Wu et al.,

56   2010). One of the crops used in this technology is the short rotation, coppiced willow. It's

57   potential for phytoextraction technologies has been described and discussed in many studies.

58   The specific willow species, *Salix smithiana* (a hybrid of *Salix viminalis* L. and *Salix caprea*

59   L.) was tested for its phytoextraction capability on an acid Cambisol, with low carbonate

60   (Fisherová et al., 2006; Iqbal et al., 2012, Tlustoš et al., 2007), and/or on industrial

61   neutral/alkaline soils (Kacálková et al., 2009) and proved successful especially in the

62   remediation of Cd and Zn. However, the introduction of this species onto heavily contaminated

63   acidic Fluvisols (originating from the alluvium of the Litavka river) resulted in serious

64   symptoms of phytotoxicity leading to plant mortality (Puschenreiter et al., 2013, Vondráčková

65   et al., 2015; Tlustoš et al., 2007), especially due to extremely high content of zinc

66   (Vysloužilová et al., 2003). When grown in polluted soils, biomass production in field

67   conditions, can be an indicator of reasonable efficiency of phytoextraction (Meers et al., 2007;

68   Mertens et al., 2005). However, in specific cases of high soil pollution, plant growth can be

69   affected by the phytotoxic levels of elements in soil. Thus, the combination of phytoextraction

70   and stabilization technologies could be considered to suppress the contaminant levels in the soil

71   solution, to improve plant growth and to increase the phytoextraction potential.

72   Trace element bioavailability and leaching can be decreased using contaminant immobilizing

73   amendments which induce various sorption processes i.e. the adsorption to mineral surfaces,

74   the formation of stable complexes with organic ligands or surface precipitation and ion

75   exchange (Kumpiene et al., 2008). Among a broad range of available soil stabilization

76   materials, including inorganic and organic substances, coal-like materials or combustion by-

77   products have been investigated. Lignite fly ash can reduce Pb, Zn and Cd solubility in tailings

78   and soils, but apart from the increase in pH, they create a sorption effect on the oxides and

79   hydroxides surfaces and bind with the hydrated fly ash compounds (Stouraiti et al., 2002). The

80   potential absorbing properties of some coal - fly ash constituents, such as the highly porous

81   combustible fractions activated by the furnace temperatures cannot be overlooked (Ciccu et al.,

82   2001). Lignite was confirmed as a suitable additive for chemical stabilisation during the

83   combined phytostabilisation of contaminated soils (Uzinger and Anton, 2008).

84   In last decade, the investigation of amendments has focused on biochar, a stable carbon rich

85   charred biomass. Its utilization has been as a soil additive for improvement of soil properties

86   (Atkinson et al., 2010) and for remediation purposes (Tang et al., 2013; Ahmad et al., 2014;

87   Zhang et al., 2013). While mechanisms of heavy metal immobilization using biochar include: i)

88   the development of hydroxides, carbonates, or phosphates, i.e. precipitation (due to high pH of

89   biochars); especially for Pb immobilization (Wang et al., 2015), ii) electrostatic interactions

90   between cations and functional groups (Wang et al., 2015) and iii) surface chemisorption

91   between d-electrons of metals and p-electrons of biochar (Cao et al., 2009). Indirectly, biochar

92   can immobilize heavy metals by increasing pH which increases the negative charge of surface

93   and thus increases the soil affinity to cations of the heavy metals (Jiang et al., 2012). The

94   biochar sorption ability is affected by the chemical properties of the individual contaminants.

95   For instance, soil pH increases after biochar applications increase the mobility of As, and due

96   to increased dissolved carbon, Cu mobility is also increased; the opposite pattern was observed

97   for Cd and Zn (Beesley et al., 2010; Jiang et al., 2012).

98   An assessment of the mobility and potential bioavailability of heavy metals is connected with

99   the determination of these elements in soil solution. Elemental transport through the soil profile

4

100   can be observed with a lysimeter (Jordan, 1968). The utilization of cylinder pots placed in the

101   laboratory, greenhouse or into field conditions were described as a suitable process for this type

102   of investigation (Trakal et al., 2011).

103   The potential benefit of the combination of biochar amendments and phytoremediation

104   technologies have been reviewed by Ferreiro et al. (2014). Fellet et al. (2014) where they found

105   biochar applications helped to sufficiently enhance the biomass yields of low-biomass plants

106   and reduced Cd and Pb accumulation in plant tissues. Similarly, Lu et al. (2014) observed that

107   red amaranth plant biomass yields increased after biochar applications, however it did not

108   increase the phytoextraction efficiency. However, the knowledge concerning the ability of

109   biochar to enhance phytoextraction on a heavily contaminated area is not so readily available,

110   and detailed research is necessary in the long-term effectivity of this measure.

111   The aim of this study was i) to evaluate the potential effect of elevated rates of biochar

112   applications on heavy metals transported through the soil profile, and ii) to assess the effect of

113   biochar amendments on plant growth as well as contaminant accumulation in willow tissues.

114   **2   Materials and Methods**

115   **2.1   Biochar and soil samples**

116   Biochar derived from coconut shells was purchased from Erspol., Ltd. (Czech Republic). It was

117   produced by fast pyrolysis (at 800°C) and activated by water steam. Soil was sampled from the

118   top layer (0-30 cm) of grassland in the vicinity of Trhové Dušníky (Czech Republic)

119   municipality, 49°71'8.8742N, 14°0'12.8814E. This locality is close to the alluvium of the

120   Litavka river and belongs to the abovementioned mining and smelting area of Příbram. Soil

121   was air-dried, homogenized and sieved to <2 mm fractions. The elemental contents, and main

122   physicochemical characteristics of the soil are presented in Table 1.

123

5

124 **Table 1. Basic soil and biochar characteristics.**

Soil			Biochar		
pH(CaCl$_2$)	-	5.93	C total	(% w/w)	93
Soil texture		clay loamy sand	Ash	(% w/w)	7
CEC	mmol$_+$ kg^{-1}	149	CEC	mmol$_+$ kg^{-1}	95
TOC	g kg^{-1}	3.5	pH (CaCl$_2$)	−	8.1
Cd total[1]	mg kg^{-1}	40.6	SA $_{BET}$	m^2 g^{-1}	486
Zn total[1]	mg kg^{-1}	5623	Cd total[2]	mg kg^{-1}	<0.1
Pb total[1]	mg kg^{-1}	3706	Zn total[2]	mg kg^{-1}	8.3
K total[1]	g kg^{-1}	6.03	Pb total[2]	mg kg^{-1}	bdl[3]
Ca total[1]	g kg^{-1}	2.11	K total[2]	g kg^{-1}	0.5
Mg total[1]	g kg^{-1}	3.13	Ca total[2]	g kg^{-1}	2.9
P total[1]	g kg^{-1}	0.53	Mg total[2]	g kg^{-1}	2.2
Fe total[1]	g kg-1	6.64	P total[2]	g kg-1	0.8
			Fe total[2]	g kg^{-1}	5.2

125 [1] determined by *Aqua regia* and HF decomposition, [2] analysed by neutron activation analysis,

126 [3]below detection limit

2.2 Experimental design

128 This experiment ran for three years. It was established in a greenhouse under controlled

129 conditions. The experiment consisted of 4 treatments: control (no applied biochar), and three

130 rates representing 5%, 10%, and 15% (w / w) biochar from total mass of soil. High rates of

131 biochar were applied due to extremely high soil contamination. Lysimeter pots (40cm tall;

132 20cm diameter) were filled with 8kg of sampled contaminated soil and/or with mixtures of oil

133 anad biochar. *Salix smithiana* Willd. (clone no. S-218) was chosen as the experimental plant.

134 For each lysimeter pot, two willow cuttings were planted. Pots were fertilized with 0.1 g N;

135 0.16 g P; 0.4 g K per 1 kg of soil, when the experiment was established and then every year at

136 the beginning of the willows regrowth in the beginning of vegetation e.i. in spring. Each year,

137 twigs and leaves of two plants in each pot were harvested in early October to allow the plants

138 regrowth in spring, to simulate usual harvest of short rotation coppice (Vondráčková et al.

6

139   2015). The pots were watered to keep 60% of water holding capacity. Every two weeks, the

140   pots were watered to reach 120% of water holding capacity and to obtain percolate.

141   **2.3   Analytical procedures.**

142   *Biochar.* Ash content (A) in the biomass sample was determined according to the

143   Czech/European standard (CSN EN 15403). Content of C, H, N, O and S was determined by

144   using the apparatus Flash EA 1112 in the CHNS/O configuration (Thermo Fisher Scientific,

145   USA). The adsorption isotherms were fitted by using the Brunauer-Emmett-Teller (BET)

146   method for specific surface area (SA) (Brunauer et al., 1938). The biochar pH (leachate of 0.01

147   mol l $^{-1}$ CaCl $_2$, 1:5 w / v) and cation exchange capacity (CEC) (ISO 11260, 1994) were

148   determined, as well. The total elemental content in biochar was determined by neutron

149   activation analysis (NAA) (Kubešová and Kučera 2010). Certified reference material, NIST

150   SRM-1574 Peach Leaves, NIST SRM-1633b Constituent Elements in Coal Fly Ash and NIST

151   SRM-1635 Trace Elements in Coal, were applied for quality assurance of the analytical data.

152   The accuracy of the analysis results is evaluated according to the "En number" described in

153   Kubešová and Kučera (2010)

154   *Soil.* Soil was sampled twice, i) at the beginning of the experiment, before the establishment of

155   experiment and ii) after three years (at the end of the experiment). For determination of the

156   total content of heavy metals in soil, 0.5 g of soil sample was decomposed in a closed system

157   with microwave heating in the device Ethos 1 (MLS GmbH, Germany) in a mixture of 8 ml

158   HNO_3, 5 ml HCl and 2 ml HF. Mobile (plant-available) portions of elements in soils were

159   determined using 0.01 mol L^{-1} $CaCl_2$ (Tlustoš et al., 1994; Quevauviller, 1998). A sequential

160   extraction protocol, or the original BCR (Ure et al., 1993), was performed for the determination

161   of Cd, Pb and Zn fractionation changes in soil after the experiment termination. The element

162   contents in the soil digests and extracts were determined by inductively coupled plasma-optical

163   emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA). Certified

164 reference material, CRM 7004 (Analytika, CZ), was applied for quality assurance of the

165 analytical data. This material was certified to contain the following: 198±6 mg Zn kg^{-1};

166 1.44±0.07 mg Cd kg^{-1} and 83.1±2.3 Pb kg^{-1} was determined: 193±4 mg Zn kg^{-1}; 1.5±0.09 mg

167 Cd kg^{-1} and 81.2±3.1 mg Pb kg^{-1} (means±SEM of entry and final samples analysis).

168 *Plant.* Total elemental contents of the plant biomass were determined using digestions obtained

169 from dry ashing procedures (Street et al., 2006) by ICP-OES. The uptake of Cd, Pb and Zn by

170 plants was calculated by equation: risk element concentration in plant tissues (mg g^{-1}) *

171 biomass yield (mg) / 1000. Certified reference material, NCS DC 73348 Bush Branches and

172 Leaves, was applied for quality assurance of the analytical data. This material was certified to

173 contain the following: 20.6 ± 2.2 mg Zn kg^{-1}; 0.14±0.06 mg Cd kg^{-1} and 7.1±1.1 Pb kg^{-1} was

174 determined: 19.4±2.1 mg Zn kg^{-1}; 0.15±0.08 mg Cd kg^{-1} and 6.9±1.4mg Pb kg^{-1} (means±SEM

175 of analysis during 3 years).

176 *Leachate*. To observe risk element transport through the soil profile, the lysimeter pots were

177 drained with gravel and leachate directed into a funnel. The leachate was collected into a

178 polyethylene laboratory bottles pitched on the funnel and analysed (ICP-OES) each 5 weeks

179 during vegetative growth. First sampling was conducted 1 month after planting in the first year

180 of vegetation, 1 month after regrowth in the second and third year of the experiment. Certified

181 reference material, AN9090 (MN-100) (Analytika, CZ), was applied for quality assurance of

182 the analytical data. This material was certified to contain the following: 100 mg Zn, Cd and L^{-1};

183 was determined: 100±2.1 mg Zn L^{-1}; 101±0.03 mg Cd L^{-1} and 99±12 mg Pb L^{-1} (means±SEM

184 of analysis during 3 years).

185

186 *Data processing*. All statistical analyses were performed using the Statistica 12.0 (Tulsa, USA).

187 All data were checked for homogeneity of variance and normality (Levene and Shapiro-Wilk

188 tests). Collected data were evaluated by one way/multi-factor ANOVA with multivariate F

189   value (Wilks' lambda). A single ANOVA was applied to identify the effect of treatments,

190   contamination and their interactions as independent variables. A single ANOVA was followed

191   by post-hoc comparison using a Tukey test ($p < 0.05$).

192   **3   Results and Discussion**

193   **3.1   Soil and biochar elemental content.**

194   Nutrients and heavy metals contents of the experimental biochar and soil at the beginning of

195   the experiment are given in Table 1. There are no legislative limits for heavy metals for land

196   applications of biochar in the Czech Republic. Comparing the content of heavy metals in used

197   biochar with the threshold limits for land application of ash (according to public notice

198   no.131/2014), the Cd content was within the limit (the limits are: Cd: 5 mg kg^{-1}, for Zn no limit

199   was established). According to suggested maximum allowed thresholds for biochar by the

200   International Biochar Initiative (for Cd: 1.4-39, Zn: 416-7400 and Pb: 121-300 mg kg^{-1}), our

201   biochar was suitable for soil application (biochar-internationl.org). Within Europe, plant-

202   available Cd and Zn concentrations in ordinary uncontaminated arable land are up to 0.05 and

203   0.2 mg kg^{-1}, respectively (Uprety et al., 2009). According to Czech legislative standard

204   153/2016 Sb., the indicative values for Cd and Pb are given (Cd = 20 mg kg^{-1}; Pb= 400 mg kg^{-1}

205   ^{1}; for zinc the indicative value iss unfortunately not given in Czech legislative) . Exceeding the

206   threshold of Cd and Pb can cause threats to humans and animals in food and feed. Exceeding

207   the threshold of Zn can influence the plant growth and the production functions of soils. In the

208   experimental soil, Cd and Pb contents were 2fold, 9.3fold, respectively, higher than these

209   indicative values. These extremely high contents of heavy metals in soil require a specific

210   approach and methods for the prevention of heavy metals spreading into food chain. Soil

211   contamination in our experiment was specific.

3.2 Biomass yield

Figure 1a shows the biomass yield of willow leaves and figure 1b shows the yield of willow twigs 3 years after the start of the experiment. The yield of leaves was higher than yield of twigs in all treatments. Although Novak et al. (2016) referred inconsistencies in biochar effects on plant biomass yield, our results revealed an improvement in biomass yield after biochar application in first and second seasons. However, different patterns were recorded for individual seasons. In the first year, the significantly lowest biomass yield (both leaves and twigs) was observed in the control with no applied biochar. The biochar dose of 5% w / w resulted in substantial improvement of biomass production, whereas with elevated rates of biochar the increment of the yield of aboveground biomass was not significant. The leaves and twigs yield in second year changed in comparison to first one. There was no significant difference in biomass yield between control and treatment with 5% of applied biochar. The highest yield was determined at treatment of 15% of applied biochar. Finally, the third year was surprisingly characterised by no differences in biomass yields among all treatments. The strong phytotoxic symptoms on willow leaves were observed at control plants each year. The top, young, leaves yellowed with green veins indicating Fe deficiency due to competition between Zn and Fe uptake. The amendment treatments did not generally show phytotoxic symptoms in the first year, however, by the second year, plants grown at 5% treatment did have phytotoxic symptoms. In the third year although there was no significant improvement in biomass production in the amended treatments; the phytotoxicity at 10 and 15% was weak compared to the control. Meers et al. (2007) planted willows on contaminated soils in a pot experiment (5 mg Cd kg^{-1}, 275 mg Zn kg^{-1}; *Aqua regia* extraction) and achieved yields (leaves and twigs) of 1.4 g per plant grown in 3 kg of soil. In our experiment, the soil was substantially more contaminated, and at the treatments with 5% w / w of biochar, willows produced 10 g of dry matter biomass per plant in the 1st year and to a lesser extent in the 2nd year. Thus, the biochar

237 application was able to overcome the phytotoxicity of the extremely contaminated soil in two

238 years following the start of the experiment.
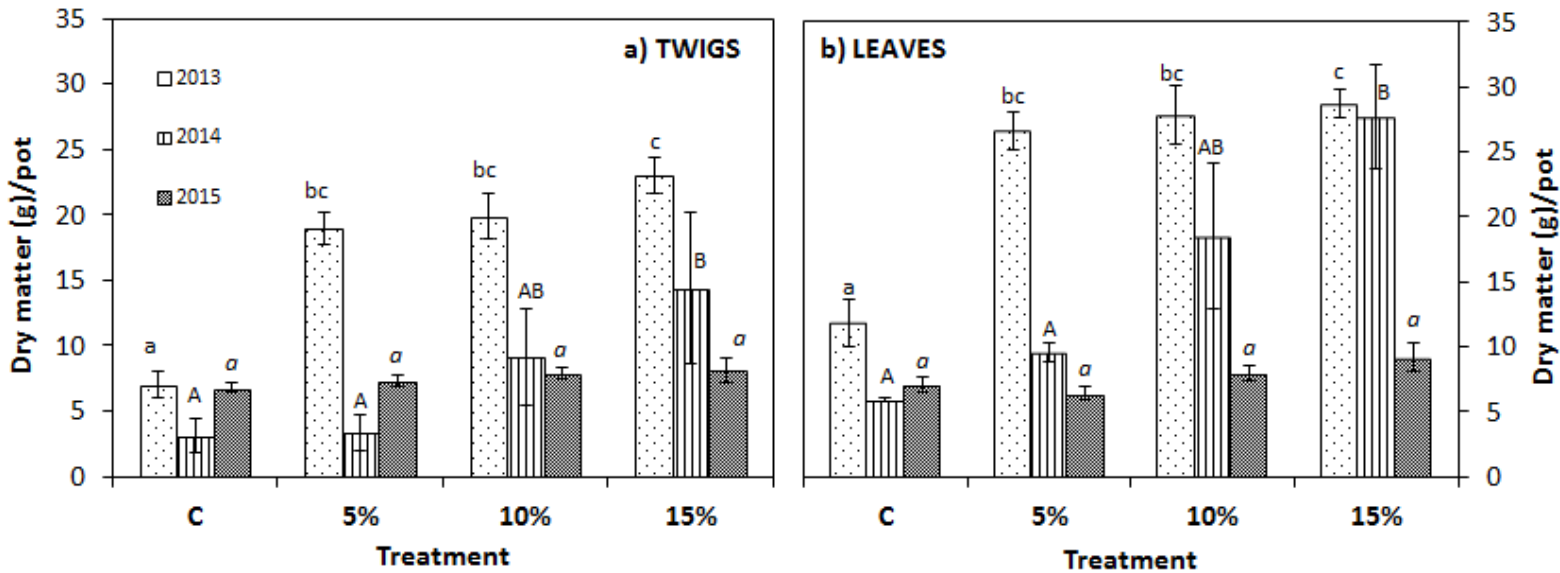
239 **Figure 1. Willow biomass yields (g / pot) within three years of the experiment.**

240 The values represent the means (±SE) of data obtained in the experiment (n=5). Different

241 letters and fonts indicate significantly different values (*P*<0.05) in each year x treatment. The

242 differences were determined by post-hoc Tukey's test. Treatments were C-control, 5, 10 and

243 15% of applied biochar.

244

245 **3.3   Heavy metal concentration and total uptake by aboveground willow biomass**

246 Cadmium concentration in leaves and twigs (sum of concentrations in leaves and twigs) was

247 without any significant differences among treatments (control, 5, 10, 15 % w / w of biochar)

248 and ranged from 176 to 194, from 181 to 254, and from 26 to 30 mg kg^{-1} in the first, second and

249 third year, respectively. Table 2 shows the heavy metals uptake by aboveground biomass, i.e.

250 concentration in leaves and twigs related to biomass yield. Cadmium uptake increased in leaves

251 and twigs with increasing dose of applied biochar in the first year of vegetation. In the second

252 year, a similar trend in uptake was observed in leaves but the only significant difference was by

11

253   twigs in the 15% of applied biochar treatment. In the third year, no significant differences were

254   observed in the heavy metal uptake among treatments. The highest uptake of a heavy metal was

255   reported for zinc, where its magnitude occurred in both soil and plants. Zinc is a plant

256   micronutrient and thus the higher concentration of this element in plant tissues in comparison to

257   cadmium and lead was expected. A similar trend was also observed by Tlustoš et al. (2007).

258   The Zn concentrations in twigs ranged from 1,828 to 2,103, from 2,070 to 3,442 and from

259   2,980-3,505 mg kg^{-1} in the first, second and third year of vegetation, respectively. There were

260   no significant differences of zinc concentration in leaves and twigs among treatments, except

261   for Zn concentration in twigs in second year, where the highest Zn concentration was detected

262   in willows planted at treatment with 15 % w / w of biochar. The most significant ability to take

263   up zinc from soil was in the 15 % applied biochar treatments in the first and second year.

264   Vondráčková et al. (2015) showed that concentrations of Cd ranged 36.5–73 mg kg^{-1} and Zn

265   concentration ranged 2,074–3,488 mg kg^{-1} in willows growing in soils from the same locality

266   as used in this experiment. Thus, the present study achieved similar values. Comparing leaves

267   and twigs, the foliar concentration was higher by 2.4, 3.6 and 1.2 - fold for cadmium (average

268   concentration of all treatments) in the leaf biomass harvested at 3-years when compared to

269   twigs. For zinc, the foliar concentration it was 3.8, 5 and 2 times higher than in twigs. This was

270   confirmed by Lettens et al. (2011) reporting increased foliar concentrations of Cd, Zn, Cu and

271   Mn towards the end of the season. High concentrations of both Cd and Zn in plant leaves

272   require annual harvesting to avoid the heavy metals return by leaf litter in autumn (Robinson et

273   al., 2000).

274   The significantly highest Pb uptake was obtained in treatments with the biochar dose of 15 % in

275   the twigs in the first and second year. However, the lead uptake was very low compared to Cd

276   and Zn, and in the 3rd year the concentrations in plant tissues were below the detection limit of

277   the analytical technique.

278 Third year of the experiment was specific. The harvested biomass was very low comparing

279 previous years and there were no differences within the tratments. There were no differences

280 among treatments concerning either Cd and Zn uptake, nor concentration. The zinc

281 concentration decreased approximately 4-fold in comparison to previous year.

282 The Zn phytotoxicity threshold was determined at 400 mg kg^{-1} (Kabata-Pendias and Pendias,

283 2001). Despite exceeding these values in the aboveground biomass of willows, plants at

284 amendment treatments did not have phytotoxic symptoms in the first and second years.

285 However, there were visible phytotoxic signs including Fe chlorosis at all treatments in the last

286 season (third year). Similar signs were observed by Vondráčková et al. (2015), at similar soil

287 Zn concentrations. Beesley et al. (2010) documented the decrease of phytotoxicity of zinc by

288 biochar soil amendment in seed phytotoxicity test. This study can confirm and expand this

289 statement on whole plants for two subsequent seasons, but the results showed that the

290 beneficial results of the biochar did not have a long-term duration. Conversely, Shen et al.

291 (2016) observed a reduction of Zn and Ni in carbonic acid leachate after three years of biochar

292 and compost + soil incorporation in field experiment. In their study, the Zn contamination was

293 lower compared to those presented here and they also suggested a higher dosage of biochar

294 (<5%) is required to prevent the longer term efficiency of remediation.

295

296 **Table 2. Uptake of cadmium, lead and zinc (mg / pot) by different parts of willow plants**

297 **at annual harvest**.

Year	Plant part	Treatment	Cd	Pb	Zn
			(mg/pot)		
1st year	Leaves	C	0.564±0.128[a]	0.024±0.005[a]	31.9±8.94[a]
		5%	1.772±0.169[bc]	0.101±0.008[ab]	87.6±8.02[bc]
		10%	1.803±0.182[b]	0.093±0.012[ab]	90.7±10.2[b]
		15%	1.939±0.142[c]	0.203±0.050[ab]	116±9.53[c]
	Twigs	C	0.175±0.022[a]	0.051±0.011[a]	4.83±0.680[a]
		5%	0.462±0.029[b]	0.090±0.006[a]	12.9±1.08[ab]
		10%	0.449±0.074[b]	0.076±0.014[a]	13.6±2.20[bc]

13

		15%	0.584 ± 0.105^b	0.195 ± 0.036^b	21.5 ± 3.18^c
2nd year	Leaves	C	0.368 ± 0.021^a	0.061 ± 0.024^a	28.2 ± 0.77^a
		5%	0.872 ± 0.090^{ab}	0.016 ± 0.007^a	47.8 ± 4.05^{ab}
		10%	1.851 ± 0.574^b	0.058 ± 0.019^a	99.6 ± 31.0^{bc}
		15%	2.096 ± 0.193^b	0.115 ± 0.039^a	127 ± 11.9^c
	Twigs	C	0.115 ± 0.013^a	0.030 ± 0.005^a	5.02 ± 0.75^a
		5%	0.103 ± 0.016^a	0.021 ± 0.003^a	3.62 ± 0.47^a
		10%	0.104 ± 0.084^a	0.026 ± 0.021^a	3.85 ± 2.52^a
		15%	0.507 ± 0.097^b	0.117 ± 0.025^b	18.5 ± 3.50^b
3rd year	Leaves	C	0.112 ± 0.026^a	0.009 ± 0.005^a	14.8 ± 2.93^a
		5%	0.108 ± 0.014^a	0.005 ± 0.005^a	14.1 ± 1.58^a
		10%	0.167 ± 0.038^a	<0.0001	21.7 ± 2.90^a
		15%	0.084 ± 0.020^a	<0.0001	17.7 ± 0.35^a
	Twigs	C	0.080 ± 0.012^a	<0.0001	7.53 ± 0.91^a
		5%	0.093 ± 0.015^a	0.003 ± 0.0003^a	7.79 ± 0.40^a
		10%	0.122 ± 0.022^a	0.019 ± 0.009^b	8.93 ± 0.68^a
		15%	0.089 ± 0.020^a	0.018 ± 0.007^b	8.23 ± 0.69^a

298 The values represent the means ($\pm$SE) of data obtained in the experiment (n=5). Different

299 letters indicate significantly different values ($P<0.05$) in each year x treatment by Tukey's post-

300 hoc test. Treatments were C-control, 5, 10 and 15% of applied biochar.

301

302

303 **3.4 Heavy metals in leachate**

304 Figures 2 a, b and c show Cd, Pb, and Zn concentrations in leachate collected during the 3-

305 years of willow vegetation. As it was mentioned in Materials and Methods, in the first and

306 second year, leachate was sampled five times during the vegetative stage. In the third year, the

307 leachate was sampled only twice, due to the early termination of the experiment caused by

308 limited plant growth. Thus we obtained data from 12 leachate samples for Cd, Zn and 10

309 samples for PB and pH determination. In the first year, there was a visible decrease of cadmium

310 content in leachate with increasing rates of biochar at first and second sampling. After three

311 months, the reduction of cadmium content was by 97%, 99% and 99% at treatments with 5 %,

312 10 % and 15 % of applied biochar, respectively, in comparison to concentrations determined

14

after one month of willow growth. In stronger acetic acid extract, Jiang et al. (2012) observed, the Cd content decreased by 86 % after the addition of 3 % biochar with no significant difference between 3 % and 5 % of biochar addition. Reduction of zinc was by 97 %, 97 % and 98 % at treatments, where 5 %, 10 % and 15 % of biochar was applied and reduction of lead was by almost 100 % at all amended treatments. Alkaline materials like coal fly ash and red mud (alkaline waste from the aluminium processing) also decreased Zn leaching by 99.7 % and 99.6 %, respectively (Ciccu et al., 2001), thus, the biochar provides a similar pattern to Zn leaching. After one year of the experiment (sampling I – V), it may have been concluded that a lower dose of biochar was a sufficient stabilization agent. In the second year, the content of cadmium and zinc in leachate was stable and lower at treatments, where 10 and 15% of biochar was applied, in comparison to the control (sampling V – X). At treatment, where 5% of biochar was applied, the content of Cd and Zn slightly increased since the 8th sampling. At all amended treatments, however, lower concentrations of Cd, Pb and Zn were determined in comparison to the control.

