UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Maša JELUŠIČ

KAKOVOST IN FUNKCIONIRANJE ONESNAŽENIH VRTNIH TAL PO REMEDIACIJI

DOKTORSKA DISERTACIJA

Ljubljana, 2014

UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Maša JELUŠIČ

KAKOVOST IN FUNKCIONIRANJE ONESNAŽENIH VRTNIH TAL PO REMEDIACIJI

DOKTORSKA DISERTACIJA

QUALITY AND FUNCTIONING OF CONTAMINATED GARDEN SOIL AFTER REMEDIATION

DOCTORAL DISSERTATION

Ljubljana, 2014

Doktorsko delo je zaključek interdisciplinarnega doktorskega študijskega programa Varstvo okolja, Univerza v Ljubljani. Delo je bilo opravljeno v Centru za pedologijo in varstvo okolja Oddelka za agronomijo Biotehniške fakultete Univerze v Ljubljani.

Na podlagi Statuta Univerze v Ljubljani ter po sklepu Senata Biotehniške fakultete in sklepu Senata Univerze z dne 14. 11. 2012 je bilo odločeno, da kandidatka izpolnjuje pogoje za opravljanje doktorata znanosti na Interdisciplinarnem doktorskem študiju Varstvo okolja. Za mentorja je bil imenovan prof. dr. Domen Leštan.

Komisija za oceno in zagovor

Predsednik: prof. dr. Helena Grčman

Mentor in član: prof. dr. Domen Leštan

Član: prof. dr. Marijan Veber

Datum zagovora:

Delo je rezultat lastnega raziskovalnega dela. Izjavljam, da so vsa vključena znanstvena dela identična objavljeni verziji. Podpisana se strinjam z objavo svoje naloge v polnem tekstu na spletni strani Digitalne knjižnice Biotehniške fakultete. Izjavljam, da je naloga, ki sem jo oddala v elektronski obliki, identična tiskani verziji.

Maša JELUŠIČ

KLJUČNA DOKUMENTACIJSKA INFORMACIJA

ŠD Dd
DK UDK 631.4:631.111+351.777.6(043.3)=111=163.6
KG potencialno strupene kovine (PSK)/ etilendiamintetraocetna kislina (EDTA)/ remediacija tal/ revitalizacija remediiranih tal
AV JELUŠIČ, Maša, univ.dipl. inž. agr.
SA LEŠTAN, Domen (mentor)
KZ SI-1000 Ljubljana, Jamnikarjeva 101
ZA Univerza v Ljubljani, Biotehniška fakulteta, Interdisciplinarni doktorski študij Varstvo okolja
LI 2014
IN KAKOVOST IN FUNKCIONIRANJE ONESNAŽENIH VRTNIH TAL PO REMEDIACIJI
TD Doktorska disertacija
OP VIII, 105 str., 4 sl., 114 vir.
IJ sl
JI sl/en
AI Onesnaženje tal, povzročeno z velikimi količinami antropogenih onesnažil, ki so usidrana v kmetijska in urbana tla, postaja vedno večji svetovni problem in učinkovite metode remediacije so nujno potrebne. V doktorskem delu je bil raziskan vpliv remediacije z ligandom EDTA na vrtna tla iz Mežiške doline, onesnažena s Pb, Zn in Cd, in potencialna uporaba remediiranih tal za gojenje rastlin. Onesnažena tla so bila glede na koncentracijo onesnaženosti oprana z 10, 30, 60 in 120 mmol EDTA kg^{-1} tal, s čimer se je odstranilo do 80 % Pb, 28 % Zn in 72 % Cd, ter tudi do 75 % mikrohranila Mn. V poljskem poskusu so bila na remediirana in onesnažena tla, položena v dve vrtni gredici (4 × 1 × 0,3 m), posajene vrtnine. Analiza frakcionacije potencialno strupenih kovin (PSK) na različne talne komponente je pokazala, da so v remediiranih tleh ostale predvsem nedostopne PSK. Biodostopnost PSK v remediiranih tleh za človeka (UBM test) in rastline (DTPA test) se je z remediacijo bistveno zmanjšala, prav tako pa tudi nevarnost izpiranja (TCLP test). Pridobljene fizikalno-kemijske lastnosti remediiranih tal se v času eksperimenta niso spreminjale. Vrtnine, gojene na remediiranih tleh, so vsebovale manjše, a ponekod še vedno previsoke koncentracije Pb in Cd, ter imele zaradi pomanjkanja mikrohranil, predvsem Mn, in slabših mikrobioloških parametrov (encimski testi), manjšo biomaso v primerjavi z rastlinami, gojenimi na onesnaženih tleh. Za izboljšanje remediiranih tal so jim bili primešani naslednji dodatki: gnoj, hidrogel, vermikulit in stabilizanta Slovakit in apatit ter posejana špinača. Za najobetavnejši dodatek se je izkazal hidrogel, a tudi ta v merjenih parametrih ni dosegel originalnih tal. Alternativa rabe remediiranih tal kot podlage za okrasne rastline in trave (ob dodatku mikrohranil in hidrogela) se je izkazala za uspešno. Dobljeni rezultati nakazujejo, da se tudi pri visoki onesnaženosti toksikološki parametri v remediiranih tleh signifikantno znižajo, a tla zaradi porušenega razmerja hranil (Mn) in absorpcije PSK (Cd) v rastline pri veliki onesnaženosti niso primerna za pridelavo vrtnin, medtem ko so se trave in okrasne rastline izkazale za primerno alternativo.

KEY WORDS DOCUMENTATION

DN Dd
DC UDC 631.4:631.111+351.777.6(043.3)=111=163.6
CX Potentially toxic metals (PTMs)/ Ethylenediaminetetraacetic acid (EDTA)/ soil remediation/ revitalization of remediated soils
AU JELUŠIČ, Maša
AA LEŠTAN, Domen (supervisor)
PP SI-1000 Ljubljana, Jamnikarjeva 101
PB University of Ljubljana, Biotechnical Faculty, Interdisciplinary Doctoral Programme in Environmental Protection
PY 2014
TI QUALITY AND FUNCTIONING OF CONTAMINATED GARDEN SOIL AFTER REMEDIATION
DT Doctoral dissertation
NO VIII, 105 p., 4 fig., 114 ref.
LA en
AL sl/en
AB Soil contamination, caused by the large amounts of man-made pollutants being anchored into agricultural and urban soils every day, is becoming a major problem and efficient soil remediation techniques are urgently needed. The aim of the doctoral dissertation was to examine the effect of EDTA remediation on metal (Pb, Zn, and Cd) contaminated soil from Meža Valley, Slovenia, and to assess the potential use of remediated soil for plant cultivation. Soil washing with 10, 30, 60, and 120 mmol EDTA kg^{-1} of soil, dependent on the degree of contamination, removed 80%, 28% and 72% of Pb, Zn, and Cd, respectively, but also 75% of the essential micronutrient Mn. In a field experiment, we set up two experimental garden beds (4 ×1 × 0.3 m), one filled with the contaminated and the other with the remediated soil, and planted various vegetables. Analysis of metal association with different soil components (sequential analysis) revealed that after remediation, mainly the non-available share of PTMs remained in the soil. In remediated soil, human (UBM method) and plant (DTPA method) bioaccessibility of Pb, Zn, and Cd were reduced compared to contaminated soil, and the leaching hazard (TCLP method) was also reduced. Obtained physicochemical properties of the remediated soils did not change during the time of the experiment. The vegetables cultivated on remediated soil absorbed lower, but still too high, concentrations of Pb and Cd compared to contaminated soil and had inferior growth and, consequently, yield, presumably due to a lack of micronutrients, especially Mn and depressed soil biological parameters (enzyme activity tests). To mend the impaired properties of the remediated soil several amendments were applied: manure, hydrogel, vermiculite, apatite and Slovakite, of which hydrogel proved to be the most prospective. Results indicate that remediated soil, even when highly contaminated with PTMs, retains reduced toxicological parameters. However, due to the disturbed micronutrient balance and plant absorption of PTMs when contamination is high, the soil is not suitable for vegetable production, whereas ornamental plants and grasses proved to be a successful alternative.

KAZALO VSEBINE

	Ključna dokumentacijska informacija	III
	Key word documentation	IV
	Kazalo vsebine	V
	Kazalo slik	VI
	Kazalo prilog	VII
	Okrajšave	VIII
1	**PREDSTAVITEV PROBLEMATIKE IN HIPOTEZE**	1
1.1	TLA IN POSLEDICE ONESNAŽENOSTI	1
1.2	REMEDIACIJA TAL	3
1.2.1	**Fitoekstrakcija**	3
1.2.2	**Stabilizacija/solidifikacija**	4
1.2.3	**Pranje tal**	5
1.3	REMEDIACIJA TAL Z EDTA	7
1.4	TOKSIKOLOŠKI KAZALCI IN FUNKCIONIRANJE TAL	9
1.5	HIPOTEZE	13
2	**ZNANSTVENA DELA**	14
2.1	OBJAVLJENA ZNANSTVENA DELA	14
2.1.1	**Funkcioniranje s kovinami onesnaženih vrtnih tal po remediaciji**	14
2.1.2	**Vpliv pranja z EDTA na onesnažena vrtna tla. Del I: toksična nevarnost in vpliv na talne lastnosti**	23
2.1.3	**Vpliv pranja z EDTA na onesnažena vrtna tla. Del II: ali remediirana tla lahko uporabimo kot rastlinski substrat?**	34
2.2	OSTALO POVEZOVALNO ZNANSTVENO DELO	46
2.2 1	**Revitalizacija EDTA-remediiranih tal z gnojenjem in talnimi dodatki**	46
2.2.2	**Remediacija in obnovitev s težkimi kovinami močno onesnaženih tal kot substrat za ozelenitev z okrasnimi rastlinami in travami**	69
3	**RAZPRAVA IN SKLEPI**	93
3.1	RAZPRAVA	93
3.1.1	**Optimalna koncentracija EDTA**	93
3.1.2	**Ali so remediirana tla primerna za vrtno uporabo?**	94
3.1.3	**Revitalizacija remediiranih tal z izbranimi dodatki**	98
3.1.4	**Revegetacija remediiranih tal z okrasnimi rastlinami in travami**	99
3.2	SKLEPI	99
4	**POVZETEK (SUMMARY)**	102
4.1	POVZETEK	102
4.2	SUMMARY	104
5	**VIRI**	106

KAZALO SLIK

Slika 1 Reakcija med kelatnim ligandom EDTA in kovinskim kationom ter nastanek koordinacijskega kelatnega kompleksa. 7

Slika 2 Pilotna shema pranja tal. Korak 1: pranje tal. Korak 2: separacija in spiranje skeleta, peska. Korak 3: spiranje tal in separacija tal ter pralne raztopine v komorni preši. Korak 4: drobljenje tal v enakomerne agregate. Korak 5: alkalna substitucija in oboritev PSK. Korak 6: recikliranje EDTA. Korak 7: elektrolitska degradacija EDTA, ki je preostala v pralni raztopini. Korak 8: Priprava reciklirane pralne raztopine (Voglar in Leštan, 2014). 8

Slika 3 S potencialno strupenimi kovinami močno onesnaženo naselje Žerjav v Mežiški dolini. V ospredju so vrtovi in stanovanjske hiše ter v ozadju nekdanja topilnica svinca. 11

Slika 4 Eksperimentalne gredice z lizimetri (na levi) in mlada špinača (na desni), kjer je viden manjši pridelek na remediiranih tleh in večji na originalnih tleh. 95

KAZALO PRILOG

PRILOGA A Licenčna pogodba za uporabo članka v tiskani in elektronski verziji disertacije 1
PRILOGA B Licenčna pogodba za uporabo članka v tiskani in elektronski verziji disertacije 2
PRILOGA C Licenčna pogodba za uporabo članka v tiskani in elektronski verziji disertacije 3

OKRAJŠAVE

EDTA	etilendiamintetraocetna kislina
PSK	potencialno strupene kovine
ENOP	elektrokemijski napredni oksidacijski postopek

1 PREDSTAVITEV PROBLEMATIKE IN HIPOTEZE

1.1 TLA IN POSLEDICE ONESNAŽENOSTI

Tla, koža zemlje in prepereli del zemeljske skorje, so naravni vir, nujno potreben za pridelavo hrane in industrijskih surovin ter pridobivanje energetskih virov. Tla zadržujejo hranila, organsko snov, vodo in energijo ter tako oskrbujejo rastline in jim nudijo oporo. Delujejo kot naravni filter za podtalnico, ki je glavni vir pitne vode za človeka. Tla z ozračjem izmenjujejo pline, kot so kisik, ogljikov dioksid in metan, ter so za oceani največji ponor ogljika. Omogočajo biokemično kroženje snovi in so življenjski prostor številnih organizmov (Brady in Weil, 2002). Tla so torej neprecenljiva naravna vrednota in kot nosilec prostora za obstoj in razvoj človeštva nenadomestljiva!

Dejstvo, da vsako leto po vsem svetu nepovratno izgubimo vsaj 24 milijard ton tal, rodovitna tla pa za nastanek desetih centimetrov potrebujejo približno dva tisoč let, je zato toliko bolj zaskrbljujoče (IAAS, 2013). Evropska unija je v namen zaščite tal sprejela tematsko strategijo za zaščito tal (COM, 2006) in izpostavila naslednje probleme: erozija, zmanjšanje količine organske snovi, zasoljevanje, zbijanje tal, zemeljski usadi, pozidava, zmanjšanje biološke raznovrstnosti in onesnaževanje tal. V tej doktorski nalogi smo se osredotočili na onesnaževanje, in sicer na onesnaževanje tal s potencialno strupenimi kovinami (PSK), ki so poleg olja najpogostejši onesnaževalci tal (EEA, 2007).

PSK so v nižjih koncentracijah sicer naravno prisotne v tleh, a so se v zadnjih desetletjih zaradi antropogenih dejavnosti njihove koncentracije znatno povečale. Večina PSK v tleh je posledica človeških dejavnosti, kot so rudarjenje in taljenje rude, industrija, promet, kmetijstvo in odlaganje odpadkov (Adriano, 2001). V Sloveniji so tla razmeroma neonesnažena, z izjemo posameznih žarišč: zaradi metalurške industrije so onesnažena tla v okolici Jesenic, zaradi rudnika in topilnice svinca so onesnažena tla v Litiji, stara cinkarna je vir onesnaženosti okolice Celja, rudnik živega srebra je pustil svoj pečat na področju Idrije, bakrovi pripravki onesnažujejo vinogradniška tla, najbolj znano pa je verjetno območje Mežiške doline, ki je zaznamovano zaradi tristo let trajajoče rudniške in topilniške dejavnosti. Tla v Mežiški dolini so močno onesnažena s PSK, predvsem s svincem (Pb), pa tudi s cinkom (Zn) in kadmijem (Cd), ter tako predstavljajo nevarnost za celoten ekosistem območja. PSK se namreč ne razgradijo kot nekatera druga onesnažila in lahko stoletja vztrajajo v talnem okolju, se medtem izpirajo v podtalnico in se s prašnimi delci prenašajo po zraku (Alloway, 2013).

Potencialno strupeni kovini Pb in Cd sta neesencialna elementa, ki ju organizmi za rast in razvoj ne potrebujejo, medtem ko je Zn esencialni gradnik večine organizmov. PSK v tleh so lahko trdno vezane na talne komponente ali pa so prosto dostopne v talni raztopini oz. le

rahlo vezane na izmenljiva mesta v tleh. Če so prisotni v dosegljivih oblikah in prevelikih koncentracijah, pa so Pb, Cd in tudi esencialni Zn strupeni za večino organizmov.

Številne študije so ugotovile, da PSK vplivajo na talne organizme in biološke funkcije tal (Moreno in sod., 1999; Chander in sod., 2001). Khan in sod. (2007) so tla onesnažili z različnimi koncentracijami Cd in Pb ter ugotovili največje toksikološke učinke pri bakterijah, pa tudi pri aktinomicetah in glivah. Povečane koncentracije PSK zavirajo aktivnost mikroorganizmov, kar posledično zmanjša rodovitnost tal (Smejkalova in sod., 2003).

Rastline se na prisotnost PSK v tleh odzovejo različno. Koncentracije PSK, ki jih rastline absorbirajo, se tako razlikujejo glede na vrsto in kultivar (Zhang in sod., 1998), rastno fazo in dele rastlin (Liu in sod., 2003), koncentracijo dostopnih kovin v tleh in tip tal (Hardiman in sod., 1984), lastnosti tal − predvsem pH (Cieśliński in sod., 1996) ter vrsto okolijskih dejavnikov (Twining in sod., 2004). Nekatere rastline slabo prenašajo visoke vsebnosti dostopnih PSK (neodporne) v tleh, spet druge so razvile raznolike načine preživetja v onesnaženem okolju (odporne). Med odporne rastline prištevamo: indikatorske rastline (rastline, katerih koncentracija PSK v tkivu je odvisna od onesnaženosti okolja), akumulatorske rastline (rastline, ki absorbirajo velike količine PSK brez škode za njihovo zdravje) in rastline, ki lahko kljub onesnaženim tlom preprečujejo vstop PSK in tako vzdržujejo majhne koncentracije le-teh v svoji biomasi.

Ne glede na način prilagoditve rastlin pa v prevelikih količinah PSK lahko zavirajo rast, ovirajo delovanje encimov in različnih metabolnih poti (Gill in sod., 2012), vplivajo na respiracijo in vsebnost koencima ATP (Reese in Roberts, 1985), zmanjšajo aktivnost fotosinteznega aparata (Gill in sod., 2012), povzročijo oksidativni stres (Mobin in Khan, 2007) in pojav kloroz (Ebbs in Kochain, 1997; Burton in sod., 1984). PSK v tleh lahko porušijo razmerje sprejema nujno potrebnih/esencialnih elementov v rastlino (Sharma in Dubey, 2005; Jiang in sod., 2004). Kljub dejstvu, da rastline Pb in Cd ne potrebujejo, ju vseeno lahko privzamejo prek poti esencialnih elementov s podobnimi kemijskimi lastnostmi (Pb in Ca, Cd in Zn). Znano je na primer, da lahko prisotnost dostopnega Zn v tleh zmanjšuje vstop Cd v rastlino (Chakravarty in Srivastava, 1997).

Rastline lahko absorbirajo relativno velike količine PSK brez vidnih zunanjih sprememb in onesnažila se tako tiho prenašajo v prehranjevalno verigo in biološko cirkulacijo. Tako pridelana zelenjava, »obogatena« s PSK, ima lahko negativne učinke na človekovo zdravje (Bešter, 2013).

