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Katedra agroenvironmentální chemie a výživy rostlin

SPECIACE RTUTI V ANTROPOGENNĚ KONTAMINOVANÉ PŮDĚ

Disertační práce

Autor: **Ing. Adéla Šípková**

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

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

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Prohlášení

Prohlašuji, že jsem předloženou disertační práci na téma Speciace rtuti v antropogenně kontaminované půdě vypracovala samostatně, s využitím citovaných literárních pramenů a pod odborným vedením školitelky prof. Jiřiny Szákové, CSc.

v Praze dne: _____ _____

Ing. Adéla Šípková

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OBSAH

1. ÚVOD

Kontaminace životního prostředí je široce studovanou problematikou z důvodu přímého vlivu na kvalitu života a možného ovlivnění přírodních biogeochemických cyklů. Zdroje znečištění bývají většinou spojeny se širokým spektrem lidských činností (intenzivní zemědělství, rostoucí objem dopravy a průmyslovou aktivitou). Mimo antropogenních zdrojů existují také zdroje geogenní, a to například zvětrávání matečních hornin. Znečištění prostředí rizikovými prvky způsobuje omezenou využitelnost ostatních přírodních zdrojů z důvodu jejich toxicity, kdy kromě znečištění různých přírodních sfér mohou vstupovat také do potravního řetězce. Významným představitelem rizikových prvků je rtuť.

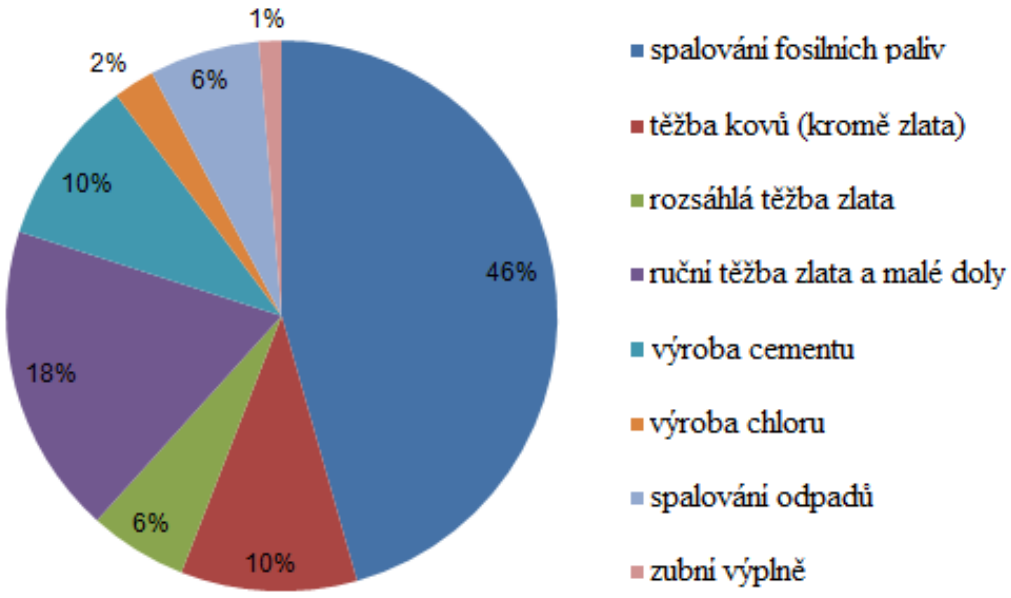
Rtuť a její sloučeniny patří mezi látky s vysokou potenciální toxicitou. Vyskytuje se ve velkém množství fyzikálních a chemických forem, které vykazují rozdílné vlastnosti a mají různě významný vliv na životní prostředí. Tyto sloučeniny se posuzují podle toxicity, která je závislá na mobilitě a bioakumulaci, se zvyšující se mobilitou roste i jejich toxicita. Nejtoxičtější jsou organokovové sloučeniny, zvláště methylrtuť, a rozpustné anorganické soli rtuti. V zemské kůře se rtuť vyskytuje v horninách, sedimentech a půdách. V nekontaminované půdě obvykle nepřesahuje koncentrace Hg 200 $\mu g.kg^{-1}$ (Adriano, 2001), pokud je koncentrace vyšší, hledá se zdroj znečištění. Tím antropogenním bývá většinou těžební průmysl a hutnictví, skládky a spalování odpadů (Lasat, 2002).

Zjištění celkového obsahu Hg v půdě může posloužit jako základ posouzení rizik, která může kontaminace způsobit. Důležitější je ovšem stanovení jednotlivých sloučenin tohoto prvku, na nichž je závislý transport, akumulace a biodostupnost Hg. To do jisté míry souvisí také s typem a složením půdy. Proto byly v této práci popsány jednotlivé vzorky půd, zvláště pak obsah a složení organické hmoty a byla řešena závislost transformace a bioakumulace rtuti právě na těchto charakteristikách. Další experimenty byly zaměřeny na popsání vazeb rtuti na jednotlivé složky půdy.

2. LITERÁRNÍ PŘEHLED

2.1 Zdroje rtuti a její koloběh v prostředí

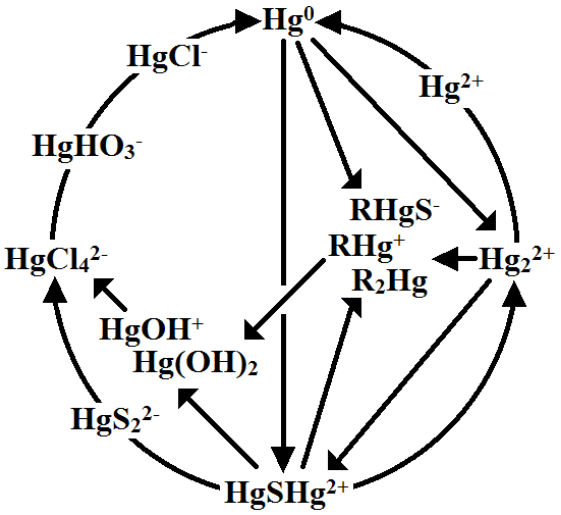
Přírodními zdroji rtuti jsou tektonická činnost, zvláště sopečné plyny, a vypařování z oceánů (Fthenakis et al., 1995). Těmi antropogenními jsou spalování fosilních paliv, těžební průmysl a zpracování rud, výroba chloru, spalování komunálního i lékařského odpadu a úprava čistírenských kalů. Poměrně významné zastoupení měly dříve také sloučeniny rtuti používané v zemědělství, jako jsou pesticidy, konzervační prostředky a prostředky používané k moření osiva (Pitter, 1999). Tyto látky jsou většinou založeny na organokovových sloučeninách Hg a z důvodu vysoké toxicity je jejich využívání již ve větší míře zakázáno. Zastoupení jednotlivých antropogenních zdrojů znečištění v globálním měřítku je uvedeno na Obr. 1.



Obr. 1 Hlavní zdroje znečištění rtutí (AMAP/UNEP, 2008).

Rtuť se jako polutant vyskytuje ve všech složkách životního prostředí, kde může být transformována, akumulována a snadno se dostává do potravních řetězců. Cyklus rtuti zahrnuje uvolňování elementární Hg a těkavých sloučenin do atmosféry, její transport a transformace. Sloučeniny rtuti jsou z atmosféry uvolňovány s prachovými částicemi, sněhem a deštěm (EPA, 2006) a dostávají se zpět do půd a povrchových vod. Zde jsou sorbovány a mohou být následně adsorbovány a bioakumulovány živými složkami přírody

a opět přeměňovány na jiné formy. Cyklus je neustále opakován a jen malé množství Hg je navázáno v nerozpustných sloučeninách. Za silné vazby je zodpovědná zvláště síra v thiolových funkčních skupinách a rozpuštěný uhlík v organické hmotě (Houserová et al., 2006). V půdě může být rtuť oxidována, redukována, hydratována, případně biologicky přeměňována na organické sloučeniny (Kabata-Pendias a Pendias, 2001). Tyto transformace jsou znázorněny na Obr. 2.



Obr. 2 Přeměny mezi jednotlivými speciemi Hg v půdě (Kabata-Pendias a Pendias, 2001).
R: CH_3^-, $CH_3CH_2^-$, $C_6H_5^-$.

Lidskou činností se v horninách a půdách stále snižuje množství stabilních a ve vodě nerozpustných forem Hg (rumělka) a zvyšuje se podíl rtuti, která se účastní koloběhu, který zahrnuje atmosféru, povrchové vody, rostliny a zvířata, a při kterém se mění její chemické i fyzikální vlastnosti. Rtuť může být uvolňována do vzduchu, zůstávat v atmosféře a šířit se i na velké vzdálenosti nebo může být uložena v místě znečištění rozpuštěná ve vodě případně navázaná v půdě a rostlinách. Předpokládá se, že v dnešní době je množství mobilní rtuti až pětkrát vyšší než před průmyslovou revolucí (Atkeson a Axelrad, 2003).

2.2 Toxicita rtuti a rizika vstupu Hg do potravních řetězců

V potravních řetězcích se hromadí hlavně organická rtuť, zatímco anorganická do nich vstupuje pouze omezeně. Vstup sloučenin Hg do potravních řetězců je významný zejména ve vodním ekosystému, kdy nejvyšší obsahy rtuti jsou nacházeny v tělech ryb (Pitter, 1999; Balarama Krishna et al., 2010). U vodních rostlin je negativní vliv pozorovatelný již při

koncentraci 1 $mg.l^{-1}$ anorganické rtuti, u organických sloučenin se jedná o koncentraci mnohem nižší. Suchozemské rostliny jsou ve většině případů vůči škodlivým účinkům rtuti odolné, ačkoliv se může vyskytovat ve všech jejich částech. Nejvyšší koncentrace se vyskytují v kořenech, skrz které je rtuť přijímána. Na rozdíl od vyšších rostlin pochází u mechů rtuť zvláště z atmosférické depozice. Hlavními projevy negativních účinků Hg jsou snížení syntézy chlorofylu, dýchání a příjmu vody, což vede k redukci fotosyntézy (Boening, 2000). Rtuť se může ukládat spíše v houbách, vyšší rostliny Hg příliš neakumulují.

Ve vodním prostředí je toxicita rtuti ovlivněna teplotou, množstvím rozpuštěných solí a rozpuštěného kyslíku a rychlostí proudění. I zde ovšem platí, že pro vodní organismy a ptáky jsou organické sloučeniny Hg více toxické než formy anorganické. Bezobratlí živočichové na rtuť reagují velice rozdílně, obecně ovšem platí, že nejvíce citliví jsou v larválním stavu. Množství Hg, které ryby přijmou, je kromě již zmíněných parametrů ovlivněno také pH vody. Obsahy rtuti v tělech ryb jsou při nižším pH vyšší (Wren a MacCrimmon, 1983). Činností bakterií probíhá v žábrách nebo ve střevech ryb methylace Hg a dochází u nich pak k fyziologickým, reprodukčním a biochemickým odchylkám, kdy se navenek nejdříve objevuje úbytek hmotnosti. U ptáků se rtuť akumuluje zvláště v játrech a ledvinách a otrava se projevuje snížením příjmu potrav a následně špatným růstem, případně zhoršením kardiovaskulární funkce, snížení imunitní reakce a změny v chování. U druhů, které se neživí rybami, jsou nalezené úrovně rtuti podstatně nižší (WHO, 1989; Boening, 2000). Methylované sloučeniny se akumulují ve svalové tkáni, proto mohou být u starších jedinců nebo ve vyšších stupních potravního řetězce nalezena také vyšší množství Hg (EPA, 2006).

Do těla člověka může rtuť vstupovat dýchacími cestami, orálně nebo přes pokožku, míra toxických účinků pak závisí na formě, ve které se vyskytuje. Co se týče zvýšeného příjmu rtuti s potravou, v nejvyšší míře k němu přispívá konzumace mořských plodů, korýšů a ryb (Driscoll et al., 2013). V Číně v současné době probíhají studie zkoumající akumulaci organických sloučenin rtuti v rýži. Ukazuje se, že semena rýže mají vysokou schopnost hromadit methylované formy Hg a rtuť se tak může dostávat do potravního řetězce lidí (Meng et al., 2011).

Při požití kovové rtuti se do těla vstřebá přes žaludek a střeva jen velice malé množství, a to v řádech setin procenta. Většina je z těla vyloučena s močí a stolicí. Naproti tomu při vdechnutí par rtuti vstupuje až 80 % do krevního řečiště a do plic a šíří se do dalších částí těla, včetně mozku a ledvin, kde se může hromadit. U anorganických sloučenin je ve

většině případů požití absorbováno méně než 10 %, přes kůži se do těla většinou nedostávají. Ovšem až z 95 % jsou absorbovány organické formy Hg, vstupují do krevního řečiště a přechází do většiny tkání, včetně mozku. Snadno se do těla dostávají přes dýchací soustavu, neboť k jejich výparu dochází již při pokojové teplotě, a také přes kůži je vstup do těla jednodušší než v případě anorganických forem (ATSDR, 1999).

Páry kovové rtuti nebo organických forem ovlivňují zvláště centrální nervový systém a může docházet ke změnám chování, třesu, poruše vidění, hluchotě, problémy s koordinací pohybů a s pamětí. Anorganické soli Hg se k mozku nedostávají tak snadno, a proto je jejich toxické působení na tento orgán poměrně malé. Dalším velice ohroženým orgánem jsou ledviny, protože se v nich rtuť akumuluje. Jejich poškození způsobují všechny formy Hg, ovšem pokud je množství nízké, mohou se ledviny opět vyčistit a jejich funkce je zachována. Krátkodobé působení Hg se projevuje poškozením sliznice úst a podrážděním plic, poruchami trávicího ústrojí, zvýšení krevního tlaku a srdeční frekvence, dlouhodobá expozice nízkými dávkami Hg pak vede k poškození nervové soustavy a ledvin. Methylrtuť má navíc také silné teratogenní účinky (ATSDR, 1999). Studie účinků Hg nejen na lidský organismus můžeme nalézt v pracích mnoha autorů (EPA, 2006; Virtanen et al., 2007; Burger, 2009; Nance et al., 2012; Driscoll et al., 2013).

2.3 Rtuť a její sloučeniny v půdě

V půdě můžeme nalézt rtuť v anorganických nebo organických sloučeninách, případně jako elementární, ve třech oxidačních stavech Hg^0, Hg^{+1} a Hg^{+2}. Rozpustné anorganické specie rtuti jsou oproti těm nerozpustným snadněji transportovány přírodními procesy a mohou podléhat methylačním procesům (Miretzky et al., 2005). Právě s alkylovanou rtutí přispívají nejvyšší mírou k potenciální toxicitě Hg v půdě. V organických sloučeninách je rtuť vázaná přímo na uhlík. Jsou více mobilní než anorganické formy, čímž mají i vyšší toxické účinky (Boszke et al., 2003). Koncentrace methylrtuti (CH_3Hg) se většinou pohybuje kolem 1 % celkového obsahu rtuti v půdě (Ullrich et al., 2001). Elementární rtuť se v nekontaminovaných půdách většinou nevyskytuje, v těch kontaminovaných se může její obsah pohybovat až kolem 20 % celkového obsahu Hg (Lechler et al., 1997). Je chemicky více stabilní, a proto méně toxická než například rozpustné anorganické formy (Han et al., 2003).

Jiné dělení specií rtuti může být podle prvku, na který je Hg v půdě navázaná, a z toho vyplývající vlastnosti a síla vazby.

Ve vodě rozpustné formy rtuti. Jedná se rtuť vyskytující se v kapilární vodě v půdách, většinou vázanou na organickou hmotu, ne však přímo na uhlík (Biester a Scholz, 1997). Jsou snadno transportovány a podléhají methylačním procesům (Boszke et al., 2003). Těchto ve vodě rozpustných forem bývá většinou méně než 1 % z celkového obsahu Hg, navíc bývá jejich absolutní koncentrace vyšší než v nekontaminovaných půdách (Boszke et al., 2008).

Formy rtuti rozpustné v kyselinách. Jsou to silně vázané specie Hg, které lze získat extrakcí kyselinami. Jde o biologicky dostupnou anorganickou rtuť navázanou na sulfidy železa, hydroxidy a uhličitany manganu, nebo Hg navázanou na minerály (Lechler et al., 1997). Obsah v kontaminované půdě bývá kolem 1,5 % (Boszke et al., 2008).

Formy rtuti vázané na huminové látky. Jedná se o Hg^{2+} v komplexech s huminovými kyselinami, fulvokyselinami a aminokyselinami. Rtuť není navázána přímo na uhlík, většinou však na síru ve funkčních skupinách thiolů, sulfidů a síranů, případně na kyslík a dusík (Xia et al., 1999). Těchto specií bývá 20 – 30 % z celkového obsahu rtuti (Boszke et al., 2008).

Formy rtuti vázané na sulfidy. Rtuť se na sulfidy váže velice pevně za vzniku nerozpustného HgS a nepodléhá procesu methylace. Sulfid se ovšem může oxidovat na síran a Hg^{2+} je uvolňována a následně methylována (Ullrich et al., 2001; Boszke et al., 2003). Z celkového obsahu rtuti může být takto vázáno až 60 % (Boszke et al., 2008).

S tímto rozdělením souvisí také mobilita jednotlivých forem rtuti. Elementární a dvojmocná rtuť vázaná na huminové látky a organickou matrici se nazývá semimobilní (Han et al. 2003), stejně tak část specií rtuti rozpustných ve vodě (Tabulka 1). Reis et al. (2010) popsali vyšší mobilitu rtuti v průmyslově znečištěných půdách, nemobilní rtuť nalezli například v důlních půdách s vyšším obsahem síry. V půdě s nízkým obsahem organických látek je rtuť více reaktivní a náchylné k methylaci (Skyllberg et al., 2006). Pro transformace rtuti mezi jednotlivými formami jsou určující také zdroje kontaminace a doba expozice. Han et al. (2006) ukázali, že Hg vázaná na síru je hlavní součástí pevné fáze půdy kontaminované HgS, zatímco organicky vázaná rtuť je přítomna v půdě čerstvě kontaminované rozpustnými sloučeninami Hg.

Tabulka 1 Rozdělení nejčastějších specií rtuti a jejich mobilita (Han et al, 2003).

definice frakce rtuti		specie
mobilní a toxická rtuť	organické formy rtuti	MeHgCl
		EtHgCl
	rozpustná anorganická rtuť	$HgCl_2$
		$Hg(OH)_2$
		$Hg(NO_3)_2$
		$HgSO_4$
		HgO
		Hg^{2+} komplexy
neextrahovatelná rtuť	semimobilní rtuť	Hg nebo Hg-M (=amalgám)
		Hg^{2+} komplexy
		Hg_2Cl_2 (menší podíl)
	nemobilní rtuť	Hg_2Cl_2 (hlavní podíl)
		HgS
		HgSe

2.4 Rtuť vázaná v půdní organické hmotě a vliv obsahu S a pH

Z hlediska potenciálního toxického působení rtuti na životní prostředí je důležité popsat a určit míru sorpce Hg v půdě. Tu ovlivňují především fyzikálně-chemické vlastnosti půd a sedimentů jako je minerální složení, velikost pevných částic a pH (Schlüter, 1997; Rodrigues et al., 2006). Z hlediska mobility a biodostupnosti Hg je významné zvláště množství a složení organické hmoty. Ta se skládá z hydrofobních sloučenin s vysokou molekulovou hmotností, které se souhrnně nazývají huminové látky a dělíme je na huminové kyseliny (HA) a fulvokyseliny (FA), a jednodušší hydrofilní sloučeniny (Hy) a hydrofobní neutrální (HON) organická hmota (Stevenson, 1994). Vazba Hg na organické látky pravděpodobně silně přispívá k jejímu hromadění v mělkých horizontech půd bohatých na organickou hmotu (Fujikawa a Fukui, 2001). Nicméně chemická afinita k jejím jednotlivým frakcím je různá (Milne et al., 2003) a význam těchto sloučenin je spojován s vyšší biologickou rozložitelností nízkomolekulárních látek a na druhé straně nízkou mobilitou huminových kyselin. Za komplexaci až 50 % z vyluhovaného množství rtuti jsou většinou zodpovědné hydrofilní sloučeniny (Laborda et al., 2009).

Vliv různých frakcí humusu (fulvokyselin a huminových kyselin) na obsah rtuti v půdě zkoumali Yao et al. (2006). Vztah sloučenin rtuti a huminových látek závisí na komplexotvorné kapacitě a stabilitě vzniklých komplexů. Vysoká komplexotvorná kapacita pro Hg a nízká stabilita komplexů zvyšuje odpar rtuti. To je ovlivněno také sorpční kapacitou rtuti a sorpční sílou minerálů. Přidání fulvokyselin do kontaminovaných půd může zvýšit mobilitu rtuti, zatímco huminové kyseliny s vyšší komplexní stabilitou můžou redukovat její těkání. Silnou interakci Hg s huminovými kyselinami a stabilitu komplexů vysvětlují Chai et al. (2012) vysokým počtem kyslíkových ligandů přítomných v HA. Naproti tomu vyšší komplexotvornou kapacitu u fulvokyselin zajišťuje relativně vysoké zastoupení karboxylových skupin. Zdá se tedy, že FA hrají důležitou roli na počátku procesu stabilizace množství Hg v půdě. Obecně lze ovšem říci, že vyšší množství organické hmoty zvyšuje adsorpční kapacitu půdy, jak ve svých pracích ukázali například Miretzky et al. (2005) nebo Schlüter (1997). V povrchových vrstvách, kde je jí více, je také koncentrace Hg vyšší (Boszke et al., 2008). Nepřímo úměrné na obsahu organické hmoty jsou pak pohyb rtuti v půdě a její desorpce. Větší množství Hg se uvolňuje z půdy chudší na organické složky, která může být nalezena na místech, kde byl přírodní pokryv odstraněn lidskou činností (Miretzky et al., 2005).

Vedle organické hmoty ovlivňuje množství mobilní rtuti také obsah síry. Jako účinné adsorbenty Hg jsou známé sulfidické minerály (Barnet et al., 1997). Vazbu rtuti na sloučeniny síry v organické hmotě blíže popsali Hesterberg et al. (2001) a Remy et al. (2006). Obě tyto práce ukázaly, že rtuť se v půdách váže na sulfidy velmi pevnou vazbou a její množství negativně koreluje právě s obsahem síry. Akerblom et al. (2013) zjistili, že dlouhodobá depozice SO_4^{2-} v rašeliništích zvyšuje jejich kapacitu pro methylaci a množství nasorbované MeHg.

Jak již bylo řečeno, dalším rozhodujícím faktorem pro sílu sorpce rtuti v půdě je pH. Na rozdíl od jiných stopových prvků, se množství mobilizovatelné rtuti snižuje při $pH < 3$ a při $pH > 12$, vzhledem k extrémně vysoké pufrační kapacitě organické hmoty, a to jak v kyselém, tak alkalickém prostředí (Kabata-Pendias a Pendias, 2001). Nicméně, v kyselých půdách se Hg váže zejména na huminové kyseliny (Schwesig et al., 1999; Zhang et al., 2009). Závislost sorpce na pH byla studována například v práci Bernause et al. (2005), kteří zhodnocovali dostupnost organických forem rtuti z kontaminovaných půd za různých podmínek. Stejně tak ovlivňuje chování rtuti v půdě obsah organických kyselin (Jing et al., 2007). V nízkých koncentracích desorpci potlačují, při vyšších (10^{-4} M) se uvolňování Hg

zvyšuje. Nejsnáze dochází k desorpci v prostředí kyseliny citronové o koncentraci > 10^{-3} M, dále kyseliny vinné a kyseliny jablečné, u kyseliny šťavelové je účinek nejnižší.

Vliv těchto faktorů, obsahu organické hmoty a hodnotu pH, na uvolňování rtuti z půdy zkoumali Yang et al. (2007). Ve své práci ukázali, že emise Hg je nepřímo úměrná na obsahu organické hmoty v půdě. Dochází totiž ke snížení její dostupnosti a mobilitě a nejsou tak vhodné podmínky pro redukci rtuti ve vyšších oxidačních stavech a jejímu těkání v elementární formě. Bylo ukázáno, že v kontrolních půdách bez přídavku organické hmoty je uvolňování Hg nejvyšší. Pokud byly srovnávány půdy se stejným množstvím organické hmoty, těkání Hg se snižovalo s nižším pH.

2.5 Vliv mikroorganismů

Je známo, že pro mobilitu a bioakumulaci je důležitá půdní organická hmota, úloha mikroorganismů v hromadění nebo transformaci rtuti však není zatím podrobněji popsána. Methylační proces s následným transportem Hg přes buněčnou membránu představuje jeden z ochranných mechanismů mikroorganismů (Wood, 1984). Již v nízkých koncentracích představuje rtuť pro mikroorganismy vážné riziko. U anorganických sloučenin rtuti mohou být účinky pozorovány již při koncentraci Hg v kultivačním mediu 5 $\mu g.l^{-1}$ (Boening, 2000). U organických sloučenin může být vliv pozorován již při koncentracích desetkrát nižších.

Změny půdní mikroflóry ve třech kontaminovaných půdách s různými obsahy rtuti studovali například Casucci et al. (2003). Snížení mikrobiální aktivity bylo pozorováno při zvýšení koncentrace Hg o 0,5 – 10 mmol na g vysušené zeminy. Mikrobiální aktivita je však závislá také na typu a složení půd. Vliv dlouhodobého působení rtuti na půdní mikroorganismy zkoumali Müeller et al. (2001). Nejvyšší pokles populace bakterií a prvoků byl pozorován v nejvíce kontaminované půdě, v biomase hub tak významné rozdíly nebyly.

Holtze et al. (2006) zkoumali odolnost bakterií *Pseudomonas* ve znečištěných půdách. Rezistence vůči Hg byla pozorována pouze u *P. frederiksbergensis* a *P. migulae*. Navíc přítomnost rtuti způsobila změny v zastoupení jednotlivých druhů tohoto rodu. Sas-Nowosielska et al. (2008) pak zjistili, že studovaná mikroflóra, s výjimkou *Streptomyces*, je v kontrolních půdách ve vyšších obsazích než v půdách kontaminovaných. Je tedy zřejmé, že reakce půdních mikroorganismů na zvýšené hladiny Hg v půdě jsou dle dosavadních poznatků nejednoznačné a pro přesnější popis akumulace a transformace důležité.

Dá se říci, že rtuť v půdě je stabilní prvek, nebezpečím je ovšem fakt, že jednotlivé formy Hg mohou podléhat mnoha transformacím (Comino et al., 2009). Znalost půdních vlastností, vazby rtuti a její mobility by mohla napomoci s výběrem možných postupů použitelných k imobilizaci Hg v kontaminovaných oblastech nebo určit nejvhodnější remediační postup. Použity by mohly být například specifické ionexy, amalgamační filtry (Huttenloch et al., 2003; Bollen et al., 2008), nanočástice pyritu (Xiong et al., 2009) nebo přidání redukované S k vytvoření nerozpustného HgS (Piao a Bishop, 2006). Jako levný, dostupný a poměrně silný sorbent lze použít i organickou hmotu (Zhang et al., 2009). V menší míře jsou k fytoremediacím využívány i rostliny (Patra a Sharma, 2000). Ke zvýšení dostupnosti Hg při fytoextrakčních metodách je pak používán například $Na_2S_2O_3$ (Moreno et al., 2005). Pokles mobility rtuti je spojený se snížením jeho toxických účinků na ostatní složky životního prostředí díky omezení vstupu tohoto prvku do potravních řetězců.

2.6 Stanovení rtuti

2.6.1 Frakcionace a speciace prvků

Speciace je aktivní proces identifikace a kvantifikace forem nebo fází, ve kterých se prvek nachází (Ure, 1991). Pro jejich rozlišení se využívají rozdíly ve vlastnostech chemických i fyzikálních. V kontextu s půdou mohou být specie definovány různými způsoby

- funkčně – popis role a dostupnosti v půdě, například specie dostupné pro rostliny nebo mobilní (Lechler et al., 1997; Bloom et al., 2003; Lin et al., 2010)
- pracovně – pomocí reakčních činidel a postupů, kterými jsou jednotlivé specie identifikovány, izolovány a kvantifikovány (Das et al., 1995; Issaro et al., 2009)
- jako specifické chemické sloučeniny nebo oxidační stavy (dos Santos et al, 2009; Jongwana a Crouch, 2012).

2.6.2 Extrakční metody pro frakcionaci a speciaci jednotlivých forem rtuti

Většina z běžně používaných instrumentálních analytických metod vyžaduje pro vlastní měření přítomnost analytu v roztoku. Pokud studujeme nejrůznější biologické, zemědělské či geologické materiály, musíme analyzovaný prvek nebo jeho sloučeninu převést

do roztoku. Je nutné vybrat takový postup, při kterém je daný analyt převeden kvantitativně, bez případných chemických změn a ve formě, která je vhodná pro použitou měřící techniku. Pro stanovení celkových obsahů prvků ve vzorcích volíme destrukci matrice vzorku a převedení analytů do roztoku, tedy rozklad nebo mineralizaci vzorku. Problematika rozkladů byla jak po teoretické, tak i po praktické stránce mnohokrát souborně zpracována. Podrobný přehled metod rozkladu, počínaje jejich teoretickým základem a konče vybranými praktickými aplikacemi, podávají Krakovská a Kuss (2001) nebo Bock (1979). Všechny tyto metody ale umožňují pouze stanovení celkového obsahu prvků v analyzovaných materiálech. V případě, že máme v daném materiálu stanovit ne celkový obsah prvku, ale jeho sloučeniny, valenční stav nebo způsob vazby na jiné složky, volíme namísto metod rozkladu metody extrakce do vhodného média (Nolan et al. 2003; Hlavay et al. 2004).

Nejméně vázané specie Hg mohou být uvolněny jednoduchou extrakcí deionisovanou vodou (Séguin et al., 2004; Rodrigues et al., 2010) a představují již zmíněnou rtuť v kapilárních vodách. Slabě vázaná rtuť se dále uvolňuje do půdního roztoku. Pro přesnější odhad rostlinám dostupného podílu sloučenin rtuti v půdách lze využít i techniky difuzního gradientu v tenkém filmu (DGT), jak popsali například Cattani et al. (2008). Použití zředěné CH_3COOH jako extrakčního činidla může sloužit k simulaci přibližného složení půdního roztoku, stejně jako například $CaCl_2$ (Menzies et al., 2007; Novozamsky et al., 1993; Quevauviller et al., 1993). Extrakční roztoky na bázi chelatačních činidel jako je ethylendiamintetraoctová kyselina (EDTA) nebo diethylentriaminpentaoctová kyselina (DTPA) mají vyšší efektivitu a díky nim lze získat rtuť i z nerozpustných organických nebo organokovových komplexů (Rao et al., 2008). Specie Hg pevně vázané na železo nebo mangan se nejčastěji získávají extrakcí HCl (Lechler et al., 1997).

Nemobilní frakce rtuti vázané na síru lze stanovit extrakcí za pomoci lučavky královské a mikrovlnného rozkladu (Fernández-Martínez and Rucandio, 2003) nebo použitím roztoku $Na_2S_2O_3$ (Revis et al., 1989; Issaro et al., 2010). Extrakce thiosíranem sodným se ke speciační analýze používá často, neboť během ní nedochází k žádným transformacím Hg.

Podíl rtuti, který není vázaný přímo na silikátovou matrici půdy, se získává pomocí HNO_3 jako extrakčního činidla (Reis et al., 2010). Koncentrace takto extrahované rtuti se navíc dá použít pro odhad množství Hg z antropogenních zdrojů. V některých případech se koncentrovaná kyselina dusičná ve směsi s HCl (Bollen et al., 2008; Teršič et al., 2011) nebo H_2SO_4 (Mailman a Bodaly, 2005) používá pro stanovení celkového obsahu rtuti v půdě. Může být také použita v rámci sekvenčních extrakcí pro získání elementární Hg (Bloom et al., 2003,

Liu et al., 2006). Sekvenční extrakce jsou vhodnými metodami pro speciační analýzu pevných vzorků a velké množství jednotlivých postupů je studováno mnoha autory (Renneberg a Dudas, 2001; Sladek a Gustin, 2003; Han et al., 2006).

2.6.3 Detekce rtuti

K samotné detekci rtuti ve vzorku může posloužit několik instrumentálních metod. Nejčastěji se používá atomová absorpční spektrometrie (AAS), atomová fluorescenční spektrometrie (AFS) a hmotnostní spektrometrie s indukčně vázaným plazmatem (ICP-MS). Díky vysoké těkavosti Hg lze použít metodu studených par v kombinaci s některou z uvedených spektrometrických metod, případně termický rozklad.

Atomová absorpční spektrometrie. Podstatou metody je absorpce vhodného elektromagnetického záření volnými atomy v plynném stavu. Ke stanovení rtuti se využívá měření absorpce záření na rezonanční čáře rtuti 253,7 nm. Rtuť ve vzorku lze stanovit různými technikami, které se navzájem liší citlivostí a způsobem atomizace vzorku. Jsou jimi plamenná AAS, AAS s elektrotermickým atomizátorem a nejčastěji používaná metoda studených par (CV-AAS), která se vyznačuje velmi dobrou citlivostí a vysokou selektivitou. Při této technice se Hg^{2+} v roztoku redukuje chloridem cínatým nebo tetrahydridoboritanem sodným na elementární rtuť. Ta je vedena proudem nosného plynu (argon, dusík) ve formě monoatomové páry přes sušící vrstvu ($CaCl_2$, $Mg(ClO_4)_2$, silikagel) do křemenné absorpční průtokové kyvety (Klouda, 2003; Houserová et al., 2006).

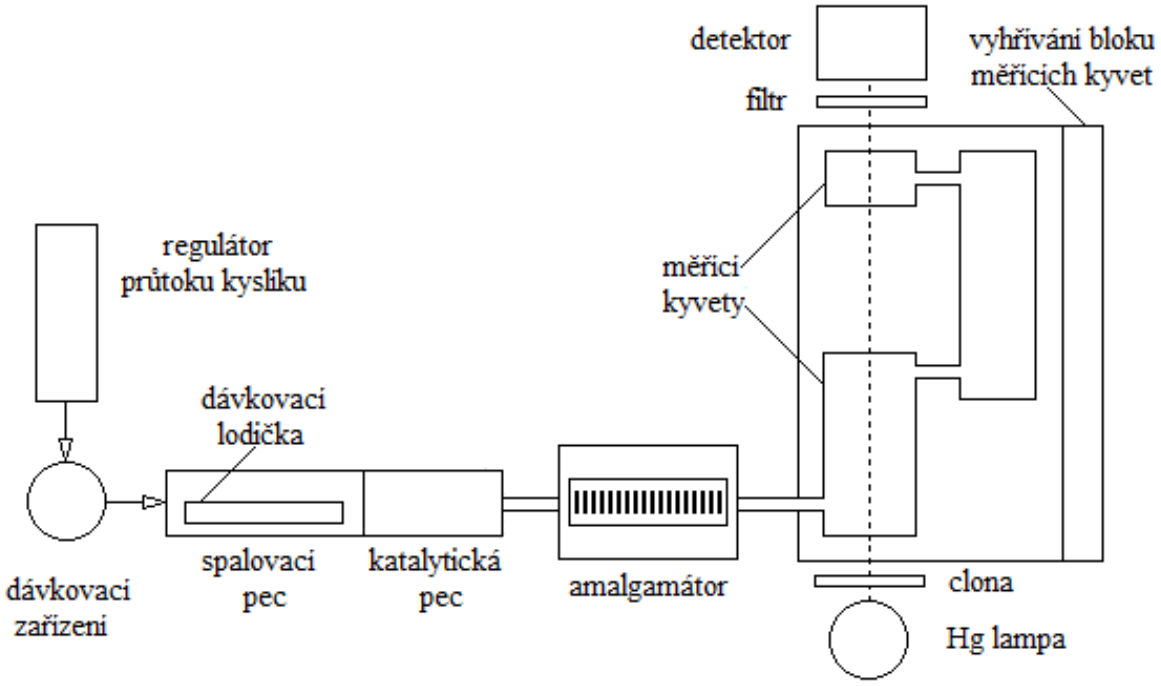
V případě obsahů Hg nižších než 0,1 $mg.kg^{-1}$, což jsou koncentrace běžné v biologických materiálech, je nutno zařadit krok prekoncentrace analytu. Před vlastním měřením hodnoty absorbance v průtokové kyvetě je tedy většinou zařazena fokusace na amalgamátoru. Na tenké vrstvě kovu (zlata nebo stříbra) umístěné na keramickém nosiči (amalgamátoru), který se nachází mezi generátorem rtuťových par a atomizátorem, dojde k významnému zakoncentrování rtuti z velkého objemu vzorku do malého objemu amalgamátoru (Welz a Schubert-Jacobs, 1988; Horvat et al., 1991; Brandvold et al., 1993). Na základě metody CV-AAS doplněné krokem amalgamace byla vyvinuta celá řada komerčních zařízení (Urba et al., 1995; Livardjani et al., 1995). Tyto systémy ale vyžadují před vlastním stanovením převedení vzorku do roztoku a separaci rtuti od matrice vzorku. Tento krok je v případě rtuti extrémně obtížný. Ukazuje se, že pouze kompletní rozklad

organické matrice vzorku (např. ve směsi HNO_3 + H_2SO_4) vede ke správným výsledkům (Clevenger et al., 1997). V případě neúplného rozkladu můžeme zaznamenat při stanovení interference těkavých organických sloučenin (Coles et al., 1985). Stanovení velmi nízkých obsahů rtuti také vyžaduje použití velmi čistých kyselin, přičemž se běžně používají metody rozkladu na mokré cestě za atmosférického či zvýšeného tlaku s konvenčním nebo mikrovlnným ohřevem. Byly popsány i automatizované systémy propojující on-line mikrovlnný rozklad s CV-AAS s dávkováním do proudu (Hanna a McIntosh, 1995; Noh et al., 1998). Velmi vzácně se používají pro rozklad vzorku pro stanovení rtuti i suché metody rozkladu, například v uzavřeném systému v prostředí kyslíku (Bock, 1979; Koops et al., 1984).

AMA-254. Nejjednodušším způsobem stanovení celkového obsahu rtuti v pevných a kapalných vzorcích je použití jednoúčelového atomového absorpčního spektrometru AMA-254. Tento přístroj byl vyvinut na základě předchozího poznání, že kritickým krokem při stanovení stopových koncentrací rtuti je rozklad vzorku, který musí ve většině případů vlastnímu stanovení předcházet. Nedostatečně rozložené organické sloučeniny rtuti, které nejsou redukovatelné chloridem cínatým, ztráty rtuti během tepelného rozkladu, paměťové efekty plastových nádob, které se často používají zejména při rozkladu za zvýšeného tlaku, nebo možnost sekundární kontaminace vzorku během rozkladu jsou častými důvody nesprávných výsledků. Jedná se tedy o metodu, která nevyžaduje chemickou předúpravu vzorku a dá se použít i ve speciační analýze, kdy dávkujeme roztok s požadovanou extrahovanou frakcí Hg. Jedná se o využití techniky generování par kovové rtuti a následné zachycení a nabohacení na zlatém amalgamátoru (Anonym, 2002; Száková et al., 2004). Rtuť je ze vzorku uvolněna termickým rozkladem, kdy je vzorek spálen v proudu kyslíku a páry rtuti jsou zachyceny na amalgamátoru (Houserová et al., 2006). Metoda je to velice citlivá a její další výhodou je, že výsledky stanovení nejsou závislé na matrici vzorku. Detekční limity mohou dosahovat hodnot nižších než 0,0002 $mg.kg^{-1}$.

Schéma analyzátoru je znázorněno na Obr. 3. Vzorek je umístěn na dávkovací lodičku a zaveden do spalovací pece. Zde je vzorek ohřevem vysušen a následně spálen. Ve druhé části spalovací pece, které je vyhřívaná na konstantní teplotu 550°C, procházejí rozkladné produkty přes katalyzátor a je dokončena jejich oxidace. Páry rtuti jsou zachyceny na zlatém amalgamátoru, odkud jsou uvolněny krátkým ohřevem a vedeny do bloku měřících kyvet. Je měřena absorbance záření atomy Hg na vlnové délce 253,7 nm. Jako zdroj záření je použita

rtuťová výbojka, záření prochází interferenčním filtrem a je detekováno pomocí polovodičové UV diody. Celým přístrojem trvale protéká kyslík, jehož průtok je udržován na konstantní hodnotě**.**

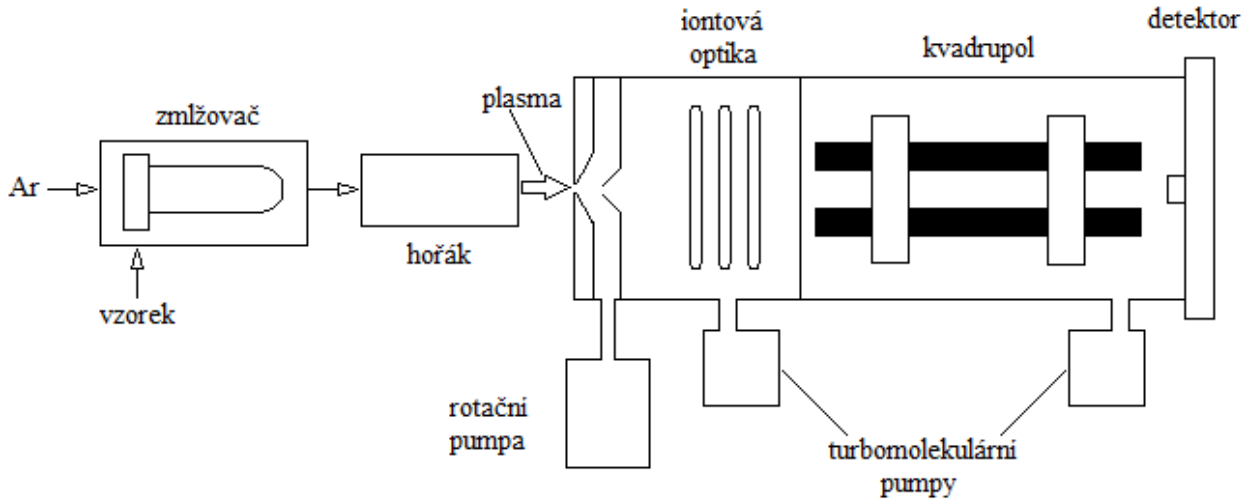


Obr. 3 Schéma analyzátoru AMA-254.

Atomová fluorescenční spektrometrie. Tato metoda sleduje emisi záření plynnými atomy, které byly excitovány absorpcí elektromagnetického záření. Využívá se rovněž rezonanční čára 253,7 nm a zdrojem záření může být bezelektrodová výbojka (EDL) nebo nízkotlaká rtuťová výbojka. Také při AFS se nejčastěji používá metoda generování studených par, kdy jsou přístroje často vybaveny amalgamační prekoncentrační jednotkou pro zvýšení citlivosti metody. Technikou atomové fluorescence se stanovuje elementární rtuť. Je velice důležité vhodně zvolit podmínky měření k dosažení symetrického a vhodného píku. Hlavními parametry, které ovlivňují stanovení, jsou zvolení optimální teploty a rychlosti průtoku plynů. Mezi nevýhody této metody patří zhášení fluorescence a samoabsorpce záření při vysokých koncentracích rtuti (Houserová et al., 2006; Carrasco et al., 2009).

Hmotnostní spektrometrie s indukčně vázaným plazmatem. Jedná se o separační techniku, při které je vzorek převeden na ionizovanou plynnou fázi pomocí vysokoteplotního ICP zdroje a vzniklé ionty jsou separovány podle hodnoty podílu jejich hmotnosti a náboje *m/z*. Je to metoda velice přesná s vysokou selektivitou, nízkými mezemi detekce a velkým lineárním rozsahem.

Nejprve jsou vzorky převedeny do kapalného stavu a dávkovány pomocí zmlžovače do indukčně vázaného plazmatu, kde nastane rozpad molekul, atomizace a ionizace. Ionizace nastává zpravidla nárazem letících elektronů nebo je využita chemická reakce. Spojení mezi plazmatem a vlastním spektrometrem je tvořeno expanzní komorou, která je od okolního prostředí ohraničena dvěma děliči tlaku, tzv. sampling a skimmer konem. Ionty vstupují do vakuového prostoru s elektromagnetickými čočkami. Úlohou iontové optiky je rozostření iontového svazku tak, aby obešel pohlcovač fotonů, který slouží jako ochrana před jejich dopadem na detektor. Následně je paprsek znovu zaostřen a urychlen do kvadrupólového separátoru. Kvadrupól obsahuje čtyři rovnoběžné tyčové elektrody, na které je přiváděna stejnosměrná složka napětí a radiofrekvenční pole. Frekvence oscilací polarity na kvadrupólových tyčích je konstantní, ale mění se amplituda napětí, která umožní průchod iontu v závislosti na jeho náboji a hmotnosti. Ostatní ionty k detektoru neprojdou, jsou buď odstraněny vakuovou pumpou, nebo se vybijí nárazem na tyč kvadrupólu. Kvadrupólem prošlé ionty dopadají na detektor, jejich signál je dále zesilován v elektronovém násobiči a elektronicky zpracován (Klouda, 2003; Mihaljevič et al., 2004). Schéma hmotnostního spektrometru s indukčně vázaným plazmatem je znázorněno na Obr. 4.



Obr. 4 Schéma hmotnostního spektrometru s indukčně vázaným plazmatem.

2.6.4 Instrumentace pro speciaci rtuti - spřažené techniky

Pro speciační analýzu se mohou využívat také spojení separační a následně detekční techniky. Oproti extrakčním postupům poskytují přesnější informace nejen o jednotlivých speciích, ale i o struktuře sloučenin, ve kterých se prvek nachází. Nejčastěji používanou separační metodou je plynová (GC) nebo kapalinová (LC) chromatografie. Vzhledem k vysoké těkavosti rtuti se plynová chromatografie jeví jako vhodnější (Hirner et al., 2000; Dzurko et al., 2009), ovšem spojení s vysoce účinnou kapalinovou chromatografií je v současné době velice hojně využívané (Han et al., 2003; He et al., 2011). Výběr detektoru je založen na požadované citlivosti stanovení a dostatečné selektivitě přístroje. Nejběžnějšími detekčními technikami, využívanými v tandemovém zapojení, jsou již zmíněná atomová absorpční spektrometrie (AAS), atomová fluorescenční spektrometrie (AFS) nebo hmotnostní spektrometrie s indukčně vázaným plazmatem (ICP-MS). Bylo popsáno i použití metody izotopového zřeďování v souvislosti s těmito technikami, eventuálně i s využitím multikolektoru (Foucher a Hintelmann, 2006; Inagaki et al., 2008; Monperrus et al., 2008).

CV-AAS je detektor rychlý a jednoduchý, s nízkými pracovními náklady, nevýhodou je však nízká citlivost (D´Haese et al., 1995; Jagtap et al., 2011). Relativně levná technika s nízkými provozními náklady, velice citlivá pro hydridy a páry rtuti je HPLC-CV-AFS (Chen a Belzile, 2010; Gao a Liu, 2011), ovšem vývoj této metody není ve srovnání s ostatními detekčními technikami tak rychlý. Nejcitlivějším, a i přes vysokou cenu nejpoužívanějším prvkově selektivním detektorem je ICP-MS (Chen et al., 2009; dos Santos et al., 2009; Yin et al., 2010). Jeho nevýhodou je však menší tolerance k vyšším obsahům solí a organických rozpouštědel v mobilní fázi.

3. HYPOTÉZA A CÍLE PRÁCE

Transformace mezi jednotlivými speciemi rtuti se liší v antropogenně znečištěných půdách a půdách nekontaminovaných. Míru těchto transformací lze ovlivnit změnami vlastností půdy, fyzikálně chemickými a biologickými, zvláště pak obsahem a druhy půdních mikroorganismů.

CÍLE PRÁCE

- Vývoj analytických metod stanovení anorganických a organických specií rtuti.
- Sledování pohybu a transformace rtuti v půdním prostředí při různých podmínkách daných vlastnostmi půdy.
- Zpřesnění a kvantifikace sorpce Hg na organickou složku půdy.
- Zhodnocení vlivu obsahu a složení půdní mikroflóry na transport a přeměny jednotlivých specií rtuti.
- Modelování různých půdních vlastností a sledování případných změn v zastoupení jednotlivých specií.

4. METODIKA

V této kapitole jsou uvedeny základní informace k použitým materiálům, postupům jednotlivých experimentů a analytickým metodám. Detailní popisy jsou pak prezentovány v publikovaných pracích.

4.1 Materiál

4.1.1 Půdy

Byly odebrány vzorky antropozemě z okolí bývalé spalovny odpadů nedaleko Hradce Králové, kde se mezi lety 1993 a 2002 spaloval nebezpečný odpad obsahující ropné látky, zemědělské odpady, konzervační látky, odpady z chemických procesů a perkolace, odpady obsahující rozpouštědla, kovy, halogeny, síru, barviva, hnojiva, pesticidy a jiné. Nejvyšší obsahy rtuti nalezené v této oblasti dosáhly až 12 $mg.kg^{-1}$ (Kacálková et al., 2009).

Pro experimenty zaměřené na půdní mikroorganismy byly odebrány vzorky půd u Příbrami, jednalo se o kambizem. Toto území je známo výskytem ložisek nerostných surovin, zvláště Pb-Ag-Zn, jejich těžbou a tavením. Emise z olověných hutí jsou zodpovědné za vysoké koncentrace Pb, Zn a Cd v půdách (Ettler et al., 2007). Pozorovány byly také zvýšené obsahy Hg. Látky na bázi organického chloridu fenyl-rtuťnatého se v České republice dříve používaly k moření osiva, ochraně proti houbovým chorobám, známé pod jménem Agronal. V roce 1990 bylo jeho používání zakázáno a nahrazeno jinými prostředky. Nicméně bývalá výrobna Agronalu může být také zdrojem kontaminace prostředí touto látkou. Taková bývalá výrobna se nachází nedaleko Příbrami a je pravděpodobné, že zvýšené obsahy Hg v půdě v dané lokalitě mohou s tímto případem souviset.

Inkubační experimenty byly prováděny na vzorcích půd s rozdílnými fyzikálně-chemickými vlastnostmi z oblastí, které nebyly znečištěny antropogenními zdroji rtuti. Konkrétně luvizem z Hněvčevse, fluvizem z Píšťan a černozem z okolí České zemědělské univerzity v Praze.

4.1.2 Půdní aditiva

Během inkubačních experimentů byly k půdám přidávány anorganické a organické látky, které ovlivňovaly mobilitu rtuti v jednotlivých vzorcích. Jednalo se o digestát z anaerobní fermentace bioodpadu, konkrétně směsi cukrové drti (50 %), ovoce (42 %) a kukuřice (8 %), dále popílek ze spalování štěpky a síran amonný.

Dále byly ve speciálních fermentorech na pokusné stanici Fakulty agrobiologie, potravinových a přírodních zdrojů ČZU na Červeném Újezdě připraveny tři vermikomposty (Pilař, 2011) s vysokým obsahem organické hmoty, které se lišily skladbou výchozích surovin na bázi bioodpadů. Prvním byl digestát, druhý byl reprezentován směsí sídlištního bioodpadu a štěpky a třetí byl připraven ze zahradního bioodpadu.

4.2 Laboratorní experimenty

4.2.1 Frakcionace organické hmoty

Jednou z oblastí, kterou se tato disertační práce zabývá, je detailnější popis sorpce rtuti na organickou hmotu, a to konkrétně na jednotlivé její frakce. Pro tento účel byla provedena frakcionace organické hmoty podle van Zomerena a Comanse (2007) za použití pryskyřice DAX-8 (SUPELCO Analytical, USA) na huminové kyseliny (HA), fulvokyseliny (FA), hydrofilní sloučeniny (Hy) a hydrofobní neutrální organickou hmotu (HON). Docházelo k sorpci na zmíněnou pryskyřici a pomocí slabých roztoků HCl, KOH a NaOH k následné desorpci. V každém extraktu byly poté měřeny obsahy vybraných prvků pomocí ICP-OES, v případě rtuti pak ICP-MS.

4.2.2 Inkubační pokusy

Pro posouzení intenzity sorpce rtuti na jednotlivé složky půdy byly provedeny inkubační pokusy na nekontaminovaných půdách, luvizem, fluvizem a černozem. K těm byly ve třech dávkách přidávány vermikomposty, přičemž ta nejnižší odpovídala doporučené dávce pro aplikaci kompostu na zemědělskou půdu, další pak dvojnásobná a trojnásobná. Jako kontrolní vzorky byly využity půdy bez přídavku vermikompostu. Jelikož byla u vzorků vermikompostů provedena výše popsaná frakcionace organické hmoty, byl znám i příspěvek

jejích jednotlivých frakcí. Byl posuzován také vliv zdroje rtuti, a proto byly vzorky uměle kontaminovány dvěma různými sloučeninami Hg. V prvním případě to byla anorganická forma, chlorid rtuťnatý, která simulovala průmyslové znečištění Hg, ve druhém pak organický chlorid fenyl-rtuťnatý. Koncentrace přidávané Hg byla 12 $mg.kg^{-1}$. Inkubační pokusy probíhaly při konstantní teplotě 25°C po dobu 56 dní a během pokusu byly vzorky pro následné analýzy odebírány desetkrát.

Další fáze experimentů byla zaměřena na vliv síry na mobilitu Hg. Použity byly dvě nekontaminované půdy, luvizem a černozem jako v předchozích experimentech, které byly uměle kontaminovány $HgCl_2$ na koncentraci rtuti 440 $mg.kg^{-1}$. K takto upraveným půdám byly dodány anorganické a organické látky, digestát z anaerobní fermentace bioodpadu, popílek ze spalování štěpky a síran amonný tak, aby celkové množství S byl ve všech případech stejný. U kontrolních vzorků nebyly tyto přídavky využity. Inkubační pokusy probíhaly za konstantních podmínek při teplotě 28°C po dobu 21 dní a vzorky byly odebrány sedmkrát.

V obou případech bylo hodnoceno množství biodostupné formy rtuti. Proces začínal extrakcí pomocí 0,11 M roztoku kyseliny octové, která probíhala přes noc. Následně byly vzorky centrifugovány rychlostí 3000 ot/min a k samotné detekci obsahu Hg bylo využito ICP-MS.

4.2.3 Sorpční experimenty

Pro podrobnější popis sorpce rtuti v půdě a vermikompostech použitých při inkubačních pokusech byly provedeny ještě sorpční experimenty. Nejprve byly provedeny loužící experimenty, které slouží k popsání kinetiky sorpce Hg na jednotlivé vzorky. Půdy i vermikomposty byly louženy v roztoku Hg s koncentrací 12 $mg.kg^{-1}$ po dobu od 10 minut do 36 hodin. Ve všech případech bylo k 40 ml roztoku Hg přidáno 0,4 g vzorku a mícháno (250 ot/min) při pokojové teplotě po požadovanou dobu. Jako základní elektrolyt byl použit 0,01 M roztok $NaNO_3$. Vzorek byl následně centrifugován rychlostí 3600 ot/min po dobu 15 minut a filtrován (0,45 μm). Obsah rtuti v roztoku byl stanoven pomocí ICP-MS.

Na základě výsledků z loužících pokusů byly zvoleny nejvhodnější podmínky pro následující vsádkové sorpční testy. K 0,4 g každého vzorku bylo přidáno 40 ml roztoku o různých koncentrací Hg v rozmezí 1 - 21 $mg.kg^{-1}$. Na ICP-MS byly poté měřeny koncentrace Hg v roztoku po sorpci a vypočítány obsahy Hg v kg půdy nebo vermikompostu.

Ze získaných dat byly sestrojeny dva základní modely sorpčních izoterm – Langmuirův a Freundlichův model, a porovnány maximální sorpční kapacity jednotlivých materiálů.

4.2.4 Vliv mikroorganismů

V rámci ucelenějšího popisu transformace a hromadění rtuti v půdě a její transformaci půdními mikroorganismy byly výsledky doplněny o analýzy provedené na Mikrobiologickém ústavu AV ČR, kde bylo stanoveno zastoupení jednotlivých kmenů mikroorganismů. Kultury izolované z půd z okolí Příbrami byly kultivovány v kapalném agaru s přídavkem roztoku $HgCl_2$, aby bylo dosaženo koncentrace Hg 0,1 $mol.l^{-1}$. Bakterie, které v tomto prostředí přežily a byly schopné růstu *Rhodanobacter, Frateuria, Luteibacter, Mycobacterium, Bacillus, Bradyrhizobium, Beijerinckia, Staphylococcus, Sphingomonas, Paenibacillus, Burkholderia* a *Pseudomonas.*

Pro hodnocení akumulační schopnosti půdních mikroorganismů byly využity tzv. rhizoboxy, ve kterých jsou kořeny rostlin odděleny od půdy permeabilní membránou. To umožňuje sledovat změny půdních charakteristik a koncentrací živin a rizikových prvků v oblasti rhizosféry. Vzhled a vlastnosti jednoho typu rhizoboxů popisují například Wenzel et al. (2001). V našem experimentu byla kořenová zóna nahrazena kulturou mikroorganismů na tenké vrstvě agaru, která byla od půdy oddělena pouze zmíněnou membránou. Z půdních mikroorganismů byly vybrány ty, které vykazovaly značnou rezistence vůči vysokým koncentracím rtuti v půdě, *Paenibacillus alginolyticus, Burkholderia glathei, Burkholderia sp.* a *Pseudomonas sp*. Použity byly dvě sady rhizoboxů, se dvě půdami s rozdílným množstvím celkové Hg (0,5 a 7 $mg.kg^{-1}$), a pokus probíhal 90 dní ve skleníku při teplotě 20°C. Pro stanovení celkových a mobilních podílů Hg v oblasti rhizosféry byla půda rozřezána na jednotlivé sekce v závislosti na vzdálenosti od agarové vrstvy pomocí speciálního zařízení, které popisují Fitz et al. (2003). V půdě z jednotlivých segmentů i ve vzorku okolní půdy byly stanoveny celkové obsahy rtuti, množství biodostupné Hg a ve spolupráci s Masarykovou univerzitou i zastoupení jednotlivých specií rtuti pomocí sekvenční extrakce.

4.3 Analytické metody

4.3.1 Stanovení půdních charakteristik

Odebrané vzorky půd byly usušeny při laboratorní teplotě a byly u nich stanoveny vybrané půdní parametry, například pH_{CaCl_2} (Novozamsky et al., 1993), obsah organické hmoty (Sims a Haby, 1971) a kationtová výměnná kapacita (ISO, 1994).

Byly změřeny také celkové obsahy dalších vybraných toxických i esenciálních prvků. Celkový obsah Hg byl stanoven za použití jednoúčelového atomového absorpčního spektrometru AMA-254 (LECO model, Altec, Česká republika), S a Mg rentgenovou fluorescenční spektrometrií (XRF, Spectro IQ, Kleve, Germany) a ostatní prvky jako je Mg, Fe, Cu, Zn a Pb pomocí emisní spektrometrie s indukčně vázaným plazmatem (ICP-OES, Varian, VistaPro, Australia).

4.3.2 Extrakční postupy

K popisu zastoupení jednotlivých forem Hg ve vzorcích odebraných u Hradce Králové bylo provedeno několik jednoduchých extrakcí a na Masarykově univerzitě v Brně jedna sekvenční, která je založena na pracích Bloom et al. (2003) a Boszke et al. (2008).

K uvolnění mobilních frakcí byla použita tři extrakční činidla, CH_3COOH, EDTA a $Na_2S_2O_3$. V případě kyseliny octové bylo k 0,5 g vzorku přidáno 10 ml 0,11 M roztoku CH_3COOH a zkumavky se nechaly třepat přes noc. Následně byly 10 min centrifugovány rychlostí 3000 ot/min, zfiltrovány a extrakt okyselen směsí kyselin (HNO_3 : HCl = 4 : 1). Ve druhém případě byl použit 0,05 M roztoku EDTA, který byl pomocí NaOH upraven na pH 7. K 1 g vzorku bylo přidáno 10 ml extrakčního činidla a extrakce probíhala 1 h na třepačkách. Následně byly vzorky 10 min centrifugovány rychlostí 3000 ot/min, zfiltrovány a extrakt okyselen směsí kyselin (HNO_3 : HCl = 4 : 1). Extrakce thiosíranem sodným probíhala přes noc v 10 ml 0,01 M roztoku, který byl přidán k 1 g vzorku. Extrakt byl opět centrifugován rychlostí 3000 ot/min, zfiltrován a následně okyselen směsí kyselin (HNO_3 : HCl = 4 : 1).

Byl také proveden mikrovlnný rozklad 0,25 g vzorku, ke kterému bylo přidáno 5 ml HNO_3. Vzorek byl rozložen při 280°C během 75 min pomocí MLS ultraCLAVE IV (Milestone, Germany) a následně doplněn vodou na konečný objem 50 ml. Před vlastním měřením byla přidána HCl. Tímto postupem bylo získáno množství potenciálně

mobilizovatelných specií rtuti.

Sekvenční extrakce se skládá z několika kroků a jako první je použit chloroform, kterým získáme množství organických sloučenin Hg. Následuje destilovaná voda – rtuť extrahovatelná vodou, 0,5 M HCl – rtuť uvolňovaná v kyselých podmínkách, 0,2 M KOH – rtuť vázaná na organickou hmotu a 50 % HNO_3, které uvolní elementární rtuť a Hg navázanou v komplexech. Reziduální Hg je vázána zvláště na S a Si. V případě sekvenční extrakce byl pro stanovení koncentrací Hg v roztocích využit analyzátor AMA-254.

4.3.3 Stanovení Hg pomocí ICP-MS

Byla vyvinuta a ověřena analytická metoda stanovení rtuti pomocí hmotnostní spektrometrie s indukčně vázaným plazmatem (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA). Pro detekci obsahů rtuti v roztocích po jednotlivých extrakcích, bylo využito právě ICP-MS. Měřen byl vždy izotop Hg(202) a Pt(195) o koncentraci 100 $\mu g.kg^{-1}$ byl použit jako interní standard.

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5.1 Publikovaná práce 1

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THE EFFECTIVITY OF VARIOUS EXTRACTION AGENTS TO RELEASE MERCURY FROM ANTHROPOGENICALLY CONTAMINATED SOILS

Adéla Šípková[1], Jiřina Száková[1], Pavel Coufalík[2], Pavel Tlustoš[1]
sipkovaa@af.czu.cz
[1] Department of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Czech Republic
[2] Department of Chemistry, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic

ABSTRACT

The potential bioavailability of the Hg from the soil might by characterized by variety of chemical processes, differing in the extraction agent, its concentration, the sample weight or the time of extraction. In this study, a comparative analysis of several extraction methods, commonly used for obtaining the mobile phase of the mercury from anthropogenically contaminated soils, was carried out. The aim was to estimate the rate of mercury sorption by soil, especially by its organic matter. Concentrated HNO_3, 0.01 M $Na_2S_2O_3$, 0.05 M EDTA and 0.11 M CH_3COOH were used as extraction agents. The inductively coupled plasma mass spectroscopy (ICP-MS) was used to estimate the mobile phase of the mercury within each extract and the Advanced Mercury Analyzer (AMA-254) for the determination of total Hg, respectively. The results showed that even strong acid HNO_3 is unable to release the mercury tightly bound to the soil matrix. This particular method with microwave digestion is commonly used for the estimation of this type of anthropogenic pollution. Conversely, the lowest mercury yield was obtained using the acetic acid. In all experiments, the concentrations were below 0.15 % of the total Hg content, which is a proportion generally defined as biologically available to plants.

KEY WORDS: mercury, contaminated soil, single extraction, inductively coupled plasma mass spectroscopy, Advanced Mercury Analyzer AMA-254

1. INTRODUCTION

Soil is a heterogenous system containing colloid inorganic and organic matter in the form of small particles, water, and various gases. The natural origin of mercury depends particularly on the character of bedrock (Závadská et al., 1999). The Hg concentration in uncontaminated soil does not usually exceed 200 $\mu g.kg^{-1}$ (Adriano, 2001). If the concentration is higher, the source of pollution is the point of interest. The anthropogenic sources may result from the proximity of the mining industry or metallurgy, agriculture, sewage sludge treatment or incinerators, and also landfills (Lasat, 2002). In the Czech Republic, the permissible content of the harmful substances in the soil are determined by the directive of Ministry of the Environment no. 13/1994. The maximum acceptable Hg content is 800 $\mu g.kg^{-1}$; however, the concentration of mercury in anthropogenically contaminated soils can be substantially higher. For instance, according to Kacálková et al., 2009 the mercury content near the former waste incineration plant in Hradec Kralove, Czech Republic, was found to be up to 12 $\mu g.g^{-1}$.
Mercury can be released from soil by different extraction procedures. These procedures enable the determination of particular species present, the varying amounts of Hg bound to the soil, and also the bioavailability or toxicity. Numerous of these procedures are described in the literature. The least tightly bound water-soluble fraction of mercury is obtained by the simple extraction using deionised water (Séguin et al., 2004, Rodrigues et al., 2010). It represents mercury present in pore water in soil. This form of mercury is usually not in the form of the water-soluble ionic species but as a species bound to organic matter; nevertheless, not directly on carbon (Biester and Scholz, 1997). Biologically available mercury is further released into the soil solution. The extraction with CH_3COOH is commonly used to simulate the approximate composition of this solution. Beside this most common approach other extraction agents, such as $CaCl_2$ solution, might be used (Novozamsky et al., 1993). The extraction solutions based on the chelating agents such as EDTA or DTPA represent another more efficient possibility. These extraction agents simulate well the interaction of plant´s roots with the dissolved organic acids, which further leads to the dissolution of some forms of particular elements, which are not originaly present in the soil solution (Cibulka, 1991).
In the soil, mercury can be bound very tightly to the sulfur forming the insoluble HgS. The portion of this mercury bound to sulfide may be up to 60 % of the total Hg content (Boszke et al., 2008). This phase of mercury can be obtained either by aqua regia extraction in a microwave oven (Fernándes-Martínez and Rucandio, 2003), or using the saturated Na_2S solution from the residue remaining in the second stage (Revis et al, 2003). Extraction with $Na_2S_2O_3$ is used for speciation analysis, because it is causing no Hg transformations. Issaro et al. (2010) studied the effect of the concentration of sodium thiosulphate on the extraction efficiency. The $Na_2S_2O_3$ is also used for increasing the Hg availability for fytoextraction methods (Moreno et al., 2005).

The proportion of Hg, which is not firmly bound in the silicate matrix of the soil, is often obtained by using HNO_3 as an extraction agent (Reis et al., 2010). The mercury concentration in these extracts enables to estimate the amount of Hg from anthropogenic sources. It might also be used in sequential extractions to obtain elemental mercury (Bloom et al., 2003, Coufalík et al., 2011). In this work, four various extraction agents were applied to assess Hg mobility in anthropogenically contaminated soils.

2. Materials and Methods

2.1 Samples

Ten soil samples were collected from the former waste incineration plant in the suburb of Hradec Kralove, Czech Republic. The hazardous waste containing: oil, agricultural waste, preservatives, waste from chemical processes and percolation, degreasing waste containing solvents, waste containing metals, halogens, sulphur, dyes, fertilizers, pesticides and others were burnt there between the years 1993 and 2002. This hazardous waste was stored without protection during the running period of the incinerator (Kacálková et al., 2009).

2.2 Total and extractable portions of mercury

Total contents of mercury were determined, without chemical pretreatment of the samples, by thermal decomposition atomic absorption spectroscopy (AAS) with gold amalgamation (LECO model AMA-254), a rapid total mercury determination method.
Four extraction reagents HNO_3, $Na_2S_2O_3$, EDTA, and CH_3COOH were used to determine the mobile and mobilizable phases of Hg. For determination of potentially mobilizable mercury portions, 0.25 g of each sample was decomposed in 5 ml of HNO_3. The reaction mixture was digested at 280°C during 75 min by using microwave heating in MLS ultraCLAVE IV system (Milestone, Germany) and then milli-q water was added to a final volume of 50 ml.
The mild extraction procedures were performed as follows: i) Sodium thiosulfate extraction proceeded overnight in 10 ml of 0.01 M solution, which was added to 1 g of the sample. ii) 0.05 M EDTA was adjusted with NaOH to pH 7. Subsequently, 1 g of soil was added to 10 ml of extraction solution and shaken for 1 h. iii) 0.5 g of sample was added to 10 ml of 0.11 M solution of CH_3COOH and shaken overnight. Subsequently, all the the samples were centrifuged for 10 minutes at 3000 rpm, filtered and the extract was acidified by the mixture of acids (HNO_3 : HCl = 4 : 1). Mercury content in all extracts was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA).

3. Results and discussion

The measurements of the total Hg content indicate that in all ten samples from the vicinity of the former waste incineration plant in Hradec Králové there are places where the concentration of mercury is relatively low; however, sites with above limits concentrations are present as well. In the most polluted sample the amount of mercury reaches almost 29 $mg.kg^{-1}$, which exceeds the required limit 36 times. Kacálková et al. (2009) report the same variability of the mercury content present in the same area showing the concentrations ranging from 0.15 to 12 $mg.kg^{-1}$.
The extraction yields by individual extraction agents are shown in Table I representing the total amount of mercury and also the its rate to total Hg content. The nitric acid proved to be the most efficient extraction agent. Concentrations of Hg range between 48 % and 56 % of total content in 8 of 10 samples using nitric acid. In the case of the most contaminated sample the concentration was approximately 70 %. In the last sample, where the total content exceeded the limit 13 times, the extraction yield was using nitric acid was almost 96 %. The mercury concentration obtained by this extraction method should correspond to the rate of anthropogenic contamination.
Using $Na_2S_2O_3$ as an extraction agent the mercury yield was also highest in two last samples. The average yield of these particular samples attains approximately 20 %. In other samples, the content of mercury ranged from 1.2 % to 3.4 %. Thus, it might be inferred that in places with higher anthropogenic contamination, the presence of mercury species bound to sulfur is significantly higher than in less contaminated samples. In the case of the most contaminated site the rate of extractable Hg using $Na_2S_2O_3$ corresponds with the results reported by Issaro et al. (2010). They showed that the extraction yield of $Na_2S_2O_3$ usually reaches 50 ± 5% of mercury obtained by nitric acid. Moreover, they showed that the rate of extractable Hg by sodium thisulphate is decreasing with decreasing total Hg content. On the other hand, Subiréz-Munoz et al. (2011) obtained by this type of extraction only approximately 20 % of the total Hg content.

Table 1: Extractable contents of mercury after individual extraction ($\mu g.kg^{-1}$).

Sample	total	HNO_3	$Na_2S_2O_3$	EDTA	CH_3COOH
1	1070	578	14.3	6.92	0.493
2	236	132	7.04	1.99	0.339
3	415	201	7.82	5.21	0.373
4	419	234	8.03	8.80	0.480
5	550	273	10.4	6.27	0.450
6	2050	1132	52.1	9.37	0.727
7	396	223	13.5	2.95	0.436
8	580	300	6.95	2.94	0.784
9	28800	20108	5877	481	11.3
10	10500	10040	2094	64.7	8.64

Further, using chelating agent EDTA, the values of extractable Hg ranged between 0.46 % and 2.1 % of the total Hg content, in all experimental samples. These results correspond to those reported by Subiréz-Munoz et al. (2011) who obtained less than 2 % of the total Hg using the same 0.01 M EDTA solution. This small variability suggests that the amount of mercury bound to organic matter, which might serve as a source for plant uptake, is similar both in more and less anthropogenically contaminated places. Coinciding results were also obtained by extraction with a solution of CH_3COOH, which simulates natural conditions of soil solution. The yields of CH_3COOH confirmed no significant differences between more and less contaminated samples. However, contrastingly to the EDTA, the average mercury yield was below 0.15 % of the total Hg.

The obtained results are also shown in Fig. 1. The mercury concentrations of individual samples are plotted in log-normal scale due to its comparability, because the differences between the various extraction agents are in order of several orders. The graph illustrates the differences between the Hg concentration of the majority of samples and the two most contaminated sites using nitric acid and sodium thiosulphate. While in the case of extractions with EDTA and CH_3COOH, the differences between samples from various sites are not reaching up to these vales.

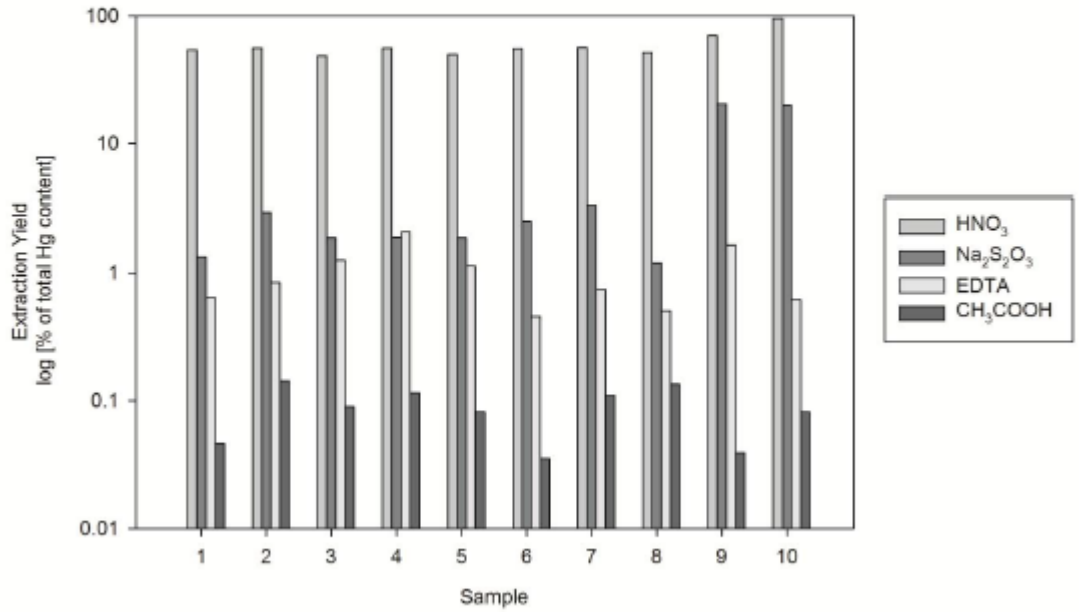

Figure 1: Extraction yield using individual extraction agents.

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5.2 Publikovaná práce 2

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Affinity of Selected Elements to Individual Fractions of Soil Organic Matter

A. Šípková · J. Száková · P. Tlustoš

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Abstract The distribution of selected elements in individual fractions of organic matter from anthropogenically contaminated soils was investigated. The attention was paid especially at Hg. Furthermore, contents of S, Mg, Mn, Fe, Cu, Zn and Pb were also measured. The decomposition of organic matter to particular fractions was carried out by the resin DAX-8. Ten soil samples were collected, and the Advanced Mercury Analyzer (AMA-254) was used for the determination of the total Hg content. The two highest Hg values reached up to the concentration 10.5 mg kg^{-1}, and in the highest one, it was almost 29 mg kg^{-1}. In each extract, mercury was measured by inductively coupled plasma mass spectrometry (ICP-MS), for other elements, inductively coupled plasma optical emission spectrometry (ICP-OES) was applied. Results of the analysis show that the Hg content bound to the humic acids is inversely proportional to the content of Mg, Mn, Fe and Cu. However, this dependence was not confirmed by the samples with the mercury content above 10 mg kg^{-1}. In the case of fulvic acids, the relationship between Hg and S was observed and has again an inverse character.

Keywords Sorption · Soil · Organic matter · Mercury · Sulphur · Trace elements

A. Šípková (✉) · J. Száková · P. Tlustoš
Department of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Life Sciences, Prague, Czech Republic
e-mail: sipkovaa@af.czu.cz

1 Introduction

The behaviour of heavy metals in soil is influenced by the presence of organic matter, its quantity and especially by its composition. Organic matter consists of high molecular weight hydrophobic compounds, collectively termed humic substances, which are comprised of humic acids (HA), fulvic acids (FA) and more simple low molecular weight hydrophilic (Hy) compounds (Stevenson 1994). The binding of metals with organic matter has probably contributed to the accumulation of particular metals in organic-rich shallow horizons of soil (Fujikawa and Fukui 2001). However, chemical affinity of various metals to fractions of organic matter is different (Milne et al. 2003). The environmental significance of these compounds is related both to the higher biodegradability of low molecular mass hydrophilic compounds and the lower mobility of humic acids. In general, the hydrophilic compounds accounted for the complexation of around 50 % of the leached metals, with variable contributions of humic and fulvic acids, depending on the nature of the samples and the metals (Laborda et al. 2009).

In the case of mercury, species bound to organic matter may represent 20–30 % of the total content in soil (Boszke et al. 2008). Mercury is not tied directly to carbon but mostly to sulphur in functional groups of thiols, sulphides and, possibly, nitrogen (Xia et al. 1999). The sorption to soil depends on complex capacity and complex stability of the humus fraction (Yao et al. 2006). A high complex capacity for Hg but weak complex stability promotes volatilization. Adding fulvic

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acid to contaminated soil increases mobility, while humic acids, which have lower complex capacity, reduce evaporation. Generally, the higher content of organic matter in soils results in higher adsorption capacity (Schlüter 1997).

The amount of organic matter plays a central role not only in the mobilization of the Hg content but also in the case of Cu and Pb. Moreover, its composition also influences the mobility of these metals within the soil (Linde et al. 2007). High ratio of association with humic acid in the case of Cu and less mobility in the case of Pb suggested that retention in humus-rich layer may last longer than that of the other metals (Fujikawa and Fukui 2001).

Some elements, including Cu, Zn, Mn and Fe, are essential for plant growth and are called trace elements (He et al. 2005). Although in some cases, their toxicity is probably more of a problem than their deficiency. The solubility of these metals depends mainly on organic matter, the metal loading over soil sorbents, the concentration of inorganic ligands, and pH (Weng et al. 2002). Humic acid and fulvic acids caused significant Zn and Pb immobilization in the acid soil, while Cu and Fe were slightly mobilized. In the calcareous soil, these effects were observed in the lesser extent. These results suggest that components of humic-rich materials may be useful amendments for the soil remediation involving stabilization, although currently mild mobilization of Zn, Pb and Cu may be provoked in acidic soils (Clemente and Bernal 2006; Terbouche et al. 2011). In contrast to other trace metals, the amount of mobilized mercury decreases at pH<3 and at pH>12, due to the extremely high buffering capacity of humics, both in acidic and alkaline states (Kabata-Pendias and Pendias 2001). However, in the acid soils, Hg bounds particularly to humic substances (Schwesig et al. 1999).

The movement of Hg in soils and desorption rate are indirectly related to the organic matter content. Higher amounts of mercury will be desorbed from the soil with a lower content of organic substances, which is typical in places where the natural coverage of the soil was removed due to anthropogenic activities (Miretzky et al. 2005).

The content of some elements, especially Hg (Xia et al. 1999), Cu, Zn and Pb is also often associated with sulphur (Thornton 1981). However, the after-effect of contamination depends not only on the concentration of metals but also on their combinations and ratios.

In this work, the relationship between selected elements bound to organic matter will be studied. Moreover, the contents of selected elements in the various fractions of organic matter will be correlated.

2 Materials and Methods

2.1 Samples

Ten soil samples were collected from the former waste incineration plant in the suburb of Hradec Králové, Czech Republic. The hazardous waste containing: oil, agricultural waste, preservatives, waste from chemical processes and percolation, degreasing waste containing solvents, waste containing metals, halogens, sulphur, dyes, fertilizers, pesticides and others were burnt there between the years 1993 and 2002. This hazardous waste was stored without protection during the running period of the incinerator (Kacálková et al. 2009).

The selection of sampling sites used in this work was based on the experiment of Kacálková et al. (2009). Samples were collected in the vicinity of points 3 and 5 from their aforementioned work. The plot 3 represents the average values of contamination (our samples 1–5), while extreme mercury concentrations were measured in the plot 5. This plot is placed closer to the incineration plant, thus highest amounts of observed elements and oxidable carbon were obtained. On the other hand, also the lowest pH from all the samples was received. In our experiment, the samples taken from this plot are labelled from 6 to 10. Samples were taken from the top layer (0–30 cm) of soil, and all experiments were carried out in three repetitions.

2.2 Total Content of Selected Elements

Total mercury contents were determined, without chemical pre-treatment of the samples, by advanced mercury analyser AMA-254 (LECO model, Altec, Czech Republic). Initially, the samples were dried and subsequently burned. The resulting Hg vapor was trapped onto gold amalgamator and then subsequently released as Hg^0 by heating. The released mercury vapor was measured in atomic absorption spectrophotometer at wavelength of 253.65 nm. The low-pressure mercury discharge lamp was used as a source of radiation (Száková et al. 2004). CRM020 Trace Metals—Sandy Loam 2 CRT USA was used as a reference material. Certified value for Hg content is given 1.12 ± 0.03 mg kg^{-1}, the determined value was 1.16 ± 0.03 mg kg^{-1}.

The pseudo-total concentrations of Mn, Fe, Cu, Zn and Pb in the soils were determined in the digests obtained by the following decomposition procedure: Aliquots (~0.5 g) of air-dried soil samples were decomposed in a digestion vessel with 10 ml of Aqua Regia (i.e., nitric and hydrochloric acid mixture in ratio 1 :3). The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave assisted wet digestion system for 33 min at 210 °C. After cooling, the digest was quantitatively transferred into a 25-ml glass tube, topped up by deionised water, and kept at laboratory temperature until measurement. The element concentrations in the digests were determined by inductively coupled plasma - atomic emission spectrometry (ICP-OES, Varian, VistaPro, Australia) equipped by a two channel peristaltic pump, a Struman-Masters spray chamber and a V-groove pneumatic nebulizer made of inert material (the experimental conditions were as follows: power 1.2 kW, plasma flow 15.0 L min^{-1}, auxiliary flow 0.75 L min^{-1}, nebulizer flow 0.9 L min^{-1}). For ICP-OES, the following spectral lines were considered: λ=308.2 nm for Al, λ=327.4 nm for Cu, λ=238.3 nm for Fe, λ=257.6 nm for Mn, λ=220.3 nm for Pb and λ=206.2 nm for Zn. Calibration solutions were prepared in diluted Aqua regia as follows: 50–500 μg L^{-1} for Cu and Pb, 1–10 mg L^{-1} for Mn and Zn, and 5–50 mg L^{-1} for Fe. The elements' contents were measured on two spectral lines, and no significant differences were observed. Hence, the calibration curve was used for the evaluation of the analytical signal of each element. A certified reference material, RM 7001 Light Sandy Soil, was used for the analytical data verification. Certified values for the Aqua regia soluble element contents are 479±18 mg kg^{-1} of Mn, 108±3.5 mg kg^{-1} of Zn, 28.9±0.8 mg kg^{-1} of Cu, (31.8±1.2) mg kg^{-1} of Ni and 24.1±1.7 mg kg^{-1} of Pb. The determined values were as follows: 468 mg kg^{-1} of Mn, 110 mg kg^{-1} of Zn, 28.7 mg kg^{-1} of Cu and 25.2 mg kg^{-1} of Pb.

Total content of sulphur was determined by X-ray fluorescence (XRF) spectrometry (Spectro IQ, Kleve, Germany), where the target material was palladium and the target angle from the central ray was 90°. The focal point was a 1 mm×1 mm^2, and the maximum anode dissipation was 50 W with 10-cfm forced-air cooling. The tested samples were pressed into pellets; mixing 4.0 g of soil (particle size 15–20 μm) with 0.9 g of the binding additive (HWC Hoechst wax, Germany) for 10 min. The pressing power was 80 kN.

2.3 Fractionation of Organic Matter

Contents of humic acid (HA), fulvic acid (FA), hydrophilic compounds (Hy) and hydrophobic neutral organic matter (HON) were determined using the Superlite™ DAX-8 (SUPELCO Analytical, USA). This resin is comprised of a poly(methyl methacrylate) matrix with following characteristics: particle size=40–60 mesh, pore size=225 Å and surface area=160 m^2 g^{-1} and which is slightly polar. Sorption, as well as desorption, of individual organic fractions on the resin was observed (Van Zomeren and Comans 2007).

2.3.1 Preparation of DAX-8

The preparation of the resin consisted from several extractions by different extraction agents. The first step was five 0.1 M HCl extractions (24 h), and this cycle was repeated with 0.1 M NaOH. Further, the resin was cleaned by two 24-h extractions by acetonitrile followed by two extractions with methanol. The cleaned DAX-8 was stored in methanol. Before use, washing of the resin by water (20 volumes of DAX-8) and 0.1 M HCl (10 volumes of DAX-8) was necessary.

2.3.2 Total Content of HA, FA, Hy and HON

First, 5 g of each sample was added to 50 ml of 0.1 M HCl and shaken for 1 h. Subsequently, samples were centrifuged for 10 min at 3,000 rpm and the supernatant was filtered. Part of the supernatant was removed for the analysis and 12.5 ml was added to 2.5 g DAX-8. After 1 h of shaking, the solution was filtered and the filtrate was submitted to the analysis.

This was followed by the FA desorption from the resin by adding 10 ml of 0.1 M KOH. The solution was then shaken for 1 h and then 9 ml were collected. Nine millilitres 0.1 M KOH was added to resin, and the procedure was repeated three more times. The total volume of the solution with released fulvic acids was 37 ml, and just part of it was removed for analysis.

The soil sample after removing the first supernatant was added to 48 ml 0.1 M NaOH and 2 ml 1 M NaOH to adjust pH>12 and left to shake overnight. The solution was subsequently centrifuged at 3,000 rpm (10 min). The supernatant was removed, acidified by 6 M HCl, and the suspension was allowed to stand overnight. There has been a precipitation of HA. After 10-min centrifugation at 3,000 rpm, the supernatant was

removed, filtered, and part of it was submitted to analysis. Moreover, desorption from the resin was carried out as in the previous case. The precipitated HA were dissolved in 45 ml of 0.1 M KOH.

2.4 Selected Elements in the Organic Matter

In extracts, the content of selected elements was obtained. Mercury was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA), for other elements, inductively coupled plasma optical emission spectrometry (ICP-OES) was applied. Auto-sampler ASX-500, a three channel peristaltic pump and MicroMist nebulizer equipped ICP-MS. Calibration solutions were prepared in diluted single element ICP standard as 0.1–10 μg L^{-1} for mercury and the isotope Hg(202) was measured. As internal standard, Pt(195) was used and its concentration was 100 μg L^{-1}.

The limit of quantification for S was 8 μg kg^{-1}, for Pb a Zn 1 μg kg^{-1}, for Cu, Fe, Mg, Mn 0.1 μg kg^{-1} and for Hg 0.003 μg kg^{-1}. All limits of quantification were calculated as fold standard deviation of blanks.

2.5 pH of Soil Samples

Measurements of pH were made on samples mixed with 0.01 M solution of $CaCl_2$ (1:10 *w/v*) by WTW pH 340 i (WTW, Germany) according to Novozamsky et al. (1993).

3 Results and Discussion

3.1 Characteristic of Experimental Soil and Total Content of Selected Elements

The pH of our samples equalled to 7.18±0.28 and the oxidizable carbon content ranged between 1.2 and 3.4 %. Similar characteristics of soil were showed by Kacálková et al. (2009) in the same area in Hradec Králové, Czech Republic. Regulation N°13/1994 Collection of Laws of Ministry of the Environment of the Czech Republic (Czech Ministry of the Environment 1994) provides maximum admissible risk element contents in soil as follows: 0.8 mg kg^{-1} Hg, 100 mg kg^{-1} Cu, 200 mg kg^{-1} Zn and 140 mg kg^{-1} Pb.

The measurements of total contents of investigated trace metals and macronutrients indicate significant differences in amounts of contamination considering all ten samples, collected from the vicinity of the former waste incineration plant (Table 1). In the case of mercury, sites with both relatively low and above limit concentrations were present. Especially for the two most polluted samples (9 and 10), the amount of mercury reached almost 29 mg kg^{-1}, which exceeded the required limit 36 times. Higher levels of particular elements in samples 9 and 10 were found also in the case of Cu, Zn and Pb. Concentrations of the rest of elements were less different from those gained in other samples. Kacálková et al. (2009) reported similar variability of total contents. Namely, Hg concentration ranges from 0.15 to 12 mg kg^{-1}, Cu from 12 to 212 mg kg^{-1}, and from 102 to 2,766 mg kg^{-1} in the case of Zn. Only the high level of zinc contamination

Table 1 Total content of selected elements in the soil (mg kg^{-1})

Sample	Hg	S	Mg	Mn	Fe	Cu	Zn	Pb
1	1.07±0.02	3,797±9	6,680±15	217±5	11,595±866	30.5±1.1	116±1	35.6±1.9
2	0.236±0.003	3,001±8	7,890±15	210±3	12,993±152	34.1±1.2	108±4	31.1±2.2
3	0.415±0.008	2,788±8	7,030±15	242±7	12,148±523	26.1±0.5	131±12	40.4±4.4
4	0.419±0.008	3,471±8	7,030±15	208±1	12,348±692	29.4±2.3	128±1	29.5±0.8
5	0.550±0.009	4,652±10	6,600±17	218±42	13,020±2174	28.3±0.3	116±5	36.5±0.3
6	2.05±0.01	5,566±10	7,650±15	243±8	19,219±34	91.4±3.8	230±8	72.8±8.9
7	0.396±0.008	3,946±8	7,000±14	282±37	25,201±977	71.3±0.7	119±16	30.1±5.6
8	0.580±0.003	3,044±8	7,440±15	314±4	20,559±1,157	55.4±1.0	124±13	42.1±2.0
9	28.8±0.5	4,398±11	6,610±15	412±15	24,050±616	149±9	453±4	773±12
10	10.5±0.2	2,983±8	7,960±15	551±53	22,028±3,524	136±17	501±21	374±43

was not found in our samples. Similarly, Römkens et al. (2009) studied the soil contamination in paddy fields in Taiwan and published total metal levels for copper, zinc and lead corresponding to our measured values. Romic and Romic (2003) studied the metal distribution in agriculture topsoil in urban area and found also comparable or lower values of Cu, Zn and Pb. Additionally, they have shown higher Mn amounts, and on the other hand, the Fe content was several times higher in our samples, ranging from approximately 12 to 25 g kg^{-1}. Differences in total contents were also found, in the case of macronutrients. However, the variability of values was significantly smaller. Total sulphur and magnesium contents range from 2.8 to 5.6 g kg^{-1} and from 6.6 to 8.0 g kg^{-1}, respectively. Lower sulphur concentrations were discovered in soil from Guangzhou, China (Sun et al. 2009) and Queensland, Australia (Liaghati et al. 2003). Nevertheless, the similar S content was detected by Linde et al. (2007) in their study of trace metals mobility affected by the change of soil conditions.

Differences observed in total contents of selected elements in our work, especially in samples 6–10, refer to an extreme heterogeneity of pollution in a small-scale area. This fact implies the essential need for collecting a larger number of samples, taken from a single site, in order to obtain representative values of contamination.

3.2 Proportion of Elements in the Organic Matter

The ratio of elements bound to organic matter with relation to their total content is shown in Fig. 1. In the case of Hg, the percentages are relatively low, not exceeding 8 %. The lowest amounts bound to organic matter were found in the case of S and Mg with an average of 2 and 2.5 %, respectively. Similar low percentage of sulphur bound to organic matter was detected by Calace et al. (2005). In the study, investigating the binding of particular sulphur fractions to long fertilized soil, 6 % of the total S was bound to carbon (Yang et al. 2007). The ratio of Fe bound to the organic matter attained only 3 %. Similarly or lower values were found by Fujikawa and Fukui (2001). They have investigated associations of trace metals with organic matter in Japanese soil using acidified hydrogen peroxide (H_2O_2) as an extraction agent. In this work, comparable contents of manganese, copper, zinc and lead were found. The percentages of Cu and Zn bound to organic matter with respect to their total content were relatively high, with the mean of 20 and 25 %, respectively.

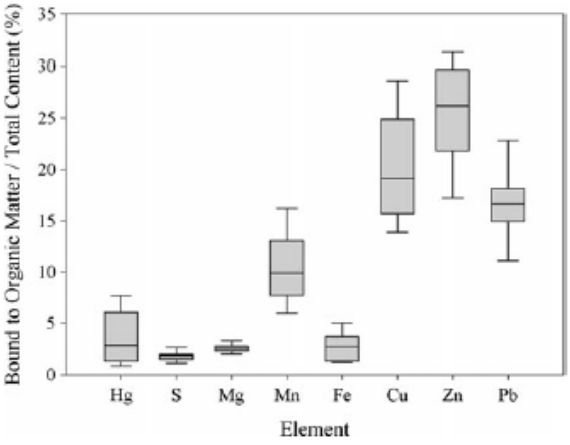

Fig. 1 The ratio of elements bound to organic matter with respect to the total concentration in soil

3.3 Distribution of Elements in the Individual Fractions of the Organic Matter

Exact contents of elements bound to organic matter are recorded in Table 2, and the distribution of bounds to particular fractions of organic matter (humic acids, fulvic acids and hydrophilic compounds) is shown in Fig. 2. In all cases, amounts of elements bound to hydrophobic neutral organic matter were less than 0.4 % of the total amount bound to the organic material. Mostly, the values were not more than 0.1 %; hence, this fraction was excluded from the calculation.

Manganese, copper, zinc and lead were bound particularly to hydrophilic compounds by an average of more than 87 %. The amount of Mn bound to this fraction reached almost up to 92 %. Laborda et al. (2009) reported in their study that in general, the hydrophilic compounds accounted for the complexation of circa 50 % of leached metals. The determination by the size exclusion chromatography and ICP-MS detection was used in their experiment. As it might be observed in Fig. 2, the values obtained in our study are even higher. It corresponds with Kabata-Pendias and Pendias (2001) who have founded that some elements such as Hg, Cu and Pb rather form stable organic complexes with fulvic acids and, most probably, also with other organic compounds. Also, Fest et al. (2008) described the important role of hydrophilic acid in the complexation of Cu in soil. In the case of zinc and lead, the element distribution between humic acid and fulvic acid was determined by Borůvka and Drábek (2004)

Table 2 Content of elements bound to organic matter (mg kg^{-1})

Sample	Hg	S	Mg	Mn	Fe	Cu	Zn	Pb
1	0.015±0.002	60.5±9.5	175±19	27.5±1.7	378±68	8.06±0.33	31.5±1.7	5.51±0.30
2	0.011±0.001	54.6±2.6	213±7	34.5±0.9	628±32	6.82±0.62	34.1±0.6	5.28±0.28
3	0.009±0.001	55.7±2.3	199±5	34.3±0.6	612±19	7.58±1.08	34.9±0.6	5.38±0.14
4	0.033±0.006	56.2±1.9	151±7	25.4±1.7	415±23	7.04±0.23	38.1±2.8	4.99±0.71
5	0.031±0.007	51.2±2.3	160±11	25.0±0.6	431±14	7.04±0.84	29.9±0.5	5.97±0.21
6	0.042±0.013	66.4±3.0	157±8	20.2±1.3	237±25	16.7±2.1	55.6±2.2	12.4±0.6
7	0.029±0.004	74.4±3.9	179±16	21.4±1.2	325±64	8.35±0.34	19.9±0.8	2.81±0.23
8	0.020±0.002	57.8±1.9	189±9	24.8±1.7	452±24	8.98±0.48	25.6±2.2	6.56±1.66
9	0.216±0.009	87.1±5.1	174±14	24.0±1.1	290±34	21.1±1.5	135±5	167±3
10	0.118±0.012	80.6±10.8	243±37	43.1±3.9	446±77	25.8±1.7	112±7	89.6±7.6

showing that amounts of metals bound to humic acids were relatively low. In our samples, the sulphur and magnesium distribution between HA and Hy was nearly equal, and only a small proportion was bound to FA. In the case of mercury and iron, the situation was different so that only a minor portion of the element was bound to Hy because over 45 % of Hg was bound to HA and in the case of iron even circa 80 %. The bounds to fulvic acids constituted almost 40 % of Hg, being the largest value of all elements of interest. Many authors did not take into account the Hg adsorption to hydrophilic compound of organic matter at all and its distribution in organic matter may differ significantly. Zhang et al. (2009), who were using acidic soils, showed that Hg is more prone to bound to HA. This was confirmed, e.g., by Schwesig et al. (1999). Contrarily, Chai et al. (2012) reported a stronger binding of Hg to FA and also discussed the influence of pH thoroughly.

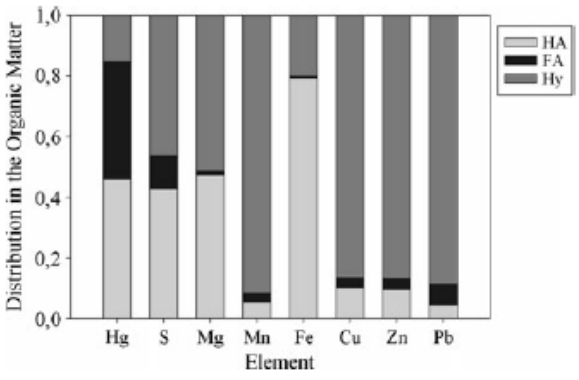

Fig. 2 Distribution of elements in the individual fractions of organic matter

3.4 Correlations Between Elements Bound to the Organic Matter

The correlation analysis among elements bound to the organic matter was carried out. Amount of these elements proportions were calculated as sum of contents bound to the individual fractions. Values of the correlation coefficient c_c, which are recorded in Table 3, show that some of the relations among selected elements are relatively strong. In particular, a match between Hg and Pb or Zn has been observed with the correlation coefficient (c_c) reaching values of 0.99 and 0.95, respectively. Also in the case of sulphur, the correlation coefficient with mercury attains 0.84. This corresponds to the fact that mercury is not tied directly to carbon in the organic material, but mostly to sulphur in functional groups of thiols and sulphides (Xia et al. 1999). The importance of this particular binding was also demonstrated by Chai et al. (2012), who divided organic matter only into two categories (HA and FA) though. For the rest of elements (Mg, Mn and Fe), values of c_c were lower than 0.5. However, if samples 9 and 10 with a higher content of Hg from the anthropogenically contamination were excluded from the correlation, the correlation coefficients substantially increased. Their values reached then the range from 0.82 to 0.87, and relations were inversely proportional. In the case of copper, c_c decreased from 0.85 to 0.61 after the removal of samples 9 and 10 from the calculation.

The sulphur content was significantly correlated with aforementioned Hg and also with copper, zinc and lead. Correlation coefficients are higher than 0.8, which corresponds to the theory that these elements are particularly bound to S (Thornton 1981). Focusing on iron, c_c equals to 0.55 and the relation observed has again an

Table 3 Correlation coefficients between elements in total organic matter

	Hg	Hg*	S	Mg	Mn	Fe	Cu	Zn	Pb
Hg	1.00								
Hg*	1.00	1.00							
S	*0.84*	0.36	1.00						
Mg	0.12	*−0.85*	0.25	1.00					
Mn	0.06	*−0.87*	0.05	*0.89*	1.00				
Fe	−0.43	*−0.82*	*−0.55*	*0.55*	*0.67*	1.00			
Cu	*0.85*	*0.61*	*0.85*	0.27	0.16	−0.50	1.00		
Zn	*0.95*	0.43	*0.81*	0.30	0.29	−0.31	*0.92*	1.00	
Pb	*0.99*	0.50	*0.83*	0.22	0.16	−0.33	*0.84*	*0.96*	1.00

Asterisk "*" indicates without two samples with the highest concentration of Hg; *Italic text* shows strong correlations 0.60–1.00=strong correlation; 0.50–0.59=moderate; 0.40–0.49=weak; 0.00–0.39=little or no association; The correlation coefficients higher than 0.37/0.41 are statistically significant at least at the 95 % percentile level using ten/eight samples

inverse character. Correlation coefficients with other elements are insignificant. The amount of magnesium coincided closely only with manganese (c_c=0.89). Certain connection was further found with Fe where the c_c attains −0.55. Additionally, the content of these two elements (Fe and Mn) corresponded to a certain extend to each other (c_c=0.67). Generally, iron and manganese have a close relationship, which was also shown by Liaghati et al. (2003). Even better link was found among Cu, Pb and Zn. Specifically, the value of c_c of 0.84 was observed between Cu and Pb, c_c=0.92 between Cu and Zn, and 0.96 between Zn and Pb. Similar trends of metal levels in soil were observed by Römkens et al. (2009), who were not particularly focused on organic matter but have investigated the total content of selected elements instead. They have reported the correlation coefficients ranging up to 0.9 concerning the contents of Cu, Zn and Pb in their samples. As it might be inferred from the Table 3, the relations concerning other elements are of less importance. The correlation coefficients are significantly lower than reported for the abovementioned elements or no connection was found at all.

3.4.1 Correlations Between Elements Bound to Humic Acids in the Organic Matter

The similar relations, as those found in the case of the total organic matter, were found also revealed for elements bound to humic acids (Table 4). Strong relations of mercury with lead and zinc were observed where the c_c is 0.94 and 0.77, respectively. Correlation coefficient between Hg and S is lower, but still a moderate dependence was found. Further, same correlations, as in the case of total organic matter, are observed after the elimination of samples 9 and 10 from the group of elements assessed. Mercury content was inversely proportional to the amount of Mg, Mn and Fe, and the values describing this relation are ranging from 0.83 to 0.86. Nevertheless, the new inverse correlation with Cu was found where c_c equals to 0.82.

The links among S and selected elements were confirmed even focusing on humic acids; albeit, the correlation coefficients are generally lower. The strongest dependence was found between S and Cu, where the decrease in c_c values is rather insignificant (from 0.85 to 0.83). On the other hand, considering Zn and Pb, the value of c_c decreased to a 0.61 and 0.65, respectively. Despite of this decrease, the dependence might still be considered tight. Taking into account links among Mg, Mn and Fe, the correlations found in the total content were found as well and even raised above the value of 0.94. The new relation between Mn and Cu was revealed with the c_c attaining 0.61. Further, moderately strong dependences were found between Mg and Cu and between Mn and Zn. On the other hand, the correlations among Cu, Zn and Pb slightly dropped. However, correlation coefficients were still above the value of 0.78, implying strong relation.

3.4.2 Correlations Between Elements Bound to Fulvic Acids in the Organic Matter

The smallest amount of stronger linkages was found with respect to bindings to fulvic acids. Moreover,

Table 4 Correlation coefficients between elements in humic acids

	Hg	Hg*	S	Mg	Mn	Fe	Cu	Zn	Pb
Hg	1.00								
Hg*	1.00	1.00							
S	0.53	−0.45	1.00						
Mg	−0.29	−0.86	0.11	1.00					
Mn	−0.15	−0.83	0.23	0.96	1.00				
Fe	−0.36	−0.85	0.07	0.98	0.94	1.00			
Cu	−0.04	−0.82	0.83	0.51	0.61	0.44	1.00		
Zn	0.77	−0.70	0.61	0.35	0.51	0.27	0.86	1.00	
Pb	0.94	−0.62	0.65	0.03	0.17	−0.06	0.78	0.92	1.00

Asterisk "*" indicates without two samples with the highest concentration of Hg

taking into account all elements, with the exception of mercury, the amounts bound to FA were smaller than to humic acid or hydrophilic compound. The previously found relation of Hg and S has only in the case of fulvic acids an inverse character. This concerns all samples even when the specifically contaminated samples 9 and 10 were excluded.

The relation of Mn and Fe was confirmed even in the FA with the c_c attaining the value of 0.61. Additional significant link between Mg and Cu was found (c_c=0.75). Besides another aforementioned dependency among Cu, Zn and Pb, no other reliable correlations were revealed (Table 5).

3.4.3 Correlations Between Elements Bound to Hydrophilic Compounds in the Organic Matter

Table 6 represents the lack of stronger relation of mercury to other studied elements bound to hydrophilic compounds of organic matter. Only a limited amount of these relations might be observed. Nevertheless, it is important to mention that in the majority of cases, the dependency had a direct character. Taking all samples into account (without excluding samples 9 and 10), only the relation to Cu proved to be significant (c_c=0.85). For the rest of the elements, these two samples had to be neglected in order to find some dependency. As in the previous compounds of organic matter, the amount of Hg was correlated with the amount of sulphur, but the dependency was weaker. Higher correlation coefficients were revealed in the case of relation of Hg to Mg and Cu with the values of the c_c attaining 0.77 and 0.81, respectively.

In the case of S, similar relations were observed as those found in HA and the total content in organic matter. Namely, S was related to Cu, Zn and Pd with c_c ranging from 0.69 to 0.85. Nevertheless, new possible relation between S and Mg was discovered (c_c=0.80). Focusing on magnesium, in all compounds of organic

Table 5 Correlation coefficients between elements in fulvic acids

	Hg	Hg*	S	Mg	Mn	Fe	Cu	Zn	Pb
Hg	1.00								
Hg*	1.00	1.00							
S	−0.86	−0.93	1.00						
Mg	0.13	0.12	0.12	1.00					
Mn	−0.32	−0.40	0.42	0.15	1.00				
Fe	−0.52	−0.53	0.47	0.14	0.60	1.00			
Cu	0.14	−0.01	0.32	0.75	0.36	0.15	1.00		
Zn	0.18	0.34	0.18	0.47	0.27	−0.08	0.91	1.00	
Pb	0.07	0.09	0.32	0.26	0.07	−0.13	0.77	0.90	1.00

Asterisk "*" indicates without two samples with the highest concentration of Hg

Table 6 Correlation coefficients between elements in hydrophilic compounds

	Hg	Hg*	S	Mg	Mn	Fe	Cu	Zn	Pb
Hg	1.00								
Hg*	1.00	1.00							
S	0.17	0.62	1.00						
Mg	0.24	0.77	0.80	1.00					
Mn	−0.46	−0.48	−0.03	0.26	1.00				
Fe	−0.12	−0.13	−0.46	−0.23	0.48	1.00			
Cu	0.85	0.81	0.85	0.85	0.17	−0.02	1.00		
Zn	−0.18	0.48	0.75	0.68	0.30	0.10	0.92	1.00	
Pb	−0.25	0.54	0.69	0.59	0.19	−0.01	0.83	0.96	1.00

Asterisk "*" indicates without two samples with the highest concentration of Hg

matter, the relation with Cu was observed. However, the relation is strongest in the case of hydrophilic compounds. Newly established connection was observed between magnesium and zinc having the c_c=0.68. Contrarily, another relation observed in all compounds concerning Mn and Fe is weakest in the case of hydrophilic acids as the correlation coefficient equals only to 0.48. Aforementioned strong correlation among Cu, Zn and Pb was also confirmed investigating hydrophilic compound. The correlation coefficients are of the same magnitude as observed in total organic matter.

3.5 Correlations Between Total Contents of Elements

Comparing correlations gained from the total contents of investigated elements with the results from particular compounds of organic matter, a good correspondence among Hg and Cu, Zn and Pb was found (Table 7). Moreover, the relation of Hg to Mn and Fe has emerged. The correlation coefficients are higher with samples 9 and 10 being a part of the analysis, and in all cases, the relation was of a direct character.

In the case of sulphur, correlation coefficients are significantly weaker and the relations to the rest of studied elements were not observed. The relations among Cu, Zn and Pb were also confirmed, and the values of correlation coefficients correspond to those from organic matter. The most significant differences between the correlations of total contents and those gained from organic matter were found in the case of Mn and Fe. For these two elements, the correlation coefficients are higher in their total contents. Moreover, Fe was always directly related to the rest of the elements. Liaghati et al. (2003) reported the same character of Fe relations. Cu correlations to other elements were comparable or higher. However, the aforementioned relation with sulphur was not found. Similar relations were described by many other authors (Salizzato et al. 1998; Calace et al. 2005).

Table 7 Correlation coefficients between element total contents

	Hg	S	Mg	Mn	Fe	Cu	Zn	Pb
Hg	1.00							
S	0.56	1.00						
Mg	0.22	−0.30	1.00					
Mn	0.66	−0.18	0.31	1.00				
Fe	0.54	0.20	0.16	0.68	1.00			
Cu	0.83	0.25	0.20	0.86	0.81	1.00		
Zn	0.83	0.08	0.21	0.92	0.58	0.93	1.00	
Pb	0.99	0.13	−0.13	0.73	0.56	0.86	0.87	1.00

4 Conclusions

Most authors divide organic matter only into humic and fulvic acids. However, in this study, organic matter is considered as composed of humic acids, fulvic acids, hydrophilic compounds and hydrophobic neutral. Mn, Cu, Zn and Pb were bound particularly to hydrophilic compounds by an average of more than 87 %. In the case of iron, the situation was different so that only a minor portion of the element was bound to Hy and almost 80 % was bound to HA. S and Mg were distributed equally between humic acids and hydrophilic compounds and only a small proportion was bound to fulvic acids. Hg was distributed approximately uniformly in the organic matter. In all cases, amounts of elements bound to hydrophobic neutral organic matter were less than 0.4 % of the total amount bound to the organic material.

Further, the relations of various strengths between selected elements bound to individual fractions were observed. Results of the analysis show that the Hg content bound to the humic acids is inversely proportional to the content of Mg, Mn, Fe and Cu. However, this dependence was not confirmed by the samples with the mercury content above 10 mg kg^{-1}. In the case of fulvic acids, the relationship between Hg and S was observed and has again an inverse character. Correlations of the sulphur content with Cu, Zn and Pb were found also. They are pronounced in the total content in organic matter and also in all fractions except fulvic acids. Furthermore, strong relations were found among Cu, Zn and Pb. Moreover, in hydrophilic compounds, these elements are related to macronutrients (S and Mg). Finally, high correlation coefficients (>0.94) among Mg, Mn and Fe bound to humic acids were revealed.

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5.3 Publikovaná práce 3

Šípková A., Száková J., Coufalík P., Zvěřina O., Kacálková L., Tlustoš P. (2014): Mercury distribution and mobility in contaminated soils from vicinity of waste incineration plant. Plant, Soil and Environment, *60*: 87-92.

Mercury distribution and mobility in contaminated soils from vicinity of waste incineration plant

A. Šípková[1], J. Száková[1], P. Coufalík[2], O. Zvěřina[2], L. Kacálková[3], P. Tlustoš[1]

[1]*Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic*
[3]*Department of Biology, Faculty of Science, University of Hradec Králové, Hradec Králové, Czech Republic*

ABSTRACT

The potential bioavailability of Hg from soil might be estimated by a variety of chemical extraction procedures, differing in the extraction agent, its concentration, the sample weight, and the time of extraction. In this study, a comparative analysis of several extraction methods, commonly used for obtaining the mobile and potentially mobilizable phase of the mercury was carried out. Concentrated HNO_3, 0.01 mol/L $Na_2S_2O_3$, 0.05 mol/L EDTA and 0.11 mol/L CH_3COOH were used as the single extraction agents. Moreover, the sequential extraction was performed. This procedure involved the following fractions: water soluble Hg, Hg extracted in acidic conditions, Hg bound to humic substances, elemental Hg and mercury bound to complexes, and residual Hg. The results showed that even strong acid HNO_3 is unable to release the mercury tightly bound to the soil matrix. This particular method with microwave digestion is commonly used for the estimation of anthropogenic pollution. Conversely, the lowest mercury yield was obtained using the acetic acid as the single extraction agent. In this case, the concentrations were below 0.15% of the total Hg content, which is a proportion generally defined as bioavailable to plants.

Keywords: bioavailability; extraction methods; inductively coupled plasma mass spectroscopy; advanced mercury analyzer AMA-254

Mercury can be released from soil by different extraction procedures. These procedures enable the determination of particular species present, the varying amounts of Hg bound to soil, and also the bioavailability and toxicity. The least tightly bound water-soluble fraction is obtained by the simple extraction using deionised water (Rodrigues et al. 2010). It estimates Hg portion present in soil pore water. This fraction of mercury is usually not in the form of the water-soluble ionic species but as species bound to dissolved organic matter; nevertheless, not directly on carbon (Biester and Scholz 1996). The application of diluted CH_3COOH as an extraction agent belongs to the methods simulating approximately composition of the soil solution similarly as other mild extraction procedures such as $CaCl_2$ solution (Novozamsky et al. 1993). The extraction solutions based on the chelating agents such as EDTA or DTPA represent another more efficient possibility. These agents are able to displace metals from insoluble organic or organometallic complexes in addition to those adsorbed on inorganic soil components (Rao et al. 2008). The other species are mercury fractions bound on iron sulphides, manganese hydroxides and carbonates, and Hg bound to the minerals. This strongly bound mercury species can be obtained by acids, e.g. HCl (Lechler et al. 1997).

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In soil, mercury can be bound very tightly to sulphur forming the insoluble HgS (Boszke et al. 2008). This phase of mercury can be obtained either by aqua regia extraction in a microwave oven (Fernándes-Martínez and Rucandio 2003), or using the saturated Na_2S solution from the residue remaining after the extraction procedures (Revis et al. 1989). The effect of the concentration of $Na_2S_2O_3$ on the extraction efficiency was in detail studied by Issaro et al. (2010).

The proportion of Hg, which is not firmly bound to the silicate matrix of soil, is often obtained by using HNO_3 as an extraction agent (Reis et al. 2010). The mercury concentration in these extracts enables an estimation of the amount of Hg from anthropogenic sources. In some cases, concentrated nitric acid combined with HCl (Teršič et al. 2011) or H_2SO_4 (Mailman and Bodaly 2005) is employed for total mercury content determination. It might also be used in sequential extraction procedures to obtain elemental Hg (Bloom et al. 2003). Sequential extractions are suitable methods for the mercury speciation analysis of solid samples. However, there is no universal sequential extraction concerning the individual Hg fraction determination. Several approaches were demonstrated by many authors (Renneberg and Dudas 2001, Sánchez et al. 2005, Han et al. 2006, Liu et al. 2006).

In this work, four various extraction agents as well as sequential extraction were applied for the assessment of Hg mobility and fractionation in one anthropogenically contaminated soil.

MATERIAL AND METHODS

Samples. Ten soil samples were collected from the former waste incineration plant in the suburb of Hradec Králové, Czech Republic. The selection of sampling sites was based on the experiment of Kacálková et al. (2009). Samples were collected in the vicinity of points 3 and 5. The plot 3 represented the average values of contamination (our samples 1–5), while extreme Hg content was measured in the plot 5 closer to the plant (our samples 6–10). Samples were taken from the top layer (0–30 cm), air-dried, sieved < 2 mm and kept at 4°C for several weeks. Following characteristics of soil were measured: pH_{CaCl_2} (Novozamsky et al. 1993), organic matter content (Sims and Haby 1971) and cation exchange capacity (ISO 1994). Total content of S was determined by X-ray fluorescence spectrometry (Spectro IQ, Kleve, Germany), mercury analyser AMA-254 (LECO model, Altec, Czech Republic, Plzeň) was used for total Hg determination. All experiments were carried out in three repetitions.

Extractable fractions of mercury. Four extraction agents HNO_3, $Na_2S_2O_3$, EDTA, and CH_3COOH were used to determine the mobile and mobilizable phases of Hg. For determination of potentially mobilizable mercury portions, 0.25 g of each sample was decomposed in 5 mL of concentrated HNO_3. The reaction mixture was digested at 280°C during 75 min by using microwave heating in MLS ultraCLAVE IV system (Milestone, Leutkirch im Allgäu, Germany) and then milli-q water was added to a final volume of 50 mL. The mild extraction procedures were performed as follows: (i) $Na_2S_2O_3$ extraction proceeded overnight in 10 mL of 0.01 mol/L solution, which was added to 1 g of the sample; (ii) 0.05 mol/L EDTA was adjusted with NaOH to pH 7. Subsequently, 1 g of soil was added to 10 mL of extraction solution and shaken for 1 h; (iii) 0.5 g of sample was added to 10 mL of 0.11 mol/L solution of CH_3COOH and shaken overnight. Subsequently, all the samples were centrifuged for 10 min at 3000 rpm.

Hg content in all extracts was measured by inductively coupled plasma mass spectrometry (Agilent 7700x, Agilent Technologies Inc., Santa Clara, USA). The isotope Hg(202) was measured and Pt(195) was used as an internal standard in concentration 10 µg/L. As reference material, San Joaquin Soil (SRM 2709) was utilized (theoretical Hg content is 1.4 ± 0.08 mg/kg; obtained recovery was 98%).

Sequential extraction. The sequential extraction procedure was designed by modifying the existing extraction schemes (Bloom et al. 2003, Boszke et al. 2008). 0.1 g of each sample was leached into 10 mL of chloroform, shaken for 3 h and centrifuged. This step was considered as F0 and residue obtained after the extractions was used in the next procedure. The soil/liquid ratio was the same for all extraction reagents. The extraction procedure was performed on the bulk samples according to the following scheme: F1 with redistilled water – Hg leachable in water, F2 with 0.5 mol/L HCl – Hg leachable under acidic conditions, F3 with 0.2 mol/L KOH – Hg bound to humic substances, F4 with 50% HNO_3 – elemental Hg and complexes, and F5 is solid residue. Experiments were carried out at laboratory temperature on shakers GFL 3006 (Burgwedel, Germany) at 300 rpm and the extraction

time was 18 h in all fractionation steps. Subsequently, extracts were separated from a solid phase by centrifugation for 10 min at 4000 rpm. The extraction agents from each single step were used as blank samples and the mercury content in all extracts was determined by using the AMA-254 analyser.

RESULTS AND DISCUSSION

Soil characteristic. The pH_{CaCl_2} of our samples equalled 7.18 ± 0.28 and the cation exchange capacity was 131.7 ± 3.8 $mmol_+$/kg. An oxidizable carbon and sulphur content ranged between 1.2–3.4% and 0.28–0.56%, respectively. The organic matter composition and/or content as well as mercury affinity to the individual fractions were described in the other works (Kacálková et al. 2009, Šípková et al. 2014). The measurements of the total Hg content indicated that in the vicinity of the former waste incineration plant there are places with relatively low Hg concentration; however, the sample with the amount of mercury reached even almost 29 mg/kg (Table 1). Kacálková et al. (2009) reported the same variability of the mercury content present in the same area showing the concentrations ranging from 0.15 to 12 mg/kg. Moreover, the highest organic matter content was observed in the samples representing the highest Hg values.

Single extractions. The extraction yields by individual extraction agents are shown in Table 1 and summarized in Figure 1 as relative Hg portions extractable from the total content. HNO_3 released around 50% of total Hg content in 8 of 10 samples. In the case of the most contaminated sample the concentration was approximately 70% and even 96%. Using $Na_2S_2O_3$ as an extraction agent the mercury yield was also highest in the last two samples. The average yield of these particular samples attains approximately 20% of the total. In other samples, the content of mercury ranged from 1.2% to 3.4%. Thus, it might be inferred that in places with higher anthropogenic contamination, the presence of mercury species bound to sulphur is higher than in less contaminated samples. In the case of the most contaminated site the rate of extractable Hg using $Na_2S_2O_3$ corresponds with the results reported by Issaro et al. (2010). They showed that the extraction yield of $Na_2S_2O_3$ usually reaches 50 ± 5% of Hg obtained by HNO_3 extraction from soils with high Hg levels from agricultural processes near Paris, France. Moreover, they showed that the rate of extractable Hg by $Na_2S_2O_3$ is decreasing with decreasing total Hg content. On the other hand, Subirés-Munoz et al. (2011) obtained by this type of extraction approximately 20% of the total Hg content. Their soils originated from the mining district of Almadén, Spain with high background Hg levels and influence of anthropogenic activities.

Further, using chelating agent EDTA, the values of extractable Hg ranged between 0.5% and 2% of the total Hg content, in all the experimental samples. These results correspond to those reported by Subirés-Munoz et al. (2011) who obtained less than 2% of the total Hg. This small variability suggests that the amount of mercury, which might serve as a source for plant uptake, is similar both in more and less contaminated places. Coinciding results were also obtained by extraction with a solution of CH_3COOH, which simulates natural conditions of soil solution. The yields of CH_3COOH were below 0.15% and confirmed no significant differences. The low availability therefore seems to indicate

Table 1. Total and extractable contents of mercury after single extractions (µg/kg)

Sample	Total	HNO_3	$Na_2S_2O_3$	EDTA	CH_3COOH
1	1070	578	14.3	6.92	0.49
2	236	132	7.04	1.99	0.34
3	415	201	7.82	5.21	0.37
4	419	234	8.03	8.80	0.48
5	550	273	10.4	6.27	0.45
6	2050	1132	52.1	9.37	0.73
7	396	223	13.5	2.95	0.44
8	580	300	6.95	2.94	0.78
9	28 800	20 108	5877	481	11.3
10	10 500	10 040	2094	64.7	8.64

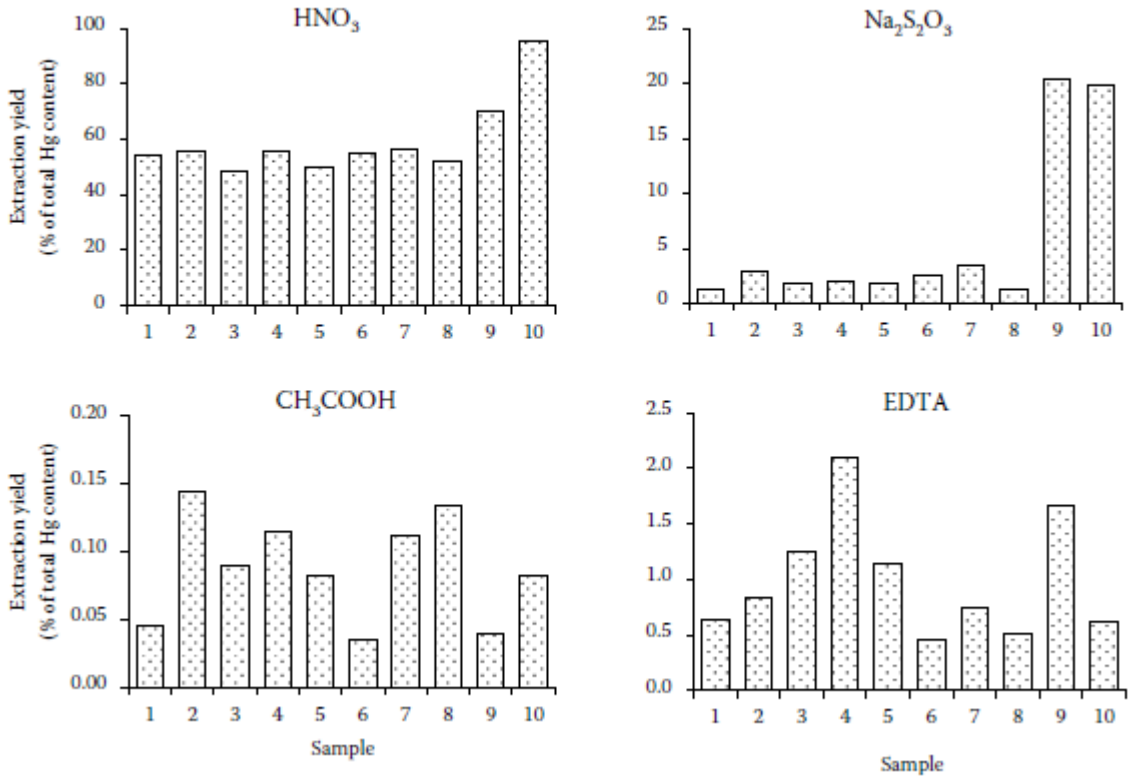

Figure 1. Extraction yield after single extraction using individual chemical agents

that mercury is strongly bound to sulphide phases and/or to insoluble clay minerals and organic matter in the samples (Rodrigues et al. 2010).

Sequential extraction. Mercury contents extracted in each step (F1–F5) are reported in Table 2. The sum of the amount removed by each extraction was in good agreement with the total amounts obtained by AMA-254. Values of recoveries ranged from 93% to 107%.

First fraction representing the total content of organomercury compounds (F0) was below the quantification limit (2 µg/kg) in all samples. Mobile fractions F1 and F2 were also very low for the majority of samples. Hg leachable in water was detectable only in the samples No. 9 and 10, containing the highest level of the total mercury content. Rodrigues et al. (2010) found similar value in samples from vicinity of chlor-alkali plant. The mercury leachable under acid conditions was measured in the aforementioned most contaminated samples and its content was less than 8%. Such high mercury content discovered in soil from the cinnabar refinery and mine by Miller et al. (1995). Low amount of this Hg species was found also in sample 6, which is the third site with high mercury concentration.

In the case of F3, Hg species values obtained were substantially higher. The semi-mobile mercury contents ranged from 18% to 30%. These Hg species bound to organic matter were regarded as stronger complexes and thus have limited mobility (Liu et al. 2006). Almost identical scale of the mercury fractions observed in the study of mercury mobility and bioavailability Boszke et al. (2008). The organic carbon content was also similar in this particular soil. Teršič et al. (2011) described mercury distribution in very contaminated soil from mining district of Idrija, Slovenia and in their study, Hg bound to organic or mineral soil matter reached from 35% to 40% of the total mercury content. These higher values can be connected with acidic pH because mercury is particularly bound to organic matter under the low pH (Schwesig et al. 1999). On the contrary, in our samples 9 and 10 the percentage ratios of this species were approximately 9% and differences among the amounts of mobile and semi-mobile fractions were relatively low.

Table 2. Mercury contents in individual fractions after sequential extraction (µg/kg)

Sample	F1	F2	F3	F4	F5
1	*	*	270	610	150
2	*	*	70	130	20
3	*	*	120	250	50
4	*	*	120	240	50
5	*	*	160	320	30
6	*	30	360	1400	220
7	*	*	80	280	30
8	*	*	130	350	70
9	400	2300	2500	16 200	5300
10	160	620	960	4900	3200

*data below the quantification limit (2 µg/kg). F1 – redistilled water; F2 – 0.5 mol/L HCl; F3 – 0.2 mol/L KOH; F4 – 50% HNO_3; F5 – residue

The highest Hg content was found in the case of non-mobile fraction, i.e. elemental mercury and Hg bound to complexes (F4). Although the sequential extraction by Lechler et al. (1997) was carried out with distinct extraction agent, results of Hg speciation showed the highest proportion of elemental mercury in the soil samples from former amalgamation milling of Ag-Au ores in Nevada, USA. Reis et al. (2010) divided the mercury species in a different way and their semi-mobile fraction included also elemental mercury. Thus, their values obtained from samples from the industrial complex and sulphide mine in Portugal, were similar to the results obtained in this study and the proportion ranged between 63% and 97%.

The content of mercury in solid residues after the extraction was in the majority of samples below 12%. However, in more contaminated samples these values were higher. Obviously, the substantial proportions of residues content are species bound to silica or Hg sulphides. Liu et al. (2006) found around 10% of Hg species bound to sulphur, which corresponds to our hypothesis.

In order to describe the mobility and bioavailability of mercury, several extraction agents and the sequential extraction described above were applied on soil collected near Hradec Králové, Czech Republic. In the area, several samples with high Hg concentration were found and the highest amount reached almost 29 mg/kg. Nevertheless, the total Hg content mostly achieved less than 2 mg/kg.

Based on the results of analyses, only a low amount of a mobile fraction, having the highest toxicity, was determined. In the majority of samples, which originated from the surroundings of the former waste incineration plant, less that 2% of the total Hg content was found. The lowest mercury yield was obtained using the acetic acid as a single extraction agent, which is a proportion generally defined as biologically available to plants (Quevauviller et al. 1993). In all experiments, the concentrations were below 0.15%.

Conversely, elemental Hg and mercury complexes were present in the highest amount and the proportion of this fraction ranged between approximately 50% and 70%. In the case of Hg bound to humic acids representing the semi-mobile species was determined as the second highest. Contrarily, higher Hg amounts were measured in the residuals of the two most contaminated samples. The results of this study concern on specific site and therefore the outcomes should not be taken as general characteristics of all anthropogenically contaminated soils.

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Corresponding author:

Ing. Adéla Šípková, Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, Katedra agroenvironmentální chemie a výživy rostlin, Kamýcká 129, 16521 Praha 6-Suchdol, Česká republika
e-mail: sipkovaa@af.czu.cz

5.4 Publikovaná práce 4

García-Sánchez M., Šípková A., Száková J., Kaplan L., Ochecová P., Tlustoš P. (2014): Applications of organic and inorganic amendments induce changes in the mobility of mercury and macro- and micronutrients of soils. The Scientific World Journal. DOI: 10.1155/2014/407049.

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Research Article

Applications of Organic and Inorganic Amendments Induce Changes in the Mobility of Mercury and Macro- and Micronutrients of Soils

Mercedes García-Sánchez, Adéla Šípková, Jiřina Száková, Lukáš Kaplan, Pavla Ochecová, and Pavel Tlustoš

Department of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Suchdol, 16521 Prague 6, Czech Republic

Correspondence should be addressed to Jiřina Száková; szakova@af.czu.cz

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Both soil organic matter and sulfur (S) can reduce or even suppress mercury (Hg) mobility and bioavailability in soil. A batch incubation experiment was conducted with a Chernozem and a Luvisol artificially contaminated by 440 $mg \cdot kg^{-1}$ Hg showing wide differences in their physicochemical properties and available nutrients. The individual treatments were (i) digestate from the anaerobic fermentation of biowaste; (ii) fly ash from wood chip combustion; and (iii) ammonium sulfate, and every treatment was added with the same amount of S. The mobile Hg portion in Chernozem was highly reduced by adding digestate, even after 1 day of incubation, compared to control. Meanwhile, the outcome of these treatments was a decrease of mobile Hg forms as a function of incubation time whereas the contents of magnesium (Mg), potassium (K), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and phosphorus (P) were stimulated by the addition of digestate in both soils. The available calcium (Ca) contents were not affected by the digestate addition. The experiment proved digestate application as the efficient measure for fast reduction of mobile Hg at extremely contaminated soils. Moreover, the decrease of the mobile mercury portion was followed by improvement of the nutrient status of the soils.

1. Introduction

Industrial activities have increased the proportion of Hg in the atmosphere and oceans and have contaminated a number of local environments [1]. From the ecotoxicological point of view, critical limits of Hg (given as soil element contents above which unacceptable effects are expected) are substantially lower than values derived for other metals such as Cd, Cu, Ni, Pb, and Zn [2]. Li et al. [3] compared the mobility and plant-availability of risk elements from industrially contaminated soil where the soil-to-plant transfer coefficients were in the order of Cd > Zn > Cu > Hg > As > Pb, confirming the relatively low availability of soil Hg for various vegetables. Rodrigues et al. [4] observed the water-soluble contents of Hg in highly contaminated sediment and soil samples (total Hg contents even higher than 3000 $mg \cdot kg^{-1}$) to be less than 1.2% of the total Hg content. Boszke et al. [5] classified the divalent and elemental Hg bounds to humic matter/organic matter as the "semimobile" element portion and observed low portions of the water-soluble Hg species as well.

Luo et al. [6] suggested that soil organic matter and nitrogen were the important sinks for Hg in the soils. The good capacity of Hg for adsorption and complexation in the solid media resulted in limited bioaccessibility of this element, which was reported by Hassen et al. [7]. Distribution coefficients for Hg^{2+} binding by humic acids were determined by Khwaja et al. [8], confirming that the calculated concentration of free Hg^{2+} at equilibrium is very low. Also, Heeraman et al. [9] observed decreasing Hg mobility and plant-availability in the organic matter-treated soil. The importance of soil organic matter for Hg mobility and bioavailability in soil samples is known and well described [5, 10]. As observed by Yao et al. [11], the addition of humus can either suppress or promote Hg bioavailability depending on the soil composition. In

this context, the effect of a particular humus fraction on Hg bioavailability is related to its ability to convert Hg bound by solid phases into soluble complexes, as well as the stability of the released complexes. On the contrary, the presence of dissolved organic matter (DOM) can significantly reduce maximum Hg adsorption capacity and even promote Hg desorption from the soils [12].

Zagury et al. [13] evaluated the potential mobility and plant-availability of Hg in the highly contaminated soils by chlor-alkali plants, where the total Hg contents in soil reached up to 11500 mg·kg^{-1}. Although the water extractable Hg portion was relatively low with regard to the high total content, it represented significant concentrations correlating with Hg uptake by experimental plants.

Reis et al. [14] observed that the presence of Hg in the mobile phase could be related to Mn and aluminum (Al) soil contents. A positive relation between Hg in the semimobile fraction and the Al content was also observed. On the contrary, organic matter and S contents contributed to Hg retention in the soil matrix, reducing the mobility of the metal. Sulfide minerals are known to be effective adsorbents for Hg(II) being the primary sink for Hg in the environment [15]. In this context, Hesterberg et al. [16] demonstrated the preferential binding of Hg(II) to reduced organic S sites. Subsequently, similar observations were provided and described in soils as mentioned by Remy et al. [17]. Concentration of MeHg is negatively correlated with soil total organic matter and total S and is influenced by the soil total Hg concentration [17]. Åkerblom et al. [18] highlighted that long-term chronic SO_4^{2-} deposition at rates similar to those found in polluted areas of Europe and North America increase the capacity of peatlands to methylate Hg and store MeHg. Competitive relationships between Hg and other metals in soil were observed by Jing et al. [19], where desorption of adsorbed Hg increased with elevated concentrations of added Cu or Zn.

In our investigation, a laboratory batch incubation experiment was conducted with Chernozem and Luvisol differing in their physicochemical parameters and the available nutrient contents. Digestate, the bio-waste originating from biogas production plants, was applied as a S-rich source of organic matter. Alternatively, wood ash from biomass combustion plants was applied as a different source of S and other macro- and micronutrients. As proven by Ochecová et al. [20], the plant-availability of the risk elements in the contaminated soil decreased after ash application, whereas the nutrient contents tended to increase. To separate the effect of organic matter and S in the soil, inorganic source of S, ammonium sulfate, $(NH_4)_2SO_4$, was applied, as well. The main objectives of the study were as follows: (i) to assess the ability of the individual treatments to immobilize Hg in the artificially contaminated soil and (ii) to evaluate the potential interactions between Hg sorption in the experimental soil and the mobility of the essential macro- and microelements in these soils.

2. Materials and Methods

2.1. Soils and Ameliorative Materials. The following two soils, differing in their physicochemical characteristics, were selected for the experiment: (i) uncontaminated Chernozem with a cation exchange capacity (CEC) of 230 mmol kg^{-1}, a pH level of 7.5, and an oxidizable carbon content (Cox) of 2.6%, and (ii) uncontaminated Luvisol with a CEC of 145 mmol kg^{-1}, a pH level of 6.5, and a Cox of 1.7%. Nutrient contents and other characteristics in both soil samples are summarized in Table 1. Soils were sampled from a depth of 20 cm, immediately after which they were homogenized, sieved through a 5 mm diameter mesh, and kept at room temperature. For the experimental incubation soils, samples were sieved again using a 2 mm mesh and kept at 4°C until use. The fly ash (pH 12.1) was produced by the combustion of wood ash produced in two reactors (1.8 MW and 0.6 MW). The digestate sample (pH 8.2) originated from a biogas station (1732 kW/h), where the digested material consisted of sugar beet pulp (50%), marc of fruit (42%), and silage maize (8%). The macro- and micronutrient contents in both ameliorative materials are summarized in Table 2. As an inorganic amendment, solid particles of $(NH_4)_2SO_4$ were used (Reagent from Fisher Scientific).

Table 1: Main physicochemical characteristics of the experimental soils.

Soil type	Luvisol	Chernozem
NRSC Soil Texture	Silt loam	Silt loam
Clay (<0.002 mm) [%]	5.38	2.18
Silt (0.002–0.05 mm) [%]	68.14	71.80
Sand (0.05–2 mm) [%]	26.48	26.03
Location	50°4′22″N, 14°10′19″E	50°7′40″N, 14°22′33″E
Altitude (m a.s.l.)	410	286
P Mehlich III* (mg kg^{-1})	100	91
K Mehlich III* (mg kg^{-1})	80	230
Mg Mehlich III* (mg kg^{-1})	110	240
Ca Mehlich III* (mg kg^{-1})	3600	9000

*Šípková et al. [23].

2.2. Experimental Design. For the experimental incubation soils, 100 g of Chernozem and Luvisol soils were placed into polypropylene bottles and immediately after were brought to 60% moisture saturation. Then, half of the soil samples were artificially contaminated with Hg by adding 60 mg of $HgCl_2$ to reach a concentration of 440 mg·kg^{-1} of Hg. Subsequently, organic and inorganic amendments: (1) ash, (2) digestate, and (3) $(NH_4)_2SO_4$, were applied both to contaminated and noncontaminated soils. The rate of amendment was calculated for 600 mg S per kg of soil as follows: (1) ash: 1.5 g, (2) digestate: 10 g, and (3) NH_4SO_4: 0.25 g per bottle.

Soils that were contaminated and noncontaminated with Hg were thoroughly mixed and incubated at 28°C for 21 days. To evaluate the mobility of Hg in both soils and interactions with macro- and micronutrients as well, soil samples were collected after 1, 2, 3, 4, 7, 14, and 21 days of incubation. Three replicates were set up per treatment.

Table 2: Nutrient contents in the dry matter of ameliorative materials.

Element	Fly-ash	Digestate
P (%)	1.29 ± 0.01	1.20 ± 0.01
K (%)	7.74 ± 0.02	2.12 ± 0.01
Mg (%)	1.44 ± 0.02	0.49 ± 0.02
Ca (%)	13.4 ± 0.1	3.15 ± 0.01
S (%)	4.07 ± 0.01	0.60 ± 0.01
Cu (%)	0.020 ± 0.001	0.004 ± 0.001
Fe (%)	2.79 ± 0.01	0.18 ± 0.01
Mn (%)	1.29 ± 0.01	0.02 ± 0.00
Zn (%)	3.58 ± 0.08	0.03 ± 0.00

2.3. Extraction of Soluble Portions of Hg and Macro- and Micronutrients. For the determination of bioavailable element portions in soils during the experiment, 0.5 g of each sample was added to 10 mL of 0.11 mol L^{-1} solution of CH_3COOH and shaken overnight [21]. Each extraction was carried out in three replicates. For the centrifugation of extracts, a Hettich Universal 30 RF (Germany) instrument was used. The reaction mixture was centrifuged at 3000 rpm (i.e., 460 g) for 10 minutes at the end of each extraction procedure, and the supernatants were kept at 6°C prior to measurements. Prior to the analysis, extracts were acidified with a mixture of acids (HNO_3 : HCl = 3 : 1). For the determination of nutrient status in the experimental soils before the experiment, the Mehlich III extraction procedure (0.2 mol L^{-1} of CH_3COOH + 0.25 mol L^{-1} of NH_4NO_3 + 0.013 mol L^{-1} of HNO_3 + 0.015 mol L^{-1} of NH_4F + 0.001 mol L^{-1} of EDTA) at the ratio of 1 g of soil per 10 mL of the extraction mixture for 10 min [22] was applied.

2.4. Determination of Hg. Hg content in the extracts was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA). The auto-sampler ASX-500, a three-channel peristaltic pump, and MicroMist nebulizer equipped the ICP-MS. Calibration solutions were prepared in diluted single element ICP-MS standards as 0.1–100 μg L^{-1} for Hg and the isotope Hg(202) was measured. As an internal standard, Pt(195) was used at the concentration of 100 μg L^{-1}.

2.5. Determination of Macro- and Micronutrients. Inductively coupled plasma-atomic emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) equipped with a two-channel peristaltic pump, a Struman-Masters spray chamber, and a V-groove pneumatic nebulizer made of inert material was applied for the determination of Cu, Fe, Mn, Zn, P, and S in the extracts (the experimental conditions were as follows: power of 1.2 kW, plasma flow of 15.0 L·min^{-1}, auxiliary flow of 0.75 L·min^{-1}, nebulizer flow of 0.9 L·min^{-1}), whereas flame atomic absorption spectrometry (F-AAS, Varian 280FS, Varian, Australia; air flow of 13.5 L·min^{-1}, acetylene flow of 2.2 L·min^{-1}, burner height of 13.5 mL, nebulizer uptake rate of 5 mL·min^{-1}) was used for Ca, Mg, and K determination in the extracts.

2.6. Determination of Total Nutrient Contents in the Ameliorative Materials. For determination of total element contents in the ash, nondestructive X-ray fluorescence (XRF) spectrometry (Spectro IQ, Kleve, Germany) was used; the target material was palladium and the target angle from the central ray was 90°. The focal point was a 1 mm × 1 mm square, and the maximum anode dissipation was 50 W with 10 cfm forced-air cooling. The instrument was equipped with the Barkla crystal HOPG. The tested samples were pressed into pellets; this involved mixing 4.0 g of ash (particle size 15–20 μm) with 0.9 g of the binding additive (HWC Hoechst wax, Germany) for 10 min with a pressing power of 80 kN. The determination was performed in the Institute of Rock Structure and Mechanics, Academy of Sciences of the Czech Republic.

The digestate sample was decomposed by pressurized wet ashing as follows: aliquots (~0.5 g) of air-dried samples were decomposed in a digestion vessel with 10 mL of *Aqua regia* (i.e., nitric and hydrochloric acid mixture in the ratio 1 : 3). The mixture was heated in an Ethos 1 (MLS, Germany) microwave assisted wet digestion system for 33 min at 210°C. ICP-OES and F-AAS were then applied as described in the previous subchapter.

2.7. Statistics. The data obtained were subjected to Dixon's test for the identification of outliers (significance level α = 0.05) using Microsoft Office Excel 2007 (Microsoft Corporation, USA). Subsequently, one-way analysis of variance was used at the significance level α = 0.05 using the Statistica 12 program (StatSoft, USA).

3. Results and Discussion

3.1. Changes in Hg Mobility in the Treated Soils. The mobile Hg contents affected by the individual treatments and their variability during the incubation experiment are summarized in Figure 1. In the treatments without artificial Hg application, the mobile Hg contents were under the detection limit of ICP-MS. Similarly to our previous observations [23], Ruggiero et al. [24] also documented that most of the Hg in the long-term polluted soils was scarcely mobile and available. The Hg contents in digestates and fly ash are usually low as well [25, 26] and did not affect the mobile portions of Hg in our experiment. The extractable Hg contents differed according to the physicochemical parameters of the used soils and to the individual treatments. In Chernozem, the extractable Hg contents were low regardless of the treatment at the beginning and end of incubation. Within the 3rd and 7th day of incubation, the mobile Hg portions increased in all treatments (including control) except for the digestate. Similar course of Hg mobility changes were observed by Bower et al. [27] in the experiments studying the mercury adsorption onto pyrite indicating the formation of nonmobile sulfides over time. In the Luvisol, the mobile Hg portions decreased during the incubation, whereas they dropped to the levels reached in Chernozem by the end of the experiment. As stated by Müller et al. [28] soil Hg contamination can cause reduced microbial biomass at the contaminated sites.

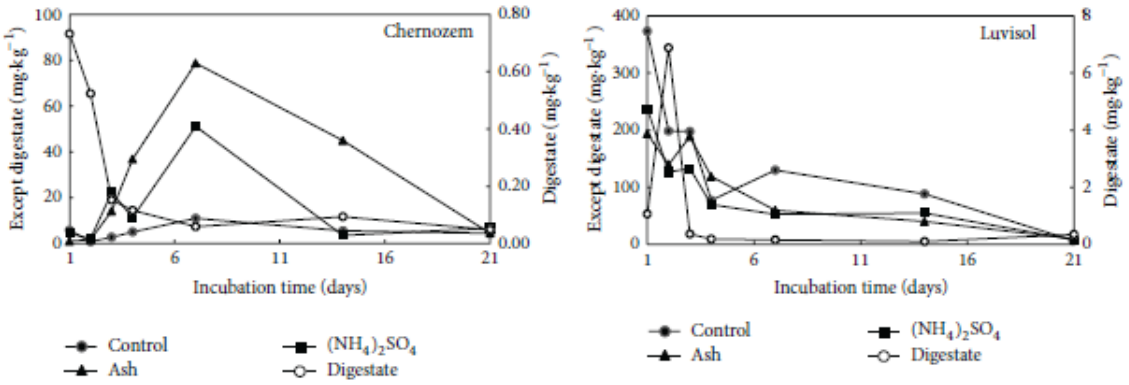

FIGURE 1: The concentrations of Hg extractable with 0.11 mol L^{-1} acetic acid within the incubation experiment (mg·kg^{-1}) according to the individual treatments.

However, some microorganisms have developed mechanisms to adapt to Hg, that is, Hg-resistant bacteria. Thus, the changes in Hg mobility observed throughout the experiment could be partially attributed to different communities of soil microorganism present in both Chernozem and Luvisol.

Therefore, among the individual treatments, digestate was shown to be the most effective Hg immobilizing agent, whereas fly ash seemed to be less effective, and no significant difference was reported comparing the ammonium sulfate treatment and untreated control except the faster increase of mobile Hg content in 3rd and 4th days of the incubation indicating potential role of increased portion of mobile sulfur as mentioned below. The effectiveness of individual treatments as well as the temporal changes in mobile Hg portions were also affected predominantly by the soil where higher sorption capacity and organic matter content in Chernozem resulted in lower mobile portions of Hg in all the treatments except increased mobility of Hg after ash application in 7th and 14th day of the incubation. These results also indicated that S content in the ameliorative materials was not the main factor controlling the Hg mobility in the soils. Luo et al. [6] reported a low relationship between S and Hg contents in soils with low total organic carbon (~2%), as in our case, where the carbon content was not increased by the addition of ammonium sulfate and ash. In the opposite, the Hg behaviour in soils strongly differed if digestate with high content of both S and total carbon content was applied.

Higher organic carbon content in the soil can enhance both soil microbial activities and the retention of total Hg and MeHg in soil [29]. Soil microorganisms need essential metals for their metabolism, which are often required in low concentrations and act as enzyme cofactors [30]. Therefore, high contents of macro- and micronutrients in both ash and digestate (Table 2) can be beneficial for the enhancement of the microbial activity in soils. Limited Hg mobility *via* complexation with soil organic matter was already described [9]. Ravichandran [31] reviewed the formation of extremely strong ionic bonds between Hg and reduced S sites in soil organic matter supporting the importance of the mutual role of S and organic matter in Hg immobilization in soil. Therefore, the Hg desorption increased with elevating concentrations of dissolved organic matter [10]. In our case, the dissolved organic matter after 1 day of incubation varied between 71.9 mg·kg^{-1} (control) and 1070 mg·kg^{-1} (digestate) in Chernozem and between 21.2 mg·kg^{-1} (control) and 56.4 mg·kg^{-1} (digestate) in Luvisol. After 7 days of incubation, the DOM contents increased even 22-fold in the digestate-treated Luvisol, whereas the maximum 1.5-fold increase was observed in Chernozem. Therefore, our results indicate that more complex factors can change the Hg mobility in soil than solely the content and solubility of organic carbon in soil. For example, the affinity of Hg to bind to metal oxides should be taken into account [32]. Also, the role of some soil bacteria which are able to degrade Hg compounds into metallic Hg by the action of specific enzymes encoded by the *mer* genes and then be released into the surrounding environment should be considered [33]. Thus, the decrease of mobile Hg in soil could be partially figured in the volatilization of this element during incubation. This assumption remains to be verified in further research. In our investigation, the experiments were concerned on the description of potential decrease of mobile Hg content without exact resolution between immobilization/volatilization ratios after the individual treatments.

3.2. The Effect of Hg and/or Ameliorative Materials on Mobile Contents of Macro- and Micronutrients in the Soil. The mobile macro- and microelement contents affected by the individual treatments and/or duration of incubation are summarized in Tables 3, 4, 5, 6, 7, 8, 9, 10, and 11. The presence of digestate showed a predominant effect on the mobile portions of most of the elements among all the treatments. The mobile element contents significantly increased after digestate application for most of the determined nutrients, except Ca and Cu in Chernozem (because of its low availability in the ameliorative materials). More apparent increase of mobile element contents after application of digestate was observed for Luvisol compared to Chernozem due to higher sorption capacity of Chernozem in accordance with their higher CEC level. For example, Table 4 shows 5-fold increase of mobile Mg

Table 3: The concentrations of Ca extractable with 0.11 mol L^{-1} acetic acid within the incubation experiment (mg·kg^{-1}); the averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns; data are presented as mean ± standard deviation ($n = 3$).

Chernozem	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	7555 ± 331^{a}	7655 ± 183^{a}	8390 ± a213^{a}	9674 ± 219^{a}	9034 ± 238^{ab}	9856 ± 1191^{ab}	6040 ± 270^{a}
Control + Hg	8074 ± 260^{a}	7694 ± 359^{a}	7467 ± a677^{a}	9607 ± 193^{a}	8891 ± 702^{a}	8951 ± 236^{ab}	7865 ± 983^{abc}
Digestate	8282 ± 735^{a}	8094 ± 155^{a}	8049 ± a687^{a}	10122 ± 206^{a}	8886 ± 362^{a}	11004 ± 926^{b}	12402 ± 2330^{bc}
Digestate + Hg	8108 ± 232^{a}	9656 ± 282^{a}	7824 ± a454^{a}	11360 ± 1521^{a}	10104 ± 205^{b}	10232 ± 499^{ab}	11286 ± 1243^{c}
Ash	8448 ± 548^{a}	8246 ± a282^{a}	8746 ± a272^{a}	9898 ± 213^{a}	9661 ± 466^{ab}	9633 ± 203^{ab}	9835 ± 2134^{abc}
Ash + Hg	8164 ± 921^{a}	8072 ± a511^{a}	8717 ± a611^{a}	10910 ± 898^{a}	9862 ± 300^{ab}	10599 ± 387^{ab}	9359 ± 1118^{abc}
$(NH_4)_2SO_4$	7330 ± 57^{a}	7409 ± a201^{a}	8037 ± a311^{a}	10134 ± 1290^{a}	8942 ± 307^{a}	8893 ± 990^{a}	10045 ± 2115^{abc}
$(NH_4)_2SO_4$ + Hg	8041 ± 796^{a}	7393 ± a359^{a}	8350 ± 1151^{a}	9820 ± 207^{a}	8991 ± 382^{ab}	10238 ± 790^{ab}	7031 ± 3008^{abc}
Luvisol	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	1404 ± 96^{a}	1401 ± 73^{a}	1372 ± 56^{a}	1691 ± 51^{ab}	1671 ± 90^{a}	1764 ± 223^{a}	1273 ± 125^{a}
Control + Hg	1450 ± 93^{a}	1407 ± 38^{a}	1396 ± 45^{a}	1635 ± 52^{a}	1575 ± 107^{a}	1655 ± 89^{a}	1274 ± 283^{a}
Digestate	3558 ± 121^{b}	2593 ± 574^{b}	2733 ± 492^{b}	4086 ± 116^{d}	3776 ± 264^{c}	3294 ± 380^{b}	3757 ± 428^{ab}
Digestate + Hg	3455 ± 569^{b}	3190 ± 282^{b}	3139 ± 328^{b}	3804 ± 613^{d}	4462 ± 644^{c}	3975 ± 206^{c}	4758 ± 327^{b}
Ash	2335 ± 433^{a}	2382 ± 197^{a}	2281 ± 321^{a}	2540 ± 234^{bc}	2917 ± 164^{b}	2686 ± 281^{b}	2121 ± 286a^{b}
Ash + Hg	1981 ± 254^{a}	1908 ± 149^{a}	2044 ± 161^{a}	2574 ± 512^{c}	2717 ± 117^{b}	3092 ± 190^{b}	2784 ± 467^{ab}
$(NH_4)_2SO_4$	1514 ± 57^{a}	1356 ± 36^{a}	1411 ± 102^{a}	1651 ± 69^{a}	1597 ± 79^{a}	1684 ± 135^{a}	1663 ± 92^{ab}
$(NH_4)_2SO_4$ + Hg	1655 ± 54^{a}	1577 ± 246^{a}	1431 ± 59^{a}	1742 ± 108^{abc}	1616 ± 32^{a}	1666 ± 124^{a}	1690 ± 107^{ab}

Table 4: The concentrations of Mg extractable with 0.11 mol L^{-1} acetic acid within the incubation experiment (mg·kg^{-1}); the averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns; data are presented as mean ± standard deviation ($n = 3$).

Chernozem	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	389 ± 20^{a}	401 ± 2^{ab}	483 ± 8^{a}	562 ± 18^{a}	503 ± 48^{a}	546 ± 23^{a}	371 ± 50^{a}
Control + Hg	347 ± 111^{a}	406 ± 20^{abc}	442 ± 32^{a}	556 ± 16^{a}	502 ± 69^{a}	505 ± 34^{a}	533 ± 135^{ab}
Digestate	544 ± 50^{c}	544 ± 30^{d}	603 ± 65^{b}	753 ± 52^{bc}	656 ± 76^{ab}	781 ± 54^{c}	963 ± 155^{c}
Digestate + Hg	519 ± 37^{c}	603 ± 24^{e}	610 ± 45^{b}	809 ± 44^{d}	734 ± 73^{b}	742 ± 22^{bc}	859 ± 77^{bc}
Ash	442 ± 24^{ab}	458 ± 27^{c}	522 ± 22^{ab}	612 ± 22^{ac}	566 ± 56^{a}	574 ± 66^{a}	636 ± 147^{abc}
Ash + Hg	432 ± 57^{ab}	447 ± 9^{bc}	518 ± 35^{ab}	681 ± 72^{ab}	555 ± 31^{a}	626 ± 62^{ab}	585 ± 122^{ab}
$(NH_4)_2SO_4$	374 ± 3^{a}	394 ± 16^{ab}	461 ± 13^{a}	572 ± 72^{a}	506 ± 59^{a}	494 ± 71^{a}	626 ± 171^{abc}
$(NH_4)_2SO_4$ + Hg	406 ± 30^{ab}	390 ± 10^{a}	468 ± 32^{a}	566 ± 10^{a}	505 ± 45^{a}	553 ± 39^{a}	489 ± 119^{a}
Luvisol	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	65 ± 5^{a}	72 ± 5^{a}	71 ± 2^{a}	89 ± 3^{a}	120 ± 47^{a}	129 ± 61^{a}	89 ± 19^{a}
Control + Hg	70 ± 4^{a}	72 ± 2^{a}	73 ± 1^{a}	86 ± 3^{a}	108 ± 43^{a}	115 ± 45^{a}	82 ± 11^{a}
Digestate	362 ± 27^{c}	259 ± 70^{c}	325 ± 52^{b}	446 ± 5^{c}	408 ± 40^{b}	391 ± 51^{b}	423 ± 11^{c}
Digestate + Hg	364 ± 89^{c}	336 ± 38^{c}	363 ± 92^{b}	460 ± 54^{c}	476 ± 28^{b}	416 ± 33^{b}	366 ± 90^{c}
Ash	144 ± 32^{b}	156 ± 19^{b}	162 ± 34^{ab}	176 ± 21^{b}	231 ± 51^{a}	214 ± 66^{a}	174 ± 40^{ab}
Ash + Hg	116 ± 19^{b}	121 ± 14^{ab}	140 ± 21^{ab}	178 ± 47^{b}	214 ± 52^{a}	243 ± 31^{a}	240 ± 33^{b}
$(NH_4)_2SO_4$	73 ± 0^{a}	68 ± 0^{a}	72 ± 5^{a}	85 ± 1^{a}	120 ± 49^{a}	114 ± 43^{a}	120 ± 33^{a}
$(NH_4)_2SO_4$ + Hg	85 ± 9^{a}	74 ± 5^{ab}	76 ± 2^{ab}	92 ± 7^{a}	115 ± 46^{a}	119 ± 50^{a}	117 ± 38^{a}

contents in the digestate treated Luvisol compared to up to 40% increase of mobile Mg in Chernozem.

Möller and Müller [34] reviewed recent research about nutrient availability after the field application of digestate and stated that there is no available information concerning the availability of S, although digestate seems to be a good source of S in soil; this was also observed in our case (Table 2) where the S content in the digestate sample reached up to 0.6%. Similarly, they stated that there were many published studies describing the effect of anaerobic digestion on micronutrient distribution and bioavailability in sewage sludge, but rarely any concerning digestates. Moreover, the availability of micronutrients in the digestate can be affected by the wide complex of various factors such as precipitation as sulfide, carbonate, phosphates, and hydroxides, sorption to the solid fraction, either biomass or inert suspended matter, and the formation of complexes in solution with intermediates and compounds produced during anaerobic digestion [34]. The application of digestate results in an improvement of crop yields compared to inorganic fertilizer. Moreover, analysis of soil solution showed that there was less potential for the loss of nutrients *via* leaching [35] in the digestate treated soil.

Table 5: The concentrations of K extractable with 0.11 mol L^{-1} acetic acid within the incubation experiment (mg·kg^{-1}); the averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns; data are presented as mean ± standard deviation ($n = 3$).

Chernozem	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	68 ± 5^{a}	78 ± 4^{a}	78 ± 2^{a}	84 ± 2^{a}	92 ± 4^{a}	95 ± 0^{a}	53 ± 3^{a}
Control + Hg	83 ± 17^{a}	76 ± 4^{a}	77 ± 10^{a}	87 ± 3^{a}	90 ± 6^{a}	94 ± 3^{a}	76 ± 12^{a}
Digestate	2882 ± 906^{b}	2931 ± 491^{b}	3026 ± 304^{b}	3547 ± 163^{b}	3615 ± 177^{c}	3842 ± 277^{c}	4408 ± 506^{b}
Digestate + Hg	2461 ± 510^{b}	3163 ± 128^{b}	2961 ± 261^{b}	3915 ± 370^{b}	3780 ± 164^{d}	3697 ± 191^{c}	4283 ± 367^{b}
Ash	269 ± 66^{a}	284 ± 40^{a}	294 ± 59^{a}	317 ± 78^{a}	374 ± 35^{cd}	362 ± 18^{ab}	406 ± 146^{a}
Ash + Hg	260 ± 61^{a}	305 ± 77^{a}	310 ± 47^{a}	467 ± 101^{a}	362 ± 25^{bc}	446 ± 63^{b}	359 ± 82^{a}
$(NH_4)_2SO_4$	97 ± 5^{a}	119 ± 11^{a}	107 ± 6^{a}	130 ± 13^{a}	126 ± 6^{ab}	117 ± 13^{ab}	109 ± 6^{a}
$(NH_4)_2SO_4$ + Hg	105 ± 6^{a}	109 ± 4^{a}	112 ± 6^{a}	135 ± 2^{a}	130 ± 5^{abc}	145 ± 10^{ab}	1568 ± 207^{a}
Luvisol	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	117 ± 7^{a}	126 ± 7^{a}	117 ± 3^{a}	140 ± 4^{a}	138 ± 2^{a}	147 ± 9^{a}	111 ± 12^{a}
Control + Hg	122 ± 6^{a}	130 ± 6^{a}	125 ± 4^{a}	139 ± 4^{a}	138 ± 5^{a}	146 ± 3^{a}	122 ± 21^{a}
Digestate	3324 ± 201^{b}	2684 ± 425^{b}	3173 ± 54^{b}	3857 ± 252^{b}	3936 ± 37^{c}	3665 ± 83^{c}	4063 ± 418^{c}
Digestate + Hg	3728 ± 541^{b}	3306 ± 214^{b}	3351 ± 540^{b}	4065 ± 150^{b}	3927 ± 308^{c}	3655 ± 64^{c}	3304 ± 180^{b}
Ash	431 ± 146^{a}	487 ± 78^{a}	431 ± 111^{a}	419 ± 70^{a}	548 ± 66^{b}	446 ± 71^{b}	387 ± 18^{a}
Ash + Hg	316 ± 70^{a}	343 ± 41^{a}	370 ± 39^{a}	454 ± 102^{a}	489 ± 15^{b}	546 ± 66^{b}	553 ± 118^{a}
$(NH_4)_2SO_4$	152 ± 3^{a}	145 ± 5^{a}	143 ± 11^{a}	162 ± 1^{a}	161 ± 5^{a}	167 ± 9^{a}	166 ± 17^{a}
$(NH_4)_2SO_4$ + Hg	159 ± 6^{a}	158 ± 9^{a}	152 ± 4^{a}	176 ± 9^{a}	169 ± 4^{a}	162 ± 4^{a}	170 ± 14^{a}

Table 6: The concentrations of Cu extractable with 0.11 mol L^{-1} acetic acid within the incubation experiment (mg·kg^{-1}); the averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns; data are presented as mean ± standard deviation ($n = 3$).

Chernozem	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	0.045 ± 0.007^{a}	0.039 ± 0.008^{a}	0.015 ± 0.003^{a}	0.025 ± 0.003^{a}	0.162 ± 0.018^{abc}	0.064 ± 0.013^{a}	0.017 ± 0.008^{a}
Control + Hg	0.047 ± 0.012^{a}	0.017 ± 0.001^{a}	0.017 ± 0.004^{a}	0.023 ± 0.003^{a}	0.039 ± 0.019^{a}	0.098 ± 0.025^{a}	0.034 ± 0.015^{a}
Digestate	0.224 ± 0.092^{b}	0.230 ± 0.098^{b}	0.187 ± 0.037^{c}	0.299 ± 0.010^{c}	0.362 ± 0.051^{c}	0.517 ± 0.089^{c}	0.911 ± 0.141^{b}
Digestate + Hg	0.120 ± 0.010^{a}	0.094 ± 0.026^{a}	0.089 ± 0.028^{b}	0.166 ± 0.038^{b}	0.264 ± 0.031^{bc}	0.334 ± 0.031^{b}	0.763 ± 0.065^{b}
Ash	0.053 ± 0.019^{a}	0.059 ± 0.014^{a}	0.032 ± 0.007^{a}	0.054 ± 0.012^{a}	0.065 ± 0.042^{ab}	0.073 ± 0.043^{a}	0.148 ± 0.029^{a}
Ash + Hg	0.079 ± 0.011^{a}	0.042 ± 0.003^{a}	0.026 ± 0.005^{a}	0.060 ± 0.013^{a}	0.055 ± 0.035^{a}	0.095 ± 0.027^{a}	0.141 ± 0.069^{a}
$(NH_4)_2SO_4$	0.047 ± 0.035^{a}	0.048 ± 0.013^{a}	0.018 ± 0.006^{a}	0.022 ± 0.006^{a}	0.081 ± 0.031^{ab}	0.091 ± 0.038^{a}	0.090 ± 0.060^{a}
$(NH_4)_2SO_4$ + Hg	0.038 ± 0.020^{a}	0.029 ± 0.007^{a}	0.008 ± 0.008^{a}	0.029 ± 0.002^{a}	0.029 ± 0.013^{a}	0.066 ± 0.048^{a}	0.379 ± 0.093^{ab}
Luvisol	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	0.095 ± 0.003^{a}	0.084 ± 0.019^{a}	0.061 ± 0.007^{a}	0.134 ± 0.021^{a}	0.142 ± 0.042^{a}	0.226 ± 0.008^{a}	0.252 ± 0.055^{a}
Control + Hg	0.128 ± 0.020^{a}	0.118 ± 0.021^{ab}	0.098 ± 0.011^{a}	0.156 ± 0.024^{ab}	0.164 ± 0.062^{a}	0.357 ± 0.021^{ab}	0.230 ± 0.062^{a}
Digestate	0.284 ± 0.085^{a}	0.268 ± 0.022^{b}	0.316 ± 0.051^{b}	0.553 ± 0.087^{c}	0.534 ± 0.136^{b}	0.785 ± 0.032^{d}	1.200 ± 0.142^{c}
Digestate + Hg	0.166 ± 0.122^{a}	0.095 ± 0.012^{a}	0.120 ± 0.055^{a}	0.131 ± 0.092^{a}	0.119 ± 0.015^{a}	0.594 ± 0.137^{cd}	0.678 ± 0.164^{ab}
Ash	0.196 ± 0.026^{a}	0.161 ± 0.036^{ab}	0.145 ± 0.023^{ab}	0.269 ± 0.051^{ab}	0.220 ± 0.045^{a}	0.542 ± 0.046^{bc}	0.493 ± 0.081^{ab}
Ash + Hg	0.225 ± 0.048^{ab}	0.261 ± 0.024^{b}	0.193 ± 0.016^{ab}	0.298 ± 0.045^{b}	0.266 ± 0.040^{a}	0.578 ± 0.058^{c}	0.900 ± 0.042^{bc}
$(NH_4)_2SO_4$	0.101 ± 0.022^{a}	0.191 ± 0.048^{ab}	0.078 ± 0.004^{a}	0.184 ± 0.018^{ab}	0.161 ± 0.024^{a}	0.308 ± 0.051^{a}	0.658 ± 0.103^{ab}
$(NH_4)_2SO_4$ + Hg	0.140 ± 0.027^{a}	0.138 ± 0.028^{ab}	0.121 ± 0.014^{a}	0.247 ± 0.068^{ab}	0.193 ± 0.050^{a}	0.422 ± 0.101^{abc}	0.555 ± 0.062^{ab}

Also, Frøseth et al. [36] observed that the field application of digestate contributed to higher soil aggregate stability. According to Fernández-Delgado Juarez et al. [37], amending soils with digestate resulted in a higher nutrient content as well as more efficient soil microbial community relative to the variants treated with farmyard manure. Therefore, the application of digestate seemed to be the effective measure for immobilization of Hg in soil together with increase of mobile nutrients in these soils.

The application of wood fly ash as a potential source of available nutrients in the soil is widely discussed in the literature [38, 39]. The benefits on the growth of the plants as the result of an increase in available P, Ca, Mg, K, and B and a decrease in Al toxicity was described [38]. Steenari et al. [40] tested the release of macro- and microelements from various ash samples, whereas low leaching rates were observed for the important plant nutrients P and Mg, as well as for Fe, Mn, Cu, and Zn, up to 50% of total K was released during the batch leaching test. In our case, the extractable element contents in the ash amended samples differed according to the experimental soil, where Ca, Fe, and Cu levels remained unchanged compared to the control.

Table 7: The concentrations of Fe extractable with 0.11 mol L^{-1} acetic acidwithin the incubation experiment (mg·kg^{-1}); The averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns; data are presented as mean ± standard deviation ($n = 3$).

Chernozem	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	1.54 ± 0.38^{a}	20.47 ± 6.26^{a}	8.49 ± 0.70^{a}	0.28 ± 0.02^{a}	0.48 ± 0.11^{a}	4.52 ± 0.24^{a}	0.25 ± 0.06^{a}
Control + Hg	1.40 ± 0.09^{a}	8.20 ± 2.34^{a}	10.76 ± 8.02^{a}	0.18 ± 0.03^{a}	0.20 ± 0.03^{a}	2.90 ± 1.05^{a}	0.24 ± 0.04^{a}
Digestate	49.3 ± 9.4^{c}	95.4 ± 17.2^{c}	105.9 ± 2.6^{b}	142.5 ± 11.7^{c}	102.4 ± 2.7^{c}	32.4 ± 5.3^{b}	48.7 ± 6.4^{b}
Digestate + Hg	27.0 ± 4.2^{b}	61.9 ± 15.2^{b}	77.1 ± 28.8^{b}	73.1 ± 10.7^{b}	69.4 ± 5.5^{b}	48.0 ± 14.2^{c}	48.3 ± 9.6^{b}
Ash	1.31 ± 0.16^{a}	28.78 ± 5.86^{a}	6.87 ± 1.97^{a}	0.26 ± 0.10^{a}	0.35 ± 0.11^{a}	1.53 ± 0.17^{a}	0.90 ± 0.24^{a}
Ash + Hg	1.16 ± 0.46^{a}	8.17 ± 2.01^{a}	7.58 ± 4.58^{a}	0.30 ± 0.11^{a}	0.28 ± 0.04^{a}	1.07 ± 0.10^{a}	0.06 ± 0.03^{a}
$(NH_4)_2SO_4$	1.29 ± 0.15^{a}	17.20 ± 5.83^{a}	5.53 ± 1.19^{a}	0.35 ± 0.17^{a}	0.26 ± 0.06^{a}	3.91 ± 1.32^{a}	0.15 ± 0.12^{a}
$(NH_4)_2SO_4$ + Hg	1.13 ± 0.28^{a}	10.53 ± 3.15^{a}	7.21 ± 2.97^{a}	0.14 ± 0.09^{a}	0.23 ± 0.03^{a}	1.53 ± 0.19^{a}	2.37 ± 0.36^{a}
Luvisol	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	3.88 ± 2.13^{a}	20.08 ± 6.85^{a}	7.35 ± 1.27^{a}	1.31 ± 0.18^{a}	1.04 ± 0.14^{a}	7.17 ± 2.64^{a}	1.13 ± 0.66^{a}
Control + Hg	2.21 ± 0.27^{a}	10.02 ± 4.27^{a}	12.24 ± 2.19^{a}	1.38 ± 0.31^{a}	0.91 ± 0.08^{a}	5.99 ± 0.76^{a}	0.67 ± 0.47^{a}
Digestate	100.8 ± 33.1^{b}	51.9 ± 25.4^{b}	67.6 ± 40.6^{ab}	44.7 ± 14.3^{b}	77.4 ± 14.5^{b}	42.5 ± 13.0^{a}	173.5 ± 71.0^{b}
Digestate + Hg	108.0 ± 38.7^{b}	340.9 ± 82.3^{b}	329.4 ± 70.4^{b}	413.7 ± 52.8^{c}	484.7 ± 97.2^{c}	396.9 ± 54.1^{b}	315.4 ± 63.8^{c}
Ash	2.80 ± 0.70^{a}	16.71 ± 3.74^{a}	15.41 ± 3.44^{a}	1.82 ± 0.59^{a}	1.58 ± 0.77^{a}	7.44 ± 2.02^{a}	2.86 ± 1.43^{a}
Ash + Hg	2.56 ± 0.27^{a}	15.88 ± 9.53^{a}	11.24 ± 4.10^{a}	1.21 ± 0.44^{a}	0.99 ± 0.18^{a}	2.94 ± 1.18^{a}	0.59 ± 0.05^{a}
$(NH_4)_2SO_4$	3.07 ± 0.09^{a}	8.82 ± 0.53^{a}	7.06 ± 2.55^{a}	1.61 ± 0.21^{a}	1.28 ± 0.07^{a}	5.68 ± 1.15^{a}	1.64 ± 0.30^{a}
$(NH_4)_2SO_4$ + Hg	3.01 ± 0.32^{a}	10.36 ± 2.32^{a}	14.56 ± 2.55^{a}	1.59 ± 0.21^{a}	1.39 ± 0.24^{a}	2.47 ± 0.42^{a}	1.02 ± 0.23^{a}

Table 8: The concentrations of Mn extractable with 0.11 mol L^{-1} acetic acid within the incubation experiment (mg·kg^{-1}); the averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns; data are presented as mean ± standard deviation ($n = 3$).

Chernozem	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	19.5 ± 1.2^{a}	19.9 ± 0.7^{a}	22.2 ± 1.1^{a}	35.0 ± 6.1^{a}	33.9 ± 0.9^{a}	46.7 ± 16.0^{a}	34.7 ± 8.7^{a}
Control + Hg	21.4 ± 4.4^{a}	22.1 ± 3.4^{ab}	20.7 ± 3.9^{a}	36.2 ± 4.8^{a}	36.6 ± 0.3^{a}	37.3 ± 5.0^{c}	37.8 ± 11.6^{a}
Digestate	168 ± 22^{c}	168 ± 12^{c}	177 ± 10^{c}	232 ± 4^{d}	204 ± 4^{c}	218 ± 11^{a}	301 ± 68^{d}
Digestate + Hg	152 ± 4^{c}	181 ± 6^{c}	168 ± 4^{c}	229 ± 23^{d}	204 ± 5^{c}	208 ± 3^{c}	266 ± 30^{cd}
Ash	42.4 ± 7.3^{b}	38.7 ± 9.8^{ab}	57.5 ± 21.7^{b}	70.2 ± 8.5^{bc}	66.3 ± 10.6^{b}	66.9 ± 14.0^{a}	101.5 ± 53.7^{ab}
Ash + Hg	38.5 ± 9.2^{b}	40.0 ± 4.4^{b}	$46.1 \pm 7.0a^{b}$	96.8 ± 18.0^{c}	73.9 ± 6.5^{b}	93.1 ± 15.5^{ab}	83.0 ± 32.3^{ab}
$(NH_4)_2SO_4$	23.8 ± 1.5^{a}	24.1 ± 1.6^{ab}	27.0 ± 0.7^{a}	46.7 ± 5.0^{ab}	69.7 ± 16.6^{b}	143.1 ± 19.7^{b}	125.6 ± 36.1^{bc}
$(NH_4)_2SO_4$ + Hg	26.4 ± 8.5^{a}	28.0 ± 7.4^{ab}	30.3 ± 9.3^{ab}	39.2 ± 5.5^{ab}	40.7 ± 9.8^{a}	56.5 ± 13.9^{a}	70.8 ± 33.6^{ab}
Luvisol	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	27.0 ± 1.0^{a}	30.3 ± 1.0^{a}	33.6 ± 3.6^{a}	55.9 ± 1.0^{a}	50.8 ± 9.3^{a}	64.2 ± 14.0^{a}	34.7 ± 7.8^{a}
Control + Hg	43.6 ± 0.8^{abc}	43.3 ± 10.1^{ab}	45.1 ± 7.0^{abc}	67.4 ± 5.1^{ab}	61.5 ± 6.7^{a}	77.4 ± 8.5^{ab}	37.8 ± 7.1^{a}
Digestate	161 ± 9^{d}	142 ± 23^{d}	169 ± 8^{d}	191 ± 2^{c}	198 ± 25^{c}	189 ± 19^{c}	301 ± 30^{c}
Digestate + Hg	148 ± 9^{d}	176 ± 2^{e}	180 ± 5^{d}	222 ± 10^{d}	223 ± 11^{c}	229 ± 11^{d}	266 ± 81^{c}
Ash	54.9 ± 10.4^{c}	59.4 ± 8.8^{b}	53.2 ± 9.0^{bc}	87.0 ± 14.2^{b}	131.4 ± 42.3^{b}	114.0 ± 23.8^{b}	101.5 ± 34.1^{b}
Ash + Hg	54.4 ± 7.2^{c}	58.6 ± 2.4^{b}	58.9 ± 4.8^{c}	86.0 ± 12.1^{b}	81.9 ± 5.8^{ab}	108.9 ± 0.8^{b}	83.0 ± 69.1^{a}
$(NH_4)_2SO_4$	27.9 ± 0.5^{ab}	28.5 ± 1.5^{a}	29.0 ± 1.7^{a}	50.3 ± 8.3^{a}	42.8 ± 6.6^{a}	60.9 ± 12.4^{a}	125.6 ± 3.2^{b}
$(NH_4)_2SO_4$ + Hg	46.5 ± 4.6^{bc}	46.9 ± 5.2^{ab}	42.6 ± 3.2^{ab}	62.4 ± 12.4^{ab}	65.9 ± 6.0^{a}	63.6 ± 2.9^{a}	70.8 ± 5.2^{a}

On the contrary, the extremely high Zn level in the ash (Table 2) resulted in the significant increase in the extractable Zn portion of the ash-treated soil regardless of the soil type (Table 11). A similar pattern was reported for Mg, where the increase in the extractable Mg portion was more apparent in Luvisol (Table 4). Whereas in the Luvisol the mobile Mg contents increased twice after ash application, the mobile portion of Mg in Chernozem rised only by 10–15%. The low mobility of micronutrients in various ash samples was also confirmed by Száková et al. [41]. The K, Mn, and P extractable levels tended to increase compared to controls (significance of the differences at $P < 0.05$ was unambiguously proved only in the case of Mn, see Table 8) but were significantly lower compared to digestate application, not confirming the high K leachability from ash samples observed by Steenari et al. [40]. Although the total S contents added *via* the individual treatments were comparable, the mobile portions of ash-derived S were lower compared to those after the application of digestate and ammonium sulfate. Ochecová et al. [20] observed increasing mobile portions of Ca, P, K, Mg, and Mn in the fly ash-treated soil after a 3-year model pot experiment. However, the effects were significant for the 3–6 fold higher

Table 9: The concentrations of P extractable with 0.11 mol L^{-1} acetic acid within the incubation experiment (mg·kg^{-1}); the averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns; data are presented as mean ± standard deviation ($n = 3$).

Chernozem	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	90.9 ± 6.3^{a}	88.9 ± 24.9^{a}	112.3 ± 33.3^{a}	109.3 ± 7.0^{a}	93.6 ± 7.9^{a}	88.8 ± 11.7^{a}	55.9 ± 6.5^{a}
Control + Hg	85.0 ± 17.9^{a}	88.1 ± 1.9^{a}	108.4 ± 19.0^{a}	127.8 ± 14.3^{a}	91.2 ± 4.7^{a}	98.1 ± 14.1^{a}	79.3 ± 13.9^{a}
Digestate	259.0 ± 50.0^{b}	269.0 ± 49.5^{b}	235.2 ± 63.2^{b}	320.9 ± 60.5^{b}	189.1 ± 23.5^{b}	278.6 ± 30.5^{b}	246.0 ± 59.9^{b}
Digestate + Hg	250.9 ± 62.4^{b}	285.6 ± 28.3^{b}	261.6 ± 40.4^{b}	361.0 ± 14.2^{b}	293.6 ± 12.5^{c}	274.5 ± 51.7^{b}	253.5 ± 51.8^{b}
Ash	107.1 ± 6.2^{a}	102.1 ± 17.1^{a}	101.7 ± 2.9^{a}	130.0 ± 18.1^{a}	115.4 ± 17.5^{a}	98.9 ± 9.2^{a}	123.3 ± 30.4^{a}
Ash + Hg	104.2 ± 7.5^{a}	101.6 ± 5.7^{a}	105.5 ± 6.4^{a}	138.3 ± 11.9^{a}	118.4 ± 28.0^{a}	115.3 ± 6.0^{a}	96.6 ± 19.6^{a}
$(NH_4)_2SO_4$	96.9 ± 10.0^{a}	91.3 ± 1.7^{a}	93.4 ± 13.1^{a}	110.4 ± 4.8^{a}	94.4 ± 1.2^{a}	102.2 ± 12.6^{a}	116.8 ± 15.6^{a}
$(NH_4)_2SO_4$ + Hg	84.3 ± 4.7^{a}	90.2 ± 4.3^{a}	117.9 ± 22.3^{a}	118.7 ± 17.1^{a}	95.4 ± 3.1^{a}	91.8 ± 7.9^{a}	106.5 ± 37.4^{a}
Luvisol	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	62.9 ± 5.8^{a}	61.9 ± 6.3^{a}	61.2 ± 11.1^{a}	70.8 ± 0.6^{a}	65.8 ± 16.3^{a}	$56.62.7 \pm^{a}$	38.9 ± 3.8^{a}
Control + Hg	66.8 ± 9.6^{a}	63.4 ± 0.5^{a}	65.6 ± 10.4^{a}	71.5 ± 8.6^{a}	62.3 ± 1.7^{a}	67.4 ± 5.7^{a}	43.7 ± 12.0^{a}
Digestate	323.7 ± 26.2^{b}	210.2 ± 67.6^{b}	203.4 ± 58.7^{b}	323.4 ± 14.3^{b}	290.3 ± 30.1^{b}	245.8 ± 56.8^{b}	254.0 ± 24.0^{c}
Digestate + Hg	329.6 ± 82.9^{b}	286.5 ± 40.6^{b}	246.3 ± 77.2^{b}	350.0 ± 44.8^{b}	348.6 ± 90.4^{b}	262.2 ± 28.7^{b}	130.7 ± 48.9^{b}
Ash	85.0 ± 5.3^{a}	84.4 ± 8.8^{a}	80.5 ± 13.1^{a}	90.6 ± 14.1^{a}	92.6 ± 7.4^{a}	78.3 ± 4.8^{a}	55.6 ± 6.0^{a}
Ash + Hg	76.4 ± 6.3^{a}	73.7 ± 4.7^{a}	75.8 ± 3.6^{a}	90.7 ± 12.4^{a}	81.4 ± 2.5^{a}	86.5 ± 4.0^{a}	68.7 ± 1.6^{a}
$(NH_4)_2SO_4$	61.3 ± 1.1^{a}	58.3 ± 3.6^{a}	57.3 ± 4.0^{a}	69.1 ± 8.2^{a}	59.4 ± 4.8^{a}	59.3 ± 4.2^{a}	60.6 ± 9.2^{a}
$(NH_4)_2SO_4$ + Hg	60.4 ± 1.6^{a}	72.9 ± 13.7^{a}	59.6 ± 2.8^{a}	69.5 ± 1.2^{a}	63.3 ± 4.1^{a}	58.8 ± 2.5^{a}	58.7 ± 0.6^{a}

Table 10: The concentrations of S extractable with 0.11 mol L^{-1} acetic acid within the incubation experiment (mg·kg^{-1}); the averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns; data are presented as mean ± standard deviation ($n = 3$).

Chernozem	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	8.8 ± 1.0^{a}	9.7 ± 0.9^{a}	12.3 ± 5.6^{a}	14.9 ± 5.0^{a}	17.7 ± 2.1^{a}	16.1 ± 4.1^{a}	10.0 ± 2.7^{a}
Control + Hg	9.3 ± 0.6^{a}	9.6 ± 0.6^{a}	9.6 ± 1.5^{a}	13.0 ± 2.4^{a}	15.1 ± 4.3^{a}	15.2 ± 3.7^{a}	15.5 ± 4.6^{a}
Digestate	255.0 ± 114.1^{bc}	199.5 ± 54.8^{b}	129.0 ± 31.7^{b}	89.7 ± 21.3^{ab}	66.1 ± 3.5^{ab}	132.2 ± 52.9^{ab}	211.3 ± 93.9^{bc}
Digestate + Hg	141.4 ± 30.4^{b}	221.6 ± 29.6^{b}	162.7 ± 46.2^{b}	198.1 ± 63.6^{ab}	91.6 ± 6.7^{ab}	136.4 ± 49.1^{ab}	307.6 ± 110.8^{cd}
Ash	138.1 ± 37.6^{b}	140.8 ± 32.9^{b}	143.9 ± 33.0^{b}	153.8 ± 37.3^{ab}	168.1 ± 12.0^{b}	165.4 ± 16.1^{ab}	201.9 ± 62.7^{bc}
Ash + Hg	138.2 ± 20.2^{b}	158.9 ± 7.5^{b}	155.2 ± 21.9^{b}	243.6 ± 41.8^{ab}	192.6 ± 3.3^{b}	205.0 ± 28.7^{b}	202.5 ± 54.7^{bc}
$(NH_4)_2SO_4$	344.0 ± 82.0^{c}	494.3 ± 153.6^{c}	454.5 ± 79.6^{c}	602.6 ± 145.5^{c}	483.6 ± 55.2^{c}	528.4 ± 91.7^{c}	519.6 ± 104.6^{e}
$(NH_4)_2SO_4$ + Hg	366.3 ± 66.7^{c}	445.8 ± 94.6^{c}	386.7 ± 13.1^{c}	660.6 ± 189.3^{c}	483.2 ± 162.3^{c}	766.5 ± 78.7^{d}	429.7 ± 123.6^{de}
Luvisol	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	4.6 ± 1.0^{a}	4.9 ± 1.0^{a}	5.3 ± 1.1^{a}	8.6 ± 2.3^{a}	11.1 ± 3.6^{a}	11.8 ± 6.3^{a}	9.2 ± 3.3^{a}
Control + Hg	5.0 ± 1.0^{a}	4.7 ± 0.3^{a}	5.7 ± 0.4^{a}	9.8 ± 6.1^{a}	11.4 ± 5.5^{a}	10.2 ± 4.9^{a}	7.0 ± 0.6^{a}
Digestate	296.4 ± 27.4^{bc}	221.6 ± 27.5^{bc}	275.5 ± 32.2^{bc}	420.5 ± 122.4^{cd}	329.1 ± 62.0^{cd}	220.3 ± 121.1^{b}	264.9 ± 101.4^{b}
Digestate + Hg	273.2 ± 55.6^{bc}	205.9 ± 19.7^{bc}	234.2 ± 46.2^{bc}	229.7 ± 48.9^{bc}	80.3 ± 26.6^{ab}	$94.9 \pm 11.1a^{b}$	362.5 ± 93.2^{b}
Ash	183.2 ± 75.2^{b}	183.2 ± 20.0^{b}	214.5 ± 64.0^{b}	208.2 ± 15.6^{b}	223.6 ± 13.8^{bc}	198.3 ± 14.0^{b}	151.0 ± 27.1^{b}
Ash + Hg	134.5 ± 21.6^{b}	131.6 ± 11.8^{b}	152.8 ± 21.2^{b}	219.1 ± 27.2^{bc}	233.4 ± 34.5^{bc}	228.7 ± 40.6^{b}	203.4 ± 36.8^{b}
$(NH_4)_2SO_4$	443.5 ± 96.3^{c}	403.0 ± 96.0^{c}	466.6 ± 87.8^{c}	590.4 ± 130.9^{d}	430.3 ± 97.5^{d}	505.2 ± 108.6^{c}	570.8 ± 118.5^{c}
$(NH_4)_2SO_4$ + Hg	536.1 ± 61.6^{c}	564.1 ± 30.2^{c}	541.0 ± 78.0^{c}	687.8 ± 87.9^{e}	560.1 ± 24.8^{e}	564.4 ± 72.6^{c}	701.8 ± 31.8^{c}

ash rate compared to our experiment. Thus, the increase of mobile nutrient contents in soils will manifest at higher ash rates compared to our experiment.

The interrelationships between soil Hg and other soil element contents described by Reis et al. [14] indicate that the presence of Hg in the mobile phase could be related to Mn and Al soil contents. Furthermore, an antagonistic effect of Mn against Hg is suggested. Our data tended to increase of mobile Mn contents during the incubation (Table 8) as related to decreasing mobile Hg (Figure 1). Similarly, Sierra et al. [42] observed negative significant correlation between the available Mn in the rhizosphere and Hg content in plants. On the contrary, S content contributed to Hg retention in the soil matrix, reducing the mobility of the metal [14]. In our case, the changes of Hg mobility in soils (Figure 1) did not reflect the changes in mobile portion of S during the incubation experiment (Table 10). In contrast, the presence of sulfates seems to favor Hg uptake by the plant. There was a positive significant correlation between the sulfate concentration in the rhizosphere and the Hg within the

Table 11: The concentrations of Zn extractable with 0.11 mol L^{-1} acetic acid within the incubation experiment (mg·kg^{-1}); the averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns; data are presented as mean ± standard deviation ($n = 3$).

Chernozem	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	1.34 ± 0.07^a	1.37 ± 0.08^a	0.87 ± 0.03^a	1.57 ± 0.19^a	1.42 ± 0.40^a	1.32 ± 0.26^a	0.59 ± 0.02^a
Control + Hg	1.43 ± 0.29^a	1.27 ± 0.06^a	0.88 ± 0.15^a	1.36 ± 0.19^a	1.28 ± 0.08^a	1.28 ± 0.16^a	0.97 ± 0.33^a
Digestate	6.56 ± 0.81^a	6.69 ± 1.21^a	6.02 ± 1.11^a	9.02 ± 0.93^a	7.44 ± 0.26^a	11.06 ± 1.22^a	12.51 ± 1.37^a
Digestate + Hg	5.23 ± 1.36^a	5.74 ± 0.82^a	5.21 ± 0.84^a	7.76 ± 0.34^a	6.69 ± 0.26^a	7.89 ± 0.17^a	9.98 ± 1.51^a
Ash	109.3 ± 23.2^b	52.1 ± 20.0^b	85.8 ± 21.8^b	93.9 ± 23.7^b	102.9 ± 12.2^b	77.2 ± 17.3^b	126.6 ± 32.9^b
Ash + Hg	52.6 ± 25.8^b	47.1 ± 10.4^b	81.5 ± 21.4^b	153.8 ± 25.7^c	108.5 ± 31.6^b	137.6 ± 22.4^c	154.3 ± 28.8^b
$(NH_4)_2SO_4$	1.57 ± 0.29^a	1.55 ± 0.15^a	1.12 ± 0.09^a	1.67 ± 0.07^a	1.66 ± 0.20^a	2.29 ± 0.27^a	2.27 ± 0.32^a
$(NH_4)_2SO_4$ + Hg	1.65 ± 0.26^a	1.39 ± 0.08^a	1.15 ± 0.18^a	1.44 ± 0.13^a	1.45 ± 0.13^a	1.56 ± 0.06^a	5.02 ± 0.35^a
Luvisol	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21
Control	2.66 ± 0.10^a	2.92 ± 0.19^a	2.46 ± 0.21^a	3.45 ± 0.19^a	3.21 ± 0.12^a	3.15 ± 0.10^a	2.02 ± 0.39^a
Control + Hg	2.94 ± 0.06^a	2.99 ± 0.30^a	2.64 ± 0.15^a	4.84 ± 2.17^a	3.05 ± 0.04^a	3.26 ± 0.06^a	2.41 ± 0.89^a
Digestate	11.58 ± 0.67^a	8.13 ± 2.02^a	8.53 ± 1.57^a	14.04 ± 0.43^a	13.07 ± 0.99^a	12.49 ± 1.14^a	14.68 ± 1.98^a
Digestate + Hg	9.02 ± 1.29^a	8.33 ± 1.04^a	7.86 ± 0.69^a	10.24 ± 0.52^a	9.98 ± 0.73^a	11.85 ± 1.90^a	6.81 ± 4.38^a
Ash	181.6 ± 30.7^b	172.9 ± 28.8^b	161.4 ± 32.8^b	164.6 ± 31.0^b	214.2 ± 24.5^c	155.6 ± 25.8^b	115.8 ± 23.0^b
Ash + Hg	112.4 ± 31.8^b	92.2 ± 12.3^b	121.1 ± 23.3^b	165.8 ± 34.6^b	162.6 ± 4.6^b	201.9 ± 35.7^b	230.9 ± 38.7^c
$(NH_4)_2SO_4$	3.05 ± 0.18^a	3.16 ± 0.73^a	2.65 ± 0.04^a	3.74 ± 0.21^a	3.38 ± 0.13^a	3.98 ± 0.50^a	4.39 ± 1.42^a
$(NH_4)_2SO_4$ + Hg	3.37 ± 0.24^a	3.17 ± 0.28^a	2.95 ± 0.24^a	4.18 ± 0.08^a	3.18 ± 0.31^a	3.44 ± 0.07^a	3.46 ± 0.35^a

aerial and root parts of plants [42]. However, only total S extracted with the 0.11 mol L^{-1} solution of CH_3COOH was determined by the ICP-OES and a portion of mobile sulfates in the extract was not determined in our case and requires further research. The competition between Hg and Cu and Hg and Zn in soils described by Jing et al. [19] was not confirmed in our case. For Fe, Mehrotra and Sedlak [43] and Rhoton and Bennett [44] highlighted Hg immobilization *via* sorption and/or the complexation of Hg with Fe compounds in soil. This statement seems to be confirmed for Chernozem, whereas the opposite pattern was observed in Luvisol. In Chernozem, the mobile portions of Fe decreased significantly after digestate application on the Hg amended samples compared to the unamended ones. In Luvisol, the mobile Fe contents increased in the Hg + digestate amended samples since 2nd day of incubation with maximum at 7th day suggesting competitive relationships of Fe and Hg in this case. The complexity of Hg sorption on Fe/Mn oxides was documented by Liang et al. [45], where the role of amorphous/crystalline Fe and Mn hydroxides, humic acids content, and also chlorine concentrations were mentioned. Šípková et al. [46] observed a negative correlation between Hg content bound to the humic acids and the content of Mg, Mn, and Fe. Therefore, the more detailed information concerning soil components, humic acid portions in the digestate, as well as the importance of the application of $HgCl_2$ compared to the other Hg compounds remains to be elucidated in further research.

4. Conclusions

Although the response of Hg contaminated soils in different ameliorative materials was affected by the individual parameters of the soils, especially by the different soil sorption capacity and organic matter contents in these soils, digestate proved to be the most effective for the immobilization of Hg in soil. Contrary to the other S-bearing measures such as wood fly ash and ammonium sulfate, in the case of digestate, the Hg immobilizing effectiveness resulted from the cooperation of various factors such as S and organic matter content. Moreover, the digestate application can result in an improvement in the macro- and micronutrient status of the soil, where mobile and theoretically plant-available portions of these elements increased in particular. Thus, the field application of organic matter-rich biowaste such as digestate seems to be reasonable for the disposal of this type of material, leading to a decreased environmental risk of Hg contamination in soil.

Conflict of Interests

The authors declared that there is no conflict of interests.

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5.5 Publikovaná práce 5

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Effects of the soil microbial community on mobile proportions and speciation of mercury (Hg) in contaminated soil

Jiřina Száková[a], Jitka Havlíčková[a], Adéla Šípková[a], Jiří Gabriel[b], Karel Švec[b], Petr Baldrian[b], Jiřina Sysalová[c], Pavel Coufalík[d,e], Rostislav Červenka[d], Ondřej Zvěřina[d,f], Josef Komárek[d], and Pavel Tlustoš[a]

[a]Department of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Prague, Czech Republic; [b]Institute of Microbiology, Vídeňská, Prague, Czech Republic; [c]Atomic Absorption Spectrometry Laboratory, Institute of Chemical Technology, Prague, Czech Republic; [d]Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic; [e]Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, Brno, Czech Republic; [f]Department of Public Health, Faculty of Medicine, Masaryk University, Brno, Czech Republic

ABSTRACT

The precise characterization of the behavior of individual microorganisms in the presence of increased mercury contents in soil is necessary for better elucidation of the fate of mercury in the soil environment. In our investigation, resistant bacterial strains isolated from two mercury contaminated soils, represented by *Paenibacillus alginolyticus, Burkholderia glathei, Burkholderia sp.*, and *Pseudomonas sp.*, were used. Two differently contaminated soils (0.5 and 7 mg kg^{-1} total mercury) were chosen. Preliminary soil analysis showed the presence of methylmercury and phenylmercury with the higher soil mercury level. Modified rhizobox experiments were performed to assess the ability of mercury accumulating strains to deplete the mobile and mobilizable mercury portions in the soil by modification; microbial agar cultures were used rather than the plant root zone. A sequential extraction procedure was performed to release the following mercury fractions: water soluble, extracted in acidic conditions, bound to humic substances, elemental, and bound to complexes, HgS and residual. Inductively coupled plasma mass spectrometry (ICP-MS) and a single-purpose atomic absorption spectrometer (AMA-254) were applied for mercury determination in the samples and extracts. Gas chromatography coupled to atomic fluorescence spectrometry (GC-AFS) was used for the determination of organomercury compounds. The analysis of the microbial community at the end of the experiment showed a 42% abundance of *Paenibacillus* sp. followed by *Acetivibrio sp., Brevibacillus sp., Cohnella sp., Lysinibacillus sp.*, and *Clostridium sp.* not exceeding 2% abundance. The results suggest importance of *Paenibacillus* sp. in Hg transformation processes. This genus should be tested for potential bioremediation use in further research.

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Introduction

Biological methods of soil remediation represent cost-effective and environmentally friendly methods to decrease the level of risk elements in soils. In the case of Hg, phytoremediation studies are still being conducted within the laboratory setting. Until now, no typical mercury hyperaccumulating plant has been described.[1] Plant species characterized by and increased ability to accumulate mercury, such as *Rumex scutatus ssp. Induratus, Marrubium vulgare*, and several crops such as *Hordeum spp., Lens culinaris, Cicer arietinum, Lupinus polyphyllus*, and *Triticum aestivum* have been mentioned.[2–4] However, the phytoextraction efficacy of these species is relatively low, requiring a long period of time to achieve a substantial decrease in the mercury content of the soil.

Investigations describing the role of soil microbial communities in mercury accumulation and/or transformation are relatively rare, and the precise characterization of the behavior of individual microorganisms in the presence of increased mercury contents in soil is still unclear in many aspects. Increasing levels of Hg generally depress microbial activity. However, the effects of Hg on soil microbial activity depend on the soil type and composition, particularly the organic matter content. Some bacteria are able to resist heavy metal contamination through chemical transformation by reduction, oxidation, methylation, and demethylation.[5] The genetic system that evolved as the "mer operon" is in fact the only well-known bacterial metal resistance system, allowing for the transformation of its toxic target into volatile nontoxic forms. Both organic (CH_3HgX) and inorganic (HgX) forms of mercurial compounds pass via MerC and MerT inner membrane proteins into cytosol where the action of the enzyme MerA results in volatilization and cellular release of only Hg^0 or both Hg^0 and CH_4, respectively.[6] These mechanisms enable the microorganisms to survive even in an extremely Hg contaminated environment[7] and have been observed in plasmids, chromosomes, transposons, and integrons of both Gram-negative and Gram-positive bacteria.[8]

The bioavailability of nutrients as well as potential risk elements and other pollutants is predominantly driven by soil

CONTACT Jiřina Száková szakova@af.czu.cz Department of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Prague 165 21, Czech Republic.

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conditions in the rhizosphere. Root exudates play an important role in this process, as they consist of a mixture of organic acids, chelates, vitamins, amino acids, purines, nucleosides, and inorganic ions.[9] For a detailed investigation into the processes in the rhizosphere, soil rhizobox experiments[10] can be done to assess changes in soil properties up to several millimeters from plant root surfaces. Instead of the depletion of nutrients and/or changing the mobility of risk elements and other pollutants in rhizosphere soil, these experiments are applied for the investigation of changing microbial activity in the rhizosphere soil as affected by the risk element level,[11–13] the interrelationship between soil nutrients and/or organic matter transformations, and the soil microflora.[14–16] Frequently, rhizobox experiments are provided as experiments describing the potential effects of various phytoremediation measures.[17–19] The efficacy of the biodegradation of organic pollutants *via* both plants and soil microflora in rhizosphere soil can be investigated using the rhizobox approach.[20–22]

In our study, the potential effect of resistant bacteria in mercury contaminated soil on the mobility, volatilization, and speciation of mercury was evaluated in a rhizobox experiment. The resistant bacterial strains isolated from mercury contaminated soils and identified preliminarily as the most resistant bacteria in contaminated soil (more details metioned below) were used for bioaugmentation of the soil in our experiment. The main objectives of the study were i) to assess rhizobox application for microbiological experiments using agar instead of plant roots for the culture of soil bacterial strains and ii) to evaluate the ability of Hg-resistant microorganisms to accumulate and transform Hg with regard to their potential bioremediation use.

Material and methods

Experimental design

The mining and smelting district of Příbram, Czech Republic is known for its Pb-Ag-Zn polymetallic mineral deposits which were mined and processed from the Middle Ages until the 1970s. Emissions from primary and secondary lead smelters are responsible for high concentrations of metallic contaminants (Pb, Cd, and Zn) in soils.[23] Increased Hg contents, especially in forest soils, have been observed in this area as well. Organic phenyl-mercury chloride, called Agronal, has also been used for protecting seeds from fungal diseases. The employment of this process was prohibited in the 1990s, and a newly developed seed dipping process has replaced it. However, the warehouse where dipping was performed is in the vicinity of Příbram, and represents an additional source of soil Hg contamination in this area.

Preliminary analysis of these soils has shown low contents of mobile Hg forms (water-soluble and plant-available fractions), but the location can be considered as hazardous to the environment due to the high content of total Hg (varying between 0.85 and 9.8 mg//kg) and potential occurrence of organometallic compounds.[24] The maximum permissible limits of elements in the soils of the Czech Republic is publically availablev;[25] according to this notice, the total Hg concentration is set as 0.8 mg kg^{-1}, confirming the high contamination level in our sampling area. Therefore, this area was chosen for sampling of representative soil samples for the model rhizobox experiment. At the time of this study soil pH was 7.0, the oxidizable carbon content (C_{ox}) was 3.24% and the cation exchange capacity (CEC) was 175 mMl/kg. Concerning the contents of available nutrients, the contents of elements extractable with the Mehlich III extraction procedure[26] were: 0.04 ± 0.00% of P, 0.62 ± 0.07% of K, 0.31 ± 0.00% of Mg, 0.26 ± 0.03% of Ca, and 0.07 ± 0.01% of S.

The microbial community isolated from these soil samples was cultivated in liquid agar culture amended with a solution of $HgCl_2$ to reach the final Hg concentration in the medium 0.1 mol L^{-1}. The bacteria being able to survive and grow in this medium were identified as the genera *Rhodanobacter, Frateuria, Luteibacter, Mycobacterium, Bacillus, Bradyrhizobium, Beijerinckia, Staphylococcus, Sphingomonas, Paenibacillus, Burkholderia*, and *Pseudomonas*. The methods applied for the analysis of the soil microbial community are described next.

Two sets of modified rhizobox experiments with two soils colected at the area of interest with different levels of soil Hg (0.5 and 7 mg kg^{-1} total mercury as determined before start of the experiment and labeled as Experiment 1 – lower level, and Experiment 2 – higher level of Hg, respectively), each with five rhizobox units, were provided to assess the ability of mercury accumulating strains to deplete the mobile and mobilizable mercury portions from soil. The soils were collected at the area of interest according to previous experiments,[24] air-dried, sieved through 2 mm diameter mesh and thoroughly homogenized.

Before start of the experiment, soil moisture was set up at 60% of the maximum water holding capacity (MWHC) using deionized water and kept at this level for whole experiment. No sterilization of the soil was provided to keep the natural microbial community in the soil and to estimate the potential ability of the organisms to penetrate the nylon membrane and the agar layer. Special designed rhizoboxes [10] allowing sampling of the soil rhizosphere vertical profile in a thickness of 1 mm per layer, were modified to apply microbial instead of plant root zone cultures as follows: the root zone of the rhizobox was filled up with the agar inoculated by the four organisms, *Paenibacillus alginolyticus, Burkholderia glathei, Burkholderia sp.*, and *Pseudomonas sp.* in the comparable amount of the organisms.

These organisms were chosen from the organisms isolated from the contaminated soil (see above) because of their best growth characteristics among the genera able to grow for a long-time in the medium containing 0.1 mol L^{-1} Hg. The agar layer was in contact with the "rhizosphere soil compartment"[10] *via* the nylon membrane. The "rhizosphere soil compartment" of the rhizobox with the agar layer was kept in the dark to avoid potential photochemical reactions. The "soil-plant compartment" was filled with the contaminated soil, as well to simulate the real conditions. The duration of the experiment was 90 days. The experiment was carried out in the greenhouse under controlled conditions at 20°C. At the end of the experiment, the soil was cut without freezing into root-parallel sections according to the distance from plant roots using a specially designed slicing device,[27] freeze-dried and homogenized. The agar layer with the microbial colonies was separated from the membrane and freeze-dried as well.

Analytical methods

For the determination of the bioavailable element portions in soils, 0.5 g of each sample was added to 10 mL of 0.11 mol L^{-1} solution of CH_3COOH and shaken overnight.[28] Each extraction was carried out in three replicates. For the centrifugation of extracts, a Hettich Universal 30 RF (Germany) instrument was used. The reaction mixture was centrifuged at 3000 rpm for 10 min at the end of each extraction procedure, and the supernatants were kept at 6°C prior to measurements.

The sequential extraction procedure releasing the particular fractions of Hg bound to individual soil components was performed as follows: 0.1 g of each sample was leached into 10 mL of extractant and the residue obtained after the extractions was used in the next step. The soil/liquid ratio was the same for all extraction reagents. The extraction procedure was performed on the bulk samples according to the following scheme: F1 with redistilled water–Hg leachable in water, F2 with 0.5 mol L^{-1} HCl–Hg leachable under acidic conditions, F3 with 0.2 mol L^{-1} KOH–Hg bound to humic substances, F4 with 50% HNO_3–elemental Hg and complexes, F5 with a saturated solution of Na_2S–mercury sulfide, and F6–solid residue. Experiments were carried out at laboratory temperature on shakers GFL 3006 (Burgwedel, Germany) at 300 rpm and the extraction time was 18 h in all fractionation steps. Subsequently, extracts were separated from the solid phase by centrifugation for 10 min at 4000 rpm.[29]

Inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., Wilmington, DE, USA) with an ASX-500 auto-sampler, a three-channel peristaltic pump, and MicroMist nebulizer and an AMA-254 single-purpose atomic absorption spectrometer (LECO model, Altec, Czech Republic) were applied for mercury determination in the individual samples and extracts. Agar from the microbial culture was dissolved in 6 M HCl and Hg was determined using the AMA-254.

For speciation analysis of organomercury compounds, 0.5 g of the sample was placed in a glass vial and extracted with 10 mL of 6 mol/L HCl using a GFL 3006 reciprocating shaker for 12 h. Then, 5 mL of liquid extract, after centrifugation on an EBA 20 Hettich centrifuge at 3500 rpm, was placed in a 40 mL glass vial and 17 mL of 4 mol/L sodium acetate was added to reach pH 5. The vial was closed immediately after the addition of 1 mL of hexane and 1 mL of 2% sodium tetraethylborate ($NaBEt_4$) and shaken on IKA Vortex Genius 3 shaker for 5 min. After phase separation, 2 μL of the extract were injected into the column of the chromatograph. Gas chromatography coupled to atomic fluorescence spectrometry (GC-AFS; Agilent Technologies 6890 N Network GC System with a PSA 10.750 fluorescence detector) was used for the determination of organomercury compounds, i.e., methylmercury (MeHg) and phenylmercury (PhHg). The GC separations were performed on a 30 m × 0.32 mm I.D. HP-5 capillary column. Optimized GC parameters were: splitless injection mode; injection port temperature, 220°C; argon flow, 2 mL/min; oven temperature programme, from 50°C to 130°C at ramp rate 15°C/min; from 130°C to 230°C at ramp rate 30°C/min; oven final temperature, 230°C; final time, 1 min.

Gaseous elemental mercury Hg^0 was measured at 253.7 nm by a portable single-purpose Lumex RA-915+ mercury analyzer (Lumex Ltd., St. Petersburg, Russia). The analyzer is based on Zeeman atomic absorption spectrometry with high-frequency modulation of light polarization. The radiation EDL source (mercury electrodeless discharge lamp at $\lambda = 253.7$ nm) absorbs only gaseous Hg^0. The measurements were repeated twice during the incubation, one and two months after the beginning of the experiment.

For microbial community analysis, DNA was extracted using the modified method of Miller[30] and purified as described previously.[31] The V4 region of bacterial 16S rRNA was amplified as a service by the Argonne National Laboratory with the barcoded primers 515F and 806R.[32] Amplicons were sequenced on an Illumina MiSeq. The amplicon sequencing data were processed with SEED 1.2.1.[33] Briefly, pair end reads were merged, chimeric sequences were deleted, and the remaining sequences were clustered using UPARSE[34] positioned at a 97% similarity level. Consensus sequences were constructed, and the closest hits at the genus or species level were identified using BLASTn search against the GenBank database. Full taxonomy was assigned to the identified hits using the SEED 1.2.1 permanent magnetic field.

Results and discussion

The high total Hg contents found in our anthropogenically contaminated soils did not differ from their contents in other industrial areas. For instance, Bloom et al.[35] determined soil Hg contents in various industrial locations reaching up to 73 mg kg^{-1}. Similarly, Millán et al.[36] determined the soil Hg contents up to 1710 mg kg^{-1} in the vicinity of a cinnabarite mine. Total contents of Hg in the individual rhizobox sections (Fig. 1) did not show any unambiguous changes with distance from the agar layer for both soil contamination levels. For acetic acid extractable Hg, the first experiment with lower total Hg content led to a similar conclusion, most probably due to the low extractable Hg contents falling close to the detection limits of the analytical method. However, at the higher Hg level in experiment 2, the results suggested a slight decrease in mobile Hg in the soil segments closest to the agar layer.

Similarly, Li et al.[37] investigated dynamic changes in the rhizosphere properties and antioxidant enzyme responses of wheat plants (*Triticum aestivum* L.) grown in three levels of Hg-contaminated soils and found that the soluble Hg in the rhizosphere soil solutions of the wheat plants decreased over time, especially in the highly Hg polluted soil compared to the slightly Hg polluted soil. They explained these changes by the decreasing pH in rhizosphere soil due to plant root activity. In our case, the effect of the exudates of the microbial community can be speculated on in this context.

The results of detailed Hg fractionation in the individual soil segments are summarized in Tables 1 and 2. As for total Hg, the results suggest ambiguous behavior of the Hg fractions as affected by the distance from the microbial culture, most probably due to the low mobile mercury pool in the soil regardless of the anthropogenic source of contamination. Water soluble Hg, mimicking the bioavailable Hg pool in soil, was low in accordance with other investigations where the most mobile Hg portions in industrially contaminated soils represented up

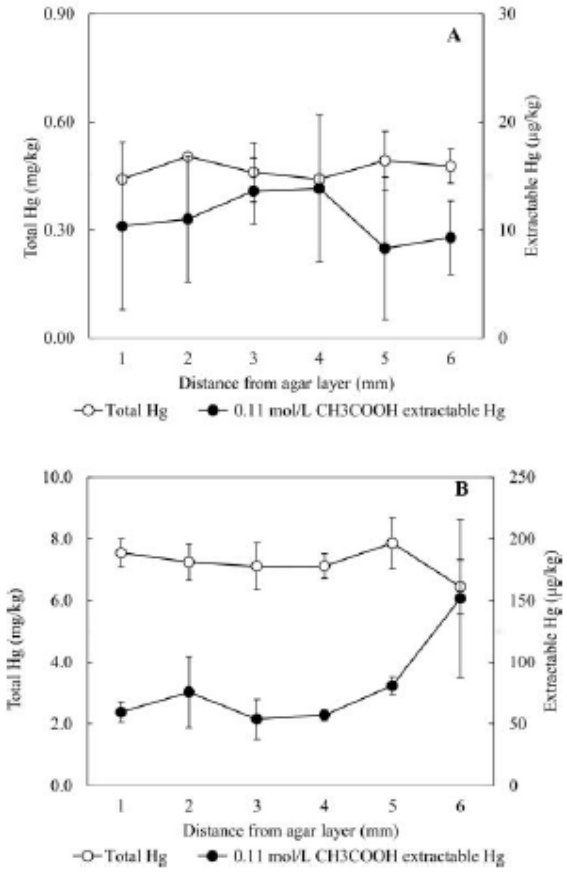

Figure 1. The total and mobile contents of Hg in soil according to distance from agar layer; A - experiment 1, B - experiment 2; data are presented as mean ± standard deviation, n = 5.

to 5% of the total Hg.[38] The distribution of Hg in the soil segments differed with the different soil contamination levels. With a lower Hg content in experiment 1, Table 1) the predominant Hg fraction was F3, i.e., the fraction representing the low mobile Hg pool bound to humic substances. In the more contaminated soil, the Hg was distributed predominantly among three fractions in the order F4 > F3 > F5. Thus, the most abundant Hg fraction was the semi-mobile elemental pool, as usually observed in highly contaminated industrial soils.[39] The high Hg pool was bound to sulfides, according to Liu et al.,[5] presenting a sulfidic Hg pool of around 10%.

The speciation of mercury in soils affected by industrial activity connected with a chlor-alkali plant in the Thur River basin (Alsace, France) was investigated by Remy et al.[40] Concentrations of MeHg reached up to 0.027 mg kg^{-1} and total Hg up to 29 mg kg^{-1}, confirming the low proportion of organomercury compounds in soils. In our case, detectable concentrations of organomercury compounds were observed only in experiment 2 with a higher soil Hg content (Fig. 2). The results show low levels of MeHg with no apparent trend according to the distance from the agar layer, whereas the PhHg levels tended to decrease with the distance from the agar layer. It has already been shown that the organic forms of mercury are more mobile than inorganic forms, and thus more toxic and more readily bio-accumulated.[41] Thus, we can speculate on the role of microbial exudates resulting in the mobilization of organic Hg species. The presence of PhHg is not surprising in the vicinity of a seed-dipping warehouse where phenylmercury chloride was applied, as documented by Hintelmann et al.[42]

The volatile pool of Hg was also detected in experiment 2 with the higher Hg level. The concentrations of volatile Hg^0 determined above the upper part of the rhizoboxes varied between 13 and 20 ng m^{-3} in the first measurement and from 16 to 19 ng m^{-3} in the second one, whereas the Hg° concentrations in the ambient air varied from 5 to 12 ng m^{-3}. Although abiotic reduction in soils occurs with the help of reductants (electron donors) such as Fe^{2+} and humic and fulvic com-

Table 1. Mercury contents in individual fractions after sequential extraction – experiment 1; F1 – Hg leachable in water, F2 – Hg leachable under acidic conditions, F3 – Hg bound to humic substances, F4 – elemental Hg and complexes, F5 - mercury sulfide, and F6 –residual H.

Distance from surface (mm)	F1 mg/kg	F2 mg/kg	F3 mg/kg	F4 mg/kg	F5 mg/kg	F6 mg/kg	Recovery %
1	<0.003	0.035 ± 0.007	0.29 ± 0.02	0.105 ± 0.021	0.070 ± 0.042	0.015 ± 0.007	95.5 ± 3.5
2	<0.003	0.040 ± 0.014	0.30 ± 0.03	0.100 ± 0.014	0.065 ± 0.021	0.015 ± 0.007	96.5 ± 2.1
3	<0.003	0.040 ± 0.014	0.31 ± 0.06	0.085 ± 0.021	0.080 ± 0.028	0.015 ± 0.007	91.5 ± 2.1
4	<0.003	0.040 ± 0.014	0.30 ± 0.06	0.090 ± 0.014	0.060 ± 0.028	0.015 ± 0.007	96.5 ± 3.5
5	<0.003	0.045 ± 0.007	0.28 ± 0.06	0.105 ± 0.007	0.050 ± 0.014	0.010 ± 0.000	92.0 ± 7.1
6	<0.003	0.050 ± 0.014	0.27 ± 0.03	0.135 ± 0.049	0.120 ± 0.085	0.010 ± 0.000	92.0 ± 1.4

Table 2. Mercury contents in individual fractions after sequential extraction – experiment 2; F1 – Hg leachable in water, F2 – Hg leachable under acidic conditions, F3 – Hg bound to humic substances, F4 – elemental Hg and complexes, F5 - mercury sulfide, and F6 –residual Hg.

Distance from surface (mm)	F1 mg/kg	F2 mg/kg	F3 mg/kg	F4 mg/kg	F5 mg/kg	F6 mg/kg	Recovery %
1	0.036 ± 0.006	0.062 ± 0.043	2.95 ± 0.68	3.28 ± 1.01	1.00 ± 0.52	0.034 ± 0.004	93.8 ± 4.1
2	0.038 ± 0.006	0.047 ± 0.036	2.30 ± 0.50	3.38 ± 0.97	1.16 ± 0.70	0.035 ± 0.006	90.7 ± 2.6
3	0.042 ± 0.008	0.032 ± 0.022	2.30 ± 0.49	3.35 ± 0.85	1.12 ± 0.65	0.049 ± 0.009	92.6 ± 7.0
4	0.040 ± 0.014	0.042 ± 0.034	2.15 ± 0.42	3.29 ± 0.94	1.05 ± 0.77	0.050 ± 0.015	90.3 ± 6.8
5	0.041 ± 0.009	0.034 ± 0.025	2.15 ± 0.35	3.48 ± 0.86	1.03 ± 0.78	0.056 ± 0.018	90.4 ± 1.4
6	0.039 ± 0.011	0.033 ± 0.028	2.15 ± 0.51	3.48 ± 0.85	0.97 ± 0.72	0.055 ± 0.010	95.5 ± 10.4

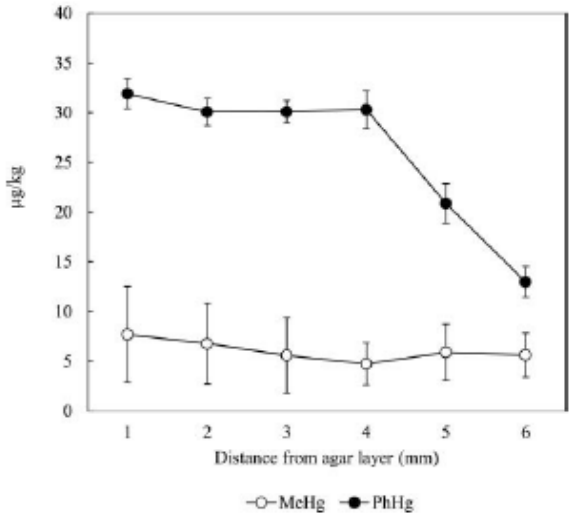

Figure 2. The contents of organomercury compounds (MeHg and PhHg) in soil according to distance from agar layer – experiment 2; data are presented as mean ± standard deviation, n = 5.

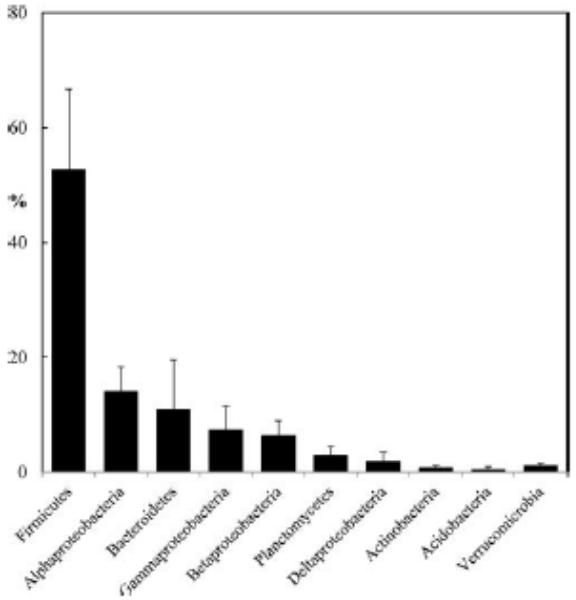

Figure 3. Relative abundance of individual phyla (for phyla representing > 1% abundance within the community) of microorganisms in the agar layer identified in the end of rhizobox experiment; data are presented as mean ± standard deviation, n = 5.

pounds,[43] the potential role of various soil microbial strains has been widely investigated. Under model laboratory conditions, organisms such as *Pseudomonas putida*, *Acidithiobacillus ferrooxidans*, or *Lysinibacillus fusiformis* are able to volatilize from 50% to almost 100% of Hg.[44–46]

For the estimation of Hg accumulation in the microbial biomass, the exposed agar layer was analyzed at the end of the experiment. The total Hg contents varied in the range from 0.11 to 0.36 mg kg^{-1} at the end of the first experiment and from 2.09 to 15.7 mg kg^{-1} after the second experiment (the pure agar contained 0.0034 mg kg^{-1} of Hg). In the second experiment, detectable values of organomercurials were found, ranging from 2.2 to 12.1 μg/kg of MeHg, and from the levels below the detection limit to 8.4 μg kg^{-1} of PhHg. Therefore, the microorganisms showed a high ability to accumulate Hg, especially in highly contaminated soil. Karunasagar et al.[47] investigated the Hg and MeHg biosorption ability of *Aspergillus niger* where the accumulated Hg contents reached up to 3.2 mg g^{-1} of the sorbent without any toxicity symptoms for the microorganisms. Similarly, the high Hg accumulation capacity of *Pseudomonas sp.* and *Bacillus sp.* was observed by Grassi and Netti[48] in extremely Hg contaminated coastal water.

To describe more precisely the soil microbial community at the agar layer in the end of the second experiment, the diversity of the individual strains was analyzed. The organisms representing 27 phyla were identified; those with more than 1% abundance are summarized in Fig. 3. Sorkhoh et al.[49] identified *Gammaproteobacteria*, *Actinobacteria*, and *Firmicutes* as the dominant mercury-resistant phyla. In our case, *Firmicutes* was the predominant phylum at the end of the experiment, and the detailed analysis of these organisms showed 42% abundance of *Paenibacillus* sp. followed by *Acetivibrio sp.*, *Brevibacillus sp.*, *Cohnella sp.*, *Lysinibacillus sp.*, and *Clostridium sp.* where their abundances varied between 0.5 and 1.8%. Other genera of *Firmicutes* did not exceed 0.6% of the total amount of the organisms.

Within the *Paenibacillus* genus, *Paenibacillus agaridevorans* was the most abundant species, with abundance ranging from 7 to 32% of sequences.[50] The characteristics of the microbial community before start of the experiment and after 90 days of cultivation are substantially different. Among the four microorganisms inoculated to the agar layer *Paenibacillus* sp. abundance exceeded the remaining organisms where the abundance of *Pseudomonas* sp. (*Gammaproteobacteria*) reached 3.5%, and *Burkholderia* sp. (*Betaproteobacteria*) only 0.1%. Comparing the agar-dwelling microbial community with the community identified in the soil before start of the experiment, from the organisms able to grow in the 0.1 mol L^{-1} solution of Hg, only *Luteibacter* (*Gammaproteobacteria*), *Mycobacterium* (*Actinobacteria*), *Bacillus* (*Firmicutes*), *Staphylococcus* (*Firmicutes*), *Bradyrhizobium* (*Alphaproteobacteria*), and *Sphingomonas* (*Alphaproteobacteria*) in abundance between 0.01 and 0.6% were identified. Therefore, only *Paenibacillus sp.* proved to be the predominant organism growing for a long time in the specific conditions of the rhizobox experiments. The evaluation of the microbial strains behavior without presence of dominating *Paenibacillus sp.* should be provided in further research to assess which organisms are able to replace it.

Conclusions

Paenibacillus sp. are Gram-positive, aerobic or facultatively anaerobic, spore-forming bacteria and are known as promising organisms for the bioremediation of soil contaminated by polyaromatic hydrocarbons (particularly naphtalene) in soil.[51] However, their potential ability to accumulate and transform Hg in soil has never been tested. Microorganisms suitable for

bioremediation should be characterized by developed resistance mechanisms against the target pollutant, thereby avoiding potential cell damage.[52] Simultaneously, the bacteria should be able to absorb the pollutant and transform it into volatile compounds. For mercury, this represents the reduction of Hg^{2+} to Hg° followed by passive volatilization without a loss of energy.[53]

In such cases, the microbial biomass works as a catalyst without mercury accumulation in the biomass. In this context, *Deinococcus geothermalis, Cupriavidus metallidurans, Enterobacter cloacae, Alkaligenes faecalis*, and *Pseudomonas putida* has been suggested as promising organisms after model laboratory experiments.[54,55] Our results suggest that *Paenibacillus* sp. should be tested for potential bioremediation use as well. However, their ability to accumulate, transform and potentially volatilize mercury needs to be investigated in further research.

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5.6 Publikovaná práce 6

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SOILS, SEC 3 • REMEDIATION AND MANAGEMENT OF CONTAMINATED OR DEGRADED LANDS • RESEARCH ARTICLE

Mobility of mercury in soil as affected by soil physicochemical properties

Adéla Šípková[1] · Jiřina Száková[1] · Aleš Hanč[1] · Pavel Tlustoš[1]

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Abstract
Purpose The interaction of mercury with organic matter was studied on three soils with distinct physical-chemical compositions (Fluvisol, Luvisol, and Chernozem) and three vermicomposts based on various bio-waste materials (digestate, kitchen waste with woodchips, and garden bio-waste).
Materials and methods Laboratory batch experiments, in which organic matter composition was modeled by adding graded doses of vermicompost to individual soils, were carried out. The composition of organic matter in these vermicomposts was assessed via fractionation of humic acids, fulvic acids, hydrophilic compounds, and possible hydrophobic neutral organic matter. Furthermore, the samples were artificially contaminated with inorganic and organic mercury. Prepared samples were stored under constant temperature of 25 °C. The incubation experiments lasted for 56 days, in which the samples were taken ten times. During the experiments, the changes in mercury mobile phase amount were observed, and the influence of the source of contamination was evaluated.
Results and discussion The amount of mobile mercury increased and then decreased during the time. In most of the soils and vermicompost combinations, the content of mercury bound to the soil was stable after 21 days. The effects of the mercury source on the exchangeable portion of Hg in the soils were most obvious in samples without added vermicompost. Nevertheless, differences between mobile inorganic and organic forms of Hg were lower in the case of Fluvisol compared to other soils. Moreover, in this soil, the content of available mercury was higher than from others.
Conclusions In general, the smallest differences between mobile inorganic and organic forms of Hg were observed in the case of soil with the highest content of organic matter. Also higher doses of vermicomposts decreased the amount of mercury mobile phase available. Additionally, the largest positive influence of vermicompost dose on Hg mobility was measured in soils combined with vermicompost with the highest portion of humic acids.

Keywords Bioavailability · Incubation experiments · Mercury · Organic matter · Vermicompost

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✉ Adéla Šípková
sipkovaa@af.czu.cz

[1] Department of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Czech Republic

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1 Introduction

Because of the potential toxic influence of mercury on the environment, the most accurate evaluation and description of mercury sorption by soil is essential. Sources of anthropogenic contamination could be miscellaneous, e.g., mining and smelting activities (Fernández-Martínez et al. 2005), industrial production, waste incineration, and agriculture (Cattani et al. 2008; Kacálková et al. 2009). The fate of mercury in soil depends on its physicochemical parameters such as mineral compositions and pH (Schlüter 1997; Rodrigues et al. 2006). In particular, the quantity and quality of soil organic matter are important for Hg mobility and bioavailability. However, chemical affinity to individual fractions of organic matter is different (Milne et al. 2003) and depends on the complexing capacity and complex stability of these fractions (Yao et al. 2006). Organic matter consists of high molecular-weight

hydrophobic compounds, collectively termed humic substances. They are comprised of humic acids (HA), fulvic acids (FA), simpler low-molecular weight hydrophilic compounds (Hy), and hydrophobic neutral (HON) organic matter (Stevenson 1994). In general, hydrophilic compounds account for the complexation of around 50 % of leached metals, with variable contributions of humic and fulvic acids (Laborda et al. 2009). Humic acids have a weak complex capacity for Hg but high complex stability, thereby causing a decrease in its mobility (Yao et al. 2006). The strong interaction of mercury with humic acids was explained by Chai et al. (2012) as a result of the abundant O-ligands present. The stability of fulvic acid complexes may be ascribed to its relatively high content of carboxylic groups. Nevertheless, compounds with fulvic acids can be comparatively soluble (Wallschläger et al. 1998). This implies that fulvic acids are important early in the process of stabilizing mercury. Additionally, the amount of mercury mobilized is reduced at pH <3 and at pH >12, due to the extremely high buffering capacity of humics both in acidic and alkaline states (Wallschläger et al. 1996; Kabata-Pendias and Pendias 2001). In acidic soils, Hg binds primarily to humic substances (Schwesig et al. 1999). Nevertheless, mercury cannot be bound directly to C but will bind to functional groups in organic matter, particularly sulfur (Xia et al. 1999). Moreover, these thiol binding sites associate stronger with Hg than they bind other heavy metals such as Cd or Zn (Mousavi 2015).

Generally, mercury is present in numerous chemical forms with different mobilities and toxicities. Non-mobile Hg is found, e.g., in mining soils with higher sulfur content (Martinez-Coronado et al. 2011; Gosar and Teršič 2012), because the Hg is tied mainly to the sulfur and constitutes insoluble HgS (Boszke et al. 2008). In the case of elemental mercury, these forms are less hazardous because of their reduced mobility and potential toxic impact on the environment. However, inorganic forms can be converted into organic mercury, especially methyl mercury, one of the most toxic compounds known with high bioaccumulation as well (Miretzky et al. 2005). In soils with low organic matter content, mercury is more reactive and prone to methylation (Skyllberg et al. 2006). The transformation of mercury among individual forms is significantly influenced by the source of the contamination and terms of exposition. Han et al. (2006) showed that Hg bound to sulfur is the major solid phase fraction in HgS-contaminated soil, while organically bound mercury is present in soil freshly contaminated with soluble Hg compounds.

The main goal of this study was to assess the influence of different sources of contamination as well as the amount and composition of organic matter on the fraction of mobile mercury. Better description of the influence of soil characteristics, mercury binding, and mobility could help to define patterns of Hg immobilization at contaminated sites or determine the most suitable remediation strategy. For instance, specific resins, amalgamating filters (Bollen et al. 2008), iron sulfide nanoparticles (Xiong et al. 2009), or the addition of reduced S in order to form HgS (Piao and Bishop 2006) may be applied. Moreover, cheap and strongly sorbent organic matter can be employed (Zhang et al. 2009). Decreasing the mobility of mercury is associated with a reduction in its toxic effects on other environmental components, e.g., groundwater or atmosphere.

2 Materials and methods

2.1 Samples

Surface soil samples with different physical-chemical compositions were used in this study. Fluvisol and Luvisol were collected from Píšťany and Hněvčeves, respectively, in the Czech Republic. Chernozem originates from an experimental field near the Czech University of Life Sciences in Prague. All areas of interest were uncontaminated anthropogenically by mercury. Three types of vermicompost were prepared in a specially adapted laboratory under controlled conditions (temperature 22 °C, relative humidity 80 %) using earthworms of the genus *Eisenia* (Hanč and Vašák 2015). These vermicomposts were based on various bio-waste materials with a high organic matter content including digestate (V1), kitchen waste with woodchips (V2), and garden bio-waste (V3).

2.2 Characteristics of soil and vermicompost samples

Measurements of pH were made using samples mixed with a 0.01 M solution of $CaCl_2$ 1:10 (*w/v*) by a WTW pH 340 i meter (WTW, Germany), and organic matter content was determined colorimetrically in a 0.33 M solution of $K_2Cr_2O_7$ and H_2SO_4. The cation exchange capacity (*CEC*) is defined as a sum of the extractable Ca, Mg, K, Na, and Al in 0.1 M $BaCl_2$. The concentrations of these elements were determined by inductively coupled plasma atomic emission spectrometry (ICP-OES, Varian, VistaPro, Australia).

2.3 Fractionation of vermicompost organic matter

The humic acid (HA), fulvic acid (FA), hydrophilic compounds (Hy), and hydrophobic neutral organic matter (HON) composition of each vermicompost was determined using the Superlite™ DAX-8 (SUPELCO Analytical, USA) according to Van Zomeren and Comans (2007). This slightly polar resin is comprised of a poly(methyl methacrylate) matrix with the following characteristics: particle size = 40–60 mesh, pore size = 225 Å and surface area = 160 $m^2\ g^{-1}$. Sorption as well as desorption of individual organic matter fractions on the resin were observed.

2.4 Incubation experiments

All three different soils and three vermicomposts with variable composition were submitted to incubation experiments. Each dried soil sample was mixed with three different dosages of each vermicompost. The first of three vermicompost dosages corresponded to that ordinarily recommended batch for soil (3.5 g kg^{-1}), the others were twofold (7 g kg^{-1}) and threefold larger (10.5 g kg^{-1}). Moreover, soils without any vermicompost were also used in incubation experiments for a comparison. Altogether, thirty combinations of soils and vermicomposts were evaluated (3 soils × 3 vermicomposts × 3 vermicompost dosages and 3 untreated soils). Concerning these vermicomposts, organic matter fractionation was executed primarily; therefore, additions of individual fractions were characterized. In all soil samples, the maximum water holding capacity was determined by gravimetric method and afterwards moistened to 60 % of this value.

Further, the samples were spiked with mercury. In this study, two main anthropogenic sources of mercury in the Czech Republic were compared: waste incineration (inorganic Hg) and dipping seeds (organic Hg). In the first case, inorganic mercury chloride was employed. Subsequently, organic phenyl-mercury chloride was used in the second case. Materials based on this compound were formerly used for dipping seeds called Agronal. The application of this kind of dipping seed was prohibited in the 1990s, and newly developed dipping seeds have replaced it. However, phenyl-mercury remained in soil for a long time after its use (Száková et al. 2016). The amount of mercury added artificially was equal to 12 mg kg^{-1}. This dose corresponded to the highest concentration of Hg found in a former waste incineration plant in the suburb of Hradec Králové, Czech Republic (Kacálková et al. 2009). Soils without any addition of vermicompost were used as control samples. Both forms of mercury (inorganic or organic) were supplemented to soil dissolved in water used for samples moisturizing. A large volume of solution suppressed problems with homogeneity. Moreover, all combinations were carried out in three repetitions, and relative standard deviation was less than 14 % in all cases.

The prepared samples were stored in the dark in sealed plastic vessels under a constant temperature of 25 °C. The incubation experiments were continued for 56 days. Samples were taken ten times during the entire period of the experiment, and they were subsequently subjected to following procedures.

2.5 Extraction of the mercury mobile phase

For the determination of portions of mobile mercury, the method of Quevauviller et al. (1993) was used. 0.5 g of each sample was added to 10 mL of a 0.11 M solution of CH_3COOH and shaken overnight. Subsequently, all samples were centrifuged for 10 min at 3000 rpm, and the extracts were acidified with a mixture of acids (HNO_3 : HCl = 3 : 1).

In all extracts, mercury content was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA) employing an autosampler ASX-500, a three-channel peristaltic pump and a MicroMist nebulizer. Calibration solutions of mercury, isotope Hg(202), were prepared as dilutions of single element ICP standards, 0.1–10 μg L^{-1}. Detection limit of ICP for mercury was 0.08 μg L^{-1}. Pt(195) was used at a concentration of 10 μg L^{-1} as an internal standard. For interference suppression, the collision cell was applied. Moreover, the method of standard additions was used to verification of potential interferences and/or accuracy of the measurements. Differences in slopes between external calibration and standard addition modes did not exceed 3 % in any case.

Table 1 Main physicochemical characteristic of experimental soils, pH, total carbon (TC), inorganic carbon (IC), organic carbon (OC) contents, and cation exchange capacity (CEC)

Soil	Fluvisol	Luvisol	Chernozem
Soil texture	Clay-loamy sand	Silt loam	Silt loam
Clay (<0.002 mm) (%)	11.63	4.36	2.18
Silt (0.002–0.05 mm) (%)	22.93	76.99	71.80
Sand (0.05–2 mm) (%)	65.43	18.65	26.03
pH	6.8	5.6	7.2
TC (%)	4.9	2.8	4.2
IC (%)	0.2	0.1	0.3
OC (%)	4.7	2.7	3.9
CEC (mmol kg^{-1})	201	79	255

Table 2 Characteristics of vermicomposts: pH, total carbon (TC), inorganic carbon (IC), organic carbon (OC) contents and distribution of individual organic matter fractions in vermicomposts: humic acids (HA), fulvic acids (FA), hydrophilic compounds (Hy), and hydrophobic neutral organic matter (HON)

Vermicompost	V1	V2	V3
pH	7.3	8.1	7.3
TC (%)	33.5	23.3	33.7
IC (%)	0.1	0.7	0.3
OC (%)	33.4	22.6	33.4
HA (%)	50.6	45.4	57.3
FA (%)	13.3	16.7	12.3
Hy (%)	25.0	26.8	21.2
HON (%)	11.2	10.8	9.3

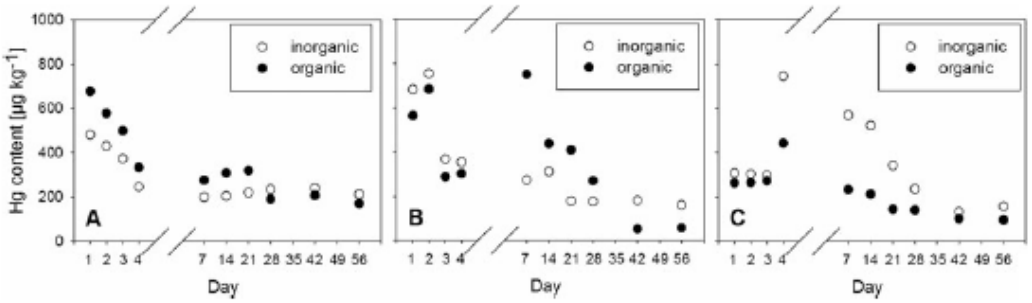

Fig. 1 Comparison of inorganic and organic mercury sources and their influence on the amount of mobile Hg in soils without added vermicompost. **a** Fluvisol, **b** Luvisol, **c** Chernozem

3 Results and discussion

3.1 Characteristics of soils and vermicomposts

Elementary chemical properties of all the soil samples are recorded in Table 1. The soil samples had neutral to slightly acidic pH and low organic matter content. The highest cation exchange capacity was measured in the case of Chernozem; in contrast, the lowest was found in Luvisol. Another important factor is represented by an oxidation state of the studied soils. Although the redox-potential was not measured, we assume that it was certainly positive. Since all soil samples were moistened to 60 % of the maximum water holding capacity. Concerning the binding abilities of specific samples, the maximum sorption capacity of all soils and vermicomposts ranged between 85 and 130 mg g^{-1}. The thorough description of the sorption properties of all samples will be an object of our future interest.

The organic matter composition of the vermicomposts is summarized in Table 2. Only minor variations concerning the organic matter fractions in all the vermicomposts were noted. In the case of garden bio-waste vermicompost (V3), the highest content of humic acids and the lowest content of fulvic acids were determined, approximately 57 and 12 %, respectively. In contrast, the vermicompost prepared from kitchen waste with woodchips (V2) contained only 45 % humic acids and the highest level of fulvic acids. In samples prepared from digestate and kitchen waste with woodchips, the hydrophobic neutral organic matter content ranged around 11 %. In the third vermicompost, hydrophobic neutral organic matter represented a slightly lower portion of the total organic matter. A similar trend was also observed in the case of hydrophilic compounds. In all samples, soils and vermicomposts, the mobile mercury contents were under the detection limit of ICP-MS.

3.2 Comparisons of inorganic and organic mercury sources and individual soils

The effects of the mercury source on the exchangeable portion of Hg in the soils were most obvious in samples without added vermicompost (Fig. 1). The initial values representing the mobile mercury portion released at the first day of incubation. The smallest differences in the amount of mercury released from inorganic and organic compounds were observed in the case of Fluvisol. This soil has the highest contents of oxidizable carbon and clay. This smallest grain size fraction (<2 µm) associates with the highest concentration of Hg (Boszke et al. 2004; Chakraborty et al. 2014). In the case of Chernozem, differences were measured during the incubation, especially in the control soils. Nevertheless, their mercury contents were similar at the end of the experiment (Fig. 1). In these two soils,

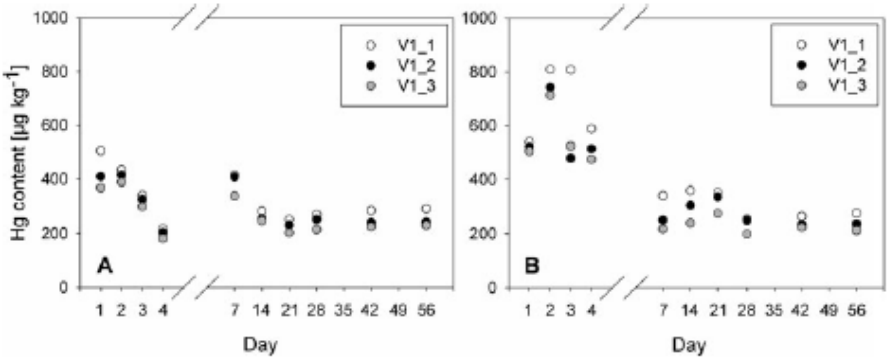

Fig. 2 Fluvisol treated with three dosages (1, 2, 3) of vermicompost—a comparison of the amount of mobile mercury released during the experiment from **a** inorganic and **b** organic sources

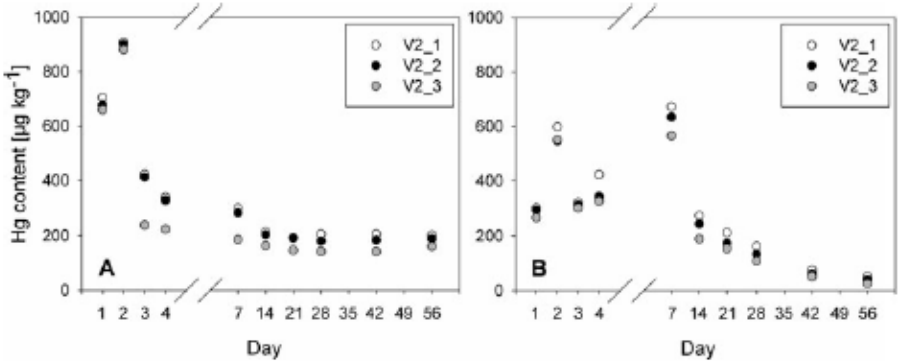

Fig. 3 Luvisol treated with three dosages (1, 2, 3) of vermicompost—a comparison of the amount of mobile mercury released during the experiment from **a** inorganic and **b** organic sources

the differences between inorganic and organic Hg did not exceed 130 µg kg^{-1} (in 75 % of samples up to 90 µg kg^{-1}). In contrast, the greatest difference was found in the case of Luvisol, where the variability in Hg content ranged between 105 and 155 µg kg^{-1} (only in one case, the value was 85 µg kg^{-1}). The amounts of mobile mercury portion released during the entire experiment are plotted in Figs. 2, 3, and 4 for all three soil samples in combination with selected examples of vermicomposts. Figure 2 shows the combination of Fluvisol with vermicomposts from digestate (V1). This figure demonstrates the overall lowest differences between released mobile mercury amount from inorganic and organic compounds, which concerned Fluvisol soil sample. Hence, this soil exhibit similar behavior both with (Fig. 2) and without (Fig. 1) the addition of vermicomposts. In contrast, Fig. 3 shows Luvisol treated by vermicomposts from kitchen waste with the highest different. The lowest measured organic mercury content at the end of experiment was observed in this soil. Thus, the difference between released organic and inorganic Hg was most pronounced. In 80 % of cases, the values were less than 60 µg kg^{-1}. This may be explained by the lowest pH being measured in Luvisol, due to the high adsorption of mercury onto organic matter under acidic conditions (Schwesig et al. 1999; Zhang et al. 2015). Moreover, at low pH values, the highest formation of insoluble mercuric sulfide can lead to a low solubility of mercury (Winfrey and Rudd 1990).

During the experiment, the amount of mobile mercury increased and then decreased. In most of the soils and vermicompost combinations, the content of mercury bound to the soil was stable after 21 days. Bower et al. (2008) observed a similar timeframe in their experiments focusing on mercury adsorption onto pyrite. Organic matter complexes strongly with Hg and reduces its mobility (Wang et al. 1997), so that over time non-mobile sulfides may be formed (Schuster 1991; Hirner et al. 2000). Because of the anaerobic conditions and slightly acidic pH, methylation can also occur (Hirner et al. 2000). The difference between inorganic and organic Hg sources was seen as a shift in the maximum Hg released. With organic compounds, the maximum amount of mercury was released a few days later as seen for instance in Fig. 4 in the case of Chernozem in combination with vermicomposts from digestate. Moreover, lower Hg contents were observed at the end of the experiment, especially in the case of Luvisol, with the lowest pH. This was plotted in Fig. 5 on example of Luvisol in combination with vermicomposts from digestate. These variations might be explained by different reactions of individual mercury species in soil. While the

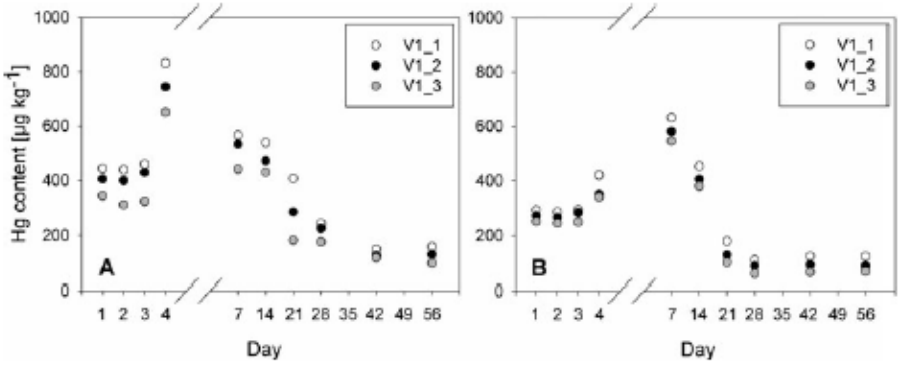

Fig. 4 Chernozem treated with three dosages (1, 2, 3) of vermicompost—a comparison of the amount of mobile mercury released during the experiment from **a** inorganic and **b** organic sources

can have a positive influence on the microbial population (Kiikkilä et al. 2001), and mercury can be mobilized by complexation with biomolecules of microbial metabolites (Carrillo-Gonzáles et al. 2006). Another explanation might be represented by the competition between organic matter and Hg on the adsorption sites on soil (Yang et al. 2008; Miller et al. 2009). This may reduce the number of Hg adsorption sites and therefore decrease the rate of mercury adsorption on soil. In the case of higher doses of vermicomposts, the effect of organic matter was more significant, and the amount of mercury released was reduced due to greater sorption.

4 Conclusions

The quantity and quality of the organic matter play an important role in mercury mobility in soil. The smallest differences in the amount of mercury released from inorganic and organic compounds were observed in the case of Fluvisol, with the highest content of organic matter. The largest positive influence of vermicompost dose on Hg mobility was measured in soils combined with vermicompost V3 from garden biowaste. Conversely, the smallest differences were found in vermicompost V2 from kitchen waste with woodchips. The highest amount of bound mercury was found at low pH and high humic acid content.

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5.7 Podaná práce

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The sorption abilities of soils and vermicomposts for mercury

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Corresponding Author:	Adéla Šípková Czech University of Life Sciences, Prague CZECH REPUBLIC	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Czech University of Life Sciences, Prague	
Corresponding Author's Secondary Institution:		
First Author:	Adéla Šípková	
First Author Secondary Information:		
Order of Authors:	Adéla Šípková	
	Hana Šillerová	
	Jiřina Száková	
	Pavel Tlustoš	
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Abstract:	In this study, the effectiveness of mercury (Hg) sorption by three different soils (Fluvisol, Luvisol, and Chernozem) and three vermicomposts was assessed. The kinetics of Hg sorption onto the materials was studied by static sorption experiments employing two basic models of sorption isotherms - Langmuir and Freundlich. The results showed that Chernozem had the best sorption properties among the studied soils, with the highest cation exchange capacity. The greatest amount of Hg was adsorbed by the vermicompost originating from garden bio-waste. This vermicompost contained the most humic acids and the smallest amounts of other organic matter fractions. The experiment showed that the soils had greater Hg sorption ability than vermicomposts, probably due to the mutual effects of clay minerals and soil organic matter. In the vermicomposts, Hg seems to be bound into less stable organic compounds (such as thiol groups) and the detailed description of these associations in further research will be necessary to better understand the fate of Hg in such materials.	
Suggested Reviewers:	Markus Puschenreiter markus.puschenreiter@boku.ac.at	
	Walter Wenzel walter.wenzel@boku.ac.at	
	Fangjie Zhao fangjie.zhao@bbsrc.ac.uk	
Opposed Reviewers:		

The sorption abilities of soils and vermicomposts for mercury

Adéla Šípková[1], Hana Šillerová[2], Jiřina Száková[1], Pavel Tlustoš[1]

[1] Department of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Czech Republic

[2]Department of Environmental Geosciences, Czech University of Life Sciences Prague, Czech Republic

Abstract In this study, the effectiveness of mercury (Hg) sorption by three different soils (Fluvisol, Luvisol, and Chernozem) and three vermicomposts was assessed. *The kinetics of Hg sorption onto the materials was studied by static sorption experiments employing two* basic models of sorption isotherms - Langmuir and Freundlich. The results showed that Chernozem had the best sorption properties among the studied soils, with the highest cation exchange capacity. The greatest amount of Hg was adsorbed by the vermicompost originating from garden bio-waste. This vermicompost contained the most humic acids and the smallest amounts of other organic matter fractions. The experiment showed that the soils had greater Hg sorption ability than vermicomposts, probably due to the mutual effects of clay minerals and soil organic matter. In the vermicomposts, Hg seems to be bound into less stable organic compounds (such as thiol groups) and the detailed description of these associations in further research will be necessary to better understand the fate of Hg in such materials.

Key words Mercury; Sorption Experiments; Organic Matter; Soil; Vermicompost

Introduction

The amount of Hg in the environment has increased because of industrial, mining and agricultural activities. Its mobility in soil depends on the Hg species and the soil properties, especially the pH and organic matter content (Tipping et al., 2010). Organic matter (OM) interacts very strongly with Hg, affecting its speciation, solubility, mobility and toxicity (Xu et al., 2015). Many organic compounds have a high affinity for Hg by means of their functional groups, such as hydroxyl-, carboxylic-, aromatic- and S-containing ligands. The percentage of Hg mercury bound to organic matter can range from 2.34 to 73.70 % of the total mercury and can be correlated with the amounts of clay and Fe oxides, depending on the

soil properties (Różański et al., 2016). As reported by Boszke et al. (2008), the sorption capacity of OM depends on its characteristics. On the one hand, immobilized OM soil particles may provide additional sorption sites, which may enhance Hg immobilization, while on the other hand, OM can increase Hg mobility in soil through the formation of dissolved OM (Xu et al. 2015). Soil OM consists of hydrophobic compounds; humic acids (HA) and fulvic acids (FA); simpler low-molecular weight hydrophilic compounds (Hy); and hydrophobic neutral (HON) organic matter (Stevenson, 1994). The size of OM particles affects the abundance of the functional groups where the alkyl hydrophobic components are mainlydistributed in the largest molecular-size-fraction, whereas the amount of oxidized carbons increased with decreasing size of fractions (Conte et al. 2007).

A high proportion of fulvic acids can intensify Hg volatilization, while the presence of humic acids can decrease Hg mobility in soil because of the high stability of their complexes (Yao et al., 2006). Slightly more Hg tends to bind to humic acids than to fulvic acids, comprising around 45 % of the Hg content, while bonds to fulvic acids and hydrophilic compounds constitute the rest (Laborda et al., 2009; Šípková et al., 2013). Lower molecular-weight compounds, such as fulvic acids and hydrophilic compounds, can remain in the soil solution and thus increase the mobility of their bound metals (Naidu and Harter, 1998). In general, organic matter may enhance Hg binding by providing additional sorption sites, but it may also reduce Hg sorption in soil through the formation of stable Hg-organic matter complexes in the soil solution (Skyllberg et al., 2000). Moreover, soil organic matter, especially humic acid, is thought to be the dominant factor influencing the spatial distribution and depth distribution of soil Hg (Zhang et al. 2015). Fernandez-Martinez et al. (2014) determined both humic and fulvic acids, as well as elemental Hg, as the primary variables controlling Hg methylation in the soils.

Because of the relatively low plant-availability of Hg in soil (Liu et al., 2010), bioremediation is not a suitable method for minimizing the negative influence of Hg on the environment. Immobilization may be a more appropriate method. Adsorption on mineral surfaces, formation of stable complexes with organic matter, electrostatic interactions, and ion-exchange were identified as the main mechanisms responsible for changes in Hg mobility in soil (Boszke et al., 2008; Yang et al., 2008; Calace et al., 2009). The most common mechanism for sorption onto the solid phase is the formation of stable insoluble inorganic and organic complexes (Schuster, 1991). Decreased soil Hg mobility followed by suppressed plant uptake have been documented in organic matter amended soils, as well as the high Hg

sorption ability of soil organic matter (Heeraman et al., 2001; Linde et al., 2007; Zhang et al.,2009). Moreover, the mobility of Hg is affected by interactions between the mineral and organic phases (do Nascimento and Masini, 2014).As sources of organic matter, bio-waste and composts or vermicomposts can be used. Hg mobility is also affected by pH, being reduced at pH < 3 and at pH > 12 because of the high buffering capacity of humic compounds both in acidic and alkaline states (Yin et al., 1996; Kabata-Pendias and Pendias, 2001; Miretzky et al., 2005). In acidic soil, adsorption on humic materials is favorable (Schwesig et al., 1999). At higher pH, Hg is predominantly bound to clay and oxides (McBride, 2004). Hg is also mainly bound to minerals in soils with a low content of organic matter (Biester et al., 2002).

In previous work, incubation experiments with three soils, Fluvisol, Luvisol, and Chernozem, treated with three different vermicomposts were carried out (Šípková et al. 2016). Better sorption properties were obtained in the case of soils without vermicompost addition, although composts are generally considered to reduce the mobility and plant-availability of Hg (Restrepo-Sánchez et al., 2015). Therefore, this study concerned the effectiveness of Hg sorption in these individual soil samples and vermicomposts, studied separately to assess their sorption behavior. Kinetic and equilibrium sorption experiments were used to evaluate the potential of the individual vermicomposts to adsorb Hg as affected by their organic matter composition.

Materials and Methods

Samples

For this study, three soil samples with different physical-chemical compositions were used. Fluvisol (F) and Luvisol (L) were collected from Píšťany and Hněvčeves, respectively, in the Czech Republic. Chernozem (Ch) originated from an experimental field near the Czech University of Life Sciences in Prague. Samples were taken from the top layer (0 – 30 cm, air-dried and sieved to obtain a fraction < 2 mm in diameter). These areas were not affected by Hg contamination. Additional samples are represented by three vermicomposts based on various bio-waste materials with a high organic matter content including digestate (V1), kitchen waste with woodchips (V2), and garden bio-waste (V3). These vermicomposts were prepared in a specially adapted laboratory under controlled conditions (temperature 22 °C, relative humidity 80 %) using earthworms of the genus *Eisenia* (Hanč and Vašák, 2015).

Sample characterization

In soil samples, measurements of pH were performed using a WTW pH 340 i pH meter (WTW, Germany) in a 0.01 M solution of $CaCl_2$1:10 (w/v, Novozamsky et al. 1993).In the case of vermicomposts, pH was measured in samples mixed with deionized water (1:10 w/v, Hanč and Vašák 2015). The cation exchange capacity (CEC) was determined as the sum of the extractable Ca, Mg, K, Na, and Al in 0.1 M $BaCl_2$solution(w/v = 1+20 for 2 hours, ISO 1994). The concentrations of these elements were measured by inductively coupled plasma atomic emission spectrometry (ICP-OES, Varian, VistaPro, Australia).Total carbon content was determined by thermal decomposition at 680°C and inorganic carbon by decomposition at 280°C. The inorganic carbon samples were acidified with phosphoric acid. After decomposition, CO_2 was detected using an NDIR detector (Shimadzu SSM 5000, Japan). The organic carbon content was calculated as the difference between the total and inorganic carbon. For the determination of total sulfur in soils and vermicomposts, a CHNOS Vario MACRO cube (Elementar Analysensysteme GmbH, Germany) analyzer was applied.

The composition of the used vermicomposts was further evaluated by fractionation of humic acid (HA), fulvic acid (FA), hydrophilic compounds (Hy), and hydrophobic neutral organic matter (HON). The fractionation was performed using Superlite^{TM} DAX-8 resin (SUPELCO Analytical, USA) according to van Zomeren and Comans (2007).

The characteristics of the soil and vermicompost samples were presented and discussed in a previous paper (Šípková et al., 2016). The most important characteristics of the materials are summarized in Table 1; the properties of the vermicomposts and the proportions of individual fractions of organic matter are shown in Table 2.

Kinetic and equilibrium sorption experiments

The effect of time on Hg sorption onto the materials of interest was investigated using batch sorption experiments. *Samples were exposed to the solution of $HgCl_2$ of defined Hg concentration of* 12 mg kg^{-1}*for times ranging from* 10 minutes to 36 hours(0.16; 0.5; 1; 3; 12; 18; 24; 36 h.). In all cases, 0.4 g of sample was added to 40 mL of Hg solution and agitated (250 rpm) at room temperature for the appropriate time. A 0.01 $mol.L^{-1}$ solution of $NaNO_3$was used as background electrolyte. Samples were prepared in duplicate for each tested time interval. *After exposure, the samples were centrifuged at 3600 rpm for 15 min and filtered (0.45 μm). Hg content in the solutions was* determined *by inductively coupled plasma mass spectrometry (ICP-MS).*

The experimental kinetic data were fitted to the commonly applied pseudo-first-order and pseudo-second-order equations. These equations can be written as follows:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (1)$$

$$\frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (2)$$

where k_1 (min^{-1}) is the first order rate constant of adsorption, k_2 (g mg^{-1} min^{-1}) is the second order adsorption rate constant, q_e (mg g^{-1}) the Hg adsorbed on the sorbent at the equilibrium time and q_t (mg g^{-1}) is the Hg amount adsorbed at time t (Mohan et al., 2011).

The percentage of Hg removed from the solution at concentration equilibrium was calculated according to the following equation:

$$Hg, sorption\ (\%) = \frac{C_i - C_e}{C_i} \cdot 100 \quad (3)$$

where C_i is the initial Hg(II) concentration and C_e is the Hg concentration at equilibrium (mg L^{-1}).

All solutions were acidified with a mixture of acids (HNO_3 : HCl = 3 : 1), and the Hg content was determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA). An ASX-500auto-sampler, a three-channel peristaltic pump, and a MicroMist nebulizer equipped ICP-MS comprised the experimental setup. A calibration curve was constructed using aqueous solutions with Hg concentrations of 0.1–10 µg L^{-1} and the isotope Hg(202) was measured. Pt(195) at a concentration of 10 µg L^{-1} was usedas an internal standard.

Based on the kinetic sorption experiments the time necessary to reach the concentration equilibrium was determined and used for equilibrium batch experiments. Samples were prepared in duplicates by mixing 0.4 g of each sample with 40 mL of initial solutions with varying Hg concentrations between 1 and 21 mg kgL^{-1} and agitated on 250 rpm under room temperatureuntil the equilibrium time. Equilibrium concentration values were measured by ICP-MS in the solution after sorption and the calculated values for Hg content per kilogram of soil or vermicompost were plotted. Two basic models of sorption isotherms— Langmuir and Freundlich — were used to evaluate the Hg sorption properties. These equilibrium isotherms

were constructed from the obtained data using nonlinear least squares regression (Bolster and Hornberger, 2007; Bolster, 2008).

The Langmuir isotherm is expressed as:

$$Q_{eq} = \frac{Q_{\max} b C_{eq}}{1 + b C_{eq}} \quad (4)$$

where C_{eq} is the equilibrium Hg concentration (mg l^{-1}), Q_{eq} is the amount of Hg adsorbed on a sorbent at equilibrium (mg g^{-1}), Q_{max} corresponds to the maximum sorption capacity and *b* is a Langmuir constant related to the energy of adsorption.

The Freundlich equation can be expressed as:

$$Q_{eq} = K_F C_{eq}^{\frac{1}{b}} \quad (5)$$

where Q_{eq} is the amount of Hg adsorbed per unit of biomaterial (mg g^{-1}), C_{eq} is the equilibrium concentration of Hg (mg L^{-1}), K_F a constant indicative of the relative adsorption capacity of the adsorbent (mgg^{-1}) and the constant 1/b indicates the intensity of the adsorption (Mohan and Pittman, 2006).

Results and Discussion

Kinetic experiments

The Hg concentrations as a function of time when vermicompost or soil samples are brought into contact with the initial Hg solution are shown in Figure 1 and Figure 2, respectively. In general, the sorption process can be divided into two phases: i) a very rapid (minutes) sorption of the majority of the Hg resulting in a substantial decrease in Hg concentration in aqueous solution; followed by ii) a slower sorption of the remaining Hg lasting 12–18 hours, depending on the soil or vermicompost type. This pattern can be explained as a rapid initial phase where Hg ions easily occupy the available space and bind to functional groups and a subsequent slower phase where Hg ions might be hampered in their diffusion into the deeper pores (Zeroual et al., 2003; Wang et al., 2006; Wang et al., 2014).No desorption was observed during the kinetic batch experiments. Zhang et al. (2012) investigated the effect of ionic strength on Hg adsorption by soils and showed no effect of sodium nitrate solution, or sulfate ions, whereas the presence of chloride ions significantly

decreased the adsorption capacity of Hg on soils. Moreover, the effect of chloride ions varied according to the soil type. A similar effect of chloride was observed by Miretzky et al. (2005). Thus, sodium nitrate solution was chosen as the background electrolyte to avoid the potential side effect of the other equilibrium solutions. Table 3 shows the kinetic parameters of the adsorption of Hg onto vermicomposts and soils. The results suggest that a pseudo-second order equation best describes the kinetic data in all cases. This model assumes that the chemical sorption is the rate-limiting process and that the reaction rate is directly proportional to the number of active sites on the adsorbent surface (Bayramoğlu et al., 2006; Farooq et al., 2010).

The second phase was more noticeable in the case of vermicomposts. The concentration equilibrium time occurred at about 18 hours. Among the used vermicomposts, the lowest sorption capacity was observed in the case of vermicompost V2 (kitchen waste and woodchips), in which the lowest TOC was measured. The other two vermicomposts had approximately the same TOC, nevertheless vermicompost V3 (garden bio-waste) exhibited stronger sorption for Hg. This vermicompost contained the most humic acids. Zhang et al. (2009) investigated the different surface structures of HA, FA, and the complexes involved in Hg-humic substances, which can help to explain the complexation behavior of Hg on these humic substances. HA have a higher adsorption capacity and lower desorption ration for Hg than other organic matter compounds (Zhang et al., 2009).On the other hand, the Hg sorption onto soils reached a concentration equilibrium after approximately 12 hours suggesting that in a sample with a higher organic content, a longer time is needed for the reaction to reach equilibrium (Yin et al., 1997).

The differences in the amount of Hg adsorbed onto the soil samples were small, and the sorption equilibrium was reached after a couple of hours. The lowest values of bound Hg were determined in the case of Luvisol, which had the lowest content of organic matter. Conversely, the best sorption capability was observed in Chernozem, which had the highest CEC and a high organic matter content. Zhu and Zhong (2015) described soil organic matter-clay mineral cooperativity in the Hg sorption process, where various clay minerals exhibit different binding affinities toward Hg due to their different characteristics *e.g.* specific surface area, cation exchange capacity, and mechanism of organic matter or Hg sorptionon these minerals (such as cation exchange or complexation). Thus, the soil type is the determining factor responsible for Hg sequestration in soil (Zhang et al., 2015), as also confirmed also by the present study. In the case of composts, Hg sorption onto the components of organic matter

plays the most important role. For better understanding of the different mechanisms of Hg sorption on the soils and/or composts more detailed description of individual Hg species bound onto particular functional groups of the vermicompost derived organic matter will be necessary.

One of the most important agents in Hg binding, dissolved organic matter (DOM) could also be one of the determining factors affecting the fate of Hg in soil and vermicomposts. DOM may enhance Hg sorption by providing additional sorption sites. However, it may also reduce Hg sorption via the formation of stable complexes in soil solution (Skyllberg et al., 2000).Thus, the addition of DOM-rich vermicompost into a soil can alter the fraction of mobile Hg in the amended soil. Zhang et al. (2014) assessed the effect of dissolved organic matter from wheat straw and swine manure on Hg adsorption in the different soils, in which the maximum adsorption capacity of Hg decreased after DOM addition. The effect of different DOM concentrations on Hg adsorption depended on the soil type and DOM source; the effect of DOM from wheat straw on Hg adsorption in the soils was higher than that of swine manure. In agreement with these findings, Šípková et al. (2016) measured the largest positive influence of vermicompost dose on Hg mobility in soil treated by vermicompost V3, based on garden bio-waste. Thus, although the DOM content in the vermicomposts was not determined in the present experiment, it can be speculated that DOM content will be at least partially responsible for the higher mobility of Hg in vermicomposts compared with soils.

Equilibrium sorption experiments

The results showed that the Hg(II) adsorption isotherms fitted well the Freundlich equation. Freundlich-type adsorption is considered to be a multi-layer process in which the amount of adsorbed solute per unit of adsorbent mass increases gradually. However, the application of the Langmuir equation resulted in a very low correlation coefficient in most cases. For this reason, the parameter Q_{max}, which represents the maximum sorbent capacity, could not be calculated for V2 and Fluvisol, so these materials could not be compared in terms of Q_{max}. The parameters of both models can be found in Table 4. A strong tendency of Hg(II) for sorption has been shown in previous research (Miretzky et al., 2005; Liu et al., 2010; Xue et al., 2013). In this study, more than 98 % of the Hg was removed from solution in the case of V1, V3 and the three types of soil; and 93% of the Hg was removed by V2. Although the V1 and V3 samples originated from different feedstocks, their fate in contact with Hg was similar. HA has a higher adsorption capacity and lower desorption ratio for Hg

than FA (Zhang et al. 2009). The lower percentage removal in the case of V2 can be linked to its lower organic carbon content. It can be assumed that the adsorption of Hg on the surface of adsorbents took place in multiple layers and that sorption intensity is higher in soil samples (which also corresponds with the faster kinetics, as mentioned above).

However, the literature shows that the sorption ability of Hg on organic matter components can be affected by many other factors. Canellas et al. (2010) separated humic matter of different molecular dimensions and evaluated the relationships between the chemical properties and size-fractions. They found that larger nominal molecular size-fractions contained larger relative amounts of aromatic C, while the smaller size-fractions were predominantly composed of oxidized carbons and less aromatic C compounds. Moreover, Conte et al. (2007) suggested that the HA and its larger molecular-size-fractions are composed by few hydrophobically stable aggregates with poor mutual molecular mobility, while the smaller fractions contained a larger number of hydrophilic and mobile molecular associations. Ma et al. (2015) showed that the dominant C components for Hg complexation in DOM were aromatic C, O-alkyl C, alkyl C, and carboxyl C. Thus, the particle size of the vermicomposts and soils are among the factors affecting the Hg sorption parameters. Whereas the soil samples were homogenized and sieved through a 2 mm diameter mesh, the vermicomposts were milled to the fine particles of undefined size. These differences could also result in different sorption parameters of soils *vs.* vermicomposts.

The desorption of Hg as affected by oxalate and cysteine, representing ligands with carboxylic and thiol groups with different affinities for Hg, was investigated by Senevirathna et al. (2011). They found that oxalate did not affect the desorption of Hg from kaolinite, but cysteine strongly inhibited the desorption, where at all tested pHs the inhibition became less prominent in the later stages of the desorption tests. They explained the effect of the organic acids on Hg sorption and desorption by the formation of ternary surface complexes involving the mineral, ligand, and Hg. Concerning the fate of Hg in contaminated soils, Leterme and Jacques (2015) showed that the critical parameters are dissolved organic matter (DOM) concentration in soil solution, the binding constant for DOM thiol groups, and Hg sorption to humic and fulvic acids in solid organic matter. The sequestration of Hg in crystalline Hg sulfides or bound to thiol groups in macromolecular natural organic matter was considered as impossible in the oxidative conditions. However, Manceau et al. (2015) showed that that Hg sulfide forms from thiol-bound Hg alone in contact with air. Additionally, thiol-containing organic acids can significantly affect Hg mobility in soils (Gondikas et al., 2010;

Senevirathna et al., 2011; Száková et al., 2016).In the present experiment, the individual vermicomposts differed in sulfur content, where the total S was 0.590 ± 0.045 %, 0.335 ± 0.013 %, and 0.403 ± 0.006 % for V1, V2 and V3, respectively. The vermicomposts can be suggested as S-rich materials compared with the soils, of which the highest S content was determined in Fluvisol (0.128 ± 0.001 %).Thus, the interaction of Hg with thiol groups in the vermicomposts will result in a different distribution of bound Hg in the vermicomposts compared with the soils.

Conclusions

Our study showed that the adsorption of Hg on the surface of adsorbents took place in multiple layers and that the sorption intensity is higher in soil samples compared with vermicomposts. An assessment of the Hg bonds in the vermicomposts seemed to be more complex due to the variable character of the vermicompost organic matter, where a detailed description of the organic matter composition will be necessary to fully understand the mechanisms of sorption/desorption of Hg on these materials.Thus, the potential application of vermicomposts for immobilization of Hg in the contaminated soil needs to be associated with detailed description of the vermicompost characteristics to estimate the potential sorption ability of this material in the soil.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflictof interest.

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458 **Table 1** Main physicochemical characteristics of experimental soils,pH, total carbon (TC),
459 inorganic carbon (IC), organic carbon (OC)contents and cation exchange capacity (CEC)
460 (Šípková et al. 2016)
461

Soil	Fluvisol	Luvisol	Chernozem
Soil texture	Clay-loamy sand	Silt loam	Silt loam
Clay (<0.002 mm) [%]	11.63	4.36	2.18
Silt (0.002–0.05 mm) [%]	22.93	76.99	71.80
Sand (0.05–2 mm) [%]	65.43	18.65	26.03
pH	6.8	5.6	7.2
TC [%]	4.9	2.8	4.2
IC [%]	0.2	0.1	0.3
OC [%]	4.7	2.7	3.9
CEC [mmol kg^{-1}]	201	79	255

462
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464 **Table 2** Characteristics of vermicomposts (Šípková et al. 2016): pH, total carbon (TC),
465 inorganic carbon (IC), organic carbon (OC) contents and distribution of individual organic
466 matter fractions in vermicomposts: humic acids (HA), fulvic acids (FA), hydrophilic
467 compounds (Hy), and hydrophobic neutral organic matter (HON).
468
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Vermicompost	V1	V2	V3
pH	7.3	8.1	7.3
TC [%]	33.5	23.3	33.7
IC [%]	0.1	0.7	0.3
OC [%]	33.4	22.6	33.4
HA [%]	50.6	45.4	57.3
FA [%]	13.3	16.7	12.3
Hy [%]	25.0	26.8	21.2
HON [%]	11.2	10.8	9.3

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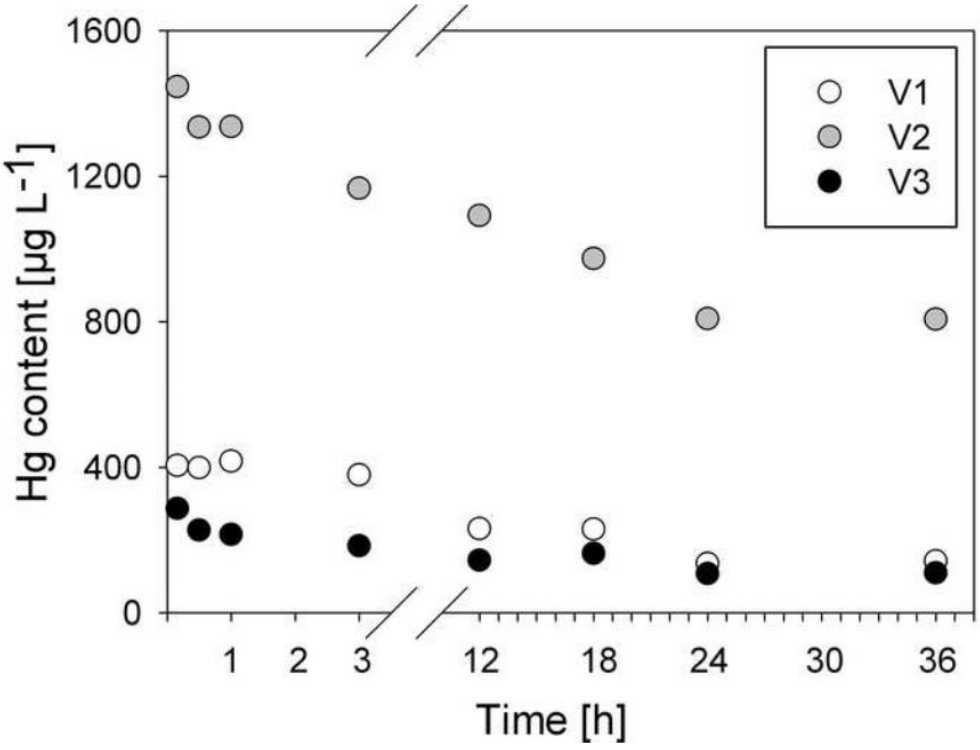
Table 3 The kinetic parameters of the adsorption of Hg onto vermicomposts and soils.

Vermicompost / Soil		Pseudo-first-order kinetic model			Pseudo-second-order kinetic model		
	q_e, exp mg g^{-1}	k_1 min^{-1}	q_e, cal mg g^{-1}	R^2	k_2 g $mg^{-1}min^{-1}$	Q_e, cal mg g^{-1}	R^2
V1	29.677	0.0016	1.015	0.882	0.0128	29.676	1.000
V2	27.999	0.0023	2.783	0.749	0.0061	28.000	0.999
V3	29.748	0.0016	0.469	0.821	0.0309	29.742	1.000
Fluvisol	29.750	0.0033	1.234	0.960	0.0363	29.762	0.998
Luvisol	29.909	0.0025	0.344	0.915	0.0962	29.912	0.999
Chernozem	29.959	0.0025	0.235	0.946	0.1426	29.961	1.000

Table 4 Fitted isotherm models for adsorption of Hg in vermicomposts and soils.

	Langmuir			Freundlich		
Vermicompost / Soil	Q_{max} mg g^{-1}	r^2	b	b	r^2	K_f mg g^{-1}
V1	100.30	0.920	0.001	0.950	0.944	108.4
V2	e	e	e	0.861	0.947	58.23
V3	93.50	0.884	0.003	1.07	0.980	123.2
Fluvisol	e	e	e	1.46	0.940	163.8
Luvisol	84.47	0.877	0.002	1.63	0.925	140.1
Chernozem	130.7	0.674	0.002	2.38	0.967	195.8

480 **Fig.1** Hg concentration during the sorption experiment by individual vermicomposts.



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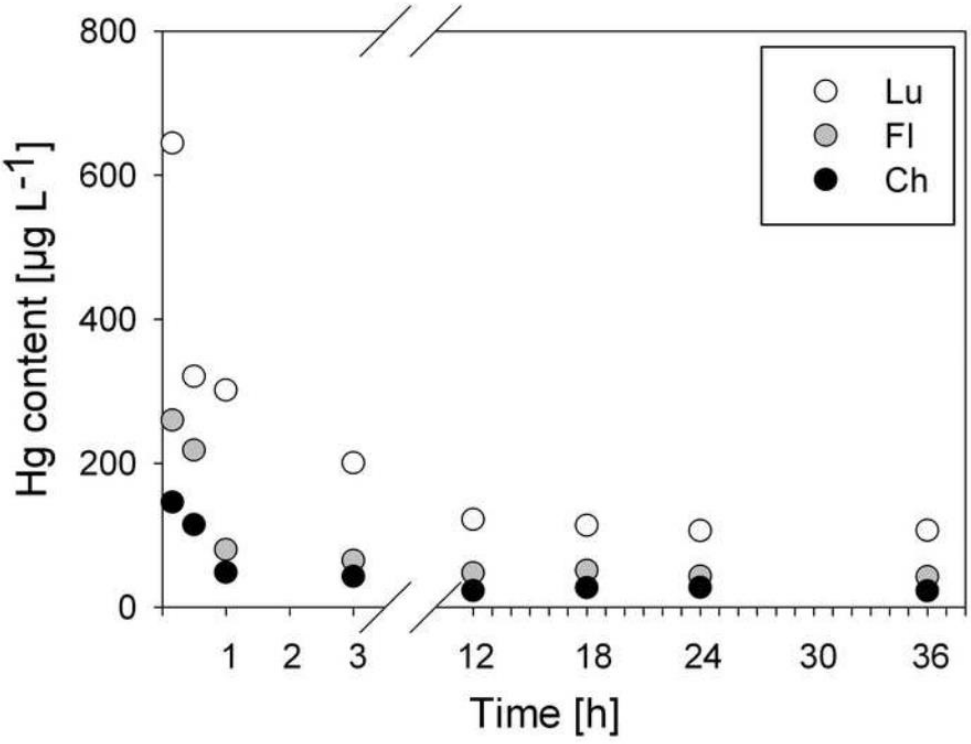
484 **Fig. 2** Hg concentration during the sorption experiment by individual soils (Luvisol, Fluvisol,
485 and Chernozem).



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6. SUMÁRNÍ DISKUZE

Tato kapitola shrnuje nejvýznamnější výsledky studie speciace rtuti, určení jednotlivých specií a vliv zdroje kontaminace půdy Hg. Podrobné výsledky jsou shrnuty v přiložených rukopisech.

6.1 Frakcionace a speciace Hg v kontaminovaných půdách

Extrahovatelnost Hg závisela na použitém extrakčním činidle. Pomocí HNO_3 bylo u 8 vzorků z 10 získáno 50 % z celkového obsahu Hg, v případě dvou nejvíce kontaminovaných pak 70 % a dokonce 96 %. Jednalo se o množství potenciálně mobilizovatelných specií rtuti. Také při použití $Na_2S_2O_3$ byl výtěžek nejvyšší u dvou nejkontaminovanějších vzorků, a to přibližně 20 % z celkového obsahu Hg. U ostatních vzorků se hodnoty pohybovaly mezi 1,2 a 3,4 %. Z toho lze usuzovat, že v místech s vyšší antropogenní kontaminací je množství rtuti vázané na S vyšší než v místech méně kontaminovaných. V případě nejvíce kontaminovaných vzorků podíl Hg extrahovatelný $Na_2S_2O_3$ koresponduje s výsledky, které publikovali Issaro et al. (2010), kde toto množství klesá s celkovým obsahem Hg.

Při použití chelatačního činidla EDTA se množství získané rtuti pohybovaly od 0,5 do 2 % ve všech vzorcích. Takové hodnoty byly pozorovány i ve studii Subirés-Munos (2011) na půdách pocházejících z těžební oblasti Almadén, Španělsko s vysokými obsahy Hg. Tato nízká variabilita naznačuje, že podíl Hg, která by potenciálně mohla být dostupná rostlinám, je velice podobný v místech s vysokou i nízkou mírou kontaminace Hg. Také v případě roztoku CH_3COOH byly výtěžky extrakce ve všech případech velice podobné a nepřekračovaly 0,15 % z celkového obsahu rtuti. Nízká dostupnost Hg extrakcí simulující půdní roztok nasvědčuje tomu, že rtuť je v půdě silně vázaná na sloučeniny síry nebo případně nerozpustné jílové minerály a organické látky (Rodrigues et al., 2010).

Výtěžky sekvenční extrakce potvrzují výsledky jednoduchých extrakcí. Obsah rtuti v prvním kroku extrakce reprezentujícím organické sloučeniny Hg byl pod detekčním limitem AMA-254 (2 $\mu g.kg^{-1}$). Také frakce F1 a F2 byly velmi nízké. Rtuť rozpustná ve vodě byla detekována pouze u dvou nejvíce kontaminovaných vzorků. Specie rozpustné v kyselinách byly naměřeny také v těchto dvou vzorcích a jejich obsah byl menší než 8 % z celkového množství Hg. Podobné hodnoty rozpustných podílů Hg získali také Rodrigues et al. (2010)

a Miller et al. (1995) v okolí dolu na rumělku. Nízké koncentrace této frakce byly nalezeny také u vzorku se třetím nejvyšším celkovým obsahem Hg. Množství semimobilních frakcí Hg (F3) se pohybovala od 18 do 30 %. Jedná se o rtuť silně vázanou na organickou hmotu s nízkou mírou mobility v půdě (Liu et al., 2006). Téměř stejný podíl Hg nalezli také Boszke et al. (2008), kteří se zabývali mobilitou a biodostupností Hg v půdách. Zastoupením rtuti v jednotlivých půdních frakcích ve velmi kontaminované půdě z těžební oblasti Idrija ve Slovinsku se věnovali Tešič et al. (2011). Zde podíl rtuti vázané na organickou hmotu a minerály dosahoval i 35 až 40 % z celkového obsahu. Tyto vyšší hodnoty mohou být spojeny s kyselým pH, protože rtuť se na organickou hmotu váže zejména při nízkém pH (Schwesig et al., 1999). V našich experimentech byly naproti tomu obsahy této frakce v nejvíce kontaminovaných vzorcích jen přibližně 9 % celkového obsahu a rozdíly mezi mobilními a semimobilními speciemi byly relativně nízké.

Nejvyšší obsah Hg byl nalezen v případě nemobilní frakce, tj. rtuť elementární a vázaná v komplexech (F4). Obsah rtuti v pevných zbytcích po extrakci byl ve většině vzorků nižší než 12 %. U více kontaminovaných vzorků však byly tyto hodnoty vyšší. Je zřejmé, že podstatné části reziduí jsou formy rtuti vázané na oxid křemičitý nebo sulfidy (Liu et al., 2006).

Dále bylo sledováno rozložení rtuti a některých vybraných prvků (S, Mg, Mn, Fe, Cu, Zn a Pb) v jednotlivých frakcích organické hmoty a případné korelace mezi nimi. Množství rtuti vázané na organickou hmotu nepřesáhlo u žádného vzorku 8 %. Organická hmota byla rozdělena na 4 frakce, huminové kyseliny (HK), fulvokyseliny (FK), hydrofilní sloučeniny (HS) a hydrofobní neutrální organickou hmotu (HN). Přes 45 % Hg bylo vázáno na HK, téměř 40 % na FK a 15 % na HS. V případě HN nepřesáhlo množství navázané rtuti ani ostatních sledovaných prvků 0,4 % z celkového obsahu, většinou to však bylo méně než 0,1 % a pro korelace nebyla tato frakce hodnocena.

Při hledání korelací mezi množstvími prvků navázaných v organické hmotě celkově byl nalezen významný vztah mezi rtutí a Pb nebo Zn, kde korelační koeficienty (r) dosahovaly hodnot 0,95 - 0,99. Také v případě síry byla zaznamenána silná závislost, kde r = 0,84. To koresponduje s faktem, že v organické hmotě není Hg vázaná přímo na uhlík, ale síru (Xia et al., 1999). U ostatních prvků (Mg, Mn a Fe) byly r se rtutí nižší než 0,5, pokud ovšem byly z výpočtů vyloučeny dva prvky s nejvyšší mírou znečištění, korelační koeficienty se zvýšily na hodnoty v rozmezí od 0,82 do 0,87 a vztahy byly nepřímo úměrné. Množství

síry významně korelovalo s již zmíněnou Hg, ale také Cu, Zn a Pb. Korelační koeficienty byly vyšší než 0,8, což odpovídá tomu, že tyto prvky jsou vázány především na S (Thornton, 1981).

V případě huminových kyselin byly nalezeny velice podobné vztahy jako při hodnocení organické hmoty celkově. Pouze korelace rtuti se sírou byla méně silná (0,53). Naproti tomu u fulvokyselin byl nalezen vztah Hg pouze se S, jednalo se ovšem o zápornou korelaci (-0,86). Málo vztahů rtuti s ostatními prvky bylo objeveno také v hydrofilních sloučeninách organické hmoty. Významný byl pouze s mědí (0,85), u ostatní prvků nepřekročil 0,5.

Porovnáním korelací mezi celkovými obsahy jednotlivých prvků byl zjištěn vztah mezi Hg a Cu, Zn a Pb s korelačním koeficientem r = 0,83, v případě olova dokonce r = 0,99. Stejné vztahy byly nalezeny taky v pracích Salizzato et al. (1998) a Calace et al. 2005).

6.2 Vliv aditiv na mobilitu Hg v půdě

6.2.1 Inkubační pokusy

Byly provedeny dvě sady inkubačních pokusů. První studie byla zaměřena na vliv jednotlivých složek organické hmoty na mobilitu rtuti v půdě a vliv zdroje kontaminace, ta druhá pak hodnotila zvláště vliv síry.

Vliv zdroje kontaminace rtutí byl nejvíce patrný ve vzorcích půd bez přídavku vermikompostu. Nejmenší rozdíl v mobilní frakci rtuti uvolněné z organických nebo anorganických látek byl pozorován u fluvizemě. Je to půda s nejvyšším obsahem uhlíku a zároveň jílu. Na tuto frakci s velikostí zrna menší než 2 μm se váže nejvíce rtuti (Boszke et al. 2004; Chakraborty et al. 2014). U černozemě byly rozdíly naměřeny, ovšem na konci experimentu byly obsahy mobilní rtuti velmi podobné. Největší rozdíly byly pozorovány u luvizemě, kde se koncentrace Hg lišily v rozmezí od 105 do 155 $\mu g.kg^{-1}$. To by mohlo být vysvětleno nejnižším naměřeným pH právě u luvizemě, kvůli vysoké adsorbci rtuti na organickou hmotu v kyselých podmínkách (Schwesig et al, 1999; Zhang et al. 2015). Navíc se při nižších hodnotách pH tvoří více nerozpustného sulfidu rtuťnatého (Winfrey a Rudd, 1990).

Během experimentů množství mobilní rtuti stoupalo a poté klesalo. Ve většině kombinací půd a vermikompostů byl však obsah rtuti vázané v půdě stabilní po 21 dnech. Podobný časový průběh pozorovali také Bower et al. (2008) v práci, která byla zaměřena na adsorpci rtuti na pyrit. Rozdíl mezi anorganickým a organickým zdroje rtuti se projevil také v posunu maxima uvolněné rtuti. V případě organické sloučeniny k němu docházelo o několik dní později. Navíc byly na konci pokusu pozorovány nižší obsahy mobilní rtuti právě v případě organického zdroje. Lze to vysvětlit různými reakcemi jednotlivých specií Hg v půdě. Zatím co mobilita anorganické rtuti je závislá především na tvorbě komplexů s organickou hmotou, rozpustnost organické Hg závisí více na iontové výměně (Schlüter, 1997). Kromě toho mikroorganismy zpracovávají spíše organické sloučeniny, což může být také důvod nižšího množství rtuti uvolněné na konci inkubačních pokusů.

Při porovnávání jednotlivých vermikompostů na mobilitu rtuti byl nejvyšší vliv pozorován u vermikompostu ze zahradního bioodpadu (V3), který obsahoval nejvíce huminových kyselin a to 57,3 %. Naproti tomu u vermikompostu V2 (kuchyňský odpad a štěpka) byly nalezeny nejmenší rozdíly. Tento vermikompost obsahoval jen 45,4 % HA. To lze vysvětlit jak rozdělením rtuti mezi jednotlivé frakce organické hmoty, tak vlastnostmi těchto frakcí. Významný je především vyšší podíl biologického rozkladu u sloučenin s nižší molekulovou hmotností a nízkou mobilitou huminových kyselin (Milne et al., 2003). Fulvokyseliny a hydrofilní sloučeniny, tedy složky s nižší molekulovou hmotností, mohou také spíše zůstávat v půdním roztoku a tím zvyšovat mobilitu navázaných kovů (Naidu a Harter, 1998).

Ve druhé studii byly hodnoceny změny v mobilitě rtuti v půdě s přídavkem sloučenin obsahujících síru. U černozemě byl obsah extrahovatelné Hg nízký ve všech variantách bez ohledu na přidané látce a to na začátku i na konci experimentu. Třetí a sedmý den ovšem bylo pozorováno zvýšení množství uvolněné rtuti, podobně jako v naší předchozí studii. V případě luvizemě klesal obsah extrahovatelné rtuti v průběhu pokusu a na jeho konci se dostal na úroveň dosaženou u černozemě. Znečištění půdy rtutí může snížit mikrobiální biomasu, nicméně některé bakterie se dokáží na tyto podmínky přizpůsobit (Muller et al., 2001). Znamená to, že pozorované změny v množství mobilní rtuti v průběhu experimentu mohou být přičteny různým komunitám žijícím v jednotlivých půdách. Na jejím konci se pak biomasa ustálí a s ní i množství mobilní Hg.

Po přidání digestátu bylo pozorován nejrychlejší pokles mobility Hg, a to již během prvního dne, u popílku byl vliv nižší. V případě síranu amonného nebyl ve srovnání s neošetřenou půdou zaznamenán významný rozdíl, kromě rychlejšího nárůstu množství mobilní rtuti ve 3. a 4. dnu inkubačních pokusů, což by mohlo být vysvětleno vlivem zvýšeného množství mobilní síry. U černozemě byl vliv přídavků na mobilitu rtuti nižší než vliv samotné půdy, která má vyšší sorpční kapacitu a obsah organické hmoty. Z těchto výsledků plyne, že obsah S v přidaných sloučeninách nebyl řídícím faktorem imobilizace rtuti. Luo et al. (2009) pozorovali jen slabý vztah mezi množstvím S a Hg v půdách s nízkým obsahem celkového organického uhlíku (~2%), jako tomu bylo v našich půdách. To může také vysvětlit jen malý vliv přidání síranu amonného, díky kterému se obsah uhlíku nezvyšuje.

Míra desorpce Hg se zvyšuje s množstvím rozpuštěné organické hmoty – DOM (Linde et al., 2007). V naší práci se ovšem obsah DOM měnil velice výrazně, v případě luvizemě ošetřené digestátem se sedmý den inkubace zvýšil 22 krát. Naproti tomu u černozemě bylo pozorováno pouze 1,5 krát zvýšení. To ukazuje, že mobilitu Hg v půdě neovlivňuje jen obsah a rozpustnost organického uhlíku v půdě. Důležitá je i afinita Hg k oxidům kovů (Kabata-Pendias, 2001), role půdních mikroorganismů (Mathema et al., 2011) nebo případné těkání rtuti během pokusu.

6.2.2 Sorpční experimenty

V rámci loužících experimentů byla pozorována velice rychlá sorpce, první fáze trvala řádově minuty a během ní byla nasorbována většina Hg. Ve druhé fázi byla sorpce pozvolná a po 12 až 18 hodinách již sorpce téměř neprobíhala, bylo dosaženo rovnovážného stavu. V případě fluvizemě a luvizemě byla rovnováha pozorována již během prvních hodin. Vysvětlením může být, že v rychlé počáteční fázi ionty Hg snadno obsadí volný prostor a naváží se na funkční skupiny. V následující pomalejší fázi mohou být ionty Hg zpomalovány difúzi do hlubších pórů (Zeroual et al., 2003; Wang et al., 2006; Wang et al., 2013). Výsledky naznačují, že reakce pseudo-druhého řádu popisuje kinetická data ve všech případech lépe. Tento model předpokládá, že chemická sorpce je proces omezující rychlost a že reakční rychlost je přímo úměrná počtu aktivních míst na povrchu adsorbentu (Bayramoğlu et al., 2006; Farooq et al., 2010). Druhá fáze byla výraznější u vermikompostů. Doba dosažení rovnováhy byla přibližně 18 hodin. Z použitých vermikompostů byla nejnižší

sorpční schopnost zjištěna u V2 (kuchyňský odpad a dřevěné štěpky), u kterého byla změřeno nejméně TOC. Zbývající dva měly přibližně stejné TOC, nicméně u vermikompostu V3 (zahradní biologický odpad) byla pozorována silnější sorpce Hg. Tento vermikompost obsahuje nejvíce huminových kyselin. V případě půd bylo rovnovážné koncentrace Hg dosaženo po přibližně 12 hodinách, což naznačuje, že při vyšším obsahu organické hmoty je čas potřebný k dosažení rovnováhy delší (Yin et al., 1997). Nejméně navázané rtuti bylo pozorováno v případě Luvizemě s nejnižším obsahem organických látek. Naopak, nejvyšší sorpční schopnost byla zjištěna u Chernozemě s nejvyšším CEC a vysokým obsahem organických látek.

Výsledky vsádkových experimentů ukázaly, že pro naše vzorky se lépe hodí Freundlichův model a adsorpce Hg na povrch sorbentů probíhala ve více vrstvách. U dvou vzorků, vermikompost V2 a fluvizem, nebylo možné Langmuirův model vůbec použít. Intenzita sorpce byla vyšší u vzorků vermikompostů, což odpovídá také rychlejší kinetice pozorované i u loužících pokusů. Nicméně sorpce Hg na složkky organické hmoty může být ovlivněna řadou faktorů. V práci Canellas et al. (2010) byla organická hmota rozdělena a byl hodnoceny vlastnosti jednotlivých velikostních frakcí. Bylo zjištěno, že větší frakce obsahují větší množství aromatické C, zatímco menší velikostní frakce byly složeny převážně z oxidovaných uhlíků a méně aromatických sloučenin C. Ma et al. (2015) ukázaly, že pro tvorbu komplexů Hg v organické hmotě jsou dominantní složky obsahující aromatický C, O-alkyl C, alkyl C a karboxyl C. Velikost částic vermicompostů a půd tedy patří k faktorům ovlivňujícím sorpci Hg. Zatímco vzorky půdy byly homogenizovány a prosévány sítem o průměru 2 mm, vermicomposty byly rozemleté na jemné částice s nedefinovanou velikostí. Tyto rozdíly by mohly vést také k rozdílným sorpčním parametrům půd a vermikompostů. Určujícím faktorem je také množství thiolových skupin ve vzorcích, které mohou ovlivnit pohyblivost Hg (Száková et al., 2016). Jednotlivé vermikomposty se lišily obsahem síry, kdy množství S se pohybovalo mezi 0,335 a 0,590 %. V případě půd bylo nejvíce síry zjištěno v případě fluvizemě (0,128 %).

6.3 Vliv půdy kontaminované Hg na společenstva půdních mikroorganismů

V poslední práci byl hodnocen vliv mikroorganismů na mobilitu rtuti. Celkový obsah Hg v jednotlivých segmentech rhizosferní půdy se nelišil u obou úrovní kontaminace,

u varianty s nižším obsahem Hg pak ani množství mobilní rtuti, extrahovatelné kyselinou octovou. Nicméně v experimentu s vyšším obsahem rtuti byl pozorován mírný pokles směrem k agarové vrstvě. Frakcionace Hg v jednotlivých segmentech rhizosferní půdy pak byla ovlivněna obsahem Hg v jednotlivých půdách. U experimentu 1, s nižším obsahem Hg, byla převládající frakcí F3, tedy semimobilní rtuť vázaná na huminové kyseliny. Ve více znečištěné půdě byla rtuť rozdělena především mezi frakce F4 > F3 > F5. Nejvíce tedy bylo rtuti elementární a vázané na komplexy, což bývá pozorováno u vysoce kontaminovaných průmyslových půd (Covelli et al., 2009). Vysoký podíl Hg byl také vázán na sulfidy. Liu et al. (2010) se domnívají, že tato frakce může představovat až 10 % z celkového obsahu Hg. V experimentu 2 byla nalezena také fenylrtuť, což není vzhledem k blízkosti bývalé mořírny osiva, překvapující. Její obsah klesal se vzdáleností od agarové vrsty. To by mohlo být vysvětleno vlivem mikrobialních exudátů na mobilizaci organické Hg. Dále byla u pokusu s vyšší obsahem Hg zjištěna těkavá frakce rtuti, jejíž koncentrace se pohybovala mezi 13 a 20 $ng.m^{-3}$.

Na konci experimentu byla analyzována vrstva agaru a bylo zjištěno, že celkový obsah Hg v experimentu 1 se pohyboval v rozmezí od 0,11 do 0,36 $mg.kg^{-1}$, u experimentu 2 to bylo od 2,09 do 15,7 $mg.kg^{-1}$. Je tedy zřejmé, že mikroorganismy dokázaly akumulovat Hg zejména v půdách s vysokou mírou kontaminace. Mikroorganismy jako *Pseudomonas putida, Acidithiobacillus ferrooxidans* nebo *Lysinibacillus fusiformis* jsou schopny vypařit až 50 - 100 % rtuti (Cabral et al., 2013; Gupta et al., 2012; Takeuchi et al., 2001). N našem případě byl podrobnou analýzou společenstva mikroorganismů jako převládající kmen identifikován *Firmicutes*, kde 42 % bylo *Paenibacillus sp.* následované *Acetivibrio sp.*, *Brevibacillus sp.*, *Cohnella sp.*, *Lysinibacillus sp.,* a *Clostridium sp.*. V rámci rodu *Paenibacillus* byl nejhojnější *Paenibacillus agaridevorans*.

7. ZÁVĚR

V této práci bylo zkoumáno rozložení rtuti v půdách, její mobilita a míra sorpce a také vliv některý anorganických a organických aditiv. Na základě výsledků jednoduchých i sekvenčních extrakcí bylo pro další měření vybrána extrakce 0,11 M roztokem kyseliny octové pro hodnocení mobility Hg. K podrobnějšímu popisu rozložení Hg v půdě pak sekvenční extrakční postup popsaný v publikovaných pracích.

Dále byla zkoumána distribuce rtuti v organické hmotě, kde je rtuť vázána především na huminové kyseliny, a to až 45 % z celkového obsahu. Na rozdíl od většiny kovů je z velké části, přibližně 40 %, vázána také na fulvokyseliny, zbytek pak na hydrofilní sloučeniny. Jen méně než 0,4 % je vázán na hydrofobní neutrální organickou hmotu. Výsledky analýz ukázaly, že množství rtuti vázané na huminové kyseliny je nepřímo úměrné obsahu Mg, Mn, Fe a Cu, ovšem pouze v půdách s množství rtuti do 10 $mg.kg^{-1}$. V případě fulvokyselin byla nalezena záporná korelace mezi Hg a S ($r = -0,86$), ovšem při hodnocení organické hmoty jako celku měl vztah mezi těmito prvky pozitivní korelaci ($r = 0,84$).

Množství a kvalita organické hmoty hrají důležitou roli v mobilitě rtuti v půdě. Z výsledků inkubačních pokusů se ukázalo, že nejmenší rozdíly v množství rtuti uvolněné z anorganický a organický sloučenin byly pozorovány v případě fluvizemě. Tato půda obsahovala nejvíce organické hmoty. Z porovnání jednotlivých vermikompostů byly pak největší rozdíly v mobilitě rtuti změřeny po přidání vermikompostu ze zahradního bioodpadu, nejmenší u vermikompostu z kuchyňského odpadu a štěpky. Jednalo se o vermikomposty s nejvyšším a nejnižším obsahem huminových kyselin.

V dalších experimentech hodnotících vliv síry na sorpci rtuti bylo zjištěno, že největší imobilizační účinek z hodnocených sloučenin obsahujících S mělo přidání digestátu. Na rozdíl od popílku a síranu amonného zde k navázání Hg došlo nejenom díky zvýšení množství S v půdě, ale také vysokému obsahu organické hmoty.

Pro odhad akumulace Hg v mikrobiální biomase a nalezení vhodných mikroorganismů pro bioremediaci kontaminované půdy byly provedeny pokusy s upravenými rhizoboxy. Výsledky experimentu ukázaly, že u *Paenibacillus sp*. by bylo vhodné dále testovat jejich schopnost akumulovat, transformovat, odpařovat Hg a případně využití k bioremediacím formou bioaugmetace půdy těmito organismy.

Našimi experimenty bylo potvrzeno, že adsorpce Hg na povrch adsorbentů probíhá ve více vrstvách a u půd je intenzita sorpce rtuti vyšší než v případě vermikompostů. Na

množství navázané rtuti mělo vliv také pH. Největší množství vázaného rtuti bylo zjištěno při nízkých hodnotách pH a vysokém obsahu huminových kyselin. Ukázalo se, že na chování rtuti v půdách mají větší vliv jednotlivé parametry půdy než sloučeniny obsahující síru použité k jejich ošetření, zejména sorpční kapacita půdy a obsah organické hmoty. Také vliv přídavku vermikompostu byl výraznější až po přidání násobných dávek, než jsou obecně přidávány, mobilita rtuti však díky nim byla snížena.

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