In the last year (3rd year), the concentration of Cd increased relatively to the previous years. The Cd content increased at the last sampling by 37%, 300% and 207% and Zn concentration increased by 15%, 150% and 124% in treatments 5%, 10% and 15% of applied biochar, respectively. Lead was under the detection limit in last year. Figure 2d shows the pH of sampled leachates. The pH value may be the key factor why the Cd and Zn content in leachate increased at the beginning of third year. Whereas in previous years the leachate pH increased with elevating rates of biochar and leachate from control treatment was slightly acidic. By the third year the pH was similar across all treatments (amended ones and control) and moreover in comparison to previous years, the pH decreased by 1. Table 3 also confirms the decrease of the soil pH by the end of the experiment. Other research by Laird (2010) found the effect of the biochar application (0.5, 1, 2 % of biochar) resulted in higher pH values (up to 1 pH unit)

338 relative to the un-amended control in soil, however the experiment was 500–days column

339 experiment, without plants. Doerge and Garden (1984) explained reacidification of limed soils

340 by the acidifying processes such as nitrification, CO_2 release via plant and microbial

341 respiration, mineralization of organic matter and dissociation of organic acids, it is expected

342 that some of these aspects were prevalent in the decrease of soil pH in our experiment as well.

343 Bradl (2004) noted, as the metals ions are sorbed, the H^+ ions are released; this can influence

344 pH of soil and leachate in our case. Houben et al. (2013) showed that the application of biochar

345 resulted into Cd, Zn and Pb immobilization (documented as decrease of 0.01 mol L^{-1} $CaCl_2$

346 extractable element portions and phytoavailability). However, when they acidified the soil by

347 pH dependent on treatment, and assessed the leachate, they found that the metal release at a

348 defined pH was not affected by the presence of biochar. This indicates that biochar applications

349 did not lead to the new metal-bearing phase formation (more resistant to pH change) and that

350 the reaction between the heavy metals and biochar were thus pH-dependent and reversible at

351 acidic pH.

352 Questionable and worthy of further observation was that Cd and Zn reduction in leachate from

353 biochar treatments was substantial, whereas the concentration of these elements in the

354 aboveground biomass only changed a little. The reason for this can be explained by the plant

355 rhizosphere and its ability to make Cd, Zn available to plants. Rees et al. (2015) observed that

356 *Nocaea caerulescens*, a Cd- and Zn-hyperaccumulator, increased uptake of metals as a

357 response to the biochar amendment in contaminated soil, may be due to immobilization of

358 major cations.
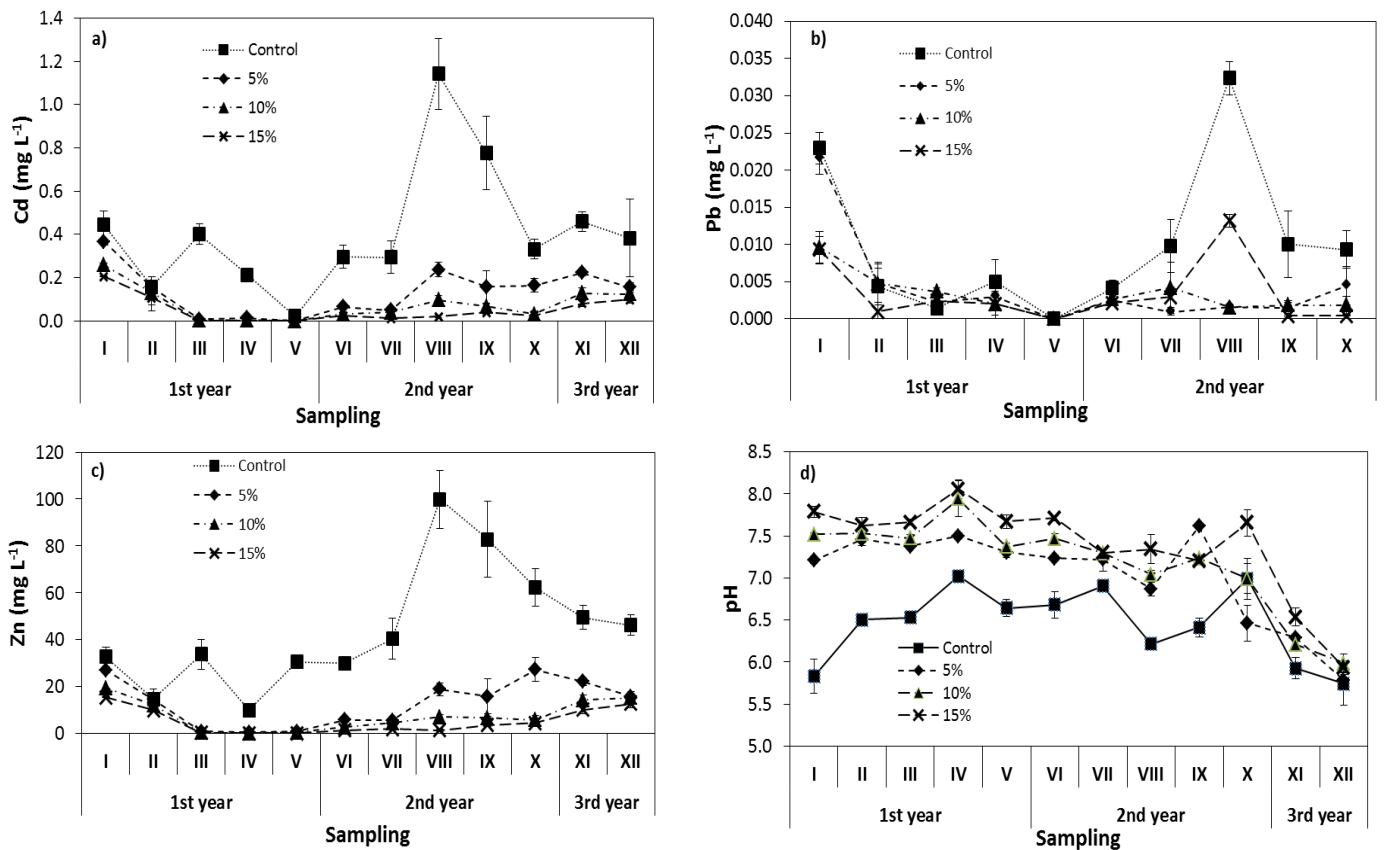
Figure 2 Content of cadmium, lead and zinc in leachate and pH of leachate collected during vegetative growth over three years of the experiment. Treatments were C-control, 5, 10 and 15% of applied biochar.

3.5   Balance of Cd and Zn removal and their leachability.

An evaluation of the total Cd and Zn removal by the willow plants was calculated by using a remediation factor (Rf) derived as a ratio of element removed by harvested biomass, to total content of element in soil (Figure 3). The relative removal of Cd by willow plants was higher than Zn removal. The lowest Rf for both Cd and Zn was observed in the control. For cadmium the Rf was 2 and 3fold lower to the average Rf in the amended treatments in the first two years, whereas in the 3rd year the Rf values dropped down and did not differ significantly among all the treatments. Rf of zinc in the control treatment was 2 and 2.6fold lower in first and second year and in the third year the control Rf value was also comparable with Rf in amended

17

373 treatments as was also observed for cadmium. The highest removal of both elements was

374 detected at 15% treatment in each year, e.g. in 1st year the values were: 2.7% for Cd and 1.3%

375 for Zn. Tlustoš et al. (2007) investigated willow phytoextraction potential on highly

376 contaminated Fluvisol without any immobilization measure, resulting in phytoextraction

377 efficiency not exceeding 1% for both Cd and Zn. Jensen et al. (2009) concluded

378 phytoextraction by willows on heavily polluted soils was unsuitable, because of poor willow

379 growth. Thus, it seems that biochar did enhance the phytoextraction potential of willow in the

380 first and second year of our experiment, mainly due to increased biomass growth. The total

381 outflow of cadmium and zinc was calculated as ratio of total element in soil to amount of

382 leached water during whole willows vegetative period. The restriction of the heavy metal

383 transport through the soil profile in comparison to control was remarkable. Beesley et al. (2010)

384 observed a reduction of water-soluble Zn and Cd in an incubation experiment, where they

385 applied 1:1 (biochar:soil). In our experiment 5% of added biochar provided a significant

386 reduction of Cd and Zn leaching. Thus, biochar application was proved as an effective measure

387 for improvement of phytoremediation efficiency in extremely contaminated soils.
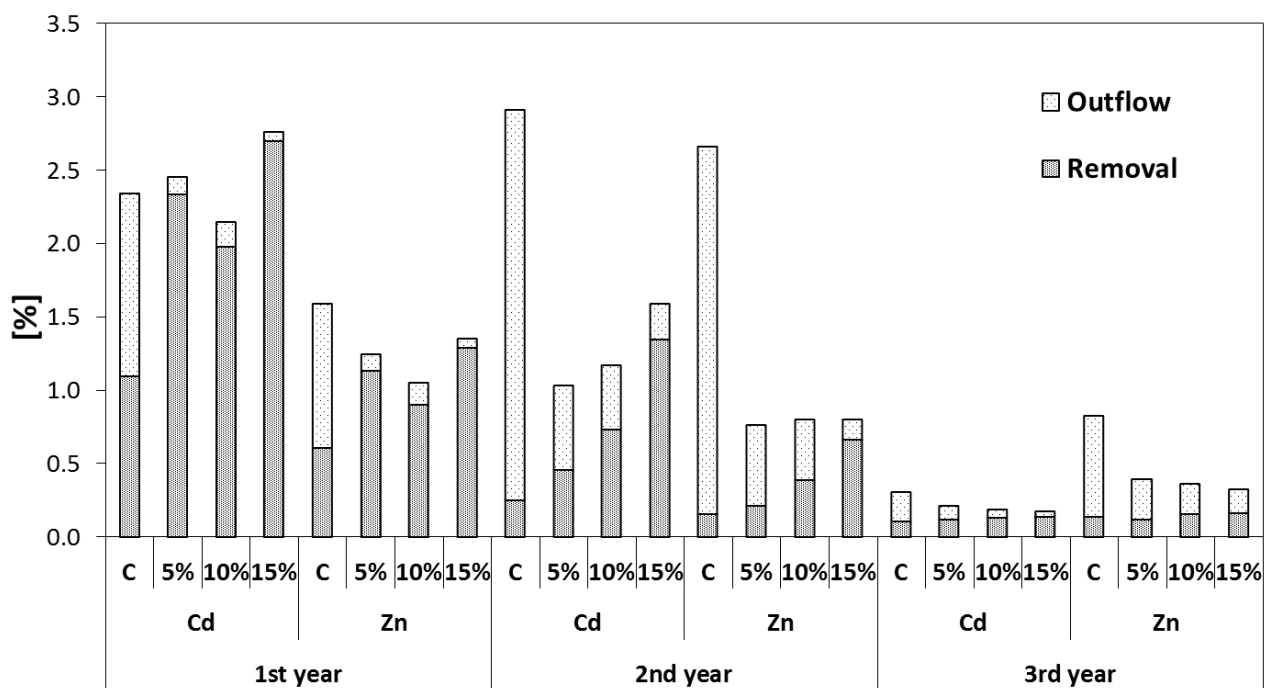
388

389 **Figure 3 Comparison of relative concentrations of leached and removed Cd, Zn by willow**

390 **plants.** Treatments were C-control, 5, 10 and 15% of applied biochar.

391 **3.6   The changes in soil physicochemical characteristics and element distribution after**

392   **three years of the experiment**

393 Figure 4 showed the changes in heavy metal contents in different soil fractions. The highest

394 contents of Cd and Zn were in the soluble fraction in heavily contaminated soil. This is

395 confirmed by Pustišek et al. (1996), who described different distribution of Cd, Zn and Pb in

396 artificially contaminated and natural soils. In natural soils, Cd and Zn are mainly bound onto Fe

397 / Mn oxides (reducible fraction) and silicates, respectively, whereas in contaminated soil, these

398 elements are distributed mainly in easily soluble fraction. Our results revealed decreasing Cd

399 and Zn content in the soluble fraction with increasing biochar rates after three years of willow

400 growth. The cadmium content decreased by 4, 11 and 12% while Zn content decreased by 3.5 6

401 and 7.5% at treatments with 5, 10 and 15% of applied biochar into soil. After 3-years of the

402 experiment, cadmium was predominately transferred onto Mn / Fe oxides. Zinc accumulation

403 was higher in the reducible and oxidizable fraction. Usually, in non-contaminated soil, lead is

404 bound onto oxidizable fraction, silicates and sulphides (Pustišek et al., 1996). In contaminated

405 soil, lead distribution was mainly in association with carbonates and the reducible (Mn / Fe

406 oxides) fraction (Stouraiti et al., 2000) or exchangeable fraction (Pustišek et al., 1996). In our

407 case, lead was mainly bound to the Mn / Fe oxides and there were only slight changes in Pb

408 distribution within the observed soil fractions. The lead affinity to Mn / Fe oxides has already

409 been described (McKenzie, 1980; Vaněk et al., 2008), and in our case, there was a high

410 concentration of iron and manganese in the experimental soil (6.0 g kg^{-1}, 3.2 g kg^{-1},

411 respectively). Moreover, the portion of organically bound lead increased by 2% in the treatment

412 with 15% of applied biochar and residual lead increased by 3% in the same case compared to

413   the original soil. Liang et al. (2014) applied 5% of dairy-manure derived biochar into neutral

414   pH contaminated soils at a rate of 5% and observed similar transformation in the fate of lead.

415
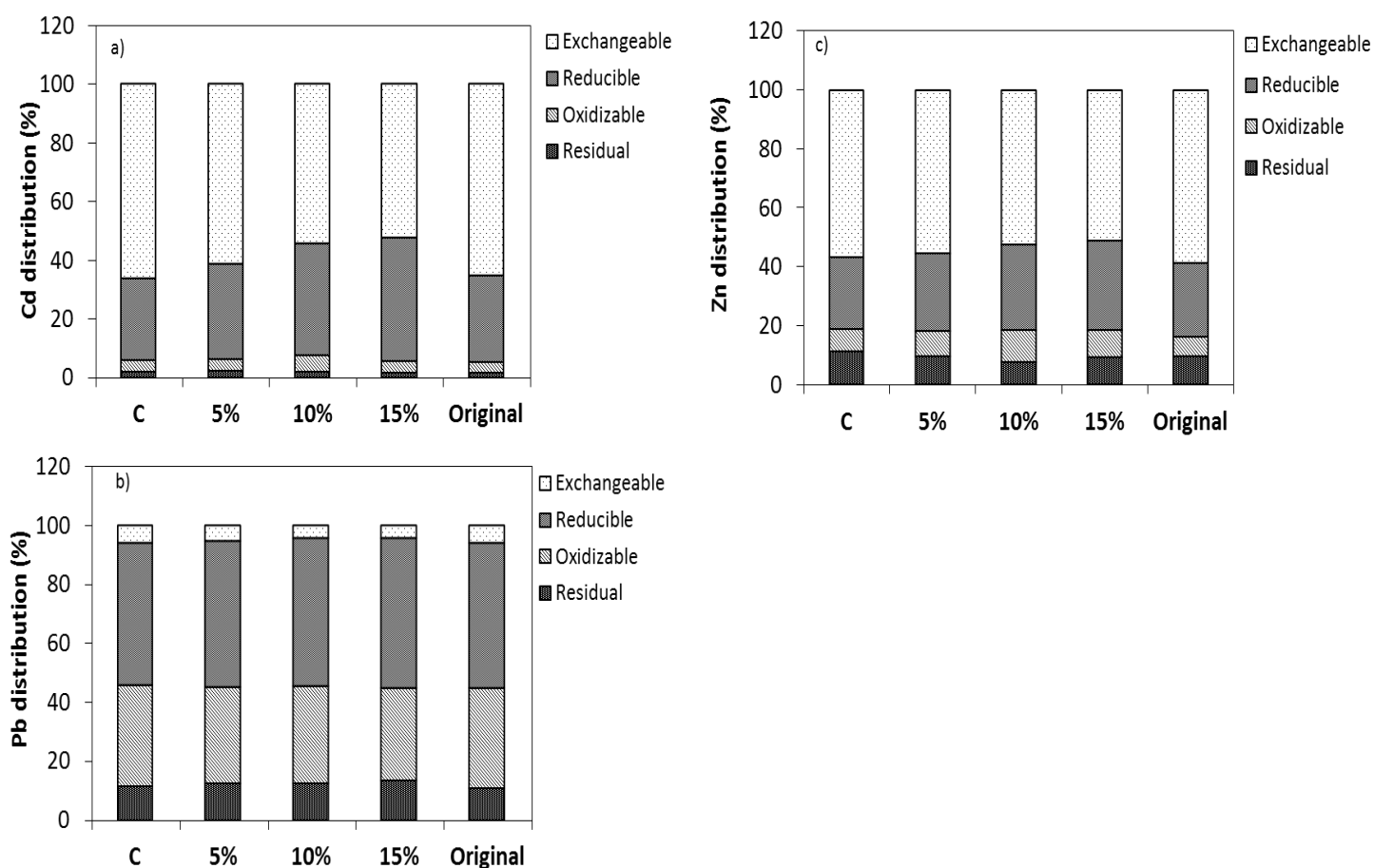
Figure 4 Distribution of cadmium, lead and zinc in soil fractions after three years of the experiment. (C-control; 5, 10 and 15% (w / w) treatments amended by different rates of biochar; Original – soil before experiment set up)

The effect of biochar applications on the main soil properties at the end of experiment is summarized in Table 3. Several studies describe increases in soil CEC as an effect of biochar (Liang et al. 2006), although no significant differences in CEC levels when we compared the beginning and termination soil CEC contents in this experiment. Electrical conductivity of soil was low, however increased with increasing dose of biochar. The end value of pH was similar, although lower at treatments with higher dose of applied biochar (10 and 15%). The pH of leachate during three years of vegetative growth (Figure 2) suggested an increase in soil pH. This alkaline effect of biochar has already explained by finding of Yuan et al. (2011). The X-

21

429   ray diffraction spectra and the content of carbonates in biochars suggested that carbonates were

430   the major alkaline components in the biochars generated at the high temperature. As a result,

431   while we consider that the pH changing mechanism by biochar similar to lime, for the stability

432   of pH it would be necessary to apply biochar as regularly as lime.

433   **Table 3 pH value, electric conductivity and cation exchange capacity of soil at individual**

434   **treatments at the beginning and the end of the experiment.**

Treatment	pH		EC (μS cm^{-1})		CEC (mmol$_+$ kg^{-1})
	entry	final	entry	final	final
C	5.66±0.07 [a]	5.35±0.02 [a]	45±2.8 [a]	94.8±14 [a]	160±6.4 [a]
5%	5.79±0.05 [a]	5.33±0.02 [a]	101±2.9 [ab]	135±19 [ab]	148±18 [a]
10%	5.98±0.03 [ab]	5.11±0.03 [b]	133±3.0 [b]	218±8.0 [bc]	154±21 [a]
15%	6.33±0.13 [b]	5.10±0.05 [b]	85±3.1 [ab]	257±40 [bc]	140±20 [a]

435   Treatments were C-control, 5, 10 and 15% of applied biochar. The values represent the means

436   (±SE) of data obtained in the experiment (n=5). Different letters and fonts indicate significantly

437   different values (*P*<0.05) in each year x treatment by Tukey's post-hoc test.

438   **4   Conclusions**

439   In first two seasons of a three-year pot experiment, biochar applications increased biomass

440   yield, decreased Cd, Pb and Zn contents in leachates and significantly improved the

441   phytoextraction potential of *Salix smithiana,* in heavily contaminated alluvium soil. In the last

442   season, Cd and Zn were remobilized, which resulted in limited plant growth and substantial

443   phytotoxicity symptoms. Increasing the rate of biochar application resulted in an increased pH

444   of the sampled leachate. However in third year, the leachate and soil pH decreased. The most

445   crucial factor that limited biochar effectivity on the heavy metal reduction was pH. At the end

446   of the experiment, Cd and Zn transfer from the exchangeable fraction into the reducible

447   fraction was demonstrated by using sequential extraction procedures in the amended

448   treatments. However, due to extremely high contamination of soil and the prevailing content of

449 these elements in the exchangeable fraction, the current transfer was not sufficient for Cd and

450 Zn immobilization. Therefore, although this preliminary investigation shows potential for this

451 technique in the remediation of soils contaminated with heavy metals, it is recommended that

452 further observation and evaluation of the efficiency and usefulness of biochar application into

453 heavily contaminated alluvium soil is required.

454

455 **Acknowledgment:**

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459 **5   References**

460 Ahmad, M., Rajapaksha, A. U., Lim, J. E., Zhang, M., Bolan, N., Mohan, D., Ok, Y. S., 2014.

461   Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere.

462   99, 19-33.

463 Atkinson, C. J., Fitzgerald, J. D., Hipps, N. A., 2010. Potential mechanisms for achieving

464   agricultural benefits from biochar application to temperate soils: a review. Plant Soil. 337(1-

465   2), 1-18.

466 Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste

467   compost amendments on mobility, bioavailability and toxicity of inorganic and organic

468   contaminants in a multi-element polluted soil. Environ. Pollut.. 158 (6). 2282-2287.

469 Borůvka, L., Huan − Wei, Ch., Kozák, J., Krištoufková, S., 1996. Heavy Contamination of Soil

470   with Cadmium, Lead and Zinc in the Alluvium of the Litavka River. Rostl. Výr. 42 (12),

471   543-550.

472 Borůvka, L., Vácha, R. 2016. Litavka river alluvium as a model area heavily polluted with

473   potentially heavy metals, *in* J.-L.Morel et al., (Eds.), Phytoremediation of Metal -

474   Contaminated Soils, Netherland: Springer, pp. 267 − 298.

475 Bradl, H.B., 2004. Adsorption of heavy metal ions on soils and soils constituents. J. Colloid.

476   Interface Sci. 277 (1), 1-18.

477 Brunauer, S., Emmett, P. H., Teller, E., 1938. Adsorption of gases in multimolecular layers. J.

478   Am. Chem. Soc. 60 (2), 309-319.

479 Cao, X., Ma, L., Gao, B., Harris, W., 2009. Dairy-manure derived biochar effectively sorbs

480   lead and atrazine. Environ. Sci. Technol.. 43 (9), 3285–3291.

481 Ciccu, R., Ghiani, M., Serci, A., Fadda, S., Peretti, R., Zucca, A., 2003. Heavy metal

482   immobilization in the mining-contaminated soils using various industrial wastes. Miner.

483   Eng. 16, 187–192.

484 ČSN EN 14775. Solid biofuels - Determination of ash content. 1.5.2010. Prague ÚNMZ [In
485   Czech].

486 Doerge, T.A., Garden, H., 1984. Reacidification of Two Lime Amended Soils in Western
487   Oregon1. Soil Sci. Soc. Am. J. 49(3). DOI: 10.2136/sssaj1985.03615995004900030031

488 European Environmental Agency, Progress in management of contaminated sites, [online,
489   update: 2015.09.04].

490 Evidence of contaminated places, Ministry of Environment, Czech Republic [In Czech, online].

491 Fellet, G., Marmiroli, M., Marchiol, L., 2014. Elements uptake by metal accumulator species
492   grown on mine tailings amended with three types of biochar. Sci. Total Environ. 468, 598-
493   608.

494 Fischerová, Z., Tlustoš, P., Száková, J., Šichorová, K., 2006. A comparison of
495   phytoremediation capability of selected plant species for given trace elements. Environ.
496   Pollut. 144(1), 93-100.

497 Houben, D., Evrard, L., Sonnet, P., 2013. Mobility, bioavailability and pH-dependent leaching
498   of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere.
499   92(11), 1450-1457.

500 Iqbal, M., Puschenreiter, M., Oburger, E., Santner, J., Wenzel, W. W., 2012. Sulfur-aided
501   phytoextraction of Cd and Zn by *Salix smithiana* combined with in situ metal
502   immobilization by gravel sludge and red mud. Environ. Pollut. 170, 222-231.

503 Jensen, J.K., Holm, P.E., Nejrup, J., Larsen, M.B., Borggaard, O.K., 2009. The potential of
504   willow for remediation of heavy metal polluted calcareous urban soils. Environ. Pollut. 157,
505   931–937.

506 Jiang, J., Xu, R., Jiang, T., Li, Z., 2012. Immobilization of Cu(II), Pb(II) and Cd(II) by the
507   addition of rice straw derived biochar to a simulated polluted Ultisol. J. Hazard. Mater. 229-
508   230, 145-150.

25

509   Jordan, C.F., 1968. A simple, tension-free lysimeter. Soil Sci. 105 (2), 81-86.

510   Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants, third ed., Boca

511   Raton, CRC Press.

512   Kacálková, L., Tlustoš, P., Száková, J., 2009. Phytoextraction of cadmium, copper, zinc and

513   mercury by selected plants. Plant Soil Environ. 55(7), 295-304.

514   Kubešová, M., Kučera, J., 2010. Validation of k0 standardization method in neutron activation

515   analysis – The use of Kayzero for Windows programme at the nuclear physics institute, Řež.

516   Nuclear Instruments and Methods in Physics Research, Section A: Accelerators,

517   Spetrometers, Detectors and Associated Equipment. 622, 403–406.

518   Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil

519   using amendments–a review. Waste Manage. 28 (1), 215-225.

520   Laird, D. A., Fleming, P., Davis, D. D., Horton, R., Wang, B., Karlen, D. L., 2010. Impact of

521   biochar amendments on the quality of a typical Midwestern agricultural soil. Geoderma.

522   158(3), 443-449.

523   Lettens, S., Vandecasteele, B., De Vos, B., Vansteenkiste, D., Verschelde, P., 2011. Intra- and

524   inter-annual variation of Cd, Zn, Mn and Cu in foliage of poplars on contaminated soil. Sci.

525   Total Environ. 409. 2306–2316.

526   Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'neill, B., Neves, E., 2006.

527   Black carbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J.. 70(5), 1719-

528   1730.

529   Liang, Y., Cao, X., Zhao, L., Arellano, E., 2014. Biochar-and phosphate-induced

530   immobilization of heavy metals in contaminated soil and water: implication on simultaneous

531   remediation of contaminated soil and groundwater. Environ. Sci. Pollut. Res. 21(6), 4665-

532   4674.

533   Lu, H., Li, Z., Fu, S., Méndez, A., Gascó, G., Paz-Ferreiro, J., 2014. Can biochar and

534   phytoextractors be jointly used for cadmium remediation? PloS One. 9(4), e95218.

535   McKenzie, R. M., 1980. The adsorption of lead and other heavy metals on oxides of manganese

536   and iron. Soil Res. 18(1), 61-73.

537   Meers, E., Vandecasteele B., Ruttens, A., Vangronsveld, J., Tack, F.M.G., 2007. Potential of

538   five willow species (Salix spp.) for phytoextraction of heavy metals. Environ. Exp. Bot. 60,

539   57–68.

540   Mertens, J., Luyssaert, S., Verheyen, K., 2005. Use and abuse of trace metal concentrations in

541   plant tissue for biomonitoring and phytoextraction. Environ. Pollut. 138(1), 1-4.

542   Němeček, J., Vácha, R., Podlešáková, E., 2010. Assessment of soil contamination in the Czech

543   Republic. Research Institute for Soil and Water Conservation, first ed., Prague. [In Czech].

544   Novak, J. M., Ippolito, J. A., Lentz, R. D., Spokas, K. A., Bolster, C. H., Sistani, K., Johnson,

545   M. G., 2016. Soil health, crop productivity, microbial transport, and mine spoil response to

546   biochars. BioEnergy Res. 9(2), 454-464.

547   Paz-Ferreiro, J., Lu, H., Fu, S., Méndez, A., & Gascó, G., 2014. Use of phytoremediation and

548   biochar to remediate heavy metal polluted soils: a review. Solid Earth. 5(1), 65.

549   Pulford, I.D., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by trees –

550   A review. Environ. Int. 29, 529–540.

551   Puschenreiter, M., Wittstock, F., Friesl-Hanl, W., Wenzel, W.W., 2013. Predictability of the Zn

552   and Cd phytoextraction efficiency of a *Salix smithiana* clone by DGT and conventional

553   bioavailability assays. Plant Soil. 369(1-2), 531-541.

554   Quevauviller, P., 1998. Operationally defined extraction procedures for soil and sediment

555   analysis—I. standardization. Trac-Trends. Anal. Chem.17, 289–298.

556   Rees, F., Sterckeman, T., Morel, J.L., 2016. Root development of non-accumulating and

557   hyperaccumulating plants in metal-contaminated soils amended with biochar. Chemosphere.

558   142, 48-55.

559   Rinklebe, J., Shaheen, S. M., Frohne, T., 2016. Amendment of biochar reduces the release of

560   toxic elements under dynamic redox conditions in a contaminated floodplain soil.

561   Chemosphere. 142, 41-47.

562   Robinson, B.H., Mills, T.M., Petit, D., Fung, L.E., Green, S.R., Clothier, B.E., 2000. Natural

563   and induced cadmium-accumulation in poplar and willow: Implications for

564   phytoremediation. Plant Soil. 227, 301–306.

565   Schwartz, R., Gerth, J., Neumann-Hensel, H., Förstner, U., 2006. Assessment of highly

566   polluted fluvisol in the Spittelwasser floodplain based on national guideline values and

567   MNA-criteria. J. Soils Sediments. 6(3), 145-155.

568   Shen, Z., Som, A. M., Wang, F., Jin, F., McMillan, O., Al-Tabbaa, A., 2016. Long-term impact

569   of biochar on the immobilisation of nickel (II) and zinc (II) and the revegetation of a

570   contaminated site. Sci. Total Environ. 542, 771-776.

571   Stouraiti, C., Xenidis, A., Paspaliaris, I., 2002. Reduction of Pb, Zn and Cd availability from

572   tailings and contaminated soils by the application of lignite fly ash. Water Air Soil Pollut.

573   137(1-4), 247-265.

574   Street, R., Száková, J., Drábek, O., Mládková, L., 2006. The status of micronutrients (Cu, Fe,

575   Mn, Zn) in tea and Te infusions in selected samples imported to the Czech Republic. Czech.

576   J. Food Sci. 24, 62–71.

577   Tang, J., Zhu, W., Kookana, R., Katayama, A., 2013. Characteristics of biochar and its

578   application in remediation of contaminated soil. J. Biosci. Bioeng. 116(6), 653-659.

579   Tlustoš, P, van Dijk, D., Száková, J., Pavlíková D., 1994. Cd and Zn release through the

580   selected extractants. Rostl. Výr. 40, 1107–1121.

581   Tlustoš, P., Száková, J., Vysloužilová, M., Pavlíková, D., Weger, J., Javorská, H., 2007.

582   Variation in the uptake of arsenic, cadmium, lead, and zinc by different species of willows

583   Salix spp. grown in contaminated soils. Cent. Eur. J. Biol. 2(2), 254-275.

584   Trakal, L., Neuberg, M., Tlustoš, P., Száková, J., Tejnecký, V., Drábek, O., 2011. Dolomite

585   limestone application as a chemical immobilization of metal-contaminated soil. Plant Soil

586   Environ. 57, 173–179.

587   Uprety, D., Hejcman, M., Száková, J., Kunzová, E., Tlustoš, P., 2009. Concentration of trace

588   elements in arable soil after long-term application of organic and inorganic fertilizers. Nutr.

589   Cycl. Agroecosyst. 85, 241–252.

590   Ure, A.M., Quevauviller, Ph., Muntau, H., Griepink, B., 1993. Speciation of heavy metals in

591   soils and sediments. An account of the improvement and harmonization of extraction

592   techniques undertaken under the auspices of the BCR of the commission of the European

593   Communities. Int. J. Environ. Anal. Chem. 51, 135–151.

594   Uzinger, N., Anton, A., 2008. Chemical stabilization of heavy metals on contaminated soils by

595   lignite. Cereal Res. Commun. 36, 1911-1914.

596   Vaněk, A., Borůvka, L., Drábek, O., Mihaljevič, M., Komárek, M. 2005. Mobility of lead, zinc

597   and cadmium in alluvial soils heavily polluted by smelting industry. Plant Soil Environ. 51,

598   316–321.

599   Vaněk, A., Ettler, V., Grygar, T., Borůvka, L., Šebek, O., Drábek, O., 2008. Combined

600   chemical and mineralogical evidence for heavy metal binding in mining-and smelting-

601   affected alluvial soils. Pedosphere. 18(4), 464-478.

602   Vondráčková, S., Tlustoš, P., Hejcman, M., Száková, J., 2015. Regulation of macro, micro, and

603   toxic element uptake by *Salix× smithiana* using liming of heavily contaminated soils. J.

604   Soils Sediments. 1-12.

605   Vysloužilová, M., Tlustoš, P., Száková, J., Pavlíková, D., 2003. As, Cd, Pb and Zn uptake by

606   *Salix* spp. clones grown in soils enriched by high loads of these elements. Plant Soil

607   Environ. 49, 191−196.

608   Wang, S., Gao, B., Zimmerman, A.R., Li, Y., Mad, L., Harris, W.G., 2015. Physicochemical

609   and sorptive properties of biochars derived from woody and herbaceous biomass.

610   Chemosphere. 134, 257−262.

611   Wu, G., Kang, H., Zhang, X., Shao, H., Chu, L., Ruan, C., 2010. A critical review on the bio-

612   removal of hazardous heavy metals from contaminated soils: issues, progress, eco-

613   environmental concerns and opportunities. J. Hazard. Mater. 174(1), 1-8.

614   Yuan, J. H., Xu, R. K., Zhang, H., 2011. The forms of alkalis in the biochar produced from

615   crop residues at different temperatures. Bioresour. Technol. 102(3), 3488-3497.

616   Zhang, G., Zhang, Q., Sun, K., Liu, X., Zheng, W., Zhao, Y., 2011. Sorption of simazine to

617   corn straw biochars prepared at different pyrolytic temperatures. Environ. Pollut. 159, 2594−

618   2601.

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Research article

Utilization of biochar and activated carbon to reduce Cd, Pb and Zn phytoavailability and phytotoxicity for plants

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ABSTRACT

In the present study, the content of risk elements and content of free amino acids were studied in spinach (*Spinacia oleracea* L.) and mustard (*Sinapis alba* L.) subsequently grown on uncontaminated and contaminated soils (5 mg Cd/kg, 1000 mg Pb/kg and 400 mg Zn/kg) with the addition of activated carbon (from coconut shells) or biochar (derived from local wood residues planted for phytoextraction) in different seasons (spring, summer and autumn). The results showed that activated carbon and biochar increased biomass production on contaminated site. Application of amendments decreased Cd and Zn uptake by spinach plants. Mustard significantly increased Pb accumulation in the biomass as well in subsequently grown autumn spinach. Glutamic acid and glutamine were major free amino acids in leaves of all plants (15–34% and 3–45%) from total content. Application of activated carbon and biochar increased content of glutamic acid in all plants on uncontaminated and contaminated soils. Activated carbon and biochar treatments also induced an increase of aspartic acid in spinach plants. Biochar produced from biomass originated from phytoextraction technologies promoted higher spinach biomass yield comparing unamended control and showed a tendency to reduce accumulation of cadmium and zinc and thus it is promising soil amendment.

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1. Introduction

Under the European Union (EU) Thematic Strategy for Soil Protection, the European Commission identified soil contamination; occurrence of 342 000 polluted sites was reported, most commonly polluted with heavy metals and mineral oil (Panagos et al., 2013). Key factor of elements toxicity or deficiency for plants is their plant-available concentration in soil (Alloway, 2012; Puga et al., 2015). Considering the reduction of hazardous elements bioavailability, the promising *in-situ* remediation method is the fixation of such elements by additives applied to the soil (Guo et al., 2006). These materials predominantly form complexes, precipitates with risk elements, and thus reduce their mobility (Basta and McGowen, 2004; Kumpiene et al., 2008).

Among a wide scale of available soil stabilization materials, inorganic and organic substances based on coal-like materials or combustion by-products have been investigated, e.g. coal or bio-

fuel fly ashes (Clark et al., 2001) or lignite (Uzinger and Anton, 2008). In recent years the investigation was focused on biochar, stable carbon-rich charred biomass and its utilization as a soil additive (Qayyum et al., 2014).

Biochar (BC) has been evidenced to act as an efficient sorbent of various contaminants, organic and inorganic, because of its huge surface area and specific structure (Tang et al., 2013). Its sorption capacity is given by both its property and characteristics of bind element (Uchimiya et al., 2011). Mechanisms of risk element immobilization by biochar are different: i) development of hydroxides, carbonates, or phosphates, ii) precipitation (thanks to high pH of biochars, especially occurs for Pb immobilization; Wang et al., 2015), iii) electrostatic interaction between cations and functional groups (described as main mechanism for As sorption, Wang et al., 2015), iv) surface chemisorption between d-electrons of metals and π-electrons of biochar (Cao et al., 2009). Indirectly, biochar can caused risk element immobilization by increasing of pH, which increases negative charge of surface and thus increase soil affinity to cations of risk elements (Jiang et al., 2012).

Increased growth of ryegrass on contaminated acidic soil was observed, while metal uptake into shoots was reduced after biochar

application. However, decreasing growth occurs on alkaline contaminated soil where biochar dose excessing 0.5% was applied (Rees et al., 2015).

Plant response to biochar application was observed by Macdonald et al. (2014). They observed strong relationship between biochar effect on plant yield and soil type where biochar was applied. The significant plant response was observed after biochar application into acidic soils in comparison to alkaline ones, but high contrasts occur between different acidic soil types. Negative effect of biochar on plant growth was observed in acidic Arenosol, while high biomass yield was achieved in acidic Ferrosol.

Gregory et al. (2014) observed increasing ryegrass shoot growth after application of 1 and 2% of wood biochar into acidic soil. They also showed higher arsenic extraction by ryegrass shoots at amended treatments. Authors mentioned that more studies are needed to understand the mechanisms through which these benefits are provided.

The disposal of contaminated biomass is of major concern for phytoexreaction, therefore pyrolysis of it can be suitable technique. Application of biochar from contaminated biomass into soil was described by Jones and Quilliam (2014) and Evangelou et al. (2015).

Activated carbon (AC) is the most commonly used sorbent and is characterized by enhanced surface area due to thermal or chemical activation. This material successfully adsorbs especially organic substances (Bucheli and Gustafsson, 2000). Study of Kadirvelu et al. (2001) confirmed the ability of AC to bind heavy metals. There is lack of studies comparing biochar and AC sorption ability; however Hale et al. (2011), observed comparable abilities of these materials to adsorb organic substances.

There are studies describing BC or AC ability to reduce bioavailability of risk elements, but we were not able to find answers to the following questions:

Will be the effect of BC produced from local contaminated wood, without any surface activation comparable to purchased AC in term of reduction of risk elements bioavailability?

Can we observe any changes in metabolites reflecting external influences in plants, which are grown on soil amended by BC or AC?

Will the plants respond to BC or AC application also on uncontaminated soil?

Hypothesis and objectives of our study:
Hypothesis

1) Biochar produced from local wood residue will have similar ability to immobilize risk elements compare to activated carbon.
2) Plants grown in soil amended with biochar and activated carbon change some metabolites in plants.
3) The carbonaceous amendments will improve plant growth on contaminated soil in higher extend comparing uncontaminated one.

First aim of our study was to compare the ability of purchased AC produced from coconut shells with BC produced from local wood residues of phytoextraction technology to reduce accumulation of risk elements by spinach and mustard plants. The plants will be planted in rotation spring spinach-mustard-autumn spinach to observe amendments influence in longer term.

Secondly, the target was to show the direct and subsequent molecular plant response to BC or AC application into soil.

The third aim was to compare the effectiveness of applied BC or AC on contaminated and uncontaminated soils.

2. Materials and methods

2.1. Biochar and activated carbon characterization

Biochar was derived from willow biomass by pyrolysis. Willows were harvested on medium contaminated side of old smelter area of Příbram locality - short-rotation coppice plantations (49°42′24″ N, 13°58′32″ E; Zárubová et al., 2015). The process was conducted in a muffle furnace in the inert nitrogen atmosphere (nitrogen flow 1 m³/h), at atmospheric pressure and retention time of 30 min. The process followed final temperature of 500 °C. It was characterized by: ash content = 13%, pH_{CaCl2} = 6.9, CEC = 176 $mmol_+$/kg, SSA_{BET} = 324 m²/g, particle fraction = 5 × 6 × 0.5 mm.

Activated carbon was purchased from Erspol., Ltd. (Czech Republic) and was derived from coconut shells was characterized by: ash content = 12%, pH_{CaCl2} = 8.9, CEC = 73 $mmol_+$/kg, SSA_{BET} = 486 m²/g (activated by water steam), particle fraction = 4 × 2 × 2 mm.

Content of C was determined by using the apparatus Flash EA 1112 in the CHNS/O configuration (Thermo Fisher Scientific, USA). The element content both in BC and AC were determined by neutron activation analysis with k0 standardization (k0-INAA) using short (1 min) and long irradiation (3 h) of neutrons in the reactor LVR-15 with the heat flux density of epithermal and fast neutrons (Kubešová and Kučera, 2010). Element contents of amendments are given in Table 1. The biochar is characterized by higher content of Cd, Pb, and Zn in comparison to AC.

2.2. Soil characterization

Soil was sampled from top layer (0–30 cm) of arable land in Prague - Suchdol (Czech Republic; 50°8′8″ N, 14°22′43″ E). The type of soil was modal Chernozem: pH_{KCl} = 7.2, CEC = 258 $mmol_+$/kg, $C_{org.}$ = 1.83%, contents of Cd = 0.3 mg/kg, Pb = 37.2 mg/kg and Zn = 113 mg/kg. Soil was air-dried and homogenized.

For determination of the total content of risk elements in soils 0.5 g of soil sample was decomposed in a closed system with microwave heating in the device Ethos 1 (MLS GmbH, Germany) in a mixture of 8 ml HNO_3, 5 ml HCl and 2 ml HF. The element contents in the soil digests and extracts were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA).

2.3. Experimental design

The experiment was established at greenhouse controlled conditions. Each pot was filled with 2.5 kg of soil. Spinach (*Spinacia oleracea* L. cv. Matador) and mustard (*Sinapis alba* L.) were chosen as experimental plants, while the crop rotation was spinach - mustard - spinach. Both plants are common edible crops and good accumulators, thus the prevention of risk elements uptake is necessary. The spinach (spinach 1 = S1) was sown in March and harvested after 64 days. Mustard (M) was sown in May and harvested after 35 days. Late spinach (spinach 2 = S2) was sown in

Table 1
Total element contents of activated carbon and biochar.

	K	Ca	Mg	Fe	Cd	Pb	Zn	C	N
	g/kg				mg/kg			(% w/w)	(% w/w)
AC[a]	0.5	2.9	2.2	4.1	<0.1	nd[b]	8.3	93	0.2
BC[a]	16.1	28	–	2.8	27	282[b]	950	64	1.1

[a] Determined by INAA.
[b] Determined by X-ray fluorescence; nd: levels below detection limit.

August and harvested after 64 days. The harvest time was always before blooming. The biomass production of tested plant was evaluated.

The experiment consists of 6 treatments in 4 replications, half pots were filled with contaminated soil, the next half with uncontaminated soil (12 and 12 pots). The soil was spiked with cadmium, lead and zinc in concentration of 5, 1000 and 400 mg/kg, respectively (by dilution of $Cd(NO_3)_2 \cdot 4H_2O$; $Pb(NO_3)_2$ and $Zn(NO_3)_2 \cdot 6 H_2O$) to get similar risk elements values like at Pribram site, where the biomass for biochar production was harvested. Contaminated and uncontaminated treatments consist of i) control (with no applied BC or AC), ii) 5% of applied biochar from total mass of soil (BC 5%) and iii) 5% of applied activated carbon from total mass of soil (AC 5%) thoroughly mixed at individual pots. In each pot, five plants were grown from seeds. Pots were uniquely fertilized with 0.1 g N; 0.16 g P; 0.4 g K per 1 kg of soil (applied in the form of NH_4NO_3 and K_2HPO_4).

The fertilizers were applied to each plant before sowing.

2.4. Analyses of plant biomass

2.4.1. Analyses of elements

Total element contents in plant aboveground biomass were determined in the digests obtained by dry ashing decomposition (Street et al., 2006) and Cd, Pb and Zn contents were determined using inductively coupled plasma with optical emission spectroscopy (ICP-OES, Agilent 720, Agilent Technologies Inc., USA). Contents of Ca, Mg and K were determined by flame atomic absorption spectroscopy (FAAS, VARIAN SpectrAA-280, Australia). Aliquots of the certified reference material RM NCS DC 73350, poplar leaves (purchased from Analytika, CZ), were mineralised under the same conditions for quality assurance.

2.4.2. Analysis of free amino acids

The content of free amino acids (AAs) was determined after their derivatisation by EZ:faast set (Phenomenex, USA). In this experiment, 1.0 g samples of fresh biomass were extracted by incubation in 15 mL of methanol + redistilled H_2O (7:3, v/v) for 24 h. Derivatisation of free AAs was performed according to the method of Neuberg et al. (2010) and content was measured by GC-MS using a Hewlett Packard 6890N/5975 MSD (Agilent Technologies, USA). Samples were separated on a ZB-AAA 10 m × 0.25 mm AA analysis GC column using a constant carrier gas (He) flow (1.1 mL/min). The oven temperature program was as follows: initial temperature of 110 °C, increased by 30 °C min^{-1} to 320 °C. The temperature of the injection port was 280 °C. A 1.5 μL aliquot of sample was injected in split mode (1:15, v/v). The MS conditions were as follows: MS source 240 °C, MS quad 180 °C, auxiliary 310 °C, electron energy 70 eV, scan m/z range 45–450 and sampling rate 3.5 scan s^{-1} (Pavlík et al., 2012). The complex of free amino acids was determined in biomass of S1, M and S2 (alanine, glycine, valine, leucine, isoleucine, threonine, serine, proline, asparagine, aspartic acid, methionine, hydroxyproline, glutamic acid, γ-amino-n-butyric acid, phenylalanine, thioproline, α-aminoadipic acid, glutamine, ornithine, histidine, tyrosine, tryptophane, cystine and glycylproline.

2.5. Statistics

All statistical analyses were performed using the Statistica 12.0 (www.statsoft.com) and CANOCO 4.5 (ter Braak and Smilauer, 2002) programs. All data were checked for homogeneity of variance and normality (Levene and Shapiro-Wilk tests). Collected data were evaluated by two-way ANOVA with multivariate F value (Wilks' lambda). A two-way ANOVA was applied to identify the

effect of treatments, contamination and their interactions as independent variables, and yield of biomass, contents of free AAs and elements as dependent variables. A two-way ANOVA was followed by post-hoc comparison Tukey test ($P < 0.05$). The relationship between contents of element and free AAs and the relationship between total contents of free AAs and selected free AAs were evaluated using linear regression. Principal component analysis (PCA), using the CANOCO 4.5 program, was applied to all collected data together (contents of free AAs and elements in the spinach and mustard as well as biomass yield). We used standardised "species data" because data of different character and units were analysed together. The PCA was used to make visible correlations between all analysed data and similarities of the different treatments. The results were visualised in the form of a bi-plot ordination diagram using the CanoDraw program.

3. Results

3.1. Yield of biomass

Fig. 1 reported yield of aboveground biomass of S1, M and S2. It was showed that the dry biomass of S1 increased with AC and BC application, by 55% and 114% in comparison to control at uncontaminated treatment. The positive effect of amendments was more significant at contaminated treatments, where S1 yield was higher by 308% at AC and by 359% at BC in comparison to control (Fig. 2).

The highest biomass yield was determined at S2 (autumn). At contaminated treatments, application of AC and BC increased the yield 1.5-fold and 2.6-fold respectively in comparison to control. The same effect, while AC and BC were applied was observed at uncontaminated soil, while the yield increased by 197% and 332% for AC and BC, respectively. Concerning S2, results revealed yield increased by 27% at BC treatment, in comparison to uncontaminated treatment. M was characterized by yield decrease (by 44%) at contaminated control treatment. The contamination negatively but not significantly affected the mustard yield at AC and BC treatment by 0.7% and 4% respectively. However, there is significantly higher yield at AC and BC treatments in comparison to control on contaminated soil (by 64% and 121% respectively).

3.2. Content of elements

Significant differences were observed between risk elements content in all plants at contaminated and uncontaminated treatments. Highest accumulation of Cd and Pb was determined in contaminated M (Cd − 1.4-fold higher in comparison to S1, and 1.8-fold higher than in S2, Pb − 28-fold higher than in S1, and 3-fold higher than in S2). Content of Zn was highest in contaminated S1 (21-fold higher than M and 13-fold higher than S2). AC application decreased accumulation of Cd (by 57% on average), Pb (by 44% on average) and Zn (by 44% on average) on uncontaminated soil in all plants. Application of BC decreased accumulation Cd (by 20% on average) and Zn (by 28% on average) on contaminated soil in all plants (Fig. 3).

To evaluate the BC and AC effect on risk element immobilization and prevention their phytovailability, the most significant differences were observed only in S2. In plant tissues of S2, there were significantly higher contents of Cd and Pb at control. Content of Zn did not differ at control and BC treatment, most probably due to of high content of this element in BC. Because the significance was observed in S2, the slow-acting effect of carbonaceous amendments is evident (Fig. 4).

According to results of PCA (Figs. 5−7) contents of Cd, Pb and Zn positively correlated with each other. This relationship was significant for Cd and Pb (S1: R = 0.65, M: R = 0.94, and S2: R = 0.98,
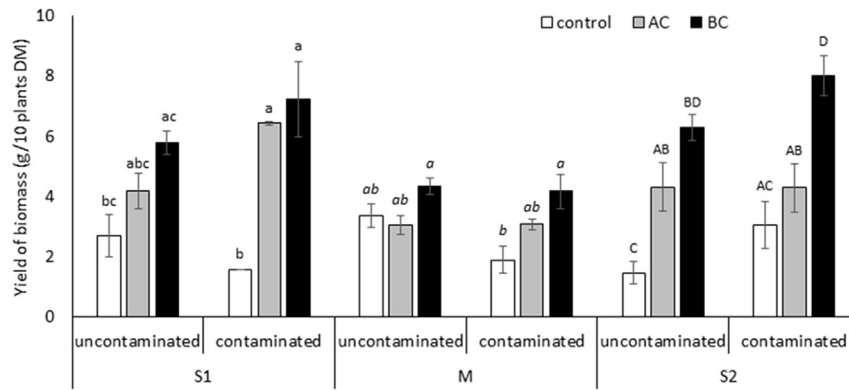
Fig. 1. Biomass yield (g dry matter of 10 plants) of spinach and mustard grown in uncontaminated and contaminated soils. The values represent the means (±SE) of data obtained in the experiment (n = 4). Different letters and fonts indicate significantly different values ($P < 0.05$) between yield and treatment × contamination. The differences were determined by post-hoc Tukey's test.
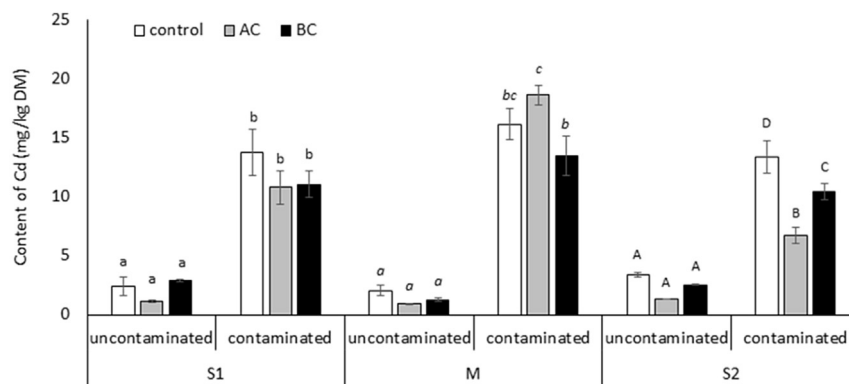
Fig. 2. Content of Cd in biomass of spinach and mustard. The values represent the means (±SE) of data obtained in the experiment (n = 4). Different letters indicate significantly different values ($P < 0.05$) between content of Cd and treatment × contamination. The differences were determined by post-hoc Tukey's test.
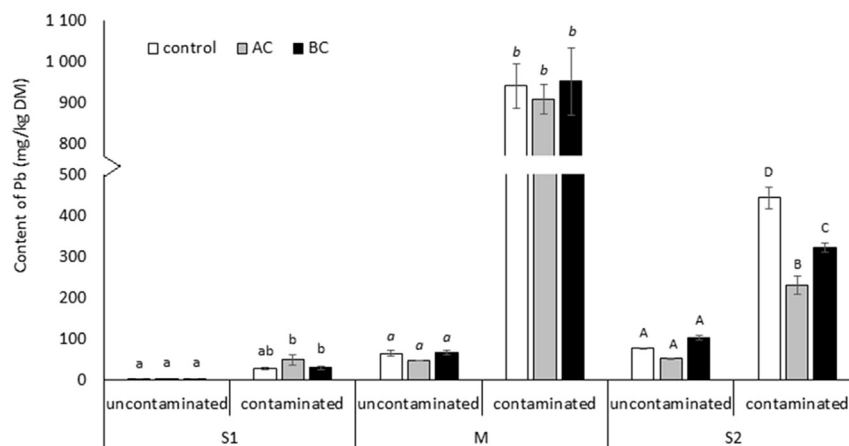
Fig. 3. Content of Pb in biomass of spinach and mustard. The values represent the means (±SE) of data obtained in the experiment (n = 4). Different letters indicate significantly different values ($P < 0.05$) between content of Pb and treatment × contamination. The differences were determined by post-hoc Tukey's test.

$P < 0.001$), Cd and Zn (S1: R = 0.97, M: R = 0.93, and S2: R = 0.80, $P < 0.001$) and Pb and Zn (S1: R = 0.71, M: R = 0.93, and S2: R = 0.81, $P < 0.001$) in all plants.

No differences were observed in soil pH between control and amended treatments at the end of experiment. The pH values at the end were lower by 0.5 in comparison to original soil.
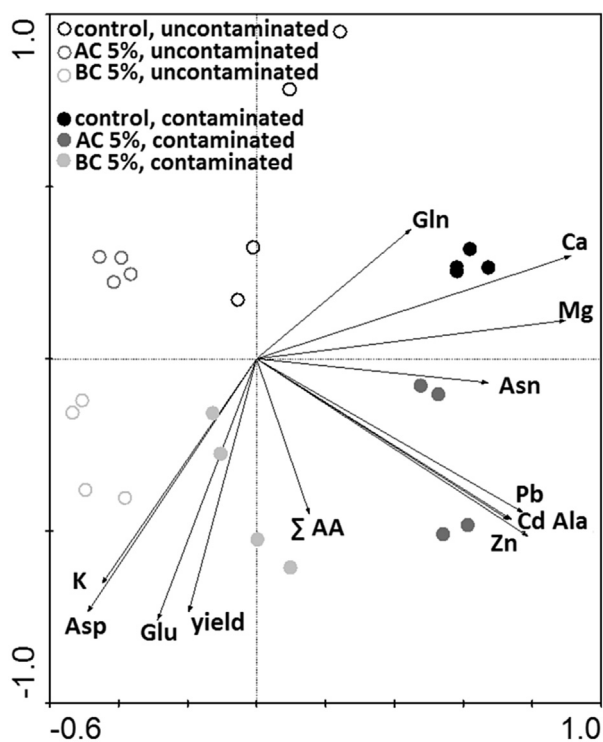
3.3. Free amino acids

The most abundant free AAs in biomass of S1, M and S2 were glutamic acid (Glu), glutamine (Gln), aspartic acid (Asp), asparagine (Asn) and alanine (Ala) (Table 2). Mentioned AAs represented approximately 73%, 35% and 83% of total content AAs in S1, M and S2, respectively.
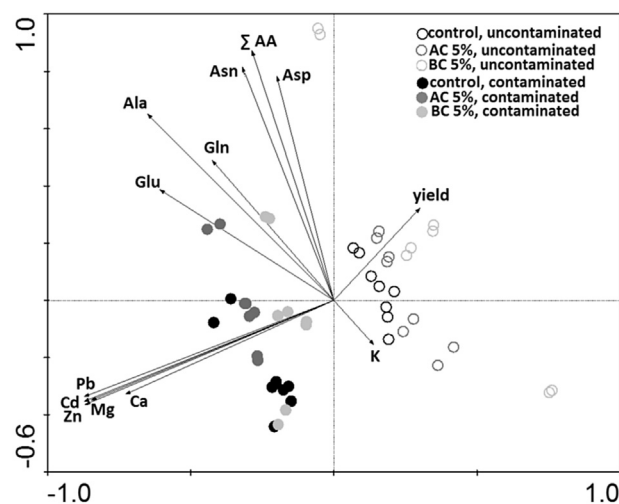
93
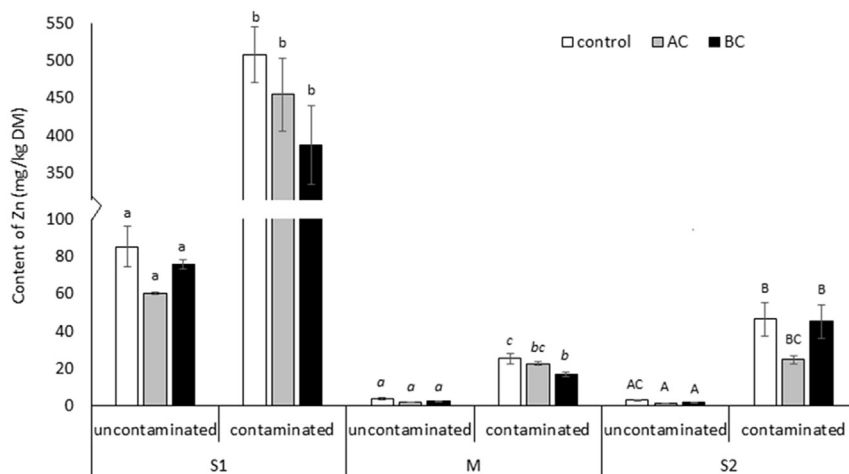
Fig. 4. Content of Zn in biomass of spinach and mustard. The values represent the means (±SE) of data obtained in the experiment (n = 4). Different letters indicate significantly different values (P < 0.05) between content of Zn and treatment × contamination. The differences were determined by post-hoc Tukey's test.

Fig. 5. Ordination diagram showing the results of PCA analysis with selected parameters in uncontaminated and contaminated treatments of spinach 1. Parameters abbreviations: yield - yield of biomass, Σ AA - total content of free amino acids, Cd - total content of Cd, Pb - total content of Pb, Zn - total content of Zn, Ca - total content of Ca, K - total content of K, Mg - total content of Mg, Asp - concentration of free aspartic acid, Asn - concentration of free asparagine, Glu - concentration of free glutamic acid, Gln - concentration of free glutamine, Ala - concentration of free alanine.

Fig. 6. Ordination diagram showing the results of PCA analysis with selected parameters of uncontaminated and contaminated treatments of mustard. Parameters abbreviations: yield - yield of biomass, Σ AA - total content of free amino acids, Cd - total content of Cd, Pb - total content of Pb, Zn - total content of Zn, Ca - total content of Ca, K - total content of K, Mg - total content of Mg, Asp - concentration of free aspartic acid, Asn - concentration of free asparagine, Glu - concentration of free glutamic acid, Gln - concentration of free glutamine, Ala - concentration of free alanine.

Glutamic acid was AA with the highest contents in S1 and M (34% and 15% from total contents of free AAs, respectively). In S2 Gln (45%) and Glu (20%) formed the largest parts from total contents of free AAs. Another AA with high contents in biomass were Asp - S1 18.5%, M 10% and S2 12% from total contents of free AAs. Contents of Gln in S1 and M were lower than in S2 (14% and 3% from total contents of free AAs, respectively). Alanine was more abundant in biomass of M (6% from total contents of free AAs) than S1

and S2 (3.5% and 2% from total contents of free AAs, respectively). The lowest contents from selected AAs were for Asn - S1 3%, M 1% and S2 4% from total contents of free AAs.

The total contents of free AAs were decreased by contamination on control and BC treatments for all plants (control by 30% and BC by 24% on average). Contamination on AC treatment increased total contents of AAs in S1 and M (by 74% and 36%, respectively) and decreased in S2 (by 41%). In all tested plants was recorded positive relationship of total contents of free AAs and Asn (S1: R = 0.81, M: R = 0.77, and S2: R = 0.61, P < 0.001).

Contents of selected AAs were characterized by four different effects of amendments (Table 2). (1) AA contents on uncontaminated and contaminated soil were increased by AC application. This effect was found in S1 for Glu, Asp, and Ala and in S2 for Glu, Asp, Asn and Ala. (2) Decrease of AA contents on uncontaminated and contaminated soil was found only in S2 for Gln. (3) Results showed decrease of AA content on uncontaminated soil and increase of AA
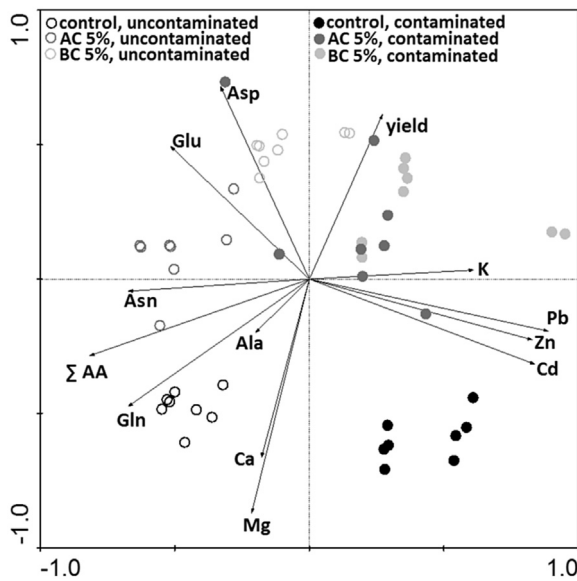
Fig. 7. Ordination diagram showing the results of PCA analysis with selected parameters in uncontaminated and contaminated treatments of spinach 2. Parameters abbreviations: yield - yield of biomass, Σ AA - total content of free amino acids, Cd - total content of Cd, Pb - total content of Pb, Zn - total content of Zn, Ca - total content of Ca, K - total content of K, Mg - total content of Mg, Asp - concentration of free aspartic acid, Asn - concentration of free asparagine, Glu - concentration of free glutamic acid, Gln - concentration of free glutamine, Ala - concentration of free alanine.

contents on contaminated soil - in S1 for Asn and Gln and in M for Glu, Asn, Gln and Ala. (4) The opposite trend was confirmed only in M for Asp.

The same effects as for AC application were reported for BC application. Asp and Glu (in S1 and S2) and Asn and Ala in M for Asn and Ala were increase by BC application. The second effect was found in S1 for Asn and Gln and in S2 only for Gln. Decrease of AA contents on uncontaminated soil and increase of AA contents on contaminated soil was confirmed in S2 for Asn and in M for Gln. The fourth effect was found in S1 and S2 for Ala and in M for Asp and Glu.

Results given in Table 2 showed variable answer of selected AAs

to contamination in all plants. However risk elements contamination led to decrease of Asp and Gln on BC treatments and increase of Glu on AC treatments in all plants. Comparing differences between uncontaminated and contaminated soil, it was observed that risk elements increased Ala content and decreased Gln content in all plants on control treatments. In S1 and S2 was found statistical significant relationship between Cd and Asn (S1: R = 0.52, P = 0.0129; S2: R = 1−0.39, P = 0.0066) and Zn and Asn (S1: R = 0.6, P = 0.0033; S2: R = 1−0.33, P = 0.0203). The negative correlation Pb and Asn was found in plants of M (R = 1−0.30, P = 0.0432).

3.4. Relationships among content of AAs and elements and treatment × contamination in spinach and mustard

Relationship was calculated using two-way analysis of variance (two-way ANOVA), contents of AAs and elements were significantly affected by treatment × contamination (S1: Wilks' lambda 0.000000, F = 1009.7, p = 0.0000; M: Wilk's lambda 0.038293, F = 9.485, p = 0.0000; S2: Wilks' lambda 0.054597, F = 7.5686, p = 0.0000). Results showed the most significant effect of contamination.

3.5. Results of principal component analysis

Spinach 1. The first axis of the PCA analysis explained 40%, the first two axes 66%, and the first four axes together 91% of the variability of all analysed data (Fig. 5). The first ordination axis divided individual pots into the uncontaminated group on the left side and contaminated on the right side of the diagram. This indicates a large effect of contamination on yield of biomass, content of elements and content of free AAs. The marks for treatments (control, AC and BC) were located in the comparatively different parts of the diagram, which indicates a high effect of treatments on all recorded data. The length and direction of the vectors of the studied parameters show links among themselves with respect to the treatments and contamination. The contents of free AAs (Σ AAs, Ala, Asn and Gln), total concentrations of Cd, Pb, Zn, Ca and Mg were accumulated more on contaminated soil. On the other hand, concentrations of Asp, Glu and total concentrations of K were accumulated more on uncontaminated soil. The content of Asp positively correlated with content of Glu as indicated by narrow

Table 2
Total content of AAs and content of selected free AAs (μmol/kg) in biomass of spinach and mustard.

	Treatment	Control		AC		BC	
	AAs	Uncontaminated	Contaminated	Uncontaminated	Contaminated	Uncontaminated	Contaminated
S1	Ala	122 ± 17[a]	318 ± 1[b]	127 ± 19[a]	324 ± 3[b]	146 ± 24[a]	174 ± 9[a]
	Asp	623 ± 143[a]	540 ± 0.4[a]	1035 ± 571[a]	1103 ± 25[a]	2048 ± 275[b]	1044 ± 64[a]
	Asn	191 ± 16[a]	223 ± 0.3[a]	86 ± 17[a]	287 ± 95[a]	91 ± 8[a]	114 ± 42[a]
	Glu	1547 ± 301[a]	1440 ± 3[a]	1694 ± 28[a]	2139 ± 264[ab]	2768 ± 107[b]	1896 ± 254[ab]
	Gln	2022 ± 259[b]	843 ± 18[a]	285 ± 25[a]	997 ± 351[a]	512 ± 86[a]	216 ± 44[a]
	ΣAAs	5908 ± 576[a]	4722 ± 14[a]	4365 ± 71[a]	7571 ± 1903[a]	6709 ± 227[a]	5119 ± 1111[a]
M	Ala	432 ± 20[ab]	442 ± 40[ab]	345 ± 36[a]	694 ± 35[b]	595 ± 138[ab]	579 ± 55[ab]
	Asp	708 ± 37[a]	857 ± 25[a]	869 ± 66[a]	742 ± 50[a]	1220 ± 323[a]	846 ± 103[a]
	Asn	87 ± 8[a]	53 ± 7[a]	74 ± 11[a]	85 ± 7[a]	106 ± 27[a]	62 ± 8[a]
	Glu	1176 ± 131[a]	1335 ± 133[a]	1142 ± 63[a]	1423 ± 83[a]	1251 ± 416[a]	1295 ± 63[a]
	Gln	286 ± 79[a]	228 ± 14[a]	260 ± 22[a]	385 ± 24[a]	262 ± 34[a]	258 ± 21[a]
	ΣAAs	8217 ± 451[ab]	6908 ± 868[ab]	6693 ± 443[a]	9131 ± 1054[ab]	13484 ± 3190[b]	9694 ± 1507[ab]
S2	Ala	185 ± 3[a]	220 ± 38[a]	178 ± 30[a]	130 ± 10[a]	201 ± 28[a]	169 ± 18[a]
	Asp	785 ± 63[bc]	500 ± 55[c]	1273 ± 185[a]	1562 ± 185[a]	1175 ± 102[ab]	1104 ± 133[ab]
	Asn	477 ± 58[ab]	243 ± 50[a]	527 ± 94[b]	460 ± 76[ab]	259 ± 43[ab]	310 ± 66[ab]
	Glu	1845 ± 119ab	945 ± 159[b]	2230 ± 165[a]	2239 ± 460[a]	1902 ± 83[ab]	2095 ± 293[a]
	Gln	11721 ± 764[d]	4953 ± 854b[c]	5104 ± 688[c]	1444 ± 361[a]	4133 ± 502[abc]	2267 ± 698[ab]
	ΣAAs	17397 ± 677c	7954 ± 823[ab]	11655 ± 1123[b]	6851 ± 1085[a]	9335 ± 802[ab]	7521 ± 1278[ab]

The values represent the means (±SE) of data obtained in the experiment (n = 8). Different letters indicate significantly different values (P < 0.05) between content of AAs and treatment × contamination. The differences were determined by post-hoc Tukey's test.

angle between them and negatively correlated with Gln. A long vector for particular parameters indicates a strong effect on the results of the analysis, and vice versa.

Mustard. The first axis of the PCA analysis explained 37%, the first two axes 65%, and the first four axes together 84% of the variability of all analysed data (Fig. 6). Similarly to spinach I. pot division of soil contamination indicates a large effect of contamination on yield of biomass, content of elements and contents of free AAs. For mustard, treatments (control, AC and BC) were located in same parts of the diagram, which indicates a low effect of treatments on all the recorded data. The length and direction of the vectors of the studied parameters indicate links among themselves with respect to the treatments and contamination. The contents of free AAs (Σ AAs, Ala, Asn, Asp, Glu and Gln), total contents of Cd, Pb, Zn, Ca and Mg were accumulated more in contaminated treatments. The contents of Asn and Asp were positively correlated with Σ AAs. The content of Cd, Pb and Zn negatively correlated with yield.

Spinach 2. The first axis of the PCA analysis explained 36%, the first two axes 58%, and the first four axes together 83% of the variability of all analysed data (Fig. 7). Again the large impact of contamination on yield of biomass, content of elements and content of free AAs was observed. The marks for treatments (control - down and AC, BC - up) were located in the comparatively different parts of the diagram, which indicates a high effect of treatments on all the recorded data. Link between studied parameters was determined similarly to previous plants. The contents of free AAs (Σ AAs, Ala, Asn, Asp, Glu and Gln), total contents of Ca and Mg were accumulated more in uncontaminated treatments. On the other hand, contents of Cd, Pb, Zn, K and yield were accumulated more on contaminated soil. The content of Cd, Pb and Zn positively correlated between them and negatively correlated with Glu and Asp. There was no effect of treatments and contamination on the content of Ala in biomass.

4. Discussion

4.1. Yield of biomass

Generally, the application of biochar produced from biomass of the phytoextraction does not have negative effect on the plant growth. Conversely, it has positive effect on biomass yield. Considering uncontaminated soil, previous studies confirmed that biochar improves plant growth on uncontaminated soils due to the improved fertility. For example Gregory et al. (2014) showed that biochar promoted a 2-fold increase in shoot dry weight (DW) and a 3-fold increase in root DW under both biochar amendments (1% and 2%). Thus we can consider the biochar improve soil properties and possible release of nutrients could support plant growth.

Observing contaminated soil, the positive effect of amendments on plant biomass production was evident in comparison to unamended control.

The lowest differences in biomass yield comparing contaminated and uncontaminated treatments were observed at M. This low effect of carbonaceous amendments on biomass yield in plants of *Brassicacea* family grown in contaminated soils was also observed in study of Rees et al. (2015).

4.2. Content of elements

Positive effect of biochar, originated from contaminated biomass to immobilize risk elements, has already been observed in laboratory batch sorption experiment (Břendová et al., 2015a). The highest trace element accumulation in plant tissues was observed in M. Previously, Indian mustard has already been considered as a plant with high phytoextraction ability of trace elements with bioconcentration ratios higher than 1 (Shaheen and Rinklebe, 2015). A good tolerance mechanism was provided by combined/concerted action of non-protein thiols, glutathione, and phytochelatins (Seth et al., 2012). In study of Rees et al. (2015), it was observed that *Nocaea caerulescens*, a Cd- and Zn-hyperaccumulator, increased uptake of metals as a respond to the biochar amendment into contaminated soil may be due to invocation of immobilization of major cations that is confirmed by PCA of S1 and S2. In Břendová et al. (2015b), the results reveals no differences in metal accumulation in willows tissues at amended treatments, moreover they provided significantly higher yields with no signs of phytotoxicity in comparison to control. However these results need further research, it seems that biochar is suitable to support the metal uptake of potential accumulating plants.

Alia et al. (2015) observed negative significant impact of heavy metal concentrations (minimal doses: Pb 300 mg/kg, Cd 0.5 mg/kg, Zn 250 mg/kg) on *Spinach olerales* biomass. Our results revealed several times higher contents of Cd, Pb, Zn at amended contaminated treatments (up to 10 mg/kg at S2 for Cd, 300 mg/kg at S2 for Pb, 450 mg/kg at S1 for Zn) while the yield was significantly higher (S1) or comparable to control and uncontaminated treatments. Gartler et al. (2013) aimed biofortification of vegetables with zinc using biochar amendment. They described higher accumulation of zinc in spinach, when they amended soil with biochar. We observed high ability to accumulate Zn in S1 (400—450 mg/kg) with higher yield of biomass in comparison to control at contaminated treatments.

AC and BC was described as reliable mediums for decreasing trace elements phytoavailability Cd content in rapeseed decreased by 23—31% at treatments amended with BC and AC, water-soluble Zn decreased by 56% with BC amendment) (Shaheen et al., 2015). These observations were also confirmed by our results. Effectiveness of amendments to immobilize trace elements was rather observed at AC treatments, e.g., contents of Cd, Pb and Zn were lowest in S2. Further according to results of pH activated carbon and biochar did not changed soil pH which could lead to decrease of risk elements mobility and the potential immobilization indicated sorption processes. However the pH determination was not conducted periodically during experiment, the opinion of Rees et al. (2015) has to be considered: biochar is mainly affecting the soil-plant system by increasing soil pH. They suggested expectation that a progressive soil acidification may slowly decrease the efficiency of biochar in future.

4.3. Free amino acids

The results of our study depicted reaction of AAs metabolism in leaves of selected plants from family *Brassicaceae* (mustard) and *Amaranthaceae* (spinach) on the presence of activated carbon and biochar in uncontaminated and contaminated soil. Several studies (e.g. Kumar et al., 2014; Sharma and Dietz, 2006; Zagorchev et al., 2013) presents important role of AAs in plant metabolism under stress condition, but effect of BC on these metabolites were published only in Younis et al. (2015a,b). These authors observed total content of AAs in spinach and fenugreek on BC-amended sewage-irrigated contaminated soil (Cd and Ni).

Pavlík et al. (2010) reported that metabolism of AAs has the central role in plant abiotic stress resistance. This confirmed results of our study for control and BC treatments, where total content of AAs was the lowest in leaves of spinach and mustard on contaminated soil. Decrease in this content indicates probable utilization of AAs for defence to risk elements. Kumar et al. (2014) reported significant roles of AAs in metal binding, antioxidant defence, and signalling in plants during heavy metal stress. According to Sharma

and Dietz (2006) AAs play pivotal role in detoxification of heavy metals. Also Tripathi et al. (2012) and Zagorchev et al. (2013) reported crucial role AAs in osmotic adjustment or as phytochelatins. Our results confirmed differences in metabolism of AAs in leaves of spinach and mustard not only depending on presence of risk elements in soil, but also depending on plant species (Table 2). Results of S1 and M showed same effect of BC on total content of AAs as results of Younis et al. (2015a). Presence of BC in soil increased total content of AAs. Opposite effect was found in leaves of S2. Similar data was found for AC amendment.

As reported by Lea and Forde (1994) AAs play pivotal role in interaction between C and N metabolisms, where N-containing metabolites are required to allow C utilization for growth. The main AAs essential for metabolism are Glu, Gln, Asp, Asn and Ala. All these AAs had high content in leaves of studied plants, especially Glu, Gln and Asp. In S1 and M was determined Glu as major AAs. Similar results found Zemanová et al. (2014, 2015) in spinach and *Noccaea caerulescens*, both stressed by Cd and Xie et al. (2014) in leaves of bermudagrass (*Cynodon dactylon* L. Pers.) under Cd stress. The major AA in leaves of S2 was Gln. Same results observed Sung et al. (2015) in leaves of tomato plants growing under N, P or K-deficient (content of Gln 58—70% of total content AAs). Aspartic acid reached approximately half the contents of Glu in all studied plants. According to results of PCA (Figs. 5—7), content of Glu and Asp in S1, M and S2 showed positive correlation. Leasure and He (2015) reported Asp as one of the first AAs formed during ammonia fixation. As already mentioned above, Glu metabolism is centrally involved in the process of assimilation and remobilization of N, but it also has important function in the development of adaptive metabolites, such as proline and γ-aminobutyric acid, developing in response to environmental stress (Planchet and Limami, 2015). Content of Gln and Asn decreased in order: S2 > S1 > M. Zhang et al. (2013) reported that Asn is the major form of N transported to sink tissues in *Arabidopsis* mutants. Although mustard and *Arabidopsis* belong to same family their content of AAs is different, mainly Asn content, which was not main AA in mustard. Asn as major AA found Pavlíková et al. (2014) in leaves of tobacco plants under Zn stress. Souza et al. (2014) founded Asn as AA with second highest content after arginine in *Calopogonium mucunoides* under Pb stress. According to Bottari and Festa (1996) and Planchet and Limami (2015) Asn has ability to bind metals as Cd, Pb and Zn by forming intracellular Asn-metal complex, which are useful for reducing the metal toxicity. This associated with accumulation of Asn in plants exposed to risk elements. However, our results confirmed this only in S1.

Ala is one of major AAs in plants and its synthesis by transfer of amino group from Glu. Ala acts as an important connector of C and N metabolism and balance (Miyashita et al., 2007; Rocha et al., 2010). Amendment of BC increased content of Ala in comparison to control. This effect was observed for AC only in leaves of S1. Increased content of Ala might be caused by a reduction in the rate of protein syntheses (Hjorth et al., 2006). According to Pavlík et al. (2010) Ala is markedly accumulated in response to stress in plants and it is especially discussed in relation to intracellular pH regulation. Content of Ala decreased in order: M > S1 >> S2.

5. Conclusion

- Biochar produced from biomass originated from phytoextraction technologies did not have negative effect on plants growth.
- Both carbonaceous materials promoted spinach biomass yield, especially at contaminated treatments.
- Metal uptake differed according to plant and series, Cd was accumulated similarly by all three plants, accumulation of Pb

was significantly highest by mustard, affecting also third plant, mobile Zn was mostly taken by first spinach
- Application of AC and BC to soil did not demonstrate significant effect on the content of AAs in mustard, while effect of BC on AAs metabolism was confirmed in spinach, this explains differences of trace element accumulation and biomass yield of spinach and mustard plants.

References

Alia, N., Sardar, K., Said, M., Salma, K., Sadia, A., Sadaf, S., Miklas, S., 2015. Toxicity and bioaccumulation of heavy metals in Spinach (*Spinacia oleracea*) grown in a controlled environment. Int. J. Environ. Res. Public Health 12, 7400—7416. http://dx.doi.org/10.3390/ijerph120707400.

Alloway, B.J., 2012. Heavy Metals in Soil, third ed. Springer, Dordrecht.

Basta, N.T., McGowen, S.L., 2004. Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. Environ. Pollut. 127, 73—82. http://dx.doi.org/10.1016/S0269-7491(03)00250-1.

Bottari, E., Festa, M.R., 1996. Asparagine as a ligand for cadmium(II), lead(II) and zinc(II). Chem. Speciat. Bioavail. 8, 75—83.

Břendová, K., Tlustoš, P., Száková, J., 2015a. Can biochar from contaminated biomass Be applied into soil for remediation purposes? Water Soil Air Pollut. 226 (6) http://dx.doi.org/10.1007/s11270-015-2456-9.

Břendová, K., Tlustoš, P., Száková, J., 2015b. Biochar immobilizes cadmium and zinc and improves phytoextraction potential of willow plants on extremely contaminated soil. Plant Soil Environ. 61 (7), 303—308. http://dx.doi.org/10.17221/181/2015-PSE.

Bucheli, T.D., Gustafsson, O., 2000. Quantification of the soot-water distribution coefficient of PAHs provides mechanistic basis for enhanced sorption observations. Environ. Sci. Technol. 34, 5144—5151. http://dx.doi.org/10.1021/es000092s.

Cao, X., Ma, L., Gao, B., Harris, W., 2009. Dairy-manure derived biochar effectively sorbs lead and atrazine. Environ. Sci. Technol. 43, 3285—3291. http://dx.doi.org/10.1021/es803092k.

Clark, R.B., Ritchey, K.D., Baligar, V.C., 2001. Benefits and constrains for use of FGD products on agricultural land. Fuel 80, 821—828.

Evangelou, M.W., Brem, A., Ugolini, F., Abiven, S., Schulin, R., 2014. Soil application of biochar produced from biomass grown on trace element contaminated land. J. Environ. Manage. 146, 100—106.

Gartler, J., Robinson, B., Burton, K., Clucas, L., 2013. Carbonaceous soil amendments to biofortify crop plants with zinc. Sci. Total Environ. 465, 308—313.

Gregory, J., Anderson, C.W.N., Camps Aberstain, M., McManus, M.T., 2014. Response of plant and soil microbes to biochar amendment of an arsenic-contaminated soils. Agric. Ecosyst. Environ. 191, 133—141. http://dx.doi.org/10.1016/j.agee.2014.03.035.

Guo, G., Zhou, Q., Ma, L.Q., 2006. Availability and assessment of fixing additives for the in situ remediation of heavy metal contaminated soils: a review. Environ. Monit. Assess. 116, 513—528. http://dx.doi.org/10.1007/s10661-006-7668-4.

Hale, S.E., Hanley, K., Lehmann, J., Zimmerman, A.R., Cornelissen, G., 2011. Effects of chemical, biological, and physical aging as well as soil addition on the sorption of pyrene to activated carbon and biochar. Environ. Sci. Technol. 45, 10445—10453. http://dx.doi.org/10.1021/es202970x.

Hjorth, M., Mathiassen, S.K., Kudsk, P., Ravn, H.W., 2006. Amino acids in loose silky-bent (*Apera spica-venti* (L.) Beauv.) responding to prosulfocarb exposure and the correlation with physiological effects. Pest. Biochem. Physiol. 86, 138—145. http://dx.doi.org/10.1016/j.pestbp.2006.02.006.

Jiang, J., Xu, R., Jiang, T., Li, Z., 2012. Immobilization of Cu(II), Pb(II) and Cd(II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. J. Hazard. Mater. 229, 145—150. http://dx.doi.org/10.1016/j.jhazmat.2012.05.086.

Jones, D.L., Quilliam, R.S., 2014. Metal contaminated biochar and wood ash negatively affect plant growth and soil quality after land application. J. Hazard. Mater. 276, 362—370.

Kadirvelu, K., Thamaraiselvi, K., Namasivayam, C., 2001. Removal of heavy metals from industrial wastewaters by adsorption onto activated carbon prepared from an agricultural solid waste. Bioresour. Technol. 76, 63—65. http://dx.doi.org/10.1016/S0960-8524(00)00072-9.

Kubešová, M., Kučera, J., 2010. Validation of k0 standardization method in neutron activation analysis — the use of Kayzero for Windows programme at the nuclear physics institute, Řež. Nucl. Instrum. Methods Phys. Res. Sect. A Accel. Spetrsom. Detect. Assoc. Equip. 622, 403—406.

Kumar, A., Singh, R.P., Singh, P.K., Awasthi, S., Chakrabarty, D., Trivedi, P.K., Tripathi, R.D., 2014. Selenium ameliorates arsenic induced oxidative stress through modulation of antioxidant enzymes and thiols in rice (*Oryza sativa* L.). Ecotoxicology 23, 1153—1163. http://dx.doi.org/10.1007/s10646-014-1257-z.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments — a review. Waste Manage. 28, 215–225. http://dx.doi.org/10.1016/j.wasman.2006.12.012.

Lea, P.J., Forde, B.G., 1994. The use of mutants and transgenic plants to study amino-acid-metabolism. Plant Cell Environ. 17, 541–556. http://dx.doi.org/10.1111/j.1365-3040.1994.tb00148.x.

Leasure, C.D., He, Z.-H., 2015. Aspartate aminotransferase. In: D'Mello, J.P.F. (Ed.), Amino Acids in Higher Plants. CABI Publishing, Wallingford, United Kingdom, pp. 57–67.

Macdonald, L.M., Farrell, M., Van Zwieten, L., Krull, E.S., 2014. Plant growth responses to biochar addition: an Australian soils perspective. Biol. Fertil. Soils 50, 1035–1045. http://dx.doi.org/10.1007/s00374-014-0921-z.

Miyashita, Y., Dolferus, R., Ismond, K.P., Good, A.G., 2007. Alanine aminotransferase catalyses the breakdown of alanine after hypoxia in Arabidopsis thaliana. Plant J. 49, 1108–1121. http://dx.doi.org/10.1111/j.1365-313X.2006.03023.x.

Neuberg, M., Pavlíková, D., Pavlík, M., Balík, J., 2010. The effect of different nitrogen nutrition on proline and asparagine content in plant. Plant Soil Environ. 56, 305–311.

Panagos, P., Van Liedekerke, M., Yigini, Y., Montanarella, L., 2013. Contaminated sites in Europe: review of the current situation based on data collected through a European Network. J. Environ. Public Health 2013, 11. http://dx.doi.org/10.1155/2013/158764.

Pavlík, M., Pavlíková, D., Staszková, L., Neuberg, M., Kaliszová, R., Száková, J., Tlustoš, P., 2010. The effect of arsenic contamination on amino acids metabolism in Spinacia oleracea L. Ecotox. Environ. Safe. 73, 1309–1313. http://dx.doi.org/10.1016/j.ecoenv.2010.07.008.

Pavlík, M., Pavlíková, D., Zemanová, V., Hnilička, F., Urbanová, V., Száková, J., 2012. Trace elements present in airborne particulate matter — stressors of plant metabolism. Ecotox. Environ. Safe 79, 101–107. http://dx.doi.org/10.1016/j.ecoenv.2011.12.009.

Pavlíková, D., Zemanová, V., Procházková, D., Pavlík, M., Száková, J., Wilhelmová, N., 2014. The long-term effect of zinc soil contamination on selected free amino acids playing an important role in plant adaptation to stress and senescence. Ecotox. Environ. Safe. 100, 166–170. http://dx.doi.org/10.1016/j.ecoenv.2013.10.028.

Planchet, E., Limami, A.M., 2015. Amino acid synthesis under abiotic stress. In: D'Mello, J.P.F. (Ed.), Amino Acids in Higher Plants. CABI Publishing, Wallingford, United Kingdom, pp. 262–276.

Puga, A.P., Abreu, C.A., Melo, L.C.A., Beesley, L., 2015. Biochar application to a contaminated soil reduces the availability and plant uptake of zinc, lead and cadmium. J. Environ. Manage. 159, 86–93. http://dx.doi.org/10.1016/j.jenvman.2015.05.036.

Qayyum, M.F., Steffens, D., Reisenauer, H.P., Schubert, S., 2014. Biochars influence differential distribution and chemical composition of soil organic matter. Plant Soil Environ. 60, 337–343.

Rees, F., Germain, C., Sterckeman, T., Morel, J.-L., 2015. Plant growth and metal uptake by a non-hyperaccumulating species (Lolium perenne) and a Cd-Zn hyperaccumulator (Noccaea caerulescens) in contaminated soils amended with biochar. Plant Soil 395, 57–73. http://dx.doi.org/10.1007/s11104-015-2384-x.

Rocha, M., Licausi, F., Araújo, W.L., Nunes-Nesi, A., Sodek, L., Fernie, A.R., van Dongen, J.T., 2010. Glycolysis and the tricarboxylic acid cycle are linked by alanine aminotransferase during hypoxia induced by waterlogging of Lotus japonicus. Plant Physiol. 152, 1501–1513. http://dx.doi.org/10.1104/pp.109.150045.

Seth, C.S., Misra, V., Chauhan, L.K.S., 2012. Accumulation, detoxification, and genotoxicity of heavy metals in indian mustard (Brassica juncea L.). Int. J. Phytoremediat. 14 (1), 1–13.

Shaheen, S.M., Rinklebe, J., Selim, M.H., 2015. Impact of various amendments on immobilization and phytoavailability of nickel and zinc in a contaminated floodplain soil. Int. J. Environ. Sci. Technol. 12 (9), 2765–2776. http://dx.doi.org/10.1007/s13762-014-0713-x.

Shaheen, S.M., Rinklebe, J., 2015. Phytoextraction of potentially toxic elements by Indian mustard, rapeseed, and sunflower from a contaminated riparian soil. Environ. Geochem. Health 37 (6), 953–967. http://dx.doi.org/10.1007/s10653-015-9718-8.

Sharma, S.S., Dietz, K.J., 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J. Exp. Bot. 57, 711–726. http://dx.doi.org/10.1093/jxb/erj073.

Souza, L.A., Camargos, L.S., Schiavinato, M.A., Andrade, S.A.L., 2014. Mycorrhization alters foliar soluble amino acid composition and influences tolerance to Pb in Calopogonium mucunoides. Theor. Exp. Plant Physiol. 26, 211–216. http://dx.doi.org/10.1007/s40626-014-0019-x.

Street, R., Száková, J., Drábek, O., Mládková, L., 2006. The status of micronutrients (Cu, Fe, Mn, Zn) in tea and Te infusions in selected samples imported to the Czech Republic. Czech J. Food. Sci. 24, 62–71.

Sung, J., Sonn, Y., Lee, Y., Kang, S., Ha, S., Krishnan, H.B., Oh, T.-K., 2015. Compositional changes of selected amino acids, organic acids, and soluble sugars in the xylem sap of N, P, or K-deficient tomato plants. J. Plant Nutr. Soil Sci. 178, 792–797. http://dx.doi.org/10.1002/jpln.201500071.

Tang, J., Zhu, W., Kookana, R., Katayama, A., 2013. Characteristics of biochar and its application in remediation of contaminated soil. J. Biosci. Bioeng. 116 (6), 653–659.

ter Braak, C.J.F., Smilauer, P., 2002. CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5). Microcomputer Power, Ithaca, p. 500.

Tripathi, P., Mishra, A., Dwivedi, S., Chakrabarty, D., Trivedi, P.K., Singh, R.P., Tripathi, R.D., 2012. Differential response of oxidative stress and thiol metabolism in contrasting rice genotypes for arsenic tolerance. Ecotox. Environ. Safe. 79, 189–198. http://dx.doi.org/10.1016/j.ecoenv.2011.12.019.

Uchiyama, M., Wartelle, L.H., Klasson, K.T., Fortier, C.A., Lima, I.M., 2011. Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. J. Agric. Food Chem. 59, 2501–2510. http://dx.doi.org/10.1021/jf104206c.

Uzinger, N., Anton, A., 2008. Chemical stabilization of heavy metals on contaminated soils by lignite. Cereal Res. Commun. 36, 1911–1914.

Wang, S., Gao, B., Zimmerman, A.R., Li, Y., Mad, L., Harris, W.G., Migliaccio, K.W., 2015. Physicochemical and sorptive properties of biochars derived from woody and herbaceous biomass. Chemosphere 134, 257–262. http://dx.doi.org/10.1016/j.chemosphere.2015.04.062.

Xie, Y., Hu, L., Du, Z., Sun, X., Amombo, E., Fan, J.B., Fu, J.M., 2014. Effects of cadmium exposure on growth and metabolic profile of bermudagrass [Cynodon dactylon (L.) Pers.]. PLoS One 9, e115279. http://dx.doi.org/10.1371/journal.pone.0115279.

Younis, U., Athar, M., Malik, S.A., Shah, M.H.R., Mahmood, S., 2015b. Biochar impact on physiological and biochemical attributes of spinach Spinacia oleracea (L.) in nickel contaminated soil. Glob. J. Environ. Sci. Manage. 1, 245–254. http://dx.doi.org/10.7508/gjesm.2015.03.007.

Younis, U., Qayyum, M.F., Shah, M.H.R., Danish, S., Shahzad, A.N., Malik, S.A., Mahmood, S., 2015a. Growth, survival, and heavy metal (Cd and Ni) uptake of spinach (Spinacia oleracea) and fenugreek (Trigonella corniculata) in a biochar-amended sewage-irrigated contaminated soil. J. Plant Nutr. Soil Sci. 178, 209–217. http://dx.doi.org/10.1002/jpln.201400325.

Zagorchev, L., Seal, C.E., Kranner, I., Odjakova, M., 2013. A central role for thiols in plant tolerance to abiotic stress. Int. J. Mol. Sci. 14, 7405–7432. http://dx.doi.org/10.3390/ijms14047405.

Zárubová, P., Hejcman, M., Vondráčková, S., Mrnka, L., Száková, J., Tlustoš, P., 2015. Distribution of P, K, Ca, Mg, Cd, Cu, Fe, Mn, Pb and Zn in wood and bark age classes of willows and poplars used for phytoextraction on soils contaminated by risk elements. Environ. Sci. Pollut. R. 22 (23), 18801–18813.

Zemanová, V., Pavlík, M., Pavlíková, D., Kyjaková, P., 2015. Changes in the contents of amino acids and the profile of fatty acids in response to cadmium contamination in spinach. Plant Soil Environ. 61, 285–290. http://dx.doi.org/10.17221/274/2015-PSE.

Zemanová, V., Pavlík, M., Pavlíková, D., Tlustoš, P., 2014. The significance of methionine, histidine and tryptophan in plant responses and adaptation to cadmium stress. Plant Soil Environ. 60, 426–432.

Zhang, Q., Lee, J., Pandurangan, S., Clarke, M., Pajak, A., Marsolais, F., 2013. Characterization of Arabidopsis serine: glyoxylate aminotransferase, AGT1, as an asparagine aminotransferase. Phytochemistry 85, 30–35. http://dx.doi.org/10.1016/j.phytochem.2012.09.017.

Effect of biochar application on the content of nutrients (Ca, Fe, K, Mg, Na, P) and amino acids in subsequently growing spinach and mustard

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ABSTRACT

Zemanová V., Břendová K., Pavlíková D., Kubátová P., Tlustoš P. (2017): Effect of biochar application on the content of nutrients (Ca, Fe, K, Mg, Na, P) and amino acids in subsequently growing spinach and mustard. Plant Soil Environ., 63: 322–327.

The objective of this study was to assess the effect of biochar on growth and metabolism of spinach (*Spinacia oleracea* L.) and mustard (*Sinapis alba* L.) planted in crop rotation: spinach (spring)-mustard-spinach (autumn). The impact of biochar soil application (5% per mass of soil) on the availability of Ca, Fe, K, Mg, Na and P to plants as well as the content of free proline and total amino acids contents were evaluated at degraded Chernozem soil. The results showed that biochar soil addition significantly increased spinach growth by 102% and 353% in spring and autumn, respectively. Biochar limited plant content of Ca, Mg and Na, however K content increased in all plants. Inconsistent effect was determined for Fe and P content in plants biomass. Total content of free amino acids was higher in plants harvested at amended treatments, except autumn spinach. Biochar increased proline content in all plants in comparison to control. The highest increase was obtained in mustard – by 186%. The results showed a more sensitive reaction of mustard to biochar application than spinach.

Keywords: carbonaceous amendment; macroelement; plant; stress metabolism

Biochar (BC) is a carbon-rich material produced by pyrolysis. In recent decade, it is widely described as a soil amendment improving soil quality. The main reason for the positive impact on soil properties, plant and microbial ecosystem is a direct BC influence on soil physical-chemical properties, nutrients available contents, and on its ability to sorb nutrients and release them slowly into soil solution (Atkinson et al. 2010). Nutrient composition of BC depends on the feedstock material and conditions of pyrolysis (Mukherjee and Zimmerman 2013). However, many studies described a positive effect of BC application to soil, whereas the responses on crop yields are not consistent (Novak et al. 2016). According to Břendová et al. (2015) BC ability to increase pH value is probably one of the crucial factors of reduction of element leachability.

As mentioned by Rizwan et al. (2016), there is a limited number of reports describing the effect of BC on the biochemical and physiological activities of plants. One of the important indicators of plant metabolism is composition and content of amino acids (AAs), which are affected by environmental conditions (Nikiforova et al. 2006). According to Singh (1999), amino acid metabolism may play an important role in plant stress resistance, by osmotic

adjustment and the accumulation of compatible osmolyte, detoxification of active oxygen species and heavy metals, and intracellular pH regulation. Proline (Pro) is specific free AA which is involved in stress metabolism and tolerance of plants (Pavlíková et al. 2014).

Our study aimed to compare the ability of spinach and mustard accumulation of nutrients and amino acids as a response to biochar application to soil.

MATERIAL AND METHODS

Experimental design and soil characteristics. The experiment was conducted at greenhouse controlled conditions. Spinach (*Spinacia oleracea* L.) and mustard (*Sinapis alba* L.) were chosen as experimental plants, while the crop rotation was spinach-mustard-spinach. Firstly, spinach was sown in March and harvested after 64 days. Mustard was sown in May and harvested after 35 days. Late spinach was sown in August, harvested after 64 days. Biomass of both spinaches was sampled once during vegetation, after 42 days. Modal Chernozem soil (2.5 kg; Prague-Suchdol, Czech Republic; pH_{KCl} = 7.2, cation exchange capacity (CEC) = 258 $mmol_+$/kg, C_{org} = 1.83%, available content of elements: Ca = 6754 mg/kg; K = 233 mg/kg; Fe = 153 mg/kg, Mg = 191 mg/kg; P = 74 mg/kg) was uniquely fertilized with 0.1 g N, 0.16 g P and 0.4 g K per 1 kg of soil (applied in the form of NH_4NO_3 and K_2HPO_4), only at the beginning of the experiment. The experiment consists of 2 treatments: (i) control (without BC) and (ii) 5% of the applied BC from total mass of soil (BC 5%) thoroughly mixed with soil volume.

Biochar characteristics. Detailed biochar properties were described in Břendová et al. (2016). Biochar was characterized by: ash content = 13%; pH_{CaCl_2} = 6.9; CEC = 176 $mmol_+$/kg; specific surface area = 324 m^2/g and particle fraction = 5 × 6 × 0.5 mm. The total contents of elements in biochar were: K = 16.1 g/kg; Ca = 28 g/kg; Fe = 2.8 g/kg; C_{total} = 64% (w/w) and N = 1.1% (w/w). Total content of Mg was not determined.

Analysis of plant biomass. Dry-ashing decomposition was used for analyses of total element contents in plants (Street et al. 2006). The total contents of elements were determined by ICP-OES (Agilent 720, Agilent Technologies Inc., Torrance, USA) for Ca, Fe, Mg and P and by FAAS (Varian

SpectrAA-280, Mulgrave, Australia) for K and Na. Aliquots of the certified reference material RM NCS DC 73350, poplar leaves (Analytika, Prague, Czech Republic), were determined under the same conditions to test quality assurance.

The contents of free AAs were determined after their derivatisation in extracts (1.0 g of fresh biomass, 15 mL of methanol + redistilled H_2O (7:3, v/v), 24 h) by EZ:faast set (Phenomenex, Santa Clara, USA). Samples were measured by GC-MS (Hewlett Packard 6890N/5975 MSD, Agilent Technologies, Torrance, USA) with a ZB-AAA 10 m × 0.25 mm AA analysis GC column (Zemanová et al. 2013).

Statistical analysis. All statistical analyses were performed using the Statistica 12.0 program (StatSoft, Tulsa, USA, www.statsoft.com). Collected data were performed using the non-parametric Kruskal-Wallis test.

RESULTS AND DISCUSSION

Biomass yield and proline content. Results presented in Table 1 showed fresh biomass yield of all treatments. Dry biomass of control and BC-amended spinaches were 9.6% and 9.7% on average, respectively. Application of BC increased dry biomass of both spinaches by 0.1% on average in comparison to control. The highest dry matter of biomass was found in mustard – 11% and was decreased by 2% at BC treatment. Application of BC significantly increased spinach biomass yields by 102% (spring) and 353% (autumn) on average, respectively in comparison to control. Same effect of BC was determined in mustard biomass yield (increase by 69%), but this effect was not statistically significant. According to Mukherjee and Lal (2014) application of BC may have positive, mixed or negative effect on yield of biomass. Jones et al. (2012) showed that BC application had no effect on maize growth, but did enhance the growth of the subsequent grass crop. Evangelou et al. (2014) using contaminated BC achieved higher yields of ryegrass in comparison to unamended treatment.

Amino acids are critically important for plant metabolism to make bridges between C and N metabolisms (Foyer et al. 2003). Plants can produce high quantities of AAs under limited stress conditions (Younis et al. 2015). In previous papers, there is a lack of information of the BC effect on composition and content of amino acids. Only

Table 1. Biomass yield (BY), total content of amino acids (Σ AAs) and free proline content (Pro) of spinach and mustard

	42 days		64 days	
	control	BC 5%	control	BC 5%
Spinach – spring				
BY (g per pot FM)	23.5 ± 2.3[aA]	46.4 ± 1.6[bA]	28.5 ± 2.5[aA]	58.7 ± 2.3[bA]
Σ AAs (µmol/kg FM)	5386 ± 789[aA]	5420 ± 279[aA]	5908 ± 364[aA]	6709 ± 144[aB]
Pro (µmol/kg FM)	349 ± 34[aB]	1052 ± 369[bB]	147 ± 13[aA]	167 ± 36[aA]
Spinach – autumn				
BY (g per pot FM)	12.4 ± 1.3[aA]	57.8 ± 2.9[bA]	15.3 ± 2.4[aA]	67.4 ± 3.5[bA]
Σ AAs (µmol/kg FM)	22 890 ± 5483[bA]	10 110 ± 2305[aA]	17 397 ± 677[bA]	9335 ± 802[aA]
Pro (µmol/kg FM)	131 ± 10[aA]	154 ± 20[aA]	149 ± 32[aA]	180 ± 60[aA]
	35 days			
	control		BC 5%	
Mustard				
BY (g per pot FM)	30.6 ± 1.4[a]		51.7 ± 2.2[a]	
Σ AAs (µmol/kg FM)	8217 ± 451[a]		13 484 ± 3190[a]	
Pro (µmol/kg FM)	2371 ± 395[a]		6771 ± 2191[a]	

The values represent the means (± standard error) of data obtained in the experiment (*n* = 4). Different letters indicate significantly different values (*P* < 0.05) between treatments (a, b) and sampling period (A, B). FM – fresh matter

Zhang et al. (2014) and Younis et al. (2015) measured AAs content in plants grown in B-amended soil. The results presented in Table 1 showed total contents of free AAs in all tested plants. In spring spinach and mustard planted at BC treatment, total content of free AAs was increased by 7% on average and by 64%, respectively, however not significantly, in comparison to control. Similarly, Younis et al. (2015) obtained increasing production of AAs by BC application under toxic levels of Cd and Ni in spinach and fenugreek. According to these authors, high production of AAs and also proteins indicated the activation of defensive mechanism against oxidative damage. Opposite effect of BC – decrease of total content of free AAs (by 51% on average) was observed in spinach-autumn. However, this decrease can be partly influence due to the inhibition of photosynthesis by lower irradiance. Synthesis of AAs depends on photosynthetic activity, which, in turn, depends on other factors e.g. nutrient availability (Weckopp and Kopriva 2015).

Proline is a multifunctional AA accumulated in plant cells in response to various stresses (Szabados and Savouré 2010). In our study, Pro found in spring spinach, mustard and autumn spinach represented 8, 40 and 1% on average of total content of free AAs (Table 1). The highest content of Pro was accumulated in mustard, and the content was

15-fold higher on average and 31-fold higher on average than in spring spinach and autumn spinach, respectively. As presented by Jogaiah et al. (2013), Pro is known as an osmoprotectant and antioxidant, playing an important role in stress management in plants. Application of BC increased Pro content in all tested plants, but significantly only in spring spinach after 42 days of growth. Different results were obtained by Kammann et al. (2011) in the study with *Chenopodium quinoa*. In these plants under drought stress, application of BC decreased Pro content in leaves. Also Zhang et al. (2014) reported a decrease of Pro content in rice by BC soil application in comparison to unamended treatments.

Elements contents in plant tissues. The results, presented in Figure 1, showed diverse accumulation of selected elements in spinach and mustard after BC application. The contents of Ca, Mg and Na were reduced by BC application in all tested plants. The content of Ca was decreased in both spinach treatments – spring and autumn – by 45% and 30% on average, and in mustard – by 34%. The highest Ca content was measured in mustard both at control (3.5%) and at BC treatment (2.3%). The same effect of BC on the Ca content was found in corn ear-leaf and soybean plants (Brantley et al. 2016, Waqas et al. 2017). In comparison to

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control, BC application decreased Mg content in spring spinach (26% on average), mustard (27%) and autumn spinach (20%). Significant changes of Mg content in lettuce plants by BC soil application were confirmed by Woldetsadik et al. (2016). These authors observed a decrease of Mg content on sandy loam soil and an increase of Mg content on silty loam soil after BC application. Increase of Mg was found in dry beans (*Phaseolus vulgaris* L.) by Gao et al. (2016). Potassium concentration in plant tissues indicated high consumption in our experiment, thus the content of Mg could be depressed by antagonistic interaction mechanism of these two elements, which is seated in the translocation step from the root to the shoot (Ohno and Grunes 1985). The highest Mg content was measured in autumn spinach at both treatments – 1.4% at

control and 0.9% at BC. The lowest Mg content was measured in mustard – 0.3% at control and 0.2% at BC. Like Mg, Na content could be reduced by high K consumption. In all plants, Na content decreased in spring spinach (88% on average), mustard (64%) and autumn spinach (79% on average) by BC application. Decrease of sodium content was found in maize (Kim et al. 2016) by BC treatment and in mixed biomass of grasses and forbs on temperate grassland (Schimmelpfennig et al. 2015).

The positive effect of BC application on plant accumulation of elements was observed only for K. Although this element was applied to the soil as a fertilizer at the beginning of the experiment, BC increased K availability and its accumulation in the tested plants during the whole experiment (Figure 1, Table 2). The same results – increase of K
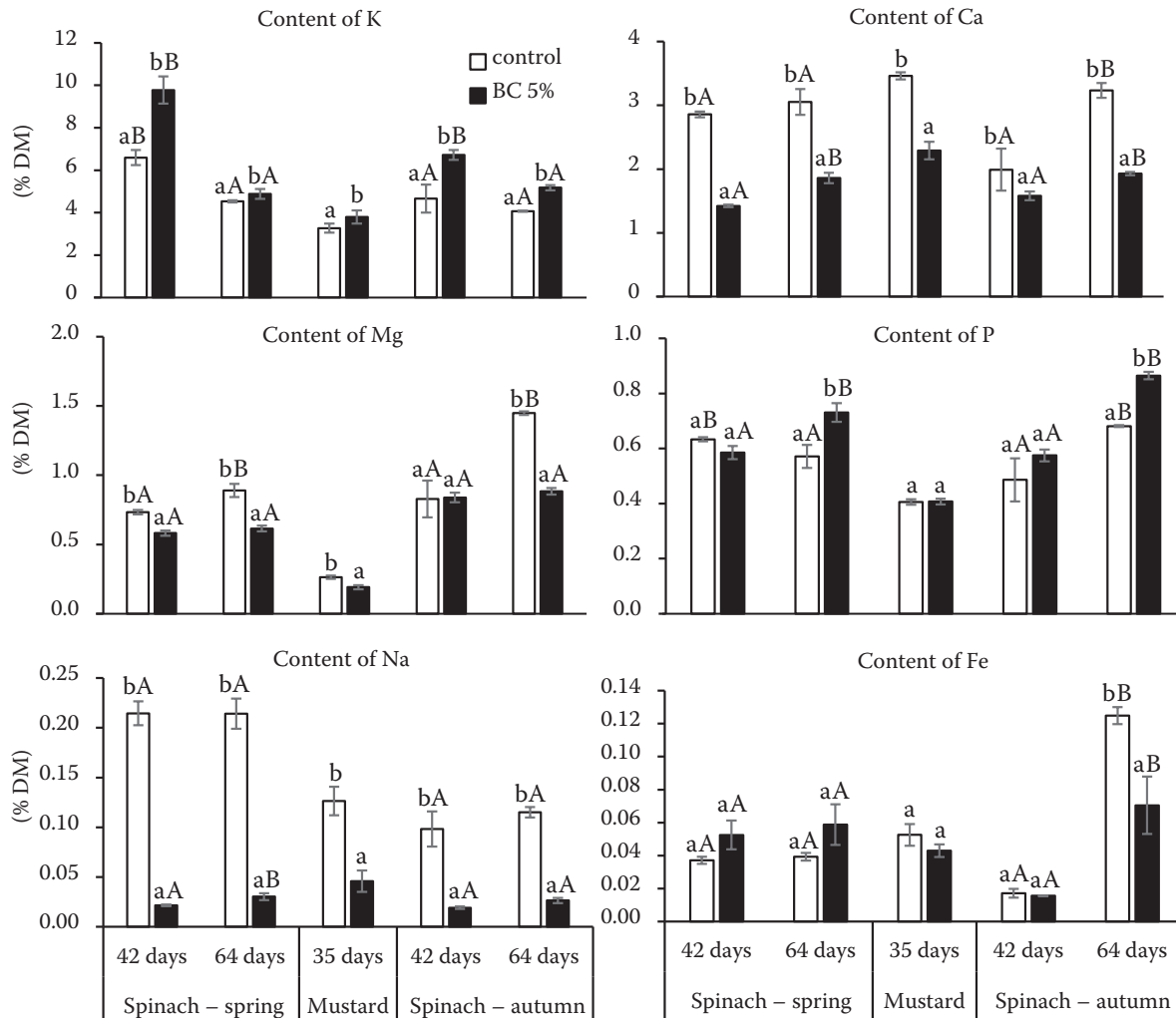

Figure 1. Content of nutrients (%) in spinach and mustard dry biomass. The values represent the means (± standard error) of data obtained in the experiment (*n* = 4). Different letters indicate significantly different values (*P* < 0.05) between treatments (a, b) and sampling period (A, B). DM – dry matter

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Table 2. Content of soil available nutrients (Mehlich III) and pH at the end of the experiment

	Ca	Fe	K	Mg	Na	P	pH
	(mg/kg)						
Control	11 784	0.26	279	225	30.3	75.1	6.51
BC 5%	13 704	0.47	480	350	34.3	199	6.61

BC – biochar

availability, was found by Kraska et al. (2016) after application of BC derived from wheat straw. The content of K was increased in spring spinach (72% on average), mustard (16%) and autumn spinach (36% on average) by BC. Significantly increased K content was found in stems, leaves and roots of green bean (*Vigna radiata* L.) by Prapagdee and Tawinteung (2017). Also Zhang et al. (2016b) observed an increase of K content by BC application. The highest K content was measured in autumn spinach in both treatments – 4.4% in control and 5.9% in BC. Evangelou et al. (2014) found that the BC application increased significantly K and Zn content in plant shoots, however no differences were found in P, Fe, Mg, Mn and Cu concentration comparing control and amended treatments.

The mixed effect of BC application on nutrients accumulation in plants was obtained for Fe and P content. The content of Fe was non-significantly affected by BC application, except autumn spinach at 64 days. The content of P was significantly affected by BC application only in spinaches (Figure 1). Similar results were found by Prapagdee and Tawinteung (2017), who reported no significant difference of P content in green bean parts at all BC treatments. Biochar can be a potential P source and some BC can also adsorb P efficiently from solutions (Peng et al. 2012, Yao et al. 2013). They suggested that BC could play a role in retaining P applied as fertilizer. However, information of the effect of BC on phosphate retention in soils is limited (Zhang et al. 2016a). In our experiment, P was applied as fertilizer – just at the beginning (before spring spinach sowing) and the positive effect of BC on the P plant content was shown in the end of spring and autumn spinach plants (increase by 27.5% on average). Gonzaga et al. (2017) observed same effect of BC derived from biosolids. Phosphorus contents in above-ground biomass of maize increased with increasing BC application rates. According to Chintala et al. (2014) and Zhang et al. (2016a), the ability of BC to increase P retention in soils is quite variable and it varies with concentration

of P in the soil solution. Although the Mehlich III – available contents of Fe and P increased with BC application (Table 2), only Fe was not accumulated into plant tissues. Similar trend was observed for Fe in Evangelou et al. (2014). Sorrenti et al. (2016) showed a decrease of Fe content in nectarine leaf. Opposite effect – Fe increase by BC was found by Gao et al. (2016) in dry beans.

Biochar from contaminated biomass has a positive effect on plant growth. Positive effect of BC application on accumulation of K and P was shown especially after longer exposure. Biochar increased proline and reduced availability of some nutrients (Ca, Mg and Na), which means that BC amendment may cause stress for plants, especially at the beginning of the growth. Our results of nutrient and proline contents showed a different response of mustard and spinach. According to the lowest yield of biomass and higher proline content, mustard is more sensitive to BC soil application than spinach.

REFERENCES

Atkinson C.J., Fitzgerald J.D., Hipps N.A. (2010): Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. Plant and Soil, 337: 1–18.

Brantley K.E., Savin M.C., Brye K.R., Longer D.E. (2016): Nutrient availability and corn growth in a poultry litter biochar-amended loam soil in a greenhouse experiment. Soil Use and Management, 32: 279–288.

Břendová K., Tlustoš P., Száková J. (2015): Biochar immobilizes cadmium and zinc and improves phytoextraction potential of willow plants on extremely contaminated soil. Plant, Soil and Environment, 61: 303–308.

Břendová K., Zemanová V., Pavlíková D., Tlustoš P. (2016): Utilization of biochar and activated carbon to reduce Cd, Pb and Zn phytoavailability and phytotoxicity for plants. Journal of Environmental Management, 181: 637–645.

Chintala R., Schumacher T.E., McDonald L.M., Clay D.E., Malo D.D., Papiernik S.K., Clay S.A., Julson J.L. (2014): Phosphorus sorption and availability from biochars and soil/biochar mixtures. Clean – Soil, Air, Water, 42: 626–634.

Evangelou M.W.H., Brem A., Ugolini F., Abiven S., Schulin R. (2014): Soil application of biochar produced from biomass grown on trace element contaminated land. Journal of Environmental Management, 146: 100–106.

Foyer C.H., Parry M., Noctor G. (2003): Markers and signals associated with nitrogen assimilation in higher plants. Journal of Experimental Botany, 54: 585–593.

Gao S., Hoffman-Krull K., Bidwell A.L., DeLuca T.H. (2016): Locally produced wood biochar increases nutrient retention and availability in agricultural soils of the San Juan Islands, USA. Agriculture, Ecosystems and Environment, 233: 43–54.

Gonzaga M.I.S., Mackowiak C.L., Comerford N.B., da Veiga Moline E.F., Shirley J.P., Guimaraes D.V. (2017): Pyrolysis methods impact biosolids-derived biochar composition, maize growth and nutrition. Soil and Tillage Research, 165: 59–65.

Jogaiah S., Govind S.R., Tran L.S. (2013): Systems biology-based approaches toward understanding drought tolerance in food crops. Critical Reviews in Biotechnology, 33: 23–39.

Jones D.L., Rousk J., Edwards-Jones G., DeLuca T.H., Murphy D.V. (2012): Biochar – Mediated changes in soil quality and plant growth in a three year field trial. Soil Biology and Biochemistry, 45: 113–124.

Kammann C.I., Linsel S., Gößling J.W., Koyro H.-W. (2011): Influence of biochar on drought tolerance of *Chenopodium quinoa* Willd and on soil-plant relations. Plant and Soil, 345: 195–210.

Kim H.-S., Kim K.-R., Yang J.E., Ok Y.S., Owens G., Nehls T., Wessolek G., Kim K.-H. (2016): Effect of biochar on reclaimed tidal land soil properties and maize (*Zea mays* L.) response. Chemosphere, 142: 153–159.

Kraska P., Oleszczuk P., Andruszczak S., Kwiecińska-Poppe E., Różyło K., Pałys E., Gierasimiuk P., Michałojć (2016): Effect of various biochar rates on winter rye yield and the concentration of available nutrients in the soil. Plant, Soil and Environment, 62: 483–489.

Mukherjee A., Lal R. (2014): The biochar dilemma. Soil Research, 52: 217–230.

Mukherjee A., Zimmerman A.R. (2013): Organic carbon and nutrient release from a range of laboratory-produced biochars and biochar-soil mixtures. Geoderma, 193–194: 122–130.

Nikiforova V.J., Bielecka M., Gakière B., Krueger S., Rinder J., Kempa S., Morcuende R., Scheible W.R., Hesse H., Hoefgen R. (2006): Effect of sulfur availability on the integrity of amino acid biosynthesis in plants. Amino Acids, 30: 173–183.

Novak J.M., Ippolito J.A., Lentz R.D., Spokas K.A., Bolster C.H., Sistani K., Trippe K.M., Phillips C.L., Johnson M.G. (2016): Soil health, crop productivity, microbial transport, and mine spoil response to biochars. BioEnergy Research, 9: 454–464.

Ohno T., Grunes D.L. (1985): Potassium-magnesium interactions affecting nutrient uptake by wheat forage. Soil Science Society of America Journal, 49: 685–690.

Pavlíková D., Zemanová V., Procházková D., Pavlík M., Száková J., Wilhelmová N. (2014): The long-term effect of zinc soil contamination on selected free amino acids playing an important role in plant adaptation to stress and senescence. Ecotoxicology and Environmental Safety, 100: 166–170.

Peng F., He P.-W., Luo Y., Lu X., Liang Y., Fu J. (2012): Adsorption of phosphate by biomass char deriving from fast pyrolysis of biomass waste. Clean – Soil, Air, Water, 40: 493–498.

Prapagdee S., Tawinteung N. (2017): Effects of biochar on enhanced nutrient use efficiency of green bean, *Vigna radiata* L. Environmental Science and Pollution Research, 24: 9460–9467.

Rizwan M., Ali S., Qayyum M.F., Ibrahim M., Zia-ur-Rehman M., Abbas T., Ok Y.S. (2016): Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: A critical review. Environmental Science and Pollution Research, 23: 2230–2248.

Schimmelpfennig S., Kammann C., Moser G., Grünhage L., Müller C. (2015): Changes in macro- and micronutrient contents of grasses and forbs following *Miscanthus × giganteus* feedstock, hydrochar and biochar application to temperate grassland. Grass and Forage Science, 70: 582–599.

Singh B.K. (1999): Plant Amino Acids: Biochemistry and Biotechnology. New York, Marcel Dekker, 227–248.

Sorrenti G., Ventura M., Toselli M. (2016): Effect of biochar on nutrient retention and nectarine tree performance: A three-year field trial. Journal of Plant Nutrition and Soil Science, 179: 336–346.

Street R., Száková J., Drábek O., Mládková L. (2006): The status of micronutrients (Cu, Fe, Mn, Zn) in tea and Te infusions in selected samples imported to the Czech Republic. Czech Journal of Food Science, 24: 62–71.

Szabados L., Savouré A. (2010): Proline: A multifunctional amino acid. Trends in Plant Science, 15: 89–97.

Waqas M., Kim Y.-H., Khan A.L., Shahzad R., Asaf S., Hamayun M., Kang S.-M., Khan M.A., Lee I.-J. (2017): Additive effects due to biochar and endophyte application enable soybean to enhance nutrient uptake and modulate nutritional parameters. Journal of Zhejiang University Science B, 18: 109–124.

Weckopp S.C., Kopriva S. (2015): Are changes in sulfate assimilation pathway needed for evolution of C_4 photosynthesis? Frontiers in Plant Science, 5: 773.

Woldetsadik D., Drechsel P., Keraita B., Marschner B., Itanna F., Gebrekidan H. (2016): Effects of biochar and alkaline amendments on cadmium immobilization, selected nutrient and cadmium concentrations of lettuce (*Lactuca sativa*) in two contrasting soils. SpringerPlus, 5: 397.

Yao Y., Gao B., Chen J.J., Zhang M., Inyang M., Li Y.C., Alva A., Yang L.Y. (2013): Engineered carbon (biochar) prepared by direct pyrolysis of Mg-accumulated tomato tissues: Characterization and phosphate removal potential. Bioresource Technology, 138: 8–13.

Younis U., Qayyum M.F., Shah M.H.R., Danish S., Shahzad A.N., Malik S.A., Mahmood S. (2015): Growth, survival, and heavy metal (Cd and Ni) uptake of spinach (*Spinacia oleracea*) and fenugreek (*Trigonella corniculata*) in a biochar-amended sewage-irrigated contaminated soil. Journal of Plant Nutrition and Soil Science, 178: 209–217.

Zemanová V., Pavlík M., Pavlíková D., Tlustoš P. (2013): The changes of contents of selected free amino acids associated with cadmium stress in *Noccaea caerulescens* and *Arabidopsis halleri*. Plant, Soil and Environment, 59: 417–422.

Zhang Z.-Y., Jun M., Shu D., Chen W.-F. (2014): Effect of biochar on relieving cadmium stress and reducing accumulation in super japonica rice. Journal of Integrative Agriculture, 13: 547–553.

Zhang H.Z., Chen C.R., Gray E.M., Boyd S.E., Yang H., Zhang D.K. (2016a): Roles of biochar in improving phosphorus availability in soils: A phosphate adsorbent and a source of available phosphorus. Geoderma, 276: 1–6.

Zhang J.X., Zhang Z.F., Shen G.M., Wang R., Gao L., Kong F.Y., Zhang J.G. (2016b): Growth performance, nutrient absorption of tobacco and soil fertility after straw biochar application. International Journal of Agriculture and Biology, 18: 983–989.

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5. Sumární diskuze

Diskuze je rozdělena podle jednotlivých sérií experimentů, které byly připraveny a prováděny tak, aby se postupně plnily vytyčené cíle práce. Nejprve byl připravován biochar z kontaminované rostlinné biomasy za různých teplot pyrolýzy (400, 450, 500, 550 a 600 °C), stejného atmosférického tlaku a doby zdržení 30 minut (nosný plyn dusík). Získané materiály, biochary, byly analyzovány a byly stanoveny fyzikální a chemické vlastnosti: obsah popela, elementární analýza, určení pH hodnoty, stanovení specifického povrchu různými metodami, určení distribuce pórů, skutečné a zdánlivé hustoty.

Následně byly vybrány tři typy biocharu: biochar připravený z kontaminovaných rostlin kukuřice, kontaminovaného dřeva vrb a nekontaminovaného dřeva topolu. Ve vsádkových inkubačních experimentech byly testovány schopnosti těchto biocharů sorbovat kadmium, olovo a zinek ve středně kontaminované půdě. Současně byla pozorována míra desorpce prvků obsažených v připravených materiálech a směsích s půdou v různých typech vyluhovadel.

Paralelně byl založen nádobový experiment v kontrolovaných podmínkách. Zde byl sledován vliv aplikace komerčně dostupného biocharu na transfer kadmia, olova a zinku půdním profilem v extrémně kontaminované půdě. Pro tento účel byly použity lyzimetrické nádoby. Dále byl testován vliv aplikace biocharu na růst rychle rostoucích dřevin v této kontaminované půdě.

V předchozích experimentech byla použita půda z Příbrami, kontaminovaná v důsledku dlouhodobé důlní a hutní činnosti. V posledním pokusu byla zvolena nekontaminovaná půda obohacená přídavkem rizikových prvků (Cd, Pb, Zn) v koncentracích srovnatelných s půdou z Příbrami. Tento přístup byl zvolen proto, aby mohla být porovnána biochemická odezva rostlin rostoucích jak v kontaminované, tak i v nekontaminované půdě. V půdě byly pěstovány rostliny ve sledu špenát – hořčice – špenát a byl sledován vliv aplikace biocharu a komerčně dostupného aktivního uhlí na přístupnost prvků rostlinám a vliv na oxidativní stres rostlin.

5.1 Fyzikální a chemické vlastnosti biocharů jako výsledek rozdílných vstupních materiálů a teplot pyrolýzy: předurčují osud těchto materiálů v půdě?

Velká diverzita vstupních materiálů a stejně tak i různé postupy pyrolýzy (teplota, doba zdržení, atd.) ovlivňují kvalitu výsledného biocharu a vedou k vysoce rozdílným chemickým (obsah uhlíku, zastoupení další prvků, množství funkčních skupin) a fyzikálním

(velikost specifického povrchu, struktura, distribuce pórů) vlastnostem (Keiluweit a kol., 2010; Sohi a kol., 2010; Mohan a kol., 2006; Fu a kol., 2009; Ahmad a kol., 2012; Lua a kol., 2004; Lua a kol., 2006; Břendová a kol., 2017). Znalost fyzikálních vlastností biocharu je nezbytná vzhledem k interakcím v půdním systému, které jsou podmíněny těmito vlastnostmi (Downie a kol., 2009). Aller (2016) shrnuje dostupná publikovaná data týkající se fyzikálních vlastností biocharů a závěrem udává, že v porovnání s ostatními vlastnostmi biocharu jsou tato data nedostatečná pro komplexní hodnocení vlastností biocharu.

Zhao a kol. (2013) hodnotili efekt vstupního materiálu a finální teploty pyrolýzy na pH biocharu. Zjistili, že tato vlastnost je především podmíněná teplotou pyrolýzy. V naší studii byla potvrzena lineární korelace teploty pyrolýzy a hodnoty pH výsledného biocharu.

Aplikuje-li se biochar do půdy, poločas rozpadu se udává v řádu tisíce let, pokud je poměr obsahu kyslíku k uhlíku nižší než 0,2. Tudíž lze předpokládat, že biochary připravené v našem experimentu budou v půdě stabilní (Spokas, 2010). Jestliže porovnáme molární poměry našich materiálů s organickými složkami vlastními půdě, jsou tyto poměry u biocharů nižší (poměr O /C u huminových je obvykle 0,5 a u fulvo kyselin 0,7 ; poměr H /C u obou typů kyselin se blíží 1,0 (Stevenson, 1994).

Fyzikální vlastnosti biocharu, jakými jsou jeho specifický povrch, objem pórů i průměrná velikost pórů, jsou obvykle spojovány s jeho schopností sorpce, vodozádržností, a tudíž souvisí s působením biocharu na půdní strukturu, mobilitu kontaminantů a jeho interakci s mikroorganismy (Zhao a kol., 2013).

Specifický povrch biocharu určený BET metodou (S_{BET}) vzrůstal logaritmicky (dřevo bez kůry: R^2=0,98; dřevo s kůrou: R^2=0,98; kukuřice: R^2=0,89; luční seno: R^2=0,99; sláma pšenice ozimé: R^2=0,94; zrno pšenice ozimé: R^2=0,55) se vzrůstající finální teplotou pyrolýzy. Například specifický povrch biocharu z kornatého dřeva vzrůstal z 124,4 $m^2 . g^{-1}$ na 428,1 $m^2.g^{-1}$, z kukuřice z hodnoty 4,75 na 105 $m^2.g^{-1.}$ Tento jev je pravděpodobně způsoben únikem těkavé organické složky ze vstupního materiálu, čímž dochází k tvorbě pórů ve výsledném produktu, biocharu (Downie a kol., 2009).

Bachmann a kol. (2016) uvádějí, že určování specifického povrchu podle BET modelu je závislé na velikosti a distribuci pórů, a tak může vést k přeceňování či podceňování celkového specifického povrchu závisejícího na poměru parciálního tlaku měření a na typu pórů dominujících v analyzovaném vzorku. Většina dostupné literatury věnující se biocharu uvádí specifický povrch určený metodou BET, je ale zapotřebí dalšího výzkumu pro určení vztahu těchto měření k množství reaktivních míst povrchu biocharu (Brewer, 2009). Tudíž by

se obecně dalo navrhnout, že specifický povrch biocharu určený BET metodou je vhodné doplnit dalšími vlastnostmi povrchu pro důkladnější určení vlastností tohoto materiálu.

Struktura pórů ovlivňuje fyzikální, chemické i biologické vlastnosti půdy, jako je difúze plynu, distribuce a zádržnost vody, mechanická odolnost, dynamika uhlíku, výskyt mikrobů a pronikání kořenů (Zaffar, a kol., 2015). Distribuce a velikost pórů je klíčový parametr v charakteristice porézního aktivního uhlí (Jiménez-Cordero a kol., 2013). Tvar adsorpčních izoterem určujících velikost pórů v materiálech získaných pyrolýzou v naší studii koresponduje s I. a III. typem dle IUPAC (2015). Profil izoterem naznačuje přítomnost velkého množství mikropórů ve všech vzorcích biocharu, a to ve větší míře v biocharech ze dřeva oproti materiálům z bylinné biomasy.

Jestliže uvažujeme o porézním biocharu pro účely půdní aplikace, lze toto aditivum srovnat s porézními jílovitými minerály běžně se vyskytujícími v půdě. Objem pórů biocharů z dřevní biomasy je srovnatelný s přírodním zeolitem ($\sim$0,3 $cm^3.g^{-1}$; Herron and Corbin, 1995) či montmorillonitem ($\sim$0,21 $cm^3.g^{-1}$; Diamond, 1970), přičemž objem pórů biocharů z bylinné biomasy je nižší.

Hustota a porozita jsou základními fyzikálními charakteristikami, které hrají jednu z hlavních rolí určujících stabilitu dřevěného uhlí v půdě (Massiello a kol., 2012). Zatímco skutečná hustota biocharů připravených v našem experimentu se zvyšovala s finální teplotou pyrolýzy, zdánlivá hustota těchto materiálů slabě klesala nebo nebyl sledován žádný vliv finální teploty pyrolýzy.

Porozita biocharu závisela na vstupním materiálu a finální teplota pyrolýzy tuto vlastnost neovlivnila. Například porozita biocharu z dřevní biomasy se pohybovala v rozmezí od 54 do 58 % napříč zkoušenými teplotami pyrolýzy. Porozita je považována jako zásadní vlastnost umožňující v dané matrici zadržovat vodu. Biochary připravené za nízkých teplot pyrolýzy se vyznačují nižší schopností poutat vodu, avšak poutají stejné množství etanolu. To naznačuje, že rozdíly v zádržnosti vody v materiálech připravených za různých teplot pyrolýzy reflektují spíše rozdíly v hydrofobním charakteru (Gray a kol., 2014). Podobně Brewer a kol. (2014) popisují výsledky měření skutečné a zdánlivé hustoty biocharu. Jejich zjištěné hodnoty skutečné hustoty se pohybují v rozmezí od 1,34 $g.cm^{-3}$ do 1,96 $g.cm^{-3}$ a jsou srovnatelné s našimi výsledky. Hodnoty zdánlivé hustoty (0,25 $g.cm^{-3}$ do 0,60 $g.cm^{-3}$) se pohybovaly v nižším rozmezí než v této práci. Navíc byly pozorovány vyšší hodnoty tohoto parametru u biocharu z dřevní biomasy než z bylinné.

5.2 Biochar a jeho schopnost sorpce kadmia, olova a zinku

Ve srovnání s výchozí surovinou byly rizikové prvky zakoncentrovány v připraveném biocharu. Čtyřikrát vyšší obsah zinku byl detekován v biocharu ze dřeva, desetkrát vyšší v biocharu z kukuřice a šestkrát vyšší v nekontaminovaném biocharu oproti obsahům ve vstupních materiálech. V porovnání se vstupními materiály je obsah kadmia nižší v připravených biocharech, což potvrzuje těkavost kadmia při vyšších teplotách pyrolýzy (Stals a kol., 2010). Problematika zachycování kadmia při pyrolýze dále zůstává otevřena dalšímu výzkumu.

V desorpčním experimentu se ze sledovaných matric nejvíce uvolňoval zinek, a to zřejmě díky jeho vysokému obsahu v biocharu v porovnání s ostatními sledovanými prvky. Extrakce zinku byla nejvyšší z biocharu z kukuřice a kontaminovaného dřeva (až 25 %) ve srovnání s biocharem z nekontaminovaného dřeva a také s půdou, především v kyselém prostředí roztoku CH_3COOH. Jestliže uvažujeme zinek nejen jako rizikový prvek, ale také jako mikroživinu, pak jeho pomalé uvolňování nemusí znamenat zpětnou kontaminaci půdy. Naopak by tento biochar mohl být využit jako hnojivo s pozvolna se uvolňující živinou, zinkem, vhodné k aplikaci na půdách s deficitem tohoto prvku. Tuto problematiku již popisují autoři Evangelou a kol. (2014) a Břendová a kol. (2016).

Pro popis sorpčních schopností biocharu ve směsích s půdou byly použity sorpční izotermy. Sorpční schopnost směsí biocharu s půdou byla porovnávána se sorpčními schopnostmi půdy samotné. Bylo pozorováno, že všechny sledované typy biocharů sorpční schopnost půdy zvyšují. Podobně jako uvádí Uchymia a kol. (2011a, b, c), i v naší studii bylo zřejmé, že sorpční schopnost biocharu silně závisí na typu sorbovaného iontu (v naší studii byl pozorován trend intenzity sorpce: $Cd^{2+}<Zn^{2+}<<Pb^{2+}$). I přes rozdílný typ biocharu (připravený z hovězího hnoje za nižší teploty pyrolýzy, tj. 350°C), sled maximální sorpční kapacity pro dané ionty byl obdobný i v dalších publikacích, např. Xu a kol. (2013) a Cao a kol. (2009) a to: $Zn^{2+}<Cd^{2+}<<Pb^{2+}$. Tyto výsledky poukazují na silnou afinitu olova na biocharovou matrici oproti ostatním sledovaným prvkům. Vysokou účinnost sorpce olova ve srovnání s kadmiem dokládá i publikace Mohan a kol. (2007). Ačkoliv nejvyšší sorpční kapacita byla zjištěna u matrice biocharu z kukuřice + půda, nelze v tomto případě namodelovat izotermy dle Langmuirova modelu. Sorpční izotermy jednotlivých směsí obou druhů dřeva a půdy jsou srovnatelné. Xu a kol. (2013) zjistili, že více než 75 % retence kovů se připisuje srážení, zvláště díky karbonátovým funkčním skupinám. Proto rozdíly v přítomnosti konkrétních funkčních skupin u jednotlivých biocharů mohou být vztaženy k jejich různému chování v půdě.

Aplikace biocharu do půdy zvyšuje její hodnotu pH (Lehmann, 2007a). Zvýšení pH půdy může vést ke zvýšení negativního povrchového náboje a tudíž lze očekávat, že afinita půdy spolu s biocharem ke kationtům vzroste (Jiang a kol., 2012). Zlepšující efekt aplikovaného biocharu do kyselé půdy – spočívající buď ve zvýšení pH půdy nebo lepší retenci živin, případně oběma faktory – popsali Yuan a kol. (2011). V naší studii jsme pozorovali vysokou afinitu sledovaných kovů na matrice biochar + půda při jejich nižších koncentracích v roztoku. Tento jev byl provázen vysokým pH systému. Následně bylo pozorováno, že sorpce kationtů kovů byla doprovázena uvolňováním H^+ iontů zpět do pozaďového elektrolytu, což zapříčinilo snížení pH systémů se vzrůstající sorpcí kationtů, podobně jako to zaznamenali Mustafa a kol. (2002). Díky schopnosti biocharu z kukuřice zvýšit pH systému až na hodnotu 9 došlo ke srážení iontů Cd a Zn (až 80 % Cd^{2+}, Zn^{2+}). Toto chování bylo vyvozeno na základě dle modelování dat softwarem *Visual* MINTEQ (množství aniontů uvolněných z půdy bylo zanedbáno). Jestliže jsme vzali v potaz obsah aniontů v půdě (SO_4^{2-}: 3,2 mmol.L$^{-1,}$ PO_4^{3-}: 1,6 mmol.L^{-1}; extrahováno KNO_3), bylo zjištěno, že až 98 % Cd bylo s nejvyšší pravděpodobností vysráženo jako komplexy se sulfáty a hydroxidy, a to v celé koncentrační řadě přidávaného kadmia. Zinek mohl být taktéž vysrážen s fosforečnany v matrici biochar z kukuřice + půda a v matrici nekontaminované dřevo + půda za nejnižších koncentrací tohoto prvku. Můžeme tedy shrnout, že biochar nepřímo, ale významně ovlivňuje sorpci rizikových prvků v půdě.

Opačný trend byl pozorován při sorpci olova na matrice biochar z kukuřice a dřeva + půda, kde pH systémů téměř nevzrůstalo při vzrůstající koncentraci sorbovaného kovu. Sorpce by v tomto případě mohla být vysvětlena přítomností amorfního SiO_2 v obou matricích, k němuž mohou mít ionty Pb^{2+} silnou afinitu. Této vlastnosti využili Hao a kol. (2012), kteří popsali kompozit SiO_2/grafen s vysokou sorpční efektivitou a schopností navození rychlé sorpční rovnováhy jako praktický sorbent Pb^{2+} iontu. Lu a kol. (2012) pozorovali při sorpci olova na biocharu z čistírenského kalu srážení tohoto kovu s fosfátosilikátovým komplexem, a tudíž potvrzují podstatnou roli silikátu při sorpci olova. Ionty Pb^{2+} v systému biochar z nekontaminovaného dřeva + půda byly zřejmě vysráženy jako $Pb(SO_4)$, a tak se pH systému snižovalo za uvolňování H^+ při srážení vyšších koncentrací tohoto kationtu.

Data získaná vsádkovým experimentem byla také modelována pomocí Langmuirovy izotermy. Dle Nash–Sutcliffova koeficientu efektivnosti modelu (E) (Nash a Sutcliffe, 1970), je Langmuirova izoterma vhodnější pro popis sledovaných matric a jejich sorpčních schopností ve srovnání s jinými modely. K podobným závěrům, dospěli i Mohan a kol.

(2007), kteří studovali vlastnosti biocharu ze dřeva, pyrolyzovaného při 450°C. Díky tomu, že naše data odpovídají danému modelu, lze shrnout, že povrch matric je homogenní, sorpce iontů kovů je monovrstevná a sorbované ionty nemohou být vzájemně ovlivňovány (Langmuir, 1916). Maximální sorpční kapacity sledovaných matric byly seřazeny dle nejvyšších hodnot pro jednotlivé kovy: biochar z kukuřice + půda> biochar z nekontaminovaného dřeva + půda>půda pro olovo; biochar ze dřeva > biochar z nekontaminovaného dřeva > biochar z kukuřice pro kadmium a biochar z kukuřice > biochar z nekontaminovaného dřeva > biochar ze dřeva pro zinek.

5.3 Biochar a jeho vliv na růst rostlin a pohyb rizikových prvků v kontaminované půdě

Legislativa zatím neupravuje limity pro obsahy rizikových prvků v biocharu při jeho aplikaci do půdy. Mezinárodní iniciativa pro biochar (International Biochar Initiative) navrhuje následující maximální obsahy vybraných prvků v biocharu, pokud je uvažován jako půdní aditivum: Cd: 1.4 -39, Zn: 416-7400 and Pb: 121-300 mg.kg$^{-1.}$ Biochar použitý v naší studii je dle těchto kritérií vhodný pro aplikaci do půdy (biochar-internationl.org). Vyhláškou 153/2016 Sb. jsou dány indikační hodnoty pro obsah kadmia, olova a zinku v půdě. Překročením těchto hodnot pro kadmium a olovo může být ohrožena zdravotní nezávadnost potravin a krmiv a překročením hodnoty obsahu zinku může být podezření z ohrožení růstu rostlin a produkční funkce půdy. Obsahy prvků v půdě zkoumané v našem experimentu převyšovaly tyto hodnoty několikanásobně (Cd: 27krát, Pb: 12krát a zinek 14krát). Aluviální území řeky Litavky, odkud zemina pro experiment prochází, bylo dříve studováno Vaňkem a kol. (2005). Tito autoři zjistili, že v povodňových oblastech byl průměrný obsah Cd, Pb a Zn v orné půdě 34; 2979; a 3363 mg.kg$^{-1.}$ K vysoké kontaminaci došlo v důsledku protržení zádržných lagun kovohutí a odpad byl vyplaven a rozšířen do nivy řeky Litavky v důsledku povodní (Borůvka and Vácha, 2006; Borůvka a kol., 1996). Rizikové prvky kontaminující fluvizemě se vyznačují vysokou rozpustností (Vácha a kol., 2002), tudíž takto extrémně zatížená půda vyžadovala specifický přístup a metody, aby se předešlo šíření těchto rizikových prvků do potravního řetězce.

Ačkoliv Novak a kol. (2016) popsali nejednoznačný vliv biocharu na výnos pěstované biomasy, naše výsledky dokazují pozitivní působení biocharu na její produkci: zvýšení výnosu biomasy v prvním a druhém roce pěstování. Meers a kol. (2007) pěstovali vrby na kontaminované půdě v nádobovém experimentu (5 mg Cd kg $^{-1,}$ 275 mg Zn kg $^{-1}$; výluh lučavkou královskou) a dosáhli výnosu (nadzemní biomasa) 1,4 g .rostlina^{-1} v 3 kg půdy. V našem experimentu jsme testovali extrémně kontaminovanou půdu a na variantě, kde bylo

aplikováno 5 % biocharu, jsme dosáhli výnosu 10 g na rostlinu v prvním roce pěstování, nižšího pak v roce druhém. Aplikace biocharu potlačila fytotoxicitu extrémně kontaminované půdy ve dvou po sobě jdoucích sezonách pěstování.

Vzhledem k tomu, že zinek je pro rostliny mikroživina, bylo možno předpokládat, že ho rostliny budou akumulovat ve vyšších koncentracích než olovo a kadmium (Tlustoš a kol., 2007). Koncentrace zinku ve větvích se pohybovaly v rozmezích 1828–2103 mg.kg^{-1} v prvním roce, 2070–3442 mg.kg^{-1} ve druhém a 2980–3505 mg.kg^{-1} ve třetím roce pěstování. Statisticky významně nejvyšší schopnost odebírat zinek z půdy do pletiv byla pozorována u rostlin vrb na variantě, kde bylo aplikováno 15 % biocharu, v prvním a druhém roce pěstování. Vondráčková a kol. (2015) pěstovali vrby na půdě ze stejné lokality jako v našem experimentu a obsah zinku v pletivech stanovali v rozmezí 2074–3488 mg.kg$^{-1,}$ tedy srovnatelné s našimi hodnotami. Obsah kadmia a zinku v listech byl několikanásobně vyšší oproti větvím, tento jev pozorovali i Lettens a kol. (2011), kteří popsali zvyšující se koncentraci prvků v listech ke konci vegetace. Robinson a kol. (2000) navrhuje kvůli vysoké koncentraci Cd a Zn každoroční sklizeň listové biomasy, aby se zabránilo zpětnému uvolňování akumulovaných prvků do prostředí rozkladem opadaných listů. Odlišné chování se projevilo ve třetím roce pěstování, kdy nebyl zaznamenán žádný rozdíl mezi variantami v akumulaci a koncentraci kadmia a zinku, a výnos biomasy byl nejnižší při srovnání všech tří sezon.

Hodnota obsahu zinku považována za hraniční pro projev fytotoxicity je 400 mg.kg^{-1} (Kabata Pendias a Pendias, 2001). Ačkoliv byla tato hodnota ve sledovaných rostlinách několikanásobně překročena, rostliny na variantách s biocharem neprojevily žádné známky fytotoxicity v prvním a druhém roce pěstování. Beesley a kol. (2010) popsali snížení fytotoxického působení zinku díky přídavku biocharu do půdy při testu fytotoxicity na semenech. Naše studie tudíž může toto tvrzení potvrdit a navíc pozitivní působení biocharu rozšířit také na celé rostliny. Ovšem pozitivní efekt neměl dlouhotrvající účinek, byl pozorován pouze ve dvou ze tří let pozorování. Naproti tomu Shen a kol. (2016) popsali snížení dostupného obsahu zinku a niklu (stanoveného ve výluhu kyselinou uhličitou) v půdě ošetřené biocharem a kompostem po třech letech spolupůsobení v polních podmínkách. V jejich studii však byla kontaminace zinku v půdě značně nižší než v naší, a tak i pozitivně působící dávka biocharu byla v jejich studii nižší (<5 %).

Redukce mobilního obsahu rizikových prvků (stanoveno ve výluzích různými extrakčními činidly) různých matric vlivem alkalických materiálů pozorovali například Jiang a kol. (2012); Ciccu a kol. (2003). Ve výluhu silnější kyselinou octovou zjistili Jiang a kol.

(2012) snížení obsahu kadmia o 86 %, po přidání 3 % biocharu a nenalezli významný rozdíl mezi dávkou 3 a 5 % biocharu. Ciccu a kol. (2003) sledovali vlivem aplikace popílku a červeného kalu do půdy snížení vyluhovatelnosti zinku až o 99 %. V našem experimentu byl v perkolátu obsah zinku a kadmia redukován o 97 % (průměr ze všech stanovení na všech variantách) na všech variantách, kde byl aplikován biochar ve srovnání s kontrolou. Obsah olova v perkolátu byl pod mezí detekce stanovení. Ovšem pouze v prvních dvou letech experimentu. Ve třetím roce pěstování se koncentrace kadmia v perkolátu zvýšila o 37 %, 300 % a 207 %, zinku o 15 %, 150 % a 124 % na variantách 5 %, 10 % a 15 % aplikovaného biocharu (první odběr ve třetím roce), při čemž koncentrace olova zůstala pod mezí detekce. Zdá se, že rozhodujícím faktorem, který ovlivnil průběh experimentu, je hodnota pH. V první a druhém roce pH perkolátu vzrůstalo se vzrůstající dávkou biocharu. Ve třetím roce nebyl zjištěn rozdíl mezi variantami a hodnota pH perkolátu klesla o 1,5. Laird a kol. (2010a, b) popsali v kolonovém experimentu zvýšení pH výluhu na variantách, kde byl aplikován biochar o jednotku v porovnání s kontrolou bez biocharu (dávka biocharu 0,5; 1,2 %; experiment bez rostlin, trvání experimentu: 500 dnů). Doerge a Garden (1984) vysvětlují reacidifikaci vápněné půdy okyselujícími procesy jako je například nitrifikace. CO_2 je uvolňován rostlinami, mikrobiální respirací, mineralizací organické hmoty a disociací organických kyselin. Všechny tyto aspekty mohou vést ke snížení pH půdy. Bradl (2004) popisuje uvolňování H^+ iontů při sorpci kovů, což v našem případě mohlo také hrát roli. Houben a kol. (2013) pozorovali imobilizaci kadmia, olova a zinku po aplikaci biocharu (snížení extrahovatelného podílu těchto prvků v 0,01 $mol.l^{-1}$ $CaCl_2$). Jakmile však hodnotu pH půdy snížili, zjistili, že uvolňovaní kovů při přesně definovaném pH nebylo ovlivněno přítomností biocharu. To naznačuje, že aplikace biocharu nutně nevede k nově vytvořeným sloučeninám odolným ke změnám pH, takže interakce mezi biocharem a sledovanými prvky je plně závislá na pH a při acidifikaci je reverzibilní.

Další otázky vyvolává naše pozorování významné redukce obsahu sledovaných prvků v perkolátu a současně jen malé změny v jejich akumulaci do pletiv rostlin ve srovnání s kontrolou. Důvodem může být schopnost rhizosféry rostlin zpřístupnit prvky rostlinám. Může také dojít k jisté kompetici prvků, kdy biochar imobilizuje některé kationty a jiné jsou pak přednostně přijímány, jak to pozorovali Rees a kol. (2015) u hyperakumulátoru *Nocaea caerulescens*, pěstovaném na kontaminované půdě, kde vlivem biocharu bylo docíleno zvýšeného příjmu kadmia a zinku.

Tlustoš a kol. (2007) stanovili fytoextrakční potenciál vrb na vysoce kontaminované fluvizemi bez jakýchkoliv imobilizačních opatření, kdy efektivnost fytoextrakce nepřekročila

1 % pro kadmium a zinek. Jako nevhodné remediační opatření těžce zatížených půd označují fytoextrační metody také Jensen a kol. (2009) s odůvodněním nedostatečné tvorby biomasy. V prvních dvou letech už 5 % biocharu aplikovaného do silně kontaminované půdy podpořilo růst vrb a tím i jejich fytoextrakční potenciál.

Po ukončení experimentu byla půda podrobena sekvenční analýze. Nejvyšší obsahy kadmia a zinku byly stanoveny ve snadno rozpustné frakci. To je ve shodě s výsledky, které publikovali Pustišek a kol. (2001). Tito autoři popsali různou distribuci kadmia, olova a zinku v uměle a přirozeně kontaminované půdě. Přirozeně se kadmium a zinek vyskytují především vázané na Fe/Mn oxidy (redukovatelná frakce) a silikáty, kdežto v kontaminovaných půdách se tyto prvky vyskytují především ve snadno rozpustné frakci. Naše výsledky ukazují snižující se obsah pozorovaných prvků v této frakci na variantách s biocharem po třech letech pěstování a navýšení zastoupení kadmia v redukovatelné frakci. Zinek byl stanoven ve vyšší míře i v oxidovatelné frakci. Zvýšením pH půdy v důsledku působení biocharu dochází k zvýšení negativního náboje na povrchu půdy, a tak dochází ke zvýšení sorpce kadmia a zinku (Chang a kol., 2013). V nekontaminovaných půdách je olovo obvykle vázáno na oxidovatelnou frakci, silikáty a sulfidy (Pustišek a kol., 2001). V kontaminovaných půdách je olovo distribuováno hlavně v karbonátech a Fe/Mn oxidech (Stouraiti a kol., 2000) nebo ve výměnné frakci (Pustišek a kol., 2001). V našem případě bylo především vázáno na Fe/Mn oxidy. Afinita olova na Mn/Fe oxidy byla popsána (McKenzie, 1980; Vaněk a kol., 2005) a v půdě našeho experimentu byl stanoven vysoký obsah manganu i železa (Mn: 6,0 g.kg^{-1}; Fe: 3,2 g.kg^{-1}). Množství organicky vázaného olova se zvýšilo o 2 % a v reziduální frakci o 3 % na variantě, kde bylo aplikováno 15 % biocharu. Liang a kol. (2014) ve své studii aplikovali 5 % biocharu z kravského hnoje do kontaminované půdy a pozorovali podobnou distribuci olova jako v našem experimentu.

Ačkoliv některé studie popisují, že biochar zvyšuje KVK půdy (Liang a kol., 2006; Novak a kol., 2009a), v našem experimentu se KVK půdy po třech letech nezměnila. Zjištěné pH perkolátu v prvních dvou letech pěstování poukazovalo na zvýšení pH půdy. Takzvaný „liming efekt“ biocharu popsali již Yuan a kol. (2011). Rentgenová difrakce biocharu poukazuje na to, že karbonáty jsou hlavní alkalickou složkou biocharu. Jestliže biochar přispívá ke změnám pH půdy podobně jako vápnění, z výsledků naší studie vyplývá, že i aplikace biocharu bude nutné pravidelně opakovat.

5.4 Biochar připravený z kontaminované biomasy a jeho působení na růst rostlin

Gregory a kol. (2014) sledovali, že aplikace biocharu v dávce 1–2 % zvýšila výnos nadzemní biomasy dvakrát a kořenové biomasy třikrát oproti kontrole bez biocharu. V naší studii dokumentujeme, že biochar připravený z kontaminované biomasy tvorbu biomasy podporuje, a to především na kontaminované půdě u špenátu. V případě hořčice byl efekt biocharu nízký, ale nízký efekt působení uhlíkatých aditiv na výnos biomasy rostlin z čeledi *Brassicaceae* zaznamenali např. i Rees a kol. (2015).

Negativní vliv rizikových prvků (v dávkách Pb 300 mg.kg^{-1}, Cd 0,5 mg.kg^{-1}, Zn 250 mg.kg^{-1}) na tvorbu biomasy špenátu popsali Ali a kol. (2015). Naše výsledky ukázaly, že aplikací biocharu bylo dosaženo vyšších výnosů na kontaminovaných variantách (Cd: 10 mg.kg^{-1}; Pb: 300 mg.kg^{-1} a Zn: 450 mg.kg^{-1}). Gartler a kol. (2013) docílili biofortifikace zeleniny zinkem při použití biocharu jako aditiva do substrátu. V našem experimentu došlo k vyšší schopnosti špenátu akumulovat zinek – při vyšším výnosu – ve variantách, kde byl aplikován biochar (400–450 mg.kg^{-1}). Biochar a aktivní uhlí byly popsány jako vhodná média snižující dostupnost kadmia i zinku rostlinám (Shaheen a kol., 2015). Schopnost imobilizovat těžké kovy byly v naší studii pozorovány především na variantách, kde bylo aplikováno aktivní uhlí. Rees a kol. (2015) uvádějí, že zvýšení pH aplikací biocharu je právě ten faktor, který ovlivňuje systém půda – rostlina. A dále vyvozují, že efektivita biocharu se vlivem reacidifikace tudíž sníží s časem. V našem experimentu bylo stanoveno pH půd až po jeho ukončení a bylo zjištěno, že biochar a aktivní uhlí nezvýšily pH sledovaných půd.

Výsledky naší studie popisují reakci metabolismu aminokyselin v rostlinách špenátu a hořčice v přítomnosti aktivního uhlí a biocharu v půdě na kontaminované i nekontaminované půdě. Významná role aminokyselin v metabolismu rostlin ve stresových podmínkách je známa (např. Kumar a kol., 2014; Sharma a Dietz, 2006; Zagorchev a kol., 2013). Efekt biocharu na tvorbu těchto metabolitů popisuje pouze Younis a kol. (2015a,b), kteří stanovovali celkový obsah aminokyselin ve špenátu a pískavici pěstovaných na kontaminované půdě (Cd a Ni) s přídavkem biocharu. Pavlík a kol. (2010) uvádějí, že metabolismus aminokyselin hraje velkou roli v rezistenci rostlin vůči abiotickým stresům. To se potvrdilo v naší studii, kde byla nalezena nižší hodnota součtu obsahu aminokyselin na kontaminované půdě ve srovnání s nekontaminovanou. Snížení obsahu aminokyselin je zřejmě způsobeno jejich využitím rostliny při obraně proti zvýšenému obsahu těžkých kovů v půdě. Funkce aminokyselin při těchto pochodech byla pozorována při poutání těžkých kovů, antioxidačních procesech, osmotických procesech a tvorbě fytochelatinů (Kumar a kol., 2014; Sharma a Dietz 2006; Zagorchev a kol., 2013). Rozdíly v metabolismu aminokyselin byly

v našem experimentu závislé nejen na přítomnosti zvýšených obsahů prvků, ale také na druhu pozorované rostliny. Přítomnost biocharu v kontaminované půdě zvýšila obsah aminokyselin ve špenátu a následně pěstované hořčici oproti kontrole, což potvrzují i Younis a kol. (2015a). Podobné výsledky byly pozorovány i pro aktivní uhlí.

6. Závěr

Přírodní zdroje jsou jedinečné a je třeba o ně náležitě pečovat. Půda je jedním z hlavních zdrojů života. Kontaminace antropogenními aktivitami jsou závažné, a to tím spíše jedná-li se o kontaminaci prvky, které nedegradují a z půdy se obtížně odstraňují. Ve své práci jsem navázala na dlouhodobý výzkum svých mentorů a kolegů z katedry týkající se výzkumu oblasti Příbramska a možnostmi ozdravění zdejší kontaminované půdy. V této lokalitě již byly zavedeny fytoextrakční a v menší míře stabilizační metody remediace. Využití moderního materiálu biocharu nabízí určitý potenciál při zavádění účinných remediačních postupů zejména na vysoce kontaminované půdě.

Při fytoextrakčních postupech byla vyprodukována kontaminovaná biomasa, kterou – bylinnou i dřevní – jsme podrobili pyrolýze a sledovali fyzikální a chemické vlastnosti biocharu. Vlastnosti připravených biocharů závisely významně na vstupní biomase a teplotě pyrolýzy. Mezi parametry vstupní biomasy a výsledného biocharu se projevily specifické vztahy, např. vztah mezi obsahem ligninu (logaritmický) a popela (lineární) ve vstupní biomase a tvorbou pórů. Biochar z dřevní biomasy vykazoval vyšší hodnoty specifického povrchu oproti biocharu z biomasy bylinné (nejvyšší specifický povrch byl stanoven u biocharu připraveného z dřevní biomasy: 511 $m^2 . g^{-1}$) a obsahoval vyšší obsah mikropórů. Jestliže se uvažuje, že bude připravený biochar aplikován do půdy pro sorpční účely, biochar z dřevní biomasy se díky svým povrchovým vlastnostem jeví jako vhodnější materiál oproti biocharu z rostlinné biomasy. Obecně by stanovené vlastnosti měli napomoci při tvorbě takzvaného biocharu na míru – pro konkrétní účely konkrétního místa.

Při studiu sorpčních vlastností biocharů připravených z kontaminované biomasy vrb, kukuřice a nekontaminovaného topolu (teplota pyrolýza 600 °C) jsme pozorovali úspěšnou imobilizaci sledovaných prvků: kadmia, olova a zinku. Pomocí modelů sorpčních izoterem a softwaru VisualMinteq byla zjištěna vysoká afinita sledovaných prvků ke směsím půdy s biocharem a jako hlavní faktor ovlivňující imobilizaci Cd, Pb a Zn bylo pH systému. Důležité zjištění bylo, že se sledované těžké kovy z biocharů v krátkodobém laboratorním testu zpětně neuvolňovaly.

Nádobový lyzimetrický experiment probíhal po dobu tří let. Do extrémně kontaminované půdy byl aplikován komerčně dostupný biochar a v nádobách pěstovány vrby. V prvních dvou letech jsme zaznamenali pozitivní účinky biocharu, růst biomasy bez fytotoxických symptomů oproti kontrole již na variantě, kde bylo aplikováno 5 % biocharu. Obsah Cd, Pb a Zn v perkolátu se s biocharem snížil, pH průsakové vody se zvýšilo. Ovšem

ve třetím roce se nárůst biomasy oproti kontrole nelišil a obsah sledovaných kovů se v perkolátu rapidně zvýšil, zatímco pH se naopak snížilo. Možná příčina byla zřejmě reacidifikace půdy. Výsledky tohoto experimentu naznačují, že biochar by bylo zapotřebí v polních – tedy reálných – podmínkách aplikovat opakovaně. Další výzkum v podobně polního experimentu je v tomto případě z hlediska praxe nutný.

Při studiu vlivu biocharu na růst rostlin byl do půdy aplikován biochar vlastní výroby, z kontaminované biomasy vrb a byly porovnány jeho účinky na růst rostlin v kontaminované půdě v porovnání s přídavkem s aktivním uhlím. Byl zjištěn pozitivní vliv obou aditiv na růst rostlin v kontaminované půdě a současně nebylo zjištěno negativní působení kontaminovaného biocharu na růst pěstovaných rostlin.

7. Seznam použité literatury vztažený k literárnímu přehledu a sumární diskuzi

Abbas, T., Rizwan, M., Ali, S., Zia-ur-Rehman, M., Qayyum, M. F., Abbas, F., Ok, Y. S. 2017. Effect of biochar on cadmium bioavailability and uptake in wheat *(Triticum aestivum L.)* grown in a soil with aged contamination. Ecotoxicology and environmental safety, 140, 37-47.

Adriano, D. C. 2001. Trace elements in terrestrial environments – Biogeochemistry, bioavailability and risk of metals, 2nd ed. Springer – Verlag, NY, Berlin, Heidelberg. p. 866. ISBN-10 0387986782.

Ahmad, M., Lee, S. S., Dou, X., Mohan, D., Sung, J. K., Yang, J. E., Ok, Y. O. 2012. Effects of pyrolysis temperature on soybean stover and peanut shell-derived biochar properties and TCE adsorption in water. Bioresource Technology, 118, 536-544.

Ahmad, M., Lee, S. S., Lee, S. E., Al-Wabel, M. I., Tsang, D. C., Ok, Y. S. 2017. Biochar-induced changes in soil properties affected immobilization/mobilization of metals/metalloids in contaminated soils. Journal of soils and sediments, 17 (3), 717-730.

Aller, M. F. 2016. Biochar properties: Transport, fate and impact. Critical Reviews in Environmental Science and Technology, 46 (14-15), 1183-1296.

Alloway, B. J. 1999. Heavy metals in soils. 2nd ed. Chapman & Hall. UK. p. 331. ISBN 0751401986.

Al-Wabel, M. A., Heil, D. M., Westfall, D. G., Barbarick, K. A. 2002. Solution chemistry influence on metal mobility in biosolids amended soils. Journal of Environmental Quality, 31, 1157-1165.

Amonette, J. E., Jospeh, S. 2009. Characteristics of biochar: microchemical properties. In: Lehmann J., Joseph S. (eds.). Bio¬char for environmental management science and technology. Earthscan. London. p. 33-43. ISBN: 978-1 -84407-658-1.

Antal, M. J., Gronli, M. 2003. The Art, Science, and Technology of Charcoal Production. Industrial and Engineering Chemistry Research, 42 (8), 1619-1640.

Apiratikul, R.; Pavasant, P. 2008. Batch and column studies of biosorption of heavy metals by *Caulerpa lentillifera*. Bioresource Technology, 99, 2766-2777.

Assunção, A. G. L., Schat, H., Aarts, M. G. M. 2003. *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. New Phytologist, 59 (2), 351-360.

Bachmann, H. J., Bucheli, T. D., Dieguez-Alonso, A., Fabbri, D., Knicker, H., Schmidt, H. P., Ulbricht, A., Becker, R., Buscaroli, A., Buerge, D. and Cross, A. 2016. Toward the standardization of biochar analysis: the COST action TD1107 interlaboratory comparison. Journal of Agricultural and Food Chemistry, 64 (2), 513-527.

Bapat, H., Manahan, S. E., Larsen, D. W. 1999. An activated carbon product prepared from milo (*Sorghum Vulgare*) grain for use in hazardous waste gasification by chemchar cocurrent flow gasification. Chemosphere, 39 (1), 23-32.

Barančíková, G., Makovníková, J. 2003. The influence of humic acid quality on the sorption and mobility of heavy metals. Plant, Soil and Environment, 49 (12), 565-571.

Basta, N.T., McGowen, S.L. 2004. Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. Environmental pollution, 127 (1), 73-82.

Beesley, L., Marmiroli, M. 2011. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environmental Pollution, 159 (2), 474-480.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J. L., Harris, E., Robinson, B., Sizmur, T. 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environmental pollution, 159 (12), 3269-3282.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L. 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental Pollution, 158 (6), 2282-2287.

Belviso, C., Cavalcante, F., Ragone, P., Fiore, S. 2012. Immobilization of Zn and Pb in polluted soil by in Situ crystallization zeolites from fly ash. Water, Air, and Soil Pollution, 223 (8), 5357-5364.

Berglund, L. M., DeLuca, T. H., Zackrisson, O. 2004. Activated carbon amendments of soil alters nitrification rates in Scots pine forests. Soil Biology and Biochemistry, 36, 2067-2073.

Bolan, N. S., Adriano, D. C. Curtin, D. 2003. Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. Advances in Agronomy, 78, 215-272.

Borůvka, L., Huan-Wei, Ch., Kozák, J., Krištoufková, S. 1996. Heavy contamination of soil with cadmium, lead and zinc in the alluvium of the Litavka river. Rostlinná výroba, 42 (12), 543-550.

Borůvka, L., Vácha, R. 2016. Litavka river alluvium as a model area heavily polluted with potentially heavy metal. In: Morel J. L. (eds.). Phytoremediation of Metal - Contaminated Soils. Netherland: Springer. p. 267-298. ISBN:10-1 -4020-468-1.

Bradl, H. B. 2004. Adsorption of heavy metal ions on soils and soils constituents. Journal of Colloid and Interface Science, 277 (1), 1 -18.

Brewer, C. E., Hu, Y.-Y., Schmidt-Rohr, K., Loynachan, T. E., Laird, D. A., Brown, R. C. 2012. Extent of pyrolysis impacts on fast pyrolysis biochar properties. Journal of Environmental Quality, 41, 1115-1122.

Brewer, C.E., Chuang, V.J., Masiello, C.A., Gonnermann, H., Gao, X., Dugan, B., Davies, C.A. 2014. New approaches to measuring biochar density and porosity. Biomass and Bioenergy, 66, 176-185.

Brewer, C.E., Schmidt-Rohr, K., Satrio, J.A. and Brown, R.C. 2009. Characterization of biochar from fast pyrolysis and gasification systems. Environmental Progress and Sustainable Energy, 28 (3), 386-396.

Brown, R.C., Stevens, C. 2011. Thermochemical processing of biomass: conversion into fuels, chemicals and power. Wiley. Chichester. p.350. ISBN:978-0 -470-72111-7.

Brunauer, S., Emmett, P. H., Teller, E. 1938. Adsorption of gases in multimolecular layers. Journal of the American Chemical Society, 60 (2), 309-319.

Břendová, K., Száková, J., Lhotka, M., Krulikovská, T., Punčochář, M., Tlustoš, P. 2017. Biochar physicochemical parameters as a result of feedstock material and pyrolysis temperature: predictable for the fate of biochar in soil? Environmental geochemistry and health, 39 (6), 1381-1395.

Břendová, K., Zemanová, V., Pavlíková, D., Tlustoš, P. 2016. Utilization of biochar and activated carbon to reduce Cd, Pb and Zn phytoavailability and phytotoxicity for plants. Journal of Environmental Management, 181, 637-645.

Cao, X., Harris, W. 2010. Properties of dairy-manure-derived biochar pertinent to its potential use in remediation. Bioresource technology, 101 (14), 5222-5228.

Cao, X., Ma, L., Gao, B., Harris, W. 2009. Dairy-manure derived biochar effectively sorbs lead and atrazine. Environmental Science and Technology, 43 (9), 3285–3291.

Ciccu, R., Ghiani, M., Serci, A., Fadda, S., Peretti, R., Zucca A. 2003. Heavy metal immobilization in the mining-contaminated soils using various industrial wastes. Minerals Engineering, 16 (3), 187-192.

Clark, R. B., Ritchey, K. D., Baligar, V. C. 2001. Benefits and constrains for use of FGD products on agricultural land. Fuel, 80 (6), 821-828.

Conesa, H. M., Wieser, M., Gasser, M., Hockmann, K., Evangelou, M. W. H., Studer, B., Schulin, R. 2010. Effects of three amendments on extractability and fractionation of Pb, Cu, Ni and Sb in two shooting range soils. Journal of Hazardous Materials, 181 (1), 845-850.

Cui, L., Li, L., Zhang, A., Pan, G., Bao, D., Chang, A. 2011. Biochar amendment greatly reduces rice Cd uptake in a contaminated paddy soil: a two-year field experiment. BioResources, 6 (3), 2605-2618.

Demirbas, A. 2004. Effects of temperature and particle size on bio-char yield from pyrolysis of agricultural residues. Journal of Analytical and Applied Pyrolysis, 72 (2), 243-248.

Diamond, S. 1970. Pore size distributions in clays. Clays and clay minerals, 18 (1), 7-23.

Diebold, J. P., Bridgwater, A. V. 1997. Overview of fast pyrolysis of biomass for the production of liquid fuels. In Developments in thermochemical biomass conversion. 1st ed. Springer Netherlands. p. 5-23. ISBN:978-94-009-1559-0.

Doerge, T. A., Garden, H., 1984. Reacidification of Two Lime Amended Soils in Western Oregon. Soil Science Society of America Journal, 49 (3), DOI: 10.2136/sssaj1985.03615995004900030031

Downie, A., Crosky, A., Munroe, P. 2009. Physical properties of biochar. Biochar for environmental management: Science and technology. In: Lehmann J., Joseph S. (eds.). Bio¬char for environmental management science and technology. Earthscan. London. p. 13-32. ISBN: 978-1 -84407-658-1.

Dube, A., Zbytniewski, R., Kowalkowski, T., Cukrowska, E., Buszewski, B. 2001. Adsorption and migration of heavy metals in soil. Polish Journal of Environmental Studies, 10 (1), 1 -10.

Dursun, A. Y. 2006. A comparative study on determination of the equilibrium, kinetic and thermodynamic parameters of biosorption of copper(II) and lead(II) ions onto pretreated *Aspergillus niger*. Biochemical Engineering Journal, 28, 187-195.

Dutta, B., Raghavan, V. G., Orsat, V., Ngadi, M. 2015. Surface characterisation and classification of microwave pyrolysed maple wood biochar. Biosystems Engineering, 131, 49-64.

Evangelou, M. W., Brem, A., Ugolini, F., Abiven, S., Schulin, R. 2014. Soil application of biochar produced from biomass grown on trace element contaminated land. Journal of Environmental Management, 146, 100-106.

Frankovská, A., Kordík, J., Slaninka, I., Jurkovič, L., Greif, V., Šottík, P., Dananaj, I., Mikita, S., Dercová, K., Jánová, V. 2010. Atlas sanačných metód environmentálnych

záťaží. Štátny geologický ústav Dionýza Štúra, Bratislava, p. 360. ISBN: 978-80-8934-39-3.

Frišták, V., Pipíška, M., Lesný, J., Soja, G., Friesl-Hanl, W., Packová, A. 2015. Utilization of biochar sorbents for Cd 2 +, Zn 2 +, and Cu 2 + ions separation from aqueous solutions: comparative study. Environmental Monitoring and Assessment, 187 (1), 4093.

Gani, A., Naruse, I. 2007. Effect of cellulose and lignin content on pyrolysis and combustion characteristics for several types of biomass. Renewable Energy, 32 (4), 649-661.

Gao, S., Walker, W. J., Dahlgren, R. A., Bold, J. 1997. Simultaneous sorption of Cd, Cu, Ni, Zn, Pb, and Cr on soils treated with sewage sludge supernatant. Water, Air, and Soil pollution, 93 (1-4), 331-345.

Gartler, J., Robinson, B., Burton, K., Clucas, L. 2013. Carbonaceous soil amendments to biofortify crop plants with zinc. Science of Total Environment, 465, 308-313.

Gaskin, J. W., Steiner, C., Harris, K., Das, K. C., Bibens, B. 2008. Effect of low-temperature pyrolysis conditions on biochar for agricultural use. Transactions of the American Society of Agricultural and Biological Engineers, 51 (6), 2061-2069.

Ghrair, A. M., Ingwersen, J., Streck, T. 2010. Immobilization of heavy metals in soils amended by nanoparticulate zeolitic tuff: Sorption-desorption of cadmium. Journal of Plant Nutrition and Soil Science, 173 (6), 852-860.

Gimeno-García E., Andreu, V., Boluda, R. 1996. Heavy metals incidence in the application of inorganic fertilizers and pesticides to rice farming soils. Environmental pollution, 92 (1), 19-25.

Gomez-Eyles, J. L., Sizmur, T., Collins, C. D., Hodson, M. E. 2011. Effects of biochar and the earthworm *Eisenia fetida* on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. Environmental pollution, 159 (2), 616-622.

Gray, M., Johnson, M. G., Dragila, M. I., Kleber, M. 2014. Water uptake in biochars: the roles of porosity and hydrophobicity. Biomass and Bioenergy, 61, 196-205.

Gregory, J., Anderson, C.W.N., Camps Aberstain, M., McManus, M.T. 2014. Response of plant and soil microbes to biochar amendment of an arsenic-contaminated soils. Agriculture, Ecosystems and Environment, 191, 133-141.

Gul, S., Whalen, J. K. 2016. Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. Soil Biology and Biochemistry, 103, 1-15.

Guo, G., Zhou, Q., Ma, L. Q. 2006. Availability and assessment of fixing additives for the in situ remediation of heavy metal contaminated soils: a review. Environmental monitoring and assessment, 116 (1-3), 513-528.

Haefele, S. M., Konboon, Y., Wongboon W., Amarante S., Maarifat, A. A., Pfeiffer, E. M., Knoblauch, C. 2011. Effects and fate of biochar from rice residues in rice-based systems. Field Crops Research, 121, 430-440.

Hao, L., Song, H., Zhang, L., Wan, X., Tang, Y., Lv, Y. 2012. SiO2/graphene composite for selective adsorption of Pb(II) ion. Journal of Colloid and Interface Science, 369 (1), 381-387.

Herron, N., Corbin, D. R., 1995. Inclusion chemistry with zeolites: Nanoscale materials by design. Dordrech: Springer Science + Business Media.

Hong, Z. Q., Hua, P. X., Qing, H. T., Bin, X. Z., Holden, N. M. 2014. Effect of biochar addition on maize growth and nitrogen use efficiency in acidic red soils. Pedosphere, 24, 699-708.

Horne, P. A., Williams, P. T. 1996. Influence of temperature on the products from the flash pyrolysis of biomass. Fuel, 75 (9), 1051-1059.

Houben, D., Evrard, L., Sonnet, P., 2013. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere, 92 (11), 1450-1457.

Houben, D., Sonnet, P. 2015. Impact of biochar and root-induced changes on metal dynamics in the rhizosphere of *Agrostis capillaris* and *Lupinus albus*. Chemosphere, 139, 644-651.

Cha, J. S., Park, S. H., Jung, S. C., Ryu, C., Jeon, J. K., Shin, M. C., Park, Y. K. 2016. Production and utilization of biochar: a review. Journal of industrial and engineering chemistry, 40, 1-15.

Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S. 2007. Agronomic values of greenwaste biochar as a soil amendment. Soil Research, 45 (8), 629-634.

Chang, Y. T., Hsi, H. C., Hseu, Z. Y., Jheng, S. L. 2013. Chemical stabilization of cadmium in acidic soil using alkaline agronomic and industrial by-products. Journal of Environmental Science and Health, Part A, 48 (13), 1748-1756.

Chen, B. L., Zhou, D. D., Zhu, L. Z. 2008. Transitional adsorption and partition of nonpolar and polar aromatic contaminants by biochars of pine needles with different pyrolytic temperatures. Environmental Science and Technology, 42 (14), 5137-5143.

Chen, T., Zhou, Z., Han, R., Meng, R., Wang, H., Lu, W. 2015. Adsorption of cadmium by biochar derived from municipal sewage sludge: Impact factors and adsorption mechanism. Chemosphere, 134, 286-293.

Chen, X., Chen, G., Chen, L., Chen, Y, Lehmann, J., McBride, M. B., Hay, A. G. 2011. Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. Bioresource Technology, 102 (19), 8877-8884.

Chowdhury, Z. Z., Karim, M. Z., Ashraf, M. A., Khalid, K. 2016. Influence of carbonization temperature on physicochemical properties of biochar derived from slow pyrolysis of durian wood (*Durio zibethinus*) sawdust. BioResources, 11 (2), 3356-3372.

Jahirul, M. I., Rasul, M. G., Chowdhury, A. A., Ashwath, N. 2012. Biofuels production through biomass pyrolysis - a technological review. Energies, 5 (12), 4952-5001.

Janoš, P., Vávrová, J., Herzogová, L., Pilařová, V. 2010. Effects of inorganic and organic amendments on the mobility (leachability) of heavy metals in contaminated soil: a sequential extraction study. Geoderma, 159 (3), 335-341.

Jensen, J. K., Holm, P. E., Nejrup, J., Larsen, M. B., Borggaard, O. K. 2009. The potential of willow for remediation of heavy metal polluted calcareous urban soils. Environmental Pollution, 157 (3), 931-937.

Jha, P., Biswas, A. K., Lakaria, B. L., Rao, A. S. 2010. Biochar in agriculture-prospects and related implications. Current Science, 99 (9), 1218-1225.

Jiang, J., Xu, R., Jiang, T., Li, Z. 2012. Immobilization of Cu(II), Pb(II) and Cd(II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. Journal of Hazardous Materials, 229-230, 145-150.

Jimenez-Cordero, D., Heras, F., Alonso-Morales, N., Gilarranz, M. A., Rodriguez, J. J. 2013. Porous structure and morphology of granular chars from flash and conventional pyrolysis of grape seeds. Biomass and Bioenergy, 54, 123-132.

Jones, D. L., Edwards-Jones, G., Murphy, D. V. 2011. Biochar mediated alterations in herbicide breakdown and leaching in soil. Soil Biology and Biochemistry, 43, 804-813.

Jones, D. L., Quilliam, R. S. 2014. Metal contaminated biochar and wood ash negatively affect plant growth and soil quality after land application. Journal of Hazardous Materials, 276, 362-370.

Jordan, C.F. 1968. A simple, tension-free lysimeter. Soil Science, 105 (2), 81-86.

Kabata-Pendias, A. 1995. Agricultural problems related to excessive trace metal contents of soils. In: Salomons, W., Förstner, U., Mader, P. (eds.). Heavy metals. Springer-Verlag. Berlin, Heidelberg. p. 3-18. ISBN: 978-3-642-79316-5.

Kabata-Pendias, A., Mukherjee, A. B. 2007. Trace elements from soil to human. 1st ed. Springer-Verlag. Berlin, Heidelberg. ISBN-10 3-540-32713-4.

Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants, third ed., Boca Raton, CRC Press. p. 331. ISBN: 0-8493-1575-1.

Keiluweit, M., Nico, P. S., Johnson, M. G., Kleber, M. 2010. Dynamic molecular structure of plant biomass-derived black carbon (biochar). Environmental Science and Technology, 44 (4), 1247-1253.

Kelly, C. N., Calderón, F. C., Acosta-Martinez, V., Mikha, M. M., Benjamin, J., Rutherford, D. W., Rostad, C. E. 2015. Switchgrass biochar effects on plant biomass and microbial dynamics in two soils from different regions. Pedosphere, 25(3), 329-342.

Khorram, M. S., Zhang, Q., Lin, D., Zheng, Y., Fang, H., Yu, Y. 2016. Biochar: a review of its impact on pesticide behavior in soil environments and its potential applications. Journal of Environmental Sciences, 44, 269-279.

Kołodyńska, D., Wnętrzak, R., Leahy, J. J., Hayes, M. H. B., Kwapiński, W., Hubicki, Z. 2012. Kinetic and adsorptive characterization of biochar in metal ions removal. Chemical Engineering Journal, 197, 295-305.

Kumar, A., Singh, R. P., Singh, P.K., Awasthi, S., Chakrabarty, D., Trivedi, P. K., Tripathi, R. D., 2014. Selenium ameliorates arsenic induced oxidative stress through modulation of antioxidant enzymes and thiols in rice (*Oryza sativa* L.). Ecotoxicology, 23, 1153-1163.

Kumpiene, J., Lagerkvist, A., Maurice, C. 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments - a review. Waste Management, 28 (1), 215-225.

Laird, D., Fleming, P., Wang, B., Horton, R., Karlen, D. 2010a. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma, 158, 436-442.

Laird, D. A., Fleming, P., Davis, D. D., Horton, R., Wang, B., arlen, D. L. 2010b. Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. Geoderma, 158 (3), 443-449.

Langmuir, I. (1916). The constitution and fundamental properties of solids and liquids. Part I. Solids. Journal of the American Chemical Society, 38 (11), 2221-2295.

Lehmann, J. 2007a. A handful of carbon. Nature, 447 (7141), 143-144.

Lehmann, J. 2007b. Bio-energy in the black. Frontiers in Ecology and the Environment, 5 (7), 381-387.

Lehmann, J., Gaunt, J., Rondon, M. 2006. Bio-char sequestration in terrestrial ecosystems - a review. Mitigation and adaptation strategies for global change, 11 (2), 395-419.

Lehmann, J., Joseph, J 2009. Biochar for environmental management: science and technology. Earthscan. London. p 416. ISBN: 978-1-84407-658-1.

Lettens, S., Vandecasteele, B., De Vos, B., Vansteenkiste, D., Verschelde, P. 2011. Intra- and inter-annual variation of Cd, Zn, Mn and Cu in foliage of poplars concontaminated soil. Science of the Total Environment, 409, 2306-2316.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'neill, B., Neves, E. G. 2006. Black carbon increases cation exchange capacity in soils. Soil Science Society of America Journal, 70 (5), 1719-1730.

Liang, Y., Cao, X., Zhao, L., Arellano, E. 2014. Biochar and phosphate-induced immobilization of heavy metals in contaminated soil and water: implication on simultaneous remediation of contaminated soil and groundwater. Environmental Science and Pollution Research, 21 (6), 4665-4674.

Lievens, C., Yperman, J., Vangronsveld, J., Carleer, R. 2008. Study of the potential valorisation of heavy metal contaminated biomass via phytoremediation by fast pyrolysis: Part I. Influence of temperature, biomass species and solid heat carrier on the behaviour of heavy metals. Fuel, 87 (10), 1894-1905.

Lim, J. E., Ahmad, M., Usman, A. R. A., Lee, S. S., Jeon, W. T., Oh, S. E., Yang, J. E., Ok, Y. S. 2013. Effects of natural and calcined poultry waste on Cd, Pb and As mobility in contaminated soil. Environmental Earth Sciences, 69 (1), 11-20.

Lin, C. W., Lian, J., Fang, H. H. 2005. Soil lead immobilization using phosphate rock.Water, Air, and Soil Pollution, 161 (1-4), 113-123.

Lone, A. H., Najar, G. R., Ganie, M. A., Sofi, J. A., Ali, T. 2015. Biochar for sustainable soil health: a review of prospects and concerns. Pedosphere, 25(5), 639-653.

Loughnan, F. C. 1969. Chemical weathering of the silicate minerals. Inc. 2nd ed. American Elsevier Publishing Company. New York. p. 154. ISBN: 978-0-415-60426-0.

Lu, H., Zhang, W., Yang, Y., Huang, X., Wang, S., Qiu, R. 2012. Relative distribution of Pb2+ sorptionmechanisms by sludge-derived biochar. Water Research, 46 (3), 854-862.

Lua, A. C., Yang, T., Guo, J., 2004. Effects of pyrolysis conditions on the properties of activated carbons prepared from pistachio-nut shells. Journal of Analytical and Applied Pyrolysis, 72 (2), 279-287.

Lua, A. C., Lau, F. Y., Guo, J., 2006. Influence of pyrolysis conditions on pore development of oil-palm-shell activated carbons. Journal of Analytical and Applied Pyrolysis, 76 (1), 96-102.

Mašek, O., Brownsort, P., Cross, A., Sohi, S. 2013. Influence of production conditions on the yield and environmental stability of biochar. Fuel, 103, 151-155.

McKenzie, R. M. 1980. The adsorption of lead and other heavy metals on oxides of manganese and iron. Soil Research, 18 (1), 61-73.

Meers, E., Vandecasteele, B.; Ruttens, A., Vangronsveld, J., Tack, F. M. G. 2007. Potential of five willow species (*Salix* spp.) for phytoextraction of heavy metals. Environmental and Experimental Botany, 60 (1), 57-68.

Mohan, D., Pittman, C. U., Jr., Bricka, M., Smith, F., Yancey, B., Mohammad, J., Steele, P. H., Alexandre-Franco, M. F., Gómez-Serrano, V., Gong, H. 2007. Sorption of arsenic, cadmium, and lead by chars produced from fast pyrolysis of wood and bark during bio-oil production. Journal of Colloid and Interface Science, 310 (1), 57-73.

Mohan, D., Pittman, C. U., Steele, P. H. 2006. Pyrolysis of wood/biomass for bio-oil: A critical review. Energy and Fuels, 20 (3), 848-889.

Moreira, M. T., Noya, I., Feijoo, G. 2017. The prospective use of biochar as adsorption matrix - A review from a lifecycle perspective. Bioresource Technology, 246, 135-141.

Morley, J. 1929. Compost and Charcoal. The National Greenkeeper, 3 (9), 8-26.

Mukherjee, A., Zimmerman, A. R. 2013. Organic carbon and nutrient release from a range of laboratory-produced biochars and biochar-soil mixtures. Geoderma, 193, 122-130.

Mulligan, C. N., Yong, R. N., Gibbs, B. F. 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Engineering geology, 60 (1), 193-207.

Mustafa, S., Dilara, B., Naeem, A., Rahana, N., Shahida, P. 2002. Sorption of metal ions on a mixed oxide [0.5 M SiO2:0.5 M Fe(OH)3]. Adsorption Science and Technology, 20 (3), 215-230.

Namgay, T., Singh, B., Singh, B.P. 2010. Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize (*Zea mays L.*). Australian Journal of Soil Research, 48 (7), 638-647.

Nash, J. E., Sutcliffe, J. V. 1970. River flow forecasting through conceptualmodels part I.: a discussion of principles. Journal of Hydrology, 10 (3), 282-290.

Nelissen, V., Saha, B. K., Ruysschaert, G., Boeckx, P. 2014. Effect of different biochar and fertilizer types on N_2O and NO emissions. Soil Biology and Biochemistry, 70, 244-255.

Němeček, J., Vácha, R., Podlešáková, E. 2010. Assessment of soil contamination in the Czech Republic. Research Institute for Soil and Water Conservation, Prague. (In Czech). ISBN: 978-80-86561-02-4.

Ngo, P. T., Rumpel, C., Ngo, Q.A., Alexis, M., Vargas, G.V., Gil, M. D.L., Jouquet, P. 2013. Biological and chemical reactivity and phosphorus forms of buffalo manure compost, vermicompost and their mixture with biochar. Bioresource Technology, 148, 401-407.

Novak, J. M., Lima, I., Xing, B., Gaskin, J. W., Steiner, C., Das, K. C., Schomberg H. 2009a. Characterization of designer biochar produced at different temperatures and their effects on a loamy sand. Annals of Environmental Science, 3 (1), 2.

Novak, J. M., Busscher, W. J., Laird, D. L., Ahmedna, M., Watts, D. W., Niandou, M. A. 2009b. Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Science, 174 (2), 105-112.

Novak, J. M., Cantrell, K. B.,Watts, D. W., Busscher,W. J., Johnson, M. G. 2014. Designing relevant biochars as soil amendments using lignocellulosic-based and manure-based feedstocks. Journal of Soils and Sediments, 14, 330-343.

Novak, J. M., Ippolito, J. A., Lentz, R. D., Spokas, K. A., Bolster, C. H., Sistani, K., Johnson, M. G. 2016. Soil health, crop productivity, microbial transport, and mine spoil response to biochars. BioEnergy Research, 9 (2), 454-464.

O'Connor, D., Peng, T., Zhang, J., Tsang, D. C., Alessi, D. S., Shen, Z., Hou, D. 2018. Biochar application for the remediation of heavy metal polluted land: A review of in situ field trials. Science of The Total Environment, 619, 815-826.

Oguntunde, P. G., Abiodun, B. J., Ajayi, A. E., van de Giesen, N. 2008. Effects of charcoal production on soil physical properties in Ghana. Journal of Plant Nutrition and Soil Science, 171 (4), 591-596.

Ochecová, P., Tlustoš, P., Száková, J. 2014. Wheat and soil response to wood fly ash application in contaminated soils. Agronomy Journal, 106, 995-1002.

Panagos, P., Van Liedekerke, M., Yigini, Y., Montanarella, L. 2013. Contaminated sites in Europe: Review of the current situation based on data collected through a European Network European Commission. Journal of Environmental and Public Health. 11. doi:10.1155/2013/158764

Park, J. H. , Choppala, G. K., Bolan, N. S., Chung, J. W., Chuasavathi, T. 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant and Soil, 348 (1-2), 439-451.

Pavlík, M., Pavlíková, D., Staszková, L., Neuberg, M., Kaliszová, R., Száková, J., Tlustoš, P. 2010. The effect of arsenic contamination on amino acids metabolism in *Spinacia oleracea* L.. Ecotoxicology and Environmental Safety, 73, 1309-1313.

Petruzzelli, G. 1997. Soil sorption of heavy metal. Ecological issues and environmental impact, 145-174.

Prasad, M. N. V. 2008. Trace elements as contaminats and nutrients.1st ed.Wiley. Hoboken p. 777. ISBN: 978-0-470-18095-2.

Prasad, M. N. V., Hagemeyer, J. 1999. Heavy metal stress in plants. 1st ed. Springer - Verlag. Berlin, Heidelberg. p. 401. ISBN:3-540-65469-0.

Pustišek, N., Milačic, R., Veber, M. 1996. Use of the BCR Three-step Sequential Extraction Procedure for the Study of the Partitioning of Cd, Pb and Zn in Various Soil Samples. Journal of Soils and Sediments, 1, 27-29.

Qiu, Y., Cheng, H., Xu, C., Sheng, G. D. 2008. Surface characteristics of crop-residue-derived black carbon and lead (II) adsorption. Water Research, 42 (3), 567-574.

Raveendran, K., Ganesh, A. 1998. Adsorption characteristics and pore-development of biomass-pyrolysis char. Fuel, 77 (7), 769-781.

Raveendran, K., Ganesh, A., Khilar, K. C. 1995. Influence of mineral matter on biomass pyrolysis characteristics. Fuel, 74, 1812-22.

Rees, F., Sterckeman, T., Morel, J.L., 2015. Root development of non-accumulating and hyperaccumulating plants in metal-contaminated soils amended with biochar. Chemosphere, 142, 48-55.

Rinklebe, J., Shaheen, S. M., Frohne, T. 2016. Amendment of biochar reduces the release of toxic elements under dynamic redox conditions in a contaminated floodplain soil. Chemosphere, 142, 41-47.

Robinson, B. H., Mills, T. M., Petit, D., Fung, L. E., Green, S. R., Clothier, B. E. 2000. Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation. Plant and Soil, 227, 301-306.

Ronsee, F., Hecke, S.V., Dickinson, D., Prins, W. 2013 Production and characterization of slow pyrolysis biochar: influence of feedstock type and pyrolysis conditions.GCB Bioenergy, 5, 104-115.

Rosa, J. M., Knicker, H., Capel, E., Manning, D. A. C., González-Perez, J. A., González-Vila, F.J. 2007. Direct detection of black carbon in soils by Py-GC/MS, carbon-13 NMR spectroscopy and thermogravimetric techniques. Soil Science Society of America Journal, 72, 258-267.

Sas-Nowosielska, A., Kucharski, R., Malkowski, E., Pogrzeba, M., Kuperberg, J. M., Krynski, K. 2004. Phytoextraction crop disposal - an unsolved problem. Environmental Pollution, 128 (3), 373-379.

Shaheen, S. M., Rinklebe, J., Selim, M. H. 2015. Impact of various amendments on immobilization and phytoavailability of nickel and zinc in a contaminated floodplain soil. International Journal of Environmental Science and Technology, 12 (9), 2765-2776.

Sharma, S. S., Dietz, K. J. 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany, 57, 711-726.

Shen, Z., Som, A. M., Wang, F., Jin, F., McMillan, O., Al-Tabbaa, A. 2016. Long-term impact of biochar on the immobilisation of nickel (II) and zinc (II) and the revegetation of a contaminated site. Science of the Total Environment, 542, 771-776.

Sohi, S. P., Krull, E., Lopez-Capel, E., Bol, R. 2010. A review of biochar and its use and function in soil. Advances in Agronomy, 105, 47-82.

Sombroek, W. 1966. Amazon soils. Centre for Agricultural Publications and Documentation. p 300.

Sparks, D. L. 1995. Environmental Soil Chemistry. Academic Press. London. p 352. ISBN: 978-149-330-196-6.

Spokas, K. A. 2010. Review of the stability of biochar in soils: predictability of O :C molar ratios.Carbon Management, 1, 289-303.

Stals, M., Carleer, R., Reggers, G., Schreurs, S., Yperman, J. 2010. Flash pyrolysis of heavy metal contaminated hardwoods from phytoremediation: Characterisation of biomass, pyrolysis oil and char/ash fiction. Journal of Analytical and Applied Pyrolysis, 89 (1), 22-29.

Stevenson, F. J. 1994. Humus chemistry: Genesis, composition, reactions. Wiley. New York. p. 512. ISBN: 978-0-471-59474-1.

Stouraiti, C., Xenidis, A., Paspaliaris, I., 2002. Reduction of Pb, Zn and Cd availability from tailings and contaminated soils by the application of lignite fly ash. Water, Air and Soil Pollution, 137 (1-4), 247-265.

Sun, F., Lu, S. 2014. Biochars improve aggregate stability, water retention, and pore-space properties of clayey soil. Journal of Plant Nutrition and Soil Science, 177 (1), 26-33.

Šichorová, K., Tlustoš, P., Száková, J., Kořínek, K., Balík, J. 2004. Horizontal and vertical variability of heavy metalsin the soil of a polluted area. Plant Soil and Environment, 50 (12), 525-534.

Šyc, M., Pohořelý, M., Kameníková, P., Habart, J., Svoboda, K., Punčochář, M. 2012. Willow trees from heavy metals phytoextraction as energy crops. Biomass and Bioenergy, 37, 106-113.

Tlustoš, P., Száková, J., Vysloužilová, M., Pavlíková, D., Weger, J., Javorská, H. 2007. Variation in the uptake of Arsenic, Cadmium, Lead, and Zinc by different species of willows *Salix* spp.grown in contaminated soils. Central European Journal of Biology, 2 (2), 254-275.

Trakal, L., Komárek, M., Száková, J., Zemanová, V., Tlustoš, P. 2011. Biochar application to metal-contaminated soil: Evaluating of Cd, Cu, Pb and Zn sorption behavior using single- and multi-element sorption experiment. Plant, Soil and Environment, 57 (8), 372-380.

Tryon, E. H. 1948. Effect of charcoal on certain physical, chemical, and biological properties of forest soils. Ecological Monographs, 18 (1), 81-115.

Uchimiya, M., Lima, I. M., Klasson, T. K., Chang, S., Wartelle, L. H., Rodgers, J. E. 2010a. Immobilization of heavy metal ions (CuII, CdII, NiII, and PbII) by broiler litter-derived biochars in water and soil. Journal of Agricultural and Food Chemistry, 58 (9), 5538-5544.

Uchimiya, M., Lima, I. M., Klasson, K. T. Wartelle, L. H. 2010b. Contaminant immobilization and nutrient release by biochar soil amendment: roles of natural organic matter. Chemosphere, 80, 935-940.

Uchimiya, M., Wartelle, L. H., Klasson, K. T., Fortier, C. A., Lima, I. M. 2011a. Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. Journal of Agriculture and Food Chemistry, 59 (6), 2501-2510.

Uchimyia, M., Klasson, K. T., Wartelle, L. H., Lima, I. M. 2011b. Influence of soil properties on heavy metal sequestration by biochar amendment: 1. Copper sorption isotherms and the release of cation. Chemospere, 82 (10), 1431-1437.

Uchimyia, M., Klasson, K. T., Wartelle, L.H., Lima, I.M. 2011c. Influence of soil properties on heavy metal sequestration by biochar amendment: 2. Copper desorption isotherms. Chemosphere, 82 (10), 1438-1447.

Uzinger, N., Anton, A. 2008. Chemical stabilization of heavy metals on contaminated soils by lignite. Cereal Research Communications, 36, 1911-1914.

Uzoma, K. C., Inoue, M., Andry, H., Fujimaki, H., Zahoor, A., Nishihara. E. 2011. Effect of cow manure biochar on maize productivity under sandy soil condition. Soil Use and Management, 27, 205-212.

Vácha, R., Němeček, J., Podlešáková, E. 2002. Geochemical and anthropogenic soil loads by potentially risky elements. Rostlinná výroba, 48, 441-447.

Vaněk, A., Borůvka, L., Drábek, O., Mihaljevič, M., Komárek, M. 2005. Mobility of lead, zinc and cadmium in alluvial soils heavily polluted by smelting industry. Plant, Soil and Environment, 51, 316-321.

Vassilev, S. V., Baxter, D., Andersen, L. K., Vassileva, C. G., Morgan, T. J. 2012. An overview of the organic and inorganic phase composition of biomass. Fuel, 94, 1-33.

Verheijen, F., Jeffery, S., Bastos, van der Velde, M., Diafas, I. 2010. Biochar application to soils a critical scientific review of effects on soil properties, processes and functions. EUR 24099 EN, Office for the Official Publications of the European Communities. Luxembourg. p. 149. ISBN: 978-92-79-14293-2.

Visual MINTEQ ver. 3.1 2014. Royal Institute of Technology, Div. of Land and Water Resources Engineering, Stockholm,Sweden. Available at: http://www2.lwr.kth.se/English/OurSoftware/vminteq/download.html.

Vondráčková, S., Hejcman, M., Tlustoš, P., Száková, J. 2013. Effect of quick lime and dolomite application on mobility of elements (Cd, Zn, Pb, As, Fe, and Mn) in contaminated soils. Polish Journal of Environmental Studies, 22, 577-589.

Vondráčková, S., Tlustoš, P., Hejcman, M., Száková, J., 2015. Regulation of macro, micro, and toxic element uptake by *Salix × smithiana* using liming of heavily contaminated soils. Journal of Soils and Sediments, 1-12.

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Vysloužilová, M., Tlustoš, P., Száková, J., Pavlíková, D. 2003. As, Cd, Pb and Zn uptake by Salix spp. clones grown in soils enriched by high loads of these elements. Plant, Soil and Environment, 49 (5), 191-196.

Wang, S., Gao, B., Zimmerman, A. R., Li, Y., Mad, L., Harris, W. G. 2015a. Physicochemical and sorptive properties of biochars derived from woody and herbaceous biomass. Chemosphere, 134, 257-262.

Wang, Z., Zong, H., Zheng, H., Liu, G., Chen, L., Xing, B. 2015b. Reduced nitrification and abundance of ammonia-oxidizing bacteria in acidic soil amended with biochar. Chemosphere, 138, 576-583.

Woolf, D., Amonette, J.E., Street-Perrott, A., Lehmann, J., Joseph, S. 2010. Sustainable biochar to mitigate global climate change. Nature Communications, 1. doi:10.1038/ncomms1053.

Xenidis, A., Stouraiti, C., Papassiopi, N. 2010. Stabilization of Pb and As in soils by applying combined treatment with phosphates and ferrous iron. Journal of Hazardous Materials, 177 (1), 929-937.

Xu, R. K., Zhao, A. Z., Yuan, J. H., Jiang, J. 2012. pH buffering capacity of acid soils from tropical and subtropical regions of China as influenced by incorporation of crop straw biochars. Journal of Soils and Sediments, 12 (4), 494-502.

Xu, X., Cao, X., Zhao, L., Wang, H., Yu, H., Gao, B. 2013. Removal of Cu, Zn, and Cd from aqueous solutions by the dairy manure-derived biochar. Environmental Science and Pollution Research, 20 (1), 358-368.

Yang, H., Yan, R., Chen, H., Lee, D. H., Zheng, C. 2007. Characteristics of hemicellulose, cellulose and lignin pyrolysis. Fuel, 86, 1781-1788.

Younis, U., Athar, M., Malik, S. A., Shah, M. H. R., Mahmood, S. 2015b. Biochar impact on physiological and biochemical attributes of spinach *Spinacia oleracea* (L.) in nickel contaminated soil. Global Journal of Environmental Science and Management, 1, 245-254.

Younis, U., Qayyum, M. F., Shah, M. H. R., Danish, S., Shahzad, A. N., Malik, S. A., Mahmood, S. 2015a. Growth, survival, and heavy metal (Cd and Ni) uptake of spinach (*Spinacia oleracea*) and fenugreek (*Trigonella corniculata*) in a biochar-amended sewage-irrigated contaminated soil. Journal of Plant Nutrition and Soil Science, 178, 209-217.

Yuan, J. H., Xu, R. K. 2011. The amelioration effects of low temperature biochar generated from nine crop residues on an acidic Ultisol. Soil Use and Management, 27 (1), 110-115.

Yuan, J. H., Xu, R. K., Qian, W., Wang, R. H. 2011. Comparison of the ameliorating effects on an acidic Ultisol between four crop straws and their biochars. Journal of Soils and Sediments, 11 (5), 741-750.

Zaffar, M., Lu, S. G. 2015. Pore size distribution of clayey soils and its correlation with soil organic matter. Pedosphere, 25(2), 240-249.

Zagorchev, L., Seal, C. E., Kranner, I., Odjakova, M. 2013. A central role for thiols in plant tolerance to abiotic stress. Internation Journal of Molecular Sciences, 14, 7405-7432.

Zhang, G., Zhang, Q, Sun, K., Liu, X, Zheng, W., Zhao, Y. 2011. Sorption of simazine to corn straw biochars prepared at different pyrolytic temperatures. Environmental Pollution, 159 (10), 2594-2601.

Zhang, Z., Solaiman, Z. M., Meney, K., Murphy, D. V., Rengel, Z. 2013. Biochars immobilize soil cadmium, but do not improve growth of emergent wetland species

Juncus subsecundus in cadmium-contaminated soil. Journal of Soils and Sediments, 13 (1), 140-151.

Zhao, G., Wu, X., Tan, X., Wang, X. 2011. Sorption of heavy metal ions from aqueous solutions: a review. Open Colloid Science Journal, 4, 19-31.

Zhao, L., Cao, X., Mašek, O., Zimmerman, A. 2013. Heterogeneity of biochar properties as a function of feedstock sources and production temperatures. Journal of Hazardous Materials, 256-257, 1-9.

Zheng, H., Wang, Z., Deng, X., Herbert, S., Xing, B. 2013a. Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. Geoderma, 206, 32-39.

Zheng, H., Wang, Z., Deng, X., Zhao, J., Luo, Y., Novak, J., Xing, B. 2013b. Characteristics and nutrient values of biochars produced from giant reed at different temperatures. Bioresource Technology, 130, 463-471.