Poleg zaužitja onesnažene zelenjave (prehrana) lahko PSK vstopijo v človeško telo z uživanjem talnih delcev (otroci), z vdihovanjem prašnih delcev in prek ran (Oliver, 1997; Adriano, 2001). Pb, Zn in Cd predstavljajo nevarnost za človekovo zdravje še posebej na

onesnaženih območjih, kot je Mežiška dolina, kjer so prebivalci kronično izpostavljeni PSK. Za Pb je značilno kopičenje v organizmu. Prizadene skoraj vse organske sisteme, najbolj pa osrednji periferni živčni sistem, prebavni trakt, ledvice, krvotvorni, srčno-žilni in endokrini sistem (Jamšek in sod., 2007; Zhang in sod., 2012). Kronična izpostavitev Cd povzroča okvare ledvic, dihal in prebavil ter lome kosti (Žukowska in Biziuk, 2008; Jamšek in Šarc, 2009). Zastrupitve z Zn so redke, a vseeno povečan vnos Zn lahko povzroči motnje metabolizma holesterola in zavira absorpcijo Cu in Fe (Bradl, 2005).

1.2 REMEDIACIJA TAL

Zaradi izboljšanja kakovosti življenja in preprečevanja tveganja na območjih, onesnaženih s PSK, je treba takšna tla sanirati, remediirati ter jim povrniti zdravje in prvotno funkcionalnost. Po podatkih Evropske unije je območij, kjer se izvajajo potencialno onesnaževalne dejavnosti, okoli 3.000.000 in okoli 250.000 izmed njih potrebuje nujno remediacijo (EEA, 2007).

Pri izbiri remediacijskega postopka je treba upoštevati značilnosti onesnaženega območja, lastnosti tal, koncentracije in vrste onesnažil, ki jih želimo odstraniti, ter končne uporabe onesnaženega območja oz. zahtevane stopnje očiščenja tal, ki jo določata zakonodaja in raba tal (Mulligan in sod., 2001; Leštan, 2002). Pristope remediacije tal, onesnaženih s PSK, lahko razdelimo na naslednje večje kategorije: **izolacija** (izkop oz. izolacija onesnažene zemljine s fizičnimi pregradami iz jekla, betona itd.), **fizična separacija** (separacija po velikosti ali gravitacijska separacija), **imobilizacija** (solidifikacija/stabilizacija, fitosolidifikacija, vitrifikacija, imobilizacija z mikroorganizmi) ter **zmanjšanje toksičnosti** in **ekstrakcija** (fitoekstrakcija, pranje tal) (Mulligan in sod., 2001). V praksi se lahko poslužujemo kombinacije omenjenih pristopov, ki jih lahko uporabimo na mestu onesnaženja (*in situ*), ali pa onesnažena tla izkopljemo, očistimo in vrnemo na mesto izkopa (*ex situ*). Najbolj razširjene in raziskane tehnike čiščenja tal, onesnaženih s PSK, so vsekakor fitoekstrakcija, solidifikacija/stabilizacija in pranje tal (Wuana in Okieimen, 2011).

1.2.1 Fitoekstrakcija

Pojem fitoremediacija zajema naslednje metode čiščenja tal (blat čistilnih naprav in odpadnih voda), onesnaženih z organskimi in anorganskimi polutanti: *rizofiltracija* (uporaba korenin rastlin, ki akumulirajo kovine, za čiščenje onesnaženih vod v čistilnih napravah), *fitostabilizacija* (uporaba rastlin, odpornih na onesnažilo, za mehansko stabilizacijo onesnaženih tal proti eroziji), *fitovolatizacija* (uporaba metabolizma rastlin za transformacijo onesnažil v hlapne snovi), *fitodegradacija* (uporaba rizosfere z mikroorganizmi za pospešeno razgrajevanje organskih onesnažil v tleh) in najbolj razširjena *fitoekstrakcija* (Gosh in Singh, 2005). Pri fitoekstrakciji izkoriščamo sposobnost

nekaterih rastlin (hiperakumulatorjev), da v nadzemne dele lahko premeščajo relativno velike koncentracije anorganskih onesnažil, še posebej PSK, a tudi metaloidov in radionukleotidov. Po žetvi odpadno biomaso, onesnaženo s kovinami, obdelajo s postopki za odstranitev nevarnih odpadkov (nadzorovano zgorevanje, uplinjanje, piroliza) (Gosh in Singh, 2005). Do sedaj so znanstveniki potrdili že več kot 500 rastlin, sposobnih hiperakumulacije različnih PSK (Sarma, 2011), vendar točni fiziološki mehanizmi in razlogi za sprejem PSK v rastlino še vedno ostajajo neznanka. Hiperakumulatorske rastline lahko akumulirajo kar 100-krat večje koncentracije PSK v primerjavi z ostalimi rastlinami, npr. 100 mg kg^{-1} Cd, 1000 mg kg^{-1} Pb in več kot 10.000 mg kg^{-1} Zn in Ni (Baker in Brooks, 1989). Znane družine hiperakumulatorjev so križnice (Brassicaceae), metuljnice (Fabaceae), mlečkovke (Euphorbiaceae), nebinovke (Asteraceae), ustnatice (Lamiaceae) in črnobinovke (Scrophulariaceae) (Salt in sod., 1998; Dushenkov, 2003). Fitoekstrakcija se večinoma izvaja *in situ* in velja za najmanj destruktivno in javnosti najbolj priljubljeno metodo, saj ne poškoduje strukture tal in njihove funkcionalnosti (Peer in sod., 2006). Na drugi strani pa je fitoekstrakcija zaradi počasne rasti rastlin in majhne biomase dolgotrajen proces. Metodo zato izboljšujejo z dodajanjem nekaterih ligandov, EDTA, HEDTA, DTPA (Peer in sod., 2006; Lombi in sod., 2001), ki povečujejo dosegljivost PSK za rastline in pospešujejo njihovo premeščanje iz korenin v nadzemne dele rastlin. Slabost pospešene fitoekstrakcije je predvsem spiranje kompleksa ligand-kovina v podtalnico. Kompleksi EDTA-kovina so namreč zelo stabilni, se v naravi počasi razgrajujejo in predstavljajo resen ekološki problem.

1.2.2 Stabilizacija/solidifikacija

V določenih primerih je PSK bolje le imobilizirati kot odstraniti iz tal. Pri imobilizaciji uporabimo organske ali neorganske dodatke, ki prispevajo k večji nedostopnosti kovin v tleh. Prek procesov sorpcije, obarjanja in kompleksacije stabiliziramo kovine v stabilnejše geokemične komplekse (Wuana in Okieimen, 2011). Metoda **stabilizacije/solidifikacije** temelji na dodajanju aditiva tlom, ki stabilizira PSK in tudi druga onesnažila iz mobilnih v fizikalno-kemijsko stabilne nemobilne oblike. Metoda je dokaj enostavna, učinkovita, večinoma izvedena *in situ* in ekonomična. Prvotno so metodo uporabljali za čiščenje onesnaženih voda in odpadnih snovi, sedaj pa se intenzivno uporablja tudi za tla (Guo in sod., 2006). Za stabilizacijo kovin v tleh so na voljo številni aditivi-stabilizanti: apno (Geebelen in sod., 2003), fosfati (Basta in McGowen, 2004), organske snovi (Brown in sod., 2004) in razni industrijski produkti (zeolit in rdeče blato) (Lombi in sod., 2002). Treba je poudariti, da v primeru stabilizacije celokupna vsebnost koncentracije kovin v tleh ostane ista, spremeni se le njihova dostopnost. Tako remediirana tla je treba dolgoročno spremljati, saj se dostopnost kovin pod vplivom talnih dejavnikov sčasoma lahko spreminja. Poleg tega se večina študij stabilizacije tal osredotoča predvsem na učinkovitost aditivov in zanemarja končni učinek remediacije na kakovost in funkcionalnost tal (Tica,

2013). Z aditivi remediirana tla tako običajno niso primerna za kmetijsko ali vrtno uporabo.

1.2.3 Pranje tal

S pranjem tal označujemo fizikalno ločitev močno onesnaženih drobnih talnih frakcij, npr. v gravitacijskem polju hidrociklonov ali z mokrim sejanjem, in izpiranje onesnažil iz tal z vodnimi raztopinami. Pri slednjem se onesnažena tla tretira na način, da kovine ločimo iz trdne faze tal v talno raztopino in jih posledično izperemo iz tal. Najpogosteje uporabljena in preučevana sredstva za pranje tal so kisline (dušikova, žveplova, klorovodikova, fluorosilikatna, citronska, vinska kislina) in razni ligandi (EDTA, DTPA, NTA, EDDS, IDSA, MGDA) (Peters, 1999; Mulligan in sod., 2001; Neale in sod., 1997; Tandy in sod., 2004).

Kisline močno raztapljajo karbonate in druga mesta v tleh, sposobna vezave kovin, ter izmenjajo kovine iz talnih koloidov s H^+ ioni. Kisli pogoji v tleh negativno vplivajo na fizikalno-kemijske lastnosti tal in biološke parametre, zato za zdravje in funkcionalnost tal kislinska metoda ni najprimernejša (Xu in Zhao, 2005). Pranje tal z ligandi ima manjši vpliv na spremembe pedoloških lastnosti (Neale in sod., 1997) in ohrani biološke lastnosti tal (Udovič in Leštan, 2012). Ligandi odstranijo PSK iz trdne faze tal tako, da z njimi tvorijo močne vodotopne komplekse, ki se nato izperejo. Pri sami izbiri liganda je pomemben ekstrakcijski potencial (ligand mora tvoriti močne in stabilne komplekse s kovinami pri različnih vrednostih pH), selektivnost liganda na elemente, ki jih želimo odstraniti, možnost ponovne uporabe liganda v primeru recikliranja, nizek redoks potencial in toksičnost ter sprejemljiva cena (Voglar, 2013).

Pranje tal z ligandi je kompleksen proces, zato je treba pred izvedbo za vsa tla določiti optimalne remediacijske pogoje (Leštan in sod., 2008) glede na **lastnosti onesnaženih tal** (pH, tekstura, koncentracije Ca, Mg in Fe, CEC, vsebnost organske snovi, mineralna zgradba, vrsta onesnaženosti in vsebnost drugih onesnaževalcev), **onesnaževalca - PSK** (vrsta in koncentracija onesnaževalca, frakcionacija v talni matriki, fizikalno-kemijske oblike in dostopnost v tleh) in **tehnologije procesa** (izbira in koncentracija liganda, čas ekstrakcije, razmerje tla-pralna raztopina in pH pralne raztopine ter izbira elektrolita) (Zou in sod., 2009).

PSK z nekaterimi talnimi elementi (organska snov, glineni minerali) tvorijo močne komplekse, ki jih ligandi zato težje izperejo. Abumaizar in Khan (1996) sta poročala o vplivu organske snovi na pranje tal z ligandi. Visoka vsebnost organske snovi namreč zmanjšuje učinkovitost remediacije, saj imajo velike humusne molekule visoko afiniteto do PSK, s katerimi tvorijo v vodi netopne komplekse (Peters, 1999). Gusiatin in Klimiuk (2012) sta preučevala vpliv detergenta saponin in učinkovitost izpiranja Cu, Zn in Cd na

teksturo različnih tal. Rezultati so pokazali, da je bila največja učinkovitost odstranitve PSK v peščenih tleh (82−90 %) in najslabša v glinenih tleh (39−62 %).

Izbira primernega liganda igra pomembno vlogo pri odstranitvi določenega elementa/onesnažila. Različni ligandi imajo različne konstante stabilnosti, različne afinitete za različne elemente. EDTA tvori močne komplekse s Ca, Fe, Cd, Cu, Mg, Ni, Pb in Zn (Tandy in sod., 2004; Begum in sod., 2012). Njegova razgradljiva različica EDDS se mu po moči približa le pri Cu in Ni. EDTA je slabo biorazgradljiv, kar je tudi razlog za številne študije, ki si prizadevajo poiskati primerljiv, a biološko razgradljiv ligand. Pri tehnologiji pranja, kjer se ligand večkrat uporabi/reciklira pa je prav ta nerazgradljivost zaželena in pomembna lastnost (Voglar, 2013).

Tandy in sod. (2004) so primerjali različne razgradljive ligande z EDTA in ugotovili, da je pri odstranitvi Cu v povprečju zaporedje učinkovitosti ekstrakcije naslednje: EDDS > NTA > IDSA > MGDA > EDTA, pri Zn NTA > EDDS > EDTA >MGDA > IDSA in pri Pb EDTA > NTA > EDDS. Izjema je bila ekstrakcija pri visokem razmerju ligand-kovina, ko je bil najučinkovitejši ligand za vse tri elemente EDTA. Poskus so izpeljali na apnenih tleh in zaključili, da je visoka vsebnost Ca ionov vplivala na ekstrakcijsko moč EDTA, ki s Ca tvori močnejši kompleks kot ostali ligandi. Merili so tudi vpliv pH in izmerili večjo učinkovitost ekstrakcije za vse merjene ligande pri nižjih pH-vrednostih. Tudi Kim in sod. (2003) so opazili zmanjšano učinkovitost EDTA pri ekstrakciji Pb, zaradi kompeticije ostalih kationov za vezna mesta liganda, predvsem Ca, Mg in Fe. EDTA je torej neselektiven ligand in poleg tarčnih PSK veže tudi druge katione, poleg tega pa EDTA v manjši meri razbija tudi strukturo tal, raztaplja organsko snov, okside in talne minerale ter s tem posredno v talno raztopino sprošča kovinske ione (Nowack in Sigg, 1997; Tsang in sod., 2007; Vulava in Seaman, 2000).

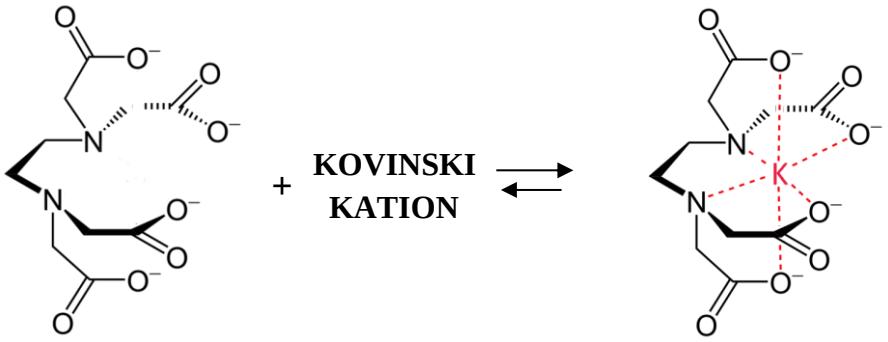
Kontaktni čas liganda in talne matrike v vodni raztopini so preučevali številni avtorji. Zou in sod. (2009) so izvedli poskus, kjer so preučevali vpliv časa na količino ekstrahiranih PSK in največjo učinkovitost dosegli pri dveh urah. S podaljševanjem časa se je dodatno izpral le še manjši delež PSK, ki je v času 4−8 ur dosegel maksimalno raven. Peters in Shem (1992) sta optimalni čas pri izpiranju Pb z EDTA dosegla že v 1 uri.

Za povečevanje koncentracije liganda velja podoben trend kot pri podaljševanju časa ekstrakcije. Zou in sod. (2009) so dodajali EDTA v koncentracijah 5−100 mmol EDTA kg^{-1} tal. Pri koncentraciji 20 mmol EDTA so dosegli optimum, saj so odstranili večino Pb, Zn in Cd. S povečevanjem koncentracije liganda so le neznatno povišali ekstrakcijsko učinkovitost. Zaključili so, da v kolikor povečujemo koncentracijo EDTA nad zahtevami tal (koncentracija dostopnih dvovalentnih kationov), le-ta nima več dodatnega učinka. O podobnih rezultatih so poročali Elliot in Brown (1989) ter Steele in Pitchel (1998).

1.3 REMEDIACIJA TAL Z EDTA

Na podlagi dosedanjih raziskav smo se pri izbiri remediacijskega sredstva odločili za ligand EDTA, ki je med ligandi najobetavnejši, predvsem zaradi visoke učinkovitosti odstranitve kovin iz tal, relativno dostopne cene in zmožnosti ponovne uporabe - recikliranja v postopku (Voglar, 2013).

EDTA ali etilendiamintetraocetna kislina je brezbarvna spojina, ki je na sobni temperaturi v trdnem agregatnem stanju in je dobro topna v vodi. EDTA je predstavnik kelatnih ligandov in kovinske ione veže v stohiometričnem razmerju 1:1 ne glede na valenco iona. Kovino veže v oktaedrični kompleks prek dveh dušikov in štirih karboksilatov (Slika 1). V kislih raztopinah prevladuje dvakrat protonirana oblika H_6EDTA^{2+}, v bazičnih medijih pa $EDTA^{4-}$. Zaradi zmožnosti vezave kovin EDTA uporabljajo v industriji, kozmetiki, medicini in pri raznih laboratorijskih tehnikah. Molekula je slabo biorazgradljiva in je zato pogosto omenjena kot vztrajni organski onesnaževalec, predvsem v vodah (Nowack in Van Briesen, 2005).



Slika 1: Reakcija med kelatnim ligandom EDTA in kovinskim kationom ter nastanek koordinacijskega kelatnega kompleksa.

Figure 1: Reaction between chelating agent EDTA and metal cation forming metal-chelant coordination complex.

Voglar in Leštan (2012) sta vrtna tla iz Mežiške doline, onesnažena s PSK, očistila s kelatnim ligandom EDTA (*ex situ*) po v nadaljevanju predstavljenem postopku. Za pripravo remediiranih tal, vodno raztopino Na_2-EDTA v koncentracijah 10, 30, 60 in 120 mmol kg^{-1} tal (odvisno od onesnaženosti tal) zamešamo s 60−70 kg tal in suspenzijo dve uri tretiramo v betonskem mešalniku (Slika 2/1). Med mešanjem ligand EDTA iz talne matrice odstrani kovine in z njimi tvori močne vodotopne komplekse (Slika 1). Mešanico nato precedimo skozi 2-milimetersko sito in odstranimo večje delce (pesek in skelet), ki jih nato speremo z vodo (Slika 2/2). Preostalo suspenzijo prečrpamo v komorno filter prešo (Slika 2/3), kjer tla toliko časa spiramo z vodo, dokler v pralni vodi ne zaznamo več PSK

(Slika 2/3). Nato iz preše iztisnemo/ločimo »vodno raztopino z EDTA kovinskimi kompleksi« od tal, ki jih nato naribamo skozi 5-milimetrsko sito ter jim s tem delno povrnemo strukturo (Slika 2/4). Na koncu v remediirana tla ponovno zamešamo pesek (delce, večje od 2 mm).

Pri opisanem postopku se porabi velika količina procesne vode in liganda EDTA, ki sta v posebnem postopku ločena in ponovno uporabljena v procesu remediacije. Za ponovno uporabo procesne raztopine je treba iz nje odstraniti PSK, EDTA pa ohraniti v aktivni obliki za ponovno uporabo pri spiranju onesnaženih tal. Voglar in Leštan (2014) sta razvila alkalno/kislinski postopek, kjer iz raztopine najprej odstranimo PSK (alkalna faza) (Slika 2/5) in nato v kislinski fazi izoborimo EDTA, ki jo od procesne vode ločimo z manjšo komorno filter stiskalnico (Slika 2/6). Na ta način se odstrani do 76 % liganda EDTA, okoli 20 % ga ostane v tleh, del pa se ga uniči pri recikliranju pralne raztopine med elektrokemijskim naprednim oksidacijskim postopkom (ENOP) (Slika 2/8). Z ENOP se z obarjanjem na jekleno nerjavečo katodo odstrani še preostali del PSK (do 99,9 % Pb, 98,9 % Zn in do 99,4 % Cd) (Slika 2/7). Tako lahko celotno pralno raztopino ponovno uporabimo v procesu pranja/spiranja tal. Metoda je ekonomična, relativno enostavna in učinkovita (Voglar, 2014).

Slika 2: Pilotna shema pranja tal. Korak 1: pranje tal. Korak 2: separacija in spiranje skeleta, peska. Korak 3: spiranje tal in separacija tal ter pralne raztopine v komorni preši. Korak 4: drobljenje tal v enakomerne agregate. Korak 5: alkalna substitucija in oboritev PSK. Korak 6: recikliranje EDTA. Korak 7: elektrolitska degradacija EDTA, ki je preostala v pralni raztopini. Korak 8: Priprava reciklirane pralne raztopine (Voglar in Leštan, 2014).

Figure 2: Pilot-scale soil washing plant. Step 1: soil washing. Step 2: separation and rinsing of the process oversize material. Step 3: soil rinsing and phase separation in a chamber filter press. Step 4: treatment of remediated soil. Step 5: alkaline substitution and metal precipitation. Step 6: acid precipitation and EDTA recovery. Step 7: electrolytic degradation of EDTA remaining in the process solution. Step 8: preparation of the recycled washing solution (Voglar and Leštan, 2014).

1.4 TOKSIKOLOŠKI KAZALCI IN FUNKCIONIRANJE TAL

Vsebnost PSK v tleh, remediiranih po opisanem postopku, se zmanjša tudi za 80 % (Voglar, 2013), a kljub vsemu EDTA ne odstrani vseh kovin iz talne matrike. PSK, ki v tleh preostanejo, so večinoma trdno vezane na talne komponente ter tako nemobilne in nedostopne za organizme v tleh, prav tako pa se ne izpirajo. Poraja se vprašanje, ali je dobljena zmanjšana mobilnost/dostopnost trajna ali le začasna. V kolikor remediirana tla izpostavimo živim (rastline, mikro in makro favna, mikroorganizmi) in neživim (temperatura, osončenost, letni časi, padavine) okolijskim dejavnikom, bi le-ti lahko tekom časa vznemirili na novo pridobljeno ravnotežje in spodbudili premeščanje trdno vezanih kovin v mobilnejše dostopne kemijske oblike. Učinkovitost remediacije in varnost očiščenih tal bi se s tem močno zmanjšala. Uporaba modelnih postopkov staranja tal je pokazala, da vsaj v laboratorijskih eksperimentih v manjših merilih lahko med staranjem tal pride do povečanja mobilnosti in biološke dostopnosti, s tem pa tudi toksičnosti kovin v tleh (Udovič in Leštan, 2007, 2009).

Test frakcionacije tal pokaže, na katere talne frakcije so PSK v tleh vezane, in s tem posredno njihovo okvirno dostopnost. Frakcionacijo kovin določamo s selektivnimi sekvenčnimi ekstrakcijami, kjer v več zaporednih stopnjah izpostavljamo tla vedno močnejšim reagentom (ekstrahantom), ki pa niso popolnoma specifični in lahko odstranijo PSK tudi iz drugih frakcij. Pogosto uporabljena je šeststopenjska modificirana Tessier-jeva (1979) metoda, kjer ločimo naslednje frakcije: I - v vodi topne PSK, II - izmenljive PSK, III – PSK, vezane na karbonate, IV – PSK, vezane na Fe in Mn okside, V – PSK, vezane na organsko snov in sulfide ter VI - preostanek PSK, običajno trdno vezan v kristalne rešetke glinenih mineralov. Prvi dve frakciji in pod posebnimi pogoji tudi tretja se v literaturi običajno povezujejo s PSK, ki so dostopne za organizme (Tsang, 2007). Ligand EDTA ima sposobnost odstranitve PSK iz vseh frakcij, po nekaterih poročilih tudi tistih trdno vezanih v glinene minerale (Barona in sod., 2001; Zhang in sod., 2010a; Elliot in Brown, 1989). Udovič in Leštan (2009) sta preučevala frakcionacijo tal Mežiške doline pred in po remediaciji z ligandom EDTA in ugotovila, da je ligand spral do 85 % Pb, vezanega na karbonate in organsko snov, ter do 91 % Zn, vezanega na karbonate in Fe-Mn−okside. Ligand je spral tudi Cd iz vseh frakcij, izjemoma iz preostanka.

Okvirno dostopnost PSK lahko nadgradimo s specifično ekstrakcijo z metodo DTPA (dietilentriaminpentaocetna kislina), ki se uporablja za določanje biodostopnosti PSK za rastline (Lindsay in Norwell, 1978) in z ekstrakcijsko metodo TCLP (Toxicity Characteristic Leaching Procedure), ki se po standardiziranem postopku uporablja za določanje mobilnosti organskih in anorganskih onesnažil (US EPA, 1995). Slednjo uporabljamo predvsem za določevanje vodotopnih kovin v tleh, ki lahko potencialno prehajajo v vodne vire in posledično ogrozijo zdravje prebivalstva na širšem območju.

Pri remediiranih tleh je nujno treba določiti tudi biodostopnost PSK za človeka in s tem oceniti varnost očiščenih tal. Iz talnih delcev, ki vstopijo v prebavila, se lahko v prebavne sokove izločijo kovine (biodostopna frakcija), ki se skozi nadaljnji metabolizem iz telesa bodisi izločijo bodisi preidejo v krvni obtok (biodosegljiva frakcija), kjer postanejo strupene za organe in tkiva (Oomen in sod., 2003). Merjenje *biodosegljivosti* (*in vivo* raziskave na živalih) je dolgotrajno, drago in sporno, zato se večja pozornost namenja predvsem *biodostopnosti* in razvoju *in vitro laboratorijskih* testov. Omenjeni testi, ki temeljijo na simulaciji človeškega prebavnega trakta, so hitri, enostavni, ponovljivi, cenovno ugodni in etično sprejemljivi (Patinha in sod., 2012).

Poleg dostopnosti PSK pa je za funkcionalnost tal pomembna tudi dostopnost esencialnih elementov. Drugo vprašanje se torej nanaša na že omenjeno neselektivnost liganda EDTA, ki poleg PSK iz tal odstrani tudi esencialne ione, kot so Mn, Cu, Mg, Ca in Fe, predvsem njihove dostopne oblike. Zhang in sod. (2010b) poročajo o raztapljanju Ca ionov in v manjši meri tudi Mg, Mn in Fe ionov ob prisotnosti EDTA v tleh. Voglar in Leštan (2012) pa sta izmerila kar 44-95 % odstranitev Mn, 5 % Ca in 7 % Fe pri remediaciji tal z EDTA. Pri tako visoki koncentraciji odstranjenega Mn, ki je esencialni element za večino organizmov, bi bilo treba izgubo elementa v remediiranih tleh nadomestiti, bodisi s sintetičnimi ali naravnimi gnojili.

V kolikor se pri dostopnosti PSK ukvarjamo predvsem z varnostjo remediiranih tal za okolje, človeka in organizme, pa se pri pomanjkanju mikroelementov posvečamo predvsem kakovosti in funkcionalnosti tal. Dosedanje raziskave čiščenja onesnaženih tal so bile osredotočene predvsem na zmanjšanje celokupne in/ali dostopne koncentracije kovin v očiščenih tleh in so zanemarile vpliv ligandov in procesnega postopka na kakovost in funkcionalnost tal. Kakovost tal je definirana kot sposobnost kompleksnega živega sistema v mejah naravnega ali uravnavanega ekosistema, da opravlja funkcije, ki podpirajo zdravje rastlin, živali in mikroorganizmov, da vzdržuje ali povečuje kvaliteto zraka in voda ter da podpira in omogoča življenje ljudi (Pankhurst in sod., 1997). Tako kompleksen pojem je težko neposredno izmeriti, zato ga lahko zgolj ocenimo s pomočjo t.i. indikatorjev kakovosti. Encimi veljajo za ene najbolj občutljivih indikatorjev kakovosti, saj se na spremembe v ravnanju s tlemi odzovejo skoraj hipoma (Mijangos in sod., 2006). Ko govorimo o encimski aktivnosti, ta običajno izhaja iz mikrobne komponente tal. Mikroorganizmi igrajo ključno vlogo pri razgradnji organske snovi in pri kroženju hranil ter močno vplivajo na rodovitnost tal. Z izbranim naborom encimskih testov, odzivnih na različne pogoje v tleh (vlaga, temperatura, organska snov in PSK), tako lahko posredno izmerimo biološko aktivnost tal.

Kmetijstvo in vrtnarstvo na onesnaženih območjih (urbana območja in območja nekdanjih rudnikov ter topilnic) se zaradi pomanjkanja prostora in popularizacije urbanega vrtnarstva hitro povečuje. V Združenem kraljestvu tako kar 87 % domovanj skrbi za domači urbani

vrt (Gibbons in sod., 2011). Ena izmed funkcij mestnega vrtička je vsekakor pridelava sveže zelenjave (Autumn in Pikai, 2000) in območje Mežiške doline ni izjema (Slika 3). Uživanje zelenjave, pridelane na s PSK onesnaženih tleh, ima lahko negativne posledice na zdravje prebivalstva, zato je onesnažena območja vsekakor potrebno remediirati. Vsakršna remediacija kmetijskih in vrtnarskih površin pa mora proizvesti tla, na katerih je omogočena rast zdravih in za uživanje varnih rastlin s konkurenčnim pridelkom.

Prvi takoj vidni kazalec kakovosti vrtnin je vsekakor pridelek/biomasa, ki jo lahko neposredno izmerimo s tehtanjem. Nadalje lahko z analizami izmerimo fiziološko stanje rastlin prek merjenja fotosinteznih kazalcev. Ko rastlina doživi stresno stanje, namreč pride do motenj v fotosinteznem aparatu, ki jih lahko zaznamo z meritvami fluorescence, prek katerih pa lahko sklepamo na učinkovitost fotosinteze. Tako se npr. merjenje fluorescence pogosto uporablja za spremljanje fiziološkega stanja rastlin (Valcke in sod., 1999). Ruley in sod. (2006) so prek fluorescence »klorofila a« proučevali učinek Pb in različnih ligandov EDTA, HEDAT, DTPA, NTA na rastlino *Sesbania drummondii.*

Poleg zadovoljivega pridelka in ugodnega fiziološkega stanja pa morajo biti vrtnine, gojene na remediiranih tleh, tudi varne za uživanje. Uredba Evropske unije »o določitvi mejnih vrednosti nekaterih onesnaževal« (ES 1881/2006) določa minimalne vsebnosti nekaterih kovin (Pb, Cd, Hg, Sn) v užitnih delih nekaterih rastlin. Za varovanje zdravja je bistveno, da v rastlinah oz. delih rastlin, namenjenih zaužitju, ohranimo toksikološko sprejemljive koncentracije (ES 1881/2006). Pelfrene in sod. (2013) so ugotavljali vsebnost Pb in Cd v rastlinah, gojenih na urbanih vrtovih na območju, kjer so pred časom potekale talilniške dejavnosti. Večina pridelane zelenjave je presegla zakonsko dovoljenje meje. Izračunali so tudi oceno tveganja za Pb in Cd, in sicer prek vdiha prašnih delcev in zaužitja onesnaženih rastlin, ter ugotovili, da kovini potencialno ogrožata zdravje otrok na onesnaženem območju.

Raziskav o funkcionalnosti in varnosti s PSK onesnaženih tal, opranimi z EDTA ali drugimi reagenti, v literaturi nismo zasledili. Redke so raziskave, ki poleg zmanjšanja celokupne oz. biodosegljive frakcije PSK v tleh po remediaciji preučijo tudi druge kazalce. Warren in sod. (2003) so preučevali vsebnost arzena v zelenjavi, pridelani na remediiranih (z dodatkom železovega sulfata in apna) in onesnaženih tleh. Boisson in sod. (1999) so preučevali remediacijski potencial hidroksiapatita in določili biomaso in vsebnost PSK v koruzi in fižolu, zraslih na remediiranih tleh. V literaturi tako nismo zasledili celostnega pristopa remediacije tal, ki bi proučeval tako varnost (mobilnost PSK) in stabilnost (spremembe skozi čas) remediiranih tal kot tudi njihovo funkcionalnost, kakovost in sposobnost, da bi ponovno zaživela kot varen in trajnostni vrtni substrat.

Slika 3: S potencialno strupenimi kovinami močno onesnaženo naselje Žerjav v Mežiški dolini. V ospredju so vrtovi in stanovanjske hiše ter v ozadju nekdanja topilnica svinca (Foto: Marko Cvetko)

Figure 3: Settlement of Žerjav in Meža Valley contaminated with potentially toxic metals. Forefront, there are gardens and houses and in the background the former smelter is pictured (Photo: Marko Cvetko).

V tej doktorski disertaciji smo s PSK onesnažena vrtna tla iz Mežiške doline, ki jih domačini uporabljajo za pridelavo vrtne zelenjave, očistili z ligandom EDTA in preučili številne parametre, ki vplivajo na zmožnost, da bi remediirana tla lahko ponovno zaživela kot kakovosten vrtni substrat.

1.5 HIPOTEZE

Pri raziskovalnem delu smo si zastavili sledeče hipoteze:

− vsebnost PSK v tleh in rastlinah na remediiranih tleh bo manjša v primerjavi z onesnaženimi tlemi.

− manjša bo tudi mobilnost in dostopnost PSK v remediiranih tleh in med staranjem remediiranih tal ne bo prišlo do bistvenih sprememb.

− postopek remediacije ne bo bistveno spremenil fizikalno-kemijskih in bioloških lastnosti tal.

− postopek remediacije ne bo poslabšal lastnosti tal kot rastlinskega substrata.

2 ZNANSTVENA DELA

2.1 OBJAVLJENA ZNANSTVENA DELA

2.1.1 Funkcioniranje s kovinami onesnaženih vrtnih tal po remediaciji

JELUŠIČ Maša, GRČMAN Helena, VODNIK Dominik, SUHADOLC Metka, LEŠTAN Domen

Functioning of metal contaminated garden soil after remediation.

Environmental Pollution, 2013, 174: 63−70

V kolonskem poskusu s kitajskim kapusom (*Brassica rapa* L.) smo ocenili učinek remediacije pri treh različnih odmerkih EDTA (10, 30, 60 mmol kg^{-1}). Iz tal, onesnaženih s 1378 mg kg^{-1} Pb, 578 mg kg^{-1} Zn in 8,5 mg kg^{-1} Cd, smo s pranjem tal odstranili do 77 % Pb, 29 % Zn in 72 % Cd. Sekvenčna ekstrakcija je pokazala odstranitev kovin iz karbonatne frakcije. Oralna dostopnost kovin v želodčni fazi se je zmanjšala do 75 %, v malem črevesju pa do 79 % (Pb). Del kovin (do 0,8 % Cd) se je iz tal izpral. Remediacija tal je zmanjšala prehajanje kovin iz tal v rastlino za 61 %, a ni preprečila kopičenja kovin v zelene dele rastlin kitajskega kapusa. Splošno stanje rastlin, gojenih na tleh, opranih z EDTA (izmenjava plinov, fluorescenca), ni bilo spremenjeno. Vsebnost mikrobne DNA takoj po remediaciji je bila zmanjšana, prav tako se je spremenila struktura mikrobne populacije.

Environmental Pollution 174 (2013) 63–70

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Functioning of metal contaminated garden soil after remediation

Masa Jelusic, Helena Grcman, Dominik Vodnik, Metka Suhadolc, Domen Lestan*

Agronomy Department, Centre for Soil and Environmental Science, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 7 June 2012
Received in revised form
12 October 2012
Accepted 26 October 2012

Keywords:
EDTA soil washing
Toxic metals
Metal bio-availability
Metal phyto-accumulation
Soil functioning

ABSTRACT

The effect of remediation using three EDTA doses (10, 30, 60 mmol kg^{-1}) on soil functioning was assessed using column experiment and *Brassica rapa*. Soil washing removed up to 77, 29 and 72% of metals from soil contaminated with 1378, 578 and 8.5 mg kg^{-1} of Pb, Zn and Cd, respectively. Sequential extraction indicated removal from the carbonate soil fraction. Metal oral-accessibility from the stomach phase was reduced by up to 75 and from the small intestine by up to 79% (Pb). Part of metals (up to 0.8% Cd) was lost due to leaching from columns. Remediation reduced toxic metal soil-root transfer by up to 61% but did not prevent metal accumulation in leaves. The fitness of plants grown on EDTA washed soils (gas exchange, fluorescence) was not compromised. Remediation initially reduced the soil DNA content (up to 29%, 30 mmol kg^{-1} EDTA) and changed the structure of microbial population.

1. Introduction

Pedogenesis is an extremely slow natural process and soil is considered to be an irreplaceable natural resource. Contamination with toxic metals is one of the most important threats to which soils are exposed. The need for agricultural production is increasing due to the population explosion and food demand, growing needs for biofuels and the negative consequences of climate change, e.g., topsoil erosion due to extreme weather. More contaminated agricultural land and urban gardens are therefore expected to be brought into use and soil remediation methods are urgently needed.

Soil washing with chelating agents, among which EDTA is the most frequently used, permanently removes a variety of toxic metals from contaminated soils by forming water-soluble complexes. We recently introduced a novel EDTA-based method (Pociecha and Lestan, 2012), where chelant and process waters are recycled in a closed loop, with no wastewater, that aims at sustainable reclamation of contaminated gardens and farmland and effectively removes toxic soil metals. In fact, most studies on EDTA-based soil washing focus on the effectiveness of toxic metal removal. However, the bio-availability of metals still remaining in the washed soil and the effect of the remediation process on soil properties and functioning as a plant and microbial (consequently soil fauna) substrate are the factors that finally decide the success or failure of soil washing and other remediation technologies.

Scientific literature on the effect of EDTA-soil washing on soil properties, however, is scarce. Although soil treatment with EDTA is much more soil gentle than using acids (Udovic and Lestan, 2012). Tsang et al. (2007) reported that EDTA washing solution dissolves indigenous oxides, carbonates and organic matter and appreciably alters both the soil physical structure and the chemical properties.

The objective of this study was to examine the effect of EDTA-soil washing of Pb, Zn and Cd contaminated soil on fractionation, oral-availability, plant (*Brassica rapa*) uptake and leaching of soil residual toxic metals, to assess the fitness of *B. rapa* for growing on remediated soils and impact of remediation on soil microbial biomass and structure of microbial populations. Recently developed pilot-scale soil-washing facility (Voglar and Lestan, 2012) was used to provide a sufficient amount of remediated garden soil rich in organic matter and fines.

2. Materials and methods

2.1. Soil properties

The contaminated soil used in this experiment was collected from the upper 30 cm layer of a managed vegetable garden near the abandoned lead smelter in Mežica Valley, Slovenia ($x = 489{,}300$ m and $y = 152{,}300$ m, Gauß-Krüger coordinate system). For soil analyses, samples were air-dried and sieved to 2 mm (ISO 11464, 2006). Soil pH was measured in a 1/2.5 (w/v) ratio of soil and 0.01 M $CaCl_2$ suspension (ISO 10390, 2005). Soil samples were analyzed for organic matter by modified Walkley–Black titrations (ISO 14235, 1998), cation exchange capacity (CEC) as the sum of base cations measured after soil extraction with ammonium acetate (pH = 7) and extractable acidity determined by the $BaCl_2$ method (Soil Survey laboratory methods manual, 1992), and soil texture by the pipette method (ISO 11277, 2009). Carbonates were determined manometrically after soil reaction with HCl (ISO 10693, 1995), easily extractable P (P_2O_5) and K (K_2O) colorimetrically

* Corresponding author.
E-mail address: domen.lestan@bf.uni-lj.si (D. Lestan).

0269-7491/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.envpol.2012.10.027

according to the Olsen method (Kalra and Maynard, 1991) and total nitrogen was determined after dry combustion (ISO 13878, 1987) The following values were obtained for the original soil: pH 6.9, organic matter 7.0%, N 43%, P 176 mg kg^{-1}, K 5.1 mg kg^{-1} and CEC 31 $cmol_c$ kg^{-1}. The soil had a high carbonate content (70.7%), and silty-loam texture with 45% sand, 52% silt and 3.3% clay according to U.S. Department of Agriculture classification scheme (Soil survey staff, 1975).

2.2. Soil remediation

Contaminated soil was remediated by washing as reported earlier by Voglar and Lestan (2012), using 10, 30 and 60 mmol Na_2–EDTA kg^{-1} of dry soil. In short, 75 kg of soil was extracted with 75 L of EDTA solution in a concrete mixer for 2 h. After extraction, the soil suspension was separated from the spent soil washing solution in a chamber filter press (filter cloth thickness 0.6 g cm^{-2}, air permeability 22 dm^3 dm^{-2} min^{-1}) and the soil rinsed within the press with pressured tap water to remove all EDTA-mobilized toxic metals species (until no Pb, Zn and Cd was detected in the used rinsing solution). The combined used washing and rinsing solution was treated in an electrolytic degradation step; the solution was flowed-through electrolytic cells equipped with a graphite anode and stainless cathode (electrode surface 0.5 m^2, distance between electrodes 10 mm, average electrical current 120 A, voltage between electrodes 11.5 V) until the EDTA was oxidatively degraded and toxic metals were removed from the solution by electrodeposition on the cathode and precipitation. The cleansed solution was discharged.

2.3. Soil column experiment

Twenty-four experimental soil columns (18 cm high × 15 cm in diameter) were filled with 3640 kg (dry weight) washed soil (10, 30, 60 mmol kg^{-1} EDTA) and (non-washed) original control soil. The pots were equipped with trapping devices for collection of the leachate and with a plastic mesh (D = 0.2 mm) placed on the bottom of the pots to retain the soil. The soil in all treatments was fertilized with 150 mg kg^{-1} N and K as NH_4NO_3 (Acros Organics, New jersey, USA) and K_2SO_4 (Kemika, Zagreb, Croatia), respectively. Before planting, the soil columns were stabilized for two weeks. Three-week old seedlings of Chinese cabbage (*Brassica rapa* L. var. pekinensis) were transplanted into the columns, one in each pot, and grown for 7 weeks. The soil in the columns was irrigated weekly with 500 mL of tap water.

2.4. Toxic metals fractionation in the soil

A modified Tessier's sequential extraction procedure (Lestan et al., 2003) was used to determine the distribution of Pb, Zn and Cd in various soil fractions in washed and non-washed original soil. The water soluble fraction in the soil solution was extracted from 1 g of air dried remediated and non-remediated soil, sieved to 2 mm and ground to 250 μm, with 10 mL of deionized water for 1 h. The exchangeable fraction from soil colloids to the soil solution was extracted from the residual soil sample with 10 mL of 1 M $MgNO_3$ for 2 h. The fraction bound to carbonates was extracted after shaking in 10 mL of 1 M NH_4OAc (pH 5) for 5 h. The fraction bound to Fe and Mn oxides was extracted with 20 mL of 0.1 M $NH_2OH{\cdot}HCl$ (pH 2) for 12 h. The fraction bound to organic matter was obtained after heating the soil suspension in 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 for 3 h at 85 °C, followed by extraction with 15 mL of 1 M NH_4OAc for 30 min. The final, residual fraction was obtained after digestion of the residual samples with *aqua regia*. Three determinations of Pb, Zn and Cd concentration were done for each fractionation sequence. The final fractional recovery of Pb, Zn, Cu and Cd was calculated by comparing the sum of their concentration in all six fractions with their pseudototal concentration (assessed by *aqua regia* digestion) in the corresponding remediated and non-remediated soil.

2.5. The physiologically based extraction test

The Physiologically Based Extraction Test (PBET) is in vitro test, designed to assess the oral bioaccessibility of metals in the human stomach and in the small intestine and thus estimates the amount of metals ready to be absorbed from the intestine into the blood (Ruby et al., 1996). The soil sample (0.5 g) was sieved through a 2 mm mesh, ground to 150 μm with an agate mill and digested in a reaction flask for 2 h at a constant temperature (37 °C) in simulated gastric fluid (50 mL) prepared by mixing 1.25 g of pepsin (porcine, Sigma), 0.50 g of citrate and malate, 420 μL of lactic acid and 500 μL of acetic acid in 1 L of deionized water and adjusting the mixture with diluted HCl to pH 2.50 ± 0.05. The pH of the reaction mixture was measured every 10 min and adjusted with HCl as necessary to keep it at a value of 2.50 ± 0.05. Samples (5 mL) were collected after 2 h and centrifuged at 2500 g for 25 min; the supernatant was stored at 5 °C. Five mL of the sample volume was replaced with gastric solution to maintain a constant volume in the reaction flask. The reaction was furthered titrated to pH 7 by the addition of $NaHCO_3$ solution; 175 mg of bile salts (porcine, Sigma) and 50 mg of pancreatin (porcine, Sigma) were added, simulating small intestine conditions. After 2 h digestion at constant temperature (37 °C), the reaction solutions were collected, centrifuged at 2500 g for 25 min and stored at 5 °C. During both phases, a constant moistened argon flow of 1 L min^{-1} at 37 °C was conducted through the reaction mixture in order to simulate peristaltic movement. The Pb, Zn and Cd in extracts were determined by AAS (Varian AA240FS). The multi-elemental PBET test was conducted in triplicate for each soil treatment.

2.6. Metal determination

Plants were harvested and the leaves and roots separated and thoroughly washed with deionized water. Samples were dried at 60 °C to a constant weight and ground in a titanium centrifugal mill. Ground plant tissues (250–300 mg dry weight) were then submitted to acid-digestion (65% HNO_3) with microwave heating and left to cool. Diluted to a volume of 25 mL with deionized water, they were stored in the cold until metal analysis. For soil metal analyses, all treatments, including the original control soil, were sampled after plant harvest. Air-dried samples of 2 mm soil fraction (1 g) were ground in an agate mill, sieved to 150 μm and digested in *aqua regia* solution, consisting of HCl and HNO_3 in a 3:1 ratio (v/v). Samples were then filtered through Whatman no. 4 filter paper and diluted with deionized water to a total 50 mL volume. The metals Pb, Zn, Cd were analyzed in both soil and plant material by flame (acetylene/air) atomic absorption spectrometry (AAS, Varian AA240FS). Metal contents in PBET and sequential extraction solutions were determined by AAS directly. Reference material used in inter-laboratory comparisons (WEPAL 2003.3/3, Wageningen University, Wageningen, Netherlands) was used in the digestion and analysis as part of the QA/QC protocol. The limits of quantification (LOQ) as given by manufacturer were 0.01, 0.02 and 0.1 mg L^{-1} for Zn, Cd and Pb, respectively. Reagent blank and analytical duplicates were used to ensure the accuracy and precision of the analysis.

2.7. Gas exchange and fluorescence measurements

Gas exchange measurements were made 12, 18, 25 and 33 days after planting (DAP) by using an LI-6400xt measuring system combined with a 6400-40 leaf chamber fluorometer and equipped with LED light source and CO_2 mixer (Li-Cor, Lincoln, NE, USA). Six plants per treatment were included in the measurements, with the first two fully developed leaves being measured on each. Chamber conditions were maintained by 400 μmol CO_2 mol^{-1}, ambient humidity and saturating PAR intensity of 1000 μmol m^{-2} s^{-1}. Chamber temperature was regulated by average ambient temperature during the measurement. Gas exchange data were registered when steady-state conditions were achieved; steady-state fluorescence was captured (F_s) at the same time. Immediately thereafter, a saturating light flash was applied over the same, light adapted, leaf area and maximum fluorescence (F_m') was recorded. Minimum fluorescence (F_0') was captured after the leaf had momentarily been darkened. Photochemical efficiency, i.e., the efficiency of energy harvesting by oxidized (open) PSII reaction centers in the light, was calculated as $F_v'/F_m' = F_m' - F_0'/F_m'$. The electron transport rate (ETR) was calculated as $ETR = ((F_m' - F_s)/F_m') \cdot f \cdot I \cdot \alpha_{leaf}$, where f is the fraction of absorbed quanta used by PSII, I is the incident photon flux density and α_{leaf} is the leaf absorbance.

2.8. Microbial biomass

All soil treatments, including the original control soil, were sampled twice, before planting *Brassica rapa* and after plant harvest. The microbial biomass was estimated by total soil DNA content after soil stabilization in soil columns, just before seedlings of Chinese cabbage were planted into the columns (week 0) and at the end of the column experiment (week 7). Total soil DNA was extracted from 0.5 g of moist sieved bulk soil from the upper 1 cm of soil columns using a BIO101 Fast DNA Spin Kit for Soil (MP-Biomedicals). Double stranded DNA (*ds*DNA) was quantified by a NanoDrop 2000/2000c Spectrophotometer.

Characterization of microbial community was studied by Terminal Fragment Lenght Polymorphism (T-RFLP) analysis of bacterial 16S rRNA and fungal ITS rRNA genes. Amplification of was performed in a 50 μL reaction mixture, which consisted of 3 μL of template DNA, buffer, dNTP (2 mM each), 1 mM $MgCl_2$, and 2.5 U of TopTaq DNA polymerase, and corresponding primers (Table 1). The forward primer was labeled with 5-phosphoramidite (FAM; Jena Bioscience, Germany). Purified PCR product rDNAs (10 μL) were double-digested with MspI and AluI restriction enzymes at 10 U of each according to the manufacturer's instructions (Fermentas, USA). The digested DNA fragments were purified by using MiniElute Reaction Clean-up kit. All reagents and kits, if not described otherwise, were obtained from Qiagen (Germany). Digested and purified DNA (1 μL) was mixed with 11 μL Hi-Di™ Formamid and 0.4 μL of DNA internal size standard (GeneScan 500 ROX Size Standard, Applied Biosystems, United Kingdom). Mixture was denaturated at 94 °C for 5 min and immediately transferred to ice. Separation and visualization of fluorescently labeled terminal restriction fragments (TRFs) was carried out with an ABI 3130xl genetic analyzer (Applied Biosystems, United Kingdom). The lengths of fluorescently labeled terminal restriction fragments (TRFs) were determined by using GeneMapper software version 4.0 (ABI, United Kingdom). Relative abundance values of peak heights were calculated for all TRFs that were between 50 and 500 bp long and had peak heights of more than 100 fluorescence units. All peaks with less than 1% of the relative abundance were not included in further statistical analyses. To analyze the

Table 1
Primers and thermal profiles used for PCR.

Gene	Primer	Reference	Sequence	Primer conc.	Conditions	Cycles
16S rRNA	B27f-FAM	Giovannoni, 1991	AGAGTTTGATCCTGGCTCAG	20 pmol/μL	95 °C, 4 min; 94 °C, 60 s; 57 °C, 60 s; 72 °C, 90 s; 72 °C, 30 s	29
	1401r	Nübel et al., 1996	CGGTGTGTACAAGAAGACCC			
ITS rRNA	ITS1f-FAM	Gardes and Bruns, 1993	TCCGTAGGTGAACCTGCGG	10 pmol/μL	95 °C, 3 min; 95 °C, 60 s; 55 °C, 60 s; 72 °C, 90 s; 72 °C, 10 min	30
	ITS4r	White et al., 1990	TCCTCCGCTTATTGATATGC			

TRFs data, between-group analysis was used (Culhane et al., 2002) based on correspondence analysis.

2.9. Statistics

The complete randomized experiment with four treatments in six replications were performed and the data were analyzed using one way analysis of variance (ANOVA). Where necessary the data was appropriately transformed to achieve equality of variances with Bartlett. In the cases where ANOVA showed statistically significant results, Duncan's multiple comparisons test was used to analyze differences between averages of variables for different treatments. Statistical analysis was done with R program (R Development Core Team, 2010). Differences between treatments were considered significant if $P < 0.05$.

3. Results and discussion

3.1. Metal removal, fractionation, mobility and bio-availability

As expected, increasing EDTA concentrations removed higher quantities of toxic metals: approx. 75% of Pb and Cd was removed from soil extracted with 60 mmol kg^{-1} EDTA (Table 2). The lower percentage of Zn removal, up to 29%, 60 mmol kg^{-1} EDTA (Table 2) can be explained by the lower affinity of EDTA for forming coordinative bonds with Zn; the stability constant of (log *Ks*) Zn–EDTA complex formation is 16.5, compared to 18.0 of the log *Ks* of Pb-EDTA (Martell and Smith, 2003). The majority of Zn was also found in the residual soil fraction (up to 64%, Table 3), encapsulated within non-solvable soil minerals and difficult to remove even under strong acidic, reducing or oxidating conditions of sequential's extractions scheme. Most Pb resided in the organic and carbonate fractions (up to 46%, and 33%, Table 3) while most Cd was bound to the labile carbonate soil fraction (up to 45%, Table 3). Reactants used in sequential extraction are not fraction specific and can remove metals also from other phases.

EDTA soil washing enhances metal mobilization from the soil solid phase by fast complexation with cationic metals and by slower EDTA-promoted dissolution (Nowack and Sigg, 1997). The former mechanism can break down weak bonds between metals and soil carbonates, as indicated by the concentrations of Pb, Zn and Cd in the carbonate fraction, which decrease with higher EDTA concentrations used for soil washing (Table 3). The latter mechanism, on the other hand, is presumably responsible for indirect mobilization and removal of Zn bound to oxides and Pb, Zn and Cd bound to the organic matter soil fraction (Table 3) by partially disrupting their structure. As also reported previously (Barona et al., 2000), EDTA was able to extract a certain amount of Pb from the silicate matrix, which implied that this part of Pb was not strongly fixed into the residual fraction, as data in Table 3 also indicate.

Fig. 1 shows the leaching of toxic metals, presumably in complex with EDTA, through the soil column during the 7 weeks of the plant

Table 2
Percentage of toxic metals removed from soil using different EDTA concentrations, concentration of Pb, Zn and Cd in roots and leaves of *B. rapa*, toxic metal translocation (TF) and bio-concentration factors (BCF). Mean ± standard deviation, $n = 6$.

	Original soil	10 mmol kg^{-1} EDTA	30 mmol kg^{-1} EDTA	60 mmol kg^{-1} EDTA
Pb				
Removed from soil (%)		35	59	77
Leaves (mg kg^{-1})	$^{a}15.0 \pm 8.1$	$^{a}17.5 \pm 12.1$	$^{a}11.5 \pm 4.5$	$^{a}16.9 \pm 8.4$
Roots (mg kg^{-1})	$^{a}107 \pm 34.2$	$^{b}53.6 \pm 18.3$	$^{c}22.0 \pm 7.8$	$^{c}13.8 \pm 1.9$
TF (leaves/roots)	0.16	0.46	0.61	1.26
BCF (roots/soil)	0.08	0.06	0.04	0.04
BCF (leaves/soil)	0.01	0.02	0.02	0.05
Zn				
Removed from soil (%)		3	11	29
Leaves (mg kg^{-1})	$^{a}120 \pm 25.7$	$^{a}126 \pm 35.9$	$^{a}115 \pm 16.3$	$^{a}126 \pm 36.2$
Roots (mg kg^{-1})	$^{a}272 \pm 31.0$	$^{bc}173 \pm 34.3$	$^{c}164 \pm 34.3$	$^{ab}225 \pm 31.1$
TF (leaves/roots)	0.44	0.77	0.74	0.57
BCF (roots/soil)	0.47	0.31	0.32	0.55
BCF (leaves/soil)	0.21	0.22	0.22	0.31
Cd				
Removed from soil (%)		45	60	72
Leaves (mg kg^{-1})	$^{a}2.5 \pm 1.3$	$^{a}3.1 \pm 1.5$	$^{a}2.2 \pm 1.1$	$^{a}3.5 \pm 1.9$
Roots (mg kg^{-1})	$^{a}9.6 \pm 3.6$	$^{b}2.1 \pm 0.3$	$^{b}1.6 \pm 0.5$	$^{b}2.1 \pm 0.6$
TF (leaves/roots)	0.28	1.45	1.41	1.91
BCF (roots/soil)	1.14	0.45	0.45	0.75
BCF (leaves/soil)	0.3	0.68	0.60	1.23

^{a,b,c} Statistically different treatments, Duncan test (data was logarithmically transformed to attain equal variances, $p < 0.05$).

Table 3
Sequential extraction of original soil and soils washed with different EDTA concentrations indicated the concentration of Pb, Zn and Cd in the soil washing solution (Fraction 1), exchangeably bound to soil colloids (Fraction 2), bound to carbonates (Fraction 3), bound to Fe- and Mn-oxides (Fraction 4), bound to soil organic matter (Fraction 5) and in the residual soil fraction (Fraction 6) after seven weeks of the *B. rapa* growth experiment. Also shown are the total metal concentrations of all treatments. Mean ± standard deviation, $n = 6$, as well as the total recovery of metals from all fractions of sequential extraction.

Fractions	Original soil (mg kg^{-1})	Remediated soil (mg kg^{-1})		
		10 mmol kg^{-1} EDTA	30 mmol kg^{-1} EDTA	60 mmol kg^{-1} EDTA
Pb				
1	LOQ	LOQ	LOQ	LOQ
2	$^{a}14.2 \pm 0.2$	$^{b}11.1 \pm 1.1$	$^{c}10.0 \pm 0.1$	$^{c}9.6 \pm 0.1$
3	$^{a}496 \pm 22$	$^{b}277 \pm 11$	$^{c}160 \pm 3$	$^{d}68.8 \pm 7.2$
4	3.5 ± 0.2	4.8 ± 0.5	1.8 ± 0.1	LOQ
5	$^{a}493 \pm 4$	$^{b}368 \pm 32$	$^{c}254 \pm 27$	$^{d}153 \pm 9$
6	$^{a}241 \pm 7$	$^{b}168 \pm 11$	$^{b}160 \pm 5$	$^{c}103 \pm 6$
Total conc.	$^{a}1378 \pm 107$	$^{b}898 \pm 41$	$^{c}564 \pm 11$	$^{d}323 \pm 7$
% Recovery	90.5	92.2	104	104
Zn				
1	$^{a}0.8 \pm 0.1$	$^{ab}0.8 \pm 0.1$	$^{a}0.7 \pm 0.1$	$^{b}1.0 \pm 0.0$
2	$^{a}7.0 \pm 0.3$	$^{b}1.9 \pm 0.1$	$^{b}1.7 \pm 0.0$	$^{c}1.4 \pm 0.0$
3	$^{a}118 \pm 17$	$^{b}68.5 \pm 3.1$	$^{c}34.3 \pm 0.5$	$^{c}24.7 \pm 0.4$
4	$^{a}18.9 \pm 0.2$	$^{b}9.3 \pm 0.5$	$^{c}4.8 \pm 0.0$	$^{d}3.2 \pm 0.1$
5	$^{a}174 \pm 3$	$^{a}178 \pm 10$	$^{b}136 \pm 11$	$^{b}114 \pm 20$
6	$^{a}244 \pm 7$	$^{b}290 \pm 6$	$^{b}291 \pm 7$	$^{a}255 \pm 10$
Total conc.	$^{a}578 \pm 38$	$^{a}561 \pm 8$	$^{a}513 \pm 7$	$^{b}412 \pm 4$
% Recovery	97.4	97.6	91.5	97.0
Cd				
1	LOQ	LOQ	LOQ	LOQ
2	$^{a}1.2 \pm 0.0$	$^{b}1.0 \pm 0.0$	$^{b}1.0 \pm 0.0$	$^{b}1.0 \pm 0.0$
3	$^{a}5.8 \pm 0.1$	$^{b}2.2 \pm 0.0$	$^{c}1.7 \pm 0.0$	$^{d}1.0 \pm 0.0$
4	0.5 ± 0.0	LOQ	LOQ	LOQ
5	$^{a}2.3 \pm 0.0$	$^{b}1.6 \pm 0.2$	$^{b}1.6 \pm 0.1$	$^{c}1.2 \pm 0.1$
6	LOQ	LOQ	LOQ	LOQ
Total conc.	$^{a}8.5 \pm 0.6$	$^{b}4.6 \pm 0.1$	$^{c}3.6 \pm 0.1$	$^{d}2.8 \pm 0.1$
% Recovery	115	104	117	112

$^{a, b, c, d}$ Statistically different treatments, Duncan test ($p < 0.05$).

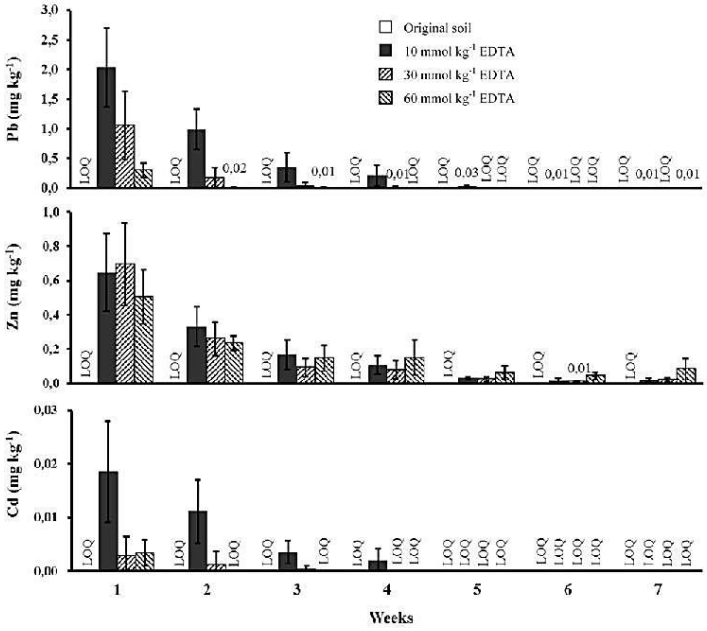


Fig. 1. Concentration of Pb, Zn and Cd in leachate from original soil and soils washed with different EDTA concentrations during the *B. rapa* growth experiment. Mean ± standard deviation, $n = 6$.

growth experiment. As expected, there were no detectable concentrations of Pb, Zn and Cd in the leachate from original, non-remediated soil, while a minor part of the initial metal soil content; up to 0.4, 0.3 and 0.8% of Pb, Zn and Cd, respectively, was leached from remediated soil, namely from 10 mmol kg^{-1} of EDTA treated soil. This indicates that, at least in this treatment, the rinsing phase of soil remediation (as explained in paragraph 2.2 and in Voglar and Lestan, 2012) was not sufficiently well performed. Although not assessed in this study, it is known that some metal-EDTA complexes are retained in the soil by forming bonds with soil iron oxides, especially goethite (Nowack and Sigg, 1996). Some of these bound complexes may be released and washed from soil column with irrigation water (Fig. 1).

As indicated in Table 3, a considerable amount of toxic metals in original and remediated soils was in a bound, non-labile form (i.e., bound to organic and residual soil fractions) rendering it non-available for biological processes. In order to evaluate soil remediation technologies, monitoring tools indicative of actual contaminant toxicity are therefore essential. For humans, the routes of exposure include inhalation of contaminated soil particles, direct soil ingestion, usually by children, ingestion of food produced on contaminated soil, or by drinking polluted water. To quantify the exposure of these pathways, a physiologically based extraction test (PBET) was used. PBET mimics the effect of the human-digestion process and incorporates different gastrointestinal tract parameters; pH, enzymes soil-to solution ratio, mixing etc. PBET simulates the gastrointestinal-tract of a 2–3 year old child to predict metal bioavailability in the stomach and small intestine (Turner and Ip, 2007). As shown in Table 4, the bioavailability of Pb from both stomach and intestinal phases decreases with a higher concentration of EDTA used for soil washing. Zn bioavailability in non-remediated compared to remediated soils is significantly different (Table 4). On the other hand, the concentration of EDTA used in the washing solution did not have a statistically significant effect on Zn bioavailability. This again is presumably the consequence of a predominate Zn association with the residual soil fraction (Table 3), which leaves a minority of the Zn in the soil (which was removed even with low EDTA concentrations) accessible to simulated gastro-intestinal solution. The concentration of Cd in the stomach and intestinal solution was below the limit of quantification (LOQ) in original and remediated soils (Table 4).

The absorption of nutrients (and toxic metals) takes place mainly in the small intestine (Ruby et al., 1996). The Pb and Zn concentrations measured in the intestinal phase, therefore, better

Table 4
Concentrations of Pb, Zn and Cd in stomach and intestinal phases of the physiologically based extraction test (PBET) in original soil and soils washed with different EDTA concentrations, after seven weeks of experiment. Mean ± standard deviation, $n = 6$.

PBET	Original soil (mg kg^{-1})	Remediated soil (mg kg^{-1})		
		10 mmol kg^{-1} EDTA	30 mmol kg^{-1} EDTA	60 mmol kg^{-1} EDTA
Pb				
Stomach	$^{a}333 \pm 29$	$^{a}286 \pm 33$	$^{b}145 \pm 58$	$^{b}81.8 \pm 9.9$
Intestine	$^{a}172 \pm 40.6$	$^{a}169 \pm 51$	$^{a}159 \pm 28$	$^{b}35.8 \pm 12.9$
Zn				
Stomach	$^{a}183 \pm 18$	$^{b}67.9 \pm 1.5$	$^{b}65.3 \pm 10.0$	$^{b}66.8 \pm 23.2$
Intestine	$^{a}78.3 \pm 16.8$	$^{b}37.9 \pm 4.7$	$^{b}31.6 \pm 4.1$	$^{b}30.1 \pm 6.8$
Cd				
Stomach	LOQ	LOQ	LOQ	LOQ
Intestine	LOQ	LOQ	LOQ	LOQ

^{a,b} Statistically different treatments, Duncan test ($p < 0.05$).

represent the oral-bioavailable fraction of metal than the concentrations in the stomach phase.

3.2. Effect of soil remediation on the test plant and soil microorganisms

Chinese cabbage (*Brassica rapa*) has been already used in metal-phytoextraction studies and was selected as the test plant in this study due to its known tendency to accumulate toxic metals (Grcman et al., 2001). As shown in Table 2, remediation significantly ($p < 0.05$) reduced the concentrations of Pb, Cd and to lesser extent also of Zn, in the roots of *B. rapa*. Generally, the concentration of toxic metals in roots decreased with increasing concentration of EDTA used for soil washing, except for Zn in the treatment with 60 mmol kg^{-1} EDTA (Table 2). Despite decreasing concentrations in the roots, concentrations of toxic metals in *B. rapa* leaves remained almost unchanged after remediation. For Zn, which is an important essential element this can be explained by active control of metal transport from roots to leaves. Differences in root Zn concentrations between non-remediated and remediated soils are also the least significant (Table 2). Higher internal translocation of Pb and Cd from roots to leaves (TF, Table 2) for plants grown in remediated soils compared to control soil (TF increased with EDTA concentration used) explain the similar accumulation of Pb and Cd in leaves. The mobile (leachable, Fig. 1) toxic metal-EDTA complexes residual in the soil after remediation (due to insufficient soil rinsing during the remediation process, in which EDTA-mobilized toxic metals species are removed from the soil) were presumably responsible for the higher TF and BCF of Pb, Cd (and also Zn) in EDTA treated soils. Similarly, Grcman et al. (2001) reported 1.7 and 3.5 and 3-times lower concentrations of Pb, Zn and Cd in the roots of *B. rapa* after the addition of 10 mmol EDTA kg^{-1} to soil, compared to plants grown in control soil. It has been suggested that enhanced plant uptake of metal-EDTA complexes can take place in points at which suberization of the root cell walls has not yet occurred and at breaks in the root endodermis and the Casparian strip (Bell et al., 1991; Nowack et al., 2006; Vassil et al., 1998). In practice, this could mean that, after EDTA treatment, *B. rapa* will accumulate some amounts of Pb and Cd even when their total concentration in the soil is low. The mechanism might, however, be species-specific and different for plants other than from the *Brassicae* family, which are known to be good toxic metal accumulators (Grcman et al., 2001).

The transfer of toxic metals from soil to roots, expressed as the bio-concentration factor (BCF), indicated reduced Pb and Cd bioavailability for *B. rapa* uptake from remediated soil (Table 2). Reduced availability is presumably related to a significantly lower concentration of toxic elements in the labile soil fractions, exchangeable and carbonate (Table 3), potentially accessible to plants. In particular, toxic metals bound into the carbonates fraction (major fraction of Pb and Cd residing in non-remediated soil, Table 3) could be made available for plants from the rhizosphere – the acidified interface between the roots and soil (Lin et al., 2004; Liao et al., 2006).

Chelating agents do not bind only to toxic metals, and soil washing also reduces the pool of soil macro and micro nutrients. Rengel (2002), for example, reported reduced growth and disturbances of mineral nutrition in EDTA treated wheat. Fe^{2+}, Fe^{3+}, Mn^{2+} and Ca^{2+}, in particular, form strong complexes with EDTA, with stability constants of log *Ks* 14.3, 25.7, 13.6 and 10.7, respectively (Martell and Smith, 2003). Consequently, 1–8.5% of Fe, 21–81% of Mn and 0.5–3% of Ca were removed from the original soil by EDTA washing.

The loss of micro-nutrients from remediated soil was not reflected in the physiological response of *B. rapa* during the course of growth, assessed using gas exchange and fluorescence measurements (Fig. 2). Photosynthetic rates during the first three measurements varied between 10.8 and 15.2 μmol CO_2 m^{-2} s^{-1} but were within a much lower range (6.8–9 μmol CO_2 m^{-2} s^{-1}, Fig. 2A) at the end of the growth period. Differences in net photosynthesis between plants grown in non-washed and EDTA washed substrate were minor. Rather surprisingly, the lowest rates were observed in plants grown in non-remediated soil. These differences were most prominent on the fourth measuring date, when they coincided with the differences found for stomatal conductance (Fig. 2B) and transpiration (Fig. 2C). On the other hand, no relation between the strength of washing media (EDTA concentration) and plant photosynthetic response, gas exchange or transpiration could be observed. Fluorescence measurements showed a stable photochemical efficiency over the entire period of

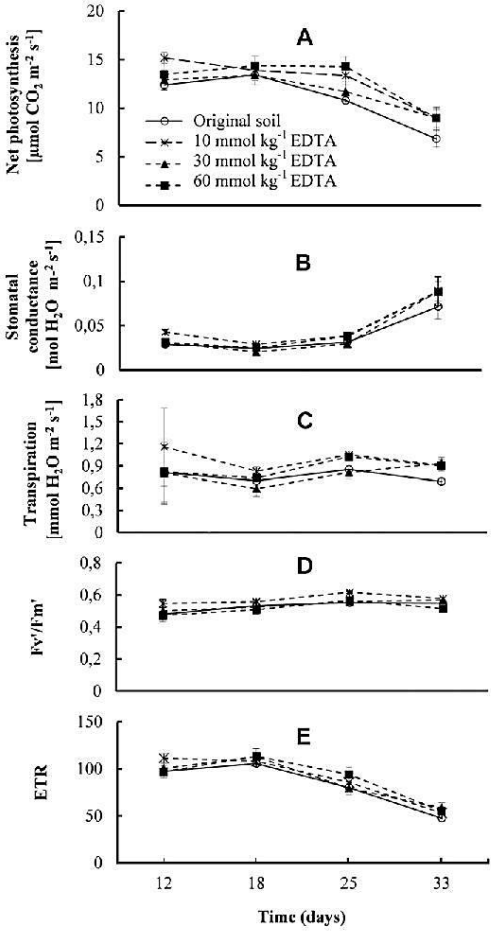


Fig. 2. Gas exchange (A, net-photosynthesis; B, stomatal conductance; C transpiration) and fluorescence parameters (D, photochemical efficiency F_v'/F_m'; E, electron transport rate ETR) of *B. rapa* grown in original soil and in soils washed with different EDTA concentrations during the course of the experiment. Mean ± standard deviation, $n = 6$.

the experiment (Fig. 2D). In contrast to F_v'/F_m', the ETR (Fig. 2E) values decreased in the second half of experiment, reaching ca. 40% of initial values. Differences between the treatments were again negligible.

Plant performance in EDTA washed soils can be affected by several factors. As explained above, some toxic metals-EDTA complexes are expected to remain bound into the soil solid phases after soil washing and rinsing. Experiments dealing with induced toxic metal phytoextraction have shown that the application of EDTA can severely inhibit plant growth, due to enhanced dissolution of metals or, directly, through free EDTA itself (Chen and Cutright, 2001; Meers et al., 2004). In addition to depression of metal ion activity, direct toxicity of EDTA caused by its interference with cellular structures and/or functions can be expected. Ruley et al. (2004, 2006) studied the physiological response of *Sesbania drummondii* exposed to EDTA or HEDTA by using chlorophyll fluorescence. A significant decrease in potential photosynthetic efficiency (F_v/F_m ratio, dark adapted plants) was found when a chelating agent was added to heavy metal free soil or nutrient solution, but not when Pb was also present. This suggests that chlorophyll fluorescence may be a valuable parameter for monitoring chelate induced stress originating from non-balanced ratios of metal and chelate after performing induced phytoextraction or soil washing by chelate. In our case, the F_v'/F_m' values, expressing the actual photochemical efficiency of light exposed plants, were similar in all four treatments. Any harmful effects of chelate residues on plant fitness can therefore be excluded. Moreover, when compared to the control, a slight promotion of both photochemical efficiency and photosynthetic activity was observed in plants grown in the soil remediated with the lowest EDTA dose.

Soil microbial biomass in the upper 1 cm soil layer was significantly higher in the original soil than in 30 and 60 mmol kg^{-1} EDTA remediated soils at the beginning of the soil column experiment, whereas no statistically significant differences between treatments were found after 7 weeks (Fig. 3). Similarly, statistical evaluation of the T-RFLP data set for bacterial 16S rRNA and fungal ITS rRNA gene fragments showed a clear difference between original soil and EDTA treated soil at the beginning of the column experiment, whereas bacterial and fungal community in all soils were clustering together after 7 weeks (Fig. 4). Numbers of different T-RFs in each soil ranged between 23 and 28 for bacterial community and between 21 and 33 for fungal with no statistical difference between treatments and sampling times. The initial loss of total (microbial) DNA from EDTA (30 and 60 mmol kg^{-1}) remediated soil, as well as structural change of bacterial and fungal community at the beginning of column experiment, may be related to the stringent physical conditions during soil extraction. A negative effect of EDTA soil treatments on soil biota has already been reported (Grcman et al., 2001; Hu et al., 2003; Mühlbachova, 2011). However, our study showed a recovery of soil microbes after 7 weeks. Moreover, microbial community switch from week 0 to week 7 was similar in original and all remediated soils, which indicates that the effect of inherent soil properties and the same conditions of soil aging overruled the initial effect of soil treatment. Microbial community could be also influenced by Chinese cabbage planted to all soils at week 0, despite only bulk soil was sampled for microbial analyses, which is expected to be less influenced than rhyzosphere soil. Interestingly, at the end of the growth period, the average total soil DNA concentrations in EDTA (30 and 60 mmol kg^{-1}) treated soils significantly increased in comparison to week 0, although differences in microbial biomass between original and remediated soil at

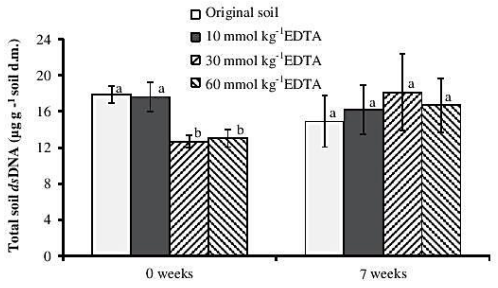


Fig. 3. Microbial biomass (in *ds*DNA concentration g^{-1} dry matter soil) in the upper 1 cm of original (non-washed) soil and in EDTA treated soils at the beginning (week 0) and end (week 7) of column leaching and *B. rapa* growth experiment. Mean ± standard deviation, $n = 6$. The letters a, b, c denote statistically different treatments according to the Duncan test ($p < 0.05$).

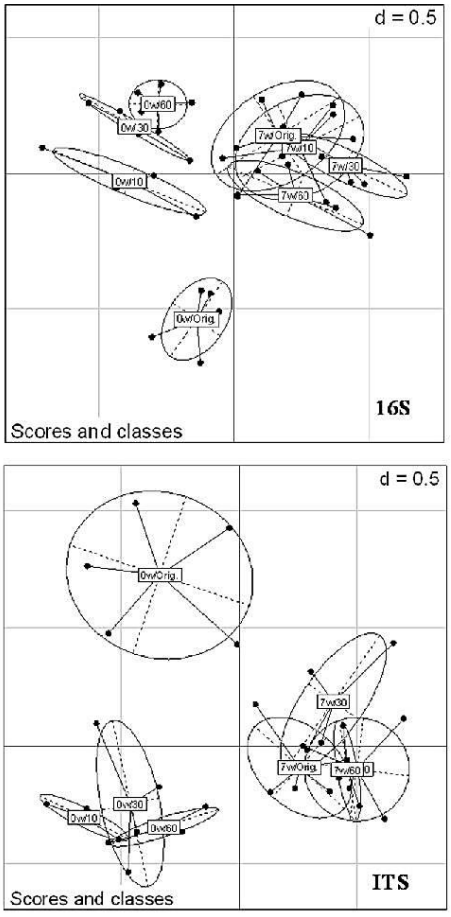


Fig. 4. Between-group analysis based on correspondence analysis of the T-RFLP data set for bacterial 16S rRNA and fungal ITS rRNA gene fragments. Ellipses surround the replicates for each treatment, showing that they cluster together.

week 7 were not statistically different. This might reflect removal of toxic metals, particularly from the carbonates soil fraction (Table 3) and/or potentially elevated concentrations of dissolved organic carbon (DOC) in EDTA treated soils, which were not followed in our study (Wang et al., 2007; Mühlbachova, 2011). The higher uptake of Pb and Cd into cabbage roots in original soil (Table 2) indicates that the metal bioavailable fraction was higher in the original than in EDTA treated soil, which is in agreement with the first hypothesis. Mühlbachova (2011) also reported a negative relationship between microbial biomass (soil microbial carbon) and available Cd and Zn fractions (NH_4NO_3 extraction) in EDTA treated arable soils, however she found no significant correlations for available Pb or for all three available metals in grassland soil.

4. Conclusions

The goal of soil remediation with EDTA is to reduce the total and bioavailable concentrations of toxic metals in soil and thus reduce the risk that polluted soil poses to the environment and human health. It is also desirable that, after reintroduction into the environment, remediated soil preserves the function of plant and microbial substrate, and, consequently, can become quickly repopulated by other soil organisms. The following conclusions can be drawn from our study:

- EDTA soil washing effectively removed Pb and Cd from contaminated soil, mainly from the carbonate fraction, and reduced oral-bio-availability of all three metallic contaminants.
- Leaching of toxic metals at the beginning of the growth experiment can be attributed to metal-EDTA complexes residual in the soil after remediation.
- EDTA soil washing significantly reduced the transfer of Pb, Zn and Cd from the soil to *B. rapa* roots but did not prevent the accumulation of toxic elements in plant green parts.
- The applied remediation technology does not limit the use of processed soil in terms of reducing the fitness of plants growing in EDTA washed substrate.
- Soil microbial biomass (indicated by total soil DNA) efficiently recovered after an initial decline due to EDTA soil washing.

This study revealed the accumulation of toxic elements in green parts of the test plant from remediated soil as a problematic aspect of the remediation technology applied. Pb, Zn and Cd phyto-accumulation and initial leaching presumably indicate the importance of efficient soil rinsing after soil extraction, in order to remove all EDTA-mobilized species of toxic metals. In addition to optimization of the soil rinsing phase of the soil remediation process, the feasibility of subsequent immobilization of residual toxic metals after soil extraction will therefore be the focus of our further studies.

Acknowledgment

This work was supported by the Slovenian Research Agency, Grant J4-3609.

References

Bell, P.F., Chaney, R.L., Angle, J.S., 1991. Free metal activity and total metal concentrations as indexes of micronutrient availability to barley (*Hordeum vulgare* L. "Klages"). Plant and Soil 130, 51–62.
Barona, A., Aranguiz, I., Elias, A., 2000. Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. Environmental Pollution 113, 79–85.
Chen, H., Cutright, T., 2001. EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. Chemosphere 45, 21–28.
Culhane, A.C., Perrière, G., Considine, E.C., Cotter, T.G., Higgins, D.G., 2002. Between group analysis of microarray data. Bioinformatics 18, 1600–1608.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for Basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113–118.
Giovannoni, S.J., 1991. The polymerase chain reaction. Nucleic acid techniques in bacterial systematics. In: Stackebrandt, E., Goodfellow, M. (Eds.), Modern Microbiological Methods: Nucleic Acids Techniques in Bacterial Systematics. John Wiley and Sons, New York, pp. 177–203.
Grcman, H., Velikonja-Bolta, S., Vodnik, D., Kos, B., Lestan, D., 2001. EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant and Soil 235, 105–114.
Hu, Z., Chandran, K., Grasso, D., Smets, B.F., 2003. Nitrification inhibition by ethylenediamine-based chelating agents. Environmental Engineering Science 20, 219–228.
ISO 10390, 2005. Soil Quality-Determination of pH. International Organization for Standardization, Genève, Switzerland.
ISO 10693, 1995. Determination of Carbonate Content. Volumetric Method. International Organization for Standardization, Genève, Switzerland.
ISO 11277, 2009. Determination of Particle Size Distribution in Mineral Soil Material-Method by Sieving and Sedimentation. International Organization for Standardization, Genève, Switzerland.
ISO 11464, 2006. Soil Quality-Pretreatment of Samples for Physico-chemical Analysis. International Organization for Standardization, Genève, Switzerland.
ISO 13878, 1987. Determination of Total Nitrogen Content by Dry Combustion ("Elemental Analysis"). International Organization for Standardization, Genève, Switzerland.
ISO 14235, 1998. Soil Quality- Determination of Organic Carbon by Sulfochromic Oxidation. International Organization for Standardization, Genève, Switzerland.
Kalra, Y.P., Maynard, D.G., 1991. Methods Manual for Forest Soil and Plant Analysis. Canadian Forest Service, Northern Forestry Centre, Edmonton.
Lestan, D., Grcman, H., Zupan, M., Bacac, N., 2003. Relationship of soil properties to fractionation of Pb and Zn in soil and their uptake into *Plantago lanceolata*. Soil and Sediment Contamination 12, 507–522.
Liao, Y.C., Chien, S.W.C., Wang, M.C., Shen, Y., Hung, P.L., Das, B., 2006. Effect of transpiration on Pb uptake by lettuce and on water soluble low molecular weight organic acids in rhizosphere. Chemosphere 65, 343–351.
Lin, Q., Chen, Y.X., He, Y.F., Tian, G.M., 2004. Root-induced changes of lead availability in the rhizosphere of *Oryza sativa* L. Agriculture, Ecosystems & Environment 104, 605–613.
Martell, A.E., Smith, R.M., 2003. NIST Critically Selected Stability Constants of Metal Complexes. Version 7.0. NIST, Gaithersburg.
Meers, E., Hopgood, M., Lesage, E., Vervaeke, P., Tack, F.M.G., Verloo, M.G., 2004. Enhanced phytoextraction: in search of EDTA alternatives. International Journal of Phytoremediation 6, 95–109.
Mühlbachova, G., 2011. Soil microbial activities and heavy metal mobility in long-term contaminated soils after addition of EDTA and EDDS. Ecological Engineering 37, 1064–1071.
Nowack, B., Sigg, L., 1996. Adsorption of EDTA and metal–EDTA complexes onto goethite. Journal of Colloid and Interface Science 177, 106–121.
Nowack, B., Sigg, L., 1997. Dissolution of Fe (III) (hydr)oxides by metal–EDTA complexes. Geochimica et. Cosmochimica Acta 61, 951–963.
Nowack, B., Schulin, R., Robinsos, B.H., 2006. Critical assessment of chelant-enhanced metal phytoextraction. Environmental Science & Technology 40, 5225–5232.
Nübel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R.I., Ludwig, W., Backhaus, H., 1996. Sequence heterogeneities of genes encoding 16S rRNAs in Paenibacillus polymyxa detected by temperature gradient gel electrophoresis. Journal of Bacteriology 178, 5636–5643.
Pociecha, M., Lestan, D., 2012. Recycling of EDTA solution after soil washing of Pb, Zn, Cd and As contaminated soil. Chemosphere 86, 843–846.
Rengel, Z., 2002. Chelator EDTA in nutrient solution decreases growth of wheat. Journal of Plant Nutrition and Soil Science 25, 1709–1725.
R Development Core Team, 2010. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. http://www.R-project.org.
Ruby, M.V., Davis, A., Schoof, R., Eberle, S., Sellstone, C.M., 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environmental Science & Technology 30, 422–430.
Ruley, A.T., Sharma, N.C., Sahi, S.V., 2004. Antioxidant defense in a lead accumulating plant, *Sesbania drummondii*. Plant Physiology and Biochemistry 42, 899–906.
Ruley, A.T., Sharma, N.C., Sahi, S.V., Singh, S.R., Sajwan, K.S., 2006. Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in *Sesbania drummondii* grown in soil. Environmental Pollution 144, 11–18.
Soil Survey laboratory methods manual, 1992. Soil Survey Investigations Report No. 42, Version 2.0.
Soil Survey Staff, 1975. Soil Taxonomy: a Basic System of Soil Classification for Making and Interpreting Soil Surveys. U.S. Government printing office, Washington, DC. USDA-SCS Agric. Handb. 436.
Tsang, D.C.W., Zhang, W.H., Lo, I.M.C., 2007. Copper extraction effectiveness and soil dissolution issues of EDTA-flushing of contaminated soils. Chemosphere 68, 234–243.
Turner, A., Ip, K.H., 2007. Bioaccessibility of metals in dust from the indoor environment: application of a physiologically based extraction test. Environmental Science & Technology 41, 7851–7856.

Udovic, M., Lestan, D. EDTA and HCl leaching of calcareous and acidic soils polluted with potentially toxic metals: remediation efficiency and soil impact. Chemosphere. Corrected Proof, Available Online 14 May 2012, in press.
Vassil, A.D., Kapulnik, Y., Raskin, I., Salt, D.E., 1998. The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiology 117, 447–453.
Voglar, D., Lestan, D., 2012. Pilot-scale washing of metal contaminated garden soil using EDTA. Journal of Hazardous Materials 215–216, 32–39.
White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols – a Guide to Methods and Applications. Academic Press, San Diego, CA, pp. 315–322.
Wang, G., Koopmans, G.F., Song, J., Temminghoff, E.J.M., Luo, Y., Zhao, Q., Japanenga, J., 2007. Mobilization of heavy metals from contaminated paddy soil by EDDS, EDTA and elemental sulphur. Environmental Geochemistry and Health 29, 221–235.

2.1.2 Vpliv pranja z EDTA na onesnažena vrtna tla. Del I: toksična nevarnost in vpliv na talne lastnosti

JELUŠIČ Maša, LEŠTAN Domen
Effect of EDTA washing of metal polluted garden soils. Part I: Toxicity hazards and impact on soil properties.
Science of the Total Environment, 2014, 475: 132−141

Ocenili smo kakovost, strupenost in funkcioniranje s Pb, Zn in Cd onesnaženih/remediiranih vrtnih tal iz Mežiške doline, Slovenija. Onesnažena tla smo očistili z EDTA in jih položili v eksperimentalne gredice, opremljene z lizimetri. Talne lastnosti smo ocenili po standardni pedološki analizi. Frakcionacijo in nevarnost izpiranja strupenih kovin smo analizirali s sekvenčno ekstrakcijo in TCLP metodo, dostopnost kovin pa s testom biodosegljivosti UBM. Kot indikatorje funkcioniranja tal smo analizirali dihanje tal in aktivnost talnih encimov. Remediacija je z omejenimi vplivi na pedologijo tal znižala vsebnost kovin za 80 % Pb, 28 % Zn in 72 % Cd. Strupene kovine so bile odstranjene iz dostopnih frakcij. V sedmih mesecih eksperimenta nismo opazili premeščanja kovin med dostopnimi in nedostopnimi frakcijami. Začetno izpiranje kovin iz remediiranih tal je po nekaj mesecih prenehalo. Remediacija je značilno znižala aktivnost talnih encimov in v celotnem trajanju poskusa nismo opazili nobenih trendov izboljšanja. S pranjem tal smo torej uspešno odstranili dosegljive oblike Pb, Zn in Cd ter tako zmanjšali nevarnost, ki jo kovine predstavljajo za človeka in okolje. Na drugi strani pa je remediacija hkrati odstranila tudi esencialne elemente, nujno potrebne za biotsko raznovrstnost tal, in zmanjšala vodno-zadrževalne lastnosti remediiranih tal. Zdravje tal ni bilo v celoti obnovljeno.

Science of the Total Environment 475 (2014) 132–141

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effect of EDTA washing of metal polluted garden soils. Part I: Toxicity hazards and impact on soil properties

Masa Jelusic, Domen Lestan *

Agronomy Department, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

HIGHLIGHTS

- EDTA extraction permanently removed labile pool of soil toxic metals.
- Levels of bioavailable Pb, Zn and Cd were drastically lowered by remediation.
- Remediation has limited impact on soil pedological properties.
- The functioning of remediated soil was not completely restored.

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 8 November 2013
Accepted 9 November 2013
Available online 7 December 2013

Keywords:
Toxic metals
Soil washing
Metal availability stripping
Soil enzyme activity

ABSTRACT

We applied a multi-level approach assessing the quality, toxicity and functioning of Pb, Zn and Cd contaminated/remediated soil from a vegetable garden in Meza Valley, Slovenia. Contaminated soil was extracted with EDTA and placed into field experimental plots equipped with lysimeters. Soil properties were assessed by standard pedological analysis. Fractionation and leachability of toxic metals were analyzed by sequential extraction and TCLP and metal bioaccessibility by UBM tests. Soil respiration and enzyme activities were measured as indicators of soil functioning. Remediation reduced the metal burden by 80, 28 and 72% for Pb, Zn and Cd respectively, with a limited impact on soil pedology. Toxic metals associated with labile soil fractions were largely removed. No shifts between labile and residual fractions were observed during the seven months of the experiment. Initial metal leaching measured through lysimeters eventually ceased. However, remediation significantly diminished potential soil enzyme activity and no trends were observed of the remediated soil recovering its biological properties. Soil washing successfully removed available forms of Pb, Zn and Cd and thus lowered the human and environmental hazards of the remediated soil; however, remediation also extracted the trace elements essential for soil biota. In addition to reduced water holding capacity, soil health was not completely restored.

1. Introduction

Soil contamination, caused by the large amounts of man-made pollutants and chemicals that are being anchored into agricultural and urban soils every day, is becoming a major problem. Potentially toxic metals (PTM) are one of the main causes of concern, since they are persistent in soils and are difficult to remove (EC, 2012). In Europe alone, an estimated 240,000 contaminated sites are in need of immediate remedial treatment and, in almost 40% of these sites, toxic metals are the most important contaminants (EEA, 2007).

Soil degradation processes are increasing the need to use contaminated land, such as former landmine sites and urban areas, for several purposes. For example, urban horticulture is currently booming in the world, fulfilling a variety of functions, including food production and community building. In some urbanized societies, up to 90% of the population resides in urban areas. In the United Kingdom, 87% of households tend a domestic garden (Gibbons et al., 2011). Living in such contaminated areas necessitates being in contact with contaminated soils in everyday life through soil dust inhalation, accidental mouth ingestion (especially by children) and consumption of foods grown in contaminated areas. In addition to the negative effect on human health, certain metals in soils also impact on the environment, creating toxicity in ground and surface waters and their organisms.

Several soil remediation approaches are being developed and studied to address this problem (Mulligan et al., 2001). One of the more practical approaches is ex situ method of soil washing with EDTA chelating agent, which promises limited changes in soil pedological properties compared to other extractants (Neale et al., 1997), preservation of soil biological properties (Udovic and Lestan, 2012) and high metal removal efficiency (Finzgar and Lestan, 2007), especially from bioavailable and labile fractions (Udovic and Lestan, 2010). Our previous results,

* Corresponding author at: Centre for Soil and Environmental Science, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia. Tel.: +386 1 3203 162; fax: +386 01 256 57 82.
E-mail address: domen.lestan@bf.uni-lj.si (D. Lestan).

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.11.049

however, were obtained from laboratory-scale experiments, where scale-up and process effects on soil, i.e. stringent extraction conditions in mixing reactor and rinsing soil under high pressure were not accounted for.

Furthermore, when returned to the site and exposed to environmental factors and agricultural use, is EDTA-remediated soil stable in terms of these newly obtained properties? Removing the labile metals from the soil could establish a new equilibrium during the process of aging, shifting residual metals into more labile fractions and thus diminishing the main effect of remediation. No long-term, large-scale scientific studies have yet been performed, to demonstrate the fate of improvements and/or deficiencies of EDTA-remediated soil.

We recently introduced a novel EDTA-based method that effectively removes PTMs (Pociecha and Lestan, 2012; Voglar and Lestan, 2013) in which chelant and process waters are recycled in a closed loop with no wastewater produced. The total cost of soil remediation using the novel technology, which includes also fixed costs for plant erection, site infrastructure, equipment, regulatory requirements etc. was evaluated in a pilot-scale remediation plant and amounted to 299 € ton^{-1} of soil for a small, 6-ton of soil per day remediation plant (paper in preparation). Fixed costs are driven by the economy of scale and decrease with plant soil treatment capacity. The cost of the technology is favorable compared to the 400 € ton^{-1} for traditional soil "dig and dump" (Dermont et al., 2008) and thus promises economical and sustainable reclamation of contaminated urban gardens and farmlands. In this study, we applied an integrated approach using well standardized chemical analyses with biological tests and with plant experiments for assessing the quality, toxicity and functioning of Pb, Zn and Cd contaminated/remediated soil from an active vegetable garden in the Meža Valley, Slovenia. This multi-level approach reduces the risk of undervaluation of some soil functions. The quality of remediated soil in terms of its physicochemical, biochemical and toxicological properties is addressed in Part I; plant performance on remediated soil, the ability to form mycorrhizae and the safety of vegetables and crops produced on remediated soil are addressed in Part II of this study. The remediated soil used in the present study was obtained from a recently developed pilot-scale soil-washing facility (Voglar and Lestan, 2012).

2. Materials and methods

2.1. Soil properties

The contaminated soil used in this experiment was collected from the upper 30 cm layer of a managed vegetable garden near an abandoned lead smelter in the Meža Valley, Slovenia (x = 489,300 m and y = 152,300 m, Gauβ–Krüger coordinate system). The Meža Valley was exposed to more than 300 years of active lead mining and smelting, which ceased in 1990 leaving behind 6600 ha of agricultural land polluted primarily with not only Pb but also Zn and Cd. For soil analyses, samples were air-dried and sieved to 2 mm (ISO11464, 2006). Soil pH was measured in a 1/2.5 (w/v) ratio of soil and 0.01 M $CaCl_2$ suspension (ISO10390, 2005). Soil samples were analyzed for organic matter and organic carbon by modified Walkley–Black titrations (ISO14235, 1998), total nitrogen was determined after dry combustion (ISO13878, 1998) and C/N was calculated from the organic carbon and total nitrogen ratio. Cation exchange capacity (CEC), as the sum of base cations, was measured after soil extraction with ammonium acetate (pH 7) and soil texture was analyzed by the pipette method (ISO11277, 2009). Easily extractable P (P_2O_5) and K (K_2O) were measured colorimetrically according to Kalra and Maynard, 1991. Soil water sorption capacity was determined by inserting soil samples, sieved through a 2 mm sieve into retaining rings placed on a ceramic plate and irrigated with deionized water for 48 h. The ceramic plate with soil sample was then placed in an extractor-pressure vessel for another 48 h. Negative pressures of 0.33, 1, 2, 5, and 15 bar were applied. Samples were weighed and then dried for 24 h at 105 °C and weighed again to determine the mass percentage of water sorbed.

2.2. Soil remediation

The original contaminated soil was remediated by chelant washing as reported by (Voglar and Lestan, 2012), using 2 h extraction of 65 kg (air dried) soil batches with 60 mM EDTA per kg of soil in a rotary mixer. The soil to washing solution weight ratio was 1:1. Extracted soil was separated from the soil washing solution in a filter chamber press and in a press rinsed with fresh water to remove EDTA-mobilized metallic species. Used washing and rinsing solutions were treated by an electrochemical advanced oxidation process using a graphite anode to degrade the EDTA oxidatively and to electro-precipitate PTMs onto a stainless-steel cathode before the cleansed waste water was discharged (Voglar and Lestan, 2012).

2.3. Experimental plot and sampling

Two experimental plots on raised garden beds 4 m × 1 m × 0.3 m, equipped with lysimeter-like devices (Fig. 1) for the collection of leachate, were filled with remediated and original soils up to 0.15 m in height. The garden beds were exposed to environmental conditions for a period of 7 months. The ten year average temperature for the location of the field experiment (Ljubljana Slovenija) with a continental climate was 10.9 °C, with a peak in July (21 °C) and a minimum in January (0.8 °C) and total annual precipitation of 1350 mm.

Both treatments were fertilized twice, in April and in August, with 300 kg ha^{-1} KNO_3 and 120 kg ha^{-1} NH_4NO_3 (Acros Organics, New Jersey, USA) and selected vegetables were then planted. The first series of crops was onion (*Allium cepa*, L.), pea (*Pisum sativum*, L.), spinach (*Spinacia oleracea*, L.) and cauliflower (*Brassica oleracea* Bortytis, L.) in spring 2011 and the second series was spinach and Chinese cabbage (*Brassica rapa*, L. Pekinensis) in fall 2011. Original and remediated soils were sampled for analyses immediately after the garden beds were filled, before fertilization and cultivation, in April 2011 (0 months). Further samples were taken under different seasonal cropping 1 month (only for enzyme activity) and 4 and 7 months after the first sampling. Each soil sampling was conducted in three replicates. One replicate consisted of ten thoroughly mixed subsamples collected from randomly selected points (depth 5–10 cm).

One lysimeter was placed under original soil and three under remediated soil (Fig. 1). Samples from each of the four lysimeters were taken after every substantial precipitation until no metals were detected in the samples. The volume of lysimeter water was measured after the leachates had been collected. Samples were then filtered through Whatman no. 4 filter paper and stored at 5 °C until the PTM concentrations in the leachates were determined.

2.4. Metal fractionation

We used a modified Tessier's sequential extraction procedure (Lestan et al., 2003) to determine the association of Pb, Zn and Cd with different soil fractions in washed remediated soils and non-washed original soil. The water soluble fraction in the soil solution was extracted from 1 g of air dried remediated and original soils, sieved to 2 mm and ground to 250 μm, with 10 mL of deionized water for 1 h. The exchangeable fraction from soil colloids to the soil solution was extracted from the residual soil sample with 10 mL of 1 M $Mg(NO_3)_2$ for 2 h. The fraction bound to carbonates was extracted after shaking in 10 mL of 1 M NH_4OAc (pH 5) for 5 h. The fraction bound to Fe and Mn oxides was extracted with 20 mL of 0.1 M $NH_2OH{\cdot}HCl$ (pH 2) for 12 h. The fraction bound to organic matter was obtained after heating the soil suspension in 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 for 3 h at 85 °C, followed by extraction with 15 mL of 1 M NH_4OAc for 30 min. The final, residual fraction was obtained after digestion of the

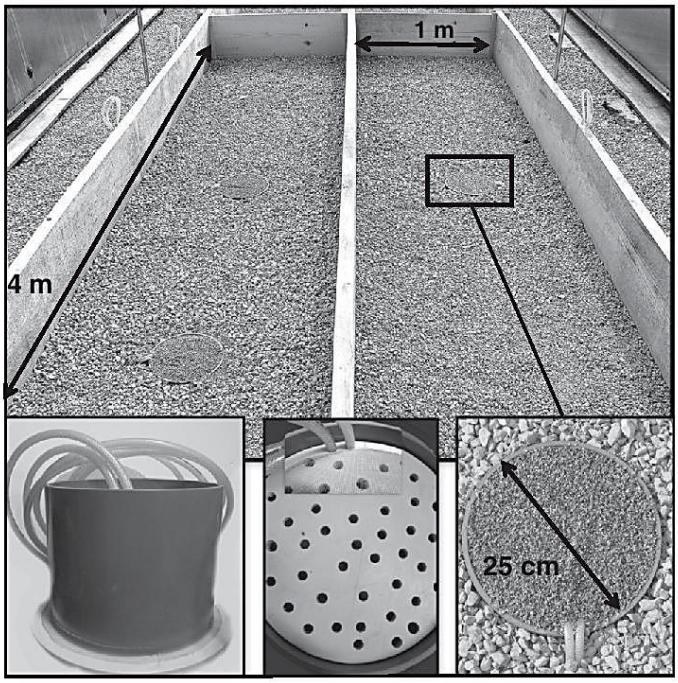


Fig. 1. Two experimental plots on raised garden beds 4 m × 1 m × 0.3 m, equipped with lysimeter-like devices (bottom of the figure). Left side of the garden bed will be filled with remediated soil and the right side with the original soil.

residual samples with *aqua regia*. Three determinations of Pb, Zn and Cd concentration were done for each fractionation sequence. The final fractional recovery of Pb, Zn, Cu and Cd was calculated by comparing the sum of their concentrations in all six fractions with their pseudototal concentration (assessed by *aqua regia* digestion) in the corresponding remediated soils and non-remediated soil.

2.5. Accessibility and mobility of metals

For the human hazard assay a unified bio-accessibility method (UBM) was applied according to Wragg et al. (2011)_ and the bioaccessibility research group of Europe (BARGE). Four digestive fluids: saliva, gastric, duodenal and bile were made for the test. Each solution was a combination of organic and inorganic solution and specific enzymes. Saliva fluid contained the following reagents: KCl, NaH_2PO_4, KSCN, Na_2SO_4, NaCl, NaOH, HCl, urea, alpha amylase, mucin and uric acid. Gastric fluid contained: KCl, NaH_2PO_4, NaCl, $CaCl_2$, NH_4Cl, HCl, urea, glucose, glucuronic acid, glucosamine hydrochloride, mucin, bovine serum albumin and pepsin. Duodenal fluid contained: KCl, NaCl, $NaHCO_3$, KH_2PO_4, $MgCl_2$, HCl, urea, bovine serum albumin, pancreatin and lipase and bile fluid contained: KCl, NaCl, $NaHCO_3$, HCl, urea, bovine serum albumin, and bile. Soil samples were sieved through a 2 mm mesh and ground to 250 μm with an agate mill. Approximately 0.6 g of soil was weighed directly into polycarbonate tubes, to which 9 mL of simulated saliva (pH 6.5 ± 0.5) was added. The suspension was manually shaken and adjusted with HCl or NaOH to pH of 1.20 ± 0.05 before simulated gastric solution (13.5 mL) of pH 0.9–1.0 was added. The extraction vessel was then placed in an end-over-end shaker in a thermostatically controlled water bath at 37 °C, thus simulating the stomach (pH 1.2–1.7). After 1 h the pH was checked whether the pH < 1.50 (if not, the procedure was restarted from the beginning with special insistence on a pH stability of 1.20 ± 0.05 every 15 min) and centrifuged at 4500 *g* for 20 min. To simulate the intestinal phase (pH 6.3 ± 0.5), the procedure was repeated and after the pH of the stomach phase had been checked, 27 mL of duodenal (pH 7.4 ± 0.2) and 9 mL of bile (pH 8.0 ± 0.2) solutions were added and the tubes were returned to the water bath for a further 4 h. The vessels were then centrifuged for 20 min at 4500 *g* and supernatant was collected by careful pipetting and stored at 4 °C until analysis.

The mobility and leachability of Pb, Zn and Cd in the soil samples were determined using the toxicity characteristic leaching procedure (TCLP) (U.S. EPA, 1995). Samples (1 g) were sieved through a 2 mm mesh and agitated in 20 mL of 0.0992 M acetic acid and 0.0643 M NaOH extraction solution (1:20 ratio) with a pH of 4.93 ± 0.05 for 18 h at 300 rpm. Samples were filtered through Whatman no. 4 filter paper, acidified with 65% HNO_3 to pH < 2 and stored at 5 °C. Pb, Zn and Cd in the extracts in the latter two tests were determined by AAS (Varian AA240FS). All extractions were prepared in triplicate and with reagent blanks.

2.6. Metal determination

Air-dried samples of the 2 mm soil fraction (1 g) were ground in an agate mill, sieved to 250 μm and digested in *aqua regia* solution, consisting of HCl and HNO_3 in a 3:1 ratio (v/v). A microwave (Mars Xpress, CEM MDS-2000) with closed vessels and temperature of 175 °C was used. Samples were then filtered through Whatman no. 4 filter paper and diluted with deionized water to a total 50 mL

volume. The inter-laboratory comparison reference material used was WEPAL 2003 and 2004 (Wageningen University, Wageningen, Netherlands) with 90–109% recovery from reference values.

Flame (acetylene/air) atomic absorption spectrometry (AAS, Varian AA240FS) was used for the metals Pb, Zn, Cd for sequential analysis, toxicological tests and for total metal analysis. The limits of quantification (LOQ) as given by the manufacturer were 0.01, 0.02 and 0.1 mg L^{-1} for Zn, Cd and Pb, respectively. Reagent blank and analytical duplicates were used to ensure the accuracy and precision of the analysis.

2.7. Glucose-induced respiration of soil organisms

The commercial equipment OxiTop Control (WTW, Wilheim, Germany) was used for assessment of substrate glucose-induced respiration (SIR). The experimental soil (corresponding to 50 g of dry soil) was placed in glass containers, moistened to 90% of field capacity. A plastic beaker containing approx. 10 mL of 25% NaOH solution was placed in the container and pressure meters installed in a plastic head were fixed on top of the glass container. The glass containers were incubated in the dark at 20 °C for 24 h. SIR was measured manometrically by alkaline (25% NaOH) absorption of the CO_2 produced during the incubation period (Zimakowska-Gnoinska and Bech, 1999) and expressed in mmol of CO_2 produced by microorganisms in 1 g of soil after 24 h of incubation at 20 °C. Five replicates of each treatment were performed.

2.8. Soil enzyme activities

Various enzymatic activity assays were performed on remediated and original soil in selected months during the seven-month period of the garden experiment. Soil samples of approximately 300 g were sieved through 5 mm sieves and incubated in glass jars for three weeks at a constant 20 °C. All measurements were carried out in three to five repetitions.

Determination of β-glucosidase activity in the soil was based on *p*-nitrophenol (PNP) formation after incubation of the soil with *p*-nitrophenyl glucoside (PNG) as substrate for 1 h at 37 °C (Eivazi and Tabatabai, 1988). The developed *p*-nitrophenol was measured spectrophotometrically at 400 nm against a blank.

Acid and alkaline phosphatase activity in the soil was determined according to Tabatabai and Bremner (1969). Formation of PNP was measured at 400 nm after 1 h incubation at 37 °C of soil with a substrate of *p*-nitrophenyl phosphate in various modified universal buffers (MUB at pH 6.5 for acid and MUB at pH 11 for alkaline phosphatase activity).

Dehydrogenase activity was determined according to the method described by Thalmann (1968) based on the reduction of triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) measured spectrophotometrically at 546 nm.

Urease activity was determined according to the method described by Hoffmann and Teicher (1961). The method is based on spectrophotometric (580 nm) determination of ammonia released after incubation of soil samples with urea solution for 3 h at 37 °C.

Soil esterase, lipase and some protease activity were assessed by hydrolysis of fluorescein diacetate according to Green et al. (2006) Fluorescein released after hydrolysis of fluorescein diacetate (FDA) was spectrophotometrically (490 nm) measured after soil samples had been incubated together with a substrate FDA at 37 °C for 3 h.

2.9. Statistical analysis

A field experiment was performed with two treatments, original and remediated, and three time period replications 0, 4 and 7 months. The objective of the statistical analysis was to observe the stability of certain soil characteristics obtained with EDTA-washing of remediated soil. The Kruskal–Wallis non-parametric test was employed to calculate the differences between time periods for remediated soils for the following: TCLP test, UBM test and sequential analysis. The non-parametric test was used since no data transformation was able to display equal variances between samples for calculating the ANOVA (analysis of variance) and possible further multiple range tests. The non-parametric test was also applied to calculate differences between time periods in original soil for the UBM test.

Another objective was to compare the enzymatic activities between original soil and remediated soil. Differences for each time period and enzyme were assessed with Student's t-test. Also assessed with the Student's t-test were the differences between the water holding capacities of original and remediated soils for the period of 0 and 7 months. Statistical analysis was done with R program (R Development Core Team, 2010). Differences between treatments were considered significant at $p < 0.05$.

3. Results

Polluted soil was remediated with 60 mM EDTA. The initial metal concentrations of 1585 mg kg^{-1} Pb, 525 mg kg^{-1} Zn and 8.8 mg kg^{-1} Cd were reduced to 313, 378 and 2.52 mg kg^{-1} for Pb, Zn and Cd respectively.

Remediation of polluted soil slightly raised the pH of the soil, which remained elevated throughout the experiment (Table 1). Organic matter was reduced by the EDTA-washing, and was only increased modestly after two crop rotations. The amounts of nutrients P and K were also reduced and a substantial amount of K was thus added before the third sampling, since it became a deficient element in the remediated soil. However, a large amount of precipitation rinsed the applied fertilizer and another dose was added a month before the fourth sampling. As expected washing did not have any effect on soil texture, which remained mostly unaltered, as did other soil properties determined by standard pedological analysis.

Soil water capacity was measured at five soil matric potentials, −0.33, −1, −2, −5 and −15 bar (Fig. 2). The difference between field capacity (−0.33 bar) and permanent wilting point (−15 bar) represents water in the soil available to plants. As shown in Fig. 2, leaching with EDTA lowered the soil water content at all measured potentials compared to the original soil. Nevertheless, differences became smaller after seven months due to the lower water potential in the original soil. The difference between original and remediated soil samples was statistically significant for all measured potentials for both time periods, except for the field capacity measured in the seventh month.

A sequential extraction procedure was used to associate the distribution of Pb, Zn and Cd for the original soil and remediated soils with six fractions: (I) first fraction: metals soluble in water; (II) second fraction: exchangeable metal forms; (III) third fraction: metals associated with carbonates and weakly complexed metals including easily soluble oxides/hydroxides under slightly acidic conditions; (IV) fourth fraction: reduced conditions, metals associated with Fe–Mn oxides of low crystallinity; (V) fifth fraction: contaminants associated with easily oxidizable compounds, including organic matter and sulfides; and (VI) sixth fraction: residual fraction containing metals strongly bound to silicates and to crystalline iron oxides.

The share of almost all metal fractions decreased after remediation and remained practically unaltered throughout the all seven months of the experiment (Table 2). Water soluble and exchangeable fractions and fractions extracted in reduced conditions even fell below the level of quantification for Pb and Cd and decreased significantly for Zn. Acid extractable metals in remediated soil varied between 14% and 2% of the original soil fractions. Extraction after the oxidation procedure reduced Pb by 85% and Zn and Cd by more than 50%. Pb and Zn were predominantly present in the last, least accessible residual soil fraction before and after leaching with EDTA. Ligand successfully removed a

Table 1
Selected pedological properties; pH, organic matter, C/N, P_2O_5 and K_2O (mg 100 g^{-1}), CEC ($mmol_c$ 100 g^{-1}) and texture in remediated and original soils 0, 4 and 7 months after the start of the experiment. Three subsamples were mixed to form a combined sample for each measurement.

Pedological analysis	Original soil Sampling time (months)			Remediated soil Sampling time (months)		
	0	4	7	0	4	7
pH ($CaCl_2$)	7.1	6.9	7	7.4	7.1	7.2
Organic matter (%)	6.5	8	8	4.9	5.4	5.8
C/N	9.7	12.4	11.5	9.7	12.4	10.3
P_2O_5 (mg 100 g^{-1})	40.1	47	45.5	33	29.5	32.9
K_2O (mg 100 g^{-1})	34.8	26.5	38.4	5.3	7.9	36
CEC ($mmol_c$ 100 g^{-1})	21.4	21.0	20.4	20.4	17.7	20.7
Sand (%)	39.7	34.8	41.7	43.8	42.7	38.6
Silt (%)	55.1	58.7	52.5	51.7	49.8	55.8
Clay (%)	5.2	6.5	5.8	4.5	7.5	5.6

substantial part of Pb and Cd from this fraction; however no change in Zn in the residual fraction was observed.

A chemical toxicity characteristic leaching procedure (TCLP) was employed to determine whether the remediated soil is potentially hazardous in terms of metal leaching. After remediation, the mobility of Pb and Zn dropped by more than 88%, and that of Cd by 71% (Fig. 3). No significant changes in the remediated soil were observed during the course of the experiment. On the other hand lysimeter measurements demonstrated that no metals were leached from the original soil and high amounts of Pb, Zn and Cd were collected from vessels under the remediated soil (Fig. 4). Leaching in the remediated soil ultimately ceased, by the second month for Pb and Cd and by the fourth month for Zn.

In order to characterize the oral bio-accessibility of Pb, Zn and Cd we applied the UBM test, which is an in vitro method simulating the human digestive procedure using synthetic digestive fluids. Values for the original soil stomach phase were very high, approximately 88%, 64% and 100% of the total metal concentration, for Pb, Zn and Cd respectively (Table 3). Bioaccessibility in the stomach phase was reduced with remediation by more than 80% for Pb and Cd and by 56% for Zn. Accessibility was much lower in the intestinal phase for the original soil and was brought under the level of determination in the remediated soil. The Kruskal Wallis statistical test showed no differences between sampling times in original and remediated soils.

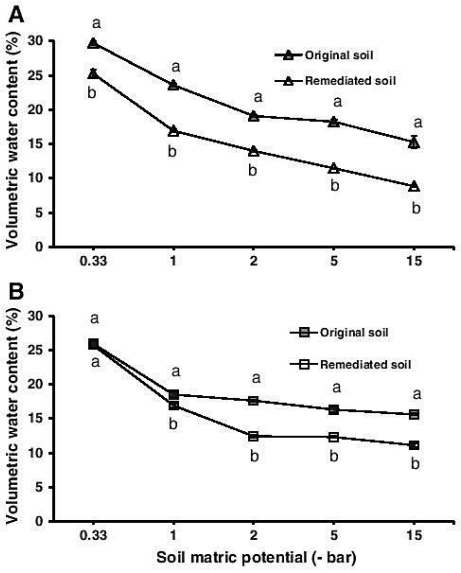


Fig. 2. Volumetric water content in original and remediated soils at different matric potentials at the beginning of the experiment (A) and after 7 months (B). Error bars represent standard deviation (n = 3). The letters "a" and "b" denote statistically different treatments according to the Student t-test ($p < 0.05$).

Substrate induced respiration (SIR) is an index of active microbial biomass and is widely used to estimate PTM toxicity for soil organisms. Although no statistically significant differences were determined except in October, the original soil demonstrated increased respiration in all measurements (Fig. 5).

Several specific enzyme activities were measured as indicators of the functional diversity of the soil microbial community (Fig. 5). All measurements revealed that the remediation process significantly diminished potential soil enzyme activity and no trends were observed of the remediated soil developing the same or better enzymatic activity than the original soil.

Responses of the same enzymes varied over time, due to different plant cover and changing seasonal and other environmental factors. Acid phosphomonoesterase activity and hydrolysis of fluorescein diacetate peaked in April, alkaline phosphomonoesterase activity and urease activity in October and dehydrogenase activity in July for both remediated and original soils. β-Glucosidase activity was highest in April for original soil and in October for remediated soil.

4. Discussion

Contaminated soil was collected from an old smelter site contaminated mainly with Pb, Zn and Cd. The area was and despite the high contamination risks, still is used for urban gardening with plant cultivation. Such areas pose a potential threat to the resident population and are in need of a soil healthy remediation technique to enable further but hazard free soil exploitation. For that purpose we remediated contaminated soil with the EDTA-washing technique and studied its impact on soil properties and possible soil re-cultivation.

4.1. Soil properties and metal fractionation

Remediation did not have any notable effect on soil properties determined by standard pedological analysis (Table 1). The observed drastic depletion of K in the remediated soil was probably caused by washing the soil with Na_2-EDTA, whereby the abundance of Na^+ ions replaced the K^+ ions on the soil binding sites. Recent technological development has led to the use of EDTA, recycled as Ca salt, which should at least partially alleviate the problem (Voglar and Lestan, 2013).

Soil washing with EDTA first forms complexes with cationic metals, which are thermodynamically favorable (Martell and Smith, 2003) and thus depletes the soil of loosely bound metals. Second by an EDTA promoted dissolution mechanism, soil washing also partially breaks

Table 2
Sequential extraction indicated the concentrations of Pb, Zn and Cd in the soil washing solution (Fraction 1), exchangeably bound to soil colloids (Fraction 2), bound to carbonates (Fraction 3), bound to Fe- and Mn-oxides (Fraction 4), bound to soil organic matter (Fraction 5) and in the residual soil fraction (Fraction 6), as well as the total recovery of metals from all fractions of sequential extraction 0, 4 and 7 months after the start of the experiment for remediated soil and an average of all three periods (0, 4 and 7) was calculated for the original soil.

Fractions	Original soil (mg kg^{-1})	Remediated soil (mg kg^{-1}) Sampling time (months)		
		0	4	7
Pb				
1	3.4 ± 0.6	LOQ	LOQ	LOQ
2	2.0 ± 0.4	LOQ	LOQ	LOQ
3	588 ± 20	[a]80.9 ± 1.0	[a]81.4 ± 0.4	[a]81.6 ± 1.9
4	4.3 ± 0.6	LOQ	LOQ	LOQ
5	439 ± 33	[a]68.2 ± 8.5	[a]66.6 ± 9.4	[a]66.1 ± 9.2
6	681 ± 34	[a]197 ± 4	[a]178 ± 5	[a]191 ± 11
% recovery	108	110.4	110.4	110.8
Zn				
1	1.2 ± 0,0	[a]1.2 ± 0,1	[a]0.4 ± 0.1	[a]0.5 ± 0.1
2	7.0 ± 0.0	[a]1.0 ± 0.1	[a]0.6 ± 0.1	[a]0.5 ± 0.0
3	125 ± 3	[a]26.3 ± 0.6	[a]29.6 ± 0.3	[a]29.2 ± 0.7
4	15.3 ± 1.1	[a]2.9 ± 0.0	[a]2.9 ± 0.0	[a]2.9 ± 0.0
5	169 ± 17	[a]94.9 ± 0.7	[a]84.8 ± 7.8	[a]85.4 ± 5.3
6	298 ± 23	[a]303 ± 10	[a]280 ± 5	[a]300 ± 12
% recovery	117	113	109	114
Cd				
1	LOQ	LOQ	LOQ	LOQ
2	0.4 ± 0.1	LOQ	LOQ	LOQ
3	6.23 ± 0.15	[a]1.12 ± 0.01	[a]1.17 ± 0.02	[a]1.18 ± 0.01
4	0.79 ± 0.11	LOQ	LOQ	LOQ
5	2.07 ± 0.16	[a]1.04 ± 0.02	[a]0.93 ± 0.02	[a]1.02 ± 0.03
6	1.70 ± 0.22	[a]0.99 ± 0.05	[a]0.83 ± 0.03	[a]0.95 ± 0.09
% recovery	128	125	120	125

Mean ± standard deviation (n = 9 for original soil, n = 3 for remediated soil). Letter [a] denotes statistical significance between remediated soils according to the Kruskal–Wallis test ($p < 0.05$). LOQ below the level of quantification.

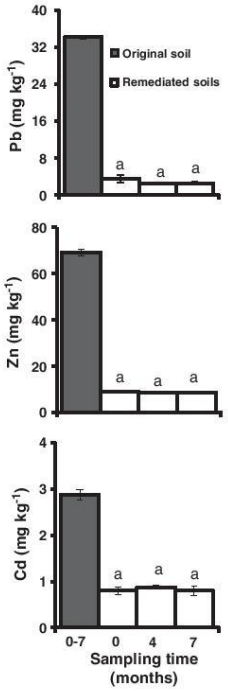


Fig. 3. Pb, Zn and Cd leachability of original and remediated soils assessed by TCLP extraction. For the original soil, the average is calculated from 0, 4 and 7-month samples. Error bars represent standard deviation (n = 9 for original soil, n = 3 for remediated soils). The letter "a" denotes statistical significance according to the Kruskal–Wallis test ($p < 0.05$).

down the soil structure, dissolving organic matter and soil minerals (Nowack and Sigg, 1997; Tsang et al., 2007; Vulava and Seaman, 2000) and thus indirectly releases more metals into the soil solution. The sum of the two activities in our case reduced the metal burden by 80, 28 and 71% for Pb, Zn and Cd, respectively.

However, it is not the total amount of metals removed that is most important, but the availability of those metals remaining in the soil matrix (Jelusic et al., 2013; Mulligan et al., 2001; Zhang et al., 2010a). We assessed the efficiency of bioavailability stripping with a sequential extraction test, TCLP and UBM.

Only sparse concentrations 0.3, 1.5 and 4.8% of Pb, Zn and Cd respectively, were found in the first and second fractions of the original soil (Table 2). This is a result of the long-term use of the garden soil, from which most of the labile forms had already been leached, absorbed by plants or more tightly bound to elemental soil structures. Even though present in small amounts, these metal forms still pose a potential threat, and EDTA washing aided by removing the Pb and Cd to below the limit of detection and by lowering Zn concentrations in the first two fractions. The original soil had a high carbonate content consequently bearing large amounts of metals on carbonate binding sites (Garcia-Delgado et al., 1996). Correspondingly most of the Cd and a large part of the Pb and Zn were found by sequential extraction to be associated with the third fraction — the fraction that under certain conditions is potentially available to organisms (Table 2). Washing lowered the metal concentrations to 14, 21 and 18% of the initial third fraction load for Pb, Zn and Cd, respectively. Together with the quick complexation of weakly complexed metals with the EDTA, the chelating agent also dissolved a portion of the soil Ca-carbonates by EDTA proton-promoted dissolution and thus indirectly released the carbonate bound metals. The data revealed approximately 15% less carbonates in the remediated soil. Fractions considered to hold a non-bioavailable pool of metals were also affected by the remediation process. EDTA promoted dissolution disturbed the fraction associated with Fe–Mn oxides and the already modest reducible fraction was thus lowered below the level of quantification for Cd and Pb and lowered for Zn (Table 2). The latter mechanism also aided in reducing metals from the oxidizable fifth fraction by disturbing the diminished organic matter content already mentioned. EDTA-washing was also able to extract a certain amount of Pb and Cd from the silicate matrix, as previously observed by Barona et al. (2001), implying that the metals were not very strongly fixed into the residual fraction. Zn, however, remained firmly anchored into the crystalline soil structures (Table 2).

EDTA washing not only removes the metal content but also has the potential to disturb the soil structure (Tsang et al., 2007; Vulava and Seaman, 2000). Furthermore a substantial amount of ligand is still left in the soil after the remediation procedure (Voglar and Lestan, 2012) and its impact on remaining metals and the soil itself over long periods has not yet been studied. Udovic and Lestan (2009) performed a remediation experiment with kindred soil and a similar remediation process and observed a significant increase of metals in exchangeable fractions after exposing remediated soil to artificial aging conditions. Our results indicate the opposite. After reintroducing the soil into garden beds and exposing it to various environmental factors, absolutely no trends were observed indicating metal shifts between fractions. All six fractions for Pb, Zn and Cd revealed no statistically significant changes between the

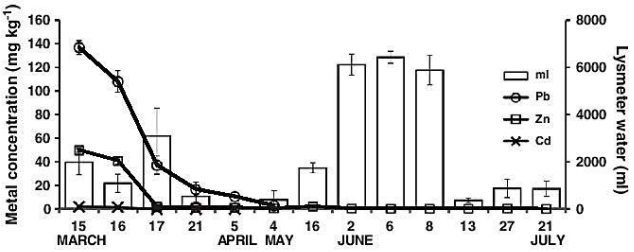


Fig. 4. Concentration of Pb, Zn and Cd (mg kg^{-1}) and amount of water in leachates (mL) from lysimeters set under the remediated garden bed during the field experiment. Error bars represent standard deviation (n = 3).

sample times of 0, 4 and 7 months, indicating no soil matrix disturbance due to metal fractionation and availability (Table 2).

4.2. Potential hazards of residual metals

As elucidated in the sequential analysis discussion, metals left in the soil after remediation remained predominantly in chemically stable forms bound to non-mobile fractions and were thus considered non-toxic. TCLP extraction in a way mirrors the second and third fractions of sequential analysis and through its one-step mild acidification indicates potentially available and leachable toxic metals in soils. The results of both analyses corresponded to fairly high levels of available Pb, Zn and Cd in the original soil, which were drastically lowered by remediation (Fig. 3). TCLP analysis also confirmed, what sequential analysis had indicated, that the impact of environmental exposure of soil to abiotic and biotic factors did not have any effect on the potential leaching of metals in remediated soil measured over time. The reduced leachability in the remediated soil thus remained unaltered throughout the 7 months of the experiment (Fig. 3).

Lysimeters set under the remediated soil trapped some measured metals only at the beginning of the field experiment and ceased by the end of mid-May for Pb and Cd and by the end of July for Zn (Fig. 4). Reasonably high metal concentrations in the first three leachates can be explained by the large amount of precipitation that fell in a relatively short time. The poorly permeable base of the garden bed prevented the precipitation from leaking into the ground and instead allowed the pouring waters from the entire surface to flow into the three lysimeters. The amount of water collected was thus higher than the amount of actual precipitation measured on the site. The metal concentrations measured in the three lysimeters actually related to the whole surface of the vegetable garden and were thus much higher. Nevertheless, the leaching of all metals did eventually cease despite further precipitation (Fig. 4).

The heavy precipitation presumably also resulted in a loss of stability of structural aggregates and, consequently, in a lower water holding capacity in the original soil after 7 months (Fig. 2). In the remediated soil, the lower water holding capacity could be attributed to the loss of soil structure due to the stringent conditions during soil extraction (Voglar and Lestan, 2012).

Other potential hazards of contaminated soils are accidental human ingestion and inhalation of soil dust and soil particles. The UBM method (unified bioaccessibility method) measures the fraction of a contaminant that is released from the soil matrix into solution by digestive fluids and thus represents the maximum amount of contaminant that is bioavailable, hence available for intestinal absorption (Wragg et al., 2011). It is also important that the absorption of nutrients (and toxic metals) takes place mainly in the small intestine (Ruby et al., 1996), so metal concentrations measured in the intestinal phase, give a better evaluation of potential risks of ingestion than the concentrations in the stomach phase.

Since all of the measured metals in the intestinal phase after remediation were under the limit of determination, it can be presumed that the remaining contaminants in soil were unlikely to be bio-available for humans through the gastro-intestinal tract (Table 3). In their lab scale study Udovic and Lestan (2009) also observed a decrease of Pb concentrations in both stomach and intestinal phases; however, after artificial aging, they observed a trend of increasing Pb bio-accessibility. In our field study, we observed no such trend for Pb, Zn or Cd. After seven

Table 3
Concentrations of Pb, Zn and Cd in stomach and intestinal phases of the UBM test in original and remediated soils measured 0, 4 and 7 months after the start of the experiment.

	Original soil (mg kg^{-1}) Sampling time (months)			Remediated soil (mg kg^{-1}) Sampling time (months)		
	0	4	7	0	4	7
Pb						
Stomach	1404 ± 10	1449 ± 40	1462 ± 42	262 ± 19	245 ± 2	262 ± 5
Intestine	9.5 ± 1.3	8.9 ± 0.6	8.9 ± 0.7	LOQ	LOQ	LOQ
Zn						
Stomach	338 ± 11	359 ± 15	354 ± 16	149 ± 13	148 ± 6	157 ± 7
Intestine	21 ± 5.6	28.1 ± 1.6	24.1 ± 0.5	LOQ	LOQ	LOQ
Cd						
Stomach	8.9 ± 0.5	9.4 ± 0.2	9.0 ± 0.1	1.7 ± 0.1	1.6 ± 0.0	1.7 ± 0.0
Intestine	6.1 ± 0.8	5.0 ± 0.3	4.8 ± 0.2	LOQ	LOQ	LOQ

Mean ± standard deