ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE
Fakulta agrobiologie, potravinových a přírodních zdrojů

Katedra agroenvironmentální chemie a výživy rostlin

Vliv půdních aditiv na regulaci příjmu rizikových prvků a živin rostlinami

doktorská disertační práce

Autorka: **Ing. Stanislava Vondráčková**

Školitel: **prof. Ing. Pavel Tlustoš, CSc.**

Konzultantka: **prof. Ing. Jiřina Száková, CSc.**

Praha 2015

Prohlášení

Prohlašuji, že jsem disertační práci na téma: **„Vliv půdních aditiv na regulaci příjmu rizikových prvků a živin rostlinami"** vypracovala samostatně a použila jen pramenů, které cituji a uvádím v přiloženém seznamu literatury.

V Praze dne 5. 11. 2015

Podpis

Poděkování

Obsah

1 Úvod

V České republice, podobně jako v mnoha dalších zemích, existují oblasti silně kontaminované několika rizikovými prvky současně. Takovéto lokality, vzhledem k potenciální toxicitě a vysoké stabilitě rizikových prvků v kontaminovaných půdách, představují environmentální problém, pro který je potřebné nalézt efektivní a dostupné řešení (do Nascimento a Xing, 2006; Alkorta et al., 2010). Na silně kontaminovaných lokalitách se ale většinou daří pouze původním tolerantním rostlinným druhům. Často se jedná o drobné rostliny s relativně jemným kořenovým systémem. Z důvodu nedostatečného vegetačního pokryvu může větrnou nebo vodní erozí docházet k šíření rizikových prvků do okolního prostředí (Friesl-Hanl et al., 2009; Bolan et al., 2014; Grobelak a Napora, 2015), ale může docházet i k vymývání rizikových prvků z půdního profilu (Vaněk et al., 2005). Rizikové prvky je nezbytné v půdě přinejmenším stabilizovat a omezit možná rizika, která jsou s nimi spojená (Vácha et al., 2002b; Tlustoš et al., 2006a). Dekontaminační metody šetrné k životnímu prostředí, jako je například fytoextrakce, však nelze ve výše uvedených případech použít, protože vysoké obsahy rizikových prvků v půdě mohou být i pro řadu tolerantních rostlin fytotoxické (Nagajyoti et al., 2010). Fytoextrakční schopnost rostlin se navíc zpravidla projevuje pro jeden, maximálně pro dva rizikové prvky a použití takových rostlin tedy neřeší problém kontaminace půdy několika prvky současně.

Vhodnou dočasnou remediační technikou pro půdy kontaminované vysokými obsahy rizikových prvků je *in-situ* chemická imobilizace. Při této metodě jsou do půdy aplikována imobilizační půdní aditiva, která snižují mobilitu rizikových prvků i jejich dostupnost pro rostliny (Kumpiene et al., 2008). Pomocí konkrétních aditiv mohou být na principu adsorpce, komplexace nebo precipitace omezovány labilní formy rizikových prvků, bez vlivu na jejich celkové obsahy (Miretzky a Fernandez-Cirelli, 2008; Bolan et al., 2014). Na mobilitu rizikových prvků v půdě má vliv celá řada půdních parametrů (např. pH, kvalita a kvantita organické hmoty, kationtová výměnná kapacita – KVK, půdní druh i půdní typ); (Violante et al., 2010; Kunhikrishnan et al., 2012; Alloway, 2013).

Po přídavku aditiv do půd silně kontaminovaných rizikovými prvky s odlišnými agrochemickými parametry můžeme očekávat rozdílné účinky aditiv na snížení mobility rizikových prvků. Ve srovnání s použitím rostlin na silně kontaminovaných půdách jsou postupy využívající půdní aditiva vhodnější pro širší spektrum rizikových prvků. Odlišnosti v mobilitě rizikových prvků nesouvisí pouze s půdními vlastnostmi nebo s konkrétním rizikovým prvkem. Důležitou roli může hrát i zvolené půdní aditivum, jeho aplikační dávka

nebo sledovaná doba působení (Yan et al., 2015). Půdní aditiva nemají vliv jen na mobilitu rizikových prvků, ale také na mobilitu živin v půdě a na vybrané půdní vlastnosti. Nevhodně zvolené půdní aditivum nebo jeho nevhodná aplikační dávka může omezit příjem živin, vyvolat jejich deficienci, znemožnit pěstování rostlin na kontaminované lokalitě (Bolan a Duraisamy, 2003) a tím i možné využití stabilizovaných lokalit (Tlustoš et al., 2007; Friesl-Hanl et al., 2009).

Na stabilizovaných půdách je proto potřebné testovat i reakce tolerantních rostlin – bylin i dřevin a pro půdy silně kontaminované rizikovými prvky hledat nejvhodnější kombinaci půdního aditiva a tolerantní rostliny.

2 Literární přehled

2.1 Kontaminace půd rizikovými prvky

Rizikové prvky je možné z hlediska jejich role v biologických systémech zařadit do dvou skupin – toxické (As, Cd, Pb, Hg) a esenciální při nižších koncentracích v půdě (Co, Cu, Fe, Mn, Ni, Zn); (Bolan et al., 2010; Nagajyoti et al., 2010; Ali et al., 2013). Specifikem rizikových prvků je, že nejsou mikrobiálně ani chemicky degradovány a na rozdíl od mnoha organických sloučenin setrvávají v půdě po dlouhou dobu (Ali et al., 2013; Bolan et al., 2014; Mahar et al., 2015).

Kontaminace půd rizikovými prvky může být geogenního a antropogenního původu (Nagajyoti et al., 2010; Vácha et al., 2013; Bolan et al., 2014). Rizikové prvky geogenního původu se na rozdíl od těch vnesených do půdy lidskou činností ve většině případů nachází ve formách velmi málo přístupných pro rostliny (Němeček et al., 2002; Lamb et al., 2009; Vácha et al., 2013). Hlavním zdrojem kontaminace půd rizikovými prvky geogenního původu je zvětrávání matečných hornin (vyvřelých, sedimentárních nebo metamorfovaných). Mezi další zdroje geogenního původu patří sopečná činnost, eroze nebo lesní požáry (Němeček et al., 2010; Ali et al., 2013; Bolan et al., 2014). V České republice byly vymezeny tři hlavní skupiny půd, vyvinutých na substrátech se zvýšenými obsahy některých rizikových prvků (Vácha et al., 2002a; Němeček et al., 2010; Rotter et al., 2013), − 1) půdy ze svahovin bazických a ultrabazických hornin se zvýšenými obsahy Co, Cr, Cu, Ni, Mn a V (čediče, hadce, diabasy, amfibolity, gabra nebo syenity), 2) půdy ze svahovin kyselých vyvřelých nebo metamorfovaných hornin se zvýšenými obsahy As, Be, Cu, Pb a Zn (žuly) a 3) půdy ze svahovin produktů zvětrávání karbonátových permokarbonských hornin se zvýšenými obsahy Cd, Cr nebo Ni (vápence nebo karbonátové břidlice, zejména flyšové). Hlavní zdroje kontaminace půd rizikovými prvky antropogenního původu jsou spojeny s průmyslovou činností, s těžbou a zpracováním surovin, s likvidací odpadů z průmyslu a z domácností, s dopravou nebo s atmosférickou depozicí. Dalším významným zdrojem je i zemědělství, především aplikace minerálních a organických hnojiv, čistírenských kalů, závlahy, v minulosti i použití pesticidů (Wuana a Okieimen, 2011; Chaney, 2012; Bolan et al. 2014).

V České republice, podobně jako v dalších státech, neexistuje komplexní zákon na ochranu veškerých půd. Výjimkou je Spolková republika Německo, která takový zákon má (Němeček et al., 2010). Kontaminace zemědělských půd v České republice je řešena vyhláškou MŽP 13/1994 Sb. (Česko, 1994). Míra kontaminace půd rizikovými prvky je hodnocena podle překročení jejich maximálních obsahů v lehkých a ostatních půdách

v extraktu lučavkou královskou (tzv. pseudocelkový obsah prvku) nebo roztokem HNO$_3$ o koncentraci 2 mol.l^{-1}. Půdy silně kontaminované rizikovými prvky několikanásobně převyšují stanovené legislativní limity. Mezi významné příklady silné kontaminace půd v České republice patří fluvizem v nivě řeky Litavky v obci Trhové Dušníky (As, Cd, Pb a Zn); (Borůvka et al., 1996; Němeček et al., 2010) nebo luvizem z břehu potoka Beránka v městské části Kutná Hora – Malín (As, Cd a Zn); (Králová et al., 2010; Horák a Hejcman, 2013).

2.2 Mobilita prvků v půdě

Celkové obsahy rizikových prvků jsou pouze měřítkem míry kontaminace půd. Nedovolují nám posoudit pohyb rizikových prvků v půdě (jejich mobilitu) ani reálné nebezpečí vstupu do potravního řetězce (jejich biodostupnost a toxicitu); (Rieuwerts, 2007; Rao et al., 2008; Abollino et al., 2011). Rizikové prvky v půdě je možné rozdělit do dvou frakcí – inertní (reziduální) a labilní (mobilní a potenciálně mobilizovatelné formy prvků); (Rachou a Sauvé, 2008). Mezi mobilní formy rizikových prvků v půdě patří vodorozpustné a iontově výměnné frakce. Nicméně pouze malý podíl rizikových prvků v půdě se vyskytuje v mobilních formách okamžitě přijatelných pro rostliny (Lasat, 2002; He et al., 2005). Potenciálně mobilizovatelné formy rizikových prvků (tj. zejména frakce prvků vázané na uhličitany, oxidy nebo na organickou hmotu); (Hen et al., 2005) jsou pro rostliny nedostupné (Sheoran et al., 2011), avšak jejich přístupnost může být ovlivněna vybranými fyzikálními, chemickými a biologickými parametry půd (Adriano, 2001).

Rizikové prvky mohou být klasifikovány podle své mobility v půdě na vysoce mobilní – Cd, Co, Mn, Zn; středně a méně mobilní – As, Be, Cu, Pb, V a nejméně mobilní – Cr, Hg a Ni (Podlešáková et al., 2001b; Němeček et al., 2002; Probst et al., 2003).

2.2.1 Metody extrakce prvků v půdě

Metody chemické extrakce půdy (jednoduché a sekvenční) slouží ke studiu mobility a biodostupnosti prvků, pomáhají definovat jednotlivé vazby (chemické formy) prvků v půdě (Němeček et al., 2010; Abollino et al., 2011).

Pomocí metod jednoduché (tzv. jednostupňové) extrakce můžeme stanovovat mobilní a potenciálně mobilizovatelné frakce prvků v půdě (Němeček et al., 2010; Abollino et al., 2011). Běžně se pro jednoduchou extrakci půd používají:

– kyseliny (silné, např. lučavka královská, HNO₃, HCl; slabé, např. kyselina octová; princip působení – okyselení)

– cheláty (např. etylendiamintetraoctová kyselina – EDTA, dietylentriaminpentaoctová kyselina – DTPA, princip účinku – komplexace)

– pufrované soli (např. octan amonný, pH = 7) a nepufrované soli (např. CaCl₂, NH₄NO₃, princip působení – iontová výměna)

(Sahuquillo et al., 2003; Meers et al., 2007; Kunhikrishnan et al., 2012).

Metody postupné extrakce (tzv. sekvenční analýzy) byly vyvinuty s cílem přesněji definovat zastoupení prvků v jednotlivých frakcích půdy (Tlustoš et al., 2005; Abollino et al., 2011). Extrakce většinou zahrnují tři až sedm samostatných kroků, jsou časově náročné, vyžadují zkušený personál a odpovídající instrumentální analytickou techniku (Tlustoš et al., 2005; Bacon a Davidson, 2008; Rao et al., 2008). Základním principem sekvenčních analýz je, že každé další použité extrakční činidlo v sekvenci musí být silnější než předchozí (Bacon a Davidson, 2008; Rao et al., 2008; Abollino et al., 2011). Mezi často využívané metody postupné extrakce jsou zařazovány metody podle Tessiera a od něho odvozené sekvenční extrakce BCR („Bureau Community of Reference", zjednodušená tříkroková extrakce); (Abollino et al., 2011). Frakce rizikových prvků, které můžeme v půdě stanovit, jsou vodorozpustné, iontově výměnné, vázané na uhličitany, vázané na oxidy Mn a Fe (redukovatelné), vázané na organickou hmotu a sulfidy (oxidovatelné) a pseudo-reziduální (např. pomocí lučavky královské) nebo reziduální (pomocí směsi kyselin HF/HClO₄, nepřístupné pro rostliny ani mikroorganismy); (Tessier et al., 1979; Rao et al., 2008).

Rizikové prvky mohou mít různou afinitu k jednotlivým složkám půdy (Száková et al., 2007; Yobouet et al., 2010). Zastoupení rizikových prvků v jednotlivých chemických formách se může lišit podle míry kontaminace půd, použitých extrakčních činidel, doby jejich působení nebo iontové síly činidel (Tlustoš et al., 2005). Řada rizikových prvků je z velké části vázána v reziduálních frakcích (např. As, Cu nebo Zn). Z hlediska regulace mobility a biodostupnosti hrají důležitou roli labilní formy rizikových prvků. Například pro Cd mají význam frakce vázané na oxidy Mn a Fe, výměnné i organicky vázané. Pro Cu a Pb jsou důležité zejména frakce vázané na organickou hmotu a pro Zn především frakce vázané na oxidy Mn a Fe (Tlustoš et al., 2005; Száková et al., 2007; Yobouet et al., 2010).

2.3 Regulace mobility prvků v půdě

Pohyblivost prvků v půdě může být ovlivněna chemickými i biologickými procesy. Mezi nejdůležitější chemické procesy regulující mobilitu prvků v půdě a jejich biodostupnost zařazujeme adsorpci/desorpci, srážení/rozpouštění a komplexaci/chelataci (Kunhikrishnan et al., 2012; Alloway, 2013; Bolan et al., 2014). Mobilitu prvků v půdě mohou ovlivňovat i biosorpce – význam pro živiny N, P, S; oxidačně-redukční reakce – význam pro rizikové prvky As, Cr, Cu, Fe, Hg, Mn, Pb nebo Se a živiny N, S nebo metylace/demetylace – význam pro rizikové prvky As, Hg nebo Se (Vaněk et al., 2012; Alloway, 2013; Bolan et al., 2014).

Sorpci můžeme rozdělit na nespecifickou adsorpci (schopnost půdy zadržovat ionty prvků na nabitém povrchu půdních koloidů pomocí elektrostatických sil) nebo na specifickou adsorpci (schopnost půdy zadržovat ionty prvků na nabitém povrchu půdních koloidů pomocí chemických vazeb); (Bolan et al., 1999; Vaněk et al., 2012). Významný vliv na sorpci prvků v půdě může mít přítomnost iontů organických a anorganických ligandů (Kunhikrishnan et al., 2012), půdní reakce (Violante et al., 2010), obsah organické hmoty (Lair et al., 2007), KVK (Kwon et al., 2010) nebo obsah Fe a Mn oxidů (Brown a Parks, 2001; Karpukhin a Ladonin, 2008). Bolan a Duraisamy (2003) zjistili, že aplikace fosforečných aditiv, vápenatých hmot nebo organických aditiv do kontaminovaných půd může vést ke zvýšené specifické adsorpci Cd.

Srážení (precipitace) převládá v alkalických půdách s přítomností aniontů SO_4^{2-}, CO_3^{2-}, OH^- a HPO_4^{2-} a s vysokou koncentrací rizikových prvků (Hong et al., 2007; Bolan et al., 2010). Nejčastěji studovaným procesem srážení je imobilizace Pb pomocí fosforečných aditiv s následnou tvorbou vysoce nerozpustného pyromorfitu (Cao et al., 2008; Fang et al., 2012). Srážení pomocí fosforečnanů nebo uhličitanů může omezovat i mobilitu dalších rizikových prvků Cd, Cu nebo Zn (McGowen et al., 2001; Bolan et al., 2014). Koprecipitace (spolusrážení) probíhá zejména v přítomnosti hydratovaných oxidů Fe, Mn, Al a má význam např. pro rizikové prvky As, Cr, Ni nebo Pb (Bolan et al., 2014).

Při komplexaci jsou prvky zadržovány na půdních koloidech pomocí komplexotvorné reakce probíhající mezi ionty prvků a ionty organických/anorganických ligandů (Bolan et al., 2010). Organická složka půdy má značnou afinitu k rizikovým prvkům díky přítomnosti organických ligandů, se kterými společně tvoří cheláty (Harter a Naidu, 1995). Se zvyšujícím se pH dochází k disociaci organické hmoty a zvyšuje se afinita funkčních skupin organických ligandů (karboxylových, fenolových, alkoholových nebo karbonylových) pro rizikové prvky (Bolan et al., 2010).

2.3.1 Půdní aditiva

Aplikace půdních aditiv neovlivňuje celkový obsah prvků, ale jednoznačně reguluje jejich labilní formy v půdě (Miretzky a Fernandez-Cirelli, 2008; Friesl-Hanl et al., 2009; Bolan et al., 2014). Půdní aditiva mohou působit na mobilitu rizikových prvků i živin. Nevhodně zvolené aditivum nebo jeho aplikační dávka může omezit příjem živin, vyvolat jejich deficienci a znemožnit tak pěstování rostlin na kontaminované lokalitě (Bolan a Duraisamy, 2003).

Půdní aditiva mohou mobilizovat nebo imobilizovat mobilní a potenciálně mobilizovatelné formy prvků v půdě (Bolan et al., 2014; Gul et al., 2015). Při mobilizačních procesech (základní princip tzv. indukované fytoextrakce; Neugschwandtner et al., 2009; Vamerali et al., 2010; Neugschwandtner et al., 2012) jsou rizikové prvky účinkem půdních aditiv přesouvány z pevné do kapalné fáze půdy, zvyšuje se jejich biodostupnost a je možné rizikové prvky za použití rostlin z půdy odstranit (Bolan et al., 2014). Naopak při imobilizačních procesech (základní princip tzv. *in-situ* chemické imobilizace; Guo et al., 2006; Kumpiene et al., 2008) jsou rizikové prvky účinkem půdních aditiv přemisťovány z kapalné do pevné fáze půdy, snižuje se jejich dostupnost pro rostliny a omezuje se jejich šíření do podzemních vod (Bolan et al., 2010).

Obr. 1 Role půdních aditiv v mobilizačních a imobilizačních procesech rizikových prvků v půdě (upraveno podle Bolana et al., 2010).
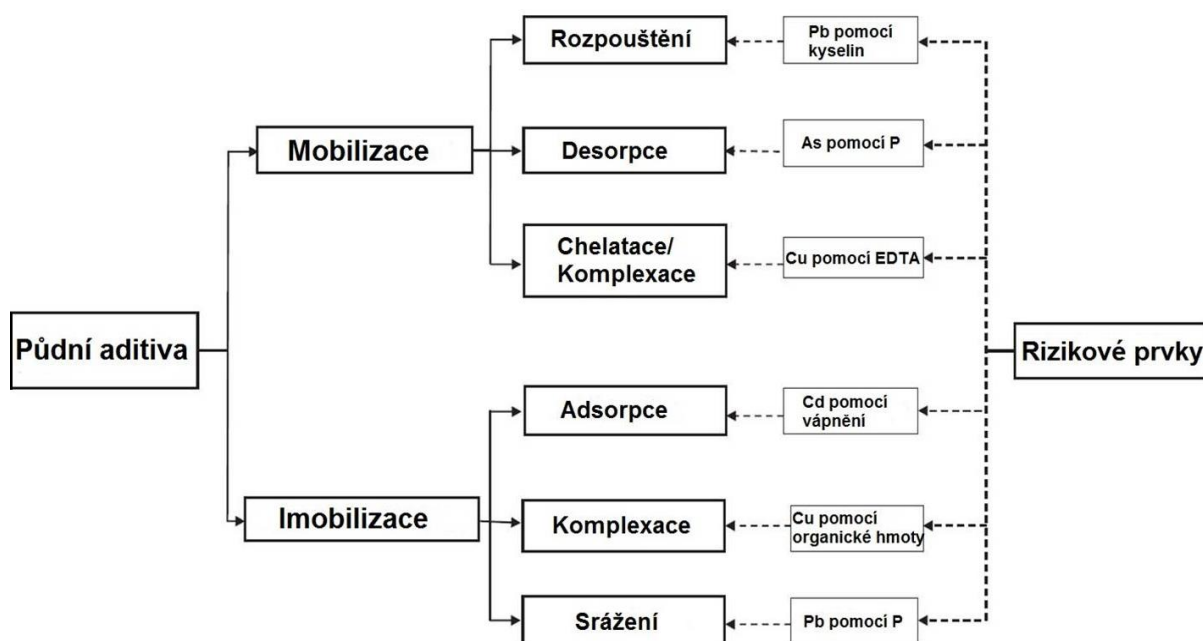
Půdní aditiva je možné rozdělit podle jejich původu na organická (např. hnůj, čistírenské kaly) nebo anorganická (např. přírodní zeolity, vápence, bentonity) nebo můžeme

použít jejich kombinace. Půdními aditivy mohou být tradiční materiály nebo odpadní látky (např. masokostní moučky produkované v kafilériích, železité kaly vznikající při čištění pitné vody nebo při výrobě hliníku; Guo et al., 2006; Gadepalle et al., 2007; Cascarosa et al., 2012). Aplikace odpadních látek je výhodná především z důvodu finanční nenáročnosti, snadné dostupnosti a omezení tvorby odpadu jeho účelným využitím (Gadepalle et al., 2007).

2.3.1.1 Mobilizační půdní aditiva

Mobilizační půdní aditiva je možné rozdělit na desorpční činidla a komplexní sloučeniny/cheláty (Bolan et al., 2014). Fungují na principu rozpouštění, desorpce a chelatace/komplexace (viz obr. 1; Bolan et al., 2010). Desorbenty (např. fosforečná aditiva – mobilizace As, Cr, Mo, Se; některá organická hnojiva – As, Cu, Se) soutěží s rizikovými prvky o sorpční místa na půdních koloidech (Bolan et al., 2014). Původně sorbovaný rizikový prvek je přídavkem činidla desorbován, uvolní se do půdního roztoku a zvýší se jeho přístupnost rostlinám (Bolan et al., 2014). Cheláty podporují tvorbu rozpustných chelátových komplexů s rizikovými prvky. Existují cheláty syntetické (např. EDTA, DTPA; Bolan et al., 2010) a přírodní (např. kyselina citrónová, elementární síra; Iqbal et al., 2012; Smolińska a Król, 2012; Bolan et al., 2014). Syntetické cheláty mohou mobilizovat rizikové prvky Cd, Cu, Fe, Mn, Pb nebo Zn (Komárek et al., 2007; Neugschwandtner et al., 2012; Bolan et al., 2014) i živiny N, P, K, Ca a Mg (Neugschwandtner et al., 2009; Zhao et al., 2011). Některé syntetické cheláty (např. EDTA) nejsou biodegradovatelné a mohou tak představovat sekundární znečištění prostředí (Zhuang et al., 2007; Zhao et al., 2011). Používání přírodních chelátů je naopak slibné opatření pro indukované fytoextrakce díky jejich snadné biologické odbouratelnosti v půdě (Smolińska a Król, 2012). V současné době se často testují lehce odbouratelná chelatační činidla (EDDS – etylendiamin disukcinát, NTA – nitrilotriacetát nebo nízkomolekulární organické kyseliny – citrónová, fumarová, jantarová octová a šťavelová); (Vamerali et al., 2010).

2.3.1.2 Imobilizační půdní aditiva

Imobilizační půdní aditiva působí na principu adsorpce, komplexace a srážení (viz obr. 1; Bolan et al., 2010). Fosforečná aditiva, vápenaté hmoty, aditiva na bázi amorfních oxidů železa, hliníku nebo manganu nebo biouhel (aktivní uhlí) patří mezi často používaná imobilizační půdní aditiva (Bolan et al., 2014).

Práce bude více zaměřena na *in-situ* imobilizační techniku a imobilizační půdní aditiva.

2.4 *In-situ* chemická imobilizace

In-situ chemická stabilizace (imobilizace rizikových prvků v půdě) patří mezi tzv. „měkké" remediace, které jsou vhodné pro půdy kontaminované rizikovými prvky. Cílem chemické stabilizace je eliminovat negativní působení rizikových prvků při zachování základních funkcí půd (Němeček et al., 2010; Trakal et al., 2011). Chemická stabilizace využívá imobilizační půdní aditiva pro snížení mobility rizikových prvků a jejich biodostupnosti na principu adsorpce, komplexace a srážení (Kumpiene et al., 2008; Hashimoto et al., 2009; Koptsik, 2014). Zvolené materiály musí být chemicky, fyzikálně a biologicky nezávadné. U použitých půdních aditiv by měla být vyhodnocena i možná rizika plynoucí z jejich aplikace na kontaminovanou půdu (Vácha et al., 2002b; Basta a McGowen, 2004; Alkorta et al., 2010).

Mezi přednosti *in-situ* imobilizačních technik patří především jednoduchý způsob aplikace, finanční nenáročnost, nízký dopad na životní prostředí, omezení tvorby odpadu nebo účinnost na široké spektrum rizikových prvků (Wuana a Okieimen, 2011; Mignardi et al., 2012). Naopak nevýhodou *in-situ* imobilizačních technik je jejich efektivita zejména v povrchové vrstvě půdy nebo dočasnost opatření. Po delší době může dojít ke snížení účinnosti aplikace půdních aditiv a k opětovné mobilizaci rizikových prvků. Z tohoto důvodu je nezbytné trvale monitorovat kontaminované lokality ošetřené imobilizačními půdními aditivy (Lee et al., 2004; Wuana a Okieimen, 2011).

2.4.1 Vápenaté hmoty

Vápnění je významné opatření pro neutralizaci půdní kyselosti na extrémně kyselých půdách vedoucí k redukci toxicity iontů Al^{3+}, Mn^{2+} a H^+ (Bolan et al., 2003; Brown et al., 2008; Farhoodi a Coventry, 2008). Vápnění je i významným zdrojem Ca popř. i Mg pro rostliny (Mayfield et al., 2004). Aplikací vápenatých hmot do silně kyselých půd omezujeme toxicitu Al^{3+}, tím můžeme podpořit růst kořenového systému rostlin i zvýšit výnosy plodin až o 70 % (Farhoodi a Coventry, 2008). K vápnění půd jsou používány materiály na bázi oxidů a hydroxidů (pálené vápno, hašené vápno), uhličitanů (vápenec, dolomitický vápenec, dolomit nebo saturační kaly produkované při výrobě cukru) nebo na bázi křemičitanů (strusky produkované při výrobě železa a oceli); (Bolan et al., 2010; Kirchmann a Eskilsson, 2010; Vaněk et al., 2012). Uváděné materiály se liší ve schopnostech neutralizovat půdní kyselost (Bolan et al., 2003).

Vápnění je jednou z nejpoužívanějších *in-situ* chemických stabilizačních technik regulující mobilitu rizikových prvků v kontaminovaných půdách (Bolan et al., 2003; Lee et al., 2004; Kumpiene et al., 2008). Mezi prvky vysoce závislé na půdní reakci se řadí Be, Cd, Co, Mn, Ni a Zn (Podlešáková et al., 2001a). Aplikací vápenatých hmot se zvyšuje pH půdy, dochází ke zvýšené adsorpci rizikových prvků na půdní koloidy a k následnému snížení mobility a biologické dostupnosti většiny rizikových prvků (Puschenreiter et al., 2005; Rieuwerts, 2007; Trakal et al., 2011).

Z vápenatých hmot je pro imobilizaci rizikových prvků nejúčinnější aplikace páleného vápna. Vazba Ca v oxidové formě způsobuje jeho okamžitou rozpustnost a reaktivitu (Bolan et al., 2003; Száková et al., 2007; Alkorta et al., 2010). Aplikací dolomitu imobilizujeme rizikové prvky jejich zvýšenou adsorpcí na povrch dolomitu (Bolan a Duraisamy, 2003).

Nevýhodou vápnění je jeho snížená účinnost po delší době od aplikace. Efektivitu vápenatých hmot je nezbytné udržovat jejich opakovanou aplikací (Friesl et al., 2003; Lee et al., 2004). Aplikací vápenatých hmot můžeme však do půd vnášet i rizikové prvky např. Cd (Nagajyoti et al., 2010). Při vápnění je nutné mít na paměti i to, že může zvyšovat mobilitu rizikových prvků As, Cr, Cu, Se nebo V v půdách (Adriano, 2001; Podlešáková et al., 2001a). Aplikací vápenatých hmot je možné mobilizovat i živiny např. Mo (Fageria et al., 2002) nebo N (Ridley et al., 1990).

2.4.1.1 Imobilizace rizikových prvků a živin

Z řady experimentů vyplývá, že vápnění je účinné imobilizační opatření pro půdy kontaminované Cd, Cu, Ni, Pb a Zn (Vácha et al., 2002b; García et al., 2004; Trakal et al., 2011). Podlešáková et al. (2001a) uvádějí, že aplikací vápenatých hmot můžeme snižovat mobilitu Be, Cd, Co, Mn, Ni a Zn o 60 až 80 %. Mobilitu Pb omezujeme zvýšením pH méně, pouze o 6 až 20 % (Podlešáková et al., 2001a).

Pomocí vápnění ale můžeme snížit mobilitu i biodostupnost mikroprvků jako B, Cu, Fe, Mn nebo Zn na nekontaminovaných půdách (Lucerna, 2000; Fageria et al., 2002; He et al., 2005; Ma a Ling, 2009). Následně může dojít i k omezení výnosu plodin. Kirchmann a Eskilsson (2010) popsali snížené výnosy zrna u obilovin po aplikaci vápenatých hmot do nekontaminovaných písčitých půd právě z důvodu omezeného příjmu mikroprvků (např. Cu a Mn) a jejich translokace do zrna obilovin. Na kyselých půdách kontaminovaných Cd, Pb a Zn aplikace vápenatých hmot naopak zvyšuje výnosy zrna jarní pšenice (o 28 % po aplikaci dolomitického vápence a o 53 % po aplikaci páleného vápna); (Tlustoš et al., 2006c), produkci nadzemní biomasy vrb (až o 80 % po druhém vegetačním období) i biomasy kořenů

vrb (Trakal et al., 2011) díky snížené fytotoxicitě rizikových prvků. Vápnění je opatření, kterým můžeme např. omezit obsahy Ca a Mg v kořenech jarní pšenice, zvýšit či snížit jejich obsahy v nadzemní biomase nebo omezit obsahy Cd a Pb v kořenech a v nadzemní biomase jarní pšenice (Tlustoš et al., 2006c).

2.4.2 Fosforečná aditiva

Přírodní fosfáty patří mezi významné zdroje fosforu (Dissanayake a Chandrajith, 2009). Fosfáty (obecný vzorec $Ca_5(PO_4)_3X$, kde X=F[-], Cl^{-}, OH^{-}, CO_3^{2-}) je možné rozdělit na apatity magmatického původu (15-20 % světových zásob fosfátů) a fosfority sedimentárního původu (75 % světových zásob fosfátů). V menší míře (1-2 %) jsou zdrojem fosforu i fosfority biogenního původu (Dissanayake a Chandrajith, 2009; Gupta et al., 2014). S původem fosfátů souvisí přítomnost rizikových prvků zejména Cd, ale také As, Cr, Cu, Ni, Hg, Pb, V nebo Zn. Apatity z Ruska z poloostrova Kola jsou téměř bez příměsí rizikových prvků, naopak fosfority z Maroka a dalších států severní Afriky nebo z USA mohou obsahovat vyšší obsahy rizikových prvků (Ramadan a Al-Ashkar, 2007; Nagajyoti et al., 2010; Chaney, 2012).

Fosforečná aditiva rozlišujeme podle míry rozpustnosti P na vysoce rozpustná (např. kyselina fosforečná, dihydrogenfosforečnan draselný nebo fosforečnany sodné), středně rozpustná (např. jednoduchý, dvojitý nebo trojitý superfosfát), málo rozpustná (např. kostní a masokostní moučky) a nejméně rozpustná (např. syntetický hydroxyapatit nebo mletý fosfát); (Hodson et al., 2000; Chrysochoou et al., 2007; Sneddon et al., 2008). Rozeznáváme i klasická (např. superfosfáty) a alternativní fosforečná aditiva (odpadní materiály, jako např. kostní a masokostní moučky); (Chrysochoou et al., 2007; Miretzky a Fernandez-Cirelli, 2008).

Aplikace fosforečných aditiv do kontaminovaných půd může přinášet řadu pozitiv (např. zdroj P pro rostliny, recyklace odpadních materiálů, imobilizace vybraných rizikových prvků). Potřebné je ale jejich aplikaci monitorovat z důvodu možného negativního dopadu např. zvýšené riziko vyplavování P a následná eutrofizace povrchových vod (Park et al., 2011; Hafsteinsdóttir et al., 2015), nebo mobilizace As, Sb, Se a W (Chrysochoou et al., 2007; Cui et al., 2010; Munksgaard a Lottermoser, 2011).

2.4.2.1 Imobilizace rizikových prvků a živin

Imobilizace rizikových prvků pomocí fosforečných aditiv je remediační metoda ohleduplná k životnímu prostředí (Ma et al., 1993; Cao et al., 2009; Yan et al., 2015).

Fosforečná aditiva přidaná do kontaminovaných půd reagují s mobilními a biodostupnými podíly rizikových prvků a přeměňují je na stabilní, méně rozpustné formy těchto prvků (Vangronsveld et al., 2009; Xenidis et al., 2010; Mignardi et al., 2012). Aplikací fosforečných aditiv je možné rizikové prvky v půdě imobilizovat různými procesy – iontovou výměnou, tvorbou nerozpustných komplexů na povrchu fosfátů, rozpouštěním původních fosfátů a následnou tvorbou fosforečných sraženin s rizikovými prvky nebo záměnou Ca ve fosfátu rizikovými prvky při spolusrážení (Ma et al., 1994; Hafsteinsdóttir et al., 2015). Fosforečnany většiny rizikových prvků (Cd, Cu, Ni, Pb a Zn) se vyznačují mimořádně nízkou rozpustností a jsou stabilní v širokém rozsahu podmínek prostředí (pH, redox potenciál); (Sneddon et al., 2006). V mnoha studiích se aplikace fosforečných aditiv ukázala jako velmi úspěšná zejména pro imobilizaci Pb díky tvorbě vysoce nerozpustného pyromorfitu (Munksgaard a Lottermoser, 2011; Fang et al., 2012; Park et al., 2012). Pyromorfity jsou fosfáty olova s chlorem, fluorem, bromem nebo s hydroxylovou skupinou (obecný vzorec $Pb_5(PO_4)_3X$), které se ve struktuře pyromorfitů mohou vzájemně zastupovat (Chrysochoou et al., 2007; Miretzky a Fernandez-Cirelli, 2008). Fosforečná aditiva mohou působit imobilizačně i na jiné rizikové prvky např. Cd, Cu a Zn, které se často vyskytují v půdách společně s Pb (Spuller et al., 2007; Baker et al., 2012; Yan et al., 2015). Nicméně imobilizace dalších rizikových prvků Cd, Cu nebo Zn pomocí různých druhů fosforečných aditiv v půdách kontaminovaných několika rizikovými prvky současně byla studována v menším rozsahu (Baker et al., 2012; Munksgaard a Lottermoser, 2013; Yan et al., 2015). Úspěšnost imobilizace rizikových prvků fosforečnými aditivy může být ovlivněna půdní reakcí (Laperche et al., 1996; Zhang a Ryan, 1998). V kyselých půdách (pH~5) dochází ke zvýšenému uvolňování rizikových prvků z minerálů, zlepšuje se i rozpustnost fosfátů. Naopak při půdní reakci vyšší než pH=6 se jejich rozpustnost snižuje (Chrysochoou et al., 2007; Miretzky a Fernandez-Cirelli, 2008; Mignardi et al., 2012). Zhang a Ryan (1998, 1999) popsali, že přeměna minerálů olova, např. galenitu (PbS), cerusitu ($PbCO_3$) a anglesitu ($PbSO_4$) v pyromorfity [($Pb_5(PO_4)_3Cl$; ($Pb_5(PO_4)_3OH$] nastává v rozmezí pH 4 až 5. Aplikace fosforečných aditiv s pomalu uvolnitelným P zvyšuje půdní reakci (Cui et al., 2010) a snižuje potenciální riziko eutrofizace (Chrysochoou et al., 2007; Mignardi et al., 2012). Přídavek syntetického hydroxyapatitu a mletého fosfátu významně snižuje vodorozpustné formy Cd, Cu, Pb a Zn (pokles o 84-99 %); (Mignardi et al., 2012). Imobilizační účinnost syntetického hydroxyapatitu byla vyšší v porovnání s mletým fosfátem (Islam et al., 2010; Mignardi et al., 2012). Aplikace fosforečných aditiv s rychle uvolnitelným fosforem mohou snižovat půdní reakci (Chrysochoou et al., 2007; Cui et al.,

2010) a tím přispět k účinné imobilizaci Pb v půdě, avšak zvyšuje se riziko eutrofizace povrchových vod (Cao et al., 2002; Basta a McGowen, 2004; Park et al., 2011).

Alternativní formy fosforečných aditiv jsou levnými a snadno dostupnými materiály se sníženým rizikem eutrofizace povrchových vod (Deydier et al., 2005; Chrysochoou et al., 2007; Miretzky a Fernandez-Cirelli, 2008). Masokostní moučky mohou imobilizovat Cd a Pb (Deydier et al., 2003; 2007; Coutand et al., 2009). V kyselém prostředí masokostní moučky převážně imobilizují Pb do nerozpustného pyromorfitu ($Pb_{10}(PO_4)_6(OH)_2$), v alkalickém prostředí naopak do více rozpustného dihydrátu uhličitanu olovnatého ($PbCO_3 \cdot 2H_2O$; Deydier et al., 2007). Masokostní moučky mohou být použity surové nebo ve formě popela (Countand et al., 2008; 2009). Surová masokostní moučka je často zařazována do kategorie nebezpečných odpadů (zejména kvůli přítomnosti Sb a Zn), proto je nezbytné provést stabilizační ošetření (Countand et al., 2008). Masokostní moučky ve formě popela se zařazují do neaktivních odpadů (Countand et al., 2008). Kostní moučky mohou imobilizovat Cu (Hodson et al., 2000), Pb i As (Sneddon et al., 2006; 2008).

2.4.3 Sloučeniny na bázi železa

I aplikací železitých sorbentů můžeme významně imobilizovat rizikové prvky v kontaminovaných půdách (Bertocchi et al., 2006; Anton et al., 2012). Materiály bohaté na Fe je možné rozdělit podle zdroje Fe na oxidy Fe (např. goethit, hematit, lepidokrokit, ferihydrit), sírany Fe (síran železnatý, síran železitý), elementární Fe, odpadní látky bohaté na Fe (např. železité kaly z čistíren odpadních vod nebo z výroby hliníku) nebo jejich kombinace (Mireztky a Fernandez-Cirelli, 2010; Komárek et al., 2013).

Červený kal (tzv. „red mud", železitý odpad z výroby hliníku) je vysoce alkalický materiál obsahující oxidy Fe (25-40 %) a Al (15-20 %); (Gray et al., 2006; Snars a Gilkes, 2009). Alkalita (pH>11) červeného kalu musí být před jeho použitím do kontaminovaných půd neutralizována (např. sádrou, zelenou skalicí, mořskou vodou); (Brunori et al., 2005; Gadepalle et al., 2007). Důvodem je omezení možné oxidace organické hmoty v půdě vlivem silně alkalického pH a následné tvorby rozpustných komplexů rizikových prvků s organickými látkami (Gadepalle et al., 2007).

2.4.3.1 Imobilizace rizikových prvků a živin

Červený kal účinně omezuje mobilitu rizikových prvků (As, Cd, Co, Cu, Ni, Mn, Pb, Zn) v kontaminovaných půdách. Aplikací červeného kalu můžeme rizikové prvky imobilizovat na principu srážení (důvod – alkalita červeného kalu) nebo adsorpce na povrch

oxidů a hydroxidů Fe a Al (Gray et al., 2006; Gadepalle et al., 2007; Garau et al., 2011). Friesl et al. (2006) popisují, že po 15 měsících od aplikace červeného kalu byla snížena mobilita Cd (o 42 %), Ni (o 50 %) a Zn (o 63 %) v půdě silně kontaminované rizikovými prvky.

Řada studií, zejména ze západní Austrálie, prokázala, že aplikací červeného kalu je možné omezovat mobilitu P v půdě a snižovat jeho příjem pro rostliny (Summers et al., 1996). Aplikací aditiv na bázi amorfních oxidů železa můžeme snižovat koncentraci rizikových prvků, ale současně imobilizovat makroprvky (např. Ca, Mg, P). Z tohoto důvodu je potřebná další úprava půdy, aby se zabránilo nežádoucímu omezení dostupných živin po aplikaci materiálů bohatých na Fe (Bleeker et al., 2002).

2.4.4 Chemofytostabilizace

Chemofytostabilizace je považována za slibné, dočasné remediační opatření pro půdy silně kontaminované rizikovými prvky (Alkorta et al., 2010). Při této metodě se nejprve do kontaminované půdy aplikují aditiva, která omezují mobilitu rizikových prvků v půdě a snižují tak jejich přístupnost pro rostliny (Kumpiene et al., 2008; Alkorta et al., 2010; Grobelak a Napora, 2015). Následně je možné na stabilizovanou lokalitu vysázet rostliny tolerantní k rizikovým prvkům (Alkorta et al., 2010) a využít lokalitu i esteticky a hospodářsky (Tlustoš et al., 2007; Friesl-Hanl et al., 2009).

2.5 Pěstování rostlin na kontaminovaných půdách

Rostliny v závislosti na rostlinném druhu nebo genotypu mohou na přítomnost rizikových prvků v půdě reagovat různými způsoby (přirozená citlivost nebo tolerance); (Tlustoš et al., 2006b). Nicméně vysoké obsahy rizikových prvků v půdě jsou pro většinu půdních mikroorganismů a rostlin toxické, vedou ke špatnému a nepravidelnému vývoji vegetace nebo dokonce k jejímu úplnému vymizení (Nagajyoti et al., 2010; Solanki a Dhankhar, 2011; Leitenmaier a Küper, 2013). Z důvodu nedostatečného vegetačního pokryvu může větrnou nebo vodní erozí docházet k šíření rizikových prvků do okolního prostředí (Bolan et al., 2014; Grobelak a Napora, 2015), ale může docházet i k vymývání rizikových prvků z půdního profilu (Vaněk et al., 2005). Nezbytné je rizikové prvky v půdě přinejmenším stabilizovat a omezit možná rizika, která jsou s nimi spojená (Vácha et al., 2002b; Puschenreiter et al., 2005; Tlustoš et al., 2006a).

Existují rostliny (tzv. metalofyty), které se na kontaminovaných půdách vyskytují přirozeně. Tyto rostliny jsou na půdy kontaminované rizikovými prvky adaptované (mají vyvinuté obranné mechanismy) a na kontaminovaných půdách prosperují (Baker, 1981; Sheoran et al., 2011). Rostliny je možné podle obsahu rizikových prvků v rostlinných pletivech rozdělit na rostliny s nízkou akumulací rizikových prvků (exkludační), s běžnou akumulací rizikových prvků (indikační) a s vysokou akumulací rizikových prvků (akumulační); (Baker, 1981). Exkludační rostliny (jednoděložné trávy – súdánská tráva, kostřava; Tlustoš et al., 2006b) udržují rizikové prvky v kořenech a do nadzemních orgánů je téměř netransportují (Seregin a Kozhevnikova, 2008; Sheoran et al., 2011; Malík a Biswas, 2012). Exkludory mohou být vhodné pro fytostabilizace (Lasat, 2002; Barceló a Poschenrieder, 2003; Yoon et al., 2006). Indikační rostliny (většina zemědělských plodin, např. kukuřice, oves nebo pšenice; Tlustoš et al., 2006b) ve svých nadzemních orgánech odrážejí koncentrace rizikových prvků v půdě (Alkorta et al., 2004; Sheoran et al., 2011) a jsou ideální pro přímou nebo nepřímou bioindikaci (Grant, 1999; Leitenmaier a Küpper, 2013). Akumulační rostliny (vhodné pro fytoextrakce; Yoon et al., 2006) obsahují ve svých nadzemních orgánech rizikové prvky ve vyšších koncentracích než v půdě, ve které rostou (Baker, 1981; Alkorta et al., 2004; Memon a Schröder, 2009). Hyperakumulační rostliny (extrémní případ akumulačních rostlin; Pollard et al., 2002) se vyznačují tím, že obsahují v nadzemní biomase více než 10 000 mg Fe (Mn, Zn).kg^{-1} sušiny, > 3000 mg Al.kg^{-1} sušiny, > 1000 mg As (Cr, Cu, Ni, Pb).kg^{-1} sušiny a více než 100 mg Cd.kg^{-1} sušiny (Baker a Brooks, 1989; Jedynak et al., 2009; Huang et al., 2009; Lorestani et al., 2011). Seznam vybraných hyperakumulačních rostlin pro rizikové prvky je uveden v řadě studií (Baker, 1981; Malík a Biswas, 2012; Ali et al., 2013).

Příjem rizikových prvků rostlinami je významně ovlivněn celkovým obsahem rizikového prvku v půdě, jeho mobilitou a biodostupností. Na příjem prvků může působit celá řada půdních parametrů – např. půdní reakce, redox potenciál, teplota půdy, KVK, kvalita a kvantita organických látek, přítomnost dalších prvků v rhizosféře, hnojení, rostlinný druh, konkurence mezi rostlinnými druhy nebo kořenový systém (Singh et al., 2003; Nagajyoti et al., 2010; Sheoran et al., 2011). Příjem a transport rizikových prvků rostlinami je hodnocen pomocí bioakumulačního faktoru (BF; poměr celkového obsahu prvku v nadzemních orgánech k celkovému nebo pseudocelkovému obsahu prvku v půdě) a translokačního faktoru (TF; poměr celkového obsahu prvku v listech k celkovému obsahu prvku v kořenech). Pro rostliny akumulující rizikové prvky jsou charakteristické hodnoty BF a TF větší než 1,

indikační rostliny mají hodnoty faktorů rovné 1. Pro exkludační rostliny jsou typické hodnoty BF a TF menší než 1 (Baker, 1981).

2.5.1 Mechanismy příjmu prvků rostlinami

Rostliny přijímají prvky ve formě iontů (kationtů nebo aniontů) primárně kořeny z půdního roztoku (Vaněk et al., 2012; Ali et al., 2013; Alloway, 2013). Příjem rizikových prvků závisí zejména na jejich biodostupnosti pro rostliny (Jabeen et al., 2009). Rizikové prvky je možné rozdělit na okamžitě biodostupné (As, Cd, Cu, Ni, Se a Zn), středně biodostupné (Co, Fe a Mn) a málo biodostupné (Cr, Pb a U); (Prasad, 2003). Řada autorů (Jabeen et al., 2009; Vaněk et al., 2012; Alloway, 2013) člení příjem prvků kořeny rostlin na přísun prvků do bezprostřední blízkosti kořenů (tzv. rhizosféry), vstup prvků do kořenů a transport prvků v rostlině. K přirozené mobilizaci prvků může docházet prostřednictvím kořenové exsudace (sekrece) nebo pomocí půdních mikroorganismů (tj. bakterií a mykorhizních hub); (Jabeen et al., 2009). Přísun prvků do rhizosféry může být zvýšen sekrecí protonů (H^+ iontů), organických kyselin (např. kyseliny jablečná, malonová, octová nebo šťavelová), chelatačních činidel (např. fytosiderofory – kyseliny mugineová a avenová) nebo enzymů (Salt et al., 1995; Sheoran et al., 2011; Vaněk et al., 2012). Po vstupu prvků do kořene jsou jejich ionty dále transportovány apoplastem nebo symplastem (Jabeen et al., 2009; Vaněk et al., 2012). Část iontů prvků může být imobilizována v buněčných stěnách kořene (Sheoran et al., 2011). Apoplastická cesta je snadno propustná pro ionty prvků od rhizodermis až po endodermis, pohyb iontů prvků se děje prostřednictvím hmotového toku nebo difúze (pasivní děje) bez nutnosti překračovat plazmatickou membránu (Jabeen et al., 2009; Vaněk et al., 2012). U endodermis je ale apoplastická cesta přerušena tzv. Caspariho proužky a ionty prvků musí překročit plazmatickou membránu a dále pokračovat symplastem (Sheoran et al., 2011; Vaněk et al., 2012). Většina iontů prvků je od svého vstupu do kořene transportována symplastem. Do buněk se ionty dostávají přes plazmatickou membránu pomocí specifických přenašečů nebo kanálků (aktivní děje); (Gaymard, 1998; Vaněk et al., 2012). Symplastickou cestou se ionty prvků postupně dostávají přes epidermis, kortex, endodermis a pericykl až do xylému (Jabeen et al., 2009; Sheoran et al., 2011; Vaněk et al., 2012). Xylémem jsou prvky následně transportovány do nadzemních částí rostlin (Jabeen et al., 2009; Vaněk et al., 2012).

2.5.2 Mechanismy tolerance rostlin

Tolerance rostlin je projevem interakce genotypu rostliny a prostředí a může být definována jako schopnost rostlin přežít na půdách kontaminovaných rizikovými prvky, které jsou pro jiné rostliny toxické (Macnair et al., 2000; Hall, 2002). Rostliny původem z kontaminovaných oblastí jsou ve většině případů mnohem tolerantnější vůči rizikovým prvkům než rostliny původem z nekontaminovaných oblastí (He et al., 2002). Tolerantní rostliny mají vyvinuté obranné mechanismy (fyziologické a biochemické), které jim pomáhají snášet vysoké koncentrace rizikových prvků v půdě (Hall, 2002; Rascio a Navari-Izzo, 2011; Revathi a Venugopal, 2013). Obranné strategie rostlin můžeme rozdělit na vnější (kořenová exsudace, event. i mykorhiza), probíhající v rhizosféře a vnitřní (vazba rizikového prvku do buněčné stěny kořenů, snížený transport rizikového prvku přes plazmatickou membránu, detoxikace rizikového prvku v buňce jeho chelatací pomocí různých ligandů nebo uložení rizikového prvku do vakuoly pomocí tonoplastových transportérů), probíhající v rostlině (Hall, 2002; Hossain et al., 2012).

Většina rostlin se snaží omezit příjem rizikových prvků již v rhizosféře (Hall, 2002; Yang et al., 2005; Hossain et al., 2012). Pomocí kořenové exsudace mohou být rizikové prvky inaktivovány srážením nebo vnější komplexací – chelatací (Hossain et al., 2012; Sytar et al., 2013). V bezprostřední blízkosti kořenů mohou být rizikové prvky sráženy zvýšením půdní reakce (Degenhardt et al., 1998; Hossain et al., 2012) nebo sekrecí fosforečnanů (Pellet et al., 1996; Hossain et al., 2012). Exsudace aniontů organických kyselin také vnější chelatací významně omezuje příjem rizikových prvků pro rostliny (Sytar et al., 2013). Pinto et al. (2008) popisují omezený příjem Cd sekrecí aniontů organických kyselin u vybraných zemědělských plodin (čirok – jablečnany, kukuřice – citráty). V řadě studií bylo potvrzeno, že exsudace aniontů organických kyselin omezuje příjem Al pro rostliny (např. u pšenice – jablečnany, Delhaize et al., 1993; u pohanky nebo u šťovíku menšího – šťavelany, Zheng et al., 1998; Schöttelndreier et al., 2001).

Mykorhiza (např. ektomykorhiza nebo arbuskulární mykorhiza) může také hrát důležitou roli při vnější obraně rostlin proti rizikovým prvkům. Ektomykorhiza brání vstupu rizikovým prvkům do hostitelské rostliny pomocí vnějších mechanismů (např. absorpce prvků povrchem hyf nebo vnějším myceliem, chelatace prvků pomocí exsudátů hub), arbuskulární mykorhiza omezuje toxicitu pro hostitelskou rostlinu pomocí vnitřních mechanismů (Jentschke a Golbold, 2000; Hall, 2002).

Vazba rizikových prvků do buněčné stěny kořenů nebo jejich omezený příjem iontovými kanálky v plazmatické membráně jsou další obranné mechanismy rostlin (Hall,

2002; Sheoran et al., 2011; Hossain et al., 2012). V buněčné stěně kořenů mohou být rizikové prvky vázány na záporné náboje karboxylových skupin polygalakturonových kyselin, které jsou součástí pektinů (Ernst et al., 1992) nebo na hystidylové skupiny (popsáno pro Cd u fazolu obecného; Leita et al., 1991). Transport rizikových prvků do nadzemních orgánů a jejich následná deaktivace je popisována především u (hyper)akumulačních rostlin (Hall, 2002). Rizikové prvky mohou být transportovány do nadzemních orgánů v komplexu s cheláty (např. s citráty, s fytochelatiny nebo s metalothioneiny); (Salt et al., 1995; Sheoran et al., 2011; Revathi a Venugopal, 2013). Existuje i několik tříd proteinů (transportérů), které se podílí na transportu rizikových prvků rostlinou (např. Nramp – „natural resistence associated macrophage protein", CDF – „cation diffusion facilitator", CPx-type ATPases – „heavy metal-transporting ATPases", ZIP – „zinc-regulated transporter, iron-regulated transporter protein"); (Guerinot, 2000; Williams et al., 2000). Rizikové prvky mohou být v nadzemní biomase rostlin detoxikovány jejich chelatací s ligandy (např. s aminokyselinami – histidin; s anionty organických kyselin – citráty, jablečnany nebo s peptidy – fytochelatiny a metallothioneiny) s vysokou afinitou v cytosolu (Rauser, 1999; Clements, 2001; Hall, 2002; Yang et al., 2005). Pomocí fytochelatinů mohou být účinně detoxikovány Cd nebo As, metallothioneny nebo histidin mohou potlačit toxicitu Cd, Cu, Hg, Ni nebo Zn (Cobbett a Goldsbrough, 2002; Jabeen et al., 2009). V nadzemních orgánech mohou být rizikové prvky inaktivovány také jejich zabudováním do buněčné stěny nebo do vakuoly, kde imobilizovaný rizikový prvek již nemůže poškodit životně důležité procesy v rostlinné buňce (Assuncao et al., 2003; Yang et al., 2005; Sheoran et al., 2011).

2.5.3 Rostliny tolerantní k rizikovým prvkům

Existuje celá řada tolerantních rostlin k rizikovým prvkům (viz kapitola 2.5). Pro experimentální část byly z důvodu mezinárodní využitelnosti vybrány celosvětově rozšířené druhy širokolistých šťovíků a rychle rostoucích dřevin. Rychle rostoucí dřeviny byly zvoleny i z důvodu potenciálního využití pro hospodářské účely.

2.5.3.1 Širokolisté šťovíky

Šťovík tupolistý (*Rumex obtusifolius* L.) i šťovík kadeřavý (*Rumex crispus* L.), patřící do čeledi rdesnovité (*Polygonaceae*), jsou považovány za významné celosvětově rozšířené plevele zejména travních porostů a orných půd (Stilmant et al., 2010; Hrdličková et al., 2011). Česká republika není v tomto ohledu výjimkou (Mikulka a Kneifelová-Korčáková, 2006; Jursík et al., 2008; Hujerová et al., 2013). Šťovík alpský (*Rumex alpinus* L.) a šťovík

dlouholistý (*Rumex longifolius* DC.) škodí na našem území zejména v horských oblastech (Jursík et al., 2008; Šťastná et al., 2010). Ostatní druhy širokolistých šťovíků (např. šťovík menší – *Rumex acetosella* L. nebo šťovík kyselý – *Rumex acetosa* L.) nejsou v České republice považovány za významné plevelné druhy (Jursík et al., 2008). Aplikace minerálních a statkových hnojiv (zvláště kejdy a chlévského hnoje) podpořila šíření těchto plevelů zejména v poválečných letech (Mikulka a Kneifelová-Korčáková, 2006).

Šťovíky jsou považovány za vysoce nitrofilní druhy (Zaller, 2004). Nicméně, v počátečním vývoji mohou být šťovíky k N velmi citlivé (Křišťálová et al., 2011; Hejcman et al., 2012). Popsána byla také prospěšnost P a K pro šťovíky (Humphreys et al., 1999; Hopkins a Johnson, 2002; Křišťálová et al., 2011). Výskyt šťovíků může být negativně ovlivněn alkalickou půdní reakcí a s tím souvisejícím vysokým obsahem Ca v půdě (Humphreys et al., 1999; Hann et al., 2012). Šťovíky jsou významné tzv. oxalátní rostliny (White a Broadley, 2003). Vysoké obsahy Ca v rostlinných pletivech šťovíků mohou být inaktivovány tvorbou vysoce nerozpustných komplexů se šťavelany zabudovaných do vakuol nebo buněčných stěn (Franceschi a Nakata, 2005; Tolrà et al., 2005). Významná je i jejich tolerance k Al popsaná v silně kyselých modelových podmínkách (Schöttelndreier et al., 2001; Tolrà et al., 2005; Myiagi et al., 2013). Širokolisté šťovíky (šťovík zahradní – *Rumex patientia* L., šťovík kyselý nebo šťovík tupolistý) mohou být používány v gastronomii jako listová zelenina nebo ve fytoterapii. Důvodem jejich prospěšnosti jsou vysoké obsahy vitamínů K_1, C, luteinu, β-karotenu, γ-tokoferolu nebo kvercetinu (Demirezer et al., 2001; Vardavas et al., 2006; da Silva et al., 2013; Tuazon-Nartea a Savage, 2013).

2.5.3.1.1 Využití ve fytoremediačních technologiích

V poslední době se řada vědců zabývá myšlenkou využít různé druhy šťovíků ve fytoremediačních technologiích (Güleryüz et al., 2008; Barrutia et al., 2009; Muhammad et al., 2013). Důvodem je nenáročnost šťovíků, jejich všudypřítomnost a u některých druhů šťovíků i značný potenciál pro akumulaci rizikových prvků zejména díky jejich vysoké produkci biomasy (Tang et al., 1999; Zhuang et al., 2007). Výhodou je také jejich přirozený výskyt v průmyslových a důlních oblastech (Wang et al., 2003; Güleryüz et al., 2008; Epelde et al., 2010). Ve fytoremediačních technologiích mohou být šťovíky uplatněny především na mírně nebo středně kontaminovaných půdách, často jsou zařazovány mezi exkludační rostliny (např. šťovík kyselý nebo šťovík menší); (Wenzel et al., 2003; Gaweda, 2009). Prokázána byla i jejich indikační schopnost zejména pro Zn (šťovík kyselý); (Barrutia et al., 2009; Epelde et al., 2010).

Obsahy rizikových prvků se v rostlinných orgánech šťovíků mohou významně lišit podle míry kontaminace půd, konkrétního rizikového prvku nebo druhu šťovíku (Zhuang et al., 2007; Güleryüz et al., 2008; Gaweda, 2009). Wang et al. (2003) testovali fytoremediační schopnosti šťovíku kyselého přirozeně rostoucího v Číně na kyselých půdách kontaminovaných Cd ($\leq$6 mg.kg^{-1}), Pb ($\leq$215 mg.kg^{-1}) a Zn ($\leq$983 mg.kg^{-1}). V kořenech i v nadzemní biomase šťovíku byly zjištěny podobné obsahy Cd (2,4 mg.kg^{-1}), Pb (86 mg.kg^{-1}), Zn (929 mg.kg^{-1}) i Cr, Cu nebo Mn. Pouze obsah Ni byl významně vyšší v kořenech než v nadzemní biomase. I Gaweda (2009) ověřoval možné využití šťovíku kyselého pro fytoremediační účely. Šťovík přirozeně rostoucí v Polsku na kyselých až alkalických půdách kontaminovaných Cd (2,4 mg.kg^{-1}) a Zn (148 mg.kg^{-1}) obsahoval nejvíce Cd (0,8 mg.kg^{-1}), Zn (27 mg.kg^{-1}) i Cr, Cu, Fe, Mn, Ni a Pb v kořenech. Nejnižší obsahy všech prvků byly stanoveny ve stoncích šťovíku (např. 0,1 mg Cd.kg^{-1}; 7 mg Zn.kg^{-1}). Ve studii Zhuanga et al. (2007) byly zjištěny odlišné fytoremediační schopnosti různých druhů šťovíků (šťovík kadeřavý a šťovík kyselý), přirozeně rostoucí v Číně na slabě kyselé půdě kontaminované Cd (7,2 mg.kg^{-1}), Pb (960 mg.kg^{-1}) a Zn (1050 mg.kg^{-1}). Šťovík kadeřavý zde projevil své indikační až akumulační schopnosti pro Cd (BF>1), Zn (BF, TF>1) a exkludační schopnosti pro Pb. U šťovíku kyselého byly popsány pouze exkludační schopnosti pro Cd, Pb a Zn. Roli v odlišných fytoremediačních schopnostech může částečně hrát i rozdílná produkce biomasy. Šťovík kadeřavý dosahuje vyšší produkce biomasy než šťovík kyselý (Zhuang et al., 2007). I další druhy šťovíků prokazují zvýšenou toleranci k rizikovým prvkům a tím i potenciální využití ve fytoremediačních technologiích, např. šťovík tupolistý (podruh *Rumex obtusifolius* L. subsp. *subalpinus*) původem z Turecka pro Cr, Cu a Ni (Güleryüz et al., 2008); šťovík původem z Pákistánu (*Rumex hastatus* D. Don) pro Fe, Mn, Cr a Ni (Muhammad et al., 2013) nebo šťovík ze Španělska (*Rumex induratus* Boiss. & Reut.) pro Hg (Moreno-Jiménez et al., 2006).

Tolerance šťovíků k rizikovým prvkům bude pravděpodobně souviset s přítomností organických kyselin. Organické kyseliny mohou hrát důležitou roli v toleranci a detoxikaci rizikových prvků rostlinami. Důvodem je možná vnější nebo vnitřní chelatace organických kyselin s rizikovými prvky (Sytar et al., 2013).

2.5.3.2 Rychle rostoucí dřeviny

Rychle rostoucí dřeviny jsou na rozdíl od bylin charakterizovány řadou důležitých vlastností (např. hlubší kořenový systém, vyšší produkce biomasy nebo vyšší transpirační aktivita); (Fischerová et al., 2006; Tlustoš et al., 2007; Capuana, 2011), díky kterým jsou

vhodné pro fytoremediační technologie (Dos Santos Utmazian a Wenzel, 2007; Meers et al., 2007). Produkci biomasy dřevin je možné navýšit hnojením N, P a K, vápněním nebo zavlažováním (Tlustoš et al., 2006a; Vamerali et al., 2009). Mezi rychle rostoucí dřeviny můžeme zařadit rody vrba (*Salix* L.), topol (*Populus* L.), bříza (*Betula* L.), olše (*Alnus* L.), javor (*Acer* L.), jasan (*Fraxinus* L.) nebo jeřáb (*Sorbus* L.); (Rosselli et al., 2003; Tlustoš et al., 2006a; Unterbrunner et al., 2007). Rozdíly mezi dřevinami se projevují nejen v nárocích na teplo, půdní texturu a strukturu, zásobenost živinami (Mrnka et al., 2011), ale i ve schopnostech přijímat rizikové prvky (olše, jasan, jeřáb – exkludační rostliny, vrba – akumulační rostlina zejména pro Cd a Zn); (Rosselli et al., 2003).

Velká pozornost z hlediska fytoremediací je zaměřována především na vrby (čeleď vrbovité − *Salicaceae*), které je možné využít pro fytoextrakce i fytostabilizace (Pulford a Watson, 2003; Meers et al., 2007). Důvodem je jejich vysoká produkce biomasy a schopnost tolerovat nebo akumulovat rizikové prvky (Pulford a Watson, 2003; Meers et al., 2007; Abhilash et al., 2012). Podle Wegera a Bubeníka (2011) patří mezi nejproduktivnější klony vrb v České republice vrba Smithova (*Salix × smithiana* Willd.); (tj. přirozený hybrid vrby jívy a vrby košíkářské). Vrby jsou rozšířené převážně v mírných a arktických oblastech, najdeme je ale i v subtropických a tropických oblastech (Kuzovkina a Volk, 2009). Chen et al. (2010) dělí rod vrba na čtyři podrody *Salix, Chosenia, Triandrae* a *Vetrix*. Významné agronomické, fyziologické a ekologické vlastnosti vrb nezbytné pro jejich využití ve fytoremediačních technologiích jsou uvedeny ve studii Kuzovkiny a Volka (2009).

2.5.3.2.1 Využití ve fytoremediačních technologiích

Některé druhy/klony vrb mohou v nadzemní biomase hromadit značná množství Cd nebo Zn. Například v listech vrby Smithovy pěstované v hydroponii s přídavkem Cd (4,45 μmol.l^{-1}) nebo Zn (76,5 μmol.l^{-1}) bylo zjištěno až 3180 mg Zn.kg^{-1} a 245 mg Cd.kg^{-1} (Dos Santos Utmazian et al., 2007). Akumulační schopnosti pro Cd a Zn byly prokázány u vrby drsnokvěté (*Salix dasyclados* Wimm.) nebo pro Zn u vrby Smithovy a Matsudovy (*Salix matsudana* Koidz.). Exkludační schopnosti pro Zn byly naopak popsány u vrby purpurové (*Salix purpurea* L.) a vrby křehké (*Salix fragilis* L.); (Dos Santos Utmazian et al., 2007). Distribuce rizikových prvků v orgánech vrb může být ovlivněna konkrétním rizikovým prvkem (Tlustoš et al., 2007). Například Cd, Ni a Zn jsou ve většině případů nejvíce obsaženy v nadzemních orgánech vrb (Pulford a Watson, 2003; Hammer et al., 2003; Tlustoš et al., 2007). Nejvyšší obsahy As, Cr, Cu a Pb jsou naopak popisovány v kořenech (Pulford a Watson, 2003; Tlustoš et al., 2007). Vamerali et al. (2009) zjistili vyšší obsahy As a Cu

v jemných kořenech v porovnání s kořeny hrubými. Míra kontaminace půdy rizikovými prvky může mít také vliv na distribuci rizikových prvků v orgánech vrb i na produkci jejich biomasy (Tlustoš et al., 2007). Na středně kontaminované půdě byly vyšší obsahy Cd zjištěny v listech, dále ve větvích a ve dřevě. Nejnižší obsahy Cd byly popsány v kořenech (Tlustoš et al., 2007). Na silně kontaminované půdě byly obsahy Cd v listech a v kořenech podobné (Tlustoš et al., 2007). Nejvyšší produkce biomasy jednotlivých orgánů vrb byla popsána na středně kontaminovaných půdách (kořeny>větve>listy), naopak nejnižší na silně kontaminovaných půdách (listy>větve>kořeny); (Tlustoš et al., 2007). Vrby mohou být využity ve fytoremediačních technologiích zejména na mírně nebo středně kontaminovaných půdách (Vysloužilová et al., 2003b; Tlustoš et al., 2007; Jensen et al., 2009). Na silně kontaminovaných půdách vrby neprosperují (Vysloužilová et al., 2006; Jensen et al., 2009). Produkce vrb je zde limitována fytotoxicitou Cd (až 80 mg.kg^{-1} v listech) a Zn (až 3000 mg.kg^{-1} v listech); (Jensen et al., 2009). Na silně kontaminovaných půdách je proto nezbytná imobilizace rizikových prvků před uplatněním fytoremediačních technologií (Vysloužilová et al., 2003a; Puschenreiter et al., 2005; Tlustoš et al., 2006a).

3 Vědecké hypotézy a cíle práce

Tématem doktorské disertační práce bylo posoudit účinek imobilizačních půdních aditiv na mobilitu rizikových prvků a živin ve dvou půdách silně kontaminovaných rizikovými prvky As, Cd, Pb a Zn s odlišnými agrochemickými parametry. Tématem práce bylo i vyhodnotit reakce rostlin, pěstovaných na takto stabilizovaných půdách a účinky porovnat s kontrolními variantami půd, bez přidaných imobilizačních aditiv.

Hypotézy práce

1) Vápenaté hmoty a fosforečná aditiva reagují s rizikovými prvky, snižují jejich mobilitu v půdě a omezují jejich přístupnost pro rostliny.

2) Aplikací vápenatých hmot a fosforečných aditiv do půdy se pravděpodobně mění přístupnost živin a tím může být negativně ovlivněn vývoj a růst rostlin.

3) Účinnost aplikace vápenatých hmot a fosforečných aditiv je pravděpodobně ovlivněna půdními vlastnostmi, intenzitou kontaminace a povahou kontaminantu.

Cíle práce

1) Sledování účinnosti aditiv (vápenaté hmoty, fosforečná aditiva) na omezení přístupnosti rizikových prvků a na změnu přístupnosti živin v půdě v modelových inkubačních experimentech.

2) Hodnocení vlivu půdních aditiv (vápenaté hmoty, fosforečná aditiva) na omezení přístupnosti rizikových prvků v půdě a na akumulaci rizikových prvků v širokolistých šťovících a rychle rostoucích dřevinách v nádobovém pokusu.

3) Posuzování účinku aditiv (vápenaté hmoty, fosforečná aditiva) z hlediska změny přístupnosti živin v půdě a akumulace živin v širokolistých šťovících a rychle rostoucích dřevinách v nádobovém pokusu.

4 Publikované práce

4.1 Hejcman et al. (2012). Účinek páleného vápna a superfosfátu na vzcházení a přežívání rostlin šťovíku tupolistého v kyselé a v alkalické půdě kontaminované As, Cd, Pb a Zn.

Název: Effect of quick lime and superphosphate additives on emergence and survival of *Rumex obtusifolius* seedlings in acid and alkaline soils contaminated by As, Cd, Pb, and Zn.

Autoři: Hejcman, M., Vondráčková, S., Müllerová, V., Červená, K., Száková, J., Tlustoš, P.

Publikováno v časopise: Plant, Soil and Environment. 58 (12). 561-567.

Effect of quick lime and superphosphate additives on emergence and survival of *Rumex obtusifolius* seedlings in acid and alkaline soils contaminated by As, Cd, Pb, and Zn

M. Hejcman[1], S. Vondráčková[2], V. Müllerová[1], K. Červená[1], J. Száková[2], P. Tlustoš[2]

[1]*Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*

ABSTRACT

Rumex obtusifolius is a troublesome weed widely spread in temperate grasslands and can be potentially used for detection of soils contaminated by trace elements. We asked how emergence and survival of its seedlings are affected by application of quick lime (Ca) and superphosphate (P) additives in soils contaminated by trace elements. We performed the pot seeding experiment with slightly acid Litavka soil contaminated by arsenic (As), cadmium (Cd), lead (Pb), and zinc (Zn) and alkaline Malín soil contaminated by As, Cd, and Zn. We used a control without any additives, Ca and P treatments in both soils. Higher and quicker emergence, together with substantially higher mortality of seedlings, was recorded in Litavka than in Malín. A positive effect of the Ca treatment on seedlings was recorded in Litavka, but a negative in Malín. Small seedlings with narrow and long leaves of reddish colour were recorded in Litavka in the control and in the P treatment both with high availability of Zn, Cd, and Pb. In the Ca treatment, leaves of seedlings were more elliptic and less reddish. In Malín, seedlings were green and substantially more vital in the control and in the P treatment than in Litavka. In the Ca treatment, small and unviable seedlings were recorded. Seedlings of *R. obtusifolius* are sensitive on high availability of Ca, Zn, Cd, and Pb in the soil.

Keywords: broad-leaved dock; calcium; cadmium; lead; zinc; metal toxicity; soil reaction

Rumex obtusifolius subsp. *obtusifolius* (broad-leaved dock) is a highly problematic and widely spread weed species particularly under organic farming in temperate grasslands (Cavers and Harper 1964, Hejcman et al. 2012). Factors that determine *R. obtusifolius* to be a successful weed are high production of long-term viable seeds, regeneration of plants from fragments of underground organs, deep growing taproot with high storage capacity, the perennial character of the species, and quick and large biomass production (Zaller 2004, Gilgen et al. 2010, Strnad et al. 2010, Hrdličková et al. 2011).

Rumex obtusifolius can be negatively related to a high Ca content in the soil and a high pH value, which was shown by Humphreys et al. (1999) and Hann et al. (2012). Bohner (2001) described *R. obtusifolius* as a 'calciphobic' species which precipitates excessive Ca into calcium oxalate crystals. On Ca and Mg rich soils, *R. obtusifolius* can suffer from insufficient K supply because of antagonism between K and Ca/Mg uptake as suggested by Hann et al. (2012).

Growth of *R. obtusifolius* can also be highly affected by the availability of trace elements, but there have not been any studies investigating the effect of As, Cd, Pb, and Zn soil contamination on emergence and survival of *R. obtusifolius* seedlings. As *R. obtusifolius* is a widely spread species, it could potentially be a suitable species for detection of soils contaminated by trace elements by field vegetation mapping.

25

Availability of many trace elements to plants, As, Cd, and Zn in particular, is also related to soil P status. In the case of As this is because of competition between phosphates and arsenates for binding sites in the soil (Száková et al. 2009, Liu et al. 2012), and in the case of Cd and Zn, because of Cd and Zn precipitation into phosphates (Dong et al. 2007). Also the soil pH is important as the availability Cd, Fe, Mn, and Zn to plants is high especially in acidic soils (Dong et al. 2007). Addition of Ca (CaO – quick lime) or P ($Ca(H_2PO_4)_2$ – superphosphate) additives can therefore substantially decrease the availability of many trace elements in the soil (Puschenreiter et al. 2005, Kumpiene et al. 2008, Hejcman et al. 2010, Trakal et al. 2011).

Using a pot experiment with Ca and P additives to two soils differing in pH, As, Ca, Cd, Mg, Pb, and Zn status, the aim of this study was to investigate how much emergence and survival of *R. obtusifolius* seedlings are affected by these soil chemical properties.

MATERIAL AND METHODS

Design of the pot experiment. In May 2011 we established the pot experiment in an outdoor weather-controlled vegetation hall in Prague-Suchdol (Czech Republic) with natural temperature and light conditions.

We used two contaminated soils: (1) slightly acidic Fluvisol termed 'Litavka' from alluvium of the Litavka river collected in the village Trhové Dušníky. The Litavka soil was contaminated with As, Cd, Zn, and Pb by wastes from smelter setting pits (Šichorová et al. 2004, Trakal et al. 2011). (2) The alkaline Luvisol termed Malín collected from a bank of the streamlet Beránka near Malín village. The Malín soil was contaminated by As, Cd, and Zn due to tailings of silver mining in the 13–16th centuries (Száková et al. 2009). Chemical properties of the soils used analysed before establishment of the experiment are given in Table 1. In addition to the effect of soil, an effect of Ca (CaO – quick lime) and P ($Ca(H_2PO_4)_2$ – superphosphate) additives

Table 1. Basic characteristics of soil collection sites and chemical properties of investigated soils (in dry matter)

Site and soil properties	Soil	
	Litavka (49°43'N, 14°0'E)	Malín (49°58'N, 15°17'E)
Altitude (m a.s.l.)	450	230
Mean annual temperature (°C)	7.3	8.5
Mean annual precipitation (mm)	623	575
Soil texture	Clay loamy sand	Loam
Soil type	Fluvisol	Luvisol
pH_{CaCl_2}	5.8	7.2
CEC ($mmol_{(+)}$/kg)	55	346
C_{org} (g/kg)	36	27
Ca (mg/kg)	1856	8914
Mg (mg/kg)	160	354
K (mg/kg)	192	234
P (mg/kg)	9	56
Cd (mg/kg)	53.8	11.3
Zn (mg/kg)	6172	1022
Pb (mg/kg)	3305	98
As (mg/kg)	354	688
Fe (mg/kg)	21193	17379
Mn (mg/kg)	2688	371

CEC – cation exchange capacity; C_{org} – content of organic carbon. Values for P, K, Ca, and Mg are plant available concentrations of nutrients determined by Mehlich III extraction procedure. Values for As, Cd, Fe, Mn, and Zn are total concentrations of elements extracted by *aqua regia*. Czech legislation limits for *aqua regia* (pseudo-total) concentrations of elements in light-textured and other soils, respectively (in mg/kg) are 0.4 and 1.0 for Cd, 130 and 200 for Zn, 100 and 140 for Pb and 30 and 30 for As

26

Table 2. Effect of soils and applied treatments on plant available concentrations of elements extracted by $CaCl_2$ on 28th day after application of additives (according to Vondráčková et al. 2012)

Element (mg/kg)	LC	LCa	LP	MC	MCa	MP
As	0.06	0.2	0.2	0.5	0.3	2.2
Cd	0.8	0.05	0.7	0.01	0.007	0.02
Pb	0.06	2.1	0.1	0.005	0.004	0.01
Zn	33	5.9	30	0.1	0.03	0.3

L – Litavka; M – Malín; C – control; Ca – application of Ca additive; P – application of P additive

on emergence of *R. obtusifolius* was investigated. Based on previous experiments with application of Ca and P additives into investigated soils (Trakal et al. 2011, Vondráčková et al. 2013), we applied to pots 7.3 g CaO per 1 kg of soil and 1.3 g $Ca(H_2PO_4)_2$ per 1 kg of soil. The pot experiment was composed of six treatments replicated five times (30 pots altogether): LC – Litavka soil without any additive as the control; LCa – Litavka soil with Ca additive; LP – Litavka soil with P additive; MC – Malín soil without any additive as the control; MCa – Malín soil with Ca additive and MP – Malín soil with P additive. Plant available concentrations in investigated treatments 28th day after establishment of the experiment are given in Table 2.

We used 5 L pots with 20 cm diameter filled with 5 kg of air dried soil sieved through a 10 mm sieve. We then applied the following fertilisers: 0.5 g N (in the form of NH_4NO_3), 0.16 g P and 0.4 g K (in the form of K_2HPO_4) into each pot. Application of N, P, and K fertilisers was performed in order to make N, P, and K availability non-limiting for growth of *R. obtusifolius* in all treatments. According to our previous experience, amount of applied P fertilizer in the form of K_2HPO_4 was not high enough to change mobility of metals, but was high enough to alleviate P deficiency for *R. obtusifolius* growth. The additives and fertilizers were mixed with the soil and then all pots were watered. The fertilisers and additives were applied on the morning of the 3rd May 2011. In the evening of the same day, we sowed 100 seeds, 1–2 cm deep, into each pot.

Seeds of *R. obtusifolius* were collected during spring 2011 from a region near Prague in central Czech Republic. The collection sites were mainly roadside ditches or abandoned fields with neutral soil pH, good P and K and low As, Cd, Pb, and Zn availability. The seeds from different mother plants were mixed to avoid any maternal effect and stored at room temperature in paper bags and in the dark. Germination of used seeds was 86% tested under laboratory conditions in a 12 h day/night regime at 25°C directly before establishment of

the experiment. Pots were regularly watered if necessary to maintain optimal growth conditions during the course of the experiment.

We recorded the number of seedlings daily in each pot from the start of the emergence on the 5th day of the experiment up to 28 day of the experiment in 8th June 2011. The field emergence was defined as the maximal number of seedlings recorded during the course of the experiment in each treatment. We used field emergence because the maximal number of seedlings was recorded on different days for each treatment. In addition to field emergence, we analysed mortality defined as the maximal number of seedlings minus the final number of seedlings on the 28th day of the experiment. Mortality was expressed as percentage from used seeds.

Soil pH $CaCl_2$ was measured in suspension of 10 g of soil and 50 mL of solution containing 0.01 mol/L $CaCl_2$ at 20 ± 1°C at the end of the experiment.

Data analysis. Repeated measures and factorial ANOVA followed by comparison using Tukey HSD test were applied to obtained data. The relationship between soil pH and field emergence was evaluated using linear regression. All analyses were performed using the Statistica 8.0 program (Statsoft, Tulsa, USA).

RESULTS

Effect of additives on soil pH. Effects of locality, additive and additive × locality interaction on soil pH were significant at the end of the experiment (Figure 1a). In Litavka soil, the pH value was highly increased after application of Ca additive, but only slightly increased in Malín soil. There was no effect of P addition on the pH value in either soil.

Seedling number during the experiment and field emergence. The number of seedlings in Litavka soil was significantly affected by day and by day × additive interaction, but there was no effect due to soil additives (Figure 2a). In Malín

27
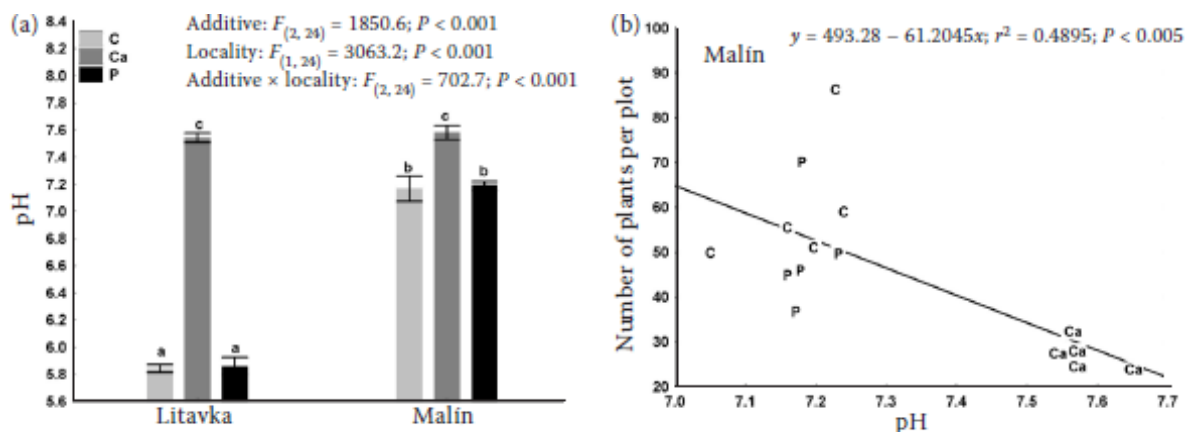
Figure 1. (a) Effect of additive, locality and additive × locality interaction on soil pH ($CaCl_2$) measured at the end of the experiment and (b) effect of soil pH ($CaCl_2$) on emergence of *Rumex obtusifolius* (maximal number of seedlings recorded during the experiment) in Malín soil. C – control treatment without any additive; Ca – application of Ca additive; P – application of P additive. Error bars represent standard errors of the means (SE). *F* and *P* values – results of ANOVA analyses for particular effects (locality, additive and additive × locality interaction) and values in brackets are degrees of freedom. According to the Tukey post-hoc test, treatments with the same letter were not significantly different at the 0.05 probability level
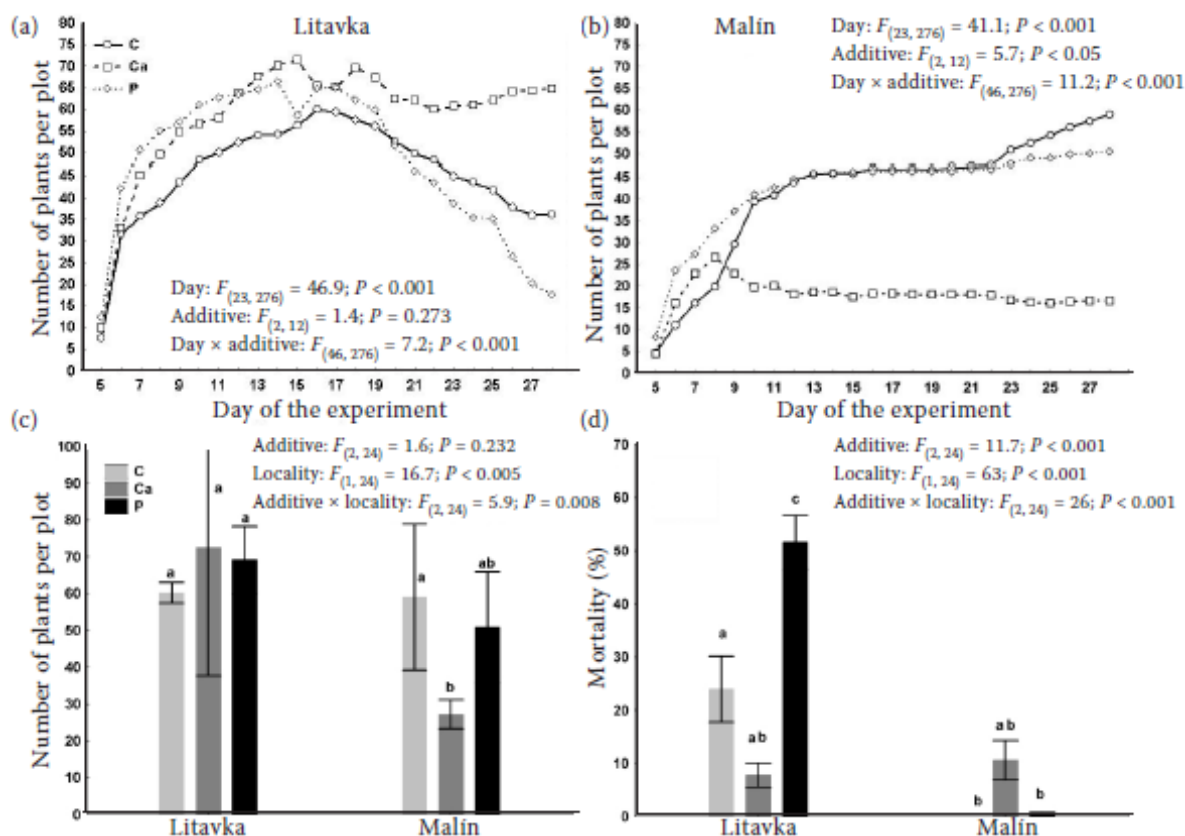
Figure 2. Effect of additives on number of *Rumex obtusifolius* seedlings during the 28 days of the experiment in (a) Litavka soil and (b) Malín soil. (c) Field emergence (maximal number of seedlings) recorded over the study period in Litavka and Malín soils and (d) mortality in Litavka and Malín soils expressed as percentage from used seeds per pot. *F* and *P* values – results of ANOVA analyses for particular effects (day, additive, day × additive interaction, locality, additive × locality interaction) and values in brackets are degrees of freedom. According to the Tukey post-hoc test, treatments with the same letter were not significantly different at the 0.05 probability level. For explanation see Figure 1

soil, the number of seedlings was significantly affected by all factors – day, additive and by day × additive interaction (Figure 2b). In Litavka soil, higher emergence values and quicker emergence was recorded than in Malín soil (Figure 2c), but in Malín soil, substantially lower mortality of seedlings was recorded than in Litavka soil (Figure 2d). In Litavka soil, there was no significant effect of Ca additive on emergence and survival of seedlings in comparison to the control. In Malín soil, there was a large negative effect of Ca additive on the emergence and survival of seedlings in comparison to the control. P addition tended to enhance speed of emergence in both soils but there were differing effects due to P addition on survival of seedlings in both soils. In Litavka soil, P addition increased mortality while there was no effect of P addition on the mortality of seedlings in Malín soil.

The overall field emergence was significantly affected by locality and by additive × locality interaction, but not by additive (Figure 2c). The field emergence 73% in Litavka soil with Ca additive was not significantly different from the other treatments except the Ca treatment in Malin soil with field emergence 27%. The emergence was not affected by the pH value in Litavka soil ($P = 0.33$, $r^2 = 0.055$), but was significantly negatively related to pH value in Malín soil (Figure 1b).

The overall mortality was significantly affected by the locality, additive and by locality × additive interaction, indicating a soil specific effect of additives (Figure 2d). No mortality of seedlings was recorded in Malín soil in C treatment and the highest mortality was in Litavka soil in P treatment (52%).

The effect of all treatments on number, vitality and size of seedlings on the 28th day of the experiment is obvious in photographs of individual pots shown in Figure 3. Very small seedlings with narrow and long leaves with a characteristic reddish colour of leaf tips were recorded in Litavka soil in the control and P treatment. In Ca treatment, leaves of seedlings were more elliptic and less reddish. In Malín soil, lower number of seedlings was recorded than in Litavka soil, but seedlings were green and substantially more vital in the control and P additive treatment than in Litavka soil. In Ca treatment, low numbers of small and unviable seedlings was recorded.

DISCUSSION

Field emergence and consequent survival of *R. obtusifolius* seedlings were highly affected by soil chemical properties. Most detrimental for emergence of *R. obtusifolius* seedlings was high pH, related to high Ca availability in the soil. This was shown clearly in the Ca and Mg rich Malín soil where addition of Ca resulted in very low emergence of *R. obtusifolius* seedlings although there was comparatively low availability of As, Cd, Pb, and Zn. Humphreys et al. (1999) reported weak negative correlation between soil Mg status and pH and abundance of *R. obtusifolius* in grasslands in UK. Hann et al. (2012) found negative correlations between plant available Ca and Mg contents in soil and the density of *R. obtusifolius*, but they did not test effects of Ca application. Bohner (2001) classified *R. obtusifolius* as a 'calciphobic' species which precipitate excessive Ca into insoluble calcium oxalate crystals. As the enhanced synthesis of oxalic acid in Ca rich soils can result in extra energy costs (Kinzel 1983), this might, together with low K supply for this highly K demanding species, decrease competitiveness and increase mortality of *R. obtusifolius* on Ca rich soils (Hann et al. 2012). We demonstrated that negative effects of high Ca availability on *R. obtusifolius* can already be recorded during early development of seedlings.

In the first days of the experiment we observed the highest emergence in P treatments in both soils. This is in accordance with results by Křišťálová et al. (2011), recording the positive effect of high P availability in the soil on early development of *R. obtusifolius* seedlings. A relatively low effect due to P addition on the emergence of seedlings in our study can be explained by P fertiliser application in all pots before the start of the experiment which removed the strong P limitation on early seedlings growth in all treatments. The consequent high mortality of seedlings in P treatment in Litavka soil was probably caused by toxic effects of too high P status.

In contrast to Malín soil, application of Ca increased the survival of *R. obtusifolius* seedlings in Litavka soil. This was due to substantially lower initial availability of Ca in this soil, and a substantial decrease in the availability of Zn and Cd and hence their toxicity on seedlings after application of the Ca additive (Vondráčková et al. 2013). In Malín soil no, or only slight, decrease in Cd and Zn availability was recorded and initial concentrations of Cd and Zn available to plants were about three fold lower than in Litavka soil. Therefore there was no Cd or Zn toxicity even in untreated Malín soil. In Litavka soil, toxicity of Cd and Zn for *R. obtusifolius* seedlings was indicated also by
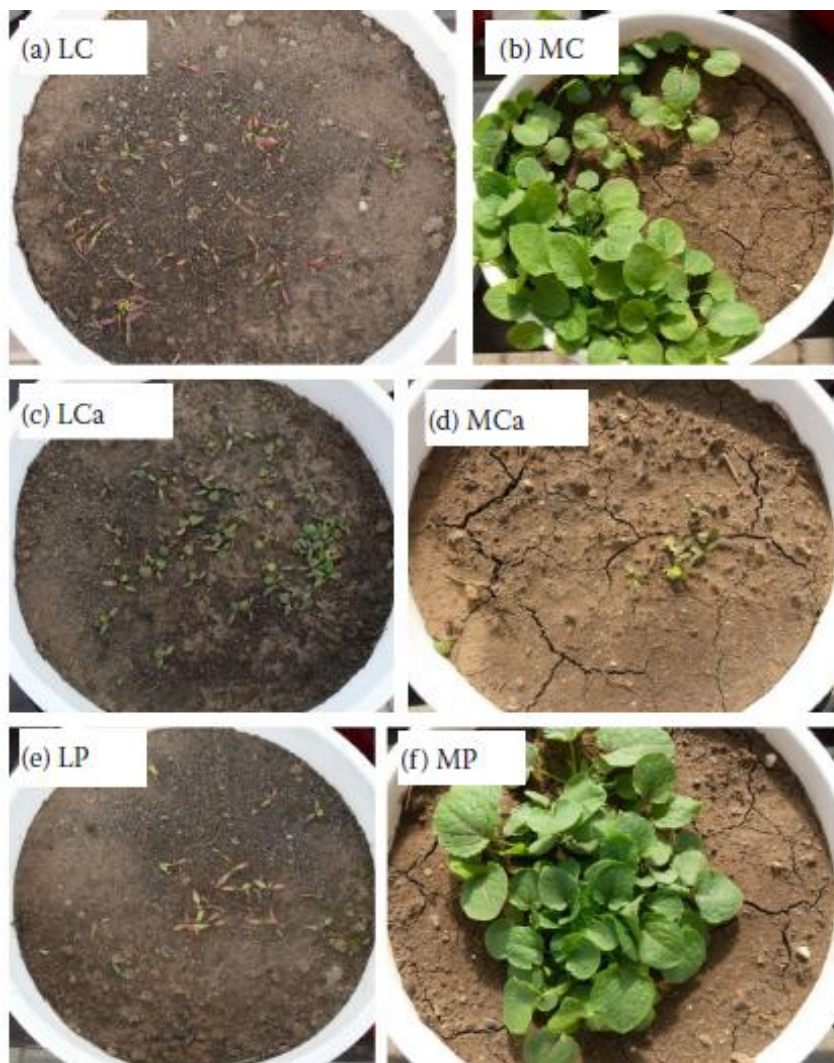
Figure 3. Photographs of *Rumex obtusifolius* plants in investigated treatments on the 28th day of the experiment. (a) LC – Litavka soil without any additive as the control; (b) MC – Malín soil without any additive as the control; (c) LCa – Litavka soil with Ca additive; (d) MCa – Malín soil with Ca additive; (e) LP – Litavka soil with P additive; (f) MP – Malín soil with P additive

substantially higher concentrations of both elements in leaves than in Malín soil (unpublished data). In Litavka soil, the concentration of Zn in leaves exceeded the critical limit for Zn toxicity, estimated by Broadley et al. (2007) for a wide range of species to be 300 mg Zn/kg. Although the concentration of Zn was lower in Ca treatment in comparison to P treatment and the control, the concentration of Zn in leaves still exceeded 500 mg/kg. In addition, toxicity of Zn was clearly visible by the reduction in biomass growth and the yellow, brown and reddish colour of leaves (Figure 3). These symptoms are connected with Zn toxicity resulting from Fe deficiency in leaves, as has been described in studies on other plant species (Sagardoy et al. 2009, Cui and Zhao 2011, Song et al. 2011).

In addition to Zn toxicity in Litavka soil, Cd and Pb toxicities were probably also highly important,

as concentrations of Cd in leaves of *R. obtusifolius* ranged from 5–14 mg/kg and concentrations of Pb were around 100 mg/kg. These concentrations were approximately one order higher than in Malín soil (unpublished data).

Finally we can conclude that *R. obtusifolius* suffers from high Ca availability in the soil since emergence of seedlings. As *R. obtusifolius* is a widely spread weed species with high seed production, restricted growth and conspicuous changes in leaf shape and colour (yellow, brown and reddish rather than green) of its seedlings can indicate soils with toxic Zn, Cd and Pb contents.

REFERENCES

Bohner A. (2001): Physiologie und futterbaulicher Wert des Ampfers. Bericht über das 7. Alpenlädische Expertenforum, 22. und 23. März 2001, BAL Gumpenstein, 39–44.

Broadley M.R., White P.J., Hammond J.P., Zelko I., Lux A. (2007): Zinc in plants. New Phytologist, 173: 677–702.

Cavers P.B., Harper J.L. (1964): Biological flora of the British Isles, *Rumex obtusifolius* L. and *Rumex crispus* L. Journal of Ecology, 52: 737–766.

Cui Y., Zhao N. (2011): Oxidative stress and change in plant metabolism of maize (*Zea mays* L.) growing in contaminated soil with elemental sulfur and toxic effect of zinc. Plant, Soil and Environment, 57: 34–39.

Dong J., Mao W.H., Zhang G.P., Wu F.B., Cai Y. (2007): Root exudation and plant tolerance to cadmium toxicity – a review. Plant, Soil and Environment, 53: 193–200.

Gilgen A.K., Signarbieux C., Feller U., Buchmann N. (2010): Competitive advantage of *Rumex obtusifolius* L. might increase in intensively managed temperate grasslands under drier climate. Agriculture, Ecosystems and Environment, 135: 15–23.

Hann P., Trska C., Kromp B. (2012): Effects of management intensity and soil chemical properties on *Rumex obtusifolius* in cut grasslands in Lower Austria. Journal of Pest Science, 85: 5–15.

Hejcman M., Strnad L., Hejcmanová P., Pavlů V. (2012): Effects of nutrient availability on performance and mortality of *Rumex obtusifolius* and *R. crispus* in unmanaged grassland. Journal of Pest Science, 85: 191–198.

Hejcman M., Száková J., Schellberg J., Tlustoš P. (2010): The Rengen Grassland Experiment: relationship between soil and biomass chemical properties, amount of elements applied, and their uptake. Plant and Soil, 333: 163–179.

Hrdličková J., Hejcman M., Křišťálová V., Pavlů V. (2011): Production, size, and germination of broad-leaved dock seeds collected from mother plants grown under different nitrogen, phosphorus, and potassium supplies. Weed Biology and Management, 11: 190–201.

Humphreys J., Jansen T., Culleton N., Macnaeidhe F.S., Storey T. (1999): Soil potassium supply and *Rumex obtusifolius* and *Rumex crispus* abundance in silage and grazed grassland swards. Weed Research, 39: 1–13.

Kinzel H. (1983): Influence of limestone, silicates and soil pH on vegetation. In: Lange O.L., Nobel P.S., Osmond C.B., Ziegler H. (eds): Physiological Plant Ecology. III. Responses to the Chemical and Biological Environment. Encyclopedia of Plant Physiology, New Series, Volume 12C. Springer-Verlag, Berlin, 201–244.

Křišťálová V., Hejcman M., Červená K., Pavlů V. (2011): Effect of nitrogen and phosphorus availability on the emergence, growth and over-wintering of *Rumex crispus* and *Rumex obtusifolius*. Grass and Forage Science, 66: 361–369.

Kumpiene J., Lagerkvist A., Maurice C. (2008): Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. Waste Management, 28: 215–225.

Liu Q.J., Zheng C.M., Hu C.X., Tan Q.L., Sun X.C., Su J.J. (2012): Effects of high concentrations of soil arsenic on the growth of winter wheat (*Triticum aestivum* L.) and rape (*Brassica napus*). Plant, Soil and Environment, 58: 22–27.

Puschenreiter M., Horak O., Friesl W., Hartl W. (2005): Low-cost agricultural measures to reduce heavy metal transfer into the food chain – a review. Plant, Soil and Environment, 51: 1–11.

Sagardoy R., Morales F., López-Millán A.F., Abadía A., Abadía J. (2009): Effects of zinc toxicity on sugar beet (*Beta vulgaris* L.) plants grown in hydroponics. Plant Biology, 11: 339–350.

Song A., Li P., Li Z., Fan F., Nikolic M., Liang Y. (2011): The alleviation of zinc toxicity by silicon is related to zinc transport and antioxidative reactions in rice. Plant and Soil, 344: 319–333.

Strnad L., Hejcman M., Křišťálová V., Hejcmanová P., Pavlů V. (2010): Mechanical weeding of *Rumex obtusifolius* L. under different N, P and K availabilities in permanent grassland. Plant, Soil and Environment, 56: 393–399.

Száková J., Tlustoš P., Goessler W., Frková Z., Najmanová J. (2009): Mobility of arsenic and its compounds in soil and soil solution: The effect of soil pretreatment and extraction methods. Journal of Hazardous Materials, 172: 1244–1251.

Šichorová K., Tlustoš P., Száková J., Kořínek K., Balík J. (2004): Horizontal and vertical variability of heavy metals in the soil of a polluted area. Plant, Soil and Environment, 50: 525–534.

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.

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. (In Press)

Zaller J.G. (2004): Ecology and non-chemical control of *Rumex crispus* and *R. obtusifolius* (Polygonaceae): a review. Weed Research, 44: 414–432.

Corresponding author:

Prof. RNDr. Michal Hejcman, Ph.D. et Ph.D., Česká zemědělská univerzita v Praze, Fakulta životního prostředí, Kamýcká 129, 165 21 Praha 6-Suchdol, Česká republika
phone: + 420 224 382 129, e-mail: hejcman@fzp.czu.cz

31

4.2 Vondráčková et al. (2013). Vliv aplikace páleného vápna a dolomitu na mobilitu prvků (Cd, Zn, Pb, As, Fe a Mn) v kontaminovaných půdách.

Název: Effect of quick lime and dolomite application on mobility of elements (Cd, Zn, Pb, As, Fe, and Mn) in contaminated soils.

Autoři: Vondráčková, S., Hejcman, M., Tlustoš, P., Száková, J.

Publikováno v časopise: Polish Journal of Environmental Studies. 22 (2). 577-589.

Effect of Quick Lime and Dolomite Application on Mobility of Elements (Cd, Zn, Pb, As, Fe, and Mn) in Contaminated Soils

Stanislava Vondráčková[1], Michal Hejcman[2]*, Pavel Tlustoš[1], Jiřina Száková[1]

[1]Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Kamýcká 129, 165 21 Prague, Czech Republic
[2]Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Prague, Kamýcká 129, 165 21 Prague, Czech Republic

Abstract

Weakly acidic Litavka and alkaline Malín soils are good examples of multi-contaminated soils in the Czech Republic. The aim of this study was to investigate the effects of different application rates of quick lime (lime) and dolomite on the mobility of cadmium, zinc, lead, arsenic, iron and manganese.

Additives were applied to soil samples at three rates and incubated for 7, 14, 28, and 42 days. Plant-available (extracted by $CaCl_2$) and acid-extractable (extracted by CH_3COOH) concentrations of elements were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). In alkaline soil, there was no effect of lime and dolomite application on concentrations of elements. In acid soil, there was a decrease in plant-available concentrations of Cd and Zn, no effect on plant-available Fe and Mn concentrations, and a slight increase in plant-available Pb and As concentrations after lime application. With the exception of a decrease in Pb and Mn concentrations, the same trends were observed for acid-extractable concentrations of elements. Dolomite application was less effective than lime application. The effect of dolomite on the immobilization of elements increased with increasing application rates. There was a weak effect of time during incubation on changes in concentrations of elements.

We concluded that high immobilization efficiency of alkaline additives on Cd and Zn can be recorded only on acid soils. Application of lime and dolomite is an ineffective measure to immobilize Pb and As in both acid or alkaline soils.

Keywords: alkaline additives, arsenic, cadmium, lead, plant-available and acid-extractable concentrations, zinc

Introduction

Excessive concentrations of trace elements in soils pose a significant health risk to humans, animals, and plants, as has been documented by many authors [1-3]. Unlike organic compounds, trace elements cannot be degraded, and the cleaning of soils usually requires their complete removal, or at least immobilization [4].

Many additives have been screened for their potential to immobilize heavy metals in soils [5]. Each of these additives has a different effect on the bioavailability of metals, micronutrient availability, soil pH, and soil microstructure [6]. Liming is the most widely used treatment, and can lead to the precipitation of metals as metal-carbonates and sig-

*e-mail: hejcman@fzp.czu.cz

nificantly decrease the exchangeable fraction of metals in contaminated soils [7]. Alkaline additives reduce heavy metal solubility in the soil by increasing soil pH and concomitantly increasing metal sorption to soil particles [3, 8]. Soil pH is one of the main parameters controlling the solubility and mobility of heavy metals in soils [9]. The available range of liming materials includes limestone ($CaCO_3$), quick lime (CaO), slaked lime [$Ca(OH)_2$], dolomite [$CaMg(CO_3)_2$], and slag ($CaSiO_3$), which vary in capacity for acid-neutralizing of a liming material [10]. The most effective is quick lime because of its high solubility and reactivity and its large effect on soil pH [11]. One of the possible mechanisms for the immobilization of heavy metals by soil additives such as limestone is enhanced metal adsorption through surface charge [10].

Bioavailability is a key factor for remediation technologies, and immobilization may be a preferred option [10]. The plant-available fraction represents the mobile portion of the trace elements that can easily be taken up by plants from the soil solution [12]. Extraction by $CaCl_2$ gives a fraction that is highly mobile in natural conditions [13]. The easily mobilizable fraction extracted by organic acids or chelates represents the portion of elements in soil that are bound on the surface of oxides and in organic matter [14]. The exchangeable and acid-extractable fractions (elements bound to carbonates) comprise elements adsorbed onto the surface of soil particles. The decrease in soil pH leads these to migrate from the solid phase to water and into plants [15].

The aim of this paper was to investigate the effect of quick lime and dolomite application on the immobilization of Cd, Zn, Pb, As, Fe, and Mn in weakly acidic and alkaline soils with regard to application rates and incubation days.

Experimental Procedures

Soil Samples Collection

Two heavily contaminated soils differing in physico-chemical parameters were selected for the incubation experiment (Table 1). Weakly acidic soil, called "Litavka," was collected from the alluvium of the Litavka River in the village of Trhové Dušníky (60 km south of Prague). Litavka soil has been contaminated by Cd, Zn, and Pb due to waste from smelter settling pits [16]. Alkaline soil, called "Malín," was collected from a bank of Beránka stream near Malín village (close to the town of Kutná Hora, 82 km east of Prague). Malín soil is contaminated by As, Cd, and Zn due to the tailings of silver mining in the 13-16th centuries [17]. Soil samples were collected in March 2010 from topsoil in the layer at 0-20 cm depth, and were then air-dried at 20ºC, ground in a mortar, and passed through a 2 mm plastic sieve before establishment of the incubation experiment.

Design of the Incubation Experiment

The incubation experiment was established in the laboratory of the Department of Agroenvironmental Chemistry and Plant Nutrition in Prague in April 2010.

Table 1. Basic characteristics of soil collection sites and chemical properties of investigated soils. Mean values calculated from three replications (n=3) together with standard error of the mean (SE) are provided for each measured property. Cation exchange capacity (CEC) was analyzed only in mixed soil samples without any replication.

Soil property	Soil	
	Litavka (49º43'N, 14º0'E)	Malín (49º58'N, 15º17'E)
Altitude (m a.s.l.)	450	230
Mean annual temperature (ºC)	7.3	8.5
Mean annual precipitation (mm)	623	575
Soil texture	Clay loamy sand	Loam
Soil type	Fluvisol	Luvisol
pH_{CaCl_2}**	6.5±0.02	7.3±0.02
CEC (mmol·kg⁻¹)	55	346
C_{org} (%)	3.6±0.1	2.7±0.1
Ca[a] (mg·kg⁻¹)**	1856±31	8914±98
Mg[a] (mg·kg⁻¹)**	160±5	354±5
K[a] (mg·kg⁻¹)*	192±8	234±4
P[a] (mg·kg⁻¹)**	9±0.3	56±3
Cd_total (mg·kg⁻¹)**	53.8±0.9	11.3±0.2
Zn_total (mg·kg⁻¹)**	6172±42	1022±18
Pb_total (mg·kg⁻¹)**	3305±85	98±31
As_total (mg·kg⁻¹)**	354±2	688±26
Fe_total (mg·kg⁻¹)**	21193±146	17379±224
Mn_total (mg·kg⁻¹)**	2688±16	371±4

[a] – plant-available concentrations of nutrients determined by Mehlich III extraction procedure [20].

total – total concentrations of elements extracted by *Aqua Regia*. Legislation limits for total concentrations of elements in light-textured/other soils (mg·kg⁻¹): Cd 0.4/1.0, Zn 130/200, Pb 100/140, As 30/30 [46]. Calculated by one-way ANOVA, differences between locations were either not statistically significant (n.s.), significant on the 0.05(*) probability level, or were significant on the 0.01 (**) probability level.

The experiment comprised seven treatments for each soil, giving 14 treatments for both soils in total (C, control without any additive; L1, L2, and L3 treatments with application of quick lime; D1, D2, D3 treatments with the application of dolomite). Each treatment was replicated ten times and soils were incubated for 7, 14, 28, and 42 days; the experiment was therefore composed of 140 bottles for each incubation time and therefore a total of 560 bottles. We applied 50 g of dry soil to each acid-clean polyethylene 250 ml plastic bottle. In the L1, L2, L3, D1, D2, and D3 treatments, the soils were mixed with a specific amount

Table 2. Basic chemical characteristics of applied alkaline additives. Mean values together with standard error of the mean (SE) are provided in the case of the chemical properties of additives. Concentrations of Ca and Mg were provided by distributors of additives and therefore they were not analyzed. All analyzed concentrations and values of pH were performed in three replications (n=3).

Property	Quick lime[a] (L)	Dolomite[b] (D)
pH_{CaCl_2}**	12.0±0.01	8.3±0.02
Ca (g·kg⁻¹)	686	220
Mg (g·kg⁻¹)	0	100
Cd (mg·kg⁻¹)	0	0.02±0.01
Zn (mg·kg⁻¹)	0	0.7±0.2
Pb (mg·kg⁻¹)	0	0.29±0.01
As (mg·kg⁻¹)	0	1.2±0.3
Fe (mg·kg⁻¹)	0	516±6
Mn (mg·kg⁻¹)	0	69.4±0.5

[a] – analytical grade purity, distributor Lach-Ner Ltd., Czech Republic
[b] – distributor Agro CS SpA., Czech Republic
Legislation limits for total concentrations of elements in mineral calcareous and magnesium-calcareous fertilizers (mg·kg⁻¹): Cd 1.5, Pb 30, As 20 [47]. Calculated by one-way ANOVA, differences between additives were either not statistically significant (n.s.), were significant on the 0.05(*) probability level, or were significant on the 0.01 (**) probability level.

of additive (see Table 2 for chemical properties of used additives and Table 3 for the quantity of elements applied by three rates for each additive). Deionized water at a volume equivalent to 60% of the maximum water holding capacity was then added to each bottle (18 ml for Litavka soil and 17 ml for Malín soil). The incubation was performed at a constant temperature of 25ºC. Bottles were opened and aerated by fresh air every week.

Chemical Analyses

The total element concentrations in investigated soils were determined using a microwave assisted wet digestion system: for details see [18]. A certified reference material RM 7004 Loam (Analytika, CZ) containing 1.52±0.15 mg Cd·kg⁻¹, 227±7 mg Zn·kg⁻¹, 93.4±3.4 mg Pb·kg⁻¹, 49.6±2.9 mg As·kg⁻¹, and 869±34 mg Mn·kg⁻¹ was used for quality assurance of the analytical data used for determining total elements, and 1.45 mg Cd·kg⁻¹, 232 mg Zn·kg⁻¹, 96.1 mg Pb·kg⁻¹, 51.2 mg As·kg⁻¹, and 852 mg Mn·kg⁻¹ were determined for this sample. The total content of element in the dolomite was determined using *Aqua Regia*. At days 7, 14, 28, and 42, plant-available and acid-extractable concentrations of elements in soils were determined. Soil samples were extracted using a 0.01 mol·L⁻¹ CaCl₂ aqueous solution (plant-available concentrations) at a solid/liquid ratio of 1/2.4 (50 g+120 ml) for six hours, and with a 0.11 mol·L⁻¹ aqueous solution of CH₃COOH (acid-extractable concentrations) at a solid/liquid ratio of 1/2.4 (50 g+120 ml) overnight. Hettich Universal 30 RF (Germany) equipment was used for centrifugation of the reaction mixtures at 3,000 rpm for 10 min. Supernatants were kept at laboratory temperature until measurement. Blank extracts representing 5% of the total number of extracts were prepared using the same batch of reagents and the same apparatus. Blank extracts were prepared and analyzed in the same way as soil extracts. All extracts were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES) (VARIAN Vista Pro, Varian, Australia) and a standard edition procedure was used for evaluating the data. The concentration of organic carbon in soil was determined colorimetrically, according to Sims and Haby [19]. Available concentrations of nutrients were determined by the Mehlich III soil extraction procedure [20], using flame atomic absorption spectroscopy (FAAS, VARIAN SpectrAA-280, Australia) (for Ca, K, and Mg) and ICP-OES (for P). Soil and dolomite pH was measured in 1/5 (10 g+50 ml) and quick lime pH in 1/20 (10 g+200 ml) 0.01 mol·L⁻¹ CaCl₂ at 20±1ºC. Cation exchange capacity (CEC)

Table 3. Amount of applied elements placed into experimental pots by three levels of applied quick lime (treatment abbreviations L1, L2, and L3) and dolomite (treatment abbreviations D1, D2, and D3).

Amount of applied elements	Treatment abbreviation (TA)					
	L1	L2	L3	D1	D2	D3
Ca (g·kg⁻¹ soil)	15	30	60	15	30	60
Mg (g·kg⁻¹ soil)	0	0	0	6.8	13.6	27.2
Cd (mg·kg⁻¹ soil)	0	0	0	0.001	0.003	0.006
Zn (mg·kg⁻¹ soil)	0	0	0	0.05	0.1	0.2
Pb (mg·kg⁻¹ soil)	0	0	0	0.02	0.04	0.08
As (mg·kg⁻¹ soil)	0	0	0	0.1	0.2	0.3
Fe (mg·kg⁻¹ soil)	0	0	0	35.2	70.4	140.7
Mn (mg·kg⁻¹ soil)	0	0	0	4.7	9.5	18.9

was calculated as the sum of Ca, Mg, K, Na, and Al extractables in 0.1 mol·L⁻¹ BaCl₂ (w/v=1:20 for 2 hours) [21]. All used reagents were of electronic grade purity (Analytika, Ltd., CZ).

Data Analyses

All univariate analyses were performed using STATIS-TICA 9.0 software (StatSoft, Tulsa, OK, USA). A repeated measures ANOVA was applied to identify the effect of treatments, time, and their interactions. A one-way ANOVA followed by a post-hoc comparison Tukey test was used to identify significant differences between treatments for incubation time. We used ANOVA because data were sufficiently homogeneous within groups and with sufficient normality.

Results

As calculated by repeated measures ANOVA, plant-available Cd, Zn, As, and Mn and acid-extractable Cd, Zn, As, Fe, and Mn concentrations were significantly affected by treatment ($p < 0.002$), time ($p < 0.001$), and by treatment × time interaction ($p < 0.004$) in both soils. Plant-available Pb concentrations were significantly affected by treatment ($p < 0.001$) and treatment × time interaction ($p < 0.001$) only in Litavka soil, and plant-available Fe concentrations by treatment × time interaction ($p < 0.001$). Acid-extractable Pb concentrations were only significantly affected by treatment ($p < 0.001$) in Litavka soil.

Concentrations of Elements in Used Soils

Total concentrations of Cd, Zn and As in soils considerably exceeded the Czech legislation limits (Table 1). Total Pb concentrations were close to the legislative limit in Malín soil and exceeded the limit by thirty-three times in Litavka soil. Total concentrations of Cd, Pb, and As in dolomite did not exceed the Czech legislative limits for fertilizers (Table 2).

Soil pH

Individual soil additives resulted in varying changes in soil pH (Fig. 1a and b). Dolomite did not affect the soil pH, which was 6.9 and 7.3 in Litavka and Malín soils, respectively. Application of lime rapidly and considerably increased the pH values to 12.3 and 12.0 in Litavka and Malín soils, respectively. The pH values were stable during the incubation period, with no effect resulting from different application rates of the additives.

Cadmium

In comparison to the control, lime application substantially and permanently decreased mobility of Cd in Litavka soil (Fig. 2a) but only slightly in Malín soil (Fig. 2b). In Malín soil, there was a substantial decrease in plant-available Cd concentrations in the control at the end of the experiment.

The effect of dolomite on plant-available Cd concentrations was not as marked as in the case of lime. In Litavka soil, a slightly significant decrease in concentrations of plant-available Cd was recorded after dolomite application (Fig. 2a) in comparison to the control, and the concentrations were only slightly affected by the application rate of dolomite. In Malín soil, the decrease in plant-available concentrations of Cd in dolomite treatments (Fig. 2b) was the same as that in the control.

In comparison to the control, lime application substantially and permanently decreased concentrations of acid-extractable Cd in Litavka soil (Fig. 2c). In Malín soil, lime application also permanently decreased concentrations of acid-extractable Cd (Fig. 2d) but, in the control, there was a decrease in acid-extractable Cd concentrations as measured on the 28th day of the experiment, though later the Cd concentrations again increased.

The effect of dolomite application on concentrations of acid-extractable Cd was not as marked as in the case of lime. In Litavka soil, there was only found to be a minimal effect of dolomite on concentrations of acid-extractable Cd (Fig. 2c). In Malín soil, there was no decrease in acid-extractable Cd concentrations in dolomite treatments
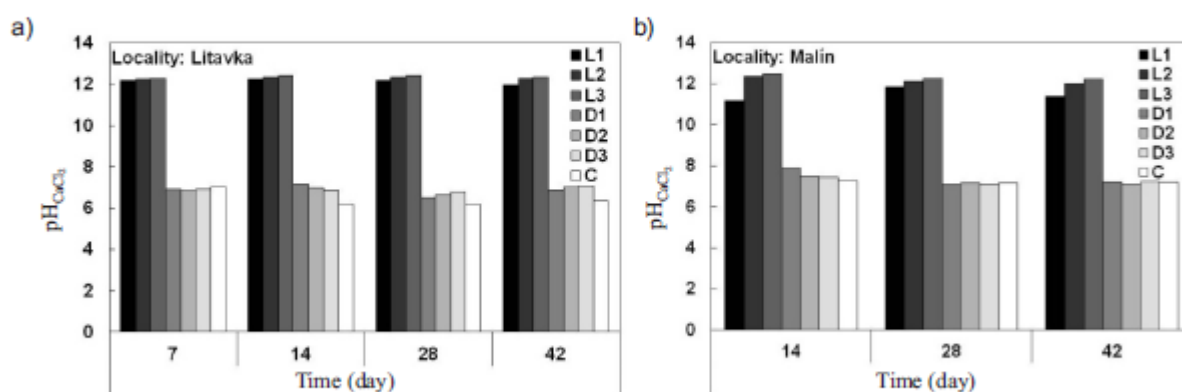
Fig. 1. Effect of treatment on pH$_{CaCl_2}$ in (a) Litavka and (b) Malín soils. Treatment abbreviations are given in Table 3. Values of pH were analyzed only in mixed soil samples without any replication.

(Fig. 2d) up to the 14th day of the experiment. Later, as measured on the 28th day, there was a decrease in Cd concentrations in the D3 treatment and in the control. On the 42nd (last) day of the experiment, an increase in Cd concentrations was recorded in the control, but there were very low concentrations in all dolomite treatments.

Zinc

In comparison to the control, lime application substantially and permanently decreased mobility of Zn in Litavka soil (Fig. 3a). In Malín soil, a decrease in plant-available Zn concentrations after lime application (Fig. 3b) was recorded on the 7th and 14th days of the experiment, but later there was found to be no effect of lime application on plant-available Zn concentrations, which were not significantly different from the control.

The effect of dolomite on concentrations of plant-available Zn was not as marked as was the case for lime. In Litavka soil, a slightly significant decrease in concentrations of plant-available Zn was recorded after dolomite application (Fig. 3a), and the Zn concentration was significantly though slightly affected by the application rate of dolomite. In Malín soil, changes in plant-available concentrations of Zn in dolomite treatments (Fig. 3b) were the same as changes in the control, and therefore there was no effect from dolomite applications on plant-available Zn concentrations.

In comparison to the control, lime application substantially and permanently decreased concentrations of acid-extractable Zn in Litavka and Malín soils (Figs. 3c and d). In Malín soil, on the 28th day a decrease in acid-extractable concentrations of Zn was recorded, as well as in the control. There were therefore no significant differences in Zn concentrations between lime treatments and control.

The effect of dolomite application on concentrations of acid-extractable Zn (Figs. 3c and d) was very similar to the case for Cd.

Lead

In comparison to the control, lime application substantially and permanently increased plant-available concentrations of Pb in Litavka soil (Fig. 4a), and Pb concentrations were only slightly affected by the lime application rate. In Malín soil, concentrations of plant-available Pb were the same in lime treatments (Fig. 4b) as they were in the control, and slightly decreased during the experiment.

There was no effect of dolomite application on plant-available concentrations of Pb in Litavka and Malín soils (Figs. 4a and b), as plant-available concentrations of Pb were found to be the same as in the control.

In comparison to the control, lime application substantially and permanently decreased concentrations of acid-extractable Pb in Litavka soil (Fig. 4c). With the exception of the 14th day of the L1 treatment for Malín soil, concen-

Fig. 2. Effect of treatment on mean concentrations of plant-available Cd (a, b) and acid-extractable Cd (c, d) in Litavka and Malín soils. Treatment abbreviations are given in Table 3. Error lines represent standard error of the mean (SE). Calculated by one-way ANOVA, differences between treatments either were not statistically significant (n.s.), were significant on the 0.05(*) probability level, or were significant on the 0.01 (**) probability level.

Fig. 3. Effect of treatment on mean concentrations of plant-available Zn (a, b) and acid-extractable Zn (c, d) in Litavka and Malín soils. Treatment abbreviations are given in Table 3. Error lines represent standard error of the mean (SE). Calculated by one-way ANOVA, differences between treatments were either not statistically significant (n.s.), were significant on the 0.05(*) probability level, or were significant on the 0.01 (**) probability level.

Fig. 4. Effect of treatment on mean concentrations of plant-available Pb (a, b) and acid-extractable Pb (c, d) in Litavka and Malín soils. Treatment abbreviations are given in Table 3. Error lines represent standard error of the mean (SE). Calculated by one-way ANOVA, differences between treatments were either not statistically significant (n.s.), were significant on the 0.05(*) probability level, or were significant on the 0.01 (**) probability level.

trations of acid-extractable Pb in lime treatments (Fig. 4d) were the same as in the control.

The effect of dolomite on acid-extractable Pb concentrations was recorded only in Litavka soil, where a slight decrease in Pb concentrations was recorded (Fig. 4c), dependent on the application rate of dolomite. In Malín soil, the decrease in acid-extractable concentrations of Pb in dolomite treatments (Fig. 4d) was the same as that for the control, and therefore there was no effect of dolomite application on acid-extractable Pb concentrations.

Arsenic

In comparison to the control, lime application increased concentrations of plant-available As in Litavka soil (Fig. 5a), and As concentrations were affected by the application rate of lime. In Malín soil, the concentration of plant-available As in lime treatments (Fig. 5b) was the same as in the control, with the exception of a high increase for the L1 treatment on the 14th and 42nd days of the experiment.

There was no effect of dolomite application on plant-available concentrations of As in either soil (Figs. 5a and b).

In both soils, there were minimal differences between concentrations of plant-available and acid-extractable As in comparison to other elements.

In comparison to the control, lime application permanently increased acid-extractable concentrations of As in Litavka soil (Fig. 5c), and As concentrations were only slightly affected by the lime application rate. In Malín soil,

the decrease in acid-extractable concentrations of As in lime treatments (Fig. 5d) was similar to the decrease found in the control.

There was no effect of dolomite application on acid-extractable concentrations of As in Litavka soil (Fig. 5c), and only a slight effect in the Malín soil (Fig. 5d). In Malín soil, the effect of dolomite application on concentrations of acid-extractable As was similar to that for Cd and Zn.

Iron

In Litavka soil, a decrease in plant-available concentrations of Fe in lime treatments (Fig. 6a) was the same as the decrease in the control, with the exception of an increase in all L1 treatments. In Malín soil, the increase in plant-available concentrations of Fe in lime treatments (Fig. 6b) was the same as that for the control.

In Litavka and Malín soils, concentrations of plant-available Fe in dolomite treatments (Fig. 6a and b) was the same as in the control; therefore there was no effect of dolomite application on plant-available Fe concentrations.

In Litavka soil, the concentration of acid-extractable Fe in lime treatments (Fig. 6c) was the same as that for the control, with the exception of a high increase in the L1 treatment in Litavka soil on the 14th, 28th, and 42nd days. In Malín soil, concentrations of acid-extractable Fe in lime treatments (Fig. 6d) were the same as in the control, in that they increased on the 14th day and then substantially decreased.
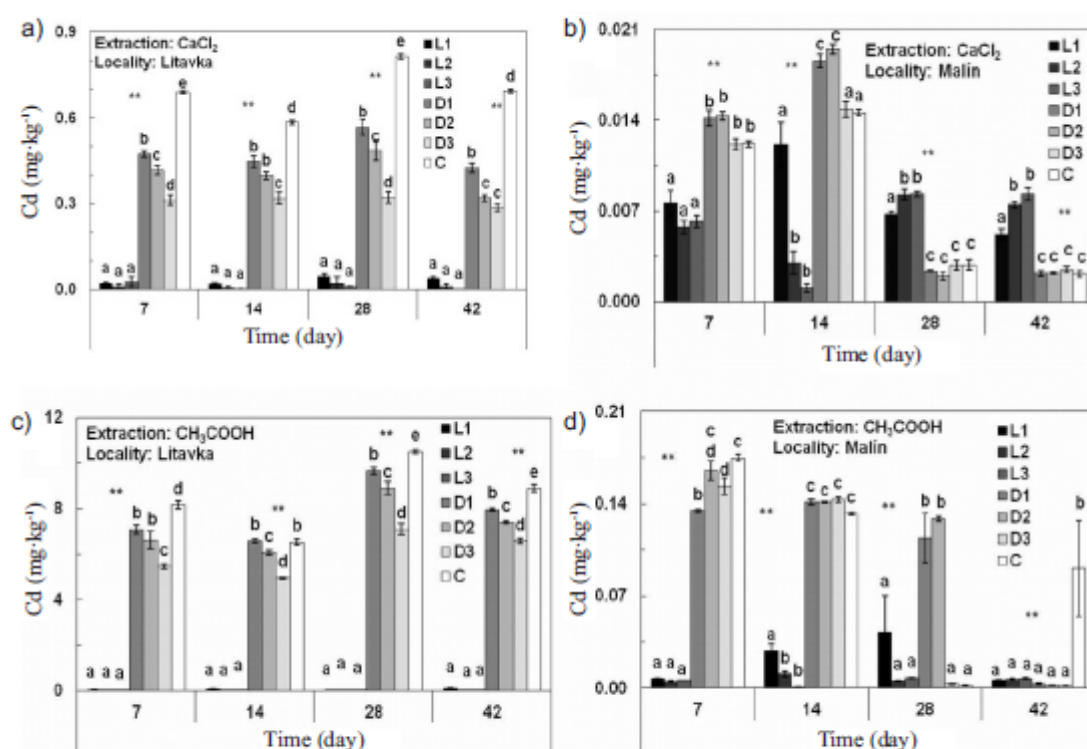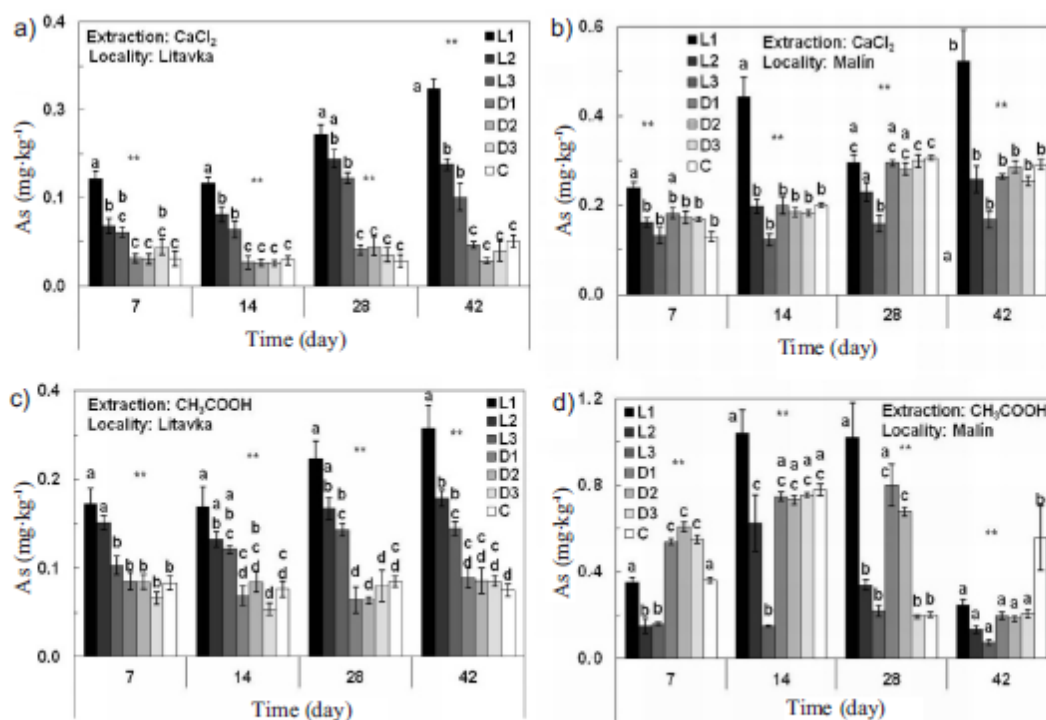

Fig. 5. Effect of treatment on mean concentrations of plant-available As (a, b) and acid-extractable As (c, d) in Litavka and Malín soils. Treatment abbreviations are given in Table 3. Error lines represent standard error of the mean (SE). Calculated by one-way ANOVA, differences between treatments were either not statistically significant (n.s.), were significant on the 0.05(*) probability level, or were significant on the 0.01 (**) probability level.
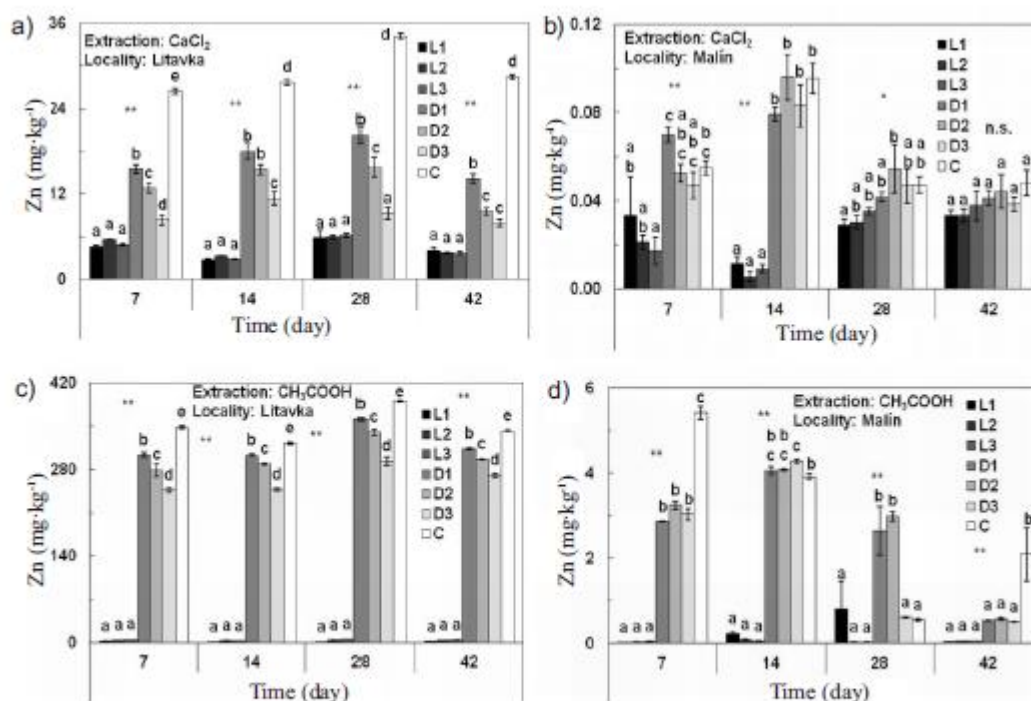
Fig. 6. Effect of treatment on mean concentrations of plant-available Fe (a, b) and acid-extractable Fe (c, d) in Litavka and Malín soils. Treatment abbreviations are given in Table 3. Error lines represent standard error of the mean (SE). Calculated by one-way ANOVA, differences between treatments were either not statistically significant (n.s.), were significant on the 0.05(*) probability level, or were significant on the 0.01 (**) probability level.
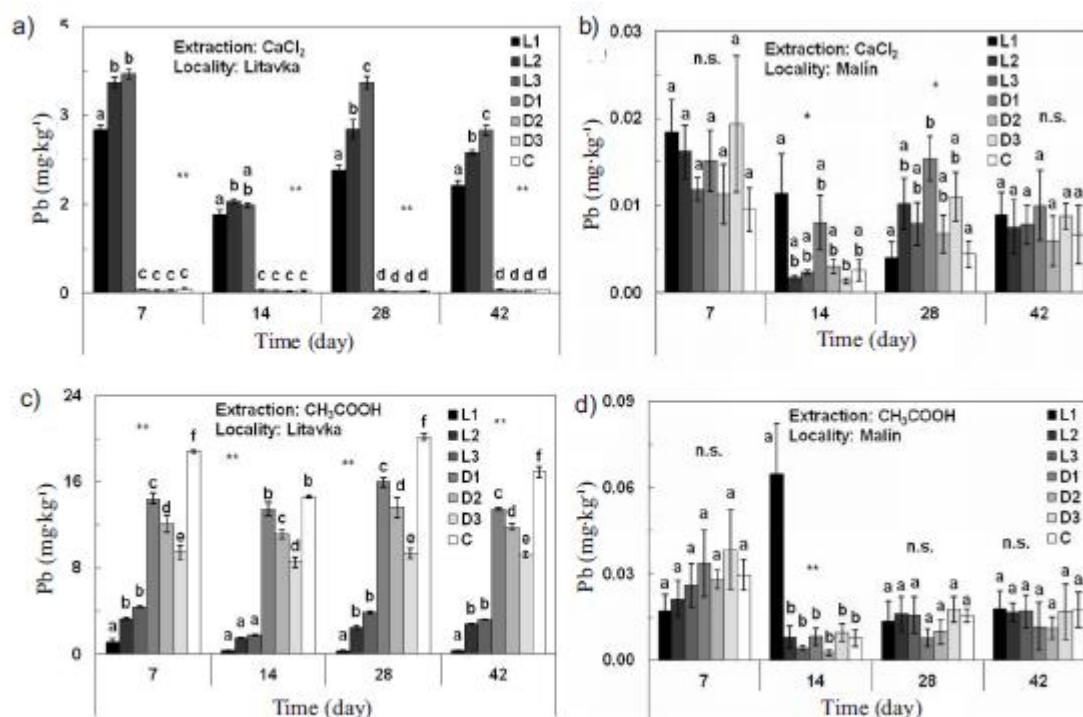

Fig. 7. Effect of treatment on mean concentrations of plant-available Mn (a, b) and acid-extractable Mn (c, d) in Litavka and Malín soils. Treatment abbreviations are given in Table 3. Error lines represent standard error of the mean (SE). Calculated by one-way ANOVA, differences between treatments were either not statistically significant (n.s.), were significant on the 0.05(*) probability level, or were significant on the 0.01 (**) probability level.
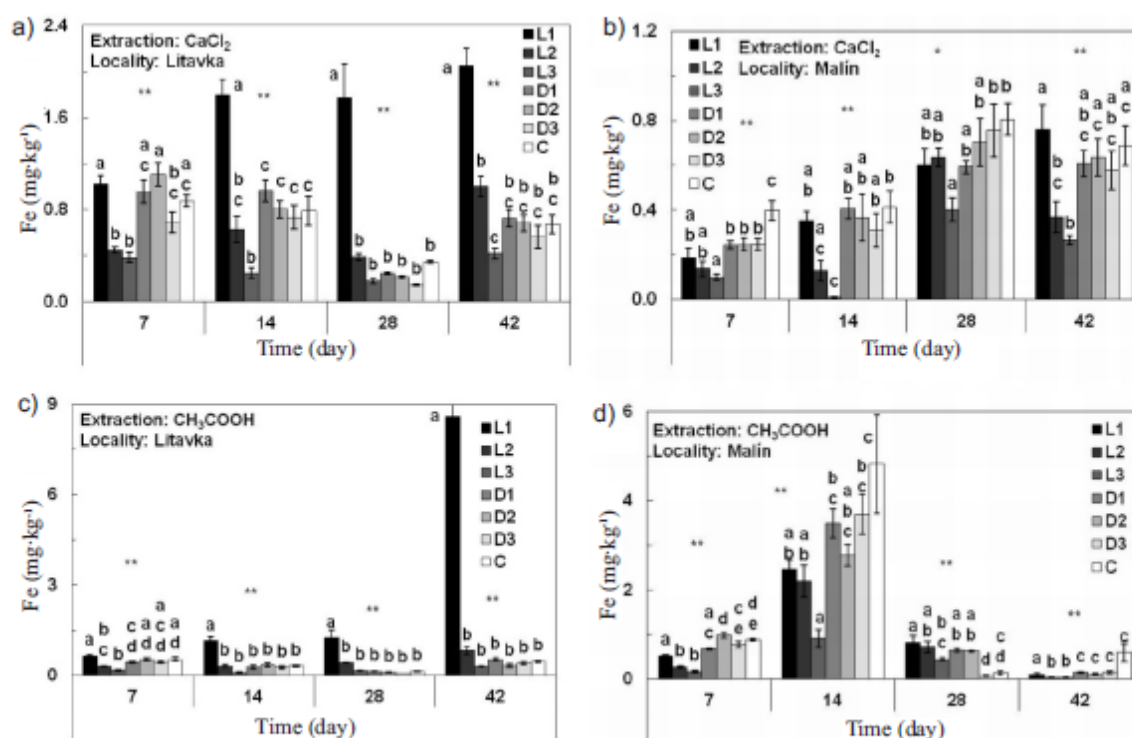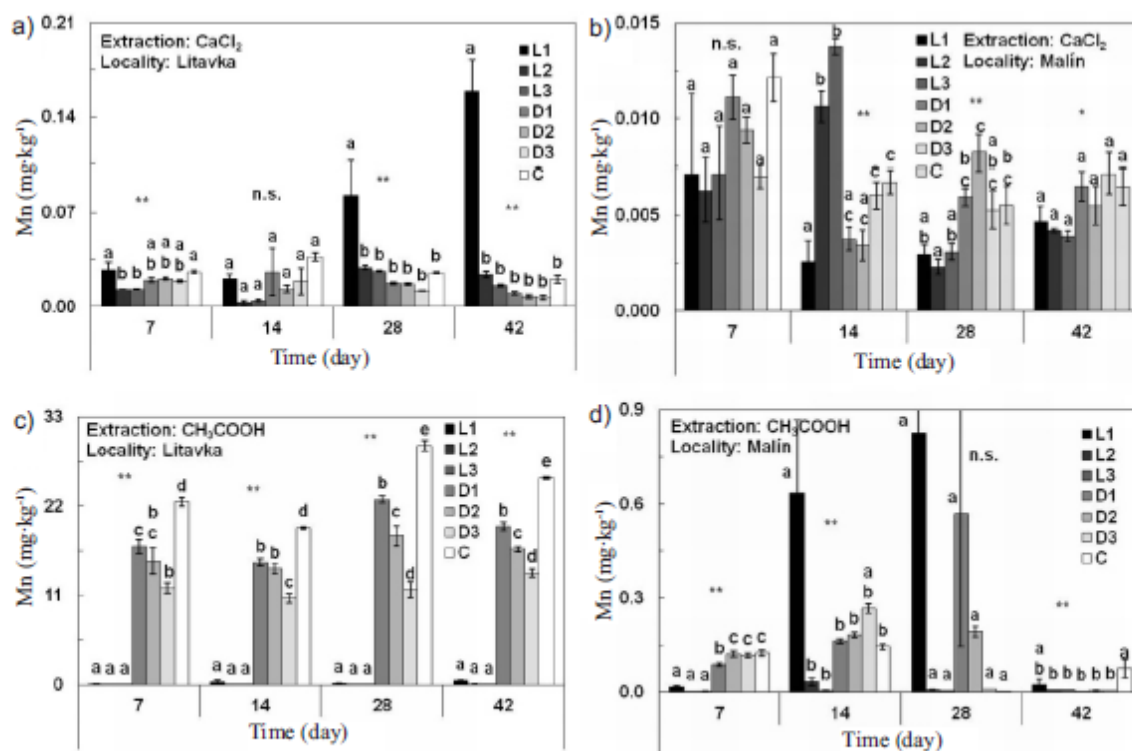
In both soils, concentrations of acid-extractable Fe in dolomite treatments (Fig. 6c and d) were the same as in the control.

Manganese

In Litavka soil, with the exception of an increase for the L1 treatment on the 28th and 42nd days, concentrations of plant-available Mn in lime treatments (Fig. 7a) and in the control remained stable during the experiment. In Malín soil, with the exception of an increase in the L2 and L3 treatments recorded on the 14th day, concentrations of plant-available Mn in lime treatments (Fig. 7b) and in the control remained stable.

There was no effect of dolomite application on plant-available concentrations of Mn in either soil (Fig. 7a and b).

In comparison to the control, lime application substantially and permanently decreased concentrations of acid-extractable Mn in Litavka soil (Fig. 7c). In Malín soil, with the exception of an increase for the L1 treatment on the 14th and the 28th days, the decrease in acid-extractable concentrations of Mn in lime treatments (Fig. 7d) was the same as the decrease in the control.

There was only a slight effect of dolomite application on concentrations of acid-extractable Mn. In Litavka soil, a slight decrease in Mn concentrations was recorded after dolomite application (Fig. 7c). In Malín soil, the recorded decrease in acid-extractable concentrations of Mn in dolomite treatments (Fig. 7d) was the same as that for the control. No effect of dolomite application on acid-extractable Mn concentrations was therefore found.

Discussion of Results

Soil pH

There was a high and immediate effect of lime but no effect of dolomite application on the pH value of the soils, although the amount of Ca supplied by both additives was the same. This result was because of the different anion form found in each of the additives. Calcium in lime is bound in an oxide form, whereas in dolomite it is in a carbonate form [22]. Anion form plays an important role in additive solubility. The carbonate form is characterized by poor solubility, while the oxide form is highly soluble [22, 23]. The addition of lime to moist soil created strongly alkaline slaked lime, which highly increased soil pH. The addition of dolomite does not usually increase soil pH above 7 [24]. Soil pH also was connected with the different buffering capacities of the soils. Buffering capacity is positively related to cation exchange capacity. In the Malín soil, there was a value of cation exchange capacity that was six times higher than for the Litavka soil, and a similar content of organic C. Therefore, Malín soil is characterized by a higher buffering capacity than Litavka soil. This is clear from the different pH value of the L1 treatment in both soils. The amount of applied Ca was very high in L2 and L3 treatments, and therefore the differences in buffering capacity between soils were not sufficiently high to affect soil pH, which was 12 in both soils.

Cadmium

In Litavka soil, the mean plant-available Cd concentration in the control was 0.7 mg·kg^{-1}, but in Malín soil, plant-available Cd concentrations were about one order lower. In Europe, plant-available Cd concentrations in common agricultural soils with low total Cd concentrations are up to 0.05 mg·kg^{-1} [25, 26]. Therefore, in Litavka soil, plant-available Cd concentrations without any additive were about one order higher than in common agricultural soils.

Plant-available Cd concentrations were about one order lower than acid-extractable Cd concentrations, in both soils. This was driven by the different leaching capacity of the used extractants. Calcium chloride is a mild extractant and behaves like an enhanced soil solution [27]. Weak acetic acid is a stronger extractant and is able to release a proportion of the elements bound onto a soil sorption complex [28] and carbonate-bound fractions. Another driver is the low pH of acetic acid, as the mobility of Cd is especially high under conditions of low soil pH [11, 27]. In Malín soil, plant-available Cd concentrations were very low, approaching the detection limit. This was probably because of the low mobility of Cd, due to the high pH value and high carbonate content of the soil [17].

Lime application decreased plant-available Cd concentrations substantially and constantly in Litavka soil. This was connected with a high increase in soil pH after lime application, as has also been recorded by other authors [22, 29-31]. The lack of any effect of lime application rates suggests that a decrease in Cd mobility can also be recorded under lower lime application rates than were tested in this study. Therefore, to detect minimal effective lime application rates, any future study must be designed with substantially lower lime application rates.

In Malín soil, there were minimal changes in plant-available Cd concentrations after lime application. This was because of an initially high pH value and the high Ca status of the control soil. At the end of the experiment, there was a slight increase in plant-available Cd concentrations in all treatments to which lime was applied. This was probably because of the presence of dissolved organic C that could minimize adsorption of Cd onto solid phases [32]. Although there was a minimal effect of lime application on Cd mobility, initial concentrations of plant-available Cd were very low and it was not necessary to decrease them further. We can therefore conclude that lime application decreases Cd mobility considerably, especially on acid soils, as has been recorded by other authors [11, 33, 34].

Dolomite application slightly decreased plant-available Cd concentrations in Litavka soil. This was probably because carbonates created with Cd^{2+} precipitate CdCO$_3$ [32]. There was a decrease of plant-available Cd concentrations with the application rate of dolomite, and therefore a future study must be designed with higher dolomite application rates so as to detect a maximal possible decrease of plant-available Cd concentrations after dolomite application.

In Malín soil, there was no effect of dolomite application on plant-available Cd concentrations. This was because of an initially high pH value in the control soil, and there was no increase in soil pH after dolomite application. Therefore, we can recommend dolomite application only on acidic soils, especially at higher application rates. This conclusion is in agreement with other authors [11, 33, 34].

Zinc

In Litavka soil, the mean plant-available Zn concentration in the control was 29 mg·kg^{-1}, but in Malín soil plant-available Zn concentrations were about three orders lower. In Europe, plant-available Zn concentrations in common agricultural soils with low total Zn concentrations are up to 0.2 mg·kg^{-1} [25, 26]. Therefore, in Litavka soil, plant-available Zn concentrations were, without any additive, about two orders higher than in common agricultural soils.

Plant-available Zn concentrations were about one order lower than acid-extractable Zn concentrations in Litavka soil and about two orders lower than acid-extractable Zn concentrations in Malín soil, as was also recorded by Száková et al. [11].

The effect of lime and dolomite applications to decrease plant-available Zn concentrations in both soils was the same as found for plant-available Cd concentrations. This was due to the similar chemical properties of Cd and Zn [35]. We can therefore conclude that the practical use of lime and dolomite applications follows the same rules as for Cd and Zn.

Lead

In Litavka soil, the mean plant-available Pb concentration in the control was 0.05 mg·kg^{-1}, but in Malín soil, plant-available Pb concentrations were about three orders lower. In Europe, plant-available Pb concentrations in common agricultural soils with low total Pb concentrations are up to 0.2 mg·kg^{-1} [25, 26]. Therefore, in Litavka soil, plant-available Pb concentrations were, without any additive, about one order lower than in common agricultural soils.

Plant-available Pb concentrations were about one order lower than acid-extractable Pb concentrations in Litavka soil. In Malín soil, plant-available and acid-extractable Pb concentrations were similar.

Lime application increased plant-available Pb concentrations in Litavka soil. This was connected to the release of Pb from soil organic matter after lime application, because Pb is bound especially to organic matter [13, 36]. Dissolved organic matter after lime application released Pb, which then formed complexes with hydroxides. These soluble hydroxide complexes are formed in highly alkaline conditions at pH > 12, as has also been recorded by other authors [32].

In Malín soil, lime application had no effect on plant-available Pb concentrations. This was connected to an initial high soil pH and the generally low mobility of Pb. Therefore, we can conclude that lime application is not suitable for decreasing the mobility of Pb on acid soils.

In both soils, dolomite application had no effect on plant-available Pb concentrations. We can therefore conclude that the application of dolomite is not a suitable measure to impact the mobility of Pb on soils with different pH values.

Lime application decreased acid-extractable Pb concentrations in Litavka soil. This was probably because of the occurrence of Pb in insoluble forms in alkaline soil conditions [37]. Therefore, we can conclude that lime application is effective in decreasing acid-extractable Pb concentrations in acid soils with weak soil sorption. This conclusion is in agreement with Friesl-Hanl et al. [38].

The lack of any effect of lime application on acid-extractable Pb concentrations in Malín soil was the same as was the case for plant-available Pb concentrations.

In Litavka soil, there was a significant decrease in acid-extractable Pb concentrations after dolomite application. This was probably because of $PbCO_3$ formation [39]. The slight effect of dolomite application rates indicates that the decrease in Pb mobility is connected to the higher adsorption capacity for Pb in $CaCO_3$-rich soils [39]. Therefore, a future study must be designed with substantially higher dolomite application rates in order to detect the most effective dolomite application rates. We can therefore conclude that dolomite application is suitable for decreasing acid-extractable Pb concentrations in acid soils at higher application rates.

The lack of any effect of dolomite application on acid-extractable Pb concentrations in Malín soil was the same as was the case for plant-available Pb concentrations. Therefore, we concluded that dolomite and lime application is not a suitable method to immobilize Pb in alkaline soil.

Arsenic

In Litavka soil, the mean plant-available As concentrations in the control was 0.05 mg·kg^{-1} and plant-available As concentrations were similar in Malín soil. In Europe, plant-available As concentrations in common agricultural soils with low total As concentrations are up to 0.3 mg·kg^{-1} [25, 26]. Therefore, in Litavka soil, plant-available As concentrations were, without any additive, similar or approximately one order lower than in common agricultural soils.

Plant-available As concentrations were similar to acid-extractable As concentrations in both soils.

Lime application slightly increased plant-available As concentrations in Litavka soil. This was probably connected with the formation of mobile arsenite, which is typical for alkaline soils, as has been recorded by other authors [11, 25, 40].

There were minimal changes in plant-available As concentrations after lime application in Malín soil. A similar trend was recorded by Hartley et al. [41]. This was probably because of the initially high pH value of the control soil. Mobile arsenite was therefore already dominant in the soil before lime application. In both soils, lime application rate slightly affected plant-available As concentrations. The high amount of applied Ca in the soil was probably precipitated as As-Ca complexes, $CaHAsO_4$ and $Ca_3(AsO_4)_2$, as has been recorded by other authors [42, 43].

In both soils, there was no effect of dolomite application on plant-available As concentrations and this is in accordance with the results of Hartley et al. [41]. This was probably because dolomite had no effect on soil pH and therefore no effect on the change of As species in the soil.

We can conclude that lime and dolomite applications are not a suitable measure to immobilize As, either in acid or in alkaline soils. This conclusion is in agreement with Száková et al. [11].

Iron

In Litavka soil, the mean plant-available Fe concentration in the control was 0.4 mg·kg⁻¹ and, in Malín soil, plant-available Fe concentrations were similar. In Europe, plant-available Fe concentrations in common agricultural soils are up to 6 mg·kg⁻¹ [25]. Therefore, in Litavka soil, plant-available Fe concentrations were, without any additive, approximately one order lower than in common agricultural soils.

Plant-available Fe concentrations were approximately one order lower than acid-extractable Fe concentrations in both soils.

In Litavka soil, there was a minimal effect of lime application on plant-available Fe concentrations. The reason for discrepancies in plant-available Fe concentrations in L1 treatment requires further research.

In Malín soil, there was no effect of lime application on plant-available Fe concentrations.

In both soils, there was no effect of dolomite application on plant-available Fe concentrations. This was because of the high soil pH in which metals are generally, with few exceptions, less soluble than in acid soil [44].

Manganese

In Litavka soil, the mean plant-available Mn concentration in the control was 0.03 mg·kg⁻¹ but, in Malín soil, plant-available Mn concentrations were approximately one order lower. In Europe, plant-available Mn concentrations in common agricultural soils are up to 5 mg·kg⁻¹ [25, 26], and therefore in Litavka soil, plant-available Mn concentrations were, without any additive, approximately two orders lower than in common agricultural soils.

Plant-available Mn concentrations were approximately three orders lower than acid-extractable Mn concentrations in both soils.

In both soils, there was in most cases no effect of lime and dolomite application on plant-available Mn concentrations. This was because of high soil pH under which the mobility of Mn is generally low [44].

Lime application substantially and permanently decreased acid-extractable Mn concentrations in Litavka soil. In Malín soil, there was no clear effect of lime application on acid-extractable Mn concentrations.

We can conclude that lime application is suitable as a method for decreasing acid-extractable Mn concentrations in acid soils.

Dolomite application slightly decreased acid-extractable Mn concentrations in Litavka soil. This can be explained by Mn chemisorptions on $CaCO_3$ and following the precipitation of $MnCO_3$, as has been recorded by other authors [32, 45]. There was a slight effect of dolomite application rates, indicating that the decrease in Mn mobility is probably connected with the amount of carbonate in soils.

In Malín soil, there was no effect of dolomite application on acid-extractable Mn concentrations. We can therefore conclude that dolomite application slightly immobilized the Mn in acid soil, but that there was no effect in alkaline soils.

Conclusions

The incubation experiment provides clear evidence of the different efficiency of lime and dolomite application on immobilization of elements in contaminated soils with different soil pH and sorption properties. Lime application is an effective measure to immobilize Cd and Zn only in acid soils. On the other hand, lime application is an ineffective measure for immobilizing Pb and As, either in acid or in neutral soils. Dolomite application is a suitable measure to immobilize Cd and Zn in acid soils, but with higher application rates than lime. Dolomite application is an ineffective measure for immobilizing Pb and As, either in acid or in neutral soils. It can be concluded that application rate plays a significant role, especially in the case of the less effective dolomite. During incubation, there was a weak effect of time on immobilization of the various elements.

Acknowledgements

References

1. ALKORTA I., BECERRIL J.M., GARBISU C. Phytostabilization of metal contaminated soils. Rev. Environ. Health. **25**, 135, **2010**.
2. DO NASCIMENTO C.W.A., XING B. Phytoextraction: a review on enhanced metal availability and plant accumulation. Sci. Agric. **63**, 299, **2006**.
3. PUSCHENREITER M., HORAK O., FRIESL W., HARTL W. Low-cost agricultural measures to reduce heavy metal transfer into the food chain – a review. Plant Soil Environ. **51**, 1, **2005**.
4. KUMPIENE J., LAGERKVIST A., MAURICE C. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments - A review. Waste Manage. **28**, 215, **2008**.
5. MISRA V., TIWARI A., SHUKLA B., SETH C.S. Effects of soil amendments on the bioavailability of heavy metals from zinc mine tailings. Environ. Monit. Assess. **155**, 467, **2009**.

6. LOMBI E., HAMON R.E., MCGRATH S.P., MCLAUGHLIN M.J. Lability of Cd, Cu and Zn in polluted soils treated with lime, beringite, and red mud and identification of a non-labile colloidal fraction of metals using isotopic techniques. Environ. Sci. Technol. **37**, 979, **2003**.

7. LEE T.-M., LAI H.-Y., CHEN Z.-S. Effect of chemical amendments on the concentration of cadmium and lead in long-term contaminated soils. Chemosphere **57**, 1459, **2004**.

8. MCBRIDE M., SAUVÉ S., HENDERSHOT W. Solubility control of Cu, Zn, Cd, and Pb in contaminated soils. Eur. J. Soil Sci. **48**, 337, **1997**.

9. BURGOS P., MADEJÓN E., PÉREZ-DE-MORA A., CABRERA F. Spatial variability of the chemical characteristics of a trace-element-contaminated soil before and after remediation. Geoderma **130**, 157, **2006**.

10. BOLAN N.S., DURAISAMY V.P. Role of inorganic and organic soil amendments on immobilisation and phytoavailability of heavy metals: a review involving specific case studies. Aust. J. Soil Res. **41**, 533, **2003**.

11. SZÁKOVÁ J., TLUSTOŠ P., PAVLÍKOVÁ D., HANČ A., BATYSTA M. Effect of addition of ameliorative materials on the distribution of As, Cd, Pb, and Zn in extractable soil fractions. Chem. Pap. **61**, 276, **2007**.

12. MORAL R., GILKES R.J., MORENO-CASELLES J. A comparison of extractants for heavy metals in contaminated soils from Spain. Comm. Soil Sci. Plant Anal. **33**, 2781, **2002**.

13. YOBOUET Y.A., ADOUBY K., TROKOUREY A., YAO B. Cadmium, copper, lead and zinc speciation in contaminated soils. Inter. J. Eng. Sci. Tech. **2**, 802, **2010**.

14. HICKEY M.G., KITTRICK J.A. Chemical partitioning of cadmium, copper, nickel and zinc in soils and sediments containing high levels of heavy metals. J. Environ. Qual. **13**, 372, **1984**.

15. ZERBE J., SOBCZYŃSKI T., ELBANOWSKA H., SIEPAK J. Speciation of heavy metals in bottom sediments of lakes. Pol. J. Environ. Stud. **8**, 331, **1999**.

16. BORŮVKA L., HUAN-WEI C., KOZÁK J., KRIŠTOUFKOVÁ S. Heavy contamination of soil with cadmium, lead and zinc in the alluvium of the Litavka River. Rostl. Výr. **42**, 543, **1996**.

17. KRÁLOVÁ L., SZÁKOVÁ J., KUBÍK Š., TLUSTOŠ P., BALÍK J. The variability of arsenic and other risk element uptake by individual plant species growing on contaminated soil. Soil Sediment Contam. **19**, 617, **2010**.

18. SZÁKOVÁ J., MIHOLOVÁ D., TLUSTOŠ P., ŠESTÁKOVÁ I., FRKOVÁ Z. Effect of soil properties and sample preparation on extractable and soluble Pb and Cd fractions in soils. Agric. Sci. **3**, 119, **2010** (doi:10.4236/as.2010.13015).

19. SIMS J.R., HABY V.A. Simplified colorimetric determination of soil organic matter. Soil Sci. **112**, 137, **1971**.

20. MEHLICH A. Mehlich 3 Soil Test Extractant: a modification of Mehlich 2 Extractant. Commun. Soil Sci. Plant Anal. **15**, 1409, **1984**.

21. ISO 11260. Soil quality – determination of effective cation exchange capacity and base saturation level using barium chloride solution; International Organization for Standardization, **1994**.

22. MAYFIELD J.L., OZANNE L., MITCHELL C.C., SIMONNE E.H., SIBLEY J.L. Laboratory and greenhouse evaluation of quicklime sources for suitability as agricultural liming materials. Comm. Soil Sci. Plant Anal. **35**, 1167, **2004**.

23. BUTORAC A., MESIĆ M., FILIPAN T., BUTORAC J., BAŠIĆ F., KISIĆ I. The influence of special natural amendments based on zeolite tuff and different lime materials on some soil chemical properties. Rostl. Výr. **48**, 133, **2002**.

24. MEDA A.R., PAVAN M.A., CASSIOLATO M.E., MIYAZAWA M. Dolomite lime´s reaction applied on the surface of a sandy soil of the Northwest Paraná, Brazil. Braz. Arch. Biol. Technol. **45**, 219, **2002**.

25. HEJCMAN M., SZÁKOVÁ J., SCHELLBERG J., ŠREK P., TLUSTOŠ P. The Rengen Grassland Experiment: soil contamination by trace elements after 65 years of Ca, N, P and K fertiliser application. Nutr. Cycl. Agroecosyst. **83**, 39, **2009**.

26. UPRETY D., HEJCMAN M., SZÁKOVÁ J., KUNZOVÁ E., TLUSTOŠ P. Concentration of trace elements in arable soil after long-term application of organic and inorganic fertilizers. Nutr. Cycling Agroecosyst. **85**, 241, **2009**.

27. SAHUQUILLO A., RIGOL A., RAURET G. Overview of the use of leaching/extraction tests for risk assessment of trace metals in contaminated soils and sediments. Trends Anal. Chem. **22**, 152, **2003**.

28. SZÁKOVÁ J., TLUSTOŠ P., BALÍK J., PAVLÍKOVÁ D., VANĚK V. The sequential analytical procedure as a tool for evaluation of As, Cd and Zn mobility in soil. Fresen. J. Anal. Chem. **363**, 594, **1999**.

29. DONG J., MAO W.H., ZHANG G.P., WU F.B., CAI Y. Root excretion and plant tolerance to cadmium toxicity – a review. Plant Soil Environ. **53**, 193, **2007**.

30. MÜHLBACHOVÁ G., TLUSTOŠ P. Effects of liming on the microbial biomass and its activities in soils long-term contaminated by toxic elements. Plant Soil Environ. **52**, 345, **2006**.

31. TRAKAL L., NEUBERG M., TLUSTOŠ P., SZÁKOVÁ J., TEJNECKÝ V., DRÁBEK O. Dolomite limestone application as a chemical immobilization of metal-contaminated soil. Plant Soil Environ. **57**, 173, **2011**.

32. BRADL H.B. Adsorption of heavy metal ions on soils and soils constituents. J. Colloid Interface Sci. **277**, 1, **2004**.

33. TLUSTOŠ P., SZÁKOVÁ J., KOŘÍNEK K., PAVLÍKOVÁ D., HANČ A., BALÍK J. The effect of liming on cadmium, lead and zinc uptake reduction by spring wheat grown in contaminated soil. Plant Soil Environ. **52**, 16, **2006**.

34. VÁCHA R., PODLEŠÁKOVÁ E., NĚMEČEK J., POLÁČEK O. Immobilisation of As, Cd, Pb and Zn in agricultural soils by the use of organic and inorganic additives. Rostl. Výr. **48**, 335, **2002**.

35. HERREN T., FELLER U. Transport of cadmium via xylem and phloem in maturing wheat shoots: comparison with the translocation of zinc, strontium and rubidium. Ann. Bot. **80**, 623, **1997**.

36. TLUSTOŠ P., SZÁKOVÁ J., STÁRKOVÁ A., PAVLÍKOVÁ D. A comparison of sequential extraction procedures for fractionation of arsenic, cadmium, lead, and zinc in soil. Cent. Eur. J. Chem. **3**, 830, **2005**.

37. GHARAIE H.A. Lead adsorption characteristics of selected calcareous soils of Iran and their relationship with soil properties. American-Eurasian J. Agric. Environ. Sci. **6**, 637, **2009**.

38. FRIESL-HANL W., PLATZER K., HORAK O., GERZABEK M.H. Immobilising of Cd, Pb, and Zn contaminated arable soils close to a former Pb/Zn smelter: a field study in Austria over 5 years. Environ. Geochem. Health. **31**, 581, **2009**.

39. ELKHATIB E.A., ELSHEBINY G.M., BALBA A.M. Lead sorption in calcareous soils. Environ. Pollut. **69**, 269, **1991**.

40. WILSON S.C., LOCKWOOD P.V., ASHLEY P.M., TIGHE M. The chemistry and behaviour of antimony in the soil environment with comparisons to arsenic: a critical review. Environ. Pollut. **158**, 1169, **2010**.

41. HUANG B., KUO S., BEMBENEK R. Availability to lettuce of arsenic and lead from trace element fertilizers in soil. Water Air Soil Pollut. **164**, 223, **2005**.

42. HARTLEY W., EDWARDS R., LEPP N.W. Arsenic and heavy metal mobility in iron oxide-amended contaminated soils as evaluated by short- and long-term leaching tests. Environ. Pollut. **131**, 495, **2004**.

43. PORTER S.K., SCHECKEL K.G., IMPELLITTERI C.A., RYAN J.A. Toxic metals in the environment: thermodynamic considerations for possible immobilization strategies for Pb, Cd, As, and Hg. Crit. Rev. Env. Sci. Technol. **34**, 495, **2004**.

44. ADRIANO D.C. Trace elements in terrestrial environments – biogeochemistry, bioavailability, and risk of metals, 2nd ed.; Springer: New York, pp. 866, **2001**.

45. OTERO X.L., FERREIRA T.O., HUERTA-DÍAZ M.A., PARTITI C.S.M., SOUZA JR. V., VIDAL-TORRADO P., MACÍAS F. Geochemistry of iron and manganese in soils and sediments of a mangrove system, Island of Pai Matos (Cananeia – SP, Brazil). Geoderma **148**, 318, **2009**.

46. ANONYMOUS. Public notice No. 13/1994 for the management of soil protection. Czech Ministry of the Environment, Prague, **1994** [In Czech].

47. ANONYMOUS. Public notice No. 271/2009, which changes Public notice No. 474/2000 about determination requirements for fertilizers. Czech Ministry of Agriculture, Prague, **2009** [In Czech].

4.3 Vondráčková et al. (2014). Chemické vlastnosti půd ovlivňují koncentrace prvků (N, P, K, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb a Zn) a jejich distribuci mezi orgány šťovíku tupolistého.

Název: Soil chemical properties affect the concentration of elements (N, P, K, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and their distribution between organs of *Rumex obtusifolius*.

Autoři: Vondráčková, S., Hejcman, M., Száková, J., Müllerová, V., Tlustoš, P.

Publikováno v časopise: Plant and Soil. 379 (1-2). 231-245.

REGULAR ARTICLE

Soil chemical properties affect the concentration of elements (N, P, K, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and their distribution between organs of *Rumex obtusifolius*

Stanislava Vondráčková · Michal Hejcman ·
Jiřina Száková · Vladimíra Müllerová · Pavel Tlustoš

Abstract

Background and aims The ionome (elemental composition) of grassland species has rarely been studied at the level of individual organs and little is known about effects of soil chemical properties on the ionome. Using the model oxalate plant *Rumex obtusifolius,* we asked how its biomass production and the distribution of elements between its organs is affected by soil chemical properties. *Methods* We established a pot experiment with *R. obtusifolius* planted in acidic non-contaminated control and in slightly acidic and alkaline soils anthropogenically contaminated by the risk elements As, Cd, Pb, and Zn. Both contaminated soils were untreated and treated by lime and superphosphate. We determined biomass production and the concentrations of elements in its organs. *Results* Biomass production was negatively related to the mobility of micro- and risk elements. Restricted transport of micro- and risk elements from belowground organs into leaves was recorded in untreated contaminated soils. In both lime-treated soils and in superphosphate-treated alkaline soil, elevated transport of micro- and risk elements from belowground organs into leaves was recorded in comparison to untreated contaminated soils. The lowest concentrations of micro- and risk elements were recorded in stems and seeds, followed by belowground organs and leaves. *Conclusions R. obtusifolius* is an As-, Cd-, Pb-, and Zn-excluder and is sensitive to high availability of micro- and risk elements in the soil. Soil chemical properties affect the distribution of essential elements within the plant greatly.

Keywords Bioaccumulation and translocation factors · Broad-leaved dock · Oxalate plants · Quick lime · Superphosphate

S. Vondráčková · J. Száková · P. Tlustoš
Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic

M. Hejcman (✉) · V. Müllerová
Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic
e-mail: hejcman@fzp.czu.cz

Introduction

The ionome is the elemental composition of cells, tissues, organs or whole organisms (Salt et al. 2008). The elemental composition of many agricultural crops has been investigated (Šrek et al. 2012; Zhao et al. 2013). In the case of grassland species, the ionome has predominantly been studied in aboveground organs (White et al. 2012; Lindström et al. 2013). Therefore, insufficient information is available concerning the elemental composition of belowground organs. Even in aboveground organs, the elemental composition of grassland species is frequently determined only in bulk biomass or in leaves of individual species, to determine their forage quality or nutritional status (Thompson et al. 1997;

Springer

Hejcman et al. 2012a). Hence, little information is available to describe the distribution of elements in individual aboveground organs, particularly in the case of micro- and risk elements (Barman et al. 2000; Guleryuez et al. 2008; Gaweda 2009).

It has long been recognised that the elemental composition of plant tissues is influenced by environmental conditions and in particular by the availability of different elements in the soil (Barman et al. 2000; Anton and Mathe-Gaspar 2005). However, it is still not well known to what degree the concentrations of elements vary in different organs and how much of this variability is determined by soil chemical properties. Using the model oxalate plant *Rumex obtusifolius* subsp. *obtusifolius* (broad-leaved dock), which is a common weedy species in temperate grasslands (Hann et al. 2012; Strnad et al. 2012; Hujerová et al. 2013), the aim of this study was to test the extent to which concentrations of macroelements (N, P, K, Ca, and Mg), microelements (Cu, Fe, Mn, Ni, and Zn) and risk elements (As, Cd, Cr, Pb, and Zn) in its organs are affected by soil chemical properties. Zinc can be classified as both a microelement and a risk element, depending upon its availability in the soil and its concentration in plant biomass. *R. obtusifolius* belongs to the group of 'oxalate plants', which regulates excessive Ca concentrations in tissues by Ca-oxalate precipitation (White and Broadley 2003). Organic acids play an important role in heavy metal(-loid) tolerance and detoxification in plants because of their external or internal chelation with risk metal(loid)s (Sytar et al. 2013). In oxalate plants with low exudation rates of di- and tri-carboxylic acids (Tyler and Ström 1995), the main detoxification mechanism is probably internal chelation. The uptake and transport of micro- and risk elements by plants can be characterised by the bioaccumulation factor (BF), which is calculated as the plant-to-soil concentration ratio of a particular micro- or risk elements (Zhuang et al. 2007; Gupta et al. 2008). The leaf-to-root concentration ratio of particular micro- or risk elements is termed the translocation factor (TF; Gupta et al. 2008; Barrutia et al. 2009). According to Baker (1981), plants that accumulate micro- and risk elements are characterised by a BF and TF > 1, indicator plants by a BF and TF = 1 and plants that exclude these elements by a BF and TF < 1. Questions that have not yet been addressed are which BF and TF values are characteristic for oxalate plants like

R. obtusifolius and to what extent are BF and TF values affected by the micro- and risk elements availability in the soil.

In this study, we asked how (1) biomass production of *R. obtusifolius*, (2) concentrations of elements in its organs and (3) BF and TF were affected by soil chemical properties.

Materials and methods

Design of the experiment

Two long-term heavily contaminated soils ('Litavka' by As, Cd, Pb, and Zn and 'Malín' by As, Cd, and Zn) and one control soil without any contamination ('Mšec') were used for a pot experiment in an outdoor university vegetation hall in Prague–Suchdol, with natural temperature and light conditions. Details concerning the history and sources of Litavka and Malín soil contamination are given in Borůvka et al. (1996) and Horák and Hejcman (2013). The physicochemical properties of all used soils are provided in Table 1.

In contaminated soils, we manipulated the availability of elements by application of lime and superphosphate according to previous findings of Vondráčková et al. (2013, 2014). We applied 7.3 g lime (CaO) per 1 kg of soil containing 686 g Ca kg^{-1} with pH$_{CaCl2}$ 12.0 ±0.01 and 1.3 g superphosphate (Ca(H$_2$PO$_4$)$_2$ · H$_2$O) per 1 kg of soil containing 246 g P kg^{-1} and 159 g Ca kg^{-1} with pH$_{CaCl2}$ 2.2±0.003. The pot experiment was established in May 2011 with seven treatments replicated five times: LC—Litavka control soil without any additive; LCa—Litavka soil with lime; LP—Litavka soil with superphosphate; MC—Malín control soil without any additive; MCa—Malín soil with lime; MP—Malín soil with superphosphate; and McC—Mšec, non-contaminated control soil. Five kg of air dried soil were passed through a 10 mm sieve and put in 5-L pots (20 cm in diameter and height). In each pot, the whole soil profile was mixed with nutrient solution, consisting of 0.5 g N as NH$_4$NO$_3$, 0.16 g P and 0.4 g K as K$_2$HPO$_4$. Application of nutrient solution was performed, to ensure that N, P, and K availability was non-limiting for growth of *R. obtusifolius* in all treatments. The lime and superphosphate additives were mixed with the soil after application of the nutrient solution. One hundred seeds of *R. obtusifolius* were sown (1–2 cm

48

Table 1 Basic characteristic of the experimental soils (mean ± SE; $n=3$). Differences between soils, calculated by Kruskal–Wallis ranks, soils with the same letter were not significantly different test, were not statistically significant ([n.s.]) or significant at 0.05 (*) probability level. Using the multiple comparisons of mean

Soil property	Soil		
	Litavka (49°43′N, 14°0′E)	Malín (49°58′N, 15°17′E)	Mšec (50°12′N, 13°51′E)
Soil texture	Clay loamy sand	Loam	Loam
Soil type	Fluvisol	Luvisol	Pararendzina
pH_{CaCl2}*	6.5 ± 0.02^{ab}	7.3 ± 0.02^{a}	5.2 ± 0.2^{b}
CEC (mmol$_{(+)}$ kg^{-1})*	109 ± 38^{ab}	333 ± 15^{a}	64 ± 12^{b}
C_{org} (%)*	3.6 ± 0.1^{a}	2.7 ± 0.1^{ab}	2.4 ± 0.2^{b}
P [a] (mg kg^{-1})*	9 ± 0.3^{a}	56 ± 3^{a}	175.5 ± 26.5^{a}
K [a] (mg kg^{-1})*	192 ± 8^{a}	234 ± 4^{a}	155 ± 3^{a}
Ca [a] (mg kg^{-1})[n.s.]	1856 ± 31^{a}	8914 ± 98^{a}	1736 ± 216^{a}
Mg [a] (mg kg^{-1})[n.s.]	160 ± 5^{a}	354 ± 5^{a}	117.5 ± 17^{a}
As $_{total}$ (mg kg^{-1})*	354 ± 2^{ab}	688 ± 26^{a}	9 ± 0.3^{b}
Cd $_{total}$ (mg kg^{-1})*	53.8 ± 0.9^{a}	11.3 ± 0.2^{ab}	0.2 ± 0.03^{b}
Cr $_{total}$ (mg kg^{-1})*	51.5 ± 0.8^{a}	45 ± 1^{ab}	18 ± 1^{b}
Cu $_{total}$ (mg kg^{-1})*	61 ± 0.4^{a}	62 ± 2^{a}	13 ± 1^{a}
Fe $_{total}$ (mg kg^{-1})*	21193 ± 146^{a}	17379 ± 224^{ab}	8469 ± 247^{b}
Mn $_{total}$ (mg kg^{-1})*	2688 ± 16^{a}	371 ± 4^{ab}	349 ± 17^{b}
Ni $_{total}$ (mg kg^{-1})*	18.5 ± 0.1^{ab}	23.5 ± 0.3^{a}	7 ± 1^{b}
Pb $_{total}$ (mg kg^{-1})*	3305 ± 85^{a}	98 ± 31^{ab}	32 ± 2^{b}
Zn $_{total}$ (mg kg^{-1})*	6172 ± 42^{a}	1022 ± 18^{ab}	40 ± 5^{b}

$_{total}$—pseudo-total concentrations of elements extracted by *Aqua Regia*

Czech legislation limits for pseudo-total concentrations of elements in light-textured/other soils (mg kg^{-1}): As 30/30, Cd 0.4/1.0, Cr 100/200, Cu 60/100, Fe not specified (n.s.), Mn n.s., Ni 60/80, Pb 100/140, Zn 130/200 (Anonymous 1994)

CEC cation exchange capacity (Schwertfeger and Hendershot 2009)

[a] Available concentrations of macro elements determined by Mehlich III extraction procedure (Mehlich 1984)

deep) in each pot (see Hejcman et al. 2012b for details about emergence and survival of seedlings in contaminated soils) and after 1 month of growth, the seedlings were thinned to three plants per pot. The pots were regularly watered with de-ionised water to maintain the optimal moisture condition for plant growth during the vegetative period. Positions of pots were changed weekly to avoid any side effect on the collected data. The plants were harvested after a growth period of 6 months and their biomass divided into below-ground organs, stems, leaves, and seeds (i.e., achenes with a perianth). The belowground organs were first washed thoroughly with tap water to remove soil adhered to belowground organs. Then, the belowground organs were washed in ultrasound-assisted bath filled with deionised water (ELMASONIC S30, Elma Ultrasonic Technology).

Chemical analyses

Soil samples were collected from the whole soil profile at the end of the experiment and were analysed for pH, plant-available and acid-extractable concentrations of elements (Table 2). For chemical analyses, soil samples were air dried at 25 °C and sieved to <2 mm. Soil pH was measured in a 1:5 (w/v) suspension of soil and 0.01 mol L^{-1} CaCl$_2$. Mobile (plant-available) and mobilisable (acid-extractable) portions of elements in soils were determined using 0.01 mol L^{-1} CaCl$_2$ and 0.11 mol L^{-1} CH$_3$COOH (hereafter abbreviated as Ca and AA, Tlustoš et al. 1994; Quevauviller 1998). The

Table 2 Effect of treatment on soil pH, plant-available (mg kg^{-1}; extracted by 0.01 mol L^{-1} CaCl$_2$; Ca) and acid-extractable (mg kg^{-1}; extracted by 0.11 mol L^{-1} CH$_3$COOH; AA) concentrations of elements (mean ± SE) at the end of the experiment

Variable	Extraction	Treatment						
		LC	LCa	LP	MC	MCa	MP	McC
pH$_{CaCl2}$**	–	5.8±0.01^c	7.5±0.01^{ab}	5.9±0.02^{bc}	7.2±0.03^{ac}	7.6±0.02^a	7.2±0.01^{ac}	5.2±0.2^c
P **	Ca	1.0±0.2^b	1.1±0.2^b	3.4±0.6^a	1.5±0.1^{ab}	1.9±0.2^{ab}	3.3±0.1^a	4.2±0.6^a
K **	Ca	246±7^a	44±8^b	205±17^a	122±4^{ab}	90±2^{ab}	111±3^{ab}	44±9^b
Mg **	Ca	54±2^{ab}	23.5±1^b	50±3^{ab}	67±2.5^a	42±1^b	67±2.5^a	80±9^a
As **	Ca	0.35±0.04^b	0.33±0.1^b	0.4±0.1^{ab}	1.1±0.1^{ab}	1.4±0.1^a	1.8±0.1^a	0.05±0.02^b
Cd **	Ca	4.2±0.2^a	0.1±0.01^{ab}	3.8±0.2^a	0.02±0.002^b	0.03±0.01^b	0.04±0.01^b	0.03±0.004^{ab}
Cr $^{n.s.}$	Ca	0.03±0.004^a	0.03±0.01^a	0.03±0.01^a	0.02±0.01^a	0.03±0.01^a	0.02±0.003^a	0.01±0.005^a
Cu **	Ca	0.1±0.01^{ab}	0.3±0.02^a	0.1±0.01^{ab}	0.1±0.004^b	0.2±0.005^a	0.1±0.01^b	0.05±0.005^b
Fe **	Ca	7.5±0.9^{ab}	6.3±1.6^b	7.8±1.2^{ab}	11.2±1.4^{ab}	18.1±1.4^a	13.3±1.2^a	4.3±0.6^b
Mn **	Ca	9.1±0.5^a	0.3±0.03^{abc}	7.8±0.5^{abc}	0.2±0.02^c	0.2±0.02^{bc}	0.2±0.03^{abc}	10.4±1.9^{ab}
Ni **	Ca	0.2±0.003^a	0.04±0.01^{ab}	0.1±0.01^a	0.03±0.01^b	0.04±0.01^{ab}	0.05±0.01^{ab}	0.1±0.04^{ab}
Pb **	Ca	0.65±0.1^a	0.5±0.1^a	0.65±0.1^a	0.04±0.01^b	0.02±0.002^b	0.06±0.01^{ab}	0.1±0.02^{ab}
Zn **	Ca	179±7^a	3.7±0.7^{abc}	174±7^{ab}	0.6±0.1^c	13±0.3^{bc}	1.4±0.6^c	3.2±0.5^{abc}
P **	AA	3.1±0.5^b	3.2±0.4^b	18±4^{ab}	14±3^{ab}	11±0.5^{ab}	38±2^a	49±6^a
K *	AA	314±21^a	463±234^{ab}	346±56^{ab}	244±3^{ab}	237±5^{ab}	237±5^{ab}	75±13^b
Ca **	AA	1789±144^b	6484±1122^{ab}	2170±278^b	7991±155^{ab}	10779±191^a	8028±195^{ab}	1000±110^b
Mg **	AA	108±5^{ab}	129±18^{ab}	109±8^{ab}	559±15^a	515±14^{ab}	549±11^a	91.5±11^b
As **	AA	1.0±0.2^{bc}	1.1±0.05^{bc}	1.4±0.3^{abc}	7.9±0.7^{ab}	7.6±0.2^{ab}	14.5±0.7^a	0.6±0.1^c
Cd **	AA	27±1^a	26±2^a	27±1^a	3.0±0.1^{ab}	3.2±0.1^{ab}	2.9±0.1^{ab}	0.1±0.01^b
Cr **	AA	0.2±0.01^{abc}	0.3±0.03^a	0.25±0.02^{ab}	0.1±0.02^{bc}	0.1±0.02^{abc}	0.1±0.04^{abc}	0.04±0.002^c
Cu **	AA	1.8±0.1^a	2.1±0.4^a	1.8±0.1^a	0.7±0.03^{ab}	0.8±0.01^{ab}	0.7±0.03^{ab}	0.2±0.01^b
Fe **	AA	50±7^{ab}	41±6^{ab}	41±6^{ab}	59±7^{ab}	74±5^a	79±16.5^a	8.6±0.5^b
Mn **	AA	160±8^a	163.5±15^a	142±8^a	67±2^b	75±3^{ab}	64.5±1^b	76±1^{ab}
Ni **	AA	2.4±0.1^a	2.2±0.2^a	2.5±0.1^a	1.5±0.1^{ab}	1.3±0.1^b	1.5±0.03^{ab}	0.7±0.1^b
Pb **	AA	68±4^a	67±8^{ab}	52±3^{ab}	0.1±0.03^c	0.9±0.1^{abc}	0.3±0.1^{abc}	0.3±0.03^{bc}
Zn **	AA	2593±68^a	2284±114^{ab}	2607±76^a	270±5^{abc}	230±10^{bc}	272±5^{abc}	14±2^c

Treatment abbreviations: *LC* Litavka control soil without any additive, *LCa* Litavka soil with lime, *LP* Litavka soil with superphosphate, *MC* Malín control soil without any additive, *MCa* Malín soil with lime, *MP* Malín soil with superphosphate, and *McC* Mšec, non-contaminated control soil. Calculated by Kruskal-Wallis test, differences between treatments were not statistically significant ($^{n.s.}$) or were significant at 0.05 (*) and 0.01 (**) probability levels. According to the multiple comparisons of mean ranks, treatments with the same letter were not significantly different

element concentrations in soil extracts were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES, VARIAN Vista Pro, Varian, Australia).

Fresh biomass was air-dried at 60 °C to total desiccation, dry matter biomass was determined and then plant samples were ground using a stainless-steel mill and subsequently analysed. The total concentrations of elements in organs were determined by ICP-OES (for P, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and flame atomic absorption spectroscopy (FAAS, VARIAN SpectrAA-280, Australia, for K, Ca, and Mg) after microwave-assisted high-pressure acid-digestion (65 % HNO$_3$:30 % H$_2$O$_2$ 4:1, Ethos 1, MLS GmbH, Germany). Certified reference material (CTA-OTL-1 oriental tobacco leaves) was mineralised under the same conditions for quality assurance of the total element concentrations in experimental plants. The concentration of N in the plant organs was determined by the Kjeldahl method using a Vapodest 50s (Gerhardt, Königswinter, Germany) after wet-digestion with concentrated H$_2$SO$_4$ (98 %).

Data analysis

All statistical analyses were performed using the Statistica 9.0 (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). Soil and biomass data did not meet assumptions for the use of ANOVA and were thus evaluated by non-parametric Kruskal-Wallis test. We assessed the effects of 1) treatment on the concentrations of elements in the soil and biomass and on the BFs and TFs, 2) organ on concentration of elements in the biomass and 3) soil on the soil properties. After obtaining significant results from the Kruskal-Wallis test, we used multiple comparisons of mean ranks for the detection of significant differences between different soils, treatments or organs. The relationship between concentrations of elements in the biomass and biomass production was analysed by linear regression. A principal component analysis (PCA), in the CANOCO 4.5 program, was applied to all collected data together (concentrations of elements in the soil and biomass, pH, and biomass of organs). 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 similarity of different treatments. The results were visualised in the form of a bi-plot ordination diagram in the CanoDraw program.

Results

Biomass production

The effect of treatment on the total biomass of *R. obtusifolius* was significant (Fig. 1), and the total biomass weight ranged from 1.3 to 43.3 g plant^{-1} in the LP and the McC treatments, respectively. Below-ground biomass was also significantly affected by the treatment, and ranged from 0.4 to 29.0 g plant^{-1} in the LC and the McC treatments, respectively (Table 3). Biomass production of all aboveground organs together was also significantly influenced by treatment. No stems or seeds were produced in the LC and LP treatments. Leaf biomass ranged from 0.1 to 5.8 g plant^{-1} in the LC and the McC treatments. In treatments with stems and seed production, stem biomass ranged from 1.1 to 4.1 g plant^{-1} in the MC and the LCa treatments, and seed
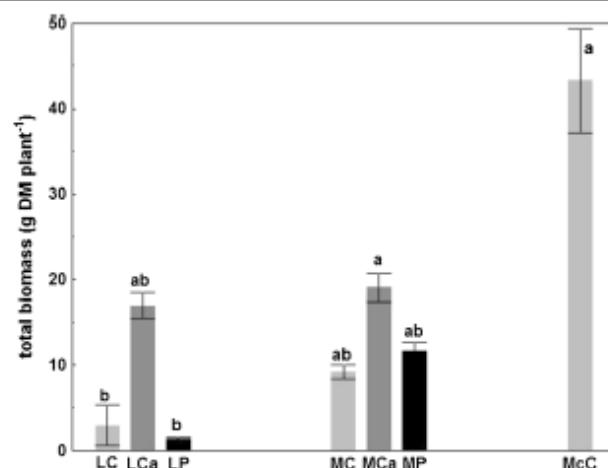
Fig. 1 Effect of treatment on the total biomass of *R. obtusifolius* (below- and aboveground biomass together) at the end of the experiment. Treatment abbreviations: *LC* Litavka control soil without any additive, *LCa* Litavka soil with lime, *LP* Litavka soil with superphosphate, *MC* Malín control soil without any additive, *MCa* Malín soil with lime, *MP* Malín soil with superphosphate, and *McC* Mšec, non-contaminated control soil. *Error bars* represent SE. Based on Kruskal–Wallis test, differences between treatments were significant based on a 0.01 (**) probability level. Using the multiple comparisons of mean ranks, treatments with the same letter were not significantly different

biomass ranged from 1.9 to 7.9 g plant^{-1} in the MC and the LCa treatments, respectively. Significant negative relationships were recorded between concentrations of micro- and risk elements and total biomass of all organs in the case of Cd ($r=-0.606$; $p<0.01$), Ni ($r=-0.373$; $p=0.033$), Pb ($r=-0.356$; $p=0.042$), and of Zn ($r=-0.552$; $p<0.01$).

Concentration of macroelements in the organs

The concentrations of N, P, and Ca were significantly affected by treatments, and the concentrations of N, P, K, Ca, and Mg differed between individual organs (see Table 3 for details). The concentration of N ranged from 3.8 g kg^{-1} in stems in the MCa treatment, to 29.9 g kg^{-1} in leaves in the LP treatment. The concentration of P ranged from 0.3 g kg^{-1} in stems in the LCa treatment to 3.5 g kg^{-1} in leaves in the MC treatment; the concentration of K ranged from 5.6 g kg^{-1} in belowground organs to 38.9 g kg^{-1} in leaves in the LC and McC treatments, respectively, and the concentration of Ca ranged from 2.8 g kg^{-1} in stems to 15.4 g kg^{-1} in leaves both in the McC treatment. The concentration of Mg lay between 0.8 g kg^{-1} in stems in the McC treatment and 5.7 g kg^{-1} in leaves in the McC treatment.

Table 3 Effect of treatment on organ biomass (belowground organs, stems, leaves, and seeds) and total concentrations of elements (mean ± SE) in organs of *R. obtusifolius* in Litavka, Malín and Mšec soils. Treatment abbreviations: *LC* Litavka control soil without any additive, *LCa* Litavka soil with lime, *LP* Litavka soil with superphosphate, *MC* Malín control soil without any additive, were not significantly different

MCa Malín soil with lime, *MP* Malín soil with superphosphate, and *McC* Mšec, non-contaminated control soil. Differences between treatments and organs were evaluated by Kruskal–Wallis test. For each element, concentrations in organs within one treatment denoted with the same letter (a–c) and concentrations in treatments within one organ denoted with the same letter (A–D)

Variable	Treatment	Organ			
		Belowground organs	Stems	Leaves	Seeds
Organ biomass (g plant^{-1})	LC	0.4±0.2^{aC}	–	0.07±0.03^{aC}	–
	LCa	11.5±1.4^{aA}	4.1±1^{abA}	3.9±0.6^{bA}	7.9±1^{abA}
	LP	1±0.3^{aBC}	–	0.4±0.2^{aBC}	–
	MC	6.6±1.2^{aAB}	1.1±0.2^{bA}	1.5±0.3^{bABC}	1.9±0.5^{abA}
	MCa	14±2.5^{aA}	2.5±0.8^{bA}	3.4±0.6^{bA}	4.9±0.9^{abA}
	MP	9.1±1.3^{aA}	1.3±0.6^{bA}	1.7±0.3^{bAB}	2.4±0.3^{abA}
	McC	29±9^{aA}	3.6±0.4^{aA}	5.8±2.2^{aA}	5.1±1.4^{aA}
N (g kg^{-1})	LC	23.3±0.5^{A}	–	–	–
	LCa	10.3±1.5^{abAB}	4.1±0.2^{bA}	17.8±1.4^{aB}	17.1±0.6^{aA}
deficient	LP	22.7±1.3^{bA}	–	29.9±1.5^{aA}	–
normal 20–50[1]	MC	7.8±0.9^{abAB}	3.95±0.5^{bA}	18.1±1.3^{aAB}	20.0±0.6^{aA}
phytotoxic	MCa	8.3±1.8^{abAB}	3.8±0.3^{bA}	15.8±1.7^{aB}	17.99±0.95^{aA}
	MP	6.6±0.3^{aAB}	5.4±0.2^{aA}	18.1±1.3^{aB}	17.6±1.1^{aA}
	McC	5.7±0.3^{aB}	6.1±0.9^{aA}	22.8±0.8^{aAB}	18.4±1.0^{aA}
P (g kg^{-1})	LC	0.6±0.04^{bB}	–	2.3±0.8^{aAB}	–
	LCa	0.96±0.05^{abAB}	0.3±0.03^{bB}	2.1±0.2^{aAB}	1.5±0.3^{aB}
deficient <2(1)[1]	LP	0.97±0.09^{aAB}	–	1.3±0.2^{aB}	–
normal 3–5[1]	MC	2.0±0.3^{abA}	0.8±0.15^{bAB}	3.5±0.9^{aAB}	2.8±0.1^{aAB}
phytotoxic >10[1]	MCa	1.7±0.2^{abA}	0.4±0.06^{bAB}	1.2±0.3^{abB}	2.2±0.2^{aAB}
	MP	1.8±0.1^{abA}	1.1±0.15^{bA}	3.2±0.2^{aA}	3.4±0.1^{aA}
	McC	0.9±0.1^{aAB}	0.6±0.1^{aAB}	1.7±0.2^{aAB}	2.4±0.4^{aAB}
K (g kg^{-1})	LC	5.6±0.1^{bA}	–	24.9±2.4^{aAB}	–
	LCa	6.4±0.3^{bA}	12.4±0.4^{abB}	23±2^{aAB}	13.7±1^{abAB}
deficient	LP	5.8±0.3^{bA}	–	24.7±3.2^{aAB}	–
normal 20–50[1]	MC	7.7±0.8^{bA}	21±2^{aAB}	30±2^{aAB}	14±1.5^{abAB}
phytotoxic	MCa	7.7±0.25^{bA}	18±2^{abAB}	32±5^{aAB}	15±1.4^{abAB}
	MP	7.2±6.2^{bA}	17±1.9^{aAB}	19±2^{aB}	11±0.9^{abB}
	McC	6.9±0.7^{bA}	32±7.5^{abA}	38.9±1.4^{aA}	22.5±4.0^{abA}
Ca (g kg^{-1})	LC	9.3±0.99^{aAB}	–	13.5±2.9^{aAB}	–
	LCa	5.9±0.3^{abAB}	3.85±0.3^{bA}	10.5±0.8^{aAB}	3.45±0.1^{bAB}
deficient	LP	9.6±0.8^{aAB}	–	11±1^{aAB}	–
normal 1–50[1]	MC	6.4±0.65^{abAB}	3.8±0.4^{bA}	6.9±0.7^{aAB}	3.8±0.4^{abAB}
phytotoxic	MCa	5.4±0.6^{abB}	3.6±0.4^{bA}	7.0±0.4^{aAB}	3.4±0.4^{bAB}
	MP	5.2±0.4^{abB}	3.6±0.6^{abA}	5.8±0.7^{aB}	3.15±0.2^{bB}
	McC	13.6±0.7^{aA}	2.8±0.3^{aA}	15.4±1.9^{aA}	6.8±0.4^{aA}
Mg (g kg^{-1})	LC	1.4±0.1^{bAB}	–	3.3±0.3^{aB}	–
	LCa	1.2±0.1^{bB}	0.9±0.1^{abA}	3.9±0.2^{aAB}	1.6±0.1^{bB}
deficient	LP	1.5±0.2^{bAB}	–	3.4±0.5^{aB}	–

Table 3 (continued)

Variable	Treatment	Organ			
		Belowground organs	Stems	Leaves	Seeds
normal 1.5–3.5[1]	MC	2.85 ± 0.5^{abA}	1.8 ± 0.2^{bA}	5.3 ± 0.2^{aA}	2.7 ± 0.3^{abA}
phytotoxic	MCa	1.7 ± 0.2^{bAB}	1.4 ± 0.2^{bA}	3.8 ± 0.15^{aAB}	2.2 ± 0.1^{abAB}
	MP	1.9 ± 0.2^{bAB}	1.5 ± 0.2^{bA}	3.9 ± 0.4^{aAB}	2.2 ± 0.1^{abAB}
	McC	1.3 ± 0.1^{abAB}	0.8 ± 0.1^{bA}	5.7 ± 0.4^{aAB}	2.8 ± 0.2^{abA}

– no material;1—Marschner (1995); 3—adapted from Pugh et al. (2002), 4—adapted from Levy et al. (1999), 5—adapted from Kabata-Pendias (2001), 6—Alkorta et al. (2004), 7—Mahler (2004), 8—Allen (1989), 9—Zhang et al. (2007), 10—Garcia-Salgado et al. (2012), 11—adapted from Lorestani et al. (2011), 12—adapted from Guleryuez et al. (2008), 13—Bose and Bhattacharyya (2008)

Concentration of microelements in the organs

The concentrations of Cu, Fe, Mn, and Ni were significantly affected by treatments, and the concentrations of Cu, Fe, Mn, Ni, and Zn differed between the individual organs (see Table 4 for details). The concentration of Cu ranged from 2.7 mg kg^{-1} in seeds in the LCa to 91 mg kg^{-1} in belowground organs in the LP treatment; the concentration of Fe ranged from 51.5 mg kg^{-1} in stems in the LCa treatment, to 5357 mg kg^{-1} in leaves in the MP treatment; the concentration of Mn ranged from 3.5 mg kg^{-1} in stems in the LCa treatment, to 228 mg kg^{-1} in belowground organs in the LC treatment; the concentration of Ni ranged from 0.5 mg kg^{-1} in stems in the LCa treatment, to 5.9 mg kg^{-1} in leaves in the MP treatment; and finally, the concentration of Zn ranged from 24 mg kg^{-1} in stems, to 83 mg kg^{-1} in belowground organs in the non-contaminated McC treatment.

Concentration of risk elements in the organs

The concentrations of As, Cd, Cr, Pb, and Zn were significantly affected by treatments and analysed plant organs (see Table 4 for details). The concentration of As ranged from 0.22 mg kg^{-1} in stems in the McC treatment to 189 mg kg^{-1} in leaves in the MCa treatment; the concentration of Cd ranged from 0.2 mg kg^{-1} in stems in the MCa treatment, to 29 mg kg^{-1} in belowground organs in the LC treatment; the concentration of Cr ranged from 0.07 mg kg^{-1} in seeds in the McC treatment, to 6.8 mg kg^{-1} in leaves in the MP treatment; the concentration of Pb ranged from 0.1 mg kg^{-1} in stems in the McC treatment to 235 mg kg^{-1} in belowground organs in the LC treatment; and finally, the

concentration of Zn, in plants grown on contaminated soil, ranged from 30 mg kg^{-1} in stems in the MC and MCa treatments, to 1479 mg kg^{-1} in belowground organs in the LC treatment.

Bioaccumulation and translocation factors

Bioaccumulation factors for all elements were significantly affected by treatments (Table 5). In the non-contaminated McC treatment, the BF was above one only for Cd and Ni and in contaminated soils of LC and MC treatments, the BF was below one for all elements. Bioaccumulation factors for As, Cd, Cu, Mn, Ni, Pb, and Zn were affected by their level of soil contamination (As: $r=0.541$, $p=0.001$; Cd: $r=-0.360$, $p=0.040$; Cu: $r=-0.377$, $p=0.031$; Mn: $r=-0.587$, $p<0.01$; Ni: $r=-0.368$, $p=0.035$; Pb: $r=-0.457$, $p<0.01$ and Zn: $r=-0.376$, $p=0.031$). Liming (MCa treatment) and application of superphosphate (LP and MP treatments) did not affect the BF in contaminated soils.

Translocation factors for As, Cu, and Ni were significantly affected by treatments (Table 5). Liming (LCa and MCa treatments) and application of superphosphate (MP treatment) affected the TF in contaminated soils.

Result of PCA analysis

The first axis of the PCA analysis explained 35 %, the first two axes 56 % and the first four axes together, 82 % of the variability of all analysed data (Fig. 2). The first ordination axis divided individual pots into the Litavka group on the right side and Malín and Mšec groups on the left side of the diagram. This indicates an effect of soil properties on the availability of elements in soil and biomass production as well as on element accumulation.

Table 4 Continuation of Table 3

Variable	Treatment	Organ			
		Belowground organs	Stems	Leaves	Seeds
As (mg kg⁻¹)	LC	50±17^aAB	–	20±8^aABC	–
	LCa	10.5±4^abAB	1.7±0.4^boAB	23±6^aABC	0.6±0.4^cdBC
deficient -[5]	LP	59±23^aAB	–	9.6±2.6^aC	–
normal 1–1.7[5]	MC	155±67^abA	12±3^bA	127±30^aAB	21±7^abA
phytotoxic 5–20[5]	MCa	60±28^abAB	7.6±1.7^bAB	189±84^aAB	6.6±3.1^bABC
hyperaccumulation level 1000[10]	MP	82±37^abAB	15.5±4.7^abA	153±30^aAB	17±10^bABC
	McC	0.81±0.17^aB	0.22±0.06^aB	0.75±0.22^aC	0.23±0.05^aABC
Cd (mg kg⁻¹)	LC	29±4.5^aA	–	14±2^bA	–
	LCa	6.8±1.1^aAB	1.5±0.3^abA	5.9±0.9^aABC	1.2±0.3^bA
deficient -[3]	LP	19±3.8^aA	–	10.5±1^bAB	–
normal 0.05–2[3]	MC	4.8±1.6^aAB	0.3±0.1^bAB	2.1±0.4^aC	0.5±0.1^abAB
phytotoxic 5–700[3]	MCa	2.1±0.6^aB	0.2±0.04^bB	1.9±0.8^bC	0.2±0.05^bB
hyperaccumulation level 100[6]	MP	2.8±0.8^aB	0.5±0.1^abAB	2.3±0.4^aABC	0.5±0.2^bAB
	McC	0.77±0.12^aBC	0.45±0.15^aAB	0.99±0.24^aC	0.29±0.15^aAB
Cr (mg kg⁻¹)	LC	3.1±1.1^aA	–	2.3±0.6^aA	–
	LCa	1.5±0.8^abA	0.1±0.03^bB	3.2±0.8^aAB	0.2±0.08^bAB
deficient -[5]	LP	3.0±1.1^aA	–	1.3±0.2^aAB	–
normal 0.1–0.5[5]	MC	6.2±2.6^abA	0.6±0.2^bAB	5.8±1.3^aAB	1.6±0.4^abA
phytotoxic 5–30[5]	MCa	2.4±13^abA	0.3±0.1^bAB	6.6±2.6^aAB	0.7±0.2^abAB
hyperaccumulation level 1000[9]	MP	3.2±14^abA	0.9±0.3^bA	6.8±1.3^aA	1.4±0.4^abA
	McC	0.82±0.25^aA	0.14±0.05^abAB	0.44±0.09^abB	0.07±0.01^bB
Cu (mg kg⁻¹)	LC	78±51^aA	–	9.5±0.8^bAB	–
	LCa	13±2^aAB	2.9±0.9^bA	8.2±1.05^abAB	2.7±0.6^bB
deficient <1–5[3]	LP	91±60^aA	–	11±2^bAB	–
normal 4–15[12]	MC	25.5±9^aAB	6.6±3.6^aA	16±2^aAB	11±3^aA
phytotoxic 20–100[3]	MCa	12.5±3^aAB	26±15^aA	15±5^aAB	4±0.6^aAB
hyperaccumulation level 1000[6]	MP	24±13^aAB	19±10^aA	17±2^aA	7±0.9^aA
	McC	4.3±0.5^aB	2.9±0.15^aA	4.3±0.3^aB	3.6±0.2^aAB
Fe (mg kg⁻¹)	LC	2463±866^aA	–	1761±703^aAB	–
	LCa	965±473^abA	51.5±13^bB	2092±607^aAB	82±34^bB
deficient <40[4]	LP	2337±961^aA	–	795±187^aB	–
normal 30–300[4]	MC	4547.5±1954^abA	251.5±97^bAB	4456±1000^aAB	926±258^abA
phytotoxic >500[4]	MCa	1857±1011^abA	171±57^bAB	5273±2231^aAB	315±124^abAB
hyperaccumulation level 10000[11]	MP	2426±1058^abA	441±144^bA	5357±1051^aA	758±387^bAB
	McC	432±135^aA	62±2^abAB	216±89^abB	52±4^bAB
Mn (mg kg⁻¹)	LC	228±96^aA	–	183±82^aA	–
	LCa	116±55^abA	3.5±0.5^bB	207±49^aA	25±19^abA
deficient	LP	118±61^aA	–	81±23^aA	–
normal 40–200[7]	MC	71±28^abA	4.65±1.4^bAB	88±33^aA	20±5^abA
phytotoxic >356[13]	MCa	30±15^abA	3.6±0.9^bAB	77±32^aA	10.5±1.8^abA
hyperaccumulation level 10000[6]	MP	39±16^abA	7.5±1.7^bAB	83±17^aA	21±8.5^abA
	McC	56±5.5^abA	17±1^bA	199±56^aA	64±3^abA
Ni (mg kg⁻¹)	LC	4.1±1^aA	–	2.3±0.6^aAB	–

54

Table 4 (continued)

Variable	Treatment	Organ			
		Belowground organs	Stems	Leaves	Seeds
	LCa	1.4 ± 0.4^{abA}	0.5 ± 0.05^{bA}	2 ± 0.4^{aAB}	0.6 ± 0.1^{bB}
deficient	LP	3.2 ± 0.8^{aA}	–	1.4 ± 0.2^{bB}	–
normal 0.5–5[8]	MC	5.3 ± 1.8^{aA}	0.7 ± 0.2^{bA}	4.8 ± 0.9^{aAB}	2.2 ± 0.7^{abA}
phytotoxic >5[8]	MCa	2.5 ± 0.85^{abA}	0.7 ± 0.1^{bA}	5.0 ± 1.8^{aAB}	1.2 ± 0.2^{abAB}
hyperaccumulation level 1000[6]	MP	3.3 ± 12^{abA}	1.0 ± 0.2^{bA}	5.9 ± 1^{aA}	1.8 ± 0.3^{abA}
	McC	1.0 ± 0.1^{aA}	0.6 ± 0.1^{aA}	1.2 ± 0.2^{aB}	1.2 ± 0.1^{aAB}
Pb (mg kg^{-1})	LC	235 ± 88^{aA}	–	142 ± 63^{aB}	–
	LCa	123 ± 54^{abAB}	2.9 ± 0.2^{bA}	166 ± 44^{aAB}	5.5 ± 3^{bA}
deficient -[3]	LP	113 ± 48^{aAB}	–	59 ± 19^{aABC}	–
normal 0.5–10[3]	MC	14 ± 5^{aAB}	1.2 ± 0.4^{aAB}	10.5 ± 2.5^{aC}	5 ± 1.9^{aA}
phytotoxic 30–300[3]	MCa	6 ± 2.5^{aB}	1.9 ± 0.6^{aAB}	13.6 ± 6.5^{aABC}	1.8 ± 0.3^{aA}
hyperaccumulation level 1000[6]	MP	7.6 ± 2.8^{aB}	2.2 ± 0.4^{aAB}	13 ± 2^{aABC}	6 ± 2.8^{aA}
	McC	1.6 ± 0.3^{aAB}	0.1 ± 0.1^{bB}	0.8 ± 0.3^{abC}	0.2 ± 0.1^{abA}
Zn (mg kg^{-1})	LC	1479 ± 360^{aA}	–	1260 ± 213^{aAB}	–
	LCa	329 ± 87^{abAB}	50 ± 7^{bA}	498 ± 105^{aABC}	55 ± 17^{bA}
deficient <10[3]	LP	809 ± 180^{aAB}	–	875 ± 66^{aB}	–
normal 10–150[3]	MC	231 ± 76^{aAB}	30 ± 7^{bA}	189 ± 39^{aC}	60 ± 9.5^{abA}
phytotoxic >100–500[5]	MCa	124 ± 46^{abB}	30 ± 8^{bA}	241 ± 100^{aABC}	32 ± 5^{bA}
hyperaccumulation level 10000[6]	MP	141 ± 48^{abB}	50 ± 9^{abA}	220 ± 40^{aABC}	54 ± 19^{bA}
	McC	83 ± 12^{aB}	24 ± 3^{aA}	74 ± 18^{aC}	28 ± 2^{aA}

In Litavka, in contrast to Malín soil, data for lime treatment (LCa) were clearly separated from all marks for control (LC) and superphosphate treatments (LP). This indicates a large effect of lime application on all the recorded data in Litavka soil and a minimal effect in Malín soil. In the majority of treatments, data for stems and seeds were grouped into the upper part of the diagram, indicating the lowest concentrations of elements in these organs, since the vectors for the majority of elements in the biomass grouped on the opposite site of the diagram.

The length and direction of the vectors relating to the individual elements indicate the association of elements with their respective treatments. For example, Zn concentration was the highest in belowground organs in the LC treatment, but the lowest in stems in the McC treatment. The concentration of Zn in plant biomass (Zn/B) was positively correlated with plant-available Zn (Zn/Ca) and also with acid-extractable Zn (Zn/AA) concentrations in the soil as indicated by an angle between the vectors for Zn/B and Zn/Ca or Zn/AA of less than 90°. The concentration of Zn in plant biomass was negatively correlated with biomass of organs (DM) as the angle between vectors for Zn/B and DM was greater than 90°. Two vectors did not positively correlate, if the angle between them is larger than 90°. A long vector for a particular variable indicates that it greatly affected the results of the analysis and the opposite was the case for a short vector. For example, there was no effect of soil and treatment on the concentrations of K and Mg in plant biomass, as vectors for these elements (K/B and Mg/B) were very short.

Discussion

Biomass production

Biomass production (i.e. total biomass of all organs) of *R. obtusifolius* was clearly negatively related to the concentrations of Cd, Ni, Pb, and Zn in its biomass, indicating their toxicity to plants. Very high concentrations of micro- and risk elements in plants (i.e. depending on the plant species, >5 mg As kg^{-1}, >5 mg Cd kg^{-1},

Table 5 Effect of treatment on bioaccumulation (BF) and translocation (TF) factors (mean ± SE). Treatment abbreviations: *LC* Litavka control soil without any additive, *LCa* Litavka soil with lime, *LP* Litavka soil with superphosphate, *MC* Malín control soil without any additive, *MCa* Malín soil with lime, *MP* Malín soil with superphosphate, and *McC* Mšec, non-contaminated control soil. Calculated by Kruskal–Wallis test, differences between treatments were not statistically significant ([n.s.]) or were significant at 0.05 (*) and 0.01 (**) probability levels. According to the multiple comparisons of mean ranks, treatments with the same letter were not significantly different

Variable	Elements	Treatment						
		LC	LCa	LP	MC	MCa	MP	McC
BF	As *	0.06±0.02[a]	0.04±0.01[a]	0.03±0.01[a]	0.1±0.03[a]	0.1±0.04[a]	0.1±0.04[a]	0.05±0.01[a]
	Cd **	0.3±0.04[ab]	0.08±0.01[b]	0.2±0.02[ab]	0.1±0.02[ab]	0.1±0.03[b]	0.1±0.04[ab]	2.9±0.7[a]
	Cr **	0.04±0.01[a]	0.04±0.01[a]	0.03±0.005[a]	0.09±0.02[a]	0.09±0.02[a]	0.1±0.03[a]	0.01±0.002[a]
	Cu *	0.2±0.01[ab]	0.1±0.01[b]	0.2±0.03[ab]	0.2±0.02[ab]	0.2±0.03[ab]	0.2±0.04[ab]	0.3±0.02[a]
	Fe **	0.08±0.03[ab]	0.06±0.02[ab]	0.04±0.01[ab]	0.2±0.05[a]	0.2±0.05[ab]	0.2±0.06[a]	0.01±0.004[b]
	Mn **	0.07±0.03[ab]	0.05±0.01[ab]	0.03±0.01[b]	0.2±0.05[ab]	0.1±0.03[ab]	0.2±0.04[ab]	0.3±0.08[a]
	Ni **	0.01±0.01[b]	0.4±0.1[ab]	0.02±0.01[ab]	0.4±0.1[ab]	0.7±0.2[a]	0.5±0.1[ab]	1.5±0.6[a]
	Pb **	0.04±0.02[a]	0.03±0.01[a]	0.02±0.01[a]	0.08±0.02[a]	0.08±0.02[a]	0.1±0.03[a]	0.01±0.005[a]
	Zn **	0.2±0.03[ab]	0.05±0.01[b]	0.1±0.01[ab]	0.1±0.03[ab]	0.1±0.03[ab]	0.2±0.04[ab]	1.0±0.1[a]
TF	As *	0.5±0.2[ab]	2.7±0.8[ab]	0.2±0.1[b]	1.5±0.7[ab]	4.5±1.7[a]	3.9±2.6[ab]	1.2±0.6[ab]
	Cd [n.s.]	0.5±0.1[a]	1.0±0.2[a]	0.5±0.04[a]	0.4±0.1[a]	0.9±0.3[a]	1.3±0.5[a]	1.2±0.1[a]
	Cr [n.s.]	0.9±0.4[a]	3.3±1.1[a]	0.4±0.1[a]	1.5±0.6[a]	3.4±1.0[a]	5.1±3.6[a]	0.7±0.3[a]
	Cu *	0.1±0.1[a]	0.7±0.1[a]	0.2±0.1[a]	0.9±0.2[a]	1.3±0.3[a]	1.4±0.4[a]	1.0±0.2[a]
	Fe [n.s.]	0.8±0.3[a]	5.2±1.8[a]	0.4±0.1[a]	2.0±1.1[a]	3.0±1.1[a]	5.1±3.6[a]	0.7±0.4[a]
	Mn [n.s.]	0.8±0.3[a]	2.5±0.9[a]	0.7±0.2[a]	1.9±0.9[a]	2.6±0.9[a]	3.6±2.2[a]	3.5±0.7[a]
	Ni *	0.5±0.02[a]	1.9±0.1[a]	0.4±0.03[a]	1.4±0.4[a]	2.4±0.8[a]	1.9±0.7[a]	1.2±0.2[a]
	Pb [n.s.]	1.0±0.4[a]	2.3±0.6[a]	0.8±0.3[a]	3.0±1.5[a]	2.0±0.4[a]	4.8±3.8[a]	0.6±0.3[a]
	Zn [n.s.]	0.8±0.2[a]	1.9±0.4[a]	1.2±0.2[a]	0.9±0.2[a]	1.6±0.4[a]	2.7±1.0[a]	0.9±0.1[a]

BF—the ratio of total concentrations of elements in aboveground plant tissues (stems, leaves and seeds together) to pseudo-total concentrations of elements in soil (BF = $c_{tissues}/c_{soil}$)

TF—the ratio of total concentrations of elements in leaves to total concentrations of elements in belowground organs (TF = $c_{leaf}/c_{belowground\ organs}$)

>5 mg Cr kg^{-1}, >5 mg Ni kg^{-1}, >30 mg Pb kg^{-1} or >100 mg Zn kg^{-1}) can reduce biomass production, because micro- and risk elements can cause inhibition of cell elongation and division (Anton and Mathe-Gaspar 2005; Chen and Wong 2006; Barrutia et al. 2009). In LC and LP treatments, the toxicity of risk elements was high enough to inhibit the development of stems and generative organs. *R. obtusifolius* is thus, a species with a high sensitivity to metal(loid) toxicity.

The highest biomass production of *R. obtusifolius* in the McC treatment was probably connected with the lower micro- and risk elements concentrations (i.e. depending on the plant species, in range of 1–1.7 mg kg^{-1} for As, 0.05–2 mg kg^{-1} for Cd, 0.1–0.5 mg kg^{-1} for Cr, 0.5–5 mg kg^{-1} for Ni, 0.5–10 mg kg^{-1} for Pb or 10–150 mg kg^{-1} for Zn), but also to better N and K nutrition as shown by the N and K concentrations in leaves. In the Litavka soil, a greater biomass production in the LCa than the LC treatment was probably connected with the obvious trend that lime substantially reduced the mobility of micro- and risk elements, in particular of Cd, Mn, and Zn (Table 2). Similar results, i.e. an increased growth of several crops and weedy species on acid soils contaminated by Cd, Cu, Ni, Pb, and Zn after lime application has also been recorded by other authors (Chen and Wong 2006; Tlustoš et al. 2006; Alvarenga et al. 2008).

Concentrations of macroelements in plant organs

In the McC treatment, the highest concentrations of N, K, Ca, and Mg were recorded as expected, in leaves,
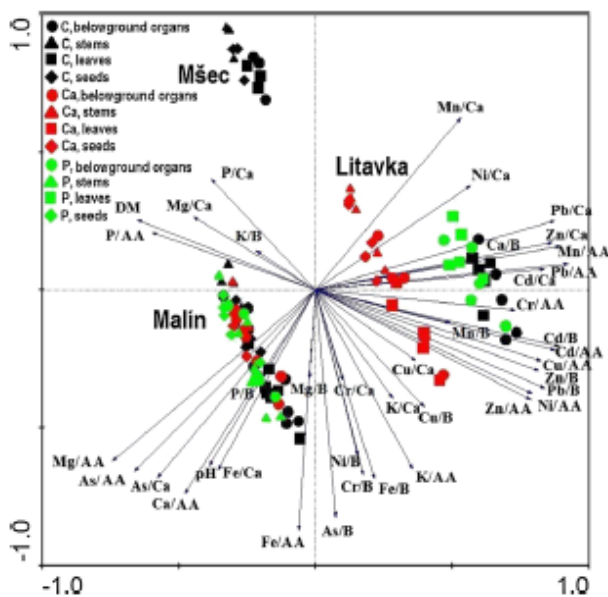
Fig. 2 Ordination diagram showing the results of PCA analysis with element concentrations in organs of *R. obtusifolius* plants grown on contaminated and non-contaminated soils. Soil abbreviations: *Litavka* slightly acidic contaminated soil, *Malín* alkaline contaminated soil, *Mšec* acidic non-contaminated soil. Treatment abbreviations: *C* control, *Ca* lime application, *P* superphosphate application; *C, belowground organs* concentrations of elements in belowground organs in control and etc. Element abbreviations:/*B* total concentrations of elements in plant biomass,/*Ca* plant-available concentrations of elements in soil (extracted by 0.01 mol L^{-1} CaCl$_2$) and/*AA* acid-extractable concentrations of elements in soil (extracted by 0.11 mol L^{-1} CH$_3$COOH). Other abbreviations: *DM* dry matter biomass per organ and *pH* mean soil pH

because leaves are the most metabolically active organs, with high nutrient requirements (López-Lefebre et al. 2001). The most surprising result was the highest Ca concentration in belowground organs, leaves and seeds in the McC treatment, despite the lowest soil Ca availability (Tables 1 and 2). Similarly, the Ca concentration in belowground organs and leaves in LC, LCa, and LP treatments was higher than in the MC, MCa, and MP treatments, despite a higher Ca availability in the MC, MCa, and MP treatments. The lowest availability of Ca in the McC treatment was reflected only by the lowest concentration of Ca in stems. The explanation for these discrepancies between Ca availability and Ca concentrations in belowground organs and leaves appears to be at least partly due to competition between Ca and Mg ions (Appenroth and Gabrys 2003). This is because of obvious trend that the highest concentrations of Mg in belowground organs were generally in the treatments where low concentrations of Ca were recorded. The highest concentration of P was recorded in seeds, due

to the high P requirements for generative reproduction (Jiang et al. 2007; White and Veneklaas 2012). With the exception of the MP treatment, the P concentration in seeds was below the critical value of 3 g kg^{-1}, below which there is a decrease in the germination ability of *R. obtusifolius* or *R. crispus* (Hrdličková et al. 2011; Hejcman et al. 2012a). The lowest concentrations of N and P were recorded in stems and belowground organs, of Ca and Mg in stems and of K in belowground organs. A low concentration of macroelements in stems is connected with their low metabolic activity and with a high mobility of N, P, K, and Mg in plants and therefore, considerable translocation into plant apices (Anton and Mathe-Gaspar 2005; Gaweda 2009).

In the LCa treatment, a significant decrease in the concentrations of N and Ca in belowground organs after liming might be associated with a dilution effect caused by greater biomass production (Chen and Wong 2006; Tlustoš et al. 2006).

No effect of liming or superphosphate application on the distribution of K, Ca, and Mg in plant biomass was recorded in either acid- or alkaline-contaminated soils. Similarly, no effect of liming on the Mg concentration in plant tissues of other weedy species was found by Alvarenga et al. (2008).

Concentrations of microelements in plant organs

In the McC treatment, the highest concentrations of Fe and Zn were recorded in belowground organs; that of Cu in belowground organs and leaves; of Mn in leaves; the lowest concentrations of Cu, Mn, and Zn in stems; and of Fe in seeds. Similar concentrations of Ni were recorded in all organs. Variability of all microelements in different organs might be due to compartmentalisation and translocation in the vascular system (Bose and Bhattacharyya 2008; Hansch and Mendel 2009). Similar results, i.e., concentrations of Cu, Fe, and Ni in the order belowground organs > leaves > stems is consistent with results for *R. acetosa*, but inconsistent for those for Cu, Fe, and Zn in *R. dentatus* when both were grown in non-contaminated soils (Barman et al. 2000; Gaweda 2009). A different distribution of Mn was recorded, with the order leaves > seeds > belowground organs > stems, which clearly separated the distribution of Mn in *R. obtusifolius* from that of other microelements. This result is inconsistent with the distribution of Mn in *R. acetosa* (belowground organs > leaves > stems,

Gaweda 2009), which shows a difference in Mn distribution within *Rumex* species.

In LC and MC treatments, a tendency for a restricted transport of microelements from belowground organs into leaves in comparison to the McC treatment was recorded. This was probably connected with protection against excessive concentrations of microelements in aboveground organs (i.e. depending on the plant species as well as organ, >20 mg Cu kg^{-1}, >500 mg Fe kg^{-1}, >356 mg Mn kg^{-1}, >5 mg Ni kg^{-1} or >100 mg Zn kg^{-1}, Hansch and Mendel 2009).

In LCa and MCa treatments, a tendency for increased transport of Cu, Fe, Mn, and Ni from belowground organs into leaves in comparison to LC and MC treatments was recorded, as also demonstrated by higher TFs. We speculate that changes in the distribution pattern of microelements are connected to the presence of organic acids (mainly oxalate) for the formation of stable complexes, similar to the internal defence mechanism of oxalate plants against excess Ca (Tolra et al. 2005; Miyagi et al. 2013). Therefore, we can speculate that micro- and risk elements are precipitated with oxalate in roots in contaminated control soils. On the other hand, in contaminated soils with lime, oxalate is precipitated with Ca and thus is not available for micro- and risk elements that can easy transport to leaves. In the MP treatment, an increased transport of all microelements from belowground organs into leaves in comparison to the MC treatment was recorded probably because of the sufficient amount of Ca available from superphosphate as well as from soil solution precipitated oxalates as Ca-oxalate and thus available microelements can be easily transported to leaves. Changes in the translocation of microelements in plants after liming and superphosphate application require further research that focuses on differences between oxalate and non-oxalate plants.

Concentrations of risk elements in plant organs

In the McC treatment, the highest concentrations of risk elements (As, Cd, Cr, and Pb) were recorded in belowground organs or leaves and the lowest were recorded in stems and seeds. This was connected with low concentrations of risk elements in reproductive organs and with their lower metabolic activity in stems (Anton and Mathe-Gaspar 2005; Bose et al. 2008; Gaweda 2009).

In the LC and MC treatments, there was a higher transport of risk elements from stems into seeds and restricted transport from belowground organs into

leaves in comparison to the McC treatment. The clear tendency for the accumulation of risk elements in belowground organs was connected with the exclusion strategy of *R. obtusifolius* and the function of roots as a barrier that limits the translocation of risk elements from the soil to the aboveground organs in soils contaminated by risk elements (Gaweda 2009; Zhang et al. 2010).

In the LCa and MCa treatments, there was a tendency for a greater transport of As, Cd, Cr, Pb, and Zn from belowground organs into leaves in comparison to the LC and MC treatments, in the most cases demonstrated also by higher TFs.

The results for Cd, Pb, and Zn transport were inconsistent with those for *Triticum aestivum* published by Tlustoš et al. (2006)—i.e. decreasing shoots/roots ratio after lime application in comparison to *R. obtusifolius*. As described above, we presume that oxalate plants possess an internal defence mechanism against risk elements, such as forming Ca-oxalate (Miyagi et al. 2013), because risk elements (mainly divalent Cd, Pb, and Zn) compete with divalent Ca for sites to form complexes with oxalate. There was a tendency for a higher transfer of Cd and Zn from belowground organs into leaves in the LP and MP treatments, in comparison to the LC and MC treatments, partly also demonstrated by higher TFs. Similar results were obtained for As and Pb, but only in alkaline-contaminated soil. This was demonstrated also by higher TFs. The results for Cd and Zn transport are inconsistent with observations for *Zea mays* and *Brassica parachinensis* (Jiang et al. 2007; Qiu et al. 2011). Therefore, we speculate that differences in distribution can be connected to the presence of oxalate (available for the formation of less toxic complexes as well as internal defence mechanism) in *R. obtusifolius*. Using lime and superphosphate application increased the in vivo mobility of As, Cd, Cr, Pb, and Zn into leaves of *R. obtusifolius*. For this reason, differences in the translocation of risk elements after liming and superphosphate application deserve closer examination, with a focus on the differences between oxalate and non-oxalate plants.

Bioaccumulation and translocation factors

In the non-contaminated McC treatment, the BF of micro- and risk elements for *R. obtusifolius* ranged from 0.01 to 2.9 and in the LC and MC treatments, ranged

from 0.01 to 0.4. A decrease in the BF with increasing pseudo-total concentrations of elements (Cd, Zn, Pb, Ni, Mn, and Cu) in soils was consistent with results of Cd and Zn published by Zhao et al. (2003). Therefore, we do not recommend the use of *R. obtusifolius* for phytoextraction in heavily contaminated soils, but it might be suitable for moderately contaminated soils, as is *R. acetosa* (Gaweda 2009). In addition, because of the sensitivity of *R. obtusifolius* to risk elements, it can be used for the identification of contaminated soils by field vegetation mapping, i.e. according to the symptoms of risk elements toxicity visible on aboveground organs in different phenological stages (Hejcman et al. 2012b). Liming tended to decrease the BF of micro- and risk elements in the contaminated Litavka soil, due to the reduction in element availability in soil and subsequently in plants. In the LP, MCa and MP treatments, no effect on the BF of micro- and risk elements was observed, because there was no reduction of element availability in the soil.

In the LC treatment, the TFs for risk elements (As, Cd, Pb, and Zn) ranged from 0.5 to 1.0, indicating an exclusion strategy by *R. obtusifolius*. In the MC treatment, the TFs for risk elements were higher, confirming that the ability to exclude risk elements was affected by their availability in the soil. The classification of *R. obtusifolius* as a metal-excluder with restricted risk element transfer to aboveground organs is consistent with observations for *R. acetosa* (Barrutia et al. 2009; Gaweda 2009). It appears that there is consistency in the physiological responses of different *Rumex* species to the availability of risk elements in the soil, but this requires further research. Plants with the ability to accumulate risk elements can be used to phytoremediate contaminated soils (Baker 1981). Therefore, the TF is only relevant for elements that exceed background concentrations in comparison to those of non-contaminated soils (see Table 1). In the LCa and MCa treatments, the TFs for As, Cd, Pb, and Zn ranged from 0.9 to 4.5, which is characteristic for indicators or accumulators. Liming substantially increased the translocation of risk elements from belowground organs into leaves. A similar result was observed for superphosphate application, but only in alkaline-contaminated soil. We conclude that the identification of plants for the phytoremediation of contaminated soils must proceed with caution, because TF values depend on the chemical properties of the soil.

Conclusions

The ionome, i.e., the elemental composition of different organs is greatly affected by soil chemical properties. Soil chemical properties affect not only the concentrations of individual elements in individual organs, but also their distribution between plant organs. Variability in the concentrations of micro- and risk elements is much greater than variability in the concentrations of macroelements, especially on metal(loid)-contaminated soils. Liming of contaminated soils as well as superphosphate application can modify the distribution pattern of elements and can increase the translocation of micro- and risk elements from belowground organs to leaves.

The oxalate plant, *R. obtusifolius*, is sensitive to Cd, Ni, Pb, and Zn toxicity, as its biomass production is reduced due to their availability in the soil and consequently due to reduction of their high concentrations in plant organs. The restricted translocation of micro- and risk elements from belowground organs to leaves in *R. obtusifolius* is consistent with this species being an As-, Cd-, Pb-, and Zn-excluder and not suitable for phytoremediation of heavily contaminated soils. However, sensitivity to risk elements can be used for identification of metal(loid)-contaminated soils by field vegetation mapping.

References

Alkorta I, Hernández-Allica J, Becerril JM, Amezaga I, Albizu I, Garbisu C (2004) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead and arsenic. Rev Environ Sci Biotechnol 3:71–90

Allen SE (1989) Analysis of ecological materials, 2nd edn. Blackwell, Oxford

Alvarenga P, Goncalves AP, Fernandes RM, de Varennes A, Vallini G, Duarte E, Cunha-Queda AC (2008) Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass. Sci Total Environ 406:43–56

Anonymous (1994) Public notice No. 13/1994 for the managemenet of soil protection. Czech Ministry of the Environment, Prague, In Czech

Anton A, Mathe-Gaspar G (2005) Factors affecting heavy metal uptake in plant selection for phytoremediation. Z Naturforsch C 60:244–246

Appenroth KJ, Gabrys H (2003) Ion antagonism between calcium and magnesium in phytochrome-mediated degradation of storage starch in *Spirodela polyrhiza*. Plant Sci 165: 1261–1265

Baker AJM (1981) Accumulators and excluders—strategies in the response of plants to heavy-metals. J Plant Nutr 3:643–654

Barman SC, Sahu RK, Bhargava SK, Chaterjee C (2000) Distribution of heavy metals in wheat, mustard, and weed grown in field irrigated with industrial effluents. Bull Environ Contam Toxicol 64:489–496

Barrutia O, Epelde L, Garcia-Plazaola JI, Garbisu C, Becerril JM (2009) Phytoextraction potential of two *Rumex acetosa* L. accessions collected from metalliferous and non-metalliferous sites: effect of fertilization. Chemosphere 74: 259–264

Borůvka L, HuanWei C, Kozák J, Krištoufková S (1996) Heavy contamination of soil with cadmium, lead and zinc in the alluvium of the Litavka river. Rostl Výr 42:543–550

Bose S, Bhattacharyya AK (2008) Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge. Chemosphere 70:1264–1272

Bose S, Chandrayan S, Rai V, Bhattacharyya AK, Ramanathan A (2008) Translocation of metals in pea plants grown on various amendment of electroplating industrial sludge. Bioresour Technol 99:4467–4475

Chen Q, Wong JWC (2006) Growth of *Agropyron elongatum* in a simulated nickel contaminated soil with lime stabilization. Sci Total Environ 366:448–455

Garcia-Salgado S, Garcia-Casillas D, Quijano-Nieto MA, Bonilla-Simon MM (2012) Arsenic and heavy metal uptake and accumulation in native plant species from soils polluted by mining activities. Water Air Soil Pollut 223:559–572

Gaweda M (2009) Heavy metal content in common sorrel plants (*Rumex Acetosa* L.) obtained from natural sites in Malopolska province. Pol J Environ Stud 18:213–218

Guleryuez G, Arslan H, Celik C, Gucer S, Kendall M (2008) Heavy metal content of plant species along Nilufer stream in industrialized Bursa City, Turkey. Water Air Soil Pollut 195:275–284

Gupta S, Nayek S, Saha RN, Satpati S (2008) Assessment of heavy metal accumulation in macrophyte, agricultural soil, and crop plants adjacent to discharge zone of sponge iron factory. Environ Geol 55:731–739

Hann P, Trska C, Kromp B (2012) Effects of management intensity and soil chemical properties on *Rumex obtusifolius* in cut grasslands in Lower Austria. J Pest Sci 85:5–15

Hansch R, Mendel RR (2009) Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Curr Opin Plant Biol 12:259–266

Hejcman M, Strnad L, Hejcmanová P, Pavlů V (2012a) Response of plant species composition, biomass production and biomass chemical properties to high N, P and K application rates in *Dactylis glomerata*- and *Festuca arundinacea*-dominated grassland. Grass Forage Sci 67:488–506

Hejcman M, Vondráčková S, Müllerová V, Červená K, Száková J, Tlustoš P (2012b) Effect of quick lime and superphosphate additives on emergence and survival of *Rumex obtusifolius*

seedlings in acid and alkaline soils contaminated by As, Cd, Pb, and Zn. Plant Soil Environ 58:561–567

Horák J, Hejcman M (2013) Use of trace elements from historical mining for alluvial sediment dating. Soil Water Res 8:77–86

Hrdličková J, Hejcman M, Křišťálová V, Pavlů V (2011) Production, size, and germination of broad-leaved dock seeds collected from mother plants grown under different nitrogen, phosphorus, and potassium supplies. Weed Biol Manag 11: 190–201

Hujerová R, Pavlů V, Hejcman M, Pavlů L, Gaisler J (2013) Effect of cutting frequency on above- and below-ground biomass production of *Rumex alpinus*, *R. crispus*, *R. obtusifolius*, and the *Rumex* hybrid (*R. patienta* x *R. tianschanicus*) in the seeding year. Weed Res 53:378–386

Jiang HM, Yang JC, Zhang JF (2007) Effects of external phosphorus on the cell ultrastructure and the chlorophyll content of maize under cadmium and zinc stress. Environ Pollut 147: 750–756

Kabata-Pendias A (2001) Trace elements in soils and plants, 3rd edn. CRC Press, Boca Raton

Levy DB, Redente EF, Uphoff GD (1999) Evaluating the phytotoxicity of Pb-Zn tailings to big bluestem (*Andropogon gerardii* Vitman) and switchgrass (*Panicum virgatum* L.). Soil Sci 164:363–375

Lindström BEM, Frankow-Lindberg BE, Dahlin AS, Wivstad M, Watson CA (2013) Micronutrient concentrations in common and novel forage species and varieties grown on two contrasting soils. Grass Forage Sci 68:427–436

López-Lefebre LR, Rivero RM, Garcia PC, Sanches E, Ruiz JM, Romero L (2001) Effect of calcium on mineral nutrient uptake and growth of tobacco. J Sci Food Agric 81: 1334–71338

Lorestani B, Cheraghi M, Yousefi N (2011) Accumulation of Pb, Fe, Mn, Cu and Zn in plants and choice of hyperaccumulator plant in the industrial town of Vian, Iran. Arch Biol Sci 63: 739–745

Mahler RL (2004) Nutrients plants require for growth. CIS1124 Publishing University of Idaho College of Agricultural and Life Sciences. http://www.cals.uidaho.edu/edcomm/pdf/CIS/CIS1124.pdf. Accessed 16 Feb 2014

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London

Mehlich A (1984) Mehlich-3 soil test extractant—a modification of Mehlich-2 extractant. Commun Soil Sci Plant Anal 15: 1409–1416

Miyagi A, Uchimiya M, Kawai-Yamada M, Uchimiya H (2013) Impact of aluminium stress on oxalate and other metabolites in *Rumex obtusifolius*. Weed Res 53:30–41

Pugh RE, Dick DG, Fredeen AL (2002) Heavy metal (Pb, Zn, Cd, Fe, and Cu) contents of plant foliage near the anvil range lead/zinc mine, Faro, Yukon Territory. Ecotoxicol Environ Saf 52:273–279

Qiu Q, Wang YT, Yang ZY, Yuan JG (2011) Effects of phosphorus supplied in soil on subcellular distribution and chemical forms of cadmium in two Chinese flowering cabbage (*Brassica parachinensis* L.) cultivars differing in cadmium accumulation. Food Chem Toxicol 49:2260–2267

Quevauviller P (1998) Operationally defined extraction procedures for soil and sediment analysis—I. standardization. TrAC Trends Anal Chem 17:289–298

Salt DE, Baxter I, Lahner B (2008) Ionomics and the study of the plant ionome. Annu Rev Plant Biol 59:709–733

Schwertfeger DM, Hendershot WH (2009) Determination of effective cation exchange capacity and exchange acidity by a one-step BaCl₂ method. Soil Sci Soc Am J 73:737–743

Šrek P, Hejcman M, Kunzová E (2012) Effect of long-term cattle slurry and mineral N, P and K application on concentration of N, P, K, Ca, Mg, As, Cd, Cr, Cu, Mn, Ni, Pb and Zn in peeled potato tubers and peels. Plant Soil Environ 58:167–173

Strnad L, Hejcman M, Hejcmanová P, Křišťálová V, Pavlů V (2012) Performance and mortality of *Rumex obtusifolius* and *R. crispus* in managed grasslands are affected by nutrient availability. Folia Geobotanica 47:293–304

Sytar O, Kumar A, Latowski D, Kuczynska P, Strzalka K, Prasad MNV (2013) Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol Plant 35:985–999

ter Braak CJF, Smilauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca

Thompson K, Parkinson JA, Band SR, Spencer RE (1997) A comparative study of leaf nutrient concentrations in a regional herbaceous flora. New Phytol 136:679–689

Tlustoš P, van Dijk D, Száková J, Pavlíková D (1994) Cd and Zn release through the selected extractants. Rostl Výr 40: 1107–1121

Tlustoš P, Száková J, Kořínek K, Pavlíková D, Hanč A, Balík J (2006) The effect of liming on cadmium, lead, and zinc uptake reduction by spring wheat grown in contaminated soil. Plant Soil Environ 53:16–24

Tolra RP, Poschenrieder C, Luppi B, Barcelo J (2005) Aluminium-induced changes in the profiles of both organic acids and phenolic substances underlie Al tolerance in *Rumex acetosa* L. Environ Exp Bot 54:231–238

Tyler G, Ström L (1995) Differing organic-acid exudation pattern explains calcifuge and acidifuge behaviour of plants. Ann Bot 75:75–78

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. Pol J Environ Stud 22:577–589

Vondráčková S, Hejcman M, Tlustoš P, Száková J (2014) Effect of rock phosphate and superphosphate application on mobility of elements (Cd, Zn, Pb, As, Fe, Mn) in contaminated soils. Environ Eng Manag J. In press.

White PJ, Broadley MR (2003) Calcium in plants. Ann Bot 92: 487–511

White PJ, Veneklaas EJ (2012) Nature and nurture: the importance of seed phosphorus content. Plant Soil 357:1–8

White PJ, Broadley MR, Thompson JA, McNicol JW, Crawley MJ, Poulton PR, Johnston AE (2012) Testing the distinctness of shoot ionomes of angiosperm families using the Rothamsted Park grass continuous hay experiment. New Phytol 196:101–109

Zhang XH, Liu J, Huang HT, Chen J, Zhu YN, Wang DQ (2007) Chromium accumulation by the hyperaccumulator plant *Leersia hexandra* Swartz. Chemosphere 67:1138–1143

Zhang XF, Xia HP, Lia ZA, Zhuang P, Gao B (2010) Potential of four forage grasses in remediation of Cd and Zn contaminated soils. Bioresour Technol 101:2063–2066

Zhao FJ, Lombi E, McGrath SP (2003) Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant Soil 249:37–43

Zhao H, Guo B, Wei Y, Zhang B (2013) Multi-element composition of wheat grain and provenance soil and their potentialities as fingerprints of geographical origin. J Cereal Sci 57: 391–397

Zhuang P, Yang QW, Wang HB, Shu WS (2007) Phytoextraction of heavy metals by eight plant species in the field. Water Air Soil Pollut 184:235–242

4.4 Vondráčková et al. (2015). Příjem a translokace hliníku ve šťovíku tupolistém – hyperakumulační rostlině Al je ovlivněna obsahem nízkomolekulárních organických kyselin a pH půdy.

Název: Aluminium uptake and translocation in Al hyperaccumulator *Rumex obtusifolius* is affected by low-molecular-weight organic acids content and soil pH.

Autoři: Vondráčková, S., Száková, J., Drábek, O., Tejnecký, V., Hejcman, M., Müllerová, V., Tlustoš, P.

Publikováno v časopise: PLoS ONE. 10 (4). 1-18. doi:10.1371/journal.pone.0123351.

Aluminium Uptake and Translocation in Al Hyperaccumulator *Rumex obtusifolius* Is Affected by Low-Molecular-Weight Organic Acids Content and Soil pH

Stanislava Vondráčková[1]*, Jiřina Száková[1], Ondřej Drábek[2], Václav Tejnecký[2], Michal Hejcman[3], Vladimíra Müllerová[3], Pavel Tlustoš[1]

1 Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic, 2 Department of Soil Science and Soil Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic, 3 Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic

* vondrackovas@af.czu.cz

Abstract

Background and Aims

High Al resistance of *Rumex obtusifolius* together with its ability to accumulate Al has never been studied in weakly acidic conditions (pH > 5.8) and is not sufficiently described in real soil conditions. The potential elucidation of the role of organic acids in plant can explain the Al tolerance mechanism.

Methods

We established a pot experiment with *R. obtusifolius* planted in slightly acidic and alkaline soils. For the manipulation of Al availability, both soils were untreated and treated by lime and superphosphate. We determined mobile Al concentrations in soils and concentrations of Al and organic acids in organs.

Results

Al availability correlated positively to the extraction of organic acids (citric acid < oxalic acid) in soils. Monovalent Al cations were the most abundant mobile Al forms with positive charge in soils. Liming and superphosphate application were ambiguous measures for changing Al mobility in soils. Elevated transport of total Al from belowground organs into leaves was recorded in both lime-treated soils and in superphosphate-treated alkaline soil as a result of sufficient amount of Ca available from soil solution as well as from superphosphate that can probably modify distribution of total Al in *R. obtusifolius* as a representative of "oxalate plants." The highest concentrations of Al and organic acids were recorded in the leaves, followed by the stem and belowground organ infusions.

63

Conclusions

In alkaline soil, *R. obtusifolius* is an Al-hyperaccumulator with the highest concentrations of oxalate in leaves, of malate in stems, and of citrate in belowground organs. These organic acids form strong complexes with Al that can play a key role in internal Al tolerance but the used methods did not allow us to distinguish the proportion of total Al-organic complexes to the free organic acids.

Introduction

Accumulation of Al by plants has been seldom studied in weakly acidic soils [1–4]. It is known that uptake of Al by plants is affected by its mobile, potentially bioavailable forms in soils [4–6]. Mobile forms of metals are often referred to as exchangeable, water-soluble, and carbonate bound fractions [4,5]. Exudation of low-molecular-weight organic acids (LMWOAs; mainly citric, malic, and oxalic acids) commonly identified in the root zone can also play an important role in the uptake of Al by plants [7–9]. These natural organic substances are able to release metals from the exchangeable, carbonate, and reducible fractions and form soluble metal-organic acid complexes that are available to plants [10–13].

Many studies have investigated plants with mechanisms to tolerate high concentrations of Al, but usually in acidic soils [14,15]. Phytotoxicity of Al depends primarily on its chemical forms and not on the total accumulated amount in plants; not all species of mobile Al present an equally high toxicity to plants [3,9,16]. Aluminium toxicity to plants decreases in the order: polymer Al_{13} (not in a form of phosphates or silicates) > Al^{3+} > $Al(OH)^{2+}$ > $Al(OH)^+_2$ > $Al(OH)^-_4$. Less or non-toxic Al species are supposed to be bound in sulphate, phosphate, silicate, fluoride or organic acids and $Al(OH)_3^0$ [17–21]. Chemical forms of Al are affected by soil pH [3,4,6,19]. Organic acids play a key role in Al tolerance mechanisms in plants; the type of organic acids and the secretion pattern depends on plant species [14,17,22–24]; distribution of organic acids between plant organs can be also crucial. Some plants detoxify Al externally, in the rhizosphere by releasing organic acids that chelate Al (i.e., *Triticum aestivum*, *Zea mays*, *Fagopyrum esculentum* or *Nicotiana tabacum*). Other plants, including species that accumulate Al in their leaves, detoxify Al internally by forming complexes with organic acids (i.e., *Camellia sinensis*, *Fagopyrum esculentum*, *Hydrangea* spp., *Melastoma malabathricum* or *Vaccinium macrocarpon*) [7,25–27].

Rumex obtusifolius subsp. *obtusifolius* (broad-leaved dock) is an important model plant with several specificities: (i) a widespread weedy species on arable land and in temperate grasslands [28], (ii) an oxalate plant with internal defence mechanism against Ca excess [29–30], (iii) an As-, Cd-, Pb-, and Zn-excluder not suitable for phytoremediation of heavily contaminated soils [31], and (iv) highly resistant to Al but the mechanism has been investigated only in hydroponics under strongly acidic conditions [30]. The high Al resistance of *R. obtusifolius* together with its ability to accumulate (>1000 mg/kg) [32] or even hyper-accumulate (>3000 mg/kg) [33] Al was not sufficiently described and explained in real soil conditions. The potential elucidation of the role of organic acids in plant organs can help to explain the mechanism of Al tolerance. The distribution of organic acids between aboveground organs in *Rumex* species has been studied [30,34] but insufficient information is available concerning the composition of organic acids in its belowground organs that are responsible for soil-plant interactions.

During our previous research [28,31], we found specific behaviour of Al in *R. obtusifolius* growing in tested soils (i.e. extremely high total concentration of Al in biomass compare to

64

common plants). Therefore, we focused present study on the Al issue in this plant-soil interaction. The aim of this paper was to investigate the effect of slightly acidic and alkaline soils untreated and treated by lime and superphosphate on 1) concentration of mobile forms of Al as determined by mild soil extractants (KCl, $CaCl_2$, and H_2O) and by organic acids identified in root exudates (AA—acetic acid, CA—citric acid, and OA—oxalic acid), 2) concentration of individual positively charged Al species ($Al(X)^{1+}$, $Al(Y)^{2+}$, and Al^{3+}) presented in exchangeable (KCl) and water-soluble (H_2O) fraction, and on 3) distribution of total and infusion Al as well as of LMWOAs between organs of *R. obtusifolius*.

Materials and Methods

Pot Experiment

Two long-term heavily anthropogenically contaminated soils were used for the pot experiment in present study as well as in previous studies [28,31,35] with main chemical properties; 'Litavka Fluvisol (49°43'N, 14°0'E)' containing 354 mg As_{AR}/kg, 54 mg Cd_{AR}/kg, 3305 mg Pb_{AR}/kg, and 6172 mg Zn_{AR}/kg; characterised by pH_{CaCl_2} 6.5, CEC 109 mmol$_{(+)}$/kg, and C_{org} 3.6% and 'Malín Luvisol (49°58′N, 15°17′E)' containing 688 mg As_{AR}/kg, 11 mg Cd_{AR}/kg, and 1022 mg Zn_{AR}/kg; characterised by pH_{CaCl_2} 7.3, CEC 333 mmol$_{(+)}$/kg, and C_{org} 2.7%. Litavka soil (forest soil) was sampled from the Ah horizon (0–15 cm) after removing the greensward layer; Malín soil (common arable soil) was sampled from the topsoil in the layer at 0–25 cm depth after removing the greensward layer.

The availability of Al in slightly acidic 'Litavka' and alkaline 'Malín' soils, which were both untreated and treated, was manipulated by lime and superphosphate application. We applied 7.3 g lime (CaO) per 1 kg of soil containing 686 g Ca/kg of material with pH_{CaCl_2} 12.0 and 1.3 g superphosphate [$Ca(H_2PO_4)_2 \ H_2O$] per 1 kg of soil containing 246 g P/kg and 159 g Ca/kg of material with pH_{CaCl_2} 2.2. The pot experiment was established in May 2011 with six treatments each with five replications: LC—Litavka control soil without any additive, LCa—Litavka soil with lime, LP—Litavka soil with superphosphate, MC—Malín control soil without any additive, MCa—Malín soil with lime, and MP—Malín soil with superphosphate. Five kg of air dried soil was passed through a 10 mm sieve then transferred to 5-L plastic pots (20 cm in diameter and height). In each pot, the whole soil profile was mixed with nutrient solution, consisting of 0.5 g N as NH_4NO_3, 0.16 g P and 0.4 g K as K_2HPO_4. Application of nutrient solution was performed, to ensure that N, P, and K availability was non-limiting for the growth of *R. obtusifolius* in all treatments. The lime and superphosphate additives were mixed with the soil after application of nutrient solution.

Rumex obtusifolius plants were grown in the pots for six months. The pots were regularly watered with deionised water to maintain the optimal moisture conditions for plant growth during the vegetation. At the harvest, plant biomass (3 plants per pot) was divided into belowground organs, stems, leaves, and seeds (i.e., achenes with a perianth) and subsequently soil samples were collected from the whole soil profile of each pot.

Soil Analysis

For all chemical analyses, soil samples were air dried at 25°C and sieved to ≤2 mm. Before establishment of the pot experiment basic parameters of the experimental soils were determined by commonly used methods; microwave assisted high pressure *Aqua regia* (*AR*)-digestion (Ethos 1, MLS GmbH, Germany) for the determination of pseudo-total concentration of Al in soils by means of inductively coupled plasma-optical emission spectrometry (ICP-OES, VARIAN Vista Pro, Varian, Australia), soil pH in a 1/5 (w/v) suspension of soil and 0.01 mol/L $CaCl_2$, cation-exchange capacity (CEC) was determined according to Schwertfeger and

65

Hendershot [36], and organic carbon content (C_{org}) colorimetrically according to Sims and Haby [37].

At the end of the experiment, soil samples were subjected to extraction with 0.5 mol/L KCl (adjusted to pH 5.8 by dilute HCl and KOH solutions; to extract the exchangeable fraction), 0.01 mol/L $CaCl_2$ (pH 5.9; exchangeable fraction), and deionised water (H_2O; pH 5.2; water-soluble fraction) in ratios 1/10 (w/v) [5,38] and by 0.11 mol/L acetic acid (AA; pH 2.8; exchangeable and carbonate fractions) [12,39], 0.11 mol/L citric acid (CA; pH 1.9; exchangeable, carbonate, and reducible fractions) [10,13], and 0.11 mol/L oxalic acid (OA; pH 1.3; exchangeable, carbonate, and reducible fractions) [10,13] in ratios 1/20 (w/v). The final concentration of AA, CA, and OA solutions was not realistic. The total concentration of Al in soil extracts (Al_{KCl}, Al_{CaCl2}, Al_{H2O}, Al_{AA}, Al_{CA}, and Al_{OA}) was determined using ICP-OES under standard conditions. Soil pH was measured in a suspension of soil and 0.01 mol/L $CaCl_2$ (1/5, w/v) and 0.11 mol/L AA, CA, and OA (1/20, w/v). Detailed speciation of the exchangeable (KCl) and water-soluble forms of Al [species: $Al(X)^{1+}$, $Al(Y)^{2+}$, Al^{3+}; and the sum of all forms of Al, Σ Al] according to the value of their positive charge was done by means of high performance liquid chromatography equipped with an ion column (HPLC/IC, Dionex, USA) [40]. Before analysis, samples were centrifuged and filtered using a 0.45-μm nylon membrane filter (Cronus Membrane Filter Nylon, GB) [5].

Plant Analysis

The total concentration of Al in organs (Al/total; air-dried at 60°C and stainless-steel milled) was determined by ICP-OES after microwave assisted high pressure acid-digestion (65% HNO_3:30% H_2O_2 4:1). Certified reference material (CTA-OTL-1 oriental tobacco leaves) was mineralised under the same conditions for quality assurance. The concentration of Al in organ infusions (Al/infusion) was determined by ICP-OES after leaching (15 min) and filtering of the suspension of organ biomass and boiled deionised water (1/50, w/v) [41] through filtration paper 'Filtrak 390' (Niederschlag, Germany) with porosity 3–5 μm and flow rate 0.1 ml/s [DIN 53137]. After filtration through a 0.45-μm nylon membrane filter, concentrations of low-molecular-weight organic acid (LMWOAs; acetate, citrate, formate, lactate, malate, maleate, propionate, tartrate, and oxalate) anions in the same organ infusions were determined by means of ion-exchange chromatography with suppressed conductivity. An 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 a gradient of 1–50 min; and flow rate was set to 1 mL/min. To suppress eluent conductivity an ASRS 300–4 mm suppressor (Dionex, USA) and Carbonate Removal Device 200 (Dionex, USA) were used.

Statistical Analysis

The statistical analysis was performed using Statistica 12.0 software (www.statsoft.com). All data were checked for homogeneity of variance and normality (Levene and Shapiro-Wilk tests). Soil and biomass data did not meet assumptions for the use of ANOVA and thus were evaluated by the non-parametric Kruskal-Wallis test. We assessed the effects of 1) treatment on soil pH, concentrations of Al in the soil and biomass and on concentration of LMWOAs in the biomass, 2) method of determination on concentration of Al, and 3) organ type on concentrations of Al and LMWOAs in the biomass. After obtaining significant results from the Kruskal-Wallis test, we used multiple comparisons of mean ranks for the detection of significant differences between different treatments or organs. The relationship between concentrations of 1) Al in different soil extracts, 2) Al/total (Al/infusion) in organs and Al in different soil extracts, and 3) Al/infusion and LMWOAs in organs was analysed by linear regression. A

66

principal component analysis (PCA), in the CANOCO 4.5 program [42], was applied to all collected data together 1) concentration of Al in the soil extracts and 2) concentrations of Al/total, Al/infusion, and LMWOAs in the biomass of organs. We used standardisation of species data because data of different character were analysed together. The PCA was used to make visible correlations between all analysed data and similarity of different treatments. Obtained results were visualised in the form of a bi-plot ordination diagram in CanoDraw program.

Results

Fractionation of Al Using Soil Extractants

The concentration of Al extracted by *AR*, $CaCl_2$, H_2O, AA, CA, and OA was significantly affected by tested soil as well as by applied treatments (see Table 1 for details).

The efficiency of KCl and $CaCl_2$ for extractability of Al was comparable in both soils (LC, LCa, LP, MC, MCa, and MP treatments). Higher efficiency of H_2O and AA for Al extractability was recorded in Litavka soil (LC, LCa, and LP treatments) and of CA and OA was recorded in Malín soil (MC, MCa, and MP treatments; see Fig 1). A significant negative relationship was recorded between the concentration of Al extracted by H_2O and CA ($r = -0.947$; $p < 0.01$), by H_2O and OA ($r = -0.944$; $p < 0.01$), by AA and CA ($r = -0.549$; $p < 0.01$), and by AA and OA ($r = -0.562$; $p < 0.01$). A significant positive relationship was recorded between concentrations of Al extracted by H_2O and AA ($r = 0.408$; $p = 0.043$) and by CA and OA ($r = 0.997$; $p < 0.01$).

Liming (LCa and MCa treatments) and application of superphosphate (LP and MP treatments) did not significantly affect the concentration of Al in soils.

Table 1. Effect of treatment on soil pH (CaCl2, AA, CA, and OA), pseudo-total (mg/kg; extracted by *Aqua regia*; *AR*), exchangeable (mg/kg; extracted by 0.5 mol/L KCl or 0.01 mol/L CaCl2), water-soluble (mg/kg; extracted by deionised water; H2O), exchangeable and carbonate (mg/kg; extracted by 0.11 mol/L acetic acid; AA), and exchangeable, carbonate, and reducible (mg/kg; extracted by 0.11 mol/L citric acid; CA or by 0.11 mol/L oxalic acid; OA) concentration of Al (mean ± SE) at the end of the experiment.

Variable	Treatment					
	LC	LCa	LP	MC	MCa	MP
pH_{CaCl2} **	$5.8^b \pm 0.01$	$7.5^a \pm 0.01$	$5.9^b \pm 0.02$	$7.2^{ab} \pm 0.03$	$7.6^a \pm 0.02$	$7.2^{ab} \pm 0.01$
pH_{AA} **	$3.9^b \pm 0.01$	$4.3^{ab} \pm 0.03$	$3.9^b \pm 0.003$	$4.6^{ab} \pm 0.03$	$5.0^a \pm 0.02$	$4.6^{ab} \pm 0.01$
pH_{CA} **	$2.3^b \pm 0.01$	$2.5^{abc} \pm 0.02$	$2.3^{bc} \pm 0.01$	$2.5^{ac} \pm 0.01$	$2.7^a \pm 0.02$	$2.5^{bc} \pm 0.02$
pH_{OA} **	$1.4^b \pm 0.01$	$1.4^{ab} \pm 0.01$	$1.4^b \pm 0.02$	$1.4^{ab} \pm 0.01$	$1.4^a \pm 0.01$	$1.4^{ab} \pm 0.01$
Al_{AR} *	$11067^b \pm 317$	-	-	$13482^a \pm 595$	-	-
Al_{KCl} n.s.	$11^a \pm 0.1$	$9^a \pm 0.1$	$10^a \pm 2$	$10^a \pm 1$	$12^a \pm 1$	$11^a \pm 1$
Al_{CaCl2} *	$12^{ab} \pm 1$	$9^b \pm 1$	$15^{ab} \pm 3$	$11^{ab} \pm 1$	$18^a \pm 1$	$13^{ab} \pm 1$
Al_{H2O} **	$81^{ab} \pm 3$	$70^{abc} \pm 2$	$106^a \pm 5$	$22^{bc} \pm 1$	$15^c \pm 1$	$21^{bc} \pm 1$
Al_{AA} *	$113^a \pm 11$	$102^{ab} \pm 21$	$86^{ab} \pm 8$	$59^a \pm 3$	$80^{ab} \pm 4$	$69^{ab} \pm 6$
Al_{CA} **	$1188^{ab} \pm 9$	$1197^{ab} \pm 7$	$1149^b \pm 5$	$1968^a \pm 8$	$1896^{ab} \pm 2$	$1974^a \pm 4$
Al_{OA} **	$3070^{ab} \pm 5$	$2940^b \pm 42$	$2972^b \pm 17$	$5119^a \pm 35$	$4908^{ab} \pm 8$	$5052^a \pm 25$

– indicates not determined

Treatment abbreviations: LC—Litavka control soil without any additive, LCa—Litavka soil with lime, LP—Litavka soil with superphosphate, MC—Malín control soil without any additive, MCa—Malín soil with lime, and MP—Malín soil with superphosphate.

Calculated by Kruskal-Wallis test, differences between treatments were not statistically significant (n.s.) or were significant at 0.05 (*) and 0.01 (**) probability levels. According to the multiple comparisons of mean ranks, treatments with the same letter were not significantly different.

doi:10.1371/journal.pone.0123351.t001

67
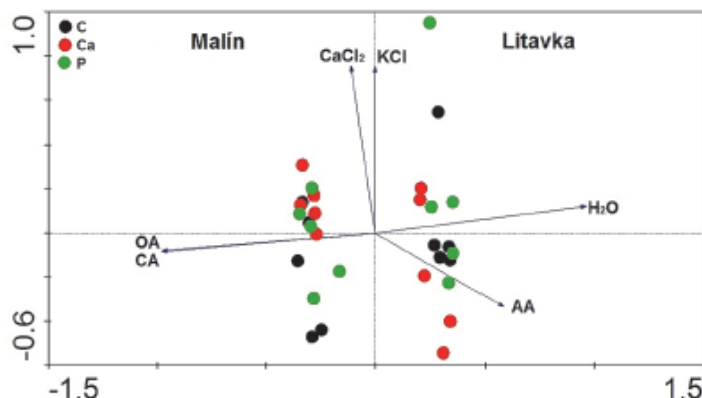
Fig 1. Ordination diagram showing the results of PCA analysis with total concentration of Al in soil extracts in contaminated slightly acidic Litavka and alkaline Malín soils. Treatment abbreviations: C—control, Ca—lime application, P—superphosphate application. Soil extractant abbreviations: KCl—exchangeable concentration of Al in soil (extracted by 0.5 mol/L KCl), CaCl₂—exchangeable concentration of Al in soil (extracted by 0.01 mol/L CaCl₂), H₂O —water-soluble concentration of Al in soil (extracted by deionised water), AA—exchangeable and carbonate concentration of Al in soil (extracted by 0.11 mol/L acetic acid), CA—exchangeable, carbonate, and reducible concentration of Al in soil (extracted by 0.11 mol/L citric acid), and OA—exchangeable, carbonate, and reducible concentration of Al in soil (extracted by 0.11 mol/L oxalic acid).

doi:10.1371/journal.pone.0123351.g001

Speciation of Exchangeable (KCl) and Water-Soluble Forms of Al in the Soil

The difference between concentrations of Σ Al and Al_{KCl} (Al_{H2O}) was significantly affected by the method of determination in all treatments (see Fig 2 for details).

Exchangeable and water-soluble concentrations of $Al(X)^{1+}$, Σ Al and water-soluble concentration of $Al(Y)^{2+}$ were significantly affected by treatments (see Table 2 for details).

Liming (LCa and MCa treatments) increased the exchangeable and water-soluble concentrations of $Al(X)^{1+}$ in both soils (LC and MC treatments). Concentrations of exchangeable Al $(Y)^{2+}$ and Al^{3+} were below the limit of detection (0.5 mg Al/kg) in all treatments.
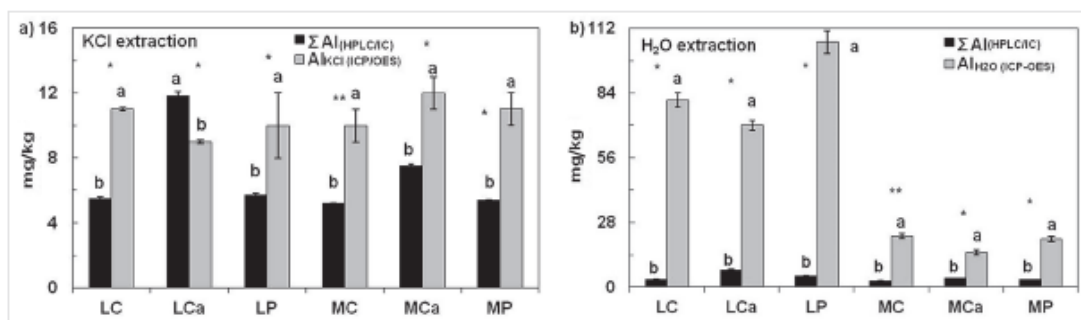
Fig 2. Effect of method of determination (HPLC/IC and ICP-OES) on mean concentration of exchangeable and water-soluble forms of Al (total AlKCl and total AlH2O; and Σ Al, all Al forms positively charged) at the end of the experiment. Treatment abbreviations: LC—Litavka control soil without any additive, LCa—Litavka soil with lime, LP—Litavka soil with superphosphate, MC—Malín control soil without any additive, MCa—Malín soil with lime, and MP—Malín soil with superphosphate. Error bars represent SE. Based on Kruskal-Wallis test, differences between methods of determination were significant at 0.05 (*) and 0.01 (**) probability levels. Using multiple comparisons of mean ranks, methods of determination with the same letter were not significantly different.

doi:10.1371/journal.pone.0123351.g002

68

Table 2. Effect of treatment on exchangeable and water-soluble forms of Al (mg/kg; species: Al(X)1+, Al(Y)2+, Al3+; and Σ Al—all Al forms positively charged; total AlKCl and total AlH2O; mean ± SE) at the end of the experiment.

Extracting reagent	Variable	Treatment					
		LC	LCa	LP	MC	MCa	MP
KCl	$Al(X)^{1+}$ **	$5.5^{abc} \pm 0.1$	$11.8^a \pm 0.3$	$5.7^{abc} \pm 0.1$	$5.2^c \pm 0.04$	$7.5^{ab} \pm 0.1$	$5.4^c \pm 0.05$
	$Al(Y)^{2+}$	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	Al^{3+}	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	Σ Al **	$5.5^{abc} \pm 0.1$	$11.8^a \pm 0.3$	$5.7^{abc} \pm 0.1$	$5.2^c \pm 0.04$	$7.5^{ab} \pm 0.1$	$5.4^c \pm 0.05$
	Al_{KCl} n.s.	$11^a \pm 0.1$	$9^a \pm 0.1$	$10^a \pm 2$	$10^a \pm 1$	$12^a \pm 1$	$11^a \pm 1$
H$_2$O	$Al(X)^{1+}$ **	$3.3^{ab} \pm 0.1$	$5.8^a \pm 0.3$	$3.5^{ab} \pm 0.1$	$2.8^b \pm 0.1$	$4.0^a \pm 0.04$	$3.2^{ab} \pm 0.1$
	$Al(Y)^{2+}$ *	<0.5	$0.9^a \pm 0.1$	$0.6^b \pm 0.05$	<0.5	<0.5	<0.5
	Al^{3+} n.s.	<0.5	$0.7^a \pm 0.1$	$0.6^a \pm 0.1$	<0.5	<0.5	<0.5
	Σ Al **	$3.3^{abc} \pm 0.1$	$7.4^a \pm 0.4$	$4.8^{ab} \pm 0.1$	$2.8^c \pm 0.05$	$4.0^{abc} \pm 0.04$	$3.2^{bc} \pm 0.1$
	Al_{H2O} **	$81^{ab} \pm 3$	$70^{abc} \pm 2$	$106^a \pm 5$	$22^{bc} \pm 1$	$15^c \pm 1$	$21^{bc} \pm 1$

limit of detection (mg/kg): 0.5

Treatment abbreviations: LC—Litavka control soil without any additive, LCa—Litavka soil with lime, LP—Litavka soil with superphosphate, MC—Malín control soil without any additive, MCa—Malín soil with lime, and MP—Malín soil with superphosphate.

Calculated by Kruskal-Wallis test, differences between treatments were not statistically significant (n.s.) or were significant at 0.05 (*) and 0.01 (**) probability levels. According to the multiple comparisons of mean ranks, treatments with the same letter were not significantly different.

doi:10.1371/journal.pone.0123351.t002

Concentrations of water-soluble $Al(Y)^{2+}$ and Al^{3+} were below the limit of detection (0.5 mg Al/kg) in most of the treatments (LC, MC, MCa, and MP). Liming (LCa treatment) and application of superphosphate (LP treatment) increased the water-soluble concentrations of $Al(Y)^{2+}$ and Al^{3+} in soil (LC treatment).

Concentration of Al in the Plant Organs

The Al/total concentration was significantly affected by treatments and analysed plant organs (see Table 3 for details). The concentration of Al/total in seeds ranged from 51±19 mg/kg in LCa treatment to 718±213 mg/kg in MC treatment. No stems or seeds were produced in the LC and LP treatments.

A tendency for higher Al/total concentration in organs was recorded in Malín soil (MC, MCa, and MP treatments) in comparison to Litavka soil (LC, LCa, and LP treatments).

A significant positive relationship was recorded between Al/total concentration in leaves and Al_{OA} concentration in soil (all treatments all together; r = 0.756, p<0.01), and between Al/total concentration in organs and Al_{CA} concentration in soil (all treatments all together; stems: r = 0.652, p = 0.012; leaves: r = 0.717, p<0.01; seeds: r = 0.560, p = 0.047).

Liming (LCa and MCa treatments) and application of superphosphate (MP treatment) affected the distribution of Al/total concentration between organs in the order: belowground organs < leaves in comparison to LC and MC treatments (belowground organs > leaves).

Concentrations of LMWOAs in the Organs

The concentrations of propionate, malate, maleate, and citrate were significantly affected by treatments, and the concentrations of acetate, citrate, formate, lactate, malate, maleate, propionate, tartrate, and oxalate differed between individual organs (see Table 4 for details).

In all treatments, a tendency for higher accumulation of citrate, maleate, and tartrate was recorded in belowground organs; a tendency for higher accumulation of malate was recorded in

69

Table 3. Effect of treatment on concentration of total and infusion Al (mg/kgDW; mean ± SE) in organs of *R. obtusifolius*.

Variable	Treatment	Organ		
		Belowground organs	Stems	Leaves
Al/total	LC	1702[aA] ± 604	-	471[aBC] ± 76
deficient	LCa	693[abA] ± 344	41[bB] ± 9	933[aABC] ± 244
normal <100–200[1]	LP	1052[aA] ± 433	-	384[aC] ± 88
phytotoxic	MC	3514[abA] ± 1583	169[bAB] ± 63	3413[aAB] ± 794
accumulation level >1000[1]	MCa	584[abA] ± 257	133[bAB] ± 49	1772[aABC] ± 528
hyperaccumulation level >3000[2]	MP	1767[abA] ± 792	198[bA] ± 25	3858[aA] ± 794
Al/infusion	LC	10.2[A]	-	-
	LCa	4.9[abA] ± 0.5	1.2[bA] ± 0.4	93[aA] ± 40
	LP	6.1[aA] ± 0.6	-	6.2[aA] ± 2.6
	MC	5.9[bA] ± 2.4	4.1[abA]	75[aA] ± 19
	MCa	3.6[bA] ± 0.5	6.3[abA] ± 3.5	38[aA] ± 14
	MP	5.9[aA] ± 1.3	-	97[bA] ± 21

References: 1– [32], 2– [33]

—indicates no material

Treatment abbreviations: LC—Litavka control soil without any additive, LCa—Litavka soil with lime, LP—Litavka soil with superphosphate, MC—Malín control soil without any additive, MCa—Malín soil with lime, and MP—Malín soil with superphosphate.

Differences between treatments and organs were evaluated by Kruskal-Wallis test. For each element, concentrations in organs within one treatment denoted with the same letter (a-b) and concentrations in treatments within one organ denoted with the same letter (A-C) were not significantly different.

doi:10.1371/journal.pone.0123351.t003

stems; and a tendency for higher accumulation of acetate, formate, lactate, propionate, and oxalate was recorded in leaves.

The distribution of total concentration of LMWOAs (65426 mg/kgDW, i.e., sum of the mean value of all organic acids concentration in the organs) between organs was recorded in the order: belowground organs (17091 mg/kgDW; 26% of total LMWOAs) < stems (17951 mg/ kgDW; 27% of total LMWOAs) < leaves (30384 mg/kgDW; 47% of total LMWOAs). Representation of LMWOAs in belowground organs was in the order: citrate (36%) > tartrate (31%) > malate (14%) > oxalate (9%) > acetate, lactate (4%) > formate (1%) > maleate (0.4%) > propionate (0.05%); in stems was in order malate (39%) > oxalate (32%) > citrate (12%) > lactate (8%) > acetate, tartrate (4%) > formate (1.5%) > propionate (0.3%) > maleate (0.1%); and in leaves was in the order: oxalate (39%) > lactate (17%) > citrate (15%) > malate, tartrate (11%) > acetate (6%) > formate (1%) > propionate (0.5%) > maleate (0.03%).

A significant positive relationship was recorded between concentrations of Al/infusion and LMWOAs in belowground organs (acetate: $r = 0.670$, $p<0.01$; formate = 0.574, $p<0.01$), between concentrations of Al/infusion and lactate in stems ($r = 0.944$, $p = 0.016$), and between concentrations of Al/infusion and LMWOAs in leaves (acetate: $r = 0.695$, $p<0.01$; lactate = 0.773, $p<0.01$).

Liming (LCa and MCa treatments) and application of superphosphate (LP and MP treatments) did not lead to the unambiguous changes in the distribution of LMWOAs between organs.

Results of PCA Analysis

Soil. The first axis of the PCA analysis explained 53%, the first two axes 75% and the first four axes together, 99% of the variability of all analysed data (Fig 1). The first ordination axis divided marks for individual pots into Litavka group on the right side and Malín group on the

70

Table 4. Effect of treatment on concentrations of LMWOAs (mg/kgDW, mean ± SE) in organs of *R. obtusifolius*.

Variable	Treatment	Organ		
		Belowground organs	Stems	Leaves
Acetate	LC	817[A]	-	-
	LCa	674[aA] ± 6	614[aA] ± 63	2028[aA] ± 491
	LP	629[aA] ± 47	-	641[aA] ± 14
	MC	623[bA] ± 96	1089[abA]	1898[aA] ± 368
	MCa	643[aA] ± 60	569[aA] ± 7	1852[aA] ± 11
	MP	662[aA] ± 52	-	1887[aA] ± 493
Citrate	LC	8825[AB]	-	-
	LCa	5845[aAB] ± 786	2028[aA] ± 858	5629[aA] ± 806
	LP	7939[aA] ± 886	-	6754[aA] ± 347
	MC	6068[aAB] ± 522	2094[aA]	4275[aA] ± 630
	MCa	5838[aAB] ± 307	2130[bA] ± 582	4742[abA] ± 550
	MP	5202[aB] ± 151	-	3875[bA] ± 487
Formate	LC	244[AB]	-	-
	LCa	184[bAB] ± 20	238[abA] ± 22	328[aAB] ± 9
	LP	153[aAB] ± 12	-	267[aB] ± 19
	MC	251[aA] ± 33	418[aA]	362[aAB] ± 27
	MCa	89[bB] ± 8	218[abA] ± 4	443[aA] ± 45
	MP	204[bAB] ± 38	-	367[aAB] ± 6
Lactate	LC	744[A]	-	-
	LCa	914[bA] ± 113	1303[abA] ± 70	5982[aA] ± 1398
	LP	646[aA] ± 70	-	2424[aA] ± 40
	MC	733[bA] ± 14	1615[abA]	6442[aA] ± 812
	MCa	707[bA] ± 57	1587[abA] ± 226	4081[aA] ± 956
	MP	723[bA] ± 25	-	6385[aA] ± 466
Malate	LC	2429[AB]	-	-
	LCa	2951[bA] ± 213	6504[aA] ± 340	3751[abAB] ± 125
	LP	1877[aB] ± 115	-	2996[aAB] ± 508
	MC	2290[aAB] ± 72	8769[aA]	2821[aAB] ± 245
	MCa	2464[bAB] ± 292	6679[aA] ± 1582	4527[aA] ± 487
	MP	2408[aAB] ± 240	-	2197[aB] ± 487
Maleate	LC	88[AB]	-	-
	LCa	139[aA] ± 19	16[abA] ± 4	<2.43[bA]
	LP	104[aAB] ± 14	-	<2.43[aA]
	MC	<2.43[aB]	<2.43[aA]	<2.43[aA]
	MCa	77[aAB] ± 25	17[aA] ± 10	12[aA] ± 7
	MP	<2.43[aAB]	-	12[aA] ± 6
Propionate	LC	<1.94[A]	-	-
	LCa	<1.94[aA]	<1.94[aA]	91[aAB] ± 57
	LP	48[aA] ± 20	-	363[aAB] ± 152
	MC	<1.94[aA]	132[aA]	<1.94[aAB]
	MCa	<1.94[bA]	<1.94[abA]	407[aA] ± 52
	MP	<1.94[aA]	-	<1.94[aB]

(Continued)

71

Table 4. (*Continued*)

Variable	Treatment	Organ		
		Belowground organs	**Stems**	**Leaves**
Tartrate	LC	6245^A	-	-
	LCa	5166^aA ± 335	659^bA ± 33	2324^abB ± 341
	LP	5362^aA ± 211	-	4063^aAB ± 372
	MC	5313^aA ± 230	1008^aA	4168^aAB ± 660
	MCa	5419^aA ± 255	550^bA ± 12	3076^abAB ± 111
	MP	4569^aA ± 85	-	4081^bA ± 165
Oxalate	LC	1510^A	-	-
	LCa	1355^bA ± 133	4427^abA ± 699	11019^aA ± 95
	LP	1401^aA ± 61	-	10884^aA ± 212
	MC	1570^bA ± 171	8449^abA	14594^aA ± 543
	MCa	1494^bA ± 45	5486^abA ± 158	13234^aA ± 1701
	MP	1569^bA ± 67	-	11180^aA ± 765

– indicates no material, limits of detection (mg/kg): acetate 1.37, citrate 3.18, formate 0.61, lactate 1.13, malate 1.85, maleate 2.43, propionate 1.94, tartrate 1.73, and oxalate 1.08.

Treatment abbreviations: LC—Litavka control soil without any additive, LCa—Litavka soil with lime, LP—Litavka soil with superphosphate, MC—Malín control soil without any additive, MCa—Malín soil with lime, and MP—Malín soil with superphosphate.

Differences between treatments and organs were evaluated by Kruskal-Wallis test. For each LMWOA, concentrations in organs within one treatment denoted with the same letter (a-b) and concentrations in treatments within one organ denoted with the same letter (A-B) were not significantly different.

left side of the diagram. This indicates a high effect of used soils on the extractability of Al. In both soils, marks for treatments (C, Ca, and P) were not clearly separated, which indicates minimal effect of treatments on all the recorded data. The length and direction of the vectors relating to the Al concentrations indicate the association of the extractants with respect of studied soil. Di- and tri-carboxylic acids such as OA and CA extracted more Al in alkaline Malín soil and mono-carboxylic acid AA and H_2O extracted more Al in slightly acidic Litavka soil. Extractants KCl and $CaCl_2$ had minimal effect on extractability of Al in both soils as shown by arrows not leading to any of the soils. Concentrations of Al_{CA} and Al_{OA} were negatively correlated with Al_{H2O} as indicated by opposing directions of vectors for CA (OA) and H_2O.

Plant Biomass. The first axis of the PCA analysis explained 42%, the first two axes 69% and the first four axes together, 86% of the variability of all analysed data (Fig 3). In the diagram, marks for treatments (LC, LCa, LP, MC, MCa, and MP) were not clearly separated indicating a weak effect of soils and treatments on all the recorded data. Marks for organs (belowground organs, stems, and leaves) were located in different parts of the diagram, which indicates a high effect of organs on all the recorded data. The length and direction of the vectors relating to the Al and LMWOAs concentrations indicate the association of the Al as well as LMWOAs with respect of organ. Concentrations of citrate, maleate, and tartrate were accumulated more in belowground organs. Concentrations of Al/infusion as well as concentrations of acetate, formate, lactate, propionate, and oxalate accumulated more in leaves and malate accumulated more in stems. Al/total accumulated more in belowground organs as well as in leaves.

72
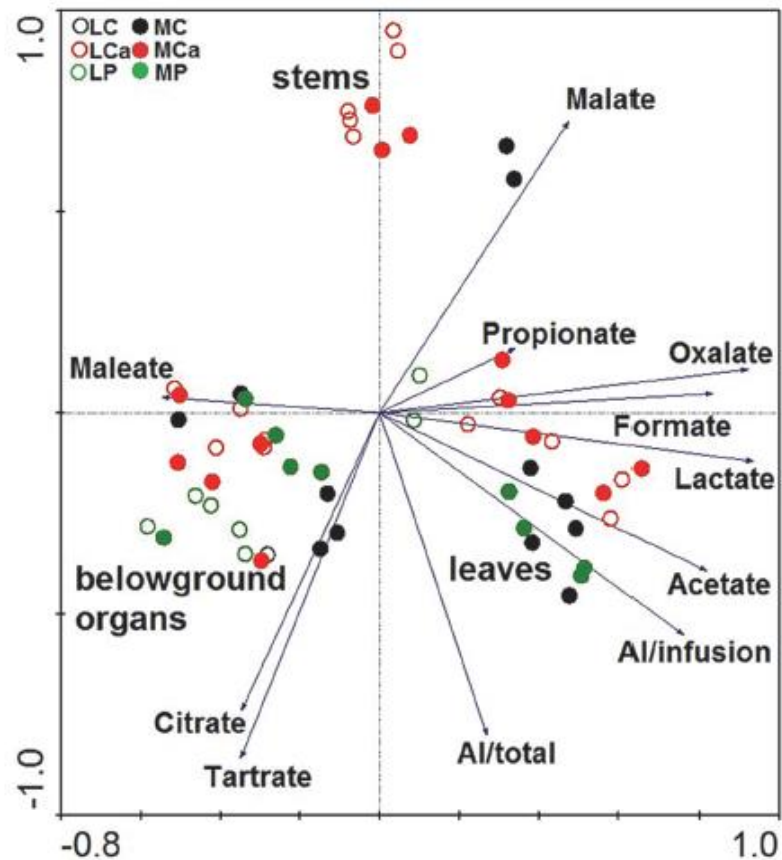
Fig 3. Ordination diagram showing the results of PCA analysis with concentrations of Al and of LMWOAs in organs (belowground organs, stems, and leaves) of *R. obtusifolius* grown on contaminated soils. Treatment abbreviations: LC—Litavka control soil without any additive, LCa—Litavka soil with lime, LP—Litavka soil with superphosphate, MC—Malín control soil without any additive, MCa—Malín soil with lime, and MP—Malín soil with superphosphate. LMWOAs abbreviations: acetate—concentration of acetate in organ infusions and etc. Al concentration abbreviations: Al/total—total concentration of Al in organs, Al/infusion—concentration of Al in organ infusions.

Discussion

Fractionation of Al Using Soil Extractants

Efficiency of extracting agents in LC and MC treatments increased in the following order: $Al_{KCl}, Al_{CaCl2} < Al_{H2O} < Al_{AA} < Al_{CA} < Al_{OA}$ representing $0.1, 0.1 < 0.2–0.7 < 0.4–1.0 < 11–15 < 28–38\%$ of pseudo-total soil Al (Al_{AR}). Exchangeable ($KCl, CaCl_2$), water-soluble (H_2O), and carbonate (AA) bound fractions of Al do not present an important role in Al mobility in slightly acidic and alkaline soils. The highest portions of Al were released by extracting with di- and tri-carboxylic acids (OA and CA), commonly identified in root exudates, which can also release Al bound in the reducible fraction [8,10–13]. The efficiency of these LMWOAs in soil can be explained by their dissociation constants: OA: pK_a 1.25 (I), 4.26 (II); CA: pK_a 3.13 (I), pK_a 4.76 (II), pK_a 6.40 (III); AA: pK_a 4.76. The number of dissociation constants corresponds to the number of COOH groups; a lower value of the negative logarithm of

73

the dissociation constant of acid—pK_a means a higher dissociation. i.e. A higher acid strength) [43] and therefore a decrease of soil pH (i.e., $pH_{AA} < pH_{CA} < pH_{OA}$; Table 1). Thus, we observed that the ability of LMWOAs to release Al from reducible fraction increased in the order AA < CA < OA. We can conclude from our experiment that 'exudation' of organic acids by *R. obtusifolius* (mainly oxalic acid) significantly affected the mobility of Al in slightly acidic and alkaline soils and helped to make a real estimate of Al uptake [12,22,44,45].

Reagents such as KCl and $CaCl_2$ were not suitable for the evaluation of available Al in all treatments. It is connected with the sorption complex with a dominant representation of the basic cations (Ca, Mg, and K) in comparison to the acidic cations (Al, Fe, and Mn) in slightly acidic and alkaline soils. Reagents such as H_2O and AA showed higher efficiency for extractability of Al in LC, LCa, and LP treatments in comparison to MC, MCa, and MP treatments. It is connected also with the exchangeable fraction, which has greater importance for Al in slightly acidic soil ('Litavka') than in alkaline soil ('Malín'). Deionised water and AA reagents with their lower solution pH (H_2O 5.2 and AA 2.8) are able to release Al into soil solution with higher efficiency than KCl and $CaCl_2$ reagents (KCl pH 5.8 and $CaCl_2$ pH 5.9) because of low representation of Al on the sorption complex. Therefore, pH probably plays a key role rather than the exchange of Al for K or Ca ions. Moreover, deionised water has high extraction power due to low ionic strength [46]. The AA reagent probably showed greater importance in releasing Al from the exchangeable fraction than from the carbonate bound fraction. It is because more carbonates were determined in the MC treatment (above 2% of carbonates) in comparison to the LC treatment (below 0.3% of carbonates; data not shown). Nevertheless, higher efficiency of the AA reagent was observed in slightly acidic soil (LC, LCa, and LP treatments). We can speculate that carbonates do not present important binding sites for Al [9] as a sorption complex in slightly acidic soils. Reagents such as CA and OA have higher efficiency for extractability of Al in MC, MCa, and MP treatments in comparison to LC, LCa, and LP treatments. It is probably connected with increased root exudates efficiency for organic acids exclusion in alkaline soil conditions (MC treatment) in comparison to slightly acidic soil conditions (LC treatment) [15]. The effect of liming (LCa and MCa treatments) on increase of soil pH was neglected with increasing strength of studied agents (see pH_{AA}, pH_{CA}, and pH_{OA} in Table 1).

Liming and application of superphosphate has an ambiguous effect on the total extractable concentration of Al (i.e. Al_{KCl}, Al_{CaCl2}, Al_{H2O}, Al_{AA}, Al_{CA}, and Al_{OA}) in soil indicating that our tested additives are not able to significantly alter the mobility of Al neither in slightly acidic nor in alkaline soils.

Speciation of Exchangeable (KCl) and Water-Soluble Forms of Al in the Soil

In most of the treatments, total extractable Al (i.e., Al_{KCl} and Al_{H2O}) was determined to be higher than the Σ Al, indicating that the derivatization agent 'Tiron (i.e., 4,5-dihydroxy-m-benzendisulfonic acid)' used in HPLC/IC did not react with certain strongly complexed Al forms with presumably zero or negative charge. However, these Al species undetectable by HPLC/IC can be determined by ICP-OES [5].

The most abundant exchangeable and water-soluble Al forms with positive charge in LC and MC treatments were $Al(X)^{1+}$ species (i.e., $Al(OH)_2^+$, $Al(SO_4)^+$, AlF_2^+, $Al(org.)^{\leq 1+}$, etc.; [5,40] representing 51% and 8.5% of total Al_{KCl} and Al_{H2O}, respectively. This can be explained by the distribution of soluble Al forms in the pH range between 5.8 and 7.2, with dominant representation of less or non-toxic Al forms with positive charge ($Al(X)^{1+}$) as well as with zero ($Al(OH)_3^0$, Al-organic complex) and negative charge ($Al(OH)_4^-$, Al-organic complex) [3,4,6,19].

74

Liming (LCa and MCa treatments) increased the exchangeable and water-soluble $Al(X)^{1+}$ form in comparison to the LC and MC treatments. Moreover, liming (LCa) and application of superphosphate (LP) caused the occurrence of new forms of Al such as $Al(Y)^{2+}$ (1.3% and 0.6% of Al_{H2O}, respectively) and Al^{3+} (1% and 0.6% of Al_{H2O}, respectively) which were identified in the H_2O-soil solution. It is because lime caused mineralisation of organic matter (representing an important binding site for Al) and decay of bound complexes to the individual forms of Al determinable using HPLC/IC [47,48]. In the case of superphosphate, the release of new forms of Al (i.e., $Al(Y)^{2+}$ and Al^{3+}) can be explained by a sufficient amount of available Ca from superphosphate that caused the decay of organic matter with lower stability.

Concentration of Al in the Plant Organs

Concentration of Al/total in aboveground organs was clearly positively related to the concentrations of Al_{CA} and Al_{OA} in soil, indicating their important role in uptake of Al by plants. Therefore, we can conclude that the Al availability for *R. obtusifolius* has been more affected by the presence of root exudates releasing organic acids (mainly oxalic acid) than by 'mobile forms' of Al (i.e., exchangeable, water-soluble, and carbonate) in slightly acidic and alkaline soils. A considerable decrease of pH down to 2.3 (CA:soil solution) and to 1.4 (OA:soil solution) is connected with possible uptake of Al by plants in the form of Al^{3+}, as has been recorded also by Ma et al. [7].

Higher concentration of Al/total in all organs was recorded in alkaline soil (MC treatment) in comparison to slightly acidic soil (LC treatment). It is probably due to a generally higher pseudo-total Al concentration in MC treatment (see Table 1) resulting in more available Al_{CA} and Al_{OA} present for plants, and in increased root exudates efficiency for organic acids exclusion in alkaline soil conditions than in slightly acidic soil conditions, as has been recorded by Arunakumara et al. [15].

A tendency for restricted transport of Al/total from belowground organs into the leaves was recorded in the LC treatment, in comparison to MC treatment. We can speculate that differences in the distribution can be connected with a different mechanism for detoxification of higher concentrations of Al/total in *R. obtusifolius* planted in slightly acidic (probably external detoxification of Al) and alkaline (probably internal detoxification of Al) soils. Low transport from belowground organ to leaf as a defence mechanism against high concentration of Al in plants was described in study of Poschenrieder et al. [21]. The effectiveness of both mechanisms for Al detoxification in the same plant is not unique (e.g. *Fagopyrum esculentum*) [49,50]. The mechanisms are known also for relatives to *R. obtusifolius*—*R. acetosella* (external) and *R. acetosa* (internal) planted in strongly acidic conditions [26,48].

R. obtusifolius is able to tolerate a high concentration of Al/total because in its leaves substantially higher Al concentration was recorded than is normal in many other plants (<200 mg/kg) in LC and MC treatments. In MC treatment, the concentration of Al/total in leaves was higher than 1000 mg/kg therefore we can speculate that *R. obtusifolius* belongs among the 'Al accumulators' or even the 'Al hyper-accumulators' (>3000 mg/kg) if planted in alkaline soils. High Al tolerance is probably connected with internal Al detoxification (formation of Al-complex, mainly with organic acids, in parts of leaves that are insensitive to Al, e.g., epidermal cells of vacuoles and cell walls) typical for 'Al-accumulators' but known mainly in acid soils [21,26,32,44,51,52].

In MC treatment, the concentration of Al/infusion in leaves was higher than in stems and belowground organs. In all treatments, higher leaching of Al from leaf and stem infusions (2–10% and 2–5% of Al/total, respectively) were recorded in comparison to belowground organ infusion (0.2–0.7% of Al/total), indicating higher representation of Al-organic complexes with

weak stability constants [16,53] in aboveground organs. The concentration of Al leached from leaf infusion of *R. obtusifolius* (in most treatments—2% of total Al) was lower in comparison to plants commonly used as beverages (i.e., *Hibiscus sabdariffa* petals—50% of total Al, *Rosa canina* receptacles—30% of total Al, *Camellia sinensis* leaves—10% of total Al, *Cymbopogon citratus* leaves—8% of total Al or *Ginkgo biloba* leaves—4% of total Al) [41,54]. Nevertheless, potential risk of a harmful effect of Al for humans remains because of the use of *R. obtusifolius* as a component of salad and soup, rather than as a beverage [55,56].

Liming (LCa and MCa treatments) and application of superphosphate (MP treatment) caused increased transport of Al/total from belowground organs into leaves in comparison to LC and MC treatments. It is probably due to the presence of oxalate (*R. obtusifolius* belongs to the group of 'oxalate plants') [29] that was precipitated with Ca as Ca-oxalate and thus available Al can be easily transported to leaves in LCa, MCa, and MP treatments. In LC and MC treatments, Al was probably immobilized as Al-oxalate in belowground organs, as has been recorded also for micro- (Cu, Fe, Mn, Ni) and risk elements (As, Cd, Cr, Pb, Zn) by Vondráčková et al. [31]. Nevertheless, the used methods did not allow us to determine the specific metal-organic complexes in plants or to distinguish the proportion of total metal-organic complexes (e.g. metal-oxalate complex) to the free organic acids (e.g. oxalic acid). Therefore, we are not able to verify the above-mentioned mechanism.

Concentrations of LMWOAs in the Organs

The LMWOAs were divided into three groups according to the detected concentration in organs. Citrate, maleate, and tartrate were recorded more in belowground organs. Malate was recorded more in stems, and acetate, formate, lactate, propionate, and oxalate were recorded more in leaves. The chemical structure of LMWOAs (position of OH/COOH groups on their main C chain) in connection with the stability constants (logKs) of Al-organic complexes can explain the ability of LMWOAs to create various strong complexes with Al in organs (higher value of the stability constant means higher stability of Al-organic complex) [16,43,53,57,58]. We can conclude that strong complexes of LMWOAs with Al (Al-citrate—logKs = 7.98 and Al-tartrate—logKs = 5.62) were recorded in belowground organs, moderate complexes of LMWOAs with Al (Al-malate—logKs = 5.40) were recorded in stems and weak complexes of LMWOAs with Al (Al-acetate—logKs = 1.60, Al-formate—logKs = 1.36, Al-lactate—logKs = 2.41, and Al-propionate—logKs = 1.78) were recorded in leaves. Nevertheless, two exceptions were recorded; Al-maleate complex with weak stability constant (logKs = 1.93) in belowground organs and Al-oxalate complex with strong stability constant (logKs = 6.16) in leaves. Concentration of Al in leaf infusions was clearly positively related to the concentrations of acetate (representing 6% of total LMWOAs) and lactate (17% of total LMWOAs); concentration of Al in stem infusions was clearly positively related to the concentration of lactate (8% of total LMWOAs); and concentration of Al in belowground organ infusions was clearly positively related to the concentrations of acetate (4% of total LMWOAs) and formate (1% of total LMWOAs), which indicates a weak stability complex of Al with these LMWOAs in organs. Therefore, possible release of Al into infusions was recorded with increasing order: belowground organs < stems < leaves.

The highest concentration of all LMWOAs all together was recorded in leaves, followed by stems and belowground organs. Oxalate, citrate, malate, and tartrate were dominant LMWOAs in leaves; malate, oxalate, and citrate were dominant LMWOAs in stems; and citrate, tartrate, and malate were dominant LMWOAs in belowground organs. Miyagi et al. [34] have recorded a similar result, i.e. oxalate as a major LMWOA in leaves of *R. obtusifolius* grown for a period of 2 and 5 weeks in a hydroponic experiment. A higher concentration of citrate in leaves in

76

comparison to stems was inconsistent with results for *R. obtusifolius* published by Miyagi et al. [34]—i.e., concentration of citrate was higher in stems. Discrepancies between concentrations of citrate in organs can be connected with the age of *R. obtusifolius* plants—i.e., 5-week-old plants have more citrate in stems and 6-month-old plants have more citrate in leaves. Citrate, oxalate, tartrate, and malate can create strong or moderate organic-Al complexes in organs [48,53]. Therefore we can speculate that these LMWOAs can partially contribute to the protection of *R. obtusifolius* against internal Al toxicity (i.e., mechanism of internal detoxification of Al known in plants that accumulate Al) [7,24,30,51].

Liming and application of superphosphate has an ambiguous effect on the concentration of LMWOAs in organs. We can thus conclude that our tested additives are not able to unambiguously alter the distribution of LMWOAs between organs of *R. obtusifolius* neither in slightly acidic nor in alkaline soils.

Conclusions

Reducible Al fraction represented by the CA and OA solution agents can serve as simulating exudation of organic acids by *R. obtusifolius* and play an important role in potential Al mobility and availability for these plants mainly in alkaline soils.

Less or non-toxic exchangeable and water soluble monovalent Al cations are dominant soluble Al forms in slightly acidic and alkaline soils with pH ranging from 5.8 to 7.2.

Rumex obtusifolius is an Al-hyperaccumulator but only in untreated alkaline soils. We can speculate that mechanism for detoxification of Al is affected by different soil chemical properties. It is possible to change distribution of total Al in plant organs by the manipulation of Al availability using lime and superphosphate in tested soils. Restricted transport of total Al from belowground organs into leaves was recorded in both untreated soils and in superphosphate-treated slightly acidic soil. On the other hand, elevated transport of total Al from belowground organs into leaves was recorded in both lime-treated soils and in superphosphate-treated alkaline soil. Therefore, we can speculate that sufficient amount of Ca available from soil solution as well as from superphosphate can change distribution of total Al in plants of *R. obtusifolius*.

The highest concentration of Al was recorded in leaf infusion, followed by stem and belowground organ infusions as a result of Al release from weak stability Al-organic complexes. Concentrations of LMWOAs were not affected by different soil chemical properties but were greatly affected by plant organs. In belowground organs more citrate, maleate, and tartrate were recorded; in stems there was more malate, and in leaves higher concentrations of acetate, formate, lactate, propionate, and oxalate were recorded. Citrate and tartrate create strong organic-Al complexes in belowground organs and conversely oxalate creates strong organic-Al complexes in leaves. The distribution of LMWOAs in plant organs can play a crucial role in internal Al tolerance but the used methods did not allow us to distinguish the proportion of total Al-organic complexes to the free organic acids. Therefore the future research should be focused on this issue.

Acknowledgments

Author Contributions

Conceived and designed the experiments: SV JS OD MH PT. Performed the experiments: SV VM. Analyzed the data: SV JS OD VT. Contributed reagents/materials/analysis tools: PT. Wrote the paper: SV JS MH PT.

References

1. Kinraide TB (1990) Assessing the rhizotoxicity of the aluminate ion, Al(OH)4-. Plant Physiol 93: 1620–1625. PMID: 16667665

2. Tyler G (1994) Plant uptake of aluminum from calcareous soils. Experientia 50: 701–703.

3. Brautigan DJ, Rengasamy P, Chittleborough DJ (2012) Aluminium speciation and phytotoxicity in alkaline soils. Plant Soil 360: 187–196.

4. Kassaye YA, Salbu B, Skipperud L, Einset J (2013) High tolerance of aluminum in the grass species *Cynodon aethiopicus*. Acta Physiol Plant 35: 1749–1761.

5. Drábek O, Mládková L, Bor vka L, Száková J, Nikodem A, N meček K (2005) Comparison of water-soluble and exchangeable forms of Al in acid forest soils. J Inorg Biochem 99: 1788–1795. PMID: 16095711

6. Namie nik J, Rabajczyk A (2010) The speciation of aluminum in environmental samples. Crit Rev Anal Chem 40: 68–88.

7. Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6: 273–278. PMID: 11378470

8. Ström L, Owen AG, Godbold DL, Jones DL (2005) Organic acid behaviour in a calcareous soil implications for rhizosphere nutrient cycling. Soil Biol Biochem 37: 2046–2054.

9. Ščančar J, Milačič R (2006) Aluminium speciation in environmental samples: a review. Anal Bioanal Chem 386: 999–1012. PMID: 16622673

10. Peters RW (1999) Chelant extraction of heavy metals from contaminated soils. J Hazard Mater 66: 151–210. PMID: 10379036

11. Gleyzes C, Tellier S, Astruc M (2002) Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures. Trac-Trends Anal Chem 21: 451–467.

12. Mucha AP, Almeida CMR, Bordalo AA, Vasconcelos MTSD (2005) Exudation of organic acids by a marsh plant and implications on trace metal availability in the rhizosphere of estuarine sediments. Estuar Coast Shelf Sci 65: 191–198.

13. Wuana RA, Okieimen FE, Imborvungu JA (2010) Removal of heavy metals from a contaminated soil using organic chelating acids. Int J Environ Sci Technol 7: 485–496.

14. Marschner H (1991) Mechanisms of adaptation of plants to acid soils. Plant Soil 134: 1–20.

15. Arunakumara KKIU, Walpola BC, Yoon MH (2013) Aluminum toxicity and tolerance mechanism in cereals and legumes—a review. J Korean Soc Appl Biol Chem 56: 1–9. doi: 10.3109/08923973.2012.736522 PMID: 23131135

16. Li JY, Xu RK, Tiwari D, Ji GL (2006) Effect of low-molecular-weight organic acids on the distribution of mobilized Al between soil solution and solid phase. Appl Geochem 21: 1750–1759.

17. Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. Annu Rev Plant Physiol Plant Mol Biol 46: 237–260.

18. Alvarez E, Fernández-Marcos ML, Monterroso C, Fernández-Sanjurjo MJ (2005) Application of aluminium toxicity indices to soils under various forest species. For Ecol Manage 211: 227–239.

19. Kubová J, Matúš P, Bujdoš M, Medve J (2005) Influence of acid mining activity on release of aluminium to the environment. Anal Chim Acta 547: 119–125.

20. Matúš P, Hagarová I, Bujdoš M, Diviš P, Kubová J (2009) Determination of trace amounts of total dissolved cationic aluminium species in environmental samples by solid phase extraction using nanometer-sized titanium dioxide and atomic spectrometry techniques. J Inorg Biochem 103: 1473–1479. doi: 10.1016/j.jinorgbio.2009.08.004 PMID: 19717191

21. Poschenrieder C, Gunsé B, Corrales I, Barceló J (2008) A glance into aluminum toxicity and resistance in plants. Sci Total Environ 400: 356–368. doi: 10.1016/j.scitotenv.2008.06.003 PMID: 18657304

22. Jones DL (1998) Organic acids in the rhizosphere—a critical review. Plant Soil 205: 25–44.

23. Ma JF (2005) Plant root responses to three abundant soil minerals: silicon, aluminum and iron. Crit Rev Plant Sci 24: 267–281.

24. Singh D, Chauhan SK (2011) Organic acids of crop plants in aluminium detoxification. Curr Sci 100: 1509–1515.

25. Shen R, Ma JF (2001) Distribution and mobility of aluminium in an Al-accumulating plant, *Fagopyrum esculentum* Moench. J Exp Bot 52: 1683–1687. PMID: 11479333

26. Tolra RP, Poschenrieder C, Luppi B, Barcelo J (2005) Aluminium-induced changes in the profiles of both organic acids and phenolic substances underlie Al tolerance in *Rumex acetosa* L. Environ Exp Bot 54: 231–238.

78

27. Reyna-Llorens I, Corrales I, Poschenrieder C, Barcelo J, Cruz-Ortega R (2015) Both aluminum and ABA induce the expression of an ABC-like transporter gene (*FeALS3*) in the Al-tolerant species *Fago-pyrum esculentum*. Environ Exp Bot 111: 74–82.

28. Hejcman M, Vondráčková S, Müllerová V, Červená K, Szákovx J, Tlustoš P (2012) Effect of quick lime and superphosphate additives on emergence and survival of *Rumex obtusifolius* seedlings in acid and alkaline soils contaminated by As, Cd, Pb, and Zn. Plant Soil Environ 58: 561–567.

29. White PJ, Broadley MR (2003) Calcium in plants. Ann Bot 92: 487–511. PMID: 12933363

30. Miyagi A, Uchimiya M, Kawai-Yamada M, Uchimiya H (2013) Impact of aluminium stress on oxalate and other metabolites in *Rumex obtusifolius*. Weed Res 53: 30–41.

31. Vondráčková S, Hejcman M, Szákovx J, Müllerová V, Tlustoš P (2014) Soil chemical properties affect the concentration of elements (N, P, K, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and their distri-bution between organs of *Rumex obtusifolius*. Plant Soil 379: 231–245. doi: 10.1007/82_2013_353 PMID: 24402712

32. Watanabe T, Osaki M (2002) Mechanisms of adaptation to high aluminium condition in native plant spe-cies growing in acid soils: a review. Commun Soil Sci Plant Anal 33: 1247–1260.

33. Huang J, Xia HP, Li Z, Xiong YM, Kong GH, Huang J (2009) Soil aluminium uptake and accumulation by *Paspalum notatum*. Waste Manage Res 27: 668–675. doi: 10.1177/0734242X09103835 PMID: 19423590

34. Miyagi A, Takahashi H, Takahara K, Hirabayashi T, Nishimura Y, Tezuka T, et al. (2010) Principal com-ponent and hierarchical clustering analysis of metabolites in destructive weeds; polygonaceous plants. Metabolomics 6: 146–155.

35. Hejcman M, Müllerová V, Vondráčková S, Száková J, Tlustoš P (2014) Establishment of *Bryum argen-teum* and concentrations of elements in its biomass on soils contaminated by As, Cd, Pb and Zn. Plant Soil Environ 60: 489–495.

36. Schwertfeger DM, Hendershot WH (2009) Determination of effective cation exchange capacity and ex-change acidity by a one-step BaCl2 method. Soil Sci Soc Am J 73: 737–734.

37. Sims JR, Haby VA (1971) Simplified colorimetric determination of soil organic matter. Soil Sci 112: 137–141.

38. Tlustoš P, van Dijk D, Száková J, Pavlíková D (1994) Cd and Zn release through the selected extrac-tants. Rostl Výr 40: 1107–1121.

39. Quevauviller P (1998) Operationally defined extraction procedures for soil and sediment analysis—I. standardization. Trac-Trends Anal Chem 17: 289–298.

40. Tejnecký V, Drábek O, Bor vka L, Nikodem A, Kopáč J, Vokurková P, et al. (2010) Seasonal variation of water extractable aluminium forms in acidified forest organic soils under different vegetation cover. Biogeochemistry 101: 151–163.

41. Malik J, Szakova J, Drabek O, Balik J, Kokoska L (2008) Determination of certain micro and macroele-ments in plant stimulants and their infusions. Food Chem 111: 520–525.

42. ter Braak CJF, Smilauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power. Ithaca.

43. Strobel BW (2001) Influence of vegetation on low-molecular-weight carboxylic acids in soil solution—a review. Geoderma 99: 169–198.

44. Barceló J, Poschenrieder C (2002) Fast roots growth responses, root exudates, and internal detoxifica-tion as clues to the mechanisms of aluminium toxicity and resistance: a review. Environ Exp Bot 48: 75–92.

45. Ettler V, Vrtišková R, Mihaljevič M, Šebek O, Grygar T, Drahota P (2009) Cadmium, lead and zinc leaching from smelter fly ash in simple organic acids—simulators of rhizospheric soil solutions. J Haz-ard Mater 170: 1264–1268. doi: 10.1016/j.jhazmat.2009.05.068 PMID: 19524363

46. Koopmans GF, Chardon WJ, van der Salm C (2005) Disturbance of water-extractable phosphorus de-termination by colloidal particles in a heavy clay soil from the Netherlands. J Environ Qual 34: 1446–1450. PMID: 15998868

47. van Hees PAW, Lundström US (2000) Equilibrium models of aluminium and iron complexation with dif-ferent organic acids in soil solution. Geoderma 94: 201–221.

48. Schöttelndreier M, Norddahl MM, Ström L, Falkengren-Grerup U (2001) Organic acid exudation by wild herbs in response to elevated Al concentrations. Ann Bot 87:769–775.

49. Ma JF, Hiradate S, Matsumoto H (1998) High aluminum resistance in buckwheat—II. Oxalic acid detox-ifies aluminum internally. Plant Physiol 117: 753–759. PMID: 9662518

50. Zheng SJ, Ma JF, Matsumoto H (1998) High aluminum resistance in buckwheat—I. Al-induced specific secretion of oxalic acid from root tips. Plant Physiol 117: 745–751. PMID: 9662517

79

51. Inostroza-Blancheteau C, Rengel Z, Alberdi M, Mora MD, Aquea F, Arce-Johnson P, et al. (2012) Molecular and physiological strategies to increase aluminium resistance in plants. Mol Biol Rep 39: 2069–2079. doi: 10.1007/s11033-011-0954-4 PMID: 21660471

52. Tolra R, Vogel-Mikuš K, Hajiboland R, Kump P, Pongrac P, Kaulich B, et al. (2011) Localization of aluminium in tea *(Camellia sinensis)* leaves using low energy X-ray fluorescence spectro-microscopy. J Plant Res 124: 165–172. doi: 10.1007/s10265-010-0344-3 PMID: 20422247

53. Hue NV, Craddock GR, Adams F (1986) Effect of organic-acids on aluminum toxicity in subsoils. Soil Sci Soc Am J 50: 28–34.

54. Malik J, Frankova A, Drabek O, Szakova J, Ash C, Kokoska L (2013) Aluminium and other elements in selected herbal tea plant species and their infusions. Food Chem 139: 728–734. doi: 10.1016/j.foodchem.2013.02.013 PMID: 23561167

55. Trichopoulou A, Vasilopoulou E, Hollman P, Chamalides C, Foufa E, Kaloudis T, et al. (2000) Nutritional composition and flavonoid content of edible wild greens and green pies: a potential rich source of antioxidant nutrients in the Mediterranean diet. Food Chem 70: 319–323.

56. Vardavas CI, Majchrzak D, Wagner KH, Elmadfa I, Kafatos A (2006) The antioxidant and phylloquinone content of wildly grown greens in Crete. Food Chem 99: 813–821.

57. McColl JG, Pohlman AA (1986) Soluble organic-acids and their chelating influence on Al and other metal dissolution from forest soils. Water Air Soil Pollut 31: 917–927.

58. Harrison WJ, Thyne GD (1992) Predictions of diagenetic reactions in the presence of organic-acids. Geochim Cosmochim Acta 56: 565–586.

80

4.5 Vondráčková et al. Vliv aplikace mletého fosfátu a superfosfátu na mobilitu prvků (Cd, Zn, Pb, As, Fe, Mn) v kontaminovaných půdách.

Iasi, February 21, 2014

Dear Dr. *STANISLAVA VONDRACKOVA*,

In your quality of *Corresponding Author*, I have the opportunity to inform you that your paper ***EFFECT OF ROCK PHOSPHATE AND SUPERPHOSPHATE APPLICATION ON MOBILITY OF ELEMENTS (Cd, Zn, Pb, As, Fe, AND Mn) IN CONTAMINATED SOILS,*** authors **Stanislava Vondráčková, Michal Hejcman, Pavel Tlustoš, Jiřina Száková** registered within the Editorial Office of ***Environmental Engineering and Management Journal*** as manuscript **55_Vondráčková_13**, has been evaluated and **accepted** for publication.

Thank you for your interest in our journal.

Sincerely yours,

Professor Maria Gavrilescu,
Executive Editor,

"Gheorghe Asachi" Technical University of Iasi, Romania

EFFECT OF ROCK PHOSPHATE AND SUPERPHOSPHATE APPLICATION ON MOBILITY OF ELEMENTS (Cd, Zn, Pb, As, Fe, Mn) IN CONTAMINATED SOILS

Stanislava Vondráčková[1], Michal Hejcman[2*], Pavel Tlustoš[1], Jiřina Száková[1]

[1]Department of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6 – Suchdol, Czech Republic
[2]Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6 – Suchdol, Czech Republic

Abstract

Weakly acidic Litavka and alkaline Malín soils are some of the most contaminated soils by multiple risk elements in the Czech Republic. The aim of this paper was to determine the effect of P sources (rock phosphate, superphosphate), each applied at three rates on the mobility of risk elements (Cd, Zn, Pb, As) and micronutrients (Fe, Mn) in soils and to compare their effectiveness with lime and dolomite tested in our previous study. In 7, 14, 28, and 42 days, we determined $CaCl_2$-extractable and acid-extractable concentrations of elements by ICP-OES. In alkaline soil, there was an increase in Cd, Zn, As, and Mn $CaCl_2$- and acid-extractable concentrations after superphosphate application but no effect on concentrations of these elements after rock phosphate application. In acidic soil, there was a decrease in $CaCl_2$-extractable concentrations of Cd and Zn and no effect on $CaCl_2$-extractable Pb, As, Fe, and Mn after rock phosphate application. With the exception of a decrease in Pb and Mn, the same trends were recorded for acid-extractable concentrations. Superphosphate was less effective than rock phosphate for immobilisation of $CaCl_2$-extractable Zn, As, and Mn. Phosphate additives were ineffective for immobilisation of all tested elements in alkaline soils. In acidic soils, phosphate additives were ineffective in immobilising As and Fe compared to lime application and were substantially less effective in Cd and Zn immobilisation than lime. Superphosphate application was a suitable measure to decrease acid-extractable Pb concentrations only in acidic soils at higher rates and was comparable with lime application at lower rates.

Key words: acetic acid-extractable concentrations, $CaCl_2$-extractable concentrations, chemical immobilisation, micronutrients, risk elements

*Author to whom all correspondence should be addressed: e-mail: hejcman@fzp.czu.cz

1. Introduction

In the Czech Republic as in many other countries, there are areas that are heavily contaminated by several risk elements and environmentally friendly cleanup methods such as phytoextraction are not possible to use because the high concentrations of risk elements are toxic for plants and consequently cause their mortality (Dan et al., 2008; Hejcman et al., 2012; Nagayjoti et al., 2010). Chemical stabilisation is a remediation method suitable for soils contaminated with high concentrations of heavy metal(loid)s that use some non-toxic materials for reducing the solubility of heavy metal(loid)s (Alkorta et al., 2010; Epelde et al., 2009; Vácha et al., 2002). Contaminated soils with high concentrations of heavy metal(loids) can induce deficiency of essential elements such as Fe and Mn for plants. Soil additives can reduce metal(loid)s mobility and bioavailability by adsorption, complexation, (co)precipitation, or their combination, although total concentrations of metal(loid)s are not significantly reduced (Bolan et al., 2010; Miretzky and Fernandez-Cirelli, 2008). The mobility and bioavailability of heavy metal(loid)s in soil depends on many parameters, e.g. pH, organic matter, cation exchange capacity, soil redox potential, the time elapsed after contamination by metal(loid)s, concentrations of heavy metal(loid)s in the soil solution or the release of heavy metal(loid) ions from the solid phase (Violante et al., 2010). The mobility of metal(loid)s in soil can be sufficiently studied using various chemical extractants, e.g. neutral salts for assessment of 'effective bioavailable metal fraction' or mild acids for 'potentially bioavailable metal fraction' (Sahuquillo et al., 2003; Száková et al., 2000; Tlustoš et al., 1997).

Phosphate compounds as non-toxic materials react with many heavy metal(loid)s and radionuclides to form secondary phosphate precipitates that are stable over a wide range of environmental conditions (Mignardi et al., 2012; Vangronsveld et al., 2009; Xenidis et al., 2010). The use of phosphate additives for Pb immobilisation has been very successful in many studies (Cao et al., 2008a; Fang et al., 2012; Munksgaard and Lottermoser, 2011). Several studies indicated that phosphate additives can mobilise As (Impellitteri, 2005; Kilgour et al., 2008; Theodoratos et al., 2002). Phosphate additives can be applied to immobilise other risk elements, e.g. Cd and Zn, which frequently occur simultaneously with Pb in soils (Baker et al., 2012; Lambert et al., 2007; Spuller et al., 2007; Wang et al., 2008). Nevertheless, the effectiveness of phosphate additives on immobilisation of other risk elements such as Cd and Zn has been investigated less, and thus the type and rate of phosphate additives on mobility of Cd and Zn should be carefully studied (Baker et al., 2012; Chen et al., 2007; Miretzky and Fernandez-Cirelli, 2008).

In our previous study (Vondráčková et al., 2013), the effect of quick lime (lime) and dolomite application on the mobility of Cd, Zn, Pb, As, Fe, and Mn was studied. Dolomite immobilised elements less than lime and only under higher application rates. Liming significantly

2

69 immobilised Cd and Zn only in weakly acidic soil. Dolomite and lime were ineffective to
70 immobilise Pb and As. Using the same contaminated soils, the aim of this paper was to study the
71 effect of rock phosphate and superphosphate application on Cd, Zn, Pb, As, Fe, and Mn mobility
72 with regard to the application rates and incubation days. The effectiveness of phosphate additives
73 was then compared with the effectiveness of alkaline additives in our previous study.
74
75 **2. Material and methods**
76
77 *2.1. Soil sample collection*
78
79   Two heavily multi-contaminated soils differing in physicochemical properties were selected
80 for the incubation experiment. Weakly acidic soil called 'Litavka' from the alluvium of the Litavka
81 River in the village of Trhové Dušníky, located 60 km south of Prague (49°43'N, 14°0'E),
82 containing 54 mg Cd/kg, 6172 mg Zn/kg, 3305 mg Pb/kg and 354 mg As/kg (extracted by *Aqua*
83 *Regia*) was contaminated by waste from smelter settling pits (Borůvka et al., 1996; Šichorová et al.,
84 2004). Alkaline soil called 'Malín' from a bank of the streamlet Beránka near Malín village located
85 82 km east of Prague (49°58'N, 15°17'E) containing 11 mg Cd/kg, 1022 mg Zn/kg and 688 mg/As
86 kg was contaminated due to tailings of silver mining in the 13–16th centuries (Horák and Hejcman,
87 2013). The specific characteristics of the soils used in this study are given in Vondráčková et al.
88 (2013).
89
90 *2.2. Design of the incubation experiment*
91
92   The incubation experiment was composed of seven treatments for each soil therefore 14
93 treatments for both soils in total (rock phosphate application in rates w/w (additive/soil), P1 – 0.2%,
94 P2 – 0.6%, and P3 – 1.7%; superphosphate application in rates w/w (additive/soil), S1 – 0.1%, S2 –
95 0.4%, S3 – 1.2%; control without any additives, C). Each treatment was replicated ten times, and
96 incubation of soils was performed for 7, 14, 28, and 42 days. Therefore the experiment was
97 comprised 140 bottles for each incubation time and 560 in total. Fifty grams of dry soil were
98 applied into each acid-clean polyethylene 250-ml plastic bottle. In P1, P2, P3, S1, S2, and S3
99 treatments, the soils were mixed with a particular amount of additive (see Table 1 for chemical
100 properties of used additives). Deionised water at a volume equivalent to 60% of maximum water
101 capacity was then applied into each bottle (18 ml for Litavka soil and 17 ml for Malín soil). The
102 incubation was performed at a constant temperature of 25°C. Bottles were opened and aerated with
103 fresh air every week.

3

104

105   **Table 1.** Basic chemical characteristics of applied phosphate additives. Mean values together with

106   standard error of the mean (SE) are provided in the case of additives chemical properties.

107   Concentrations of P and Ca were provided by distributor of superphosphate, and therefore it was not

108   analysed. Concentrations of Cd, Zn, Pb, As, Fe, and Mn were not determined (marked as dash) in

109   superphosphate because of guaranteed analytical grade purity by distributor. All analysed

110   concentrations and values of pH were performed in three replications (n=3).

Property	Rock phosphate [a] (P)	Superphosphate [b] (S)
pH_{CaCl_2}	7.8±0.01	2.2±0.003
P (g/kg)	67±11	246
Ca (g/kg)	174±22	159
Cd (mg/kg)	3.2±0.3	-
Zn (mg/kg)	83.5±13.7	-
Pb (mg/kg)	0.8±0.2	-
As (mg/kg)	5.8±1.5	-
Fe (mg/kg)	469±77	-
Mn (mg/kg)	7.9±1.3	-

111   [a] - distributor TIMAC AGRO CZECH Ltd., Czech Republic

112   [b] - analytical grade purity ($Ca(H_2PO_4)_2 \cdot H_2O$), distributor Lach-Ner Ltd., Czech Republic

113   Legislation limits for total concentrations of elements in mineral phosphate fertilizers (mg/kg): Cd

114   50, Pb 15, As 10 (Anonymous, 2009).

115

116   *2.3. Chemical analyses*

117

118   The pseudo-total concentrations of elements in soils were determined using inductively

119   coupled plasma–optical emission spectrometry (ICP-OES; VARIAN Vista Pro, Varian, Australia)

120   after *Aqua Regia* digestion in a microwave oven (Ethos 1, MLS GmbH, Germany). The details of

121   other chemical procedures before start of the incubation experiment (soil pH, cation exchange

122   capacity, concentration of organic carbon, concentrations of Ca, Mg, K, and P determined by

123   Mehlich III extraction procedure) can be found in Vondráčková et al. (2013). At days 7, 14, 28, and

124   42, $CaCl_2$-extractable and acetic acid- (acid-) extractable concentrations of elements in soils were

125   determined by ICP–OES, and a standard edition procedure was used for evaluating the data. Soil

126   samples were extracted using a 0.01 mol/L $CaCl_2$ aqueous solution at a solid/liquid ratio of 1/2.4 for

127   six hours, and with a 0.11 mol/L aqueous solution of CH_3COOH at a solid/liquid ratio of 1/2.4

4

128 overnight. Hettich Universal 30 RF (Germany) equipment was used for centrifugation of the
129 reaction mixtures at 3000 rpm for 10 min.
130
131 *2.4. Data analyses*
132   Obtained data were evaluated by repeated-measures and one-way analysis of variance
133 (ANOVA) using Statistica 9.0 program (StatSoft, Tulsa, USA) as in our previous study
134 (Vondráčková et al., 2013).
135
136 **3. Results**
137
138   Calculated by repeated measures ANOVA, $CaCl_2$-extractable Cd, As, Fe, and Mn and acid-
139 extractable Cd, Zn, and As concentrations were significantly affected by treatment, time, and by
140 treatment × time interaction in both soils. $CaCl_2$-extractable Pb concentrations were significantly
141 affected by treatment, time, and treatment × time interaction only in Litavka soil and $CaCl_2$-
142 extractable Zn concentrations by treatment and treatment × time interaction. Acid-extractable Pb
143 concentrations were significantly affected by treatment and by treatment × time interaction only in
144 Malín soil. Acid-extractable Fe concentrations were significantly affected by treatment and time in
145 Litavka soil and by time and treatment × time interaction in Malín soil. Acid-extractable Mn
146 concentrations were significantly affected by treatment and time only in Litavka soil.
147
148 *3.1. Cadmium*
149
150   In comparison to the control, $CaCl_2$-extractable Cd concentrations were decreased and
151 significantly affected by application rates of rock phosphate as well as of superphosphate with the
152 exception of an increase in S2 and S3 treatments on the 7th day of the experiment in Litavka soil
153 (Fig. 1a). In Malín soil, concentrations of $CaCl_2$-extractable Cd in rock phosphate treatments were
154 the same as in the control as well as in superphosphate treatments, with the exception of a slight
155 increase in S3 treatment on all days of the experiment (Fig. 1b).
156   Rock phosphate application did not affect or slightly decreased acid-extractable Cd
157 concentrations, and in superphosphate treatments significant decreases (strong positive effect of
158 rate) of acid-extractable Cd concentrations in Litavka soil (Fig. 1c) were recorded. In Malín soil,
159 concentrations of acid-extractable Cd in rock phosphate treatments were the same as in the control
160 but in superphosphate treatments were significantly increased (strong negative effect of rate) (Fig.
161 1d).
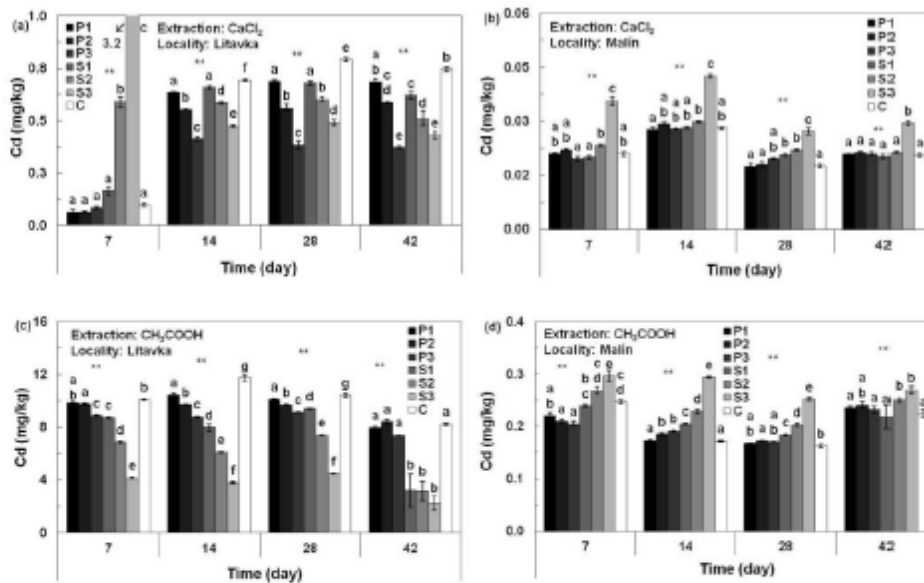
5

162

163   **Fig. 1.** Effect of treatment on mean concentrations of CaCl$_2$-extractable Cd (a, b) and acid-

164   extractable Cd (c, d) in Litavka and Malín soils. Error lines represent standard error of the mean

165   (SE). Calculated by one-way ANOVA, differences between treatments were significant based on a

166   0.01 (**) probability level. Treatment abbreviation: rock phosphate with application rates P1 – 0.2%

167   w/w, P2 – 0.6% w/w and P3 – 1.7% w/w; superphosphate with application rates S1 – 0.1% w/w, S2

168   – 0.4% w/w, S3 – 1.2% w/w; control without any additives – C.

169

170   *3.2. Zinc*

171

172   In comparison to the control, a decrease in CaCl$_2$-extractable Zn concentrations was

173   significantly affected by application rates of rock phosphate, but an effect of superphosphate

174   application on CaCl$_2$-extractable Zn concentrations was not significant as in the case of rock

175   phosphate in Litavka soil (Fig. 2a). Rock phosphate application did not affect or slightly decreased

176   CaCl$_2$-extractable Zn concentrations in Malín soil, and superphosphate application did not affect

177   (lower rates S1, S2) or increased CaCl$_2$-extractable Zn concentrations in the case of elevated

178   application rate (S3) (Fig. 2b).

179   In both soils, an effect of rock phosphate and superphosphate application on acid-extractable

180   Zn concentrations (Figs. 2c and d) was similar to the Cd.

6

Fig. 2. Effect of treatment on mean concentrations of CaCl$_2$-extractable Zn (a, b) and acid-extractable Zn (c, d) in Litavka and Malín soils. Error lines represent standard error of the mean (SE). Calculated by one-way ANOVA, differences between treatments were significant at 0.05(*) and 0.01 (**) probability levels. See Fig. 1 for more details about treatments.

3.3. Lead

In comparison to other tested elements, extraction of Pb with using solutions of CaCl$_2$ and acetic acid was very low, and therefore the usefulness of phosphate additives was negligible. In comparison to the control, no effect on CaCl$_2$-extractable concentrations of Pb was recorded after rock phosphate and superphosphate applications in both soils (Figs. 3a and b).

There was slight decrease in acid-extractable Pb concentrations in rock phosphate treatments and substantial drops in acid-extractable Pb concentrations in superphosphate treatments in Litavka soil. Concentrations of Pb were significantly affected by the application rate of rock phosphate and superphosphate (Fig. 3c). In Malín soil, concentrations of Pb were very low (Fig. 3d).

7

Fig. 3. Effect of treatment on mean concentrations of $CaCl_2$-extractable Pb (a, b) and acid-extractable Pb (c, d) in Litavka and Malín soils. Error lines represent standard error of the mean (SE). Calculated by one-way ANOVA, differences between treatments were not statistically significant ([n.s.]), were significant at 0.05([*]) and 0.01 ([**]) probability levels. See Fig. 1 for more details about treatments.

3.4. Arsenic

In both soils, concentrations of $CaCl_2$-extractable and acid-extractable As in rock phosphate treatments were the same as in the control but substantially increased in superphosphate treatments (Figs. 4a, b, c and d). $CaCl_2$-extractable and acid-extractable concentrations of As were significantly affected by application rate of superphosphate and slightly decreased during the experiment.

8
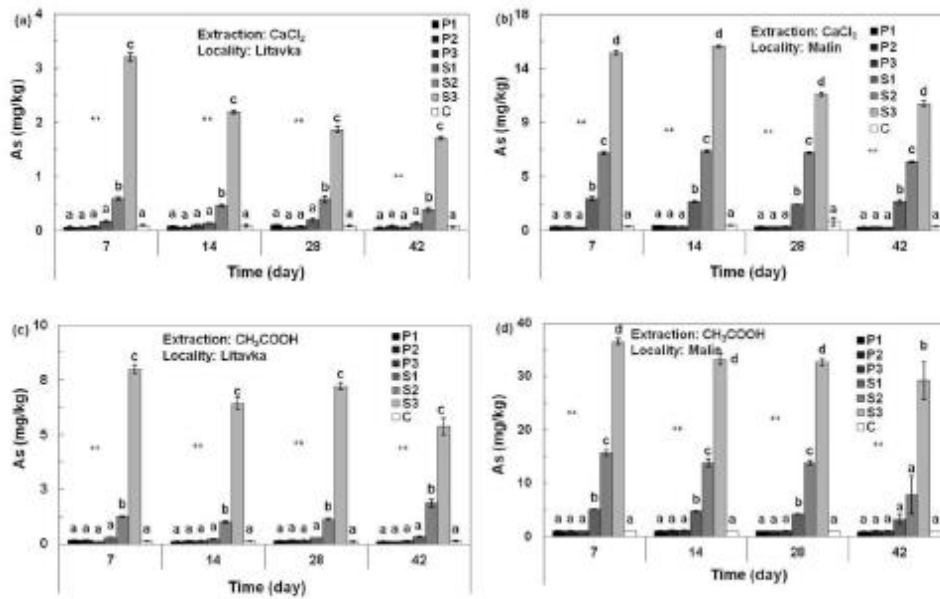
210

211   **Fig. 4.** Effect of treatment on mean concentrations of $CaCl_2$-extractable As (a, b) and acid-

212   extractable As (c, d) in Litavka and Malín soils. Error lines represent standard error of the mean

213   (SE). Calculated by one-way ANOVA, differences between treatments were significant at a 0.01

214   (**) probability level. See Fig. 1 for more details about treatments.

215

216   *3.5. Iron*

217

218   In both soils, concentrations of $CaCl_2$- and acid-extractable Fe in rock phosphate and

219   superphosphate treatments did not differ from the control (Figs. 5a, b, c and d).
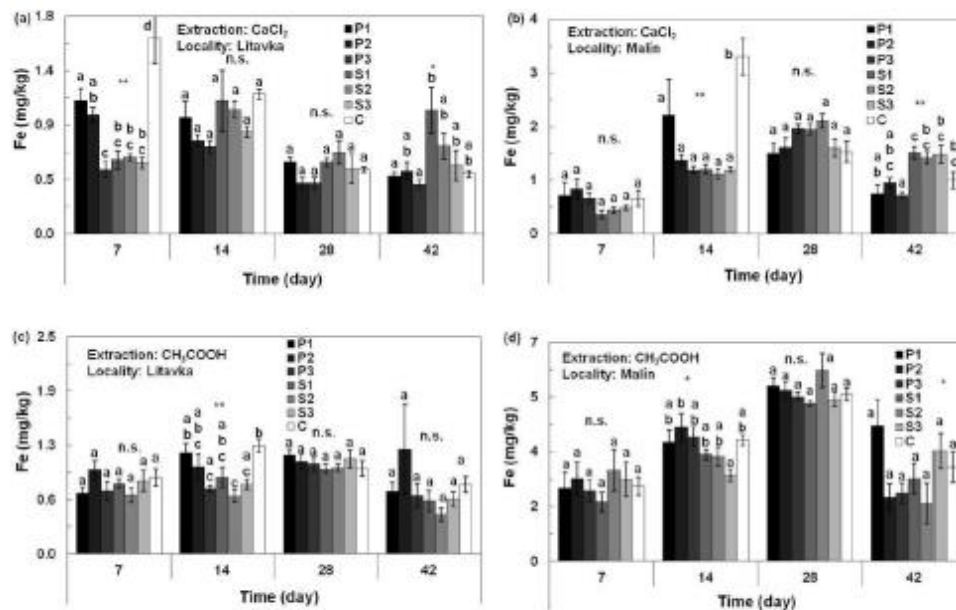
220

221   **Fig. 5.** Effect of treatment on mean concentrations of $CaCl_2$-extractable Fe (a, b) and acid-
222   extractable Fe (c, d) in Litavka and Malín soils. Error lines represent standard error of the mean
223   (SE). Calculated by one-way ANOVA, differences between treatments were not statistically
224   significant ([n.s.]), or were significant at 0.05([*]) and 0.01 ([**]) probability levels. See Fig. 1 for more
225   details about treatments.

226

227   *3.6. Manganese*

228

229   In both soils, concentrations of $CaCl_2$-extractable Mn in rock phosphate treatments were the
230 same as in the control (Figs. 6a and b). In comparison to the control, superphosphate application
231 increased concentrations of $CaCl_2$-extractable Mn in Litavka soil, and Mn concentrations were
232 considerably affected by the application rate of superphosphate (Fig. 6a). In Malín soil,
233 concentrations of $CaCl_2$-extractable Mn in superphosphate treatments were the same as in the
234 control with the exception of substantial increases in S3 treatment on all days of the experiment
235 (Fig. 6b). There was decrease in $CaCl_2$-extractable Mn concentrations in S3 treatment during the
236 experiment.

237   In comparison to the control, there was no or little effect of rock phosphate application on
238 acid-extractable concentrations of Mn in both soils (Figs. 6c and d). In comparison to the control,
239 superphosphate application significantly decreased concentrations of acid-extractable Mn in Litavka
240 soil (Fig. 6c) and substantially increased concentrations of acid-extractable Mn in S3 treatment in
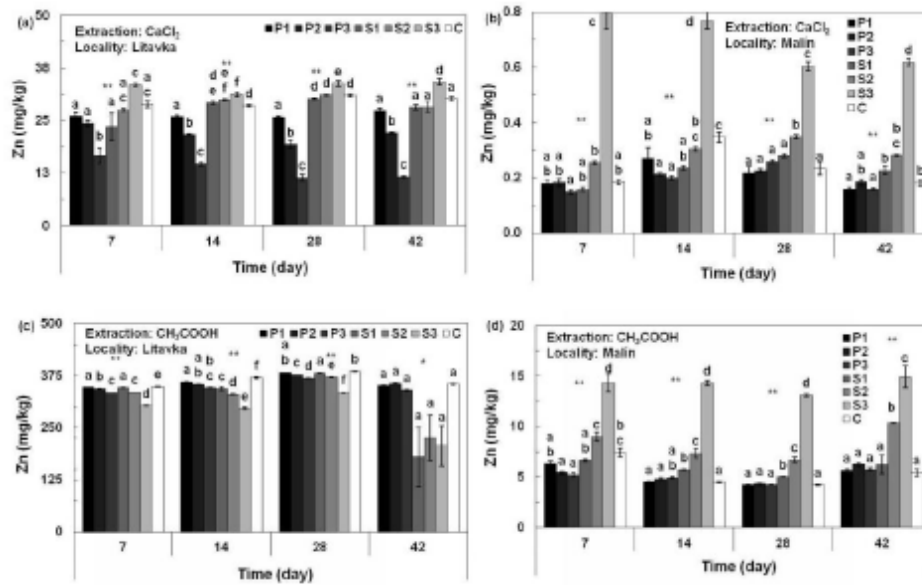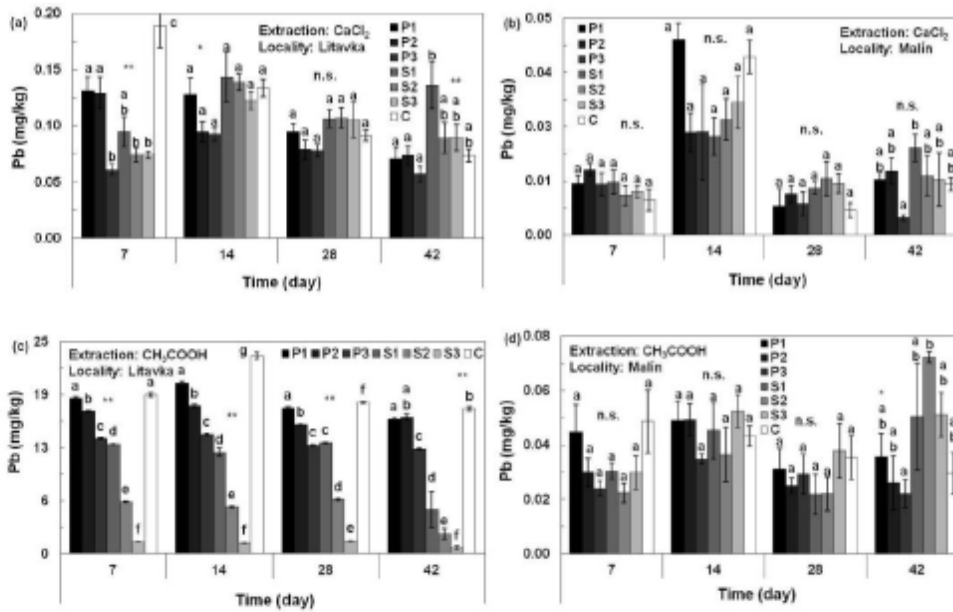241 Malín soil (Fig. 6d).
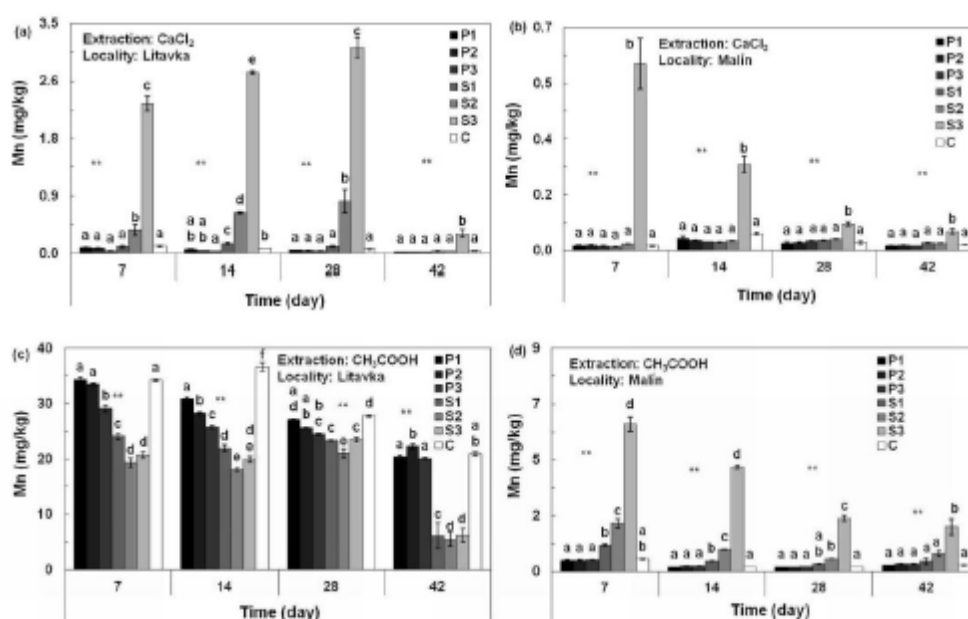
242

243   **Fig. 6.** Effect of treatment on mean concentrations of $CaCl_2$-extractable Mn (a, b) and acid-
244   extractable Mn (c, d) in Litavka and Malín soils. Error lines represent standard error of the mean
245   (SE). Calculated by one-way ANOVA, differences between treatments were significant at a 0.01
246   (**) probability level. See Fig. 1 for more details about treatments.

247

248   **4. Discussion**

249

250   *4.1. Cadmium*

251

252   Rock phosphate application decreased $CaCl_2$-extractable Cd concentrations in Litavka soil,
253   probably due to phosphate-induced Cd^{2+} adsorption and formation or co-precipitation of insoluble
254   metal phosphate in the soil such as $Cd_3(PO_4)_2$ (Hong et al., 2010; Chen et al., 2007). Slight increase
255   of rock phosphate immobilisation efficiency with application rate is in agreement with
256   Thawornchaisit and Polprasert (2009). In Malín soil, no changes in $CaCl_2$-extractable Cd
257   concentrations after application of rock phosphate were connected with less soluble phosphate input
258   into alkaline soil. In alkaline conditions, the insoluble calcium phosphates were unable to release a
259   sufficient amount of free phosphate ions for Cd immobilisation (Hong et al., 2010), but
260   simultaneously high levels of soil pH (ranging from 7.2 in C treatment to 7.2 and 7.3 in P1 and P3
261   treatments at the end of experiment, respectively) were not able to mobilise Cd. Using rock
262   phosphate is therefore ineffective for Cd immobilisation in the alkaline soil. We can recommend the
263   rock phosphate application especially at higher application rates on acid soils, but the
264   immobilisation efficiency was approximately about two orders lower than the efficiency of lime
265   (Vondráčková et al., 2013).

266   In Litavka soil, an increased concentration of Cd in S2 and S3 treatments at the beginning of
267   the experiment were probably due to the initial decrease in soil pH (ranging from 6.2 in C treatment
268   to 6.1 and 5.3 in S1 and S3 treatments, respectively) as also observed by other authors (Cui et al.,
269   2010; Wang et al., 2008). After one week of incubation experiments, Cd concentrations
270   significantly decreased, probably due to formation of insoluble Cd forms as recorded by other
271   authors (Chrysochoou et al., 2007; Spuller et al., 2007). In Malín soil, an increase in $CaCl_2$-
272   extractable Cd concentrations in S3 treatment was probably related to the formation of soluble
273   phosphate complexes such as $CdHPO_4$ that more likely occur in laboratory conditions due to high
274   phosphate inputs compared to the real field conditions (Lambert el al., 2007). Superphosphate
275   application is not effective for immobilisation of Cd on alkaline soils and can be recommended only
276   on acid soils especially at high rates. However, Cd immobilisation efficiency of superphosphate was
277   low in comparison to the efficiency of lime (Vondráčková et al., 2013). In Litavka soil, mean

11

278 CaCl$_2$-extractable and acid-extractable Cd concentrations in the control were 0.7 and 8.5 mg/kg, 0.4

279 and 7.4 mg/kg in rock phosphate, 0.4 and 2.2 mg/kg in superphosphate, 0.3 and 6.6 mg/kg in

280 dolomite, and only 0.002 and 0.003 mg/kg in lime treatments. To immobilise Cd in acid soils, use

281 of lime is therefore much more effective (more than 99% decrease in comparison to the control)

282 than the use of superphosphate (43–74% decrease). According to very low initial CaCl$_2$-extractable

283 (0.01 mg/kg) and acid-extractable (0.2 mg/kg) concentrations of Cd and no significant changes in

284 concentrations after application of additives in Malín soil, use of all additives is ineffective or

285 slightly risky in alkaline soils.

286

287 *4.2. Zinc*

288

289   A slight decrease in CaCl$_2$-extractable Zn concentrations after rock phosphate application in

290 Litavka soil was probably related to enhanced Zn adsorption on surfaces due to P inputs or Zn

291 precipitation of insoluble metal phosphate in the soil like Zn$_3$(PO$_4$)$_2$ (Lambert et al., 2007). In Malín

292 soil, no changes in CaCl$_2$-extractable Zn concentrations after rock phosphate was probably

293 connected with the same reasons as in the case of CaCl$_2$-extractable Cd because of Cd and Zn

294 chemical similarity (Jiang et al., 2007). Therefore, the use of rock phosphate is not effective to

295 immobilise either Cd or Zn in alkaline soils.

296   No or slightly increase in CaCl$_2$-extractable Zn concentration after superphosphate

297 application in Litavka soil was probably connected with formation of hopeite – Zn$_3$(PO$_4$)$_2$·4H$_2$O,

298 which is much more soluble than pyromorphite (Baker et al., 2012; Xu and Schwartz, 1994). This

299 explained significant differences between immobilisation efficiency of rock phosphate and

300 superphosphate in Litavka soil. We concluded that the use of rock phosphate in acid soils is more

301 effective for the immobilisation of Zn than the use of superphosphate, but substantially less

302 effective than the use of lime (Vondráčková et al., 2013). In Malín soil, increases in CaCl$_2$-

303 extractable Zn concentrations after superphosphate application is probably connected with lower

304 pH, especially in S3 treatments (pH ranging from 7.2 in C treatment to 7.2 and 6.4 in S1 and S3

305 treatments, respectively), and it is in agreement with Theodoratos et al. (2002). According to other

306 authors (Mignardi et al., 2012; Wang et al., 2008), superphosphate application must be carefully

307 designed because of potential soil acidification. Rock phosphate can be applied to immobilise Zn

308 only in acid soils.

309   In Litavka soil, mean CaCl$_2$-extractable and acid-extractable Zn concentrations in the

310 control were 29 and 349 mg/kg, 12 and 341 mg/kg in rock phosphate, 28 and 179 mg/kg in

311 superphosphate, 8 and 271 mg/kg in dolomite and only 4 and 2 mg/kg in lime. The most effective

312 for immobilisation is therefore lime on acid soils (86−99.4% decrease in comparison to the control).

12

313   With respect to very low initial CaCl$_2$-extractable (0.1 mg/kg) and acid-extractable (4 mg/kg)
314   concentrations of Zn and no significant effect of all additives on Zn concentrations in Malín soil, we
315   concluded that all additives were ineffective or partly risky (S3 – application rate of
316   superphosphate) in alkaline soils.

317

318   *4.3. Lead*

319

320   Rock phosphate and superphosphate applications had no effect on CaCl$_2$-extractable Pb
321   concentrations in both soils because of generally low Pb mobility (Miretzky and Fernandez-Cirelli,
322   2008). We supposed that low concentrations of CaCl$_2$-extractable Pb, even on highly Pb
323   contaminated Litavka soil, can probably indicate that the majority of Pb was in the plant-
324   unavailable fraction as indicate results by other authors (Padmavathiamma and Li, 2010). In this
325   view, we suppose that application of rock phosphate and superphosphate is ineffective in
326   immobilisation of CaCl$_2$-extractable Pb neither in acid soil nor in alkaline soils.

327   In Litavka soil, acid-extractable concentrations of Pb significantly decreased after rock
328   phosphate and superphosphate applications in contrast to CaCl$_2$-extractable Pb concentrations.
329   Acid-extractable Pb concentrations probably more reflect the dissolution–precipitation mechanisms
330   that are typical for Pb immobilisation caused by phosphate additives. In Litavka soil, significant
331   decrease in acid-extractable Pb concentrations after rock phosphate application was probably due to
332   slow dissolution of carbonate-bound Pb and quick precipitation of geochemically stable Pb
333   phosphate formation (pyromorphite) due to its low solubility (Cui et al., 2010; Chen et al., 2007;
334   Miretzky and Fernandez-Cirelli, 2008). On the other hand, superphosphate application substantially
335   decreased acid-extractable Pb concentrations in Litavka soil probably via formation of less soluble
336   PbHPO$_4$ (Cao et al., 2008a). The higher Pb immobilisation efficiency of superphosphate than rock
337   phosphate is in agreement with the findings of other authors (Cui et al., 2010; Wang et al., 2008).
338   This was because of good water solubility of superphosphate in contrast to rock phosphate.
339   Concentrations of acid-extractable Pb decreased with increases in superphosphate application rates;
340   therefore, immobilisation efficiency of superphosphate increase with application rate as was
341   recorded also by Thawornchaisit and Polprasert (2009). No effect of rock phosphate and
342   superphosphate application on acid-extractable Pb concentrations in Malín soil was provided by
343   alkaline soil pH (ranging from 7.2 in C treatment to 7.2, 7.3, 7.2 and 6.4 in P1, P3, S1 and S3
344   treatments, respectively) as solubility of Pb and P minerals is low under alkaline soil reaction
345   (Miretzky and Fernandez-Cirelli, 2008).

346   Acid soil pH is thus important for dissolution of original Pb minerals and consequent
347   immobilisation of Pb by formation of more stable Pb minerals after application of all phosphate

13

348 additives (Cao et al., 2008b). Finally, we concluded that both phosphate additives are suitable
349 measures to decrease acid-extractable concentrations of Pb only in acid soils, especially at higher
350 additive application rates. In Litavka soil, mean $CaCl_2$-extractable and acid-extractable Pb
351 concentrations were 0.08 and 17 mg/kg in the control, 0.06 and 12 mg/kg in rock phosphate, 0.09
352 and 0.7 mg/kg in superphosphate, 0.03 and 9 mg/kg in dolomite and 1.8 and 0.3 mg/kg in lime
353 (Vondráčková et al., 2013). With the exception of dolomite, application of all additives was
354 ineffective to decrease $CaCl_2$-extractable concentrations of Pb. We concluded that acid-extractable
355 concentrations of Pb can be the most decreased by lime (98% decrease in comparison to the control)
356 followed by superphosphate (96% decrease) in acid soils. With respect to very low initial $CaCl_2$-
357 extractable (0.009 mg/kg) and acid-extractable (0.02 mg/kg) concentrations of Pb and no changes in
358 concentrations after application of additives in Malín soil, the use of all additives is ineffective in
359 alkaline soils.
360
361 *4.4. Arsenic*
362
363   In both types of soil, concentrations of $CaCl_2$-extractable As in rock phosphate treatments
364 were the same as in the control because of low solubility of the rock phosphate. Probably an
365 increase in As concentration after rock phosphate application requires more than 42 days as was
366 recorded by Cao et al. (2003).
367   Superphosphate application substantially increased concentrations of $CaCl_2$-extractable As
368 in both soils due to application of soluble phosphates, which compete for sorption sites on soil
369 particles with arsenate (Impellitteri, 2005; Smith et al., 2002). Arsenates were therefore released
370 into the soil solution similarly as in other studies with superphosphate application (Theodoratos et
371 al., 2002).
372   Mean $CaCl_2$-extractable As concentrations were 0.1 and 0.3 mg/kg in the control, 0.1 and
373 0.3 mg/kg in rock phosphate, 0.1 and 2 mg/kg in superphosphate, 0.04 and 0.3 mg/kg in dolomite
374 and 0.1 and 0.2 mg/kg in lime treatments in Litavka and Malín soils, respectively (Vondráčková et
375 al., 2013). We concluded that rock phosphate and superphosphate applications can be risky because
376 they can increase mobility of As in neither acid nor in alkaline soils.
377
378 *4.5 Iron*
379
380   No effect of rock phosphate and superphosphate on $CaCl_2$-extractable Fe concentrations was
381 probably connected with Fe bound in less soluble iron phosphate (strengite – $FePO_4·2H_2O$) in acid

14

382 soils and in less soluble calcium phosphate in alkaline soils (House, 1990; Hsu, 1975; Maguire et
383 al., 2001).

384   Mean $CaCl_2$-extractable Fe concentrations were 0.7 and 0.8 mg/kg in the control, 0.4 and 0.7
385 mg/kg in rock phosphate, 0.6 and 1.4 mg/kg in superphosphate, 0.3 and 0.6 mg/kg in dolomite and
386 0.3 and 0.3 mg/kg in lime treatments in Litavka and Malín soils, respectively (Vondráčková et al.,
387 2013). We concluded that application of all additives do not affect Fe mobility in either acid or in
388 alkaline soil.

389

390 *4.6. Manganese*

391

392   N No effect of rock phosphate application on $CaCl_2$-extractable and acid-extractable Mn
393 concentrations in both soils was probably due to high rock phosphate pH. According to Hossner and
394 Richards (1967), plant Mn availability is highest when the pH of the phosphate additive is between
395 2 and 4, which is typical for superphosphate (see Table 1). Low solubility of rock phosphate in soil
396 did not allow release of sufficient amount of available phosphates for reaction with Mn.

397   Superphosphate application increased concentrations of $CaCl_2$-extractable Mn in both soils,
398 and Mn concentrations were considerably affected by the application rate of superphosphate. This
399 conclusion is in agreement with other authors (Hawkins et al., 2008; Larsen, 1964). Mobilisation of
400 Mn is probably connected with the formation of soluble manganese phosphate complex at the
401 higher application rates (Larsen, 1964). During incubation, $CaCl_2$-extractable concentrations of Mn
402 decreased in S3 treatment in Malín soil. This was probably due to the high buffering capacity of
403 Malín soil and the time required for balancing between soil pH and superphosphate pH. The slight
404 decrease in acid-extractable Mn concentrations after superphosphate application in Litavka soil was
405 probably because of formation of Mn-phosphate minerals such as $MnHPO_4$ or $Mn_3(PO_4)_2$ (Porter et
406 al., 2004; Vangronsveld et al., 2009). Superphosphate application increased concentrations of acid-
407 extractable Mn in Malín soil and Mn concentrations were considerably affected by the application
408 rate of superphosphate. This is in agreement with $CaCl_2$-extractable Mn concentrations. During
409 incubation, the substantial decrease in acid-extractable Mn concentration in S3 treatment was
410 consistent with the decrease in plant-available Mn concentrations. Similarly, as in the case of
411 $CaCl_2$-extractable Mn concentrations, superphosphate application does not decrease acid-
412 extractable concentrations of Mn in acid or in alkaline soils.

413   In Litavka soil, mean $CaCl_2$-extractable and acid-extractable Mn concentrations were 0.05
414 and 23 mg/kg in the control, 0.02 and 20 mg/kg in rock phosphate, 0.04 and 5.5 mg/kg in
415 superphosphate, 0.01 and 14 mg/kg in dolomite and 0.02 and 0.05 mg/kg in lime treatments
416 (Vondráčková et al., 2013). We concluded that acid-extractable concentrations of Mn can be most

15

417 decreased by lime (98% decrease in comparison to the control) and followed by superphosphate

418 (76% decrease) in acid soils. With respect to very low initial $CaCl_2$-extractable (0.01 mg/kg) and

419 acid extractable (0.2 mg/kg) concentrations of Mn and no changes in concentrations after

420 application of additives in Malín soil, the use of all additives is ineffective in alkaline soils.

421

422 **5. Conclusions**

423

424 The efficiency of phosphate additives with different P release was tested for Cd, Zn, Pb, As,

425 Fe, and Mn immobilisation within period of 42 days, and it was compared with lime and dolomite

426 application used in a previous study. In alkaline soils, no immobilisation effect was observed after

427 all additives application. In acidic soils, phosphate and alkaline additives ineffective at

428 immobilising As, Fe and $CaCl_2$-extractable Mn and Pb concentrations. On the other hand, higher

429 immobilisation effects on acid-extractable Cd, Zn, Pb, and Mn was observed for fast soluble

430 additives (lime>superphosphate) compare to slow soluble additives (dolomite~rock phosphate) in

431 acid soils.

432

433 **Acknowledgements**

434

438

439 **References**

440

441 Alkorta I., Becerril J. M., Garbisu C., (2010), Phytostabilization of metal contaminated soils,

442   *Reviews on Environmental Health*, **25**,135-146.

443 Anonymous, (2009), Public notice No. 271/2009, which change Public notice No. 474/2000 about

444   determination requirements for fertilizers, Czech Ministry of Agriculture, Prague (in Czech).

445 Baker L. R., Pierzynski G. M., Hettiarachchi G. M., Scheckel K. G., Newville M., (2012), Zinc

446   speciation in proximity to phosphate application points in a lead/zinc smelter-contaminated soil,

447   *Journal of Environmental Quality*, **41**, 1865-1873.

448 Bolan N., Naidu R., Choppala G., Park J., Mora M. L., Budianta D., Panneerselvam P., (2010),

449   Solute interactions in soils in relation to the bioavailability and environmental remediation of

450   heavy metals and metalloids, *Pedologist*, **53**, 1-18.

16

451 Borůvka L., Huan-Wei Ch., Kozák J., Krištoufková S., (1996), Heavy contamination of soil with
452   cadmium, lead and zinc in the alluvium of the Litavka river, *Rostlinná Výroba*, **42**, 543-550.
453 Cao X., Ma L. Q., Shiralipour A., (2003), Effects of compost and phosphate amendments on arsenic
454   mobility in soils and arsenic uptake by the hyperaccumulator, *Pteris vittata* L., *Environmental*
455   *Pollution,* **126**, 157-167.
456 Cao X., Dermatas D., Xu X., Shen G., (2008a), Immobilization of lead in shooting range soils by
457   means of cement, quicklime, and phosphate amendments, *Environmental Science and Pollution*
458   *Research,* **15**, 120-127.
459 Cao X., Ma L. Q., Singh S. P., Zhou Q., (2008b), Phosphate-induced lead immobilization from
460   different lead minerals in soils under varying pH conditions, *Environmental Pollution,* **152**, 184-
461   192.
462 Cui Y., Du X., Weng L., Van Riemsdijk W. H., (2010), Assessment of in situ immobilization of
463   lead (Pb) and arsenic (As) in contaminated soils with phosphate and iron: solubility and
464   bioaccessibility, *Water Air and Soil Pollution*, **213**, 95-104.
465 Dan T., Hale B., Johnson D., Conard B., Stiebel B., Veska E., (2008), Toxicity thresholds for oat
466   (*Avena sativa* L.) grown in Ni-impacted agricultural soils near Port Colborne, Ontario, Canada,
467   *Canadian Journal of Soil Science*, **88**, 389-398.
468 Epelde L., Becerril J. M., Mijangos I., Garbisu C., (2009), Evaluation of the efficiency of a
469   phytostabilization process with biological indicators of soil health, *Journal of Environmental*
470   *Quality*, **38**, 2041-2049.
471 Fang Y., Cao X., Zhao L., (2012), Effects of phosphorus amendments and plant growth on the
472   mobility of Pb, Cu, and Zn in a multi-metal-contaminated soil, *Environmental Science and*
473   *Pollution Research*, **19**, 1659-1667.
474 Hawkins H. - J., Hettasch H., Mesjasz-Przybylowicz J., Przybylowicz W., Cramer M. D., (2008),
475   Phosphorus toxicity in the Proteaceae: a problem in post-agricultural lands, *Scientia*
476   *Horticulturae*, **117**, 357-365.
477 Hejcman M., Vondráčková S., Müllerová V., Červená K., Száková J., Tlustoš P., (2012), Effect of
478   quick lime and superphosphate additives on emergence and survival of *Rumex obtusifolius*
479   seedlings in acid and alkaline soils contaminated by As, Cd, Pb, and Zn, *Plant Soil and*
480   *Environment,* **58**, 561-567.
481 Hong C. O., Chung D. Y, Lee D. K., Kim P. J., (2010), Comparison of phosphate materials for
482   immobilizing cadmium in soil, *Archives of Environmental Contamination and Toxicology*, **58**,
483   268-274.
484 Hossner L. R., Richards G. E., (1967), The effect of phosphorus source on the movement and
485   uptake of band applied manganese, *Soil Science Society of America Journal*, **32**, 83-85.

17

486 Horák J., Hejcman M., (2013), Use of trace elements from historical mining for alluvial sediment
487   dating, *Soil and Water Research*, **8**, 77-86.

488 House W. A., (1990), The prediction of phosphate coprecipitation with calcite in freshwaters, *Water*
489   *Research*, **24**, 1017-1023.

490 Hsu P. H., (1975), Precipitation of phosphate from solution using aluminum salt, *Water Research*,
491   **9**, 1155-1161.

492 Chen S., Xu M., Ma Y., Yang J., (2007), Evaluation of different phosphate amendments on
493   availability of metals in contaminated soil, *Ecotoxicology and Environmental Safety*, **67**, 278-
494   285.

495 Chrysochoou M., Dermatas D., Grubb D. G., (2007), Phosphate application to firing range soils for
496   Pb immobilization: the unclear role of phosphate, *Journal of Hazardous Materials*, **144**, 1-14.

497 Impellitteri C. A., (2005), Effects of pH and phosphate on metal distribution with emphasis on As
498   speciation and mobilization in soils from a lead smelting site, *Science of the Total Environment*,
499   **345**, 175-190.

500 Jiang H. M., Yang J. C., Zhang J. F., (2007), Effects of external phosphorus on the cell
501   ultrastructure and the chlorophyll content of maize under cadmium and zinc stress,
502   *Environmental Pollution*, **147**, 750-756.

503 Kilgour D. W., Moseley R. B., Barnett M. O., Savage K. S., Jardine P. M., (2008), Potential
504   negative consequences of adding phosphorus-based fertilizers to immobilize lead in soil, *Journal*
505   *of Environmental Quality*, **37**, 1733-1740.

506 Lambert R., Grant C., Sauvé S., (2007), Cadmium and zinc in soil solution extracts following the
507   application of phosphate fertilizers, *Science of the Total Environment*, **378**, 293-305.

508 Larsen S., (1964), The effect of phosphate application on manganese content of plants grown on
509   neutral and alkaline soils, *Plant and Soil*, **21**, 37-42.

510 Maguire R. O., Foy R. H., Bailey J. S., Sims J. T., (2001), Estimation of the phosphorus sorption
511   capacity of acidic soils in Ireland, *European Journal of Soil Science*, **52**, 479-487.

512 Mignardi S., Corami A., Ferrini V., (2012), Evaluation of the effectiveness of phosphate treatment
513   for the remediation of mine waste soil contaminated with Cd, Cu, Pb, and Zn, *Chemosphere*, **86**,
514   354-360.

515 Miretzky P., Fernandez-Cirelli A., (2008), Phosphates for Pb immobilization in soils: a review,
516   *Environmental Chemistry Letters*, **6**, 121-133.

517 Munksgaard N. C., Lottermoser B. G., (2011), Fertilizer amendment of mining-impacted soils from
518   Broken Hill, Australia: fixation or release of contaminants?, *Water Air and Soil Pollution*, **215**,
519   373-397.

18

520 Nagajyoti P. C., Lee K. D., Sreekanth T. V. M., (2010), Heavy metals, occurrence and toxicity for
521   plants: a review, *Environmental Chemistry Letters* 8, 199-216.

522 Padmavathiamma P. K., Li L. Y., (2010), Phytoavailability and fractionation of lead and manganese
523   in a contaminated soil after application of three amendments, *Bioresource Technology*, **101**,
524   5667-5676.

525 Porter S. K., Scheckel K. G., Impellitteri C. A., Ryan J. A., (2004), Toxic metals in the
526   environment: thermodynamic considerations for possible immobilization strategies for Pb, Cd,
527   As, and Hg, *Critical Reviews in Environmental Science and Technology,* **34**, 495-604.

528 Sahuquillo A., Rigol A., Rauret G., (2003), Overview of the use of leaching/extraction tests for risk
529   assessment of trace metals in contaminated soils and sediments, *Trac-Trends in Analytical
530   Chemistry,* **22**, 152-159.

531 Smith E., Naidu R., Alston A. M., (2002), Chemistry of inorganic arsenic in soils: II. Effect of
532   phosphorus, sodium, and calcium on arsenic sorption, *Journal of Environmental Quality,* **31**,
533   557-563.

534 Spuller C., Weigand H., Marb C., (2007), Trace metal stabilisation in a shooting range soil:
535   mobility and phytotoxicity, *Journal of Hazardous Materials,* **141**, 378-387.

536 Száková J., Tlustoš P., Balík J., Pavlíková D., Balíková M., (2000), Efficiency of extractants to
537   release As, Cd and Zn from main soil compartments, *Analusis*, **28**, 808-812.

538 Šichorová K., Tlustoš P., Száková J., Kořínek K., Balík J., (2004), Horizontal and vertical
539   variability of heavy metals in the soil of a polluted area, *Plant Soil and Environment,* **50**, 525-
540   534.

541 Thawornchaisit U., Polprasert C., (2009), Evaluation of phosphate fertilizers for the stabilization of
542   cadmium in highly contaminated soils, *Journal of Hazardous Materials,* **165**, 1109-1113.

543 Theodoratos P., Papassiopi N., Xenidis A., (2002), Evaluation of monobasic calcium phosphate for
544   the immobilization of heavy metals in contaminated soils from Lavrion, *Journal of Hazardous
545   Materials,* **94**, 135-146.

546 Tlustoš P., Balík J., Pavlíková D., Száková J., (1997), The uptake of cadmium, zinc, arsenic and
547   lead by chosen crops, *Rostlinná Výroba*, **43**, 487-494.

548 Vácha R., Podlešáková E., Němeček J., Poláček O., (2002), Immobilisation of As, Cd,-Pb and Zn in
549   agricultural soils by the use of organic and inorganic additives, *Rostlinná Výroba*, **48**, 335-342.

550 Vangronsveld J., Herzig R., Weyens N., Boulet J., Adriaensen K., Ruttens A., Thewys T., Vassilev
551   A., Meers E., Nehnevajova E., van der Lelie D., Mench M., (2009), Phytoremediation of
552   contaminated soils and groundwater: lessons from the field, *Environmental Science and
553   Pollution Research,* **16**, 765-794.

19

554   Violante A., Cozzolino V., Perelomov L., Caporale A. G., Pigna M., (2010), Mobility and
555   bioavailability of heavy metals and metalloids in soil environments, *Journal of Soil Science and*
556   *Plant Nutrition*, **10**, 268-292.

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

560   Wang B., Xie Z., Chen J., Jiang J., Su Q., (2008), Effects of field application of phosphate
561   fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (*Brassica*
562   *chinensis* L.) in a mining tailing contaminated soil, *Journal of Environmental Sciences-China*,
563   **20**, 1109-1117.

564   Xenidis A., Stouraiti C., Papassiopi N., (2010), Stabilization of Pb and As in soils by applying
565   combined treatment with phosphates and ferrous iron, *Journal of Hazardous Materials*, **177**,
566   929-937.

567   Xu Y., Schwartz F. W., (1994), Lead immobilization by hydroxyapatite in aqueous solutions,
568   *Journal of Contaminant Hydrology*, **15**, 187-206.

4.6 Vondráčková et al. Regulace příjmu makroprvků, mikroprvků a toxických prvků vrbou Smithovou vyvápněním silně kontaminovaných půd.

Název: Regulation of macro-, micro- and toxic elements uptake by *Salix × smithiana* using liming of heavily contaminated soils.

Autoři: Vondráčková, S., Tlustoš, P., Hejcman, M., Száková, J.

Odesláno do časopisu: Journal of Soils and Sediments. (v přípravě)

1 **Regulation of macro, micro, and toxic element uptake by *Salix × smithiana* using liming**

2 **of heavily contaminated soils**

3

4 **Stanislava Vondráčková[1], Pavel Tlustoš[1], Michal Hejcman[2], Jiřina Száková[1]**

5

6 [1] Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology,

7 Food and Natural Resources, Czech University of Life Sciences in Prague, Prague, Czech

8 Republic

9 [2] Department of Ecology, Faculty of Environmental Sciences, Czech University of Life

10 Sciences in Prague, Prague, Czech Republic

11

12 Corresponding author

13 E-mail: vondrackovas@af.czu.cz (Stanislava Vondráčková)

14

15 **Abstract**

16 *Purpose*

17 Willow cultivation in soils heavily contaminated by risk elements is a challenging issue due to

18 phytotoxic effects that restrict plant growth. Liming reduces the mobility of some risk

19 elements in contaminated soils and therefore can be a suitable measure for contaminated soils

20 but can also affect availability of nutrients for planted willows. We investigate how liming

21 affects concentrations of macro, micro, and toxic elements in the organs of willows planted in

22 contaminated soils.

23 *Materials and methods*

24 We established a three-year pot experiment with *S. × smithiana* planted in weakly acid and

25 alkaline soils anthropogenically seriously contaminated by As, Cd, Pb, and Zn. Soils were

1

both untreated and treated with two doses of lime and dolomite in the first year before
planting. We determined biomass production, mortality, and the concentration of macro- and
micronutrients and toxic elements in the willows' aboveground organs.

Results and discussion

Lime application increased biomass production in both soils; dose of lime played an
important role for its increase only in alkaline soil. Lime in higher dose was incompatible
with the vitality of just-planted willows in both soils. Doses of dolomite significantly affected
the biomass production and mortality of willows, where lower doses caused a permanent
decrease of biomass production and mortality in weakly acid soil. The toxicity of Cd and Zn
in leaves was recorded in both untreated soils; the latent deficiency of P and deficiency of Fe
in leaves was only recorded in weak acid untreated soil.

Conclusions

Lime application irrespective of dose with foliar Fe application seemed to be the most suitable
measure for increasing biomass production and decreasing toxic elements, especially Cd and
Zn, without decreasing the macro- and micronutrients in the aboveground organs of willows
in weakly acid soil. In alkaline soil, only higher doses of lime had a positive effect on the
studied parameters. Dolomite application is not a suitable measure for planting willows in
both contaminated soils. Dolomite in lower dose impairs the growth of willows in weakly
acid soil.

2

1 Introduction

A high concentration of risk elements in soil is potentially toxic for most plants, and can lead to their poor and irregular growth and development or even can cause their mortality (Nagajyoti et al. 2010; Solanki and Dhankhar 2011; Leitenmaier and Küpper 2013). Because of the insufficient vegetation cover of extremely contaminated areas, risk elements can be spread to the surroundings by wind or water erosion and can be leached off (Ruttens et al. 2006; Friesl-Hanl et al. 2009; Bolan et al. 2014).

Chemophytostabilisation appears as one of the most promising temporary phytoremediation techniques for heavily contaminated soils for several risk elements (Alkorta et al. 2010). The application of soil additives to reduce the mobility and bioavailability of risk elements in soil (i.e. *in situ* chemical immobilisation) is the first step of chemophytostabilisation (Kumpiene et al. 2008; Alkorta et al. 2010). Consequently, it is possible to grow plants that are tolerant to risk elements in stabilised soils and to utilise the site aesthetically and economically (Tlustoš et al. 2007; Friesl-Hanl et al. 2009; Alkorta et al. 2010). Liming of the contaminated soils can reduce the bioavailability of risk elements and is the most widely used remediation treatment (Lee et al. 2004). Quick lime can cause a large increase of soil pH and thus immobilize risk elements in soil (Alkorta et al. 2010). Dolomite can immobilize risk elements by weak pH changes and increased adsorption to the surface of dolomite (Bolan and Duraisamy 2003).

Possible differences in risk element mobility are not only affected by a wide range of soil parameters (e.g. soil pH, the quantity and quality of organic matter, cation exchange capacity, soil texture, or soil type) (Vácha et al. 2002; Kunhikrishnan et al. 2012; Alloway 2013) or by the properties of the risk elements themselves; soil additives, their application doses, and the period under consideration can also play important roles. Soil additives do not only affect toxic element mobility, but also the macro- and micronutrients availability in the soil. Unsuitable soil additives or improper application doses can reduce the macro- and

3

106

micronutrient uptake by plants, induce their deficiency, prevent plants from growing in contaminated sites, and thus make the utilisation of soils impossible (Bolan and Duraisamy 2003; Tlustoš et al. 2007; Friesl-Hanl et al. 2009).

There are a variety of plants tolerant to risk elements (Baker 1981; Sheoran et al. 2011). Considerable attention in terms of phytoremediation is focused on fast-growing trees, especially willows (*Salix* spp., *Salicaceae* family); (Pulford and Watson 2003; Meers et al. 2007). This focus is connected with the worldwide spread of willows, with their high biomass production and their ability to tolerate or accumulate risk elements in biomass, and consequently with their possible utilisation for phytoextraction or phytostabilisation (Pulford and Watson 2003; Meers et al. 2007; Tlustoš et al. 2007; Abhilash et al. 2012). Specific willow clones (e.g. *Salix × smithiana* Willd.) are able to grow and accumulate substantial concentrations of mobile elements such as Cd and Zn in their aboveground biomass in moderately contaminated soils (Vysloužilová et al. 2003b; Dos Santos Utmazian et al. 2007; Tlustoš et al. 2007). In contrast, the biomass production of willows is limited in heavily contaminated soils because of Cd and Zn phytotoxicity (Vysloužilová et al. 2006; Jensen et al. 2009). Therefore, willows can be used for phytoremediation in slightly or moderately contaminated soils (Vysloužilová et al. 2003b; Jensen et al. 2009). In heavily contaminated soils it is necessary to reduce the mobility of risk elements in the soil before planting willows (Vysloužilová et al. 2003a; Lee et al. 2004; Puschenreiter et al. 2005; Tlustoš et al. 2006).

In our previous study (Vondráčková et al. 2013), the effect of lime and dolomite application on the Cd, Zn, Pb, As, Fe, and Mn mobility was studied as the first step of chemophytostabilisation in a system with heavily contaminated soils differing in the soil parameters. Dolomite application was less effective than lime at decreasing element mobility, and only at higher doses. Liming only reduced Cd and Zn mobility in weakly acid soil and was ineffective at reducing Pb and As mobility in slightly acidic and alkaline soils. In the

4

present study, we deal with the long-term effects of liming on the concentrations of a wide range of elements, including macro- and micronutrients in willows as a main step of chemophytostabilisation. According to our knowledge, there is a gap in the literature on the long-term effect of liming on concentrations of macro- and micronutrients in the organs of willows. Using the same long-term anthropogenically contaminated soils as in our previous study, in this study we asked how (1) biomass production of *S. smithiana*, (2) its mortality, and (3) the concentration of macro (Ca, Mg, K, P), micro (Cu, Fe, Mn, Ni), and toxic elements (Al, Cd, Cr, Pb, Zn) in the aboveground organs of willows were affected by the application of two doses of lime and dolomite in the three following years. Using multivariate analysis, we also investigated how the studied parameters are related to each other.

5

2 Materials and methods

2.1 Soils

The specific characteristics of the two long-term heavily As-, Cd-, Pb-, and Zn-contaminated soils are given in previous studies (Hejcman et al. 2012, 2014; Vondráčková et al. 2014). The main chemical properties of the soils are: (1) 'Litavka Fluvisol' – 354 mg As/kg (in all elements pseudo-total concentrations were extracted with *Aqua regia*), 54 mg Cd/kg, 3,305 mg Pb/kg, and 6,172 mg Zn/kg, pH_{CaCl2} 6.5, CEC 109 $mmol_{(+)}$/kg, and C_{org} 3.6 %; and (2) 'Malín Luvisol' – 688 mg As/kg, 11 mg Cd/kg, 98 mg Pb/kg, and 1,022 mg Zn/kg, pH_{CaCl2} 7.3, CEC 333 $mmol_{(+)}$/kg, and C_{org} 2.7 %.

2.2 Pot experiment

The three-year experiment was established in May 2010. The whole soil profile of 5 kg of air-dried soil in each 5-L pot was mixed with nutrient solution, consisting of 0.1 g N as NH_4NO_3 per 1 kg of soil, and 0.032 g P and 0.08 g K as K_2HPO_4 per 1 kg of soil. Application of quick lime (hereafter abbreviated as lime) in doses – 7.3 g (dose 1) and 21.9 g (dose 2) per 1 kg of soil containing 686 g Ca/kg of material with pH_{CaCl2} 12.0 – and of dolomite in doses – 21.6 g (dose 1) and 68.1 g (dose 2) per 1 kg of soil containing 220 g Ca/kg and 100 g Mg/kg of material with pH_{CaCl2} 8.3 – was performed after the application of the nutrient solution. Therefore, we set up a pot experiment with ten treatments each with four replications: LC Litavka control soil without any additive, LL1 Litavka soil with lime in dose 1, LL2 Litavka soil with lime in dose 2, LD1 Litavka soil with dolomite in dose 1, LD2 Litavka soil with dolomite in dose 2, MC Malín control soil without any additive, ML1 Malín soil with lime in dose 1, ML2 Malín soil with lime in dose 2, MD1 Malín soil with dolomite in dose 1, and MD2 Malín soil with dolomite in dose 2.

6

134 Subsequently after liming, one 20-cm-long cutting of *Salix × smithiana* Willd (clone no. S-

135 218, the silky-leaf osier, hybrid of *S. viminalis* L. and *S. caprea* L.); (Tlustoš et al. 2007;

136 Zárubová et al. 2015) was planted in each pot (cutting was protruded approx. 1–2 cm above

137 the surface of the soil). *Salix × smithiana* plants were grown in the pots for three following

138 years. The pots were regularly watered with deionised water to maintain the optimal moisture

139 conditions for plant growth during the vegetation. Due to an Fe deficiency in the willows'

140 leaves (chlorosis) visible during the first growing season, foliar Fe application (6% solution of

141 Fe in the form of EDDHMA – i.e. ethylenediamine (*o*-hydroxy-*p*-methylphenylacetic) acid)

142 was regularly performed four times during vegetation (i.e. in July and August) from the

143 second year. Before each growing season, all the dead plants were replaced by new one of the

144 same clone.

145 At the end of each growing season before the leaves started to fall (i.e. September), plant

146 biomass was harvested, divided into twigs and leaves, weighed for the determination of dry

147 biomass (DM total – dry mass of twigs and leaves together, DM organ – dry mass of twigs

148 and leaves separately), ground, and analysed. Concurrently, soil samples were collected from

149 the whole soil profile of each pot.

150

151 **2.3 Chemical analysis**

152 At the end of each growing season, soil samples were extracted with 0.01 mol/L $CaCl_2$

153 (hereafter abbreviated as Ca; pH 5.9; mobile – plant-available portions of elements) in a 1:10

154 ratio (w/v); (Tlustoš et al. 1994) and with 0.11 mol/L acetic acid (hereafter abbreviated as

155 AA; pH 2.8; mobilisable – acid-extractable portions of elements) in a 1:20 ratio (w/v);

156 (Quevauviller 1998). The plant-available and acid-extractable concentrations of elements in

157 soil extracts (Table 4) were determined using inductively coupled plasma-optical emission

158 spectrometry (ICP-OES, VARIAN Vista Pro, Varian, Australia; for P, Cu, Fe, Mn, Ni, Al,

7

159   Cd, Cr, Pb, Zn) and flame atomic absorption spectroscopy (FAAS, VARIAN, SpectrAA-280,

160   Australia; for Ca, Mg, and K). Soil pH was measured in a suspension of soil and 0.01 mol/L

161   $CaCl_2$ (1:5, w/v; Table 1).

162   The total concentrations of elements in plant organs (twigs and leaves; air-dried at 60°C and

163   stainless-steel milled) were determined with ICP-OES and FAAS after the dry ashing of the

164   sample at 500°C under atmospheric pressure (Mader et al. 1998). Certified reference material,

165   NCS DC 73348 Bush Branches and Leaves, was applied for quality assurance of the

166   analytical data.

167

168   **2.4 Statistical analysis**

169   All statistical analyses were performed using the Statistica 12.0 (www.statsoft.com) and

170   CANOCO 4.5 (ter Braak and Smilauer 2002) software. Soil and biomass data were evaluated

171   with the non-parametric Kruskal–Wallis test. We assessed the effects of 1) treatment and

172   growing season on the soil pH, biomass production, mortality, and on the concentration of

173   elements in the soil and biomass, and 2) organ on the concentration of elements in the

174   biomass. After obtaining significant results from the Kruskal–Wallis test, we used multiple

175   comparisons of mean ranks for the detection of significant differences between different

176   treatments, growing year, and organs. The relationship between selected soil and biomass data

177   was analysed by linear regression. A principal component analysis (PCA), in the CANOCO

178   4.5 program, was applied to all collected data together (soil pH, total and organ biomass,

179   mortality, and concentration of elements in the soil and biomass) in individual soils after three

180   growing periods separately. We used the standardisation of species data because data of a

181   different character were analysed together. The PCA was used to make any correlations

182   between all the analysed data and any similarity of the different treatments visible. The results

183   were visualised in the form of a bi-plot ordination diagram in the CanoDraw program.

184

8

185　**3 Results**

186　**3.1 Soil pH**

187　Soil pH was increased in all lime and dolomite treatments (LL1, LL2, LD1, LD2) in Litavka

188　soil but in lime treatments with considerably higher effect (Table 1). On the other hand, soil

189　pH was increased only in lime treatments (ML1 and ML2) in Malín soil.

190

191　**3.2 Biomass production**

192　Total and organ (total/organ) biomass of *S. × smithiana* was significantly affected by

193　treatment, soil, growing time (time), and additive (Table 2).

194　The leaf biomass tended to have higher biomass production than twig biomass in most of the

195　treatments in the Litavka soil over time (Table 2). A significantly higher twig biomass than

196　leaf biomass was recorded in the Malín soil (all treatments all together) over time. The

197　twig:leaf biomass ratio was positively related to soil pH in both soils over time ($r = 0.413$;

198　$p < 0.01$).

199　Total/organ biomass was significantly increased in the control (MC), lime (LL1, LL2, ML1,

200　and ML2), and dolomite (LD1, LD2, MD1, and MD2) treatments over time (Table 2). A trend

201　towards decreasing twig biomass was recorded in the control treatment (LC) over time.

202　In the Litavka soil, a tendency towards a decrease in total/organ biomass after the first year

203　and a significant increase of total/organ biomass after the next two growing years were

204　recorded in the lime (LL1 and LL2) treatments compared with the control treatment (LC,

205　Table 2 and Fig. 1a,c,e). After the first year, biomass production was negatively related to soil

206　pH (total DM: $r = -0.551$, $p = 0.018$; organ DM: $r = -0.521$, $p = 0.027$) in the LC, LL1, and

207　LL2 treatments all together. A significant decrease of total/organ biomass was recorded in the

208　LD1 treatment in comparison to the LC treatment over time. In the Malín soil, all the dead

209　plants after the first growing season and a significant increase of total/organ biomass after the

9

210 next two years were recorded in the ML2 treatment in comparison to the MC treatment (Table

211 2 and Fig. 1b,d,f). After the last two growing seasons, biomass production was positively

212 related to soil pH (total DM r = 0.666, p < 0.01; and organ DM r = 0.569, p < 0.01) in the MC

213 and ML2 treatments.

214 The application dose of lime only played an important role in the total/organ biomass in the

215 Malín soil (i.e. an increase of biomass in ML2 treatment only occurred in the last two years)

216 and the application dose of dolomite only played an important role in the organ biomass in the

217 Litavka soil (i.e. decrease of biomass in the LD1 treatment).

218

219 **3.3 Mortality**

220 Mean mortality of *S. × smithiana* was significantly affected by treatment, soil, and time

221 (Table 3).

222 Higher mortality of cuttings was recorded in the Litavka soil than in the Malín soil and after

223 the first season than after the following seasons (Table 3). Higher mortality of cuttings at the

224 harvest of the first year tended to treatments with the highest pH in both soils (LL1, LL2, and

225 ML2; Tables 1, 3). The mortality of other cuttings was always recorded after winter in the

226 Litavka soil (Table 3). All the dead plants were replaced.

227 Immediate planting of the cuttings after lime application in the higher dose (LL2 and ML2

228 treatments) was recorded as incompatible with willow development in both soils (Tables 2

229 and 3). Regular mortality of cuttings was recorded only in the treatment with dolomite applied

230 in lower doses (LD1).

231

232 **3.4 Concentration of elements in biomass**

233 The concentrations of Mg, Mn, Ni, Cr, Pb, and Zn were significantly affected by treatments,

234 while the concentrations of P, Cd, Cr, Pb, and Zn were significantly affected by soil. The

10

235   concentrations of P, Cu, Fe, Mn, Ni, Al, Cd, Cr, and Pb were significantly affected by time,

236   the concentrations of Mg, Mn, Ni, and Zn were significantly affected by additive, and the

237   concentrations of Ca, Mg, K, P, Fe, Mn, Ni, Al, Cd, Cr, and Zn differed between individual

238   organs (Table 5).

239

240   **3.4.1 Macronutrients**

241   No significant differences in Ca, Mg, or K concentrations in organs (leaves and twigs

242   together) were recorded between soils (all treatments together); the concentration of P in

243   organs was significantly higher in the Malín soil than in the Litavka soil. The concentration of

244   P in leaves was positively related to acid-extractable concentrations of P in both soils over

245   time ($r = 0.458$; $p < 0.01$).

246   There was no significant effect of time on concentrations of Ca, Mg, or K in organs (leaves

247   and twigs together and all treatments in both soils together); the concentration of P in organs

248   was significantly higher in the first year than in the second year.

249   The application of lime (LL1, LL2, ML1, and ML2 treatments) and of dolomite (LD1, LD2,

250   MD1, and MD2 treatments) did not significantly affect the distribution of Ca, Mg, K, or P

251   concentrations between organs (twig < leaf) in comparison to the control treatment in both

252   soils (LC and MC).

253   The application dose of lime and of dolomite played no significant role in the potential

254   change of the concentration of all macronutrients in the organs of the willows in both

255   contaminated soils.

256

257   **3.4.2 Micronutrients**

258   No significant differences in Cu, Fe, Mn, or Ni concentrations in organs (leaves and twigs

259   together) were recorded between soils (all treatments together).

11

260   The Cu concentration in organs (leaves and twigs together) was significantly lower in the first

261   year than in the second year (all treatments in both soils together); the concentration of Fe in

262   organs was significantly higher in the last growing season than in the first season; the

263   concentration of Mn in organs was significantly higher in the last two years than in the first

264   year; and the concentration of Ni in organs was significantly lower in the last growing season

265   than in the first two years.

266   The application of lime (the LL1, LL2, ML1, and ML2 treatments) and of dolomite (the LD1,

267   LD2, MD1, and MD2 treatments) did not significantly affect the distribution of Cu, Fe, Mn,

268   or Ni concentrations between organs (Cu: twig = leaf; Fe, Mn, and Ni: twig < leaf) in

269   comparison to the control treatment in both soils (LC and MC).

270   The application dose of lime and of dolomite played no significant role in changing in Fe,

271   Mn, and Ni concentrations in the organs of willows in any of the contaminated soils. The

272   application dose of dolomite played an important role in changing in Cu concentration in the

273   organs of the willows in Litavka soil.

274

275   ### 3.4.3 Toxic elements

276   No significant differences in Al concentration in organs (leaves and twigs together) were

277   recorded between soils (all treatments together); the concentrations of Cd, Pb, and Zn in

278   organs were significantly higher in the Litavka soil than in the Malín soil; and the

279   concentration of Cr in organs was significantly higher in the Malín soil than in the Litavka

280   soil.

281   The concentrations of Al, Cd, and Cr in organs (leaves and twigs together) were significantly

282   higher in the last growing season than in the first season (all treatments in both soils together);

283   the concentrations of Cr and Pb in organs were significantly higher in the first year than in the

12

115

284   second year; no significant differences in Zn concentration in organs were recorded between

285   seasons.

286   In the Litavka soil, the application of lime (the LL1 and LL2 treatments) and of dolomite (the

287   LD1 and LD2 treatments) did not significantly affect the distribution of Al, Cd, Cr, or Zn

288   concentrations between organs (twig < leaf) in comparison to the LC treatment. The

289   application of lime (the LL1 and LL2 treatments) significantly affected the distribution of Pb

290   concentration between organs (twig = leaf) in comparison to the LC treatment (twig > leaf);

291   and the application of dolomite (the LD1 and LD2 treatments) did not significantly affect the

292   distribution of Pb concentration between organs (twig > leaf) in comparison to the LC

293   treatment. In the Malín soil, the application of lime (the ML1 and ML2 treatments) and of

294   dolomite (the MD1 and MD2 treatments) did not significantly affect the distribution of Al,

295   Cd, Cr, or Zn concentrations between organs (Al, Cd, and Zn: twig < leaf; Cr: twig = leaf) in

296   comparison to the MC treatment. The application of lime (the ML1 and ML2 treatments) and

297   of dolomite (the MD1 and MD2 treatments) significantly affected the distribution of Pb

298   concentration between organs (twig < leaf) in comparison to the MC treatment (twig = leaf).

299   The application dose of lime and of dolomite played no significant role in changing in Al, Cd,

300   Cr, and Zn concentrations in the organs of the willows in any of the contaminated soils. The

301   application dose of dolomite played an important role in changing in Pb concentration in the

302   organs of the willows in Litavka soil.

303

304   **3.5 Results of the PCA analysis**

305   In the Litavka soil, the first axis of the PCA analysis explained 31% (Fig. 1a,c) and 26%

306   (Fig. 1e) of the variability of the analysed data; the first two axes explained 48% (Fig. 1a),

307   50% (Fig. 1c), and 42% (Fig. 1e) of the variability of the analysed data; and the first four axes

308   together explained 71% (Fig. 1a), 74% (Fig. 1c), and 63% (Fig. 1e) of the variability of all the

13

309 analysed data. In the Malín soil, the first axis of the PCA analysis explained 24% (Fig. 1b),

310 28% (Fig. 1d), and 22% (Fig. 1f) of the variability of the analysed data; the first two axes

311 explained 40% (Fig. 1b), 47% (Fig. 1d), and 38% (Fig. 1f) of the variability of the analysed

312 data; and the first four axes together explained 64% (Fig. 1b), 63% (Fig. 1d), and 60% (Fig.

313 1f) of the variability of the analysed data.

314 The length and direction of the vectors of the parameters (soil pH, DM total/organ, mortality,

315 and concentration of elements in the soil and biomass) indicate links among themselves with

316 respect to the treatment. For example, the mortality of cuttings was positively correlated with

317 soil pH at the first growing year (Fig. 1a; the angle between vectors was less than 90°) and

318 was negatively correlated with soil pH at the second year in the Litavka soil (Fig. 1c). A long

319 vector for a particular variable indicates a large effect on the results of the analysis. For

320 example, there was low effect of mortality on all the analysed data after the third season in the

321 Litavka soil (Fig. 1e), as the vector for mortality was very short.

322 In the Litavka soil, the first ordination axis divided marks for individual pots into the lime

323 group on the right side and dolomite/control group on the left side of the diagram for the first

324 two growing seasons (Fig. 1 a,c). For the last growing year (Fig. 1e), the first ordination axis

325 divided marks for individual pots into positive lime groups on the left side, dolomite in dose 2

326 in the middle, and negative dolomite in dose 1/control group on the right side of the diagram.

327 This indicates a high effect of lime addition over time and the increasing efficiency of

328 dolomite in dose 2 from the last season on the analysed data in the Litavka soil. In the Malín

329 soil, the first ordination axis divided marks for individual pots into lime in the dose 2 group

330 on the right (2nd year) or the upper side (3rd year) and lime in the dose 1/dolomite/control

331 group on the left (2nd year) or the bottom side (3rd year) of the diagram (Fig. 1d,f). This

332 indicates a high effect of lime in dose 2 on the analysed data in the Malín soil in the last two

333 growing seasons. In the last two growing years in both soils (Fig. 1c,d,e,f), in contrast with

14

334   the first year (Fig. 1a,b), the data for leaves (circles) were clearly separated from all marks for

335   twigs (squares). This indicates a large effect of organs on all recorded data in the last two

336   growing seasons and a minimal effect in the first year in both soils.

337

15

4 Discussion

4.1 Biomass production

Higher leaf biomass in the slightly acidic Litavka soil and higher twig biomass in the alkaline Malín soil was connected with different soil pH and the original level of risk elements in both soils. Similar results (i.e. twig biomass > leaf biomass of willows in soil with higher pH and a lower level of risk elements) have been recorded by Tlustoš et al. (2007). A reduced twig biomass and stabile low leaf biomass with visible symptoms of Zn toxicity inducing Fe chlorosis in the Litavka control soil over time was probably caused by extremely high concentrations of Cd and Zn in the leaves (36.5–73 mg Cd/kg; 2,074–3,488 mg Zn/kg) exceeding their normal levels in plants (0.05–2 mg Cd/kg, 10–150 mg Zn/kg); (Pugh et al. 2002). A decrease of leaf biomass over time recorded in other studies with heavily acidic contaminated soils (Vysloužilová et al. 2003b; Tlustoš et al. 2007) were not observed, which is probably because of foliar Fe application from the second growing season in our experiment. Moreover, foliar Fe application in combination with lime application seems to be an appropriate measure for increasing the biomass production of willows in slightly acidic heavily contaminated soils. In heavily acidic contaminated soils, it is necessary to immobilise the risk elements before planting willow cuttings, which has also been recorded by other authors (Vysloužilová et al. 2003a; Lee et al. 2004; Puschenreiter et al. 2005; Tlustoš et al. 2006).

Lime application irrespective of dose was the most suitable measure for increasing biomass production in the slightly acidic Litavka soil from the second season (i.e. after the reduction of the negative effect of the high soil pH induced by the lime application in the first year). Similar results (i.e. increased biomass production of willows and poplars after liming) were recorded by other authors (Tlustoš et al. 2006; Vamerali et al. 2009; Trakal et al. 2011). Dolomite application in the lower dose was recorded as the least suitable measure for

16

363 increasing the biomass production in the slightly acidic Litavka soil. In the alkaline Malín

364 soil, lime application in the higher dose was recorded as the most suitable measure for

365 increasing biomass production because of the adequately high soil pH and the reduction of Ni,

366 Cd, and Zn concentrations in organs, especially in the second growing year.

367

368 **4.2 Mortality**

369 Lower mortality of willows over time as well as tripled biomass production in the third season

370 were recorded in the alkaline Malín control soil compared with the slightly acidic Litavka

371 control soil. This is in contrary to the results of Tahvanainen and Rytkönen (1999), in which

372 the optimum soil pH for cultivation of willows ranges from 5.5 to 6.5. This inconsistency is

373 connected with the high concentration of risk elements in our soils (i.e. high soil pH helps to

374 decrease risk element mobility and the mortality of plants, and to increase biomass

375 production); (Trakal et al. 2011).

376 Lime application in the higher dose in the slightly acidic Litavka soil and the alkaline Malín

377 soil was incompatible with the vitality of just-planted cuttings due to the high soil pH.

378 Therefore, an artificially increased soil pH ranging from 7.9 up to 8.3 is not appropriate for

379 willows in the early stages of their growth. This finding is in contrary to results of Hytönen

380 and Kaunisto (1999), in which it was shown that willows require high soil pH for their good

381 root development. This inconsistency is probably connected with the formation of free

382 hydroxides released from lime that can burn the roots and the presence of high loads of risk

383 elements in the soil which can be released into the soil due to the mineralisation of organic

384 matter induced by the high dose of lime (Mühlbachová and Tlustoš 2006).

385 The dose of dolomite played an important role in the mortality of willows and in their growth.

386 Dolomite application in the lower dose is not a suitable measure for increasing the growth of

387 willows in slightly acidic contaminated soils because of their high mortality. The dolomite

17

388 application in the higher dose is possible a measure for better growth of willows in slightly

389 acidic contaminated soils, but only from the third year because of its poor and gradual

390 efficiency (Mayfield et al. 2004).

391

392 **4.3 Concentration of elements in biomass**

393 **4.3.1 Macronutrients**

394 Concentrations of Ca, Mg, and K in leaves in both the contaminated control soils were in the

395 range or higher than their foliar level for the optimal growth of willows in non-contaminated

396 soils (4.5 g Ca/kg, 2–2.5 g Mg/kg, 8–18 g K/kg); (Jug et al. 1999). The concentration of P in

397 leaves in the slightly acidic contaminated control soil was usually below or at foliar P content

398 for the optimal growth of willows in non-contaminated soils (2.1 g P/kg); (Jug et al. 1999),

399 which was probably caused by an insufficient supply of P in the soil (the value of 9 mg P/kg,

400 determined with the Mehlich III extraction procedure, belongs to the category of low

401 available concentration of P in soil, i.e. <50 mg P/kg in arable land). In heavily contaminated

402 control soils differing in soil pH, there is no limit to the cultivation of willows due to Ca, Mg,

403 and K deficiency in leaves. Latent deficiency of P can be a problem for willows grown in

404 slightly acidic contaminated soils.

405 The higher concentration of P in leaves of willows grown in the Malín soil compared with the

406 Litavka soil is connected with the higher mobilisable concentration of P (i.e. acid-extractable)

407 in the Malín soil and thus with the higher P availability for plants.

408 The concentration of P in twigs was higher after the first growing year than after the second

409 year, which is probably due to the growth limitation in the first growing season.

410 Concentrations of other macronutrients in organs were stable throughout the experiment.

411 In both contaminated control soils, a tendency for higher transport of all macronutrients from

412 twigs into leaves (i.e. into the most metabolically active organ with high nutrient

18

413 requirements); (López-Lefebre et al. 2001) was recorded. Lime and dolomite applications do

414 not restrict transport from twigs into leaves of willows grown in any of the contaminated

415 soils.

416 Concentrations of Ca, Mg, and K in leaves in most cases in both lime- and dolomite-treated

417 soils were in the range or higher than their foliar level for the optimal growth of willows. The

418 ML2 treatment with 0.9 g Mg/kg in leaves was the exception. This was probably due to a

419 dilution effect because biomass production was quadrupled due to the lime application in the

420 higher dose. The concentration of P in leaves in lime- and dolomite-treated slightly acidic soil

421 was lower than the foliar P level for the optimal growth of willows because liming is not an

422 appropriate measure for increasing the concentration of P in leaves in slightly acidic

423 contaminated soil with an insufficient supply of P in the soil. The concentration of P in leaves

424 in most of the cases in lime- and dolomite-treated alkaline soil was higher than the foliar P

425 level for the optimal growth of willows. The ML2 treatment with 1.7 g P/kg in leaves was the

426 exception, and was probably because of the dilution effect. In heavily contaminated lime- and

427 dolomite-treated soils differing in soil pH there is no limit for the cultivation of willows due

428 to Ca, Mg, and K deficiency in leaves. Latent deficiency of P also remains a problem for

429 willows in lime- and dolomite-treated slightly acidic contaminated soils.

430

431 **4.3.2 Micronutrients**

432 Concentrations of Cu and Fe in leaves in most of the cases in all of the contaminated control

433 soils were in the range or slightly higher than their common foliar concentrations in willows

434 grown in acidic non-contaminated soils (3.5–9.2 mg Cu/kg; 50–1,524 mg Fe/kg); (Syso et al.

435 2014) with the exception of the LC treatment after the first growing year with 38 mg Fe/kg in

436 leaves. The visible deficiency of Fe for willows (also confirmed by the deficiency level <40

437 mg Fe/kg); (Levy et al. 1999) was probably caused by the phytotoxicity of Zn (phytotoxicity

19

438   level >100–500 mg Zn/kg); (Kabata-Pendias 2011). Similar results were recorded by other

439   authors (Vysloužilová et al.2003b; Tlustoš et al. 2007). Concentrations of Mn and Ni in

440   leaves in both contaminated control soils were lower than their common foliar concentrations

441   in willows grown in acidic non-contaminated soils (168–779 mg Mn/kg, 5.3–13 mg Ni/kg);

442   (Syso et al. 2014). The deficiency of Fe in leaves is a serious problem for the cultivation of

443   willows in slightly acidic heavily contaminated soils and can be somewhat solved by foliar Fe

444   application during vegetation.

445   Concentrations of Cu, Fe, and Mn in organs were lower after the first season than after the

446   following seasons because of their precipitation by the high soil pH caused by liming in the

447   first growing season. The higher Fe concentration in leaves after the last two years was also

448   caused by foliar Fe application from the second growing season.

449   In both contaminated control soils, a tendency for the higher transport of Fe, Mn, and Ni from

450   twigs into leaves was recorded. Lime and dolomite applications do not restrict their transport

451   from the twigs into the leaves of willows grown in any of the contaminated soils. In the

452   weakly acidic contaminated control soil, a tendency for restricted transport of Cu from twigs

453   into leaves was recorded as well as in the lime and dolomite treatments. In the alkaline

454   contaminated control soil, a tendency for higher transport of Cu from twigs into leaves was

455   recorded as well as in the lime and dolomite treatments. Similar results (i.e. higher

456   concentration of Cu in twigs than in leaves of willows in slightly acidic and soil slightly

457   contaminated by Cd, Cu, and Zn and ambiguous transport of Cu in aboveground organs of

458   willows in alkaline non-contaminated soil) have been recorded by Kacálková et al. (2015).

459   Different agrochemical characteristics of contaminated soils could probably change the

460   distribution of Cu in aboveground biomass.

461   Concentrations of Cu and Fe in leaves in most cases in the lime- and dolomite-treated soils

462   were in the range or slightly higher than the common foliar concentrations in willows grown

20

463   in acidic non-contaminated soils. The LL1 treatment with 28 mg Fe/kg in leaves, the LL2

464   treatment with 24.8 mg Cu/kg in leaves, and the LD1 treatment with 15.0–24.5 mg Cu/kg in

465   the leaves of the willows were the exceptions. It is obvious from previous results that the

466   lower dose of dolomite was connected with lower biomass production and higher mortality of

467   willows in slightly acidic contaminated soil. Concentrations of Mn and Ni in leaves in lime-

468   and dolomite-treated soils were lower than their common foliar concentrations in willows

469   grown in acidic non-contaminated soils. The dolomite application in the lower dose is not an

470   appropriate measure for the cultivation of willows in slightly acidic contaminated soils. The

471   deficiency of Fe in leaves of willows in slightly acidic contaminated soils can be partially

472   solved by lime application in combination with foliar Fe application during vegetation.

473

474   **4.3.3 Toxic elements**

475   In the present study, we are not concern with As concentration because willows are not

476   suitable plants for As uptake and accumulation (Tlustoš et al. 2007).

477   Concentrations of Cd and Zn in leaves in both contaminated control soils were considerably

478   higher (27.7–76.8 mg Cd/kg; 732–3,488 mg Zn/kg) than their common foliar concentrations

479   in willows grown in acidic non-contaminated soils (0.5 mg Cd/kg, 175–256 mg Zn/kg); (Syso

480   et al. 2014). Concentrations of Al and Cr in leaves in both contaminated control soils were

481   lower than their common foliar concentrations in willows grown in acidic non-contaminated

482   soils: <100–200 mg Al/kg valid for general plant species (Watanabe and Osaki 2002), 153 mg

483   Al/kg valid for *Salix* 'Brekkavier' (Vike 2005), 1.1–6.4 mg Cr/kg (Syso et al. 2014). The

484   concentration of Pb in leaves was higher in slightly acidic contaminated control soil and was

485   lower in alkaline contaminated control soil than its common foliar concentration in willows

486   grown in acidic non-contaminated soils (1.0–1.1 mg Pb/kg); (Lopéz-Lefebre et al. 2001). In

487   heavily contaminated control soils with different soil pH, cultivation of willows is limited by

21

488 Cd and Zn toxicity, which induces Fe deficiency in leaves, especially in slightly acidic soil

489 conditions.

490 The higher concentrations of Cd, Pb, and Zn in willow organs in the Litavka soil are

491 connected with the higher original level of these elements in the soil. The higher

492 concentration of Cr in organs in the Malín soil is connected with possible Cr uptake as anion

493 (CrO_4^{2-}) (higher soil pH is connected with higher Cr availability for plants); (Kabata-Pendias

494 2011).

495 Concentrations of Al, Cd, and Cr in organs were higher after the last growing year than after

496 the first year because of the reduced immobilisation effect of liming over time (Lee et al.

497 2004). Concentrations of Cr and Pb in organs were higher after the first year than after the

498 second year, which is probably because of the reduced effect of liming on the mineralisation

499 of organic matter and subsequently on the reduced release of Cr and Pb bound in the lime

500 over time (Yobouet et al. 2010; Kabata-Pendias 2011).

501 In both contaminated control soils, a tendency for higher transport of Al, Cd, and Zn from

502 twigs into leaves was recorded. Lime and dolomite applications do not restrict the transport of

503 Al, Cd, or Zn from twigs into leaves of willows grown in any of the contaminated soils.

504 Similar results (i.e. higher Cd and Zn concentrations in leaves than in twigs of willows) were

505 recorded by other authors (Tlustoš et al. 2007; Kacálková et al. 2015). In slightly acidic

506 contaminated control soil, a tendency for higher transport of Cr from twigs into leaves was

507 recorded as well as in the lime and dolomite treatments. In the alkaline contaminated control

508 and treated soils, a comparable concentration of Cr in twigs and in leaves was recorded. In the

509 slightly acidic contaminated control soil, a tendency for restricted transport of Pb from twigs

510 into leaves was recorded. Similar result (i.e. higher concentration of Pb in twigs than in

511 leaves) was recorded by Tlustoš et al. (2007). Lime and dolomite application increased the

512 transport of Pb from twigs into leaves at comparable level in willows (twigs = leaves) grown

22

513 in slightly acidic contaminated soil. In the alkaline contaminated control soil, a comparable

514 concentration of Pb in twigs and in leaves was recorded. Lime and dolomite application

515 increased the transport of Pb from twigs into leaves of willows grown in alkaline

516 contaminated soil.

517 The comparison of concentrations of all risk elements in leaves in lime- and dolomite-treated

518 contaminated soils with their common foliar concentrations in willows grown in acidic non-

519 contaminated soils was the same as in both contaminated control soils. The dolomite

520 application in the lower dose is not an appropriate measure for the cultivation of willows in

521 slightly acidic contaminated soils because of low biomass and higher mortality of willows.

522 Nevertheless, lime application in combination with foliar Fe application during vegetation

523 caused a reduction of Cd and Zn in leaves (not below the limit of their phytotoxicity) as well

524 as a reduction of the Zn:Fe ratio in leaves of willows, thus indicating that it can partially solve

525 the Zn phytotoxicity that induces Fe deficiency and biomass reduction in slightly acidic

526 contaminated soils.

527

23

528 **5 Conclusions**

529 Contaminated soils with different soil pH had an effect on the amount of organ biomass

530 production of willows in our three-year study. Higher twig biomass than leaf biomass was

531 recorded in alkaline soils and vice versa in slightly acidic soils.

532 Lime application was the most effective measure for increasing biomass production of

533 willows in heavily contaminated soils with slightly acidic to alkaline soil pH. The dose of

534 lime only played a significant role in increasing biomass production in alkaline contaminated

535 soils (i.e. the higher dose). The time that had passed since the lime application affected the

536 biomass production and mortality of willows in both heavily contaminated soils. Immediate

537 planting of cuttings after lime application in higher doses was fatal for them. Dolomite

538 application did not increase the biomass production of willows in heavily contaminated soils

539 with slightly acidic to alkaline soil pH. Lower doses of dolomite caused a decrease in biomass

540 production as well as regular mortality of willows in slightly acidic contaminated soils due to

541 the low immobilisation of risk elements.

542 Liming is not an appropriate measure for changing the distribution of all macro, all micro, or

543 almost all toxic elements except Pb between aboveground organs of willows in heavily

544 contaminated soils. The latent deficiency of P in leaves can be questionable in untreated as

545 well as in lime- and dolomite-treated slightly acidic contaminated soils. Willow cultivation in

546 slightly acidic contaminated soils treated with dolomite in lower dose is not suitable. The

547 toxicity of Cd as well as the toxicity of Zn, which induces Fe deficiency in leaves and is

548 connected with biomass reduction, can be partially solved by lime application with a

549 combination of foliar Fe application during vegetation in slightly acidic contaminated soils.

550

24

556 **References**

557 Abhilash PC, Powell JR, Singh HB, Singh BK (2012) Plant-microbe interactions: novel
558   applications for exploitation in multipurpose remediation technologies. Trends Biotechnol
559   30:416–420

560 Alloway BJ (2013) Bioavailability of elements in soil. In: Selinus O (eds) Essentials of
561   medical geology: revised edition. Springer, Netherlands, pp 351–373

562 Alkorta I, Becerril JM, Garbisu C (2010) Phytostabilization of metal contaminated soils. Rev
563   Environ Health 25:135–146

564 Baker AJM (1981) Accumulators and excluders - strategies in the response of plants to heavy-
565   metals. J Plant Nutr 3:643–654

566 Bolan NS, Duraisamy VP (2003) Role of inorganic and organic soil amendments on
567   immobilisation and phytoavailability of heavy metals: a review involving specific case
568   studies. Aust J Soil Res 41:533–555

569 Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB,
570   Scheckel K (2014) Remediation of heavy metal(loid)s contaminated soils – to mobilize or
571   to immobilize? J Hazard Mater 266:141–166

572 Dos Santos Utmazian MN, Wieshammer G, Vega R, Wenzel WW (2007) Hydroponic
573   screening for metal resistance and accumulation of cadmium and zinc in twenty clones of
574   willows and poplars. Environ Pollut 148:155–165

575 Friesl-Hanl W, Platzer K, Horak O, Gerzabek MH (2009) Immobilising of Cd, Pb, and Zn
576   contaminated arable soils close to a former Pb/Zn smelter: a field study in Austria over 5
577   years. Environ Geochem Health 31:581–594

578 Hejcman M, Vondráčková S, Müllerová V, Červená K, Száková J, Tlustoš P (2012) Effect of
579   quick lime and superphosphate additives on emergence and survival of *Rumex obtusifolius*
580   seedlings in acid and alkaline soils contaminated by As, Cd, Pb, and Zn. Plant Soil Environ
581   58:561–567

26

582   Hejcman M, Müllerová V, Vondráčková S, Száková J, Tlustoš P (2014) Establishment of

583   *Bryum argenteum* and concentrations of elements in its biomass on soils contaminated by

584   As, Cd, Pb and Zn. Plant Soil Environ 60:489–495

585   Hytönen J, Kaunisto S (1999) Effect of fertilization on the biomass production of coppiced

586   mixed birch and willow stands on a cut-away peatland. Biomass Bioenergy 17:455–469

587   Jensen JK, Holm PE, Nejrup J, Larsen MB, Borggaard OK (2009) The potential of willow for

588   remediation of heavy metal polluted calcareous urban soils. Environ Pollut 157:931–937

589   Jug A, Hofmann-Schielle C, Makeschin F, Rehfuess KE (1999) Short-rotation of balsam

590   poplars, aspen and willows on former arable land in the Federal Republic of Germany. II.

591   Nutritional status and bioelement export by harvested shoot axes. For Ecol Manage

592   121:67–83

593   Kabata-Pendias A (2011) Trace elements in soils and plants, 4th edn. CRC Press, Boca Raton

594   Kacálková L, Tlustoš P, Száková J (2015) Phytoextraction of risk elements by willow and

595   poplar trees. Int J Phytoremediat 17:414–421

596   Kumpiene J, Lagerkvist A, Maurice C (2008) Stabilization of As, Cr, Cu, Pb and Zn in soil

597   using amendments – a review. Waste Manage 28:215–225

598   Kunhikrishnan A, Bolan NS, Müller K, Laurenson S, Naidu R, Kim W (2012) The influence

599   of wastewater irrigation on the transformation and bioavailability of heavy metal(loid)s in

600   soil. Adv Agron 115:215–279

601   Lee TM, Lai HY, Chen ZS (2004) Effect of chemical amendments on the concentration of

602   cadmium and lead in long-term contaminated soils. Chemosphere 57:1459–1471

603   Leitenmaier B., Küpper H (2013) Compartmentation and complexation of metals in

604   hyperaccumulator plants. Front Plant Sci 4:1–13

605   Levy DB, Redente EF, Uphoff GD (1999) Evaluating the phytotoxicity of Pb-Zn tailings to

606   big bluestem (*Andropogon gerardii* Vitman) and switchgrass (*Panicum virgatum* L.). Soil

607   Sci 164:363–375

27

608 López-Lefebre LR, Rivero RM, Garcia PC, Sanches E, Ruiz JM, Romero L (2001) Effect of

609   calcium on mineral nutrient uptake and growth of tobacco. J Sci Food Agric 81:1334–1338

610 Mader P, Száková J, Miholová D (1998) Classical dry ashing of biological and agricultural

611   materials. Part II. Losses of analytes due to their retention in an insoluble residue. Analusis

612   26:121–129

613 Mayfield JL, Ozanne L, Mitchell CC, Simonne EH, Sibley JL (2004) Laboratory and

614   greenhouse evaluation of quicklime sources for suitability as agricultural liming materials.

615   J Sci Food Agric 35:1167–1183

616 Meers E, Du Laing G, Unamuno V, Ruttens A, Vangronsveld J, Tack FMG, Verloo MG

617   (2007) Comparison of cadmium extractability from soils by commonly used single

618   extraction protocols. Geoderma 141:247–259

619 Mühlbachová G, Tlustoš P (2006) Effects of liming on the microbial biomass and its activities

620   in soils long-term contaminated by toxic elements. Plant Soil Environ 52:345–352

621 Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for

622   plants: a review. Environ Chem Lett 8:199–216

623 Pugh RE, Dick DG, Fredeen AL (2002) Heavy metal (Pb, Zn, Cd, Fe, and Cu) contents of

624   plant foliage near the anvil range lead/zinc mine, Faro, Yukon Territory. Ecotoxicol

625   Environ Saf 52:273–279

626 Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees – a

627   review. Environ Int 29:529–540

628 Puschenreiter M, Horak O, Friesl W, Hartl W (2005) Low-cost agricultural measures to

629   reduce heavy metal transfer into food chain – a review. Plant Soil Environ 51:1–11

630 Quevauviller P (1998) Operationally defined extraction procedures for soil and sediment

631   analysis – I. standardization. Trac-Trends Anal Chem 17:289–298

632 Ruttens A, Mench M, Colpaert JV, Boisson J, Carleer R, Vangronsveld J (2006)

633   Phytostabilization of a metal contaminated sandy soil. I: Influence of compost and/or

634   inorganic metal immobilizing soil amendments on phytotoxicity and plant availability of
635   metals. Environ Pollut 144:524–532

636 Sheoran V, Sheoran AS, Poonia P (2011) Role of hyperaccumulators in phytoextraction of
637   metals from contaminated mining sites: a review. Crit Rev Environ Sci Technol 41:168–
638   214

639 Solanki R, Dhankhar R (2011) Biochemical changes and adaptive strategies of plants under
640   heavy metal stress. Biologia 66:195–204

641 Syso AI, Kolpashchikov LA, Ermolov YV, Cherevko AS, Siromlya TI (2014) Elemental
642   chemical composition of soils and plants in Western Taimyr. Contemp Probl Ecol 7:636–
643   642

644 Tahvanainen L, Rytkönen VM (1999) Biomass production of *Salix viminalis* in southern
645   Finland and the effect of soil properties and climate conditions on its production and
646   survival. Biomass Bioenerg 16:103–117

647 ter Braak CJF, Smilauer P (2002) CANOCO reference manual and CanoDraw for Windows
648   user's guide: software for canonical community ordination (version 4.5). Microcomputer
649   Power, Ithaca.

650 Tlustoš P, van Dijk D, Száková J, Pavlíková D (1994) Cd and Zn release through the selected
651   extractants. Rostl Výr 40:1107–1121

652 Tlustoš P, Pavlíková D, Száková J, Fischerová Z, Balík J (2006) Exploitation of fast growing
653   trees in metal remediation. In: Mackova, M., Dowling, D., Macek, T. (eds)
654   Phytoremediation and rhizoremediation. Theoretical background. Focus on biotechnology.
655   vol. 9A. Springer, Dordrecht, pp 83-102

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

29

659 Trakal L, Neuberg M, Tlustoš P, Száková J, Tejnecký V, Drábek O (2011) Dolomite

660   limestone application as a chemical immobilization of metal-contaminated soil. Plant Soil

661   Environ 57:173–179

662 Vácha, R, Podlešáková E, Němeček J, Poláček O (2002) Immobilisation of As, Cd, Pb and Zn

663   in agricultural soils by the use of organic and inorganic additives. Rostl Výr 48:335–342

664 Vamerali T, Bandiera M, Coletto L, Zanetti F, Dickinson NM, Mosca G (2009)

665   Phytoremediation trials on metal- and arsenic-contaminated pyrite wastes (Torviscosa,

666   Italy). Environ Pollut 157:887–894

667 Vike E (2005) Uptake, deposition and wash off of fluoride and aluminium in plant foliage in

668   the vicinity of an aluminium smelter in Norway. Water Air Soil Pollut 160:145–159

669 Vondráčková S, Hejcman M, Tlustoš P, Száková J (2013) Effect of quick lime and dolomite

670   application on mobility of elements (Cd, Zn, Pb, As, Fe, and Mn) in contaminated soils.

671   Pol J Environ Stud 22:577–589

672 Vondráčková S, Hejcman M, Száková J, Müllerová V, Tlustoš P (2014) Soil chemical

673   properties affect the concentration of elements (N, P, K, Ca, Mg, As, Cd, Cr, Cu, Fe, Mn,

674   Ni, Pb, and Zn) and their distribution between organs of *Rumex obtusifolius*. Plant Soil

675   379:231–245

676 Vysloužilová M, Tlustoš P, Száková J, Pavlíková D (2003a) As, Cd, Pb and Zn uptake by

677   Salix spp. clones grown in soils enriched by high loads of these elements. Plant Soil

678   Environ 49:191–196

679 Vysloužilová M, Tlustoš P, Szaková J (2003b) Cadmium and zinc phytoextraction potential

680   of seven clones of *Salix* spp. planted on heavy metal contaminated soils. Plant Soil Environ

681   49:542–547

30

682   Vysloužilová M, Puschenreiter M, Wieshammer G, Wenzel WW (2006) Rhizosphere

683   characteristics, heavy metal accumulation and growth performance of two willow (*Salix x*

684   *rubens*) clones. Plant Soil Environ 52:353–361

685   Watanabe T, Osaki M (2002) Mechanisms of adaptation to high aluminium condition in

686   native plant species growing in acid soils: a review. Commun Soil Sci Plant Anal 33:1247–

687   1260

688   Yobouet YA, Adouby K, Trokourey A, Yao B (2010) Cadmium, copper, lead and zinc

689   speciation in contaminated soils. Inter J Eng Sci Tech 2:802–812

690   Zárubová P, Hejcman M, Vondráčková S, Mrnka L, Száková J, Tlustoš P (2015) Effect of

691   aging on concentrations of P, K, Ca, Mg, Cd, Cu, Fe, Mn, Pb, and Zn in wood and bark of

692   willows and poplars used for phytoextraction on soils contaminated by risk elements.

693   Environ Sci Pollut Res doi 10.1007/s11356-015-5043-0

694

695 **Fig. 1** Ordination diagrams showing the results of the PCA analysis with element
696 concentrations in organs of *S. smithiana* grown in contaminated soils at the end of growing
697 season – 1st a, b; 2nd c, d; 3rd e, f
698 Treatment abbreviations: control C; lime application in doses (g/kg soil) L1 – 7.3 and L2 –
699 21.9, dolomite application in doses (g/kg soil) D1 – 21.6 and D2 68.1.
700 Element abbreviations: /B – total concentrations of elements in plant biomass; /Ca – plant-
701 available concentrations of elements in soil (extracted with 0.01 mol/L CaCl$_2$); and /AA –
702 acid-extractable concentrations of elements in soil (extracted with 0.11 mol/L CH$_3$COOH).
703 Other abbreviations: DM total – dry mass of twigs and leaves biomass together; DM organ –
704 dry mass of organ biomass (twigs and leaves biomass separately) and pH mean soil pH
705

32

Variable	Treatment	Growing season		
		1st **	2nd **	3rd **
pH$_{CaCl2}$	LC [n.s.]	$6.0^{bA} \pm 0.02$	$6.2^{bA} \pm 0.04$	$6.1^{bA} \pm 0.06$
	LL1 **	$7.7^{abA} \pm 0.03$	$7.6^{abAB} \pm 0.03$	$7.4^{abB} \pm 0.03$
	LL2 [n.s.]	$7.9^{acA} \pm 0.01$	$7.8^{acA} \pm 0.02$	$7.7^{aA} \pm 0.07$
	LD1 *	$6.6^{bcA} \pm 0.02$	$6.7^{bB} \pm 0.03$	$6.7^{bcAB} \pm 0.06$
	LD2 *	$6.6^{bcA} \pm 0.02$	$6.9^{bcB} \pm 0.07$	$6.7^{bcAB} \pm 0.04$
	MC [n.s.]	$7.3^{abA} \pm 0.03$	$7.2^{abA} \pm 0.02$	$7.2^{abA} \pm 0.01$
	ML1 *	$7.7^{abA} \pm 0.04$	$7.6^{abAB} \pm 0.04$	$7.4^{abB} \pm 0.02$
	ML2 **	$8.3^{aA} \pm 0.04$	$7.9^{aAB} \pm 0.02$	$7.6^{acB} \pm 0.02$
	MD1 [n.s.]	$7.4^{abA} \pm 0.03$	$7.3^{abA} \pm 0.02$	$7.3^{abA} \pm 0.02$
	MD2 [n.s.]	$7.3^{abA} \pm 0.01$	$7.4^{abA} \pm 0.03$	$7.3^{abA} \pm 0.03$

710 Treatment abbreviations: LC – Litavka control soil without any additive; LL1 –Litavka soil
711 with lime in dose 1; LL2 – Litavka soil with lime in dose 2; LD1 – Litavka soil with dolomite
712 in dose 1; LD2 Litavka soil with dolomite in dose 2; MC Malín control soil without any
713 additive; ML1 Malín soil with lime in dose 1; ML2 Malín soil with lime in dose 2; MD1
714 Malín soil with dolomite in dose 1; and MD2 Malín soil with dolomite in dose 2.
715 Calculated with the Kruskal–Wallis test, differences between treatments within the growing
716 season and differences between the growing seasons within the treatment were not
717 statistically significant ([n.s.]) or were significant at 0.05(*) and 0.01 (**) probability levels.
718 According to the multiple comparisons of mean ranks, treatments within the growing season
719 with the same letter (a–c) and growing seasons within the treatment with the same letter (A–
720 B) were not significantly different.

34

Table 2 The effect of treatment on dry mass of organ biomass (DM organ; twig and leaf separately) and on dry mass of total biomass (DM total; twig and leaf together) of *S. smithiana* (g/plant; mean ± SE) at the end of three growing seasons in the Litavka and Malín soils

Treatment	Growing season											
	1st			2nd			3rd			1st – 3rd		
	DM organ **		DM total **	DM organ **		DM total **	DM organ **		DM total **	mean DM organ **		mean DM total **
	twig **	leaf **		twig *	leaf **		twig **	leaf **		twig **	leaf **	
LC t ⁿ·ˢ·, 1 ⁿ·ˢ·, tot ⁿ·ˢ·	10.0ᵃᵇᴬ±1.3	10.2ᵃᵇᴬ±0.8	20.2ᵃᵇᴬ±1.8	7.6ᵃᵇᴬ±2.3	13.0ᵃᵇᴬ±3.5	20.6ᵃᵇᴬ±5.8	5.5ᵇᴬ±2.3	10.3ᵇᶜᴬ±3.2	15.8ᵃᵇᴬ±5.5	7.7ᵇ±1.2	11.2ᵃᶜ±1.5	18.9ᵇ±2.6
LL1 t *, 1 **, tot **	5.8ᵃᵇᴮ±3.0	6.8ᵃᵇᴮ±2.7	12.6ᵃᵇᴮ±5.6	14.6ᵃᵇᴬᴮ±3.0	19.8ᵃᵇᴬᴮ±3.5	34.4ᵃᵇᴬᴮ±6.4	32.2ᵃᵇᴬ±1.3	33.6ᵃᶜᴬ±2.2	65.8ᵃᶜᴬ±3.1	17.5ᵃ±3.6	20.1ᵃ±3.6	37.6ᵃ±7.1
LL2 t ⁿ·ˢ·, 1 ⁿ·ˢ·, tot ⁿ·ˢ·	2.6ᵃᵇᴬ	5.7ᵃᵇᴬ	8.3ᵃᵇᴬ	26.1ᵃᵇᴬ±7.8	28.5ᵃᵇᴬ±7.3	54.6ᵃᵇᴬ±15.0	26.6ᵃᵇᴬ±8.8	20.1ᵃᵇᴬ±6.7	46.7ᵃᵇᴬ±15.4	23.7ᵃ±5.5	22.2ᵃ±4.8	45.9ᵃ±10.0
LD1 t ⁿ·ˢ·, 1 ⁿ·ˢ·, tot ⁿ·ˢ·	2.0ᵇᴬ±0.5	3.4ᵇᴬ±0.5	5.4ᵇᴬ±0.9	2.6ᵇᴬ±2.3	2.4ᵇᴬ±2.0	5.0ᵇᴬ±4.3	3.3ᵇᴬ±1.5	6.1ᵇᴬ±2.2	9.4ᵇᴬ±3.6	2.6ᵇ±0.9	4.0ᶜ±1.0	6.6ᵇᶜ±1.8
LD2 t ⁿ·ˢ·, 1 *, tot *	1.7ᵇᴬ±0.5	2.2ᵇᴮ±0.7	3.8ᵇᴮ±1.1	10.1ᵃᵇᴬ±2.2	10.7ᵃᵇᴬᴮ±2.2	20.8ᵃᵇᴬᴮ±4.4	17.7ᵃᵇᴬ±5.6	18.8ᵃᵇᴬ±4.5	36.5ᵃᵇᴬ±9.8	9.8ᵇ±2.7	10.5ᵇᶜ±2.5	20.3ᵇ±5.2
MC t *, 1 *, tot **	8.1ᵃᵇᴮ±0.2	6.4ᵃᵇᴬᴮ±0.3	14.5ᵃᵇᴮ±0.4	8.6ᵃᵇᴬᴮ±0.6	6.4ᵃᵇᴮ±0.5	15.0ᵃᵇᴬᴮ±0.5	28.3ᵃᵇᴬ±1.7	16.5ᵃᵇᴬ±1.2	44.7ᵇᶜᴬ±2.5	15.0ᵇ±2.9	9.7ᵇᶜ±1.5	24.7ᵇ±4.3
ML1 t **, 1 **, tot *	15.0ᵃᵃᴬᴮ±1.0	11.7ᵃᵃᴬᴮ±0.8	26.7ᵃᵃᴬᴮ±1.7	9.6ᵃᵇᴮ±0.6	6.5ᵃᵇᴮ±0.5	16.1ᵃᵇᴮ±0.9	23.3ᵃᵇᴬ±2.6	17.5ᵃᵇᴬ±1.7	40.7ᵃᵇᴬ±3.6	16.0ᵃ±1.9	11.9ᵃᶜ±1.5	27.8ᵃ±3.3
ML2 t *, 1 ⁿ·ˢ·, tot *	died	died	died	32.6ᵃᴮ±1.6	26.1ᵃᴬ±1.2	58.7ᵃᴮ±2.6	49.9ᵃᴬ±1.2	26.3ᵃᶜᴬ±0.4	76.2ᵃᴬ±1.4	41.2ᵃ±3.4	26.2ᵃ±0.6	67.4ᵃ±3.6
MD1 t *, 1 *, tot **	8.5ᵃᵇᴬᴮ±0.4	7.5ᵃᵇᴬᴮ±0.5	16.0ᵃᵇᴬᴮ±0.7	8.5ᵃᵇᴬᴮ±0.6	5.6ᵃᵇᴮ±0.9	14.1ᵃᵇᴮ±1.3	23.3ᵃᵇᴬ±2.6	15.8ᵃᵇᴬ±2.1	39.1ᵃᵇᴬ±4.6	13.4ᵇ±2.3	9.6ᵇᶜ±1.5	23.1ᵇ±3.7
MD2 t **, 1 **, tot **	11.8ᵃᵇᴬᴮ±0.2	9.3ᵃᵇᴬᴮ±0.2	21.0ᵃᵇᴬᴮ±0.3	8.8ᵃᵇᴮ±0.3	5.2ᵃᵇᴮ±0.5	13.9ᵃᵇᴮ±0.2	23.3ᵃᵇᴬ±2.3	15.2ᵃᵇᴬ±1.6	38.5ᵃᵇᴬ±3.8	14.6ᵃ±2.0	9.9ᵇᶜ±1.3	24.5ᵃᶜ±3.3

See Table 1 for more details about the treatments.

Calculated with the Kruskal–Wallis test, differences between treatments of the same organ (i.e. twig, leaf and total DM separately) within the growing season and differences between the growing seasons within the treatment of the same organ were not statistically significant (ⁿ·ˢ·) or were significant at 0.05(*) and 0.01 (**) probability levels. According to the multiple comparisons of mean ranks, treatments between the same organ within the growing season with the same letter (a–c) and growing seasons within the treatment between the same organ with the same letter (A–B) were not significantly different.

729 **Table 3** The effect of treatment on mortality (%) of S. smithiana after winter in the Litavka
730 and Malín soils

Treatment	Mortality				
	Growing season				
	1st	2nd	3rd	1st - 3rd n.s.	2nd - 3rd n.s.
LC	0	25	0	$8^a \pm 8$	$12.5^a \pm 12.5$
LL1	50	0	0	$17^a \pm 17$	$0^a \pm 0$
LL2	75†	0	25	$33^a \pm 22$	$12.5^a \pm 12.5$
LD1	25	50	25	$33^a \pm 8$	$37.5^a \pm 12.5$
LD2	100	0	0	$33^a \pm 33$	$0^a \pm 0$
MC	0	0	0	$0^a \pm 0$	$0^a \pm 0$
ML1	0	0	0	$0^a \pm 0$	$0^a \pm 0$
ML2	100†	0	0	$33^a \pm 33$	$0^a \pm 0$
MD1	0	0	0	$0^a \pm 0$	$0^a \pm 0$
MD2	0	0	0	$0^a \pm 0$	$0^a \pm 0$

731 † mortality (%) at harvest
732 See Table 1 for more details about the treatments.
733 Calculated with the Kruskal–Wallis test, differences between treatments between growing
734 seasons were not statistically significant ([n.s.]) probability level. According to the multiple
735 comparisons of mean ranks, treatments between growing seasons were not significantly
736 different.
737

738 **Supplementary material**

739

740 **Table 4** The effect of treatment on plant-available (mg/kg; extracted with 0.01 mol/L $CaCl_2$;
741 Ca) and acid-extractable (mg/kg; extracted with 0.11 mol/L CH_3COOH; AA) concentrations
742 of elements (mean ± SE) at the end of two or three growing seasons (2nd – 3rd for Ca, Mg, K;
743 and 1st – 3rd for P, Cu, Fe, Mn, Ni, Al, Cd, Cr, Pb, and Zn) of the experiment

Variable	Treatment	Growing season			
		2nd		3rd	
Ca		Ca	AA**	Ca	AA**
	LC (AA n.s.)	–	$4,781^{bcA} \pm 147$	–	$4,556^{aA} \pm 2257$
	LL1 (AA n.s.)	–	$3,031^{bA} \pm 801$	–	$5,323^{aA} \pm 249$
	LL2 (AA*)	–	$5,048^{bcB} \pm 376$	–	$16,417^{aA} \pm 586$
	LD1 (AA n.s.)	–	$3,830^{bcA} \pm 978$	–	$4,028^{aA} \pm 1336$
	LD2 (AA n.s.)	–	$8,833^{abA} \pm 723$	–	$7,627^{aA} \pm 1251$
	MC (AA n.s.)	–	$7,888^{abA} \pm 159$	–	$8,831^{aA} \pm 1329$
	ML1 (AA*)	–	$30,087^{aA} \pm 1463$	–	$10,179^{aB} \pm 2759$
	ML2 (AA n.s.)	–	$23,435^{acA} \pm 1138$	–	$20,108^{aA} \pm 3608$
	MD1 (AA n.s.)	–	$16,901^{abA} \pm 1969$	–	$22,619^{aA} \pm 5111$
	MD2 (AA n.s.)	–	$21,062^{abA} \pm 747$	–	$23,071^{aA} \pm 3740$
Mg		Ca**	AA**	Ca**	AA**
	LC (Ca*, AA*)	$82.5^{abB} \pm 2.2$	$244^{bcA} \pm 7$	$104.5^{abA} \pm 2$	$131^{bB} \pm 14$
	LL1 (Ca*, AA*)	$41.5^{bB} \pm 1.4$	$49^{bB} \pm 12$	$57^{bcA} \pm 7$	$133^{bA} \pm 8$
	LL2 (Ca n.s., AA*)	$40.0^{bA} \pm 2.7$	$43^{bcB} \pm 1.4$	$48^{bA} \pm 2.5$	$160^{abA} \pm 3$
	LD1 (Ca*, AA n.s.)	$159.5^{abB} \pm 8.6$	$2,129^{abA} \pm 581$	$182^{aA} \pm 7$	$1,795^{abA} \pm 596$
	LD2 (Ca n.s., AA n.s.)	$187.7^{abA} \pm 8.7$	$3,303^{acA} \pm 795$	$196^{aA} \pm 15$	$3,993^{abA} \pm 799$
	MC (Ca n.s., AA n.s.)	$169^{aA} \pm 34$	$429^{abA} \pm 5$	$147^{abA} \pm 2$	$415^{abA} \pm 9.5$
	ML1 (Ca*, AA*)	$93^{abB} \pm 3$	$1,035^{abA} \pm 30$	$117^{abA} \pm 5$	$356^{abB} \pm 80$
	ML2 (Ca*, AA n.s.)	$65^{abB} \pm 0.6$	$394^{abA} \pm 8$	$104^{abA} \pm 6$	$408^{abA} \pm 11$
	MD1 (Ca*, AA n.s.)	$129^{aB} \pm 2.5$	$3,144^{aA} \pm 283$	$167^{acA} \pm 5$	$3,266^{abA} \pm 1012$
	MD2 (Ca*, AA n.s.)	$128^{aB} \pm 3$	$6,098^{aA} \pm 478$	$149^{abA} \pm 5$	$7,305^{aA} \pm 1698$
K		Ca**	AA**	Ca**	AA**
	LC (Ca n.s., AA n.s.)	$72^{abA} \pm 3$	$100^{abA} \pm 3.5$	$63^{bcA} \pm 6.5$	$101^{abA} \pm 9$
	LL1 (Ca*, AA*)	$34^{bB} \pm 6$	$58^{bB} \pm 5$	$67^{bcA} \pm 10$	$108^{abA} \pm 12$
	LL2 (Ca n.s., AA*)	$46^{bcA} \pm 9$	$70^{bcB} \pm 11.5$	$68^{abA} \pm 10.5$	$122^{abA} \pm 9$
	LD1 (Ca n.s., AA n.s.)	$82^{abA} \pm 7$	$97^{abA} \pm 14$	$89^{abA} \pm 18$	$92^{abA} \pm 31$
	LD2 (Ca*, AA n.s.)	$72^{abA} \pm 4$	$72^{abA} \pm 4$	$54^{bB} \pm 5.5$	$77^{bA} \pm 4$
	MC (Ca n.s., AA n.s.)	$402^{aA} \pm 6$	$565^{acA} \pm 14$	$396^{acA} \pm 16$	$554^{abA} \pm 20$
	ML1 (Ca n.s., AA n.s.)	$325^{abA} \pm 8$	$513^{abA} \pm 10$	$334^{abA} \pm 18$	$397^{abA} \pm 106$
	ML2 (Ca*, AA*)	$312^{abA} \pm 8$	$540^{abA} \pm 12$	$270^{abB} \pm 5$	$447^{abB} \pm 15$
	MD1 (Ca*, AA n.s.)	$379^{acB} \pm 17$	$658^{acA} \pm 84$	$456^{aA} \pm 19$	$637^{aA} \pm 28$
	MD2 (Ca n.s., AA n.s.)	$373^{acA} \pm 13$	$597^{aA} \pm 23$	$361^{abA} \pm 10$	$597^{aA} \pm 23$

744 – indicates not determine
745 See Table 1 for more details about the treatments.
746 Calculated with the Kruskal–Wallis test, differences between treatments of the same
747 extraction (i.e. Ca and AA separately) within the growing season and differences between the
748 growing seasons within the treatment of the same extraction were not statistically significant
749 ($^{n.s.}$) or were significant at 0.05(*) and 0.01 (**) probability levels. According to the multiple
750 comparisons of mean ranks, treatments between the same extraction within the growing
751 season with the same letter (a–c) and growing seasons within the treatment between the same
752 extraction with the same letter (A–B) were not significantly different.

753 **Table 4** (Continued)

Variable	Treatment	Growing season					
		1st		**2nd**		**3rd**	
P		Ca $^{n.s.}$	AA **	Ca $^{n.s.}$	AA **	Ca *	AA **
	LC (Ca *, AA **)	<0.3^{aB}	3.9^{abB} ± 1.6	2.4^{aA} ± 0.6	17.9^{abA} ± 5.1	2.1^{aAB} ± 0.6	14.5^{abAB} ± 4.3
	LL1 (Ca *, AA *)	<0.3^{aB}	1.9^{abB} ± 0.1	0.9^{aA} ± 0.1	16.4^{abA} ± 3.6	0.6^{aAB} ± 0.1	3.5^{bAB} ± 0.7
	LL2 (Ca *, AA $^{n.s.}$)	<0.3^{aB}	11.5^{abA} ± 0.5	0.7^{aAB} ± 0.4	12.7^{abA} ± 1.3	1.3^{aA} ± 0.1	10.9^{abA} ± 1.0
	LD1 (Ca $^{n.s.}$, AA *)	<0.3^{aA}	1.3^{bB} ± 0.5	1.2^{aA} ± 0.4	7.6^{bcA} ± 0.4	1.2^{aA} ± 0.3	7.0^{abAB} ± 1.7
	LD2 (Ca *, AA *)	<0.3^{aB}	1.6^{abB} ± 0.4	1.3^{aA} ± 0.4	6.4^{bAB} ± 0.5	0.9^{aAB} ± 0.3	6.4^{abA} ± 0.4
	MC (Ca $^{n.s.}$, AA $^{n.s.}$)	<0.3^{aA}	17.2^{abA} ± 7.6	0.9^{aA} ± 0.2	21^{acA} ± 2	1.1^{aA} ± 0.1	16.8^{aA} ± 1.0
	ML1 (Ca *, AA $^{n.s.}$)	<0.3^{aB}	16.6^{abA} ± 0.9	1.0^{aA} ± 0.2	17.9^{abA} ± 1.8	0.7^{aAB} ± 0.1	18.2^{aA} ± 2.0
	ML2 (Ca **, AA *)	<0.3^{aB}	14.9^{abB} ± 1.3	1.2^{aA} ± 0.2	24.0^{aA} ± 0.5	0.7^{aAB} ± 0.1	19.1^{aAB} ± 2.4
	MD1 (Ca *, AA $^{n.s.}$)	<0.3^{aB}	19.5^{aA} ± 4.1	0.7^{aA} ± 0.1	16.6^{abA} ± 1.7	0.7^{aAB} ± 0.1	15.0^{abA} ± 1.3
	MD2 (Ca *, AA $^{n.s.}$)	<0.3^{aA}	14.4^{abA} ± 1.0	0.9^{aA} ± 0.4	13.7^{abA} ± 1.3	0.8^{aA} ± 0.1	12.9^{abA} ± 1.7
Cu		Ca **	AA **	Ca **	AA **	Ca **	AA **
	LC (Ca $^{n.s.}$, AA **)	0.12^{abA} ± 0.03	0.6^{abB} ± 0.1	0.25^{abA} ± 0.03	1.2^{abA} ± 0.01	0.24^{bA} ± 0.04	1.1^{bcAB} ± 0.004
	LL1 (Ca *, AA $^{n.s.}$)	0.29^{abA} ± 0.03	1.3^{acA} ± 0.1	0.29^{abA} ± 0.04	1.6^{aA} ± 0.07	0.55^{abA} ± 0.05	1.9^{acA} ± 0.2
	LL2 (Ca $^{n.s.}$, AA $^{n.s.}$)	0.58^{aA} ± 0.02	1.9^{aA} ± 0.1	0.45^{aA} ± 0.04	2.0^{aA} ± 0.1	0.78^{abA} ± 0.15	2.6^{aA} ± 0.3
	LD1 (Ca **, AA *)	0.12^{abB} ± 0.02	0.7^{abB} ± 0.01	0.18^{bAB} ± 0.01	1.2^{abA} ± 0.03	0.25^{bA} ± 0.02	1.2^{abAB} ± 0.01
	LD2 (Ca **, AA **)	0.09^{abB} ± 0.02	0.6^{bcB} ± 0.02	0.24^{abAB} ± 0.03	1.2^{abAB} ± 0.05	0.47^{abA} ± 0.07	1.4^{abA} ± 0.06
	MC (Ca *, AA $^{n.s.}$)	0.10^{abB} ± 0.01	0.9^{abA} ± 0.1	0.4^{abAB} ± 0.07	1.2^{abA} ± 0.02	0.6^{abA} ± 0.03	0.7^{bA} ± 0.01
	ML1 (Ca *, AA **)	0.14^{abB} ± 0.02	0.8^{abB} ± 0.05	0.3^{abAB} ± 0.05	1.3^{abA} ± 0.05	0.7^{abA} ± 0.2	1.2^{abAB} ± 0.01
	ML2 (Ca *, AA $^{n.s.}$)	1.05^{aA} ± 0.16	1.5^{acA} ± 0.09	0.5^{aB} ± 0.01	1.6^{aA} ± 0.06	0.9^{aAB} ± 0.04	1.8^{acA} ± 0.08
	MD1 (Ca **, AA *)	0.06^{bB} ± 0.01	0.7^{abB} ± 0.04	0.3^{abAB} ± 0.04	1.2^{abA} ± 0.04	0.7^{abA} ± 0.1	1.2^{abAB} ± 0.1
	MD2 (Ca *, AA *)	0.06^{bB} ± 0.01	0.5^{bA} ± 0.03	0.3^{abAB} ± 0.03	0.9^{bA} ± 0.03	0.5^{abA} ± 0.1	0.9^{bcA} ± 0.09
Fe		Ca *	AA **	Ca **	AA **	Ca $^{n.s.}$	AA **
	LC (Ca *, AA $^{n.s.}$)	0.9^{abB} ± 0.4	12.4^{abA} ± 2.9	3.0^{abA} ± 0.4	15.8^{abA} ± 1.2	2.8^{aAB} ± 0.4	20.3^{aA} ± 2.1
	LL1 (Ca *, AA *)	2.8^{abAB} ± 0.4	8.8^{abB} ± 0.8	3.4^{abA} ± 0.3	13.1^{abAB} ± 1.0	1.7^{aB} ± 0.2	18.5^{aA} ± 2.9
	LL2 (Ca *, AA $^{n.s.}$)	6.2^{aA} ± 0.8	18.6^{abA} ± 1.8	5.0^{aAB} ± 0.8	16.2^{abA} ± 2.4	2.5^{aB} ± 0.2	19.7^{aA} ± 1.7
	LD1 (Ca *, AA **)	1.6^{abB} ± 0.2	3.7^{bB} ± 0.4	3.0^{abA} ± 0.2	12.1^{bAB} ± 0.4	2.4^{aAB} ± 0.5	24.5^{aA} ± 6.9
	LD2 (Ca *, AA **)	1.5^{abB} ± 0.4	3.4^{bB} ± 0.1	3.0^{abA} ± 0.1	14.5^{bAB} ± 3.8	1.6^{aAB} ± 0.3	41.6^{aA} ± 4.6
	MC (Ca *, AA *)	2.3^{abA} ± 0.1	8.9^{abB} ± 6.4	1.8^{bA} ± 0.2	43.5^{abA} ± 9.0	2.3^{aA} ± 0.1	17.6^{aAB} ± 0.8
	ML1 (Ca $^{n.s.}$, AA $^{n.s.}$)	0.8^{abA} ± 0.6	43.6^{aA} ± 7.2	1.5^{bA} ± 0.2	38.3^{abA} ± 6.1	1.6^{aA} ± 0.3	34.9^{aA} ± 9.2
	ML2 (Ca $^{n.s.}$, AA $^{n.s.}$)	1.5^{abA} ± 0.7	26.6^{abA} ± 5.8	3.1^{abA} ± 0.2	43.4^{abA} ± 5.9	2.4^{aA} ± 0.6	40.7^{aA} ± 11.9
	MD1 (Ca $^{n.s.}$, AA *)	0.9^{abA} ± 0.8	32.2^{abA} ± 4.6	2.2^{abA} ± 0.04	63.2^{aA} ± 9.2	2.5^{aA} ± 0.4	50.3^{aA} ± 6.2
	MD2 (Ca $^{n.s.}$, AA $^{n.s.}$)	0.8^{bA} ± 0.6	27.9^{abA} ± 2.6	2.4^{abA} ± 0.4	49.8^{abA} ± 8.4	1.3^{aA} ± 0.3	59.5^{aA} ± 5.6

754 limit of detection (mg/kg): P – 0.3 (Ca)

755

756 **Table 4** (Continued)

Variable	Treatment	Growing season					
		1st		2nd		3rd	
Mn		Ca**	AA**	Ca**	AA**	Ca**	AA**
	LC (Ca**, AA**)	$4.3^{aB} \pm 0.6$	$59.7^{abB} \pm 8.5$	$18.6^{aA} \pm 1.2$	$191.7^{aA} \pm 3.6$	$12.2^{aAB} \pm 1.4$	$150.4^{abAB} \pm 3.6$
	LL1 (Ca*, AA**)	$0.6^{abB} \pm 0.05$	$84.8^{aB} \pm 10.8$	$0.7^{abAB} \pm 0.1$	$149.1^{acAB} \pm 7.6$	$10.4^{abA} \pm 8.0$	$350.0^{aA} \pm 142.4$
	LL2 (Ca*, AA**)	$0.5^{abB} \pm 0.07$	$71.6^{acB} \pm 1.7$	$0.7^{abAB} \pm 0.1$	$143.9^{abAB} \pm 3.4$	$2.3^{abA} \pm 0.4$	$169.5^{abA} \pm 5.9$
	LD1 (Ca*, AA*)	$1.8^{acA} \pm 0.3$	$48.3^{abB} \pm 2.6$	$4.6^{aA} \pm 0.1$	$122.7^{abAB} \pm 6.8$	$5.5^{abA} \pm 1.1$	$128.3^{abA} \pm 11.3$
	LD2 (Ca$^{n.s.}$, AA*)	$2.3^{acA} \pm 1.3$	$47.1^{abB} \pm 2.0$	$3.8^{aA} \pm 0.3$	$113.9^{abA} \pm 6.7$	$6.2^{abA} \pm 0.4$	$111.5^{abAB} \pm 3.2$
	MC (Ca*, AA$^{n.s.}$)	$0.10^{bcB} \pm 0.01$	$26.8^{abA} \pm 7.8$	$0.9^{abAB} \pm 0.2$	$101.5^{abA} \pm 3.1$	$1.4^{abA} \pm 0.4$	$100.0^{abA} \pm 10.9$
	ML1 (Ca*, AA*)	$0.15^{abB} \pm 0.02$	$35.2^{abB} \pm 0.6$	$0.7^{abAB} \pm 0.1$	$112.7^{abAB} \pm 10.3$	$1.0^{bA} \pm 0.1$	$121.4^{abA} \pm 5.0$
	ML2 (Ca**, AA**)	$0.22^{abAB} \pm 0.01$	$33.1^{abB} \pm 0.9$	$0.1^{bB} \pm 0.01$	$82.4^{abAB} \pm 2.4$	$2.6^{abA} \pm 1.6$	$121.8^{abA} \pm 19.9$
	MD1 (Ca**, AA*)	$0.12^{bcB} \pm 0.02$	$28.7^{bcB} \pm 1.1$	$1.2^{abAB} \pm 0.1$	$120.9^{abAB} \pm 14.4$	$1.8^{abA} \pm 0.3$	$113.7^{abA} \pm 3.6$
	MD2 (Ca*, AA*)	$0.08^{bB} \pm 0.01$	$23.6^{bB} \pm 1.0$	$1.3^{abAB} \pm 0.3$	$95.0^{bcAB} \pm 11.8$	$1.6^{abA} \pm 0.1$	$98.2^{bA} \pm 13.0$
Ni		Ca$^{n.s.}$	AA**	Ca$^{n.s.}$	AA**	Ca$^{n.s.}$	AA**
	LC (Ca**, AA*)	$0.19^{aA} \pm 0.05$	$1.1^{abB} \pm 0.1$	$<0.05^{aA}$	$2.1^{aA} \pm 0.05$	$<0.05^{aA}$	$2.2^{aAB} \pm 0.2$
	LL1 (Ca**, AA*)	$0.12^{aA} \pm 0.02$	$1.2^{aB} \pm 0.07$	$<0.05^{aA}$	$1.9^{aAB} \pm 0.06$	$<0.05^{aA}$	$2.1^{aA} \pm 0.08$
	LL2 (Ca*, AA*)	$0.15^{aA} \pm 0.08$	$0.9^{abB} \pm 0.07$	$<0.05^{aA}$	$1.6^{abA} \pm 0.02$	$<0.05^{aA}$	$1.6^{abAB} \pm 0.08$
	LD1 (Ca**, AA**)	$0.15^{aA} \pm 0.03$	$1.1^{abB} \pm 0.02$	$<0.05^{aA}$	$1.8^{abAB} \pm 0.02$	$<0.05^{aA}$	$2.0^{aA} \pm 0.1$
	LD2 (Ca**, AA*)	$0.17^{aA} \pm 0.07$	$1.1^{abB} \pm 0.04$	$<0.05^{aA}$	$1.7^{abA} \pm 0.06$	$<0.05^{aA}$	$1.7^{abAB} \pm 0.1$
	MC (Ca**, AA**)	$0.11^{aA} \pm 0.01$	$0.8^{abB} \pm 0.2$	$<0.05^{aA}$	$1.8^{abA} \pm 0.04$	$<0.05^{aA}$	$1.2^{bAB} \pm 0.08$
	ML1 (Ca**, AA*)	$0.13^{aA} \pm 0.02$	$0.9^{abB} \pm 0.1$	$<0.05^{aA}$	$1.7^{abA} \pm 0.05$	$<0.05^{aA}$	$1.7^{abAB} \pm 0.1$
	ML2 (Ca**, AA*)	$0.16^{aA} \pm 0.01$	$0.8^{abB} \pm 0.06$	$<0.05^{aA}$	$1.4^{bA} \pm 0.04$	$<0.05^{aA}$	$1.3^{abAB} \pm 0.1$
	MD1 (Ca**, AA*)	$0.09^{aA} \pm 0.03$	$0.9^{abB} \pm 0.03$	$<0.05^{aA}$	$1.7^{abA} \pm 0.09$	$<0.05^{aA}$	$1.7^{abAB} \pm 0.09$
	MD2 (Ca**, AA*)	$0.19^{aA} \pm 0.1$	$0.7^{bB} \pm 0.04$	$<0.05^{aA}$	$1.4^{bA} \pm 0.07$	$<0.05^{aA}$	$1.4^{abAB} \pm 0.09$
Al		Ca**	AA**	Ca**	AA$^{n.s.}$	Ca**	AA**
	LC (Ca*, AA$^{n.s.}$)	$1.3^{bB} \pm 0.6$	$30.9^{abB} \pm 6.6$	$6.2^{abA} \pm 0.8$	$52.1^{aAB} \pm 1.9$	$5.7^{aAB} \pm 0.8$	$57.1^{abA} \pm 3.4$
	LL1 (Ca*, AA*)	$4.1^{abAB} \pm 0.7$	$29.9^{abA} \pm 4.0$	$6.5^{abA} \pm 0.6$	$50.1^{aA} \pm 2.1$	$3.3^{abB} \pm 0.4$	$54.0^{abA} \pm 5.2$
	LL2 (Ca*, AA*)	$9.6^{aA} \pm 1.5$	$37.1^{abB} \pm 3.7$	$9.6^{aA} \pm 1.4$	$54.5^{aAB} \pm 6.8$	$4.7^{acA} \pm 0.4$	$53.2^{abA} \pm 2.2$
	LD1 (Ca*, AA**)	$2.5^{abB} \pm 0.5$	$13.8^{abB} \pm 0.7$	$5.8^{abA} \pm 0.4$	$42.6^{aAB} \pm 0.8$	$4.6^{abAB} \pm 0.9$	$63.1^{abA} \pm 12.4$
	LD2 (Ca*, AA**)	$2.4^{abB} \pm 0.5$	$10.5^{bB} \pm 0.4$	$5.8^{abA} \pm 0.4$	$42.0^{aAB} \pm 6.9$	$3.5^{abAB} \pm 0.6$	$86.7^{aA} \pm 7.4$
	MC (Ca*, AA*)	$1.2^{abB} \pm 0.1$	$10.7^{abB} \pm 5.6$	$1.7^{bAB} \pm 0.2$	$55.3^{aA} \pm 8.2$	$2.2^{abA} \pm 0.1$	$18.2^{bAB} \pm 0.9$
	ML1 (Ca$^{n.s.}$, AA$^{n.s.}$)	$1.2^{abA} \pm 0.1$	$42.4^{aA} \pm 6.2$	$1.4^{bA} \pm 0.2$	$50.3^{aA} \pm 5.8$	$1.6^{bcA} \pm 0.3$	$45.2^{abA} \pm 9.2$
	ML2 (Ca$^{n.s.}$, AA*)	$1.2^{abA} \pm 0.2$	$25.4^{abB} \pm 4.6$	$2.7^{abA} \pm 0.2$	$51.7^{aA} \pm 5.4$	$2.3^{abA} \pm 0.6$	$35.3^{bAB} \pm 1.1$
	MD1 (Ca$^{n.s.}$, AA*)	$1.3^{abA} \pm 0.2$	$29.9^{abB} \pm 3.8$	$2.0^{abA} \pm 0.04$	$68.6^{aA} \pm 7.7$	$2.2^{abA} \pm 0.3$	$55.4^{abAB} \pm 6.3$
	MD2 (Ca*, AA$^{n.s.}$)	$1.1^{abB} \pm 0.1$	$22.4^{abA} \pm 2.1$	$2.3^{abA} \pm 0.4$	$52.7^{aA} \pm 7.5$	$1.3^{bAB} \pm 0.3$	$57.9^{abA} \pm 5.4$

757 limit of detection (mg/kg): Ni – 0.05 (Ca); Al – 0.5 (Ca)

758

39

759 **Table 4** (Continued)

Variable	Treatment	Growing season					
		1st		2nd		3rd	
		Ca**	AA**	Ca**	AA**	Ca**	AA**
Cd							
	LC (Ca n.s., AA**)	$3.1^{aA} \pm 0.1$	$15.9^{acB} \pm 1.8$	$3.9^{aA} \pm 0.1$	$24.9^{acA} \pm 0.4$	$4.4^{aA} \pm 0.5$	$23.4^{acAB} \pm 0.2$
	LL1 (Ca n.s., AA*)	$0.3^{abA} \pm 0.04$	$21.1^{aB} \pm 1.4$	$0.3^{abA} \pm 0.02$	$26.0^{aAB} \pm 0.4$	$0.4^{abA} \pm 0.04$	$27.1^{aA} \pm 1.1$
	LL2 (Ca n.s., AA*)	$0.1^{abA} \pm 0.01$	$16.2^{abB} \pm 0.6$	$0.1^{abA} \pm 0.01$	$22.6^{abA} \pm 0.2$	$0.1^{abA} \pm 0.01$	$22.3^{abAB} \pm 0.6$
	LD1 (Ca n.s., AA*)	$2.4^{acA} \pm 0.1$	$15.5^{abB} \pm 0.2$	$2.2^{acA} \pm 0.1$	$23.7^{abA} \pm 0.6$	$2.1^{acA} \pm 0.2$	$23.4^{acAB} \pm 0.7$
	LD2 (Ca*, AA*)	$2.4^{acA} \pm 0.6$	$14.0^{bA} \pm 0.5$	$1.6^{acB} \pm 0.1$	$21.2^{abA} \pm 0.7$	$2.0^{acAB} \pm 0.1$	$21.0^{abA} \pm 0.3$
	MC (Ca n.s., AA*)	$0.05^{abA} \pm 0.002$	$2.5^{bcB} \pm 0.2$	$0.05^{abA} \pm 0.003$	$4.3^{bA} \pm 0.1$	$0.05^{abA} \pm 0.002$	$2.8^{bAB} \pm 0.1$
	ML1 (Ca n.s., AA**)	$0.04^{abA} \pm 0.002$	$3.2^{abB} \pm 0.1$	$0.04^{bcA} \pm 0.01$	$4.9^{abA} \pm 0.2$	$0.05^{bcA} \pm 0.01$	$3.9^{abAB} \pm 0.2$
	ML2 (Ca**, AA**)	$0.05^{abA} \pm 0.004$	$2.7^{bcB} \pm 0.1$	$<0.01^{bB}$	$4.3^{bcA} \pm 0.1$	$0.02^{bAB} \pm 0.003$	$3.4^{bcAB} \pm 0.2$
	MD1 (Ca n.s., AA**)	$0.04^{bA} \pm 0.002$	$2.7^{bcB} \pm 0.01$	$0.05^{abA} \pm 0.004$	$4.7^{abA} \pm 0.1$	$0.05^{abA} \pm 0.001$	$3.9^{abAB} \pm 0.1$
	MD2 (Ca n.s., AA**)	$0.04^{bcA} \pm 0.0002$	$2.4^{bB} \pm 0.06$	$0.06^{abA} \pm 0.01$	$4.0^{bA} \pm 0.1$	$0.05^{abA} \pm 0.01$	$3.3^{bcAB} \pm 0.1$
Cr		Ca n.s.	AA n.s.	Ca n.s.	AA**	Ca n.s.	AA**
	LC (Ca**, AA n.s.)	$0.06^{aA} \pm 0.003$	$<0.1^{aA}$	$<0.05^{aA}$	$<0.1^{abA}$	$<0.05^{aA}$	$<0.1^{abA}$
	LL1 (Ca**, AA n.s.)	$0.06^{aA} \pm 0.005$	$<0.1^{aA}$	$<0.05^{aA}$	$<0.1^{acA}$	$<0.05^{aA}$	$<0.1^{abA}$
	LL2 (Ca**, AA n.s.)	$0.08^{aA} \pm 0.01$	$<0.1^{aA}$	$<0.05^{aA}$	$0.2^{aA} \pm 0.02$	$<0.05^{aA}$	$0.2^{aA} \pm 0.02$
	LD1 (Ca**, AA n.s.)	$0.07^{aA} \pm 0.01$	$<0.1^{aA}$	$<0.05^{aA}$	$<0.1^{abA}$	$<0.05^{aA}$	$<0.1^{abA}$
	LD2 (Ca**, AA n.s.)	$0.07^{aA} \pm 0.01$	$<0.1^{aA}$	$<0.05^{aA}$	$<0.1^{abA}$	$<0.05^{aA}$	$0.2^{abA} \pm 0.01$
	MC (Ca**, AA*)	$0.06^{aA} \pm 0.005$	$<0.1^{aA}$	$<0.05^{aA}$	$<0.1^{bcAB}$	$<0.05^{aA}$	$<0.1^{bB}$
	ML1 (Ca**, AA n.s.)	$<0.05^{aA}$	$<0.1^{aA}$	$<0.05^{aA}$	$<0.1^{bA}$	$<0.05^{aA}$	$<0.1^{abA}$
	ML2 (Ca**, AA n.s.)	$0.06^{aA} \pm 0.002$	$<0.1^{aA}$	$<0.05^{aA}$	$<0.1^{abA}$	$<0.05^{aA}$	$<0.1^{abA}$
	MD1 (Ca**, AA n.s.)	$0.08^{aA} \pm 0.01$	$<0.1^{aA}$	$<0.05^{aA}$	$<0.1^{abA}$	$<0.05^{aA}$	$<0.1^{abA}$
	MD2 (Ca**, AA n.s.)	$0.06^{aA} \pm 0.001$	$<0.1^{aA}$	$<0.05^{aA}$	$<0.1^{bcA}$	$<0.05^{aA}$	$<0.1^{abA}$
Pb		Ca**	AA**	Ca**	AA**	Ca n.s.	AA**
	LC (Ca n.s., AA**)	$<0.2^{abA}$	$21.8^{abB} \pm 3.3$	$<0.2^{abA}$	$46.7^{abA} \pm 2.4$	$<0.2^{aA}$	$34.9^{aAB} \pm 1.3$
	LL1 (Ca*, AA*)	$0.3^{abA} \pm 0.05$	$57.7^{aB} \pm 4.7$	$<0.2^{abAB}$	$82.0^{aAB} \pm 2.1$	$<0.2^{aB}$	$97.2^{aA} \pm 14.0$
	LL2 (Ca*, AA*)	$0.5^{aA} \pm 0.05$	$38.3^{abA} \pm 1.7$	$0.4^{aAB} \pm 0.1$	$68.0^{abA} \pm 3.0$	$<0.2^{aB}$	$66.5^{aA} \pm 0.9$
	LD1 (Ca n.s., AA*)	$<0.2^{abA}$	$39.4^{abB} \pm 1.2$	$<0.2^{abA}$	$78.1^{abA} \pm 1.9$	$<0.2^{aA}$	$76.9^{aAB} \pm 2.0$
	LD2 (Ca n.s., AA*)	$<0.2^{abA}$	$40.0^{abB} \pm 0.7$	$<0.2^{abA}$	$76.5^{abAB} \pm 0.8$	$<0.2^{aA}$	$77.5^{aA} \pm 3.2$
	MC (Ca n.s., AA n.s.)	$<0.2^{abA}$	$<0.4^{abA}$	$<0.2^{abA}$	$<0.4^{abA}$	$<0.2^{aA}$	$<0.4^{aA}$
	ML1 (Ca n.s., AA n.s.)	$<0.2^{bA}$	$<0.4^{bA}$	$<0.2^{abA}$	$<0.4^{bA}$	$<0.2^{aA}$	$<0.4^{aA}$
	ML2 (Ca n.s., AA n.s.)	$<0.2^{abA}$	$<0.4^{bA}$	$<0.2^{abA}$	$<0.4^{abA}$	$<0.2^{aA}$	$<0.4^{aA}$
	MD1 (Ca*, AA n.s.)	$<0.2^{bA}$	$<0.4^{bA}$	$<0.2^{abA}$	$<0.4^{abA}$	$<0.2^{aA}$	$<0.4^{aA}$
	MD2 (Ca n.s., AA**)	$<0.2^{bA}$	$<0.4^{abA}$	$<0.2^{bA}$	$<0.4^{abA}$	$<0.2^{aA}$	$<0.4^{aA}$

760 limit of detection (mg/kg): Cd – 0.01 (Ca); Cr – 0.05 (Ca) and 0,1 (AA); Pb – 0.2 (Ca) and 0.4 (AA)

761

40

762 **Table 4** (Continued)

Variable	Treatment	Growing season					
		1st		2nd		3rd	
		Ca [**]	AA [**]	Ca [**]	AA [**]	Ca [**]	AA [**]
Zn	LC (Ca [n.s.], AA [*])	$175^{aA} \pm 6$	$1,852^{acB} \pm 111$	$177^{aA} \pm 8$	$2,214^{aA} \pm 9$	$210^{aA} \pm 7$	$2,059^{adAB} \pm 3$
	LL1 (Ca [*], AA [n.s.])	$9.4^{abA} \pm 2.7$	$2,169^{aA} \pm 115$	$4.9^{abA} \pm 0.4$	$2,121^{acA} \pm 32$	$8.0^{abA} \pm 0.9$	$2,148^{aA} \pm 9$
	LL2 (Ca [n.s.], AA [n.s.])	$5.2^{abA} \pm 0.6$	$1,551^{abA} \pm 90$	$3.4^{abA} \pm 0.5$	$1,736^{abA} \pm 40$	$4.5^{abA} \pm 0.7$	$1,633^{abA} \pm 43$
	LD1 (Ca [*], AA [n.s.])	$110^{abA} \pm 3$	$1,883^{acA} \pm 29$	$71^{acB} \pm 2$	$1,991^{abA} \pm 32$	$62^{acB} \pm 6$	$1,983^{acdA} \pm 43$
	LD2 (Ca [**], AA [n.s.])	$119^{abA} \pm 44$	$1,623^{abA} \pm 53$	$44^{acB} \pm 3$	$1,728^{abA} \pm 39$	$58^{acAB} \pm 2$	$1,731^{abA} \pm 17$
	MC (Ca [**], AA [*])	$0.2^{abB} \pm 0.04$	$293^{abAB} \pm 34$	$0.5^{abAB} \pm 0.1$	$411^{abA} \pm 5$	$0.9^{abA} \pm 0.03$	$283^{bcdB} \pm 5$
	ML1 (Ca [*], AA [**])	$0.3^{bB} \pm 0.1$	$292^{abB} \pm 12$	$0.9^{abAB} \pm 0.4$	$398^{abA} \pm 4$	$1.6^{abA} \pm 0.5$	$352^{abAB} \pm 12$
	ML2 (Ca [*], AA [*])	$0.2^{bAB} \pm 0.1$	$185^{bB} \pm 17$	$0.1^{bB} \pm 0.01$	$278^{bA} \pm 6$	$0.4^{bA} \pm 0.02$	$220^{bAB} \pm 14$
	MD1 (Ca [*], AA [*])	$0.4^{abAB} \pm 0.1$	$296^{abB} \pm 8$	$0.4^{bcB} \pm 0.04$	$378^{bcA} \pm 10$	$0.7^{bcA} \pm 0.1$	$315^{abAB} \pm 10$
	MD2 (Ca [n.s.], AA [*])	$0.3^{bA} \pm 0.02$	$217^{bcB} \pm 13$	$1.3^{abA} \pm 0.7$	$275^{bA} \pm 7$	$0.5^{bcA} \pm 0.1$	$231^{bcAB} \pm 7$

41

763 **Table 5** The effect of treatment on the total concentrations of elements (mean ± SE) in organs
764 of *S. smithiana* at the end of two or three growing seasons (2nd – 3rd for Ca, Mg, K; and 1st –
765 3rd for P, Cu, Fe, Mn, Ni, Al, Cd, Cr, Pb, Zn) in the Litavka and Malín soils

Variable	Treatment	Growing season			
		2nd		3rd	
		twigs**	leaves**	twigs n.s.	leaves**
Ca (g/kg)	LC (t. n.s., l. n.s.)	$14.7^{aA} \pm 1.7$	$20.0^{aA} \pm 2.1$	$14.1^{aA} \pm 0.8$	$22.8^{abA} \pm 2.4$
	LL1 (t. n.s., l. n.s.)	$11.4^{aA} \pm 1.2$	$25.0^{aA} \pm 2.3$	$15.1^{aA} \pm 2.0$	$24.9^{abA} \pm 8.2$
	LL2 (t. *, l. n.s.)	$9.7^{aB} \pm 0.2$	$30.5^{aA} \pm 6.1$	$15.6^{aA} \pm 1.5$	$26.9^{aA} \pm 1.5$
	LD1 (t. n.s., l. n.s.)	$13.6^{aA} \pm 1.5$	$38.4^{aA} \pm 6.7$	$15.0^{aA} \pm 3.1$	$21.8^{abA} \pm 0.8$
	LD2 (t. n.s., l. n.s.)	$8.3^{aA} \pm 0.8$	$21.4^{aA} \pm 2.2$	$10.8^{aA} \pm 0.7$	$20.4^{abA} \pm 2.1$
	MC (t. n.s., l. n.s.)	$12.6^{aA} \pm 0.9$	$19.5^{aA} \pm 0.7$	$10.5^{aA} \pm 0.9$	$15.5^{bA} \pm 2.3$
	ML1 (t. *, l.)	$16.7^{aA} \pm 1.5$	$28.8^{aA} \pm 1.2$	$12.0^{aB} \pm 0.5$	$18.4^{abB} \pm 0.2$
	ML2 (t. n.s., l. n.s.)	$12.2^{aA} \pm 2.2$	$18.8^{aA} \pm 1.0$	$11.7^{aA} \pm 0.6$	$18.2^{abA} \pm 3.0$
	MD1 (t. *, l.)	$16.2^{aA} \pm 0.9$	$24.5^{aA} \pm 0.9$	$12.0^{aB} \pm 0.8$	$19.0^{abB} \pm 0.4$
	MD2 (t. *, l.)	$16.5^{aA} \pm 1.3$	$28.1^{aB} \pm 2.2$	$12.3^{aB} \pm 1.0$	$18.9^{abA} \pm 1.6$
Mg (g/kg)		twigs**	leaves**	twigs**	leaves**
	LC (t. *, l.)	$0.8^{abA} \pm 0.06$	$1.9^{abB} \pm 0.2$	$0.6^{abB} \pm 0.03$	$4.9^{abA} \pm 0.4$
	LL1 (t. n.s., l.)	$0.6^{bA} \pm 0.05$	$2.0^{abB} \pm 0.2$	$0.5^{abA} \pm 0.05$	$3.0^{abA} \pm 0.1$
	LL2 (t. n.s., l. n.s.)	$0.6^{bcA} \pm 0.05$	$2.7^{abA} \pm 0.6$	$0.7^{abA} \pm 0.1$	$3.4^{acA} \pm 0.3$
	LD1 (t. n.s., l. n.s.)	$1.2^{abA} \pm 0.2$	$4.8^{aA} \pm 0.8$	$1.3^{aA} \pm 0.4$	$4.3^{abA} \pm 0.5$
	LD2 (t. n.s., l.)	$0.7^{abA} \pm 0.1$	$3.9^{aB} \pm 0.2$	$0.7^{abA} \pm 0.04$	$5.6^{aA} \pm 0.5$
	MC (t. *, l.)	$0.9^{abA} \pm 0.04$	$3.7^{abA} \pm 0.04$	$0.5^{abB} \pm 0.05$	$2.0^{bB} \pm 0.5$
	ML1 (t. *, l.)	$1.0^{abA} \pm 0.1$	$3.3^{abA} \pm 0.2$	$0.5^{abB} \pm 0.03$	$2.2^{bcB} \pm 0.1$
	ML2 (t. n.s., l. n.s.)	$0.6^{abA} \pm 0.1$	$0.9^{bA} \pm 0.05$	$0.5^{bA} \pm 0.02$	$2.0^{bcA} \pm 0.4$
	MD1 (t. *, l. n.s.)	$1.1^{aA} \pm 0.06$	$3.3^{abA} \pm 0.2$	$0.6^{abB} \pm 0.05$	$2.7^{abA} \pm 0.3$
	MD2 (t. *, l.)	$1.1^{acA} \pm 0.05$	$3.8^{abA} \pm 0.1$	$0.6^{abB} \pm 0.04$	$2.6^{abB} \pm 0.1$
K (g/kg)		twigs*	leaves*	twigs n.s.	leaves*
	LC (t. n.s., l. n.s.)	$6.6^{aA} \pm 1.4$	$17.7^{aA} \pm 1.3$	$8.7^{aA} \pm 1.5$	$21.1^{aA} \pm 2.6$
	LL1 (t. n.s., l. n.s.)	$3.4^{aA} \pm 0.2$	$13.0^{aA} \pm 2.2$	$3.1^{bA} \pm 0.2$	$7.6^{aA} \pm 0.3$
	LL2 (t. n.s., l. n.s.)	$5.4^{aA} \pm 1.7$	$17.9^{aA} \pm 8.2$	$10.7^{abA} \pm 6.6$	$17.4^{aA} \pm 7.6$
	LD1 (t. n.s., l. n.s.)	$9.5^{aA} \pm 2.1$	$21.7^{aA} \pm 2.9$	$14.4^{aA} \pm 7.0$	$15.9^{aA} \pm 1.1$
	LD2 (t. n.s., l. n.s.)	$3.1^{aA} \pm 0.5$	$15.1^{aA} \pm 2.0$	$4.0^{abA} \pm 0.5$	$12.2^{aA} \pm 3.0$
	MC (t. *, l. n.s.)	$5.6^{aA} \pm 0.1$	$17.6^{aA} \pm 0.4$	$4.3^{abB} \pm 0.4$	$17.5^{aA} \pm 4.0$
	ML1 (t. *, l. n.s.)	$6.3^{aA} \pm 0.5$	$14.8^{aA} \pm 0.4$	$4.4^{abB} \pm 0.2$	$15.1^{aA} \pm 1.0$
	ML2 (t. n.s., l.)	$5.4^{aA} \pm 0.5$	$22.2^{aA} \pm 0.2$	$4.3^{abA} \pm 0.3$	$12.5^{aB} \pm 1.9$
	MD1 (t. *, l. n.s.)	$6.3^{aA} \pm 0.2$	$17.2^{aA} \pm 0.8$	$4.6^{abB} \pm 0.3$	$20.6^{aA} \pm 1.3$
	MD2 (t. *, l. n.s.)	$6.3^{aA} \pm 0.4$	$16.2^{aA} \pm 1.7$	$4.6^{abB} \pm 0.2$	$18.9^{aA} \pm 1.5$

766 The concentrations of Ca, Mg, and K were not determined after the first year because of low
767 total/organ biomass in almost all lime and dolomite treatments.
768 See Table 1 for more details about the treatments.
769 Calculated with the Kruskal–Wallis test, differences between treatments of the same organ
770 (i.e. twig and leaf DM separately) within the growing season and differences between the
771 growing seasons within the treatment of the same organ were not statistically significant (n.s.)
772 or were significant at 0.05(*) and 0.01 (**) probability levels. According to the multiple
773 comparisons of mean ranks, treatments between the same organ within the growing season
774 with the same letter (a–c) and growing seasons within the treatment between the same organ
775 with the same letter (A–B) were not significantly different.
776

42

777 **Table 5** (Continued)

Variable	Treatment	Growing season					
		1st		2nd		3rd	
P (g/kg)		twigs **	leaves **	twigs *	leaves **	twigs *	leaves n.s.
	LC (t. *, l. *)	1.1^{aAB} ± 0.07	1.3^{abB} ± 0.1	0.8^{aB} ± 0.07	1.8^{aAB} ± 0.2	1.3^{aA} ± 0.1	2.1^{aA} ± 0.2
	LL1 (t. **, l. *)	1.7^{aA} ± 0.2	1.7^{abA} ± 0.1	0.6^{aB} ± 0.06	1.5^{aAB} ± 0.2	0.8^{bAB} ± 0.05	1.1^{aB} ± 0.1
	LL2 (t. n.s., l. n.s.)	1.6^{aA}	2.2^{abA}	0.7^{aA} ± 0.1	1.5^{aA} ± 0.2	1.2^{abA} ± 0.3	1.8^{aA} ± 0.3
	LD1 (t. n.s., l. *)	0.9^{aA} ± 0.07	1.0^{bB} ± 0.04	0.7^{aA} ± 0.2	1.4^{aAB} ± 0.15	0.9^{abA} ± 0.2	1.7^{aA} ± 0.2
	LD2 (t. *, l. n.s.)	1.1^{aA} ± 0.006	1.0^{bA} ± 0.1	0.4^{aA} ± 0.1	1.4^{aA} ± 0.15	0.9^{abA} ± 0.1	1.4^{aA} ± 0.2
	MC (t. *, l. n.s.)	1.0^{aA} ± 0.04	3.2^{aA} ± 0.3	0.6^{aB} ± 0.05	2.8^{aA} ± 0.3	0.9^{abAB} ± 0.02	1.97^{aA} ± 0.4
	ML1 (t. **, l. *)	1.4^{aA} ± 0.08	2.2^{abAB} ± 0.1	0.7^{B} ± 0.02	2.7^{aA} ± 0.2	0.9^{abAB} ± 0.02	1.96^{aB} ± 0.1
	ML2 (t. *, l. n.s.)	died	died	0.6^{aB} ± 0.04	1.7^{aA} ± 0.1	1.0^{abA} ± 0.04	2.4^{aA} ± 0.5
	MD1 (t. *, l. n.s.)	1.1^{aA} ± 0.07	2.3^{abA} ± 0.09	0.8^{aB} ± 0.02	2.8^{aA} ± 0.3	0.9^{abAB} ± 0.04	2.3^{aA} ± 0.3
	MD2 (t. *, l. n.s.)	1.3^{aA} ± 0.06	2.1^{abA} ± 0.3	0.8^{aB} ± 0.05	2.8^{aA} ± 0.3	0.9^{abAB} ± 0.04	2.2^{aA} ± 0.3
Cu (mg/kg)		twigs *	leaves n.s.	twigs **	leaves n.s.	twigs *	leaves n.s.
	LC (t. *, l. *)	7.5^{aB} ± 0.8	5.1^{aB} ± 0.6	19.5^{abAB} ± 5.5	8.9^{aAB} ± 1.4	21.5^{aA} ± 2.4	14.0^{aA} ± 1.3
	LL1 (t. n.s., l. n.s.)	16.5^{aA} ± 2.7	6.4^{aA} ± 0.6	9.7^{abA} ± 1.5	11.5^{aA} ± 2.6	8.2^{abA} ± 0.9	7.6^{aA} ± 0.6
	LL2 (t. n.s., l. n.s.)	20.3^{aA}	24.8^{aA}	14.0^{abA} ± 4.5	12.2^{aA} ± 4.7	17.5^{abA} ± 9.0	12.4^{aA} ± 4.9
	LD1 (t. *, l. n.s.)	7.5^{aB} ± 1.0	15.0^{aA} ± 9.6	143^{aA} ± 15	24.5^{aA} ± 5.3	16.0^{abAB} ± 4.5	8.2^{aA} ± 1.6
	LD2 (t. n.s., l. n.s.)	6.8^{aA} ± 0.8	8.5^{aA} ± 2.1	7^{abA} ± 3.5	7.6^{aA} ± 0.9	15.3^{abA} ± 5.1	8.0^{aA} ± 1.2
	MC (t. n.s., l. *)	12.1^{aA} ± 2.4	15.4^{aA} ± 3.1	6.6^{abA} ± 0.7	12.1^{aAB} ± 2.1	9.5^{aA} ± 0.8	6.6^{aB} ± 0.9
	ML1 (t. n.s., l. *)	9.3^{aA} ± 0.3	5.9^{aB} ± 0.1	9.4^{abA} ± 0.8	11.7^{aA} ± 1.2	8.9^{abA} ± 0.7	7.3^{aAB} ± 1.0
	ML2 (t. *, l. n.s.)	died	died	6.0^{bB} ± 0.4	9.7^{aA} ± 1.5	9.7^{abA} ± 0.3	8.5^{aA} ± 0.6
	MD1 (t. *, l. *)	6.5^{aB} ± 0.2	6.7^{aB} ± 1.6	9.0^{abA} ± 0.7	12.7^{aA} ± 0.5	8.0^{bAB} ± 0.2	8.2^{aAB} ± 0.4
	MD2 (t. n.s., l. n.s.)	6.8^{aA} ± 0.6	7.4^{aA} ± 2.4	8.0^{abA} ± 0.9	13.5^{aA} ± 3.8	7.8^{abA} ± 0.6	7.8^{aA} ± 0.8
Fe (mg/kg)		twigs **	leaves *	twigs n.s.	leaves n.s.	twigs **	leaves *
	LC (t. **, l. **)	16.7^{abAB} ± 1.1	38^{abB} ± 7	10.8^{aB} ± 1.1	122^{aAB} ± 20	49^{aA} ± 10	305.5^{aA} ± 22
	LL1 (t. n.s., l. **)	17.3^{abA} ± 5.4	28^{bB} ± 3	21.5^{aA} ± 4.4	95^{aAB} ± 15.5	20^{abA} ± 3	180.5^{abA} ± 12.5
	LL2 (t. n.s., l. n.s.)	12.0^{abA}	56^{abA}	26.6^{aA} ± 8.8	108^{aA} ± 17	33^{abA} ± 13	168^{abA} ± 15
	LD1 (t. *, l. *)	8.1^{bB} ± 1.3	61^{abB} ± 14	21.1^{aAB} ± 6.5	165^{aAB} ± 34	66^{aA} ± 10.5	181^{abA} ± 20
	LD2 (t. **, l. *)	6.9^{bB} ± 0.5	55^{abB} ± 15	10.1^{aAB} ± 1.1	119^{aAB} ± 17	34^{abA} ± 9	167^{abA} ± 23.5
	MC (t. n.s., l. n.s.)	34.2^{abA} ± 23.1	157^{aA} ± 29	12.8^{aA} ± 2.7	93^{aA} ± 7	11.6^{bA} ± 0.9	92^{bA} ± 16
	ML1 (t. **, l. n.s.)	32.7^{abA} ± 4.5	61^{abA} ± 15	16.3^{aB} ± 0.8	115.5^{aA} ± 16	21.2^{abAB} ± 0.8	143^{abA} ± 39.5
	ML2 (t. *, l. n.s.)	died	died	14.0^{aB} ± 1.4	147^{aA} ± 14	20.3^{abA} ± 2.3	146^{abA} ± 28
	MD1 (t. *, l. n.s.)	46.8^{abA} ± 2.7	76^{abA} ± 22	18.9^{aAB} ± 2.2	104^{aA} ± 15	17.0^{abB} ± 1.7	142^{abA} ± 30
	MD2 (t. *, l. n.s.)	124.0^{aA} ± 23.1	168^{aA} ± 79	18.7^{aAB} ± 3.3	107^{aA} ± 10	16.5^{abB} ± 2.0	112^{bA} ± 20

778

43

779 **Table 5** (Continued)

Variable	Treatment	Growing season					
		1st		**2nd**		**3rd**	
		twigs *	**leaves** n.s.	**twigs** **	**leaves** **	**twigs** *	**leaves** n.s.
Mn (mg/kg)	LC (t. *, l. n.s.)	$2.8^{aB} \pm 0.3$	$11.8^{aA} \pm 1.9$	$10.1^{aA} \pm 5.8$	$19.3^{abA} \pm 4.9$	$3.9^{aAB} \pm 0.4$	$28^{aA} \pm 10$
	LL1 (t. n.s., l. *)	$6.4^{aA} \pm 2.5$	$17.7^{aB} \pm 0.8$	$8.7^{abA} \pm 2.2$	$49.5^{abAB} \pm 11.4$	$23.0^{aA} \pm 7.8$	$102^{aA} \pm 29$
	LL2 (t. n.s., l. n.s.)	10.5^{aA}	133^{aA}	$7.8^{abA} \pm 1.7$	$56.3^{abA} \pm 11.1$	$18.3^{aA} \pm 4.0$	$104^{aA} \pm 37$
	LD1 (t. *, l. *)	$2.7^{aAB} \pm 0.2$	$27.2^{aAB} \pm 6.8$	$2.0^{bB} \pm 0.4$	$13.6^{bB} \pm 2.2$	$29.0^{aA} \pm 12.8$	$53^{aA} \pm 14$
	LD2 (t. *, l. n.s.)	$2.7^{aB} \pm 0.1$	$29.1^{aAB} \pm 8.7$	$3.3^{abAB} \pm 0.7$	$34.9^{abA} \pm 3.9$	$12.1^{aA} \pm 5.0$	$39.5^{aA} \pm 17$
	MC (t. n.s., l. n.s.)	$2.3^{aA} \pm 0.6$	$24.6^{aA} \pm 3.5$	$3.3^{abA} \pm 0.3$	$26.9^{abA} \pm 1.8$	$4.8^{aA} \pm 1.0$	$23^{aA} \pm 7$
	ML1 (t. *, l. *)	$4.8^{aB} \pm 0.6$	$23.4^{aB} \pm 1.7$	$10.0^{aA} \pm 1.4$	$55.0^{aA} \pm 6.2$	$6.5^{aAB} \pm 1.3$	$40^{aAB} \pm 10$
	ML2 (t. n.s., l. *)	died	died	$10.1^{aA} \pm 1.7$	$64.2^{aA} \pm 8.9$	$17.1^{aA} \pm 2.8$	$33^{aB} \pm 2$
	MD1 (t. *, l. n.s.)	$2.8^{aB} \pm 0.4$	$18.6^{aA} \pm 6.4$	$7.9^{abAB} \pm 2.4$	$33.4^{abA} \pm 5.0$	$7.4^{aA} \pm 0.8$	$32^{aA} \pm 6$
	MD2 (t. n.s., l. n.s.)	$4.4^{aA} \pm 0.4$	$26.8^{aA} \pm 10.5$	$6.7^{abA} \pm 1.0$	$36.4^{abA} \pm 5.3$	$7.5^{aA} \pm 2.0$	$60.5^{aA} \pm 35$
Ni (mg/kg)		**twigs** *	**leaves** *	**twigs** n.s.	**leaves** *	**twigs** *	**leaves** **
	LC (t. n.s., l. *)	$0.55^{aA} \pm 0.11$	$0.7^{aB} \pm 0.3$	$0.93^{aA} \pm 0.46$	$1.8^{aA} \pm 0.3$	$1.29^{aA} \pm 0.2$	$1.2^{aAB} \pm 0.08$
	LL1 (t. n.s., l. n.s.)	$0.51^{aA} \pm 0.11$	$0.7^{aA} \pm 0.2$	$0.43^{aA} \pm 0.09$	$0.9^{aA} \pm 0.02$	$0.22^{abA} \pm 0.05$	$0.41^{abA} \pm 0.06$
	LL2 (t. n.s., l. n.s.)	0.94^{aA}	0.7^{aA}	$0.49^{aA} \pm 0.07$	$0.8^{aA} \pm 0.08$	$0.38^{bA} \pm 0.3$	$0.23^{bA} \pm 0.08$
	LD1 (t. n.s., l. *)	$0.34^{aA} \pm 0.08$	$1.6^{aAB} \pm 0.7$	$1.73^{aA} \pm 1.15$	$2.4^{aA} \pm 0.6$	$2.41^{abA} \pm 1.98$	$0.47^{abB} \pm 0.1$
	LD2 (t. n.s., l. *)	$0.30^{aA} \pm 0.07$	$1.1^{aAB} \pm 0.2$	$0.31^{aA} \pm 0.03$	$1.2^{aA} \pm 0.1$	$0.22^{abA} \pm 0.14$	$0.35^{bB} \pm 0.05$
	MC (t. *, l. *)	$0.54^{aA} \pm 0.12$	$1.1^{aAB} \pm 0.1$	$0.37^{aAB} \pm 0.06$	$1.2^{aA} \pm 0.16$	$0.15^{abB} \pm 0.04$	$0.56^{abB} \pm 0.1$
	ML1 (t. *, l. *)	$0.86^{aA} \pm 0.14$	$0.5^{aB} \pm 0.1$	$0.38^{aAB} \pm 0.07$	$1.6^{aA} \pm 0.13$	$0.29^{abB} \pm 0.05$	$0.602^{abAB} \pm 0.06$
	ML2 (t. n.s., l. n.s.)	died	died	$0.24^{aA} \pm 0.07$	$0.9^{aA} \pm 0.26$	$0.24^{abA} \pm 0.05$	$0.696^{abA} \pm 0.07$
	MD1 (t. n.s., l. *)	$0.34^{aA} \pm 0.08$	$0.7^{aAB} \pm 0.1$	$0.27^{aA} \pm 0.06$	$1.3^{aA} \pm 0.16$	$0.18^{abA} \pm 0.05$	$0.56^{abB} \pm 0.04$
	MD2 (t. n.s., l. *)	$0.57^{aA} \pm 0.05$	$0.6^{aB} \pm 0.1$	$0.29^{aA} \pm 0.09$	$1.15^{aA} \pm 0.04$	$0.23^{abA} \pm 0.06$	$0.696^{abAB} \pm 0.1$
Al (mg/kg)		**twigs** **	**leaves** *	**twigs** n.s.	**leaves** **	**twigs** *	**leaves** n.s.
	LC (t. *, l. *)	$10.0^{abB} \pm 1.2$	$32.4^{abB} \pm 7.1$	$10.7^{aAB} \pm 1.9$	$78.9^{abAB} \pm 9.7$	$25.0^{abA} \pm 3.8$	$108.1^{aA} \pm 8.8$
	LL1 (t. n.s., l. **)	$13.3^{abA} \pm 1.0$	$23.7^{bB} \pm 3.5$	$26.5^{aA} \pm 9.5$	$77.6^{abAB} \pm 7.7$	$18.8^{abA} \pm 5.7$	$128.5^{aA} \pm 10.0$
	LL2 (t. *, l. n.s.)	2.8^{abA}	41.7^{abA}	$20.2^{aA} \pm 6.9$	$83.6^{abA} \pm 8.8$	$22.3^{abA} \pm 9.0$	$91.6^{aA} \pm 14.2$
	LD1 (t. *, l. *)	$4.9^{bB} \pm 0.8$	$42.8^{abB} \pm 3.2$	$30.8^{aAB} \pm 17.0$	$95.6^{abA} \pm 9.9$	$66.0^{aA} \pm 12.5$	$80.4^{aAB} \pm 7.1$
	LD2 (t. *, l. n.s)	$4.6^{abB} \pm 0.5$	$51.0^{aA} \pm 15.9$	$16.6^{aAB} \pm 5.8$	$81.1^{abA} \pm 6.6$	$27.1^{abA} \pm 9.9$	$88.5^{aA} \pm 3.9$
	MC (t. n.s., l. n.s.)	$15.9^{abA} \pm 11.6$	$92.2^{aA} \pm 15.6$	$11.3^{aA} \pm 1.3$	$49.5^{abA} \pm 5.6$	$10.0^{bA} \pm 1.3$	$74.8^{aA} \pm 5.9$
	ML1 (t. n.s., l. *)	$14.3^{abA} \pm 1.8$	$33.4^{abB} \pm 9.1$	$14.4^{aA} \pm 0.6$	$54.6^{abAB} \pm 5.8$	$14.3^{abA} \pm 2.2$	$99.9^{aA} \pm 23.2$
	ML2 (t. n.s., l. n.s.)	died	died	$14.5^{aA} \pm 0.8$	$106.5^{aA} \pm 11.8$	$11.9^{abA} \pm 2.1$	$88.8^{aA} \pm 7.5$
	MD1 (t. n.s., l. *)	$18.8^{abA} \pm 1.5$	$38.3^{abB} \pm 9.5$	$13.6^{aA} \pm 3.0$	$50.8^{abAB} \pm 7.5$	$11.3^{abA} \pm 1.4$	$92.0^{aA} \pm 12.4$
	MD2 (t. *, l. n.s.)	$53.7^{aA} \pm 9.2$	$93.1^{abA} \pm 42.1$	$15.6^{aA} \pm 4.5$	$42.6^{bA} \pm 2.6$	$12.7^{abA} \pm 2.6$	$85.4^{aA} \pm 8.7$

780 limit of detection (mg/kg): Ni – 0.1

44

Table 5 (Continued)

Variable	Treatment	Growing season					
		1st		2nd		3rd	
		twigs **	leaves *	twigs **	leaves **	twigs **	leaves **
Cd (mg/kg)	LC (t. *, l. *)	$25.1^{aAB} \pm 0.5$	$36.5^{aA} \pm 3.5$	$22.8^{abB} \pm 2.2$	$69.3^{abA} \pm 10.4$	$39.5^{abA} \pm 2.8$	$73.0^{aA} \pm 8.6$
	LL1 (t. n.s., l. n.s.)	$28.0^{abA} \pm 5.2$	$53.3^{aA} \pm 8.6$	$21.7^{abA} \pm 4.6$	$67.6^{abA} \pm 2.4$	$24.5^{bA} \pm 2.3$	$66.6^{aA} \pm 5.3$
	LL2 (t. *, l. n.s.)	21.1^{abAB}	81.2^{aA}	$17.1^{abB} \pm 2.1$	$56.0^{abA} \pm 14.3$	$31.0^{abA} \pm 1.8$	$60.6^{aA} \pm 5.5$
	LD1 (t. *, l. n.s.)	$24.6^{aAB} \pm 1.6$	$58.4^{aA} \pm 15.6$	$23.7^{abB} \pm 3.3$	$105.4^{aA} \pm 19.7$	$52.5^{aA} \pm 4.0$	$58.6^{aA} \pm 2.5$
	LD2 (t. *, l. n.s.)	$20.9^{abAB} \pm 2.4$	$51.2^{aA} \pm 12.4$	$15.4^{abB} \pm 1.5$	$61.7^{abA} \pm 5.4$	$40.2^{abA} \pm 2.8$	$57.0^{aA} \pm 6.7$
	MC (t. n.s., l. *)	$17.7^{abA} \pm 0.3$	$46.6^{aAB} \pm 8.4$	$19.4^{abA} \pm 3.0$	$76.8^{abA} \pm 9.1$	$22.0^{bA} \pm 1.0$	$27.7^{aB} \pm 5.5$
	ML1 (t. *, l. *)	$13.2^{bB} \pm 1.0$	$15.2^{aB} \pm 1.4$	$27.4^{aAB} \pm 1.5$	$89.4^{abA} \pm 9.3$	$30.3^{abA} \pm 2.9$	$29.4^{aAB} \pm 6.0$
	ML2 (t. *, l. *)	died	died	$8.0^{bB} \pm 1.3$	$19.8^{bB} \pm 4.2$	$31.6^{abA} \pm 1.2$	$41.4^{aA} \pm 8.1$
	MD1 (t. n.s., l. *)	$23.8^{abA} \pm 0.7$	$32.0^{aB} \pm 7.4$	$27.4^{aA} \pm 1.1$	$88.9^{abA} \pm 8.6$	$22.4^{bA} \pm 2.8$	$44.2^{aAB} \pm 8.3$
	MD2 (t. n.s., l. *)	$19.5^{abA} \pm 1.8$	$32.0^{aB} \pm 8.4$	$21.8^{abA} \pm 1.4$	$97.2^{aA} \pm 8.8$	$23.6^{abA} \pm 5.2$	$50.1^{aAB} \pm 12.5$
Cr (mg/kg)		twigs *	leaves n.s.	twigs n.s.	leaves *	twigs n.s.	leaves n.s.
	LC (t. *, l. n.s.)	$0.2^{abA} \pm 0.03$	$0.2^{aA} \pm 0.03$	$0.1^{aA} \pm 0.02$	$0.1^{abA} \pm 0.03$	$0.1^{aA} \pm 0.04$	$0.2^{aA} \pm 0.03$
	LL1 (t. n.s., l. n.s.)	$0.1^{abA} \pm 0.04$	$0.1^{aA} \pm 0.01$	$0.1^{aA} \pm 0.03$	$0.3^{abA} \pm 0.1$	$0.1^{aA} \pm 0.02$	$0.3^{aA} \pm 0.07$
	LL2 (t. n.s., l. n.s.)	0.1^{abA}	0.3^{aA}	$0.2^{aA} \pm 0.1$	$0.2^{abA} \pm 0.1$	$0.1^{aA} \pm 0.06$	$0.3^{aA} \pm 0.05$
	LD1 (t. n.s., l. n.s.)	$0.2^{abA} \pm 0.03$	$1.1^{aA} \pm 0.9$	$0.8^{aA} \pm 0.5$	$0.6^{aA} \pm 0.1$	$1.0^{aA} \pm 0.9$	$0.4^{aA} \pm 0.1$
	LD2 (t. n.s., l. n.s.)	$0.1^{bA} \pm 0.02$	$0.7^{aA} \pm 0.3$	$0.2^{aA} \pm 0.05$	$0.3^{abA} \pm 0.0$	$0.1^{aA} \pm 0.01$	$0.2^{aA} \pm 0.04$
	MC (t. n.s., l. *)	$0.3^{abA} \pm 0.1$	$1.0^{aA} \pm 0.3$	$3.0^{aA} \pm 2.9$	$0.1^{abAB} \pm 0.03$	$0.05^{aA} \pm 0.01$	$0.1^{aB} \pm 0.02$
	ML1 (t. *, l. n.s.)	$0.2^{abAB} \pm 0.02$	$0.1^{aA} \pm 0.03$	$0.2^{aA} \pm 0.03$	$0.3^{abA} \pm 0.1$	$0.1^{aB} \pm 0.02$	$0.2^{aA} \pm 0.03$
	ML2 (t. n.s., l. n.s.)	died	died	$0.1^{aA} \pm 0.02$	$0.1^{bA} \pm 0.01$	$0.1^{aA} \pm 0.01$	$0.2^{aA} \pm 0.06$
	MD1 (t. *, l. n.s.)	$0.2^{abA} \pm 0.02$	$0.3^{aA} \pm 0.1$	$0.1^{aA} \pm 0.04$	$0.2^{abA} \pm 0.1$	$0.1^{aA} \pm 0.01$	$0.1^{aA} \pm 0.03$
	MD2 (t. *, l. n.s.)	$0.3^{bA} \pm 0.08$	$0.4^{aA} \pm 0.2$	$0.1^{aAB} \pm 0.02$	$0.1^{abA} \pm 0.02$	$0.1^{aB} \pm 0.02$	$0.15^{aA} \pm 0.04$
Pb (mg/kg)		twigs **	leaves **	twigs **	leaves **	twigs **	leaves **
	LC (t. *, l. n.s.)	$4.0^{abAB} \pm 0.4$	$2.2^{aA} \pm 0.6$	$2.7^{acB} \pm 0.2$	$1.0^{aA} \pm 0.5$	$5.9^{aA} \pm 1.5$	$3.0^{aA} \pm 0.4$
	LL1 (t. *, l. n.s.)	$6.1^{aA} \pm 1.1$	$4.6^{aA} \pm 1.0$	$2.2^{abA} \pm 0.7$	$2.7^{aA} \pm 0.4$	$1.9^{aA} \pm 0.3$	$2.6^{abA} \pm 0.2$
	LL2 (t. n.s., l. n.s.)	8.5^{abA}	11.7^{aA}	$1.5^{abA} \pm 0.6$	$2.2^{aA} \pm 1.0$	$3.1^{aA} \pm 1.5$	$3.3^{abA} \pm 1.7$
	LD1 (t. n.s., l. n.s.)	$4.4^{abA} \pm 0.5$	$3.9^{aA} \pm 0.9$	$10.5^{aA} \pm 4.0$	$3.5^{aA} \pm 0.7$	$5.8^{aA} \pm 0.1$	$2.5^{abA} \pm 0.2$
	LD2 (t. *, l. n.s.)	$4.2^{abAB} \pm 0.8$	$3.2^{aA} \pm 1.2$	$1.6^{abB} \pm 0.2$	$1.8^{aA} \pm 0.3$	$4.1^{aA} \pm 0.8$	$1.6^{abA} \pm 0.3$
	MC (t. *, l. n.s.)	$0.1^{bB} \pm 0.01$	$0.7^{aA} \pm 0.1$	$0.3^{abAB} \pm 0.06$	$0.4^{aA} \pm 0.2$	$0.4^{aA} \pm 0.09$	$0.4^{bA} \pm 0.1$
	ML1 (t. n.s., l. n.s.)	$0.1^{abA} \pm 0.03$	$0.9^{aA} \pm 0.1$	$0.1^{bA} \pm 0.05$	$0.42^{aA} \pm 0.1$	$0.3^{aA} \pm 0.08$	$1.0^{abA} \pm 0.3$
	ML2 (t. n.s., l. n.s.)	died	died	$0.3^{abA} \pm 0.07$	$0.4^{aA} \pm 0.2$	$0.4^{aA} \pm 0.08$	$0.9^{abA} \pm 0.3$
	MD1 (t. *, l. n.s.)	$0.1^{abB} \pm 0.02$	$0.9^{aA} \pm 0.2$	$0.3^{abAB} \pm 0.06$	$0.5^{aA} \pm 0.2$	$0.6^{aA} \pm 0.15$	$0.7^{abA} \pm 0.2$
	MD2 (t. n.s., l. n.s.)	$0.5^{abA} \pm 0.2$	$0.7^{aA} \pm 0.3$	$0.2^{bcA} \pm 0.07$	$0.4^{aA} \pm 0.2$	$0.3^{aA} \pm 0.10$	$0.5^{bA} \pm 0.1$

782 limit of detection (mg/kg): Pb – 0.4

45

783 **Table 5** (Continued)

Variable	Treatment	Growing season					
		1st		**2nd**		**3rd**	
		twigs[**]	leaves[**]	twigs[**]	leaves[**]	twigs[**]	leaves[**]
Zn (mg/kg)	LC (t. [*], l. [n.s.])	757[aB] ± 17	2,074[abA] ± 138	913[aAB] ± 104	3,488[aA] ± 515	1,326[aA] ± 118	3,420[aA] ± 170
	LL1 (t. [n.s.], l. [n.s.])	374[abA] ± 12.5	1,722[abA] ± 198	488[abA] ± 105	2,142[abA] ± 132	625[abA] ± 88	1,942[abA] ± 195
	LL2 (t. [n.s.], l. [n.s.])	325[abA]	2,620[abA]	337[abA] ± 35	1,819[abA] ± 384	734[abA] ± 10	1,972[abA] ± 132
	LD1 (t. [n.s.], l. [n.s.])	681.5[acA] ± 55	2,588[aA] ± 430	880[aA] ± 128	4,104[aA] ± 514	1,075[aA] ± 182	2,002[abA] ± 119
	LD2 (t. [*], l. [*])	597.5[acAB] ± 72	2,281[aAB] ± 272	448[abB] ± 67	2,831[aA] ± 169	983[aA] ± 45	2,059[abB] ± 107
	MC (t. [*], l. [**])	221.5[bcB] ± 4	1,146[abAB] ± 126	386[abAB] ± 81	2,479[abA] ± 323	387[abA] ± 13	732[bB] ± 105
	ML1 (t. [*], l. [*])	178[bB] ± 11	450[bB] ± 48	376[abA] ± 26	2,003[abA] ± 119	434[abA] ± 41	689[bAB] ± 109
	ML2 (t. [*], l. [*])	died	died	138[bB] ± 30	494[bB] ± 44	305[bA] ± 18	992[abA] ± 77
	MD1 (t. [**], l. [*])	258.5[abB] ± 8	693[abB] ± 126	459[abA] ± 16	2,141[abA] ± 279	368[abAB] ± 35	1,014[abAB] ± 112
	MD2 (t. [n.s.], l. [*])	224[abA] ± 15	692.5[abB] ± 139	358[abA] ± 8	2,248[abA] ± 194	375[abA] ± 64	968[abAB] ± 95

784

46

149

5 Sumární diskuse

V první části sumární diskuse (podkapitola 5.1) jsou shrnuty poznatky z vědeckých článků (detaily viz podkapitoly 4.2 a 4.5), ve kterých je diskutována stabilizace toxických prvků (As, Cd, Pb, Zn) a mikroprvků (Fe, Mn) ve dvou půdách silně kontaminovaných těmito rizikovými prvky. V modelových inkubačních experimentech byly vápenaté hmoty (pálené vápno, dolomit) a fosforečná aditiva (trojitý superfosfát, mletý fosfát) aplikovány do slabě kyselé fluvizemě glejové (Příbram – Litavka, kontaminace As, Cd, Pb a Zn) a alkalické luvizemě modální (Kutná Hora – Malín, kontaminace As, Cd a Zn); (detaily půd viz podkapitola 4.3). Aplikace byly realizovány ve třech dávkách. Labilní koncentrace (mobilní a potenciálně mobilizovatelné) rizikových prvků byly hodnoceny v různých dnech (7, 14, 28, 42) v systému půda a aditivum. Mobilní (extrakce roztokem $CaCl_2$ o koncentraci 0,01 mol.l^{-1}) a potenciálně mobilizovatelné koncentrace (extrakce roztokem CH_3COOH o koncentraci 0,11 mol.l^{-1}) As, Cd, Pb, Zn, Fe a Mn byly stanoveny metodou optické emisní spektrometrie s indukčně vázaným plazmatem (ICP-OES).

Ve druhé části diskuse (podkapitola 5.2) jsou sumarizovány znalosti z dalších publikovaných prací (detaily viz podkapitoly 4.1, 4.3, 4.4 a 4.6), ve kterých jsou posuzovány reakce rostlin vysetých, případně vysazených do půdy stabilizované vápněním nebo aplikací fosforečných aditiv. Pro nádobové pokusy byly vybrány zástupci bylin a dřevin (šťovík tupolistý – kosmopolitně rozšířený druh širokolistých šťovíků a vrba Smithova – vysokoprodukční, rychle rostoucí dřevina) pro rozšíření poznatků z inkubačních experimentů. V rámci pokusů byl hodnocen růst a mortalita rostlin, produkce biomasy rostlin, labilní koncentrace široké škály prvků v půdě a dále jejich celkové obsahy v rostlinné biomase. Obsahy makroprvků (P, K, Ca, Mg), mikroprvků (Cu, Fe, Mn, Ni) a toxických prvků (Al, As, Cd, Cr, Pb, Zn) byly stanoveny pomocí metod ICP-OES (mikroprvky, toxické prvky) a plamenové atomové absorpční spektrometrie – FAAS (makroprvky). Vlastnímu stanovení obsahů jednotlivých prvků předcházel vysokotlaký mikrovlnný rozklad ve směsi HNO_3 a H_2O_2 (šťovíky) nebo klasický rozklad na suché cestě, tj. zpopelnění vzorku s následným rozpuštěním popela ve zředěné HNO_3 (vrby). Obsah dusíku ve šťovících byl stanoven metodou podle Kjeldahla po mokrém rozkladu koncentrovanou H_2SO_4 za přítomnosti práškového selenu. Po výluhu rostlinných orgánů v horké vodě byly pomocí iontově-výměnné chromatografie s potlačenou vodivostí ve šťovících stanoveny i obsahy aniontů nízkomolekulárních organických kyselin.

5.1 Regulace mobility prvků v půdách silně kontaminovaných rizikovými prvky

Mobilita prvků v půdách byla regulována vápenatými hmotami (viz podkapitola 4.2) a fosforečnými aditivy (viz podkapitola 4.5).

Ve slabě kyselých půdních podmínkách byly potenciálně mobilizovatelné koncentrace Cd, Pb, Zn a Mn během 42 dnů inkubace více imobilizovány aplikací rychle rozpustných aditiv (vápno>superfosfát) než pomalu rozpustných aditiv (dolomit~mletý fosfát). Důvodem byl významný nárůst půdní reakce až na pH=12 po aplikaci vápna (viz graf 1a v podkapitole 4.2) a okamžitá reaktivita účinné složky superfosfátu ($H_2PO_4^-$) s prvky v půdním roztoku (Dermatas a Meng, 2003; Guo et al., 2006; Alkorta et al., 2010). Naopak labilní koncentrace As a Fe a mobilní koncentrace Pb a Mn nebyly stabilizovány vápněním ani aplikací fosforečných aditiv. Podle účinnosti imobilizačních půdních aditiv ve slabě kyselé půdě byly rizikové prvky rozděleny do dvou skupin:

1) **bez poklesu koncentrací**
   - mobilních a potenciálně mobilizovatelných: As, Fe
   - mobilních: Pb, Mn

2) **významný pokles koncentrací**
   - potenciálně mobilizovatelných: Pb, Mn

Účinnost půdních aditiv se zvyšovala pro prvky v následujícím pořadí:

Pb: mletý fosfát<dolomit<superfosfát<vápno. Přídavkem mletého fosfátu byly koncentrace Pb sníženy o 29 %, dolomitem o 47 %, superfosfátem o 96 % a vápnem o 98 % ve srovnání s kontrolními variantami půd.

Mn: mletý fosfát<dolomit<superfosfát<vápno. Přídavkem mletého fosfátu byly koncentrace Mn omezeny o 13 %, dolomitem o 39 %, superfosfátem o 76 % a aplikací vápna téměř o 100 % v porovnání s kontrolními variantami půd.

   - mobilních a potenciálně mobilizovatelných: Cd, Zn

Účinnost půdních aditiv stoupala pro prvky v pořadí:

Cd: mletý fosfát<dolomit<superfosfát<vápno. Aplikací mletého fosfátu byly labilní koncentrace Cd sníženy o 13-43 %, dolomitem o 22-57 %, superfosfátem o 43-74 % a aplikací vápna téměř o 100 % v porovnání s kontrolními variantami půd.

Zn: superfosfát<mletý fosfát<dolomit<vápno. Aplikací superfosfátu byly labilní koncentrace Zn omezeny o 3-49 %, mletým fosfátem o 2-59 %, dolomitem o 22-72 % a vápnem o 86-99 % ve srovnání s kontrolními variantami půd.

Aplikační dávky imobilizačních aditiv sehrály u toxických prvků důležitou úlohu v regulaci jejich mobility v půdě (viz grafy v podkapitolách 4.2 a 4.5). Nižší dávky vápna a vyšší dávky superfosfátu více mobilizovaly labilní koncentrace As. Labilní koncentrace Cd a potenciálně mobilizovatelné koncentrace Pb byly více imobilizovány vyššími aplikačními dávkami dolomitu, superfosfátu i mletého fosfátu. Podobně tomu bylo i u mobilních koncentrací Zn. Výjimkou byly pouze rostoucí dávky superfosfátu, které okyselením Zn více mobilizovaly (viz graf 2 v podkapitole 4.5). Koncentrace Pb byly regulovány i aplikačními dávkami vápna a vliv mělo též použité extrakční činidlo. Vliv aplikačních dávek aditiv na mobilitu toxických prvků v půdě potvrzují i další studie (Elkhatib et al., 1991; Thawornchaisit a Polprasert, 2009; Yan et al., 2015). Skutečnost, že nevhodně zvolené půdní aditivum nebo jeho aplikační dávka může regulovat i mobilitu živin v půdě, uvádějí Bolan a Duraisamy (2003) a potvrzují i publikované práce (viz podkapitoly 4.2 a 4.5). Aplikační dávky dolomitu a superfosfátu sehrály důležitou roli v regulaci labilních koncentrací Mn. Rostoucí dávky dolomitu snižovaly potenciálně mobilizovatelné koncentrace Mn. Vyšší dávky superfosfátu zvyšovaly mobilní koncentrace Mn. Vliv času na změny v mobilitě prvků v půdě popisují autoři (Calace et al., 2006; Yan et al., 2015) a potvrzují i publikované práce (viz podkapitoly 4.2 a 4.5).

V alkalických půdních podmínkách byla imobilizace prvků pomocí vápnění i po aplikaci fosforečných aditiv neúčinná. Tyto výsledky jsou v souladu s dalšími studiemi, které potvrzují, že při alkalickém pH půd je rozpustnost řady prvků minimální (Podlešáková et al., 2001a; Němeček et al., 2010; Kabata-Pendias, 2011). Příčinou neúspěchu imobilizace rizikových prvků po aplikaci fosforečných aditiv byla i přítomnost fosforečnanů ve velmi málo rozpustných formách (fosforečnany vápenaté); (Hong et al., 2010; Moradi et al., 2012).

Možné příčiny vzniku imobilizačních a mobilizačních změn prvků po vápnění a po aplikaci fosforečných aditiv ve slabě kyselé půdě jsou podrobněji diskutovány v následujících podkapitolách (viz 5.1.1 a 5.1.2).

5.1.1 Vápenaté hmoty

Imobilizace. Stabilizace Cd a Zn pomocí vápnění byla vyvolána podobnými imobilizačními mechanismy. Vzhledem k významné závislosti Cd a Zn na půdní reakci (Podlešáková et al., 2001a) byly jejich labilní koncentrace po aplikaci vápna sníženy. Okamžitý pokles Cd a Zn po aplikaci vápna souvisel s vysoce rozpustnou formou vápna (Mayfield et al., 2004). Labilní koncentrace Cd a Zn byly přídavkem dolomitu pozvolna snižovány precipitací dvojmocných kationtů Cd a Zn s uhličitany (Bradl, 2004). Aplikace

dolomitu omezovaly potenciálně mobilizovatelné koncentrace Pb a Mn méně než vápno. Pokles Pb a Mn po přídavku dolomitu byl vyvolán precipitací dvojmocných kationtů Pb a Mn s uhličitany (Elkhatib et al., 1991; Otero et al., 2009).

Mobilizace. Mobilní sloučeniny As zformované po aplikaci vápna mírně zvyšovaly labilní koncentrace As (Száková et al., 2007; Wilson et al., 2010). Mobilní koncentrace Pb byly po přídavku vápna zvýšeny, protože kationty Pb byly pravděpodobně uvolněny z půdní organické hmoty přísunem okamžitě přístupného Ca (Tlustoš et al., 2006c). Následně vznikly rozpustné hydroxidové komplexy Pb typické jen pro silně alkalické půdy − pH>12 (Bradl, 2004).

5.1.2 Fosforečná aditiva

Imobilizace. Stabilizace labilních koncentrací Cd pomocí fosforečných aditiv byla spojena s precipitací volných fosforečnanových iontů s dvojmocnými kationty Cd do podoby nerozpustných fosforečnanů kademnatých − $Cd_3(PO_4)_2$ (Chrysochoou et al., 2007; Spuller et al., 2007; Hong et al., 2010). Vzhledem k chemické podobnosti Cd a Zn (Kabata-Pendias, 2011) se imobilizace Zn pomocí aplikace fosforečných aditiv ve většině případů podobá imobilizaci Cd. Snížené potenciálně mobilizovatelné koncentrace Pb v porovnání s neměnnými mobilními koncentracemi Pb po aplikaci fosforečných aditiv naznačují, že potenciálně mobilizovatelné koncentrace lépe vystihují rozpouštěcí − srážecí mechanismy Pb v půdě. Vodorozpustnější superfosfát s okyselujícím účinkem byl schopný poskytnout více volných fosforečnanových iontů pro reakce s Pb v půdě (Wang et al., 2008; Thawornchaisit a Polprasert 2009; Cui et al., 2010). Potenciálně mobilizovatelné koncentrace Pb byly více omezovány aplikací superfosfátu (tvorba málo rozpustného hydrogenfosforečnanu olovnatého − $PbHPO_4$); (Cao et al., 2008) než aplikací mletého fosfátu (pozvolná formace geochemicky stabilního fosforečnanu olovnatého − pyromorfitu v rozmezí pH 4 až 5); (Zhang a Ryan, 1998, 1999; Miretzky a Fernandez-Cirelli, 2008; Cui et al., 2010). Mírné snížení potenciálně mobilizovatelných koncentrací Mn souviselo s tvorbou nerozpustných fosforečnanových komplexů s Mn − $MnHPO_4$ a $Mn_3(PO_4)_2$ (Vangronsveld et al. 2009).

Mobilizace. Mobilizace labilních koncentrací As byla vyvolána dostatečným množstvím rozpustných fosforečnanů v půdě. Fosforečnany, uvolněné ze středně rozpustného superfosfátu, byly přednostně vázány na sorpční místa půdních částic a tak byly arseničnany uvolněny do půdního roztoku (Bolan et al., 2013). Mírné zvýšení mobilních koncentrací Zn po aplikaci nejvyšší dávky superfosfátu souviselo s okyselením půdy až na pH=5,3 (viz podkapitola 4.5), což potvrzují i Thawornchaisit a Polprasert (2009). Zvýšené mobilní

koncentrace Mn po aplikaci superfosfátu byly také vyvolány okyselením půdy a pravděpodobně souvisely i s přítomností Mn v rozpustných fosforečnanových komplexech. Uvedené výsledky potvrzují ve svých studiích i další autoři (Larsen, 1964; Hossner a Richards, 1967; Munksgaard a Lottermoser, 2011; Munksgaard a Lottermoser, 2013).

5.2 Pěstování rostlin na stabilizovaných půdách

5.2.1 Širokolisté šťovíky

Šťovík tupolistý byl pěstován na půdách neošetřených (kontrolních) a ošetřených vápnem nebo superfosfátem. Okamžitá rozpustnost a reaktivita účinných složek použitých aditiv stejně jako předpokládané výraznější změny po jejich aplikaci i v alkalické půdě podpořily volbu použít pro vegetační experiment rychle rozpustná imobilizační aditiva.

Počáteční růst a mortalita rostlin. Počáteční růst a mortalita šťovíků v kontaminovaných půdách byl ovlivněn agrochemickými parametry půd i půdními aditivy. Ve slabě kyselé půdě byla prosperita šťovíků zvyšována v pořadí: kontrola~superfosfát<vápno. Naopak v alkalické půdě vzrůstalo vzcházení a přežívání šťovíků v řadě: vápno<superfosfát~kontrola.

Rychleji a ve větším počtu vzcházely drobné rostliny šťovíků s načervenalými úzkými, dlouhými listy (viz obrázky 3a, 3e v podkapitole 4.1) ve slabě kyselé kontrolní půdě a ve variantě se superfosfátem. Nicméně byla pozorována jejich výrazně vyšší mortalita (viz graf 2a v podkapitole 4.1). Důvodem úhynu šťovíků v počátečních fázích růstu byla vysoká biodostupnost Cd, Pb a Zn (viz tabulka 2 v podkapitole 4.1). Po vyvápnění slabě kyselé půdy došlo k výraznému poklesu mobility Cd o 94 % a Zn o 82 % a byl pozitivně ovlivněn vývoj raných fází šťovíků. Rostliny měly širší, méně načervenalé listy (viz obrázek 3c v podkapitole 4.1).

V alkalické kontrolní půdě a ve variantě se superfosfátem byly pozorovány zelené, více životaschopné rostliny (viz obrázky 3b, 3f v podkapitole 4.1). Počáteční vývoj šťovíků ve vyvápněné alkalické půdě byl výrazně horší. Strádání rostlin (viz obrázek 3d v podkapitole 4.1) bylo spojeno s alkalickým pH půdy (pH=7,6) a s vysokou počáteční dostupností Ca v kontrolní půdě. Nízká mobilita rizikových prvků, stanovená již v alkalické kontrolní půdě, neměla vliv na kvalitu šťovíků v počátečních fázích růstu. Citlivost dospělých rostlin šťovíků k vysokým obsahům Ca a Mg v nekontaminované půdě pozorovali již Humphreys et al. (1999) a Hann et al. (2012). Šťovíky jako tzv. oxalátní rostliny (White a Broadley, 2003) dokáží vysoké obsahy Ca ve svých pletivech inaktivovat tvorbou vysoce nerozpustných

komplexů Ca se šťavelany a jejich zabudováním do vakuol nebo buněčných stěn (Franceschi a Nakata, 2005; Tolrà et al., 2005). Novým poznatkem publikované studie (podkapitola 4.1) byla prokázaná citlivost šťovíků v raných fázích růstu k vysokým obsahům Ca ve vyvápněné alkalické půdě. Novým zjištěním také bylo, že pro počáteční růst šťovíků je nevyhovující vysoká biodostupnost Cd, Pb a Zn ve slabě kyselé kontrolní půdě i ve variantě se superfosfátem.

Produkce biomasy. Chemické vlastnosti půd a půdní aditiva významně působily také na produkci biomasy dospělých rostlin v kontaminovaných půdách. Celková produkce biomasy šťovíků i produkce jednotlivých orgánů rostla v obou půdách v pořadí: kontrola~superfosfát<vápno. Vysokými obsahy mikroprvků (>5 mg Ni.kg^{-1}; Güleryüz et al., 2008) a toxických prvků v rostlině (>5 mg Cd.kg^{-1}, >30 mg Pb.kg^{-1}, >100 mg Zn.kg^{-1}; Pugh et al., 2002; Kabata-Pendias, 2011) bylo pravděpodobně inhibováno buněčné dělení a prodlužování buněk (Barrutia et al., 2009) a tím snížena produkce biomasy šťovíku. Vysoké obsahy toxických prvků dokonce omezily vývoj stonků a generativních orgánů ve slabě kyselé kontrolní půdě a ve variantě se superfosfátem. Aplikace superfosfátu nebyla vhodným opatřením pro zvýšení produkce biomasy šťovíků ve slabě kyselé ani v alkalické půdě. Podobně i Tiecher et al. (2014) popisují pouze nepatrné navýšení biomasy pícnin a trav v nekontaminovaných půdách ošetřených superfosfátem. Šťovíky prosperovaly v půdách stabilizovaných vápnem. I další autoři (Tlustoš et al., 2006c; Alvarenga et al., 2008) popisují zvýšenou produkci biomasy polních plodin i plevelných rostlin ve vyvápněných kyselých půdách kontaminovaných Cd, Cu, Ni, Pb a Zn.

Obsahy a distribuce makroprvků, mikroprvků a toxických prvků v orgánech šťovíku. V kontrolních půdách byly nejvyšší obsahy makroprvků stanoveny v listech (N, P, K, Ca, Mg) i v semenech (N, P). Nejnižší obsahy makroprvků byly naopak zjištěny ve stoncích (N, P, Ca, Mg) a v podzemních orgánech šťovíků (K). Uvedené výsledky jsou v souladu s dalšími studiemi (López-Lefebre et al., 2001; Gaweda, 2009; White a Veneklaas, 2012). Na konci vegetačního experimentu se šťovíky byly zjištěny snížené obsahy N a Ca v podzemních orgánech ve vyvápněné slabě kyselé půdě. Projevil se zde tzv. ředící efekt obsahů N a Ca (viz tabulka 3 v podkapitole 4.3). Ke stejnému závěru se ve svých studiích přiklánějí i další autoři (Chen a Wong, 2006; Tlustoš et al., 2006c). Obsahy P v semenech šťovíku byly ve variantách bez přídavku superfosfátu (tabulka 3 v podkapitole 4.3) pod kritickým limitem 0,3 % P. Při obsahu fosforu v semenech nižším než 0,3 % P byl ve studii Hrdličkové et al. (2011) pozorován pokles klíčení semen šťovíku tupolistého i šťovíku kadeřavého. Vápněním ani aplikací superfosfátu nebyla změněna distribuce

makroprvků (K, Ca, Mg) mezi rostlinnými orgány šťovíku ve slabě kyselé ani v alkalické půdě.

Nejvyšší obsahy většiny mikroprvků byly zjištěny v podzemních orgánech v kontrolních půdách. Jejich nejnižší obsahy byly stanoveny ve stoncích. V kontrolních půdách byl také zjištěn omezený transport mikroprvků z podzemních orgánů do listů. Podzemní orgány šťovíků fungovaly jako bariéra, která omezovala přesun nadbytečných obsahů mikroprvků do nadzemních orgánů. Podobné výsledky popisují i další autoři (Gaweda, 2009; Hänsch a Mendel, 2009; Zhang et al., 2010). Ve vyvápněných půdách a v alkalické půdě po aplikaci superfosfátu byl zjištěn zvýšený transport Cu, Fe, Mn a Ni z podzemních orgánů do listů. Změny v distribuci mikroprvků byly pravděpodobně spojeny s přítomností organických kyselin. Jak již bylo uvedeno dříve, šťovíky se mohou bránit proti vysokým obsahům Ca ve svých pletivech tvorbou stabilních komplexů Ca se šťavelany (Tolrà et al., 2005; Miyagi et al., 2013). Je pravděpodobné, že na podobném principu funguje vnitřní obranný mechanismus šťovíků i proti vysokým obsahům mikroprvků v podzemních orgánech v kontrolních půdách. Šťovíky zřejmě více ohrožují vysoké obsahy Ca než vysoké obsahy mikroprvků. Ve vyvápněných půdách šťavelany přednostně tvořily stabilní komplexy s Ca než s mikroprvky. Volné mikroprvky mohly být dále transportovány do listů. Zvýšený transport mikroprvků z podzemních orgánů do listů v alkalických půdách ošetřených superfosfátem lze vysvětlit dostatečným množstvím Ca uvolněným ze superfosfátu a z půdního roztoku.

Obsahy a distribuce toxických prvků v biomase šťovíku se shodovaly s obsahy i s distribucí mikroprvků v kontrolních půdách i v půdách ošetřených vápnem a superfosfátem. Vnitřní obranná strategie šťovíků proti vysokým obsahům mikroprvků byla účinná i pro toxické prvky. Zvýšený transport toxických prvků do nadzemní biomasy po vápnění byl v rozporu s dalšími autory (Tlustoš et al., 2006c; Jiang et al., 2007; Qiu et al., 2011). Tito autoři ale pro své experimenty používali rostliny, které nepatřily do oxalátních rostlin (pšenice obecná, kukuřice setá, čínské zelí). Nízký obsah šťavelanů v neoxalátních rostlinách pravděpodobně neumožňoval uplatnit vnitřní obranný mechanismus proti vysokým obsahům vápníku.

Na základě výsledků vegetačního pokusu byl šťovík tupolistý v kontrolních půdách zařazen mezi rostliny s nízkou akumulací As, Cd, Pb a Zn (viz podkapitola 4.3), mezi tzv. exkludační rostliny (Baker, 1981). K podobnému závěru – ale u šťovíku kyselého – dospěli i další autoři (Barrutia et al., 2009; Gaweda, 2009). Nově bylo během vegetačního pokusu zjištěno, že v půdách vyvápněných nebo v půdě alkalické ošetřené superfosfátem se šťovík

tupolistý s nízkou akumulací (exkludační) choval jako rostlina s běžnou (indikační) až s vysokou akumulací (akumulační) rizikových prvků (viz podkapitola 4.3). Určování rostlin vhodných pro fytoremediace by proto mělo být prováděno obezřetně s ohledem na vlastnosti rostlin a chemické vlastnosti půd. Dalším zjištěním při pokusu byla zvýšená tolerance šťovíku tupolistého k vysokým obsahům Al ve všech variantách (viz podkapitola 4.4). Celkové obsahy Al v rostlinách se běžně pohybují do 200 mg.kg^{-1} sušiny (Kinraide, 1990). Při pěstování šťovíku v alkalické kontrolní půdě byl experimentálně zjištěn zvýšený transport Al z podzemních orgánů do listů. V listech bylo stanoveno dokonce 3413 mg Al.kg^{-1}sušiny (viz tabulka 3 v podkapitole 4.4), což vedlo k překvapivému závěru, že šťovík tupolistý patří mezi hyperakumulační rostliny Al (>3000 mg Al.kg^{-1}; Huang et al., 2009). K opačnému efektu došlo u šťovíku pěstovaného ve slabě kyselé kontrolní půdě (viz tabulka 3 v podkapitole 4.4), kde byl transport Al do listů omezen. Stanovené obsahy Al v listech byly sedmkrát nižší než v alkalické kontrolní půdě. Je pravděpodobné, že se šťovík tupolistý ve slabě kyselé půdě brání proti vysokým obsahům Al vnějším mechanismem, tzv. uvolněním organických kyselin z rhizosféry a následnou chelatací s Al (Ma et al., 2001). Vnitřní detoxikaci Al, tzv. formace stabilních komplexů Al s anionty organických kyselin uvnitř rostliny (Ma et al., 2001), šťovík pravděpodobně uplatňuje v alkalických půdách. V literatuře byly obranné mechanismy proti vysokým obsahům Al v rostlinách sledovány pouze v silně kyselých podmínkách (Arunakumara et al., 2013). Vnitřní mechanismus detoxikace Al byl popsán u šťovíku menšího (Schöttelndreier et al., 2001). Vnější mechanismus detoxikace Al uvedli Tolrà et al. (2005) u šťovíku kyselého. Využití obou obranných mechanismů proti vysokým obsahům Al u šťovíku v literatuře popsáno zatím nebylo.

Obsahy a distribuce aniontů nízkomolekulárních organických kyselin v orgánech šťovíku. Obsahy ani distribuce aniontů organických kyselin v biomase šťovíků nebyly ovlivněny agrochemickými parametry půd ani půdními aditivy. Zastoupení aniontů organických kyselin ve šťovících ovlivňovaly pouze rostlinné orgány (viz obrázek 3 v podkapitole 4.4). Celkové množství aniontů organických kyselin vzrůstalo v pořadí: podzemní orgány<stonky<listy. Při hodnocení obsahů aniontů jednotlivých kyselin v orgánech šťovíků bylo nejvíce citronanů, maleinanů a vinanů zastoupeno v podzemních orgánech. Nejvyšší obsahy jablečnanů byly zjištěny ve stoncích. Mléčnany, mravenčany, octany, propionany a šťavelany převládaly v listech. Nejvyšší obsahy šťavelanů stanovené v listech šťovíku se shodovaly s výsledky Miyagi et al. (2010). Vyšší obsah citronanů v listech než ve stoncích byl v rozporu s Miyagi et al. (2010), což může souviset se stářím rostliny. Schopnosti organických kyselin tvořit s Al komplexy o různé stabilitě souvisí

s chemickou strukturou kyselin a s konstantou stability vzniklých komplexů (Hue et al., 1986; Strobel, 2001). Komplexy aniontů organických kyselin s Al se středně silnou až silnou stabilitou byly zastoupeny ve všech orgánech šťovíků (podzemní orgány – Al-citronany, Al-vinany, stonky – Al-jablečnany, listy – Al-šťavelany). Uvedené komplexy s nízkomolekulárními organickými kyselinami pravděpodobně souvisí s vnitřní obranou šťovíku proti vysokým obsahům Al (Ma et al., 2001; Singh a Chauhan, 2011).

5.2.2 Rychle rostoucí dřeviny

Vrba Smithova byla pěstována na půdách neošetřených (kontrolních) a ošetřených vápnem a dolomitem.

Růst a mortalita rostlin. Během tříletého období vrby více hynuly ve slabě kyselé kontrolní půdě (pH=6,1) než v alkalické kontrolní půdě (pH=7,3); (viz tabulka 3 v podkapitole 4.6). Uvedený výsledek je v rozporu s optimální půdní reakcí (pH=5,5-6,5) pro růst vrb (Tahvanainen a Rytkönen, 1999). Nesrovnalost v reakci vrb na pH půdy může souviset s přítomností rizikových prvků v půdě. Vyšší hodnota pH půdy pomáhá omezovat mobilitu některých rizikových prvků v půdě a zvyšovat produkci biomasy vrb (Trakal et al., 2011). Okamžité sázení vrb do půd ošetřených vyšší dávkou vápna bylo neslučitelné s jejich životaschopností. Pro počáteční vývoj vrb byla silně alkalická reakce půdy vyvolaná aplikací vysoké dávky vápna nevhodná. Nesoulad s potřebou vysokého pH půdy pro dobrý rozvoj kořenů vrb (Hytönen a Kaunisto, 1999) pravděpodobně souvisel s destrukcí kořenů hydroxidem vznikajícím reakcí vápna s vodou. Další příčinou mohly být vysoké koncentrace rizikových prvků po mineralizaci organické hmoty ve vyvápněné půdě (Mühlbachová a Tlustoš, 2006). Dávka dolomitu sehrála důležitou roli v úhynu vrb. Zhoršený růst a zvýšená mortalita vrb byly pozorovány ve slabě kyselé půdě ošetřené nižší dávkou dolomitu.

Produkce biomasy. Vyšší výnosy listů vrb ve slabě kyselé půdě a vyšší výnosy větví v alkalické půdě byly pravděpodobně způsobeny rozdílnou půdní reakcí a mírou kontaminace půd (Tlustoš et al., 2007). Aplikace vápna v prvním roce pokusu, bez ohledu na použitou dávku, přispěla ke zvýšení výnosu nadzemní biomasy vrb ve druhém a třetím roce ve slabě kyselé půdě. Vyšší dávka vápna přispěla ke zvýšení výnosu vrb od druhého roku pokusu i v alkalické půdě. Během tříletého období byl výnos vrb omezen aplikací nižší dávky dolomitu ve slabě kyselé půdě. Naopak vyšší dávka dolomitu (pozvolná účinnost; Mayfield et al., 2004) přispěla ke zvýšenému růstu vrb až od třetí vegetační sezóny.

Obsahy a distribuce makroprvků, mikroprvků a toxických prvků v orgánech vrb. Vyšší obsahy P, K, Ca a Mg v listech než ve větvích byly stanoveny v kontrolních

půdách. Zvýšený transport makroprvků z větví do listů vrb nebyl vápněním ovlivněn. Vápnění ve většině případů nezměnilo ani obsahy makroprvků v nadzemní biomase. Vyšší obsahy P ve větvích v prvním roce pokusu souvisely s růstovým omezením vrb po vápnění. Obsahy většiny makroprvků stanovené ve vrbách v kontaminovaných kontrolních půdách se podobaly hodnotám makroprvků ve vrbách pěstovaných v nekontaminovaných půdách (4,5 g Ca.kg^{-1}; 2-2,5 g Mg.kg^{-1}; 8-18 g K.kg^{-1}; Jug et al., 1999). Pouze zjištěný průměrný obsah P v listech vrb (1,7 g.kg^{-1}) ve slabě kyselé kontrolní půdě je možné považovat za skrytý nedostatek P (< 2,1 g.kg^{-1}; Jug et al., 1999).

Vyšší obsahy Fe, Mn a Ni v listech než ve větvích vrb byly zjištěny v kontrolních půdách. Vápnění nezměnilo zvýšený transport Fe, Mn ani Ni z větví do listů. Na rozdíl od ostatních mikroprvků obsah i distribuci Cu v nadzemní biomase ovlivňovaly půdní vlastnosti. Ve slabě kyselé půdě byl zjištěn omezený transport Cu z větví do listů. V alkalické půdě byl naopak pozorován transport zvýšený. Podobné výsledky popisují i Kacálková et al. (2015). Vápnění distribuci Cu v nadzemní biomase vrb nezměnilo (viz tabulka 5 v podkapitole 4.6). Během první vegetační sezóny byly u vrb ve slabě kyselé půdě pozorovány viditelné příznaky nedostatku Fe, tzv. chlorózy, s nejvyšší pravděpodobností vyvolané fytotoxicitou Zn (>100 mg Zn.kg^{-1}; Kabata-Pendias, 2011). K podobným závěrům dospěli i další autoři (Vysloužilová et al., 2003b; Tlustoš et al., 2007). Stanovené obsahy Cu v listech (viz tabulka 5 v podkapitole 4.6) byly vyšší než běžné obsahy Cu v listech vrb v kyselých nekontaminovaných půdách (3,5-9,2 mg Cu.kg^{-1}; Syso et al., 2014). Zjištěné obsahy Mn a Ni v listech (viz tabulka 5 v podkapitole 4.6) byly nižší než běžné obsahy Mn a Ni v listech vrb v kyselých nekontaminovaných půdách (168-779 mg Mn.kg^{-1}; 5,3-13 mg Ni.kg^{-1}; Syso et al., 2014). V prvním roce pokusu byly obsahy Cu, Fe a Mn v nadzemních orgánech vrb nižší než v následných letech. Důvodem byla jejich precipitace vyvolaná alkalickým pH půdy po vápnění v první vegetační sezóně. Vyšší obsahy Fe v listech vrb souvisely také s listovou aplikací Fe prováděnou od druhé vegetační sezóny.

Vyšší obsahy Al, Cd a Zn v listech než ve větvích vrb byly zjištěny v kontrolních půdách. Podobné výsledky popisují i další autoři (Tlustoš et al., 2007; Kacálková et al., 2015). Vápnění neovlivnilo zvýšený transport Al, Cd a Zn z větví do listů vrb (tabulka 5 v podkapitole 4.6). Obsahy i distribuce Cr a Pb ve vrbách se odlišovaly od předešlých toxických prvků. Chrom v nadzemní biomase ovlivňovaly chemické vlastnosti půd. Na Pb ve vrbách významně působily půdní vlastnosti i vápnění. Vliv sledované doby působení aditiv se projevil na obsahu většiny toxických prvků. Zvýšené obsahy Al, Cd a Cr v nadzemních orgánech po třetí vegetační sezóně byly pravděpodobně vyvolány sníženou imobilizační

účinností vápnění (Lee et al., 2004). Hranice fytotoxicity pro Cd a Zn (>5 mg Cd.kg^{-1}, >100 mg Zn.kg^{-1}; Pugh et al., 2002; Kabata-Pendias, 2011) byly překročeny v obou kontrolních půdách. Stanovené obsahy Pb v listech vrb ve slabě kyselé půdě (viz tabulka 5 v podkapitole 4.6) byly vyšší než běžné obsahy Pb v listech vrb v kyselých nekontaminovaných půdách (1,0-1,1 mg Pb.kg^{-1}; Syso et al., 2014). Hranice fytotoxicity pro Pb (>30 mg.kg^{-1}; Pugh et al., 2002) však překročena nebyla.

Aplikace nižší dávky dolomitu nebyla vhodným opatřením pro pěstování vrb ve slabě kyselé půdě. Po aplikaci vápna nebyly zvýšené obsahy Pb v listech vrb zmírněny, ale nepředstavovaly pro růst vrb takové omezení, jako vysoké obsahy Zn. Stabilizace půdy vápnem v kombinaci s listovou aplikací Fe částečně pomohlo omezit vysoké obsahy Cd a Zn v listech vrb. Uvedený postup pomohl snížit poměr Zn:Fe v listech a tím zmírnit nedostatek Fe v listech vrb ve slabě kyselé půdě.

6 Závěr

Dosavadní výzkum v *in-situ* chemických imobilizací byl zaměřen především na hodnocení mobility toxických prvků a jejich dostupnosti pro rostliny. Ve vědeckých studiích byl však zřídka posuzován současně i vliv aditiv na regulaci mobility a biodostupnosti makroprvků a mikroprvků. Předložená disertační práce hodnotila obě hlediska a to toxické prvky a živiny v půdách i v rostlinách. V práci byla velká pozornost věnována zejména studiu vlastností tolerantní byliny a dřeviny pěstované na půdách stabilizovaných imobilizačními aditivy. V tomto kontextu může správné pochopení biologických i chemických vlastností tolerantních rostlin vést k jejich úspěšnému pěstování na půdách kontaminovaných vysokými obsahy rizikových prvků a tím ke smysluplnému využití takovýchto lokalit.

V regulaci labilních (mobilních a potenciálně mobilizovatelných) koncentrací mikroprvků a toxických prvků v kontaminovaných půdách sehrály důležitou úlohu půdní vlastnosti, půdní aditiva i jejich aplikační dávky. Neméně podstatnou roli představoval též samotný prvek. Během 42 dnů inkubace byla imobilizace As, Cd, Pb, Zn, Fe a Mn v alkalické půdě pomocí aplikace vápenatých hmot a fosforečných aditiv neúčinná. Ve slabě kyselé půdě byly naopak prvky přídavkem aditiv významně regulovány. Vyvolané změny u prvků byly rozděleny do dvou skupin – 1) pokles koncentrací (imobilizace) a 2) bez poklesu koncentrací (mobilizace nebo bez změny). Půdní aditiva imobilizovala labilní koncentrace Cd, Zn i potenciálně mobilizovatelné koncentrace Pb a Mn. Jako nejvhodnější pro imobilizaci většiny toxických prvků se ukázaly aplikace rychle rozpustných aditiv, především přídavek vápna.

Výše zmiňované parametry ovlivňovaly také uplatnění aditiv pro pěstování rostlin v půdách silně kontaminovaných rizikovými prvky. Podstatný byl i konkrétní rostlinný druh. Každé úspěšné pěstování rostlin začíná v raných fázích vývoje, jinak tomu není ani při pěstování šťovíků a vrb na kontaminovaných půdách. Vědecké studie se podle dostupných informací ale zatím nezabývaly vlivem imobilizačních aditiv na počáteční fáze růstu šťovíků v půdách silně kontaminovaných rizikovými prvky. Dle výsledků práce byl raný vývoj šťovíků ovlivněn půdními vlastnostmi. Půdy s vysokou dostupností Ca, Cd, Pb a Zn negativně ovlivnily vzcházení a přežívání šťovíků. Šťovíky ve slabě kyselé kontrolní půdě strádaly, naopak v alkalické kontrolní půdě prosperovaly. Přídavek vápna do slabě kyselé půdy prosperitu šťovíků zlepšoval, naopak v alkalické půdě vzcházení a přežívání rostlin zhoršoval. Počáteční fáze růstu vrb byly také významně ovlivněny půdními vlastnostmi a aditivy. Jako nejméně vhodné prostředí pro pěstování vrb se ukázala slabě kyselá půda ošetřená nižší

dávkou dolomitu. Ani půdní podmínky navozené v obou půdách okamžitě po aplikaci vyšší dávky vápna nebyly pro počáteční růst vrb vhodné. Pro efektivní pěstování vrb je podle zjištěných výsledků nezbytné vysazovat řízky do půd ošetřených vápnem až ve druhém roce po jeho aplikaci. Pro hospodářské využití kontaminovaných lokalit je nutné znát výslednou produkci biomasy pěstovaných rostlin. Vlastnosti půd ovlivnily produkci biomasy šťovíků i vrb. Vyšší produkci biomasy vykazovaly šťovíky v alkalické půdě. Přídavek vápna do obou půd produkci biomasy šťovíků výrazně zvýšil. Během vývoje šťovíků vymizel negativní účinek Ca na jejich růst, pozorovaný v počátečných fázích růstu šťovíků v alkalické půdě. Vrby produkovaly více listů ve slabě kyselé půdě a více větví v půdě alkalické. Nadzemní biomasa vrb byla zvýšena v půdách ošetřených vápnem až druhým rokem od jeho aplikace, v alkalické půdě jen po přídavku vyšší dávky vápna. Dolomit ovlivnil produkci biomasy vrb pouze ve slabě kyselé půdě. Nižší dávka dolomitu omezila produkci nadzemních orgánů vrb. Vrby začaly produkovat více biomasy až třetí rok po aplikaci vyšší dávky dolomitu. Nezbytná pro pěstování rostlin na kontaminovaných půdách je i informace o prvcích v biomase. Na rozdíl od vrb (tzv. zástupci neoxalátních rostlin) byla distribuce mikroprvků i toxických prvků mezi rostlinnými orgány šťovíku (tzv. zástupci oxalátních rostlin) ovlivněna aditivy. Omezený transport mikroprvků a toxických prvků z podzemních orgánů do listů šťovíků v kontrolních půdách byl vyvápněním půd a ošetřením alkalické půdy superfosfátem zvýšen. Změny v distribuci prvků byly pravděpodobně spojeny s přítomností organických kyselin ve šťovících. Na základě výsledků doktorské práce se nabízí možnost využít šťovík tupolistý, tzv. exkludační rostlinu As, Cd, Pb a Zn, pro vegetační mapování půd podle vizuálních symptomů pozorovaných na jeho nadzemních orgánech v odlišných fenologických fázích. Oxalátní šťovík tupolistý je možné doporučit i pro pěstování v silně kontaminovaných půdách stabilizovaných vápnem. Nově bylo zjištěno, že ve vyvápněných půdách a v alkalické půdě ošetřené superfosfátem se šťovík s nízkou akumulací (exkludační) choval jako rostlina s běžnou až s vysokou akumulací (indikační až akumulační) rizikových prvků, což vybízí k jeho využití při rekultivaci krajiny. Možnosti dalšího výzkumu a využití šťovíku nabízí i překvapivý výsledek hyperakumulační schopnosti šťovíku pro Al v alkalické půdě. Výsledky práce naznačují, že dosavadní znalosti ve zkoumané oblasti nemusí být kompletní. Budoucí výzkum by se tedy mohl s ohledem na druhy rostlin, jejich vlastnosti, půdní aditiva a chemické vlastnosti půd, zaměřit i na rozdíly mezi dalšími oxalátními a neoxalátními rostlinami, zkoumat možné souvislosti a to včetně potenciálního nebezpečí, které představuje vstup rizikových prvků do potravinového řetězce.

7 Seznam použité literatury

Abhilash, P. C., Powell, J. R., Singh, H. B., Singh, B. K. 2012. Plant-microbe interactions: novel applications for exploitation in multipurpose remediation technologies. Trends in Biotechnology. 30 (8). 416-420.

Abollino, O., Malandrino, M., Giacomino, A., Mentasti, E. 2011. The role of chemometrics in single and sequential extraction assays: a review part I. Extraction procedures, uni- and bivariate techniques and multivariate variable reduction techniques for pattern recognition. Analytica Chimica Acta. 688 (2). 104-121.

Adriano, D. C. 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals. 2nd ed. Springer-Verlag. New York. p. 871. ISBN: 9780387215105.

Ali, H., Khan, E., Sajad, M. A. 2013. Phytoremediation of heavy metals – concepts and applications. Chemosphere. 91 (7). 869-881.

Alkorta, I., Hernández-Allica, J., Becerril, J. M., Amezaga, I., Albizu, I., Garbisu, C. 2004. Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead and arsenic. Reviews in Environmental Science and Biotechnology. 3 (1). 71-90.

Alkorta, I., Becerril, J. M., Garbisu, C. 2010. Phytostabilization of metal contaminated soils. Reviews on Environmental Health. 25 (2). 135-146.

Alloway, B. J. 2013. Bioavailability of elements in soil. In: Selinus, O. (eds.). Essentials of medical geology: revised edition. Springer. Netherlands. p. 351-373. ISBN: 9789400743748.

Alvarenga, P., Goncalves, A. P., Fernandes, R. M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A. C. 2008. Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass. Science of the Total Environment. 406 (1-2). 43-56.

Anton, A., Rékási, M., Uzinger, N., Széplábi, G., Makó, A. 2012. Modelling the potential effects of the hungarian red mud disaster on soil properties. Water Air and Soil Pollution. 223 (8). 5175-5188.

Arunakumara, K. K. I. U., Walpola, B. C., Yoon, M. H. 2013. Aluminum toxicity and tolerance mechanism in cereals and legumes – a review. Journal of the Korean Society for Applied Biological Chemistry. 56 (1). 1-9.

Assuncao, 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. 159 (2). 351-360.

Bacon, J. R., Davidson, C. M. 2008. Is there a future for sequential chemical extraction? Analyst. 133 (1). 25-46.

Baker, A. J. M. 1981. Accumulators and excluders - strategies in the response of plants to heavy-metals. Journal of Plant Nutrition. 3 (1-4). 643-654.

Baker, A. J. M., Brooks, R. R. 1989. Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. Biorecovery. 1. 81-126.

Baker, L. R., Pierzynski, G. M., Hettiarachchi, G. M., Scheckel, K. G., Newville, M. 2012. Zinc speciation in proximity to phosphate application points in a lead/zinc smelter-contaminated soil. Journal of Environmental Quality. 41 (6). 1865-1873.

Barceló, J., Poschenrieder, C. 2002. Fast roots growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. Environmental and Experimental Botany. 48 (1). 75-92.

Barrutia, O., Epelde, L., García-Plazaola, J. I., Garbisu, C., Becerril, J. M. 2009. Phytoextraction potential of two *Rumex acetosa* L. accessions collected from metalliferous and non-metalliferous sites: effect of fertilization. Chemosphere. 74 (2). 259-264.

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.

Bertocchi, A. F., Ghiani, M., Peretti, R., Zucca, A. 2006. Red mud and fly ash for remediation of mine sites contaminated with As, Cd, Cu, Pb and Zn. Journal of Hazardous Materials. 134 (1-3). 112-119.

Bleeker, P. M., Assuncao, A. G. L., Teiga, P. M., de Koe, T., Verkleij, J. A. C. 2002. Revegetation of the acidic, As contaminated jales mine spoil tips using a combination of spoil amendments and tolerant grasses. The Science of the Total Environment. 300 (1-3). 1-13.

Bolan, N. S., Naidu, R., Syers, J. K. Tillman, R. W. 1999. Surface charge and solute interactions in soils. Advances in Agronomy. 67. 87-140.

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.

Bolan, N. S., Duraisamy, V. P. 2003. Role of inorganic and organic soil amendments on immobilisation and phytoavailability of heavy metals: a review involving specific case studies. Australian Journal of Soil Research. 41 (3). 533-555.

Bolan, N., Naidu, R., Choppala, G., Park, J., Mora, M. L., Budianta, D., Panneerselvam, P. 2010. Solute interactions in soils in relation to the bioavailability and environmental remediation of heavy metals and metalloids. Pedologist. 53 (3). 1-18.

Bolan, N., Mahimairaja, S., Kunhikrishnan, A., Choppala, G. 2013. Phosphorus-arsenic interactions in variable-charge soils in relation to arsenic mobility and bioavailability. Science of the Total Environment. 463-464. 1154-1162.

Bolan, N., Kunhikrishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., Kirkham, M. B., Scheckel, K. 2014. Remediation of heavy metal(loid)s contaminated soils – to mobilize or to immobilize? Journal of Hazardous Materials. 266. 141-166.

Borůvka, L., HuanWei, C., 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.

Bradl, H. B. 2004. Adsorption of heavy metal ions on soils and soils constituents. Journal of Colloid and Interface Science. 277 (1). 1-18.

Brown, G. E., Parks, G. A. 2001. Sorption of trace elements on mineral surfaces: modern perspectives from spectroscopic studies, and comments on sorption in the marine environment. International Geology Review. 43 (11). 963-1073.

Brown, T. T., Koenig, R. T., Huggins, D. R., Harsh, J. B., Rossi, R. E. 2008. Lime effects on soil acidity, crop yield, and aluminum chemistry in direct-seeded cropping systems. Soil Science Society of America Journal. 72 (3). 634-640.

Brunori, C., Cremisini, C., Massanisso, P., Pinto, V., Torricelli, L. 2005. Reuse of a treated red mud bauxite waste: studies on environmental compatibility. Journal of Hazardous Materials. 117 (1). 55-63.

Calace, N., Petronio, B. M., Pietroletti, M. 2006. Metal bioavailability: how does its significance change in the time? Annali di Chimica. 96 (3-4). 131-136.

Cao, X., Ma, L. Q., Chen, M., Singh, S. P., Harris, W. G. 2002. Impacts of phosphate amendments on lead biogeochemistry at a contaminated site. Environmental Science & Technology. 36 (24). 5296–5304.

Cao, X., Dermatas, D., Xu, X., Shen, G. 2008. Immobilization of lead in shooting range soils by means of cement, quicklime, and phosphate amendments. Environmental Science and Pollution Research. 15 (2). 120-127.

Cao, X., Wahbi, A., Ma, L., Li, B., Yang, Y. 2009. Immobilization of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid. Journal of Hazardous Materials. 164 (2-3). 555-564.

Capuana, M. 2011. Heavy metals and woody plants − biotechnologies for phytoremediation. IForest-Biogeosciences and Forestry. 4. 7-15.

Cascarosa, E., Gea, G., Arauzo, J. 2012. Thermochemical processing of meat and bone meal: a review. Renewable & Sustainable Energy Reviews. 16 (1). 942-957.

Clemens, S. 2001. Molecular mechanisms of plant metal tolerance and homeostasis. Planta. 212 (4). 475-486.

Cobbett, C., Goldsbrough, P. 2002. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annual Review of Plant Biology. 53. 159-182.

Coutand, M., Cyr, M., Deydier, E., Guilet, R., Clastres, P. 2008. Characteristics of industrial and laboratory meat and bone meal ashes and their potential applications. Journal of Hazardous Materials. 150 (3). 522-532.

Coutand, M., Deydier, E., Cyr, M., Mouchet, F., Gauthier, L., Guilet, R., Savaete, L. B., Cren, S., Clastres, P. 2009. Evaluation of laboratory and industrial meat and bone meal combustion residue as cadmium immobilizing material for remediation of polluted aqueous solutions: "chemical and ecotoxicological studies". Journal of Hazardous Materials. 166 (2-3). 945-953.

Cui, Y. S., Du, X., Weng, L. P., Van Riemsdijk, W. H. 2010. Assessment of in situ immobilization of lead (Pb) and arsenic (As) in contaminated soils with phosphate and iron: solubility and bioaccessibility. Water Air and Soil Pollution. 213 (1-4). 95-104.

Česko. Vyhláška Ministerstva životního prostředí č. 13/1994 Sb., kterou se upravují některé podrobnosti ochrany zemědělského půdního fondu. In: Sbírka zákonů České republiky. 1994. částka 4. s. 82.

da Silva, E. C., Carlos, L. D. A., Araújo, A. P., Ferraz, L. D. C. L., Pedrosa, M. W., Silva, L. S. 2013. Characterization of two types of azedinha in the region of Sete Lagoas, Brazil. Horticultura Brasileira. 31 (2). 328-331.

Degenhardt, J., Larsen, P. B., Howell, S. H., Kochian, L. V. 1998. Aluminum resistance in the *Arabidopsis* mutant alr-104 is caused by an aluminum-induced increase in rhizosphere pH. Plant Physiology. 117 (1). 19-27.

Delhaize, E., Ryan, P. R., Randall, P. J. 1993. Aluminum tolerance in wheat (*Triticum aestivum* L.) II. aluminum-stimulated excretion of malic acid from root apices. Plant Physiology. 103 (3). 695-702.

Demirezer, L. O., Kuruüzüm-Uz, A., Bergere, I., Schiewe, H. J., Zeeck, A. 2001. The structures of antioxidant and cytotoxic agents from natural source: anthraquinones and tannins from roots of *Rumex patientia*. Phytochemistry. 58 (8). 1213-1217.

Dermatas, D., Meng, X. G. 2003. Utilization of fly ash for stabilization/solidification of heavy metal contaminated soils. Engineering Geology. 70 (3-4). 377-394.

Deydier, E., Guilet, R., Sharrock, P. 2003. Beneficial use of meat and bone meal combustion residue: "an efficient low cost material to remove lead from aqueous effluent". Journal of Hazardous Materials. 101 (1). 55-64.

Deydier, E., Guilet, R., Sarda, S., Sharrock, P. 2005. Physical and chemical characterisation of crude meat and bone meal combustion residue: "waste or raw material?" Journal of Hazardous Materials. 121 (1-3). 141-148.

Deydier, E., Guilet, R., Cren, S., Pereas, V., Mouchet, F., Gauthier, L. 2007. Evaluation of meat and bone meal combustion residue as lead immobilizing material for in situ remediation of polluted aqueous solutions and soils: "chemical and ecotoxicological studies". Journal of Hazardous Materials. 146 (1-2). 227-236.

Dissanayake, C. B., Chandrajith, R. 2009. Phosphate mineral fertilizers, trace metals and human health. Journal of the National Science Foundation of Sri Lanka. 37 (3). 153-165.

do Nascimento, C. W. A., Xing, B. 2006. Phytoextraction: a review on enhanced metal availability and plant accumulation. Scientia Agricola. 63 (3). 299-311.

Dos Santos Utmazian, M. N., Wenzel, W. W. 2007. Cadmium and zinc accumulation in willow and poplar species grown on polluted soils. Journal of Plant Nutrition and Soil Science. 170 (2). 265-272.

Dos Santos Utmazian, M. N., Wieshammer, G., Vega, R. Wenzel, W. W. 2007. Hydroponic screening for metal resistance and accumulation of cadmium and zinc in twenty clones of willows and poplars. Evironmental Pollution. 148 (1). 155-165.

Elkhatib, E. A., Elshebiny, G. M., Balba, A. M. 1991. Lead sorption in calcareous soils. Environmental Pollution. 69 (4). 269-276.

Epelde, L., Becerril, J. M., Barrutia, O., González-Oreja, J. A., Garbisu, C. 2010. Interactions between plant and rhizosphere microbial communities in a metalliferous soil. Environmental Pollution. 158 (5). 1576-1583.

Ernst, W. H. O., Verkleij, J. A. C., Schat, H. 1992. Metal tolerance in plants. Acta Botanica Neerlandica. 41 (3). 229-248.

Fageria, N. K., Baligar, V. C., Clark, R. B. 2002. Micronutrients in crop production. Advances in Agronomy. 77. 185-268.

Fang, Y., Cao, X., Zhao, L. 2012. Effects of phosphorus amendments and plant growth on the mobility of Pb, Cu, and Zn in a multi-metal-contaminated soil. Environmental Science and Pollution Research. 19 (5). 1659-1667.

Farhoodi, A., Coventry, D. R. 2008. Field crop responses to lime in the mid-north region of South Australia. Field Crops Research. 108 (1). 45-53.

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

Franceschi, V. R., Nakata, P. A. 2005. Calcium oxalate in plants: formation and function. Annual Review of Plant Biology. 56. 41-71.

Friesl, W., Lombi, E., Horak, O., Wenzel, W. W. 2003. Immobilization of heavy metals in soils using inorganic amendments in a greenhouse study. Journal of Plant Nutrition and Soil Science. 166 (2). 191-196.

Friesl, W., Friedl, J., Platzer, K., Horak, O., Gerzabek, M. H. 2006. Remediation of contaminated agricultural soils near a former Pb/Zn smelter in Austria: batch, pot and field experiments. Environmental Pollution. 144 (1). 40-50.

Friesl-Hanl, W., Platzer, K., Horak, O., Gerzabek, M. H. 2009. Immobilising of Cd, Pb, and Zn contaminated arable soils close to a former Pb/Zn smelter: a field study in Austria over 5 years. Environmental Geochemistry and Health. 31 (5). 581-594.

Gadepalle, V. P., Ouki, S. K., Van Herwijnen, R., Hutchings, T. 2007. Immobilization of heavy metals in soil using natural and waste materials for vegetation establishment on contaminated sites. Soil & Sediment Contamination. 16 (2). 233-251.

Garau, G., Silvetti, M., Deiana, S., Deiana, P., Castaldi, P. 2011. Long-term influence of red mud on As mobility and soil physico-chemical and microbial parameters in a polluted sub-acidic soil. Journal of Hazardous Materials. 185 (2-3). 1241-1248.

García, M. A., Chimenos, J. M., Fernández, A. I., Miralles, L., Segarra, M., Espiell, F. 2004. Low-grade MgO used to stabilize heavy metals in highly contaminated soils. Chemosphere. 56 (5). 481-491.

Gaweda, M. 2009. Heavy metal content in common sorrel plants (*Rumex Acetosa* L.) obtained from natural sites in Malopolska province. Polish Journal of Environmental Studies. 18 (2). 213-218.

Gaymard, F., Pilot, G., Lacombe, B., Bouchez, D., Bruneau, D., Boucherez, J., Michaux-Ferriere, N., Thibaud, J. B., Sentenac, H. 1998. Identification and disruption of a plant shaker-like outward channel involved in K^+ release into the xylem sap. Cell. 94 (5). 647-655.

Grant, W. F. 1999. Higher plant assays for the detection of chromosomal aberrations and gene mutations – a brief historical background on their use for screening and monitoring environmental chemicals. Mutation Research. 426 (2). 107-112.

Gray, C. W., Dunham, S. J., Dennis, P. G., Zhao, F. J., McGrath, S. P. 2006. Field evaluation of in situ remediation of a heavy metal contaminated soil using lime and red-mud. Environmental Pollution. 142 (3). 530-539.

Grobelak, A., Napora, A. 2015. The chemophytostabilisation process of heavy metal polluted soil. PLoS ONE. 10 (6). 1-15. doi: 10.1371/journal.pone.0129538.

Guerinot, M. L. 2000. The ZIP family of metal transporters. Biochimica et Biophysica Acta. 1465 (1-2). 190-198.

Gul, S., Naz, A., Fareed, I., Irshad, M. 2015. Reducing heavy metals extraction from contaminated soils using organic and inorganic amendments – a review. Polish Journal of Environmental Studies. 24 (3). 1423-1426.

Güleryüz, G., Arslan, H., Celik, C., Gücer, S., Kendall, M. 2008. Heavy metal content of plant species along Nilüfer stream in industrialized Bursa City, Turkey. Water Air and Soil Pollution. 195 (1-4). 275-284.

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.

Gupta, D. K., Chatterjee, S., Datta, S., Veer, V., Walther, C. 2014. Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals. Chemosphere. 108. 134-144.

Hafsteinsdóttir, E. G., Camenzuli, D., Rocavert, A. L., Walworth, J., Gore, D. B. 2015. Chemical immobilization of metals and metalloids by phosphates. Applied Geochemistry. 59. 47-62.

Hall, J. L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany. 53 (366). 1-11.

Hammer, D., Kayser, A., Keller, C. 2003. Phytoextraction of Cd and Zn with *Salix viminalis* in field trials. Soil Use and Management. 19 (3). 187-192.

Hann, P., Trska, C., Kromp, B. 2012. Effects of management intensity and soil chemical properties on *Rumex obtusifolius* in cut grasslands in Lower Austria. Journal of Pest Science. 85 (1). 5-15.

Hänsch, R., Mendel, R. R. 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Ni, Mo, B, Cl). Current Opinion in Plant Biology. 12 (3). 259-266.

Harter, R. D., Naidu, R. 1995. Role of metal-organic complexation in metal sorption by soils. Advances in Agronomy. 55. 219-263.

Hashimoto, Y., Matsufuru, H., Takaoka, M., Tanida, H., Sato, T. 2009. Impacts of chemical amendment and plant growth on lead speciation and enzyme activities in a shooting range soil: an X-ray absorption fine structure investigation. Journal of Environmental Quality. 38 (4). 1420-1428.

He, B., Yang, X. E., Ni, W. Z., Wei, Y. Z., Long, X. X., Ye, Z. Q. 2002. *Sedum alfredii* – a new lead-accumulating ecotype. Acta Botanica Sinica. 44 (11). 1365-1370.

He, Z. L., Yang, X. E., Stoffella, P. J. 2005. Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology. 19 (2-3). 125-140.

Hejcman, M., Křišťálová, V., Červená, K., Hrdličková, J., Pavlů, V. 2012. Effect of nitrogen, phosphorus and potassium availability on mother plant size, seed production and germination ability of *Rumex crispus*. Weed Research. 52 (3). 260-268.

Hodson, M. E., Valsami-Jones, E., Cotter-Howells, J. D. 2000. Bonemeal additions as a remediation treatment for metal contaminated soil. Environmental Science & Technology. 34 (16). 3501-3507.

Hong, C. O., Lee, D. K., Chung, D. Y., Kim, P. J. 2007. Liming effects on cadmium stabilization in upland soil affected by gold mining activity. Archives of Environmental Contamination and Toxicology. 52 (4). 496-502.

Hong, C. O., Chung, D. Y., Lee, D. K., Kim, P. J. 2010. Comparison of phosphate materials for immobilizing cadmium in soil. Archives of Environmental Contamination and Toxicology. 58 (2). 268-274.

Hopkins, A., Johnson, R. H. 2002. Effect of different manuring and defoliation patterns on broad-leaved dock (*Rumex obtusifolius*) in grassland. Annals of Applied Biology. 140 (3). 255-262.

Horák, J., Hejcman, M. 2013. Use of trace elements from historical mining for alluvial sediment dating. Soil and Water Research. 8 (2). 77-86.

Hossain, M. A., Piyatida, P., da Silva, J. A. T., Fujita, M. 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. Journal of Botany. 2012. 1-37. doi:10.1155/2012/872875.

Hossner L. R., Richards, G. E. 1967. The effect of phosphorus source on the movement and uptake of band applied manganese. Soil Science Society of America Journal. 32 (1). 83-85.

Hrdličková, J., Hejcman, M., Křišťálová, V., Pavlů, V. 2011. Production, size, and germination of broad-leaved dock seeds collected from mother plants grown under different nitrogen, phosphorus, and potassium supplies. Weed Biology and Management. 11 (4). 190-201.

Huang, J., Xia, H., Li, Z., Xiong, Y., Kong, G., Huang, J. 2009. Soil aluminium uptake and accumulation by *Paspalum notatum*. Waste Management & Research. 27 (7). 668-675.

Hue, N. V., Craddock, G. R., Adams, F. 1986. Effect of organic acids on aluminum toxicity in subsoils. Soil Science Society of America Journal. 50 (1). 28-34.

Hujerová, R., Pavlů, V., Hejcman, M., Pavlů, L., Gaisler, J. 2013. Effect of cutting frequency on above- and belowground biomass production of *Rumex alpinus*, *R. crispus*, *R. obtusifolius*, and the *Rumex* hybrid (*R. patienta* x *R. tianschanicus*) in the seeding year. Weed Research. 53 (5). 378-386.

Humphreys, J., Jansen, T., Culleton, N., MacNaeidhe, F. S., Storey, T. 1999. Soil potassium supply and *Rumex obtusifolius* and *Rumex crispus* abundance in silage and grazed grassland swards. Weed Research. 39 (1). 1-13.

Hytönen, J., Kaunisto, S. 1999. Effect of fertilization on the biomass production of coppiced mixed birch and willow stands on a cut-away peatland. Biomass and Bioenergy. 17 (6). 455-469.

Chaney, R. L. 2012. Food safety issues for mineral and organic fertilizers. Advances in Agronomy. 117. 51-116.

Chen, Q., Wong, J. W. C. 2006. Growth of *Agropyron elongatum* in a simulated nickel contaminated soil with lime stabilization. Science of the Total Environment. 366 (2-3). 448-455.

Chen, J. H., Sun, H., Wen, J., Yang, Y. P. 2010. Molecular phylogeny of *Salix* L. (*Salicaceae*) inferred from three chloroplast datasets and its systematic implications. Taxon. 59 (1). 29-37.

Chrysochoou, M., Dermatas, D., Grubb, D. G. 2007. Phosphate application to firing range soils for Pb immobilization: the unclear role of phosphate. Journal of Hazardous Materials. 144 (1-2). 1-14.

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

Islam, M., Mishra, P. C., Patel, R. 2010. Physicochemical characterization of hydroxyapatite and its application towards removal of nitrate from water. Journal of Environmental Management. 91 (9). 1883-1891.

Jabeen, R., Ahmad, A., Iqbal, M. 2009. Phytoremediation of heavy metals: physiological and molecular mechanisms. Botanical Review. 75 (4). 339-364.

Jedynak, L., Kowalska, J., Harasimowicz, J., Golimowski, J. 2009. Speciation analysis of arsenic in terrestrial plants from arsenic contaminated area. Science of the Total Environment. 407 (2). 945-952.

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.

Jentschke, G., Goldbold, D. L. 2000. Metal toxicity and ectomycorrhizas. Physiologia Plantarum. 109 (2). 107-116.

Jiang, H. M., Yang, J. C., Zhang, J. F. 2007. Effects of external phosphorus on the cell ultrastructure and the chlorophyll content of maize under cadmium and zinc stress. Environmental Pollution. 147 (3). 750-756.

Jug, A., Hofmann-Schielle, C., Makeschin, F., Rehfuess, K. E. 1999. Short-rotation of balsam poplars, aspen and willows on former arable land in the Federal Republic of Germany. II. Nutritional status and bioelement export by harvested shoot axes. Forest Ecology and Management. 121 (1-2). 67-83.

Jursík, M., Holec, J., Zatoriová, B. 2008. Širokolisté šťovíky: šťovík tupolistý (*Rumex obtusifolius*) a šťovík kadeřavý (*Rumex crispus*). Listy cukrovarnické a řepařské. 124 (7-8). 215-219.

Kabata-Pendias, A. 2011. Trace elements in soils and plants. 4th ed. CRC Press. Boca Raton. p. 505. ISBN: 9781420093681.

Kacálková L., Tlustoš, P., Száková, J. 2015. Phytoextraction of risk elements by willow and poplar trees. International Journal of Phytoremediation. 17 (5). 414-421.

Karpukhin, M. M., Ladonin, D. V. 2008. Effect of soil components on the adsorption of heavy metals under technogenic contamination. Eurasian Soil Science. 41 (11). 1228-1237.

Kinraide, T. B. 1990. Assessing the rhizotoxicity of the aluminate ion, $Al(OH)_4^-$. Plant Physiology. 93 (4). 1620-1625.

Kirchmann, H., Eskilsson, J. 2010. Low manganese (Mn) and copper (Cu) concentrations in cereals explained yield losses after lime application to soil. Acta Agriculturae Scandinavica Section B-Soil and Plant Science. 60 (6). 569-572.

Komárek, M., Tlustoš, P., Száková, J., Chrastný, J., Ettler, V. 2007. The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. Chemosphere. 67 (4). 640-651.

Komárek, M., Vaněk, A., Ettler, V. 2013. Chemical stabilization of metals and arsenic in contaminated soils using oxides – a review. Environmental Pollution. 172. 9-22.

Koptsik, G. N. 2014. Modern approaches to remediation of heavy metal polluted soils: a review. Eurasian Soil Science. 47 (7). 707-722.

Králová, L., Száková, J., Kubík, Š., Tlustoš, P., Balík, J. 2010. The variability of arsenic and other risk element uptake by individual plant species growing on contaminated soil. Soil & Sediment Contamination. 19 (5). 617-634.

Křišťálová, V., Hejcman, M., Červená, K., Pavlů, V. 2011. Effect of nitrogen and phosphorus availability on the emergence, growth and over-wintering of *Rumex crispus* and *Rumex obtusifolius*. Grass and Forage Science. 66 (3). 361-369.

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.

Kunhikrishnan, A., Bolan, N. S., Müller, K., Laurenson, S., Naidu, R., Kim, W. I. 2012 The influence of wastewater irrigation on the transformation and bioavailability of heavy metal(loid)s in soil. Advances in Agronomy. 115. 215-279.

Kuzovkina, Y. A., Volk, T. A. 2009. The characterization of willow (*Salix* L.) varieties for use in ecological engineering applications: co-ordination of structure, function and autecology. Ecological Engineering. 35 (8). 1178-1189.

Kwon, J. S., Yun, S. T., Lee, J. H., Kim, S. O., Jo, H. Y. 2010. Removal of divalent heavy metals (Cd, Cu, Pb, and Zn) and arsenic(III) from aqueous solutions using scoria: kinetics and equilibria of sorption. Journal of Hazardous Materials. 174 (1-3). 307-313.

Lair, G. J., Gerzabek, M. H., Haberhauer, G. 2007. Sorption of heavy metals on organic and inorganic soil constituents. Environmental Chemistry Letters. 5 (1). 23-27.

Lamb, D. T., Ming, H., Megharaj, M., Naidu, R. 2009. Heavy metal (Cu, Zn, Cd and Pb) partitioning and bioaccessibility in uncontaminated and long-term contaminated soils. Journal of Hazardous Materials. 171 (1-3). 1150-1158.

Laperche, V., Traina, S. J., Gaddam, P., Logan, T. J. 1996. Chemical and mineralogical characterizations of Pb in a contaminated soil: reactions with synthetic apatite. Environmental Science & Technology. 30 (11). 3321-3326.

Larsen, S. 1964. The effect of phosphate application on manganese content of plants grown on neutral and alkaline soils. Plant and Soil. 21 (1). 37-42.

Lasat, M. M. 2002. Phytoextraction of toxic metals: a review of biological mechanisms. Journal of Environmental Quality. 31 (1). 109-120.

Lee, T. M., Lai, H. Y., Chen, Z. S. 2004. Effect of chemical amendments on the concentration of cadmium and lead in long-term contaminated soils. Chemosphere. 57 (10). 1459-1471.

Leita, L., Contin, M., Maggioni, A. 1991. Distribution of cadmium and induced Cd-binding proteins in roots, stems and leaves of *Phaseolus vulgaris*. Plant Science. 77 (2). 139-147.

Leitenmaier, B., Küpper, H. 2013. Compartmentation and complexation of metals in hyperaccumulator plants. Frontiers in Plant Science. 4. 1-13. doi: 10.3389/fpls.2013.00374.

López-Lefebre, L. R., Rivero, R. M., García, P. C., Sánches, E., Ruiz, J. M., Romero, L. 2001. Effect of calcium on mineral nutrient uptake and growth of tobacco. Journal of the Science of Food and Agriculture. 81 (14). 1334-1338.

Lorestani, B., Cheraghi, M., Yousefi, N. 2011. Accumulation of Pb, Fe, Mn, Cu and Zn in plants and choice of hyperaccumulator plant in the industrial town of Vian, Iran. Archives of Biological Sciences. 63 (3). 739-745.

Lucena, J. J. 2000. Effects of bicarbonate, nitrate and other environmental factors on iron deficiency chlorosis. A review. Journal of Plant Nutrition. 23 (11-12). 1591-1606.

Ma, Q. Y., Traina, S. J., Logan, T. J., Ryan, J. A. 1993. In situ lead immobilization by apatite. Environmental Science & Technology. 27 (9). 1803-1810.

Ma, Q. Y., Traina, S. J., Logan, T. J., Ryan, J. A. 1994. Effects of aqueous Al, Cd, Cu, Fe(II), Ni, and Zn on Pb immobilization by hydroxyapatite. Environmental Science & Technology. 28 (7). 1219-1228.

Ma, J. F., Ryan, P. R., Delhaize, E. 2001. Aluminium tolerance in plants and the complexing role of organic acids. Trends in Plant Science. 6 (6). 273-278.

Ma, J. F., Ling, H. Q. 2009. Iron for plants and humans. Plant and Soil. 325 (1-2). 1-3.

Macnair, M. R, Tilstone, G. H., Smith, S. E. 2000. The genetics of metal tolerance and accumulation in higher plants. In: Terry, N., Banuelos, G. (eds.). Phytoremediation of contaminated soil and water. CRC Press LLC. p. 235-250. ISBN: 1566704502.

Mahar, A., Wang, P., Li, R., Zhang, Z. 2015. Immobilization of lead and cadmium in contaminated soil using amendments: a review. Pedosphere. 25 (4). 555-568.

Malík, N., Biswas, A. K. 2012. Role of higher plants in remediation of metal contaminated sites. Scientific Reviews & Chemical Communications. 2 (2). 141-146.

Mayfield, J. L., Ozanne, L., Mitchell, C. C., Simonne, E. H., Sibley, J. L. 2004. Laboratory and greenhouse evaluation of quicklime sources for suitability as agricultural liming materials. Communications in Soil Science and Plant Analysis. 35 (7-8). 1167-1183.

McGowen, S. L., Basta, N. T., Brown, G. O. 2001. Use of diammonium phosphate to reduce heavy metal solubility and transport in smelter-contaminated soil. Journal of Environmental Quality. 30 (2). 493-500.

Meers, E., Du Laing, G., Unamuno, V., Ruttens, A., Vangronsveld, J., Tack, F. M. G., Verloo, M. G. 2007. Comparison of cadmium extractability from soils by commonly used single extraction protocols. Geoderma. 141 (3-4). 247-259.

Memon, A. R., Schröder, P. 2009. Implications of metal accumulation mechanisms to phytoremediation. Environmental Science and Pollution Research. 16 (2). 162-175.

Mignardi, S., Corami, A., Ferrini, V. 2012. Evaluation of the effectiveness of phosphate treatment for the remediation of mine waste soil contaminated with Cd, Cu, Pb, and Zn. Chemosphere. 86 (4). 354-360.

Mikulka, J., Kneifelová-Korčáková, M. 2006. Vliv ošetřování luk a pastvin na výskyt *Rumex* sp. In: Ferienčiková, D. (eds.). Medzinárodná vedecka konferencia pri príležitosti 70. výročia krmivinárskeho výskumu na Slovensku. Trávne porasty – súčasť horského pol´nohospodárstva a krajiny. 27. 9. – 28. 9. 2006. Bánská Bystrica. Slovensko. s. 1-6.

Miretzky, P., Fernandez-Cirelli, A. 2008. Phosphates for Pb immobilization in soils: a review. Environmental Chemistry Letters. 6 (3). 121-133.

Miretzky, P., Fernandez-Cirelli, A. 2010. Remediation of arsenic-contaminated soils by iron amendments: a review. Critical Reviews in Environmental Science and Technology. 40 (2). 93-115.

Miyagi, A., Takahashi, H., Takahara, K., Hirabayashi, T., Nishimura, Y., Tezuka, T., Kawai-Yamada, M., Uchimiya, H. 2010. Principal component and hierarchical clustering analysis of metabolites in destructive weeds; polygonaceous plants. Metabolomics. 6 (1). 146-155.

Miyagi, A., Uchimiya, M., Kawai-Yamada, M., Uchimiya, H. 2013. Impact of aluminium stress on oxalate and other metabolites in *Rumex obtusifolius*. Weed Research. 53 (1). 30-41.

Moradi, N., Sadaghiani, M. H. R., Sepehr, E., Mandoulakani, B. A. 2012. Effect of low-molecular-weight organic acids on phosphorus sorption characteristics in some calcareous soils. Turkish Journal of Agriculture and Forestry. 36 (4). 459-468.

Moreno-Jiménez, E., Gamarra, R., Carpena-Ruiz, R. O., Millán, R., Peñalosa, J. M., Esteban, E. 2006. Mercury bioaccumulation and phytotoxicity in two wild plant species of Almaden area. Chemosphere. 63 (11). 1969-1973.

Mrnka, L., Doubková, P., Habart, J., Sudová, R., Tlustoš, P., Vohník, M., Vosátka, M. 2011. Pěstování vrb a topolů formou výmladkových plantáží na půdách kontaminovaných rizikovými prvky: teoretické podklady a praktický návod pro pěstitele v ČR. Botanický ústav AVČR. Průhonice. 98 s. ISBN: 9788086188355.

Muhammad, S., Shah, M. T., Khan, S., Saddique, U., Gul, N., Khan M. U., Malik, R. N., Farooq, M., Naz, A. 2013. Wild plant assessment for heavy metal phytoremediation potential along the mafic and ultramafic terrain in northern Pakistan. BioMed Research International. 2013. 1-9. doi.org/10.1155/2013/194765.

Mühlbachová, G., Tlustoš, P. 2006. Effect of liming on the microbial biomass and its activities in soils long-term contaminated by toxic elements. Plant, Soil and Environment. 52 (8). 345-352.

Munksgaard, N. C., Lottermoser, B. G. 2011. Fertilizer amendment of mining-impacted soils from Broken Hill, Australia: fixation or release of contaminants? Water Air and Soil Pollution. 215 (1-4). 373-397.

Munksgaard, N. C., Lottermoser, B. G. 2013. Phosphate amendment of metalliferous tailings, Cannington Ag-Pb-Zn mine, Australia: implications for the capping of tailings storage facilities. Environmental Earth Sciences. 68 (1). 33-44.

Nagajyoti, P. C., Lee, K. D., Sreekanth, T. V. M. 2010. Heavy metals, occurrence and toxicity for plants: a review. Environmental Chemistry Letters. 8 (3). 199-216.

Němeček, J., Podlešáková, E., Vácha, R. 2002. Transfer of trace elements with low soil mobility into plants. Rostlinná Výroba. 48 (2). 45-50.

Němeček, J., Vácha, R., Podlešáková, E. 2010. Hodnocení kontaminace půd v ČR. VÚMOP. Praha. 148 s. ISBN: 9788086561024.

Neugschwandtner, R. W., Tlustoš, P., Komárek, M., Száková, J. 2009. Nutrient mobilization and nutrient contents of *Zea mays* in response to EDTA additions to heavy-metal-contaminated agricultural soil. Journal of Plant Nutrition and Soil Science. 172 (4). 520-527.

Neugschwandtner, R. W., Tlustoš, P., Komárek, M., Száková, J., Jakoubková, L. 2012. Chemically enhanced phytoextraction of risk elements from a contaminated agricultural soil using *Zea Mays* and *Triticum aestivum*: performance and metal mobilization over a three year period. International Journal of Phytoremediation. 14 (8). 754-771.

Otero, X. L., Ferreira, T. O., Huerta-Díaz, M. A., Partiti, C. S. M., Souza, V., Vidal-Torrado, P., Macías, F. 2009. Geochemistry of iron and manganese in soils and sediments of a mangrove system, Island of Pai Matos (Cananeia – SP, Brazil). Geoderma. 148 (3-4). 318-335.

Park, J. H., Bolan, N., Megharaj, M., Naidu, R. 2011. Comparative value of phosphate sources on the immobilization of lead, and leaching of lead and phosphorus in lead contaminated soils. Science of the Total Environment. 409 (4). 853-860.

Park, J. H., Bolan, N., Megharaj, M., Naidu, R. 2012. Relative value of phosphate compounds in reducing the bioavailability and toxicity of lead in contaminated soils. Water Air and Soil Pollution. 223 (2). 599-608.

Pellet, D. M., Papernik, L. A., Kochian, L. V. 1996. Multiple aluminum-resistance mechanisms in wheat: roles of root apical phosphate and malate exudation. Plant Physiology. 112 (2). 591-597.

Pinto, A. P., Simoes, I., Mota, A. M. 2008. Cadmium impact on root exudates of sorghum and maize plants: a speciation study. Journal of Plant Nutrition. 31 (10). 1746-1755.

Podlešáková, E., Němeček, J., Vácha, R. 2001a. Mobility and bioavailability of trace elements in soils. In: Iskandar, I. K., Kirkham, M. B. (eds.). Trace elements in soil: bioavailability, flux, and transfer. CRC Press LLC. Boca Raton. p. 21-42. ISBN: 156670507X.

Podlešáková, E., Němeček, J., Vácha, R. 2001b. The transfer of less hazardous trace elements with a high mobility from soils into plants. Rostlinná Výroba. 47 (10). 433-439.

Pollard, A. J., Powell, K. D., Harper, F. A., Smith, J. A. C. 2002. The genetic basis of metal hyperaccumulation in plants. Critical Reviews in Plant Sciences. 21 (6). 539-566.

Prasad, M. N. V. 2003. Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russian Journal of Plant Physiology. 50 (5). 686-700.

Probst, A., Hernandez, L., Probst, J. L. 2003. Heavy metals partioning in three French forest soils by sequential extraction procedure. Journal de Physique IV – Proceedings. 107 (2). 1103-1106. doi:10.1051/jp4:20030493.

Pugh, R. E., Dick, D. G., Fredeen, A. L. 2002. Heavy metal (Pb, Zn, Cd, Fe, and Cu) contents of plant foliage near the anvil range lead/zinc mine, Faro, Yukon Territory. Ecotoxicology and Environmental Safety 52 (3). 273-279.

Pulford, I. D., Watson, C. 2003. Phytoremediation of heavy metal-contaminated land by trees – a review. Environment International. 29 (4). 529-540.

Puschenreiter, M., Horak, O., Friesl, W., Hartl, W. 2005. Low-cost agricultural measures to reduce heavy metal transfer into food chain – a review. Plant, Soil and Environment. 51 (1). 1-11.

Qiu, Q., Wang, Y., Yang, Z., Yuan, J. 2011. Effects of phosphorus supplied in soil on subcellular distribution and chemical forms of cadmium in two Chinese flowering cabbage (*Brassica parachinensis* L.) cultivars differing in cadmium accumulation. Food and Chemical Toxicology. 49 (9). 2260-2267.

Rachou, J., Sauvé, S. 2008. Evaluation of affinity constants of Cu, Cd, Ca and H for active soil surfaces for a solid phase-controlled soil ligand model. Environmental Chemistry. 5 (2). 150-160.

Ramadan, M. A. E., Al-Ashkar, E. A. 2007. The effect of different fertilizers on the heavy metals in soil and tomato plant. Australian Journal of Basic and Applied Sciences. 1 (3). 300-306.

Rao, C. R. M., Sahuquillo, A., Lopez Sanches, J. F. 2008. A review of the different methods applied in environmental geochemistry for single and sequential extraction of trace elements in soils and related materials. Water Air and Soil Pollution. 189 (1-4). 291-333.

Rascio, N., Navari-Izzo, F. 2011. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? Plant Science. 180. 169-181.

Rauser, W. E. 1999. Structure and function of metal chelators produced by plants – the case for organic acids, amino acids, phytin and metallothioneins. Cell Biochemistry and Biophysics. 31 (1). 19-48.

Revathi, S., Venugopal, S. 2013. Physiological and biochemical mechanisms of heavy metal tolerance. International Journal of Environmental Sciences. 3 (5). 1339-1354. doi:10.6088/ijes.2013030500004.

Ridley, A. M., Slattery, W. J., Helyar, K. R., Cowling, A. 1990. The importance of the carbon cycle to acidification of a grazed annual pasture. Australian Journal of Experimental Agriculture. 30 (4). 529-537.

Rieuwerts, J. S. 2007. The mobility and bioavailability of trace metals in tropical soils: a review. Chemical Speciation and Bioavailability. 19 (2). 75-85.

Rosselli, W., Keller, C., Boschi, K. 2003. Phytoextraction capacity of trees growing on a metal contaminated soil. Plant and Soil. 256 (2). 265-272.

Rotter, P., Šrámek, V., Vácha, R., Borůvka, L., Fadrhonsová, V., Sáňka, M., Drábek, O., Vortelová, L. 2013. Rizikové prvky v lesních půdách: review. Zprávy lesnického výzkumu. 58 (1). 17-27.

Sahuquillo, A., Rigol, A., Rauret, G. 2003. Overview of the use of leaching/extraction tests for risk assessment of trace metals in contaminated soils and sediments. Trends in Analytical Chemistry. 22 (3). 152-159.

Salt, D. E., Blaylock, M., Kumar, N. P. B. A., Dushenkov, V., Ensley, B. D., Chet, I., Raskin, I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology. 13 (5). 468-474.

Seregin, I. V., Kozhevnikova, A. D. 2008. Roles of root and shoot tissues in transport and accumulation of cadmium, lead, nickel, and strontium. Russian Journal of Plant Physiology. 55 (1). 1-22.

Sheoran, V., Sheoran, A. S., Poonia, P. 2011. Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. Critical Reviews in Environmental Science and Technology. 41 (2). 168-214.

Schöttelndreier, M., Norddahl, M. M., Ström, L., Falkengren-Grerup, U. 2001. Organic acid exudation by wild herbs in response to elevated Al concentrations. Annals of Botany. 87 (6). 769-775.

Singh, O. V., Labana, S., Pandey, G., Budhiraja, R., Jain, R. K. 2003. Phytoremediation: an overview of metallic ion decontamination from soil. Applied Microbiology and Biotechnology. 61 (5-6). 405-412.

Singh, D., Chauhan, S. K. 2011. Organic acids of crop plants in aluminium detoxification. Current Science. 100 (10). 1509-1515.

Smolińska, B., Król, K. 2012. Leaching of mercury during phytoextraction assisted by EDTA, KI and citric acid. Journal of Chemical Technology and Biotechnology. 87 (9). 1360-1365.

Snars, K., Gilkes, R. J. 2009. Evaluation of bauxite residues (red muds) of different origins for environmental applications. Applied Clay Science. 46 (1). 13-20.

Sneddon, I. R., Orueetxebarria, M., Hodson, M. E., Schofield, P. F., Valsami-Jones, E. 2006. Use of bone meal amendments to immobilise Pb, Zn and Cd in soil: a leaching column study. Environmental Pollution. 144 (3). 816-825.

Sneddon, I. R., Orueetxebarria, M., Hodson, M. E., Schofield, P. F., Valsami-Jones, E. 2008. Field trial using bone meal amendments to remediate mine waste derived soil contaminated with zinc, lead and cadmium. Applied Geochemistry. 23 (8). 2414-2424.

Solanki, R., Dhankhar, R. 2011. Biochemical changes and adaptive strategies of plants under heavy metal stress. Biologia. 66 (2). 195-204.

Spuller, C., Weigand, H., Marb, C. 2007. Trace metal stabilisation in a shooting range soil: mobility and phytotoxicity. Journal of Hazardous Materials. 141 (2). 378-387.

Stilmant, D., Bodson, B., Vrancken, C., Losseau, C. 2010. Impact of cutting frequency on the vigour of *Rumex obtusifolius*. Grass and Forage Science. 65 (2). 147-153.

Strobel, B. W. 2001. Influence of vegetation on low-molecular-weight carboxylic acids in soil solution - a review. Geoderma. 99 (3-4). 169-198.

Summers, R. N., Guise, N. R., Smirk, D. D., Summers, K. J. 1996. Bauxite residue (red mud) improves pasture growth on sandy soils in Western Australia. Australian Journal of Soil Research. 34 (4). 569-581.

Syso, A. I., Kolpashchikov, L. A., Ermolov, Y. V., Cherevko, A. S., Siromlya, T. I. 2014. Elemental chemical composition of soils and plants in Western Taimyr. Contemporary Problems of Ecology. 7 (6). 636-642.

Sytar, O., Kumar, A., Latowski, D., Kuczynska, P., Strzalka, K., Prasad, M. N. V. 2013. Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiologiae Plantarum. 35 (4). 985-999.

Száková, J., Tlustoš, P., Pavlíková, D., Hanč, A., Batysta, M. 2007. Effect of addition of ameliorative materials on the distribution of As, Cd, Pb, and Zn in extractable soil fractions. Chemical Papers. 61 (4). 276-281.

Šťastná, P., Klimeš, L., Klimešová, J. 2010. Biological flora of Central Europe: *Rumex alpinus* L. Perspectives in Plant Ecology, Evolution and Systematics. 12 (1). 67-79.

Tahvanainen, L., Rytkönen, V. M. 1999. Biomass production of *Salix viminalis* in southern Finland and the effect of soil properties and climate conditions on its production and survival. Biomass and Bioenergy. 16 (2). 103-117.

Tang, S., Wilke, B. M., Huang, C. 1999. The uptake of copper by plants dominantly growing on copper mining spoils along the Yangtze River, the People's Republic of China. Plant and Soil. 209 (2). 225-232.

Tessier, A., Campbell, P. G. C., Bisson, M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry. 51 (7). 844-851.

Thawornchaisit, U., Polprasert, C. 2009. Evaluation of phosphate fertilizers for the stabilization of cadmium in highly contaminated soils. Journal of Hazardous Materials. 165 (1-3). 1109-1113.

Tiecher, T., Oliveira, L. B., Rheinheimer, D. S., Quadros, F. L. F., Gatiboni, L. C., Brunetto, G., Kaminski, J. 2014. Phosphorus application and liming effects on forage production, floristic composition and soil chemical properties in the Campos biome, southern Brazil. Grass and Forage Science. 69 (4). 567-579.

Tlustoš, P., Száková, J., Stárková, A., Pavlíková, D. 2005. A comparison of sequential extraction procedures for fractionation of arsenic, cadmium, lead, and zinc in soil. Central European Journal of Chemistry. 3 (4). 830-851.

Tlustoš, P., Pavlíková, D., Száková, J., Fischerová, Z., Balík, J. 2006a. Exploitation of fast growing trees in metal remediation. In: Mackova, M., Dowling, D., Macek, T. (eds.). Phytoremediation and rhizoremediation. Theoretical background. Focus on biotechnology, vol. 9A. Springer, Dordrecht, p. 83-102. ISBN: 101402049528.

Tlustoš, P., Száková, J., Hrubý, J., Hartman, I., Najmanová, J., Nedělník, J., Pavlíková, D., Batysta, M. 2006b. Removal of As, Cd, Pb, and Zn from contaminated soil by high biomass producing plants. Plant, Soil and Environment. 52 (9). 413-423.

Tlustoš, P., Száková, J., Kořínek, K., Pavlíková, D., Hanč, A., Balík, J. 2006c. The effect of liming of cadmium, lead, and zinc uptake reduction by spring wheat grown in contaminated soil. Plant, Soil and Environment. 52 (1). 16-24.

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.

Tolrà, R. P., Poschenrieder, C., Luppi, B., Barceló, J. 2005. Aluminium-induced changes in the profiles of both organic acids and phenolic substances underlie Al tolerance in *Rumex acetosa* L. Environmental and Experimental Botany. 54 (3). 231-238.

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 (4). 173-179.

Tuazon-Nartea, J., Savage, G. 2013. Investigation of oxalate levels in sorrel plant parts and sorrel-based products. Food and Nutrition Sciences. 4 (8). 838-843. doi: 10.4236/fns.2013.48109.

Unterbrunner, R., Puschenreiter, M., Sommer, P., Wieshammer, G., Tlustoš, P., Zupan, M., Wenzel, W. W. 2007. Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environmental Pollution. 148 (1). 107-114.

Vácha, R., Němeček, J., Podlešáková, E. 2002a. Geochemical and anthropogenic soil loads by potentially risky elements. Rostlinná Výroba. 48 (10). 441-447.

Vácha, R., Podlešáková, E., Němeček, J., Poláček, O. 2002b. Immobilisation of As, Cd, Pb and Zn in agricultural soils by the use of organic and inorganic additives. Rostlinná Výroba. 48 (8). 335-342.

Vácha, R., Sáňka, M., Sáňka, O., Skála, J., Čechmánková, J. 2013. The fluvisol and sediment trace element contamination level as related to their geogenic and anthropogenic source. Plant, Soil and Environment. 59 (3). 136-142.

Vamerali, T., Bandiera, M., Coletto, L., Zanetti, F., Dickinson, N. M., Mosca, G. 2009. Phytoremediation trials on metal- and arsenic-contaminated pyrite wastes (Torviscosa, Italy). Environmental Pollution. 157 (3). 887-894.

Vamerali, T., Bandiera, M., Mosca, G. 2010. Field crops for phytoremediation of metal-contaminated land. A review. Environmental Chemistry Letters. 8 (1). 1-17.

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 (7). 316-321.

Vaněk, V., Balík, J., Černý, J., Pavlík, M., Pavlíková, D., Tlustoš, P., Valtera, J. 2012. Výživa zahradních rostlin. Academia. Praha. 570 s. ISBN: 9788020021472.

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., van der Lelie, D., Mench, M. 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environmental Science and Pollution Research. 16 (7). 765-794.

Vardavas, C. I., Majchrzak, D., Wagner, K. - H., Elmadfa, I., Kafatos, A. 2006. The antioxidant and phylloquinone content of wildly grown greens in Crete. Food Chemistry. 99 (4). 813-821.

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. G., Pigna, M. 2010. Mobility and bioavailability of heavy metals and metalloids in soil environments. Journal of Soil Science and Plant Nutrition. 10 (3). 268-292.

Vysloužilová, M., Tlustoš, P., Száková, J., Pavlíková, D. 2003a. 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.

Vysloužilová, M., Tlustoš, P., Száková, J. 2003b. Cadmium and zinc phytoextraction potential of seven clones of *Salix* spp. planted on heavy metal contaminated soils. Plant, Soil and Environment. 49 (12). 542-547.

Vysloužilová, M., Puschenreiter, M., Wieshammer, G., Wenzel, W. W. 2006. Rhizosphere characteristics, heavy metal accumulation and growth performance of two willow (*Salix* x *rubens*) clones. Plant, Soil and Environment. 52 (8). 353-361.

Wang, Q. R., Cui, Y. S., Liu, X. M., Dong, Y. T., Christie, P. 2003. Soil contamination and plant uptake of heavy metals at polluted sites in China. Journal of Environmental Science and Health. Part A. 38 (5). 823-838.

Wang, B., Xie, Z., Chen, J., Jiang, J., Su, Q. 2008. Effects of field application of phosphate fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (*Brassica chinensis* L.) in a mining tailing contaminated soil. Journal of Environmental Sciences. 20 (9). 1109-1117.

Weger, J., Bubeník, J. 2011. Hodnocení výnosu a růstu domácích vrb po 14 letech výmladkového pěstování. Acta Pruhoniciana. 97. 39-46.

Wenzel, W. W., Bunkowski, M., Puschenreiter, M., Horak, O. 2003. Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. Environmental Pollution. 123 (1). 131-138.

White, P. J., Broadley, M. R. 2003. Calcium in plants. Annals of Botany. 92 (4). 487-511.

White, P. J., Veneklaas, E. J. 2012. Nature and nurture: the importance of seed phosphorus content. Plant and Soil. 357 (1-2). 1-8.

Williams, L. E., Pittman, J. K., Hall, J. L. 2000. Emerging mechanisms for heavy metal transport in plants. Biochimica et Biophysica Acta. 1465 (1-2). 104-126.

Wilson, S. C., Lockwood, P. V., Ashley, P. M., Tighe, M. 2010. The chemistry and behaviour of antimony in the soil environment with comparisons to arsenic: a critical review. Environmental Pollution. 158 (5). 1169-1181.

Wuana, R. A., Okieimen, F. E. 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. International Scholarly Research Notices. 2011. 1-20. doi:10.5402/2011/402647.

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 (1-3). 929-937.

Yan, Y., Zhou, Y. Q., Liang, C. H. 2015. Evaluation of phosphate fertilizers for the immobilization of Cd in contaminated soils. PLoS ONE. 10 (4). 1-9. doi: 10.1371/journal.pone.0124022.

Yang, X., Feng, Y., He, Z., Stoffella, P. J. 2005. Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. Journal of Trace Elements in Medicine and Biology. 18 (4). 339-353.

Yobouet, Y. A., Adouby, K., Trokourey, A., Yao, B. 2010. Cadmium, copper, lead and zinc speciation in contaminated soils. International Journal of Engineering Science and Technology. 2 (5). 802-812.

Yoon, J., Cao, X., Zhou, Q., Ma, L. Q. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Science of the Total Environment. 368 (2-3). 456-464.

Zaller, J. G. 2004. Ecology and non-chemical control of *Rumex crispus* and *R. obtusifolius* (*Polygonaceae*): a review. Weed Research. 44 (6). 414-432.

Zhang, P., Ryan, J. A. 1998. Formation of pyromorphite in anglesite-hydroxyapatite suspensions under varying pH conditions. Environmental Science & Technology. 32 (21). 3318-3324.

Zhang, P., Ryan, J. A. 1999. Transformation of Pb(II) from cerrusite to chloropyromorphite in the presence of hydroxyapatite under varying conditions of pH. Environmental Science & Technology. 33 (4). 625-630.

Zhang, X., Xia, H., Li, Z., Zhuang, P., Gao, B. 2010. Potential of four forage grasses in remediation of Cd and Zn contaminated soils. Bioresource Technology. 101 (6). 2063-2066.

Zhao, H. Y., Lin, L. J., Yan, Q. L., Yang, Y. X., Zhu, X. M., Shao, J. R. 2011. Effects of EDTA and DTPA on lead and zinc accumulation of ryegrass. Journal of Environmental Protection. 2011 (2). 932-939. doi:10.4236/jep.2011.27106.

Zheng, S. J., Ma, J. F., Matsumoto, H. 1998. High aluminum resistance in buckwheat – I. Al-induced specific secretion of oxalic acid from root tips. Plant Physiology. 117 (3). 745-751.

Zhuang, P., Yang, Q. W., Wang, H. B., Shu, W. S. 2007. Phytoextraction of heavy metals by eight plant species in the field. Water Air and Soil Pollution. 184 (1-4). 235-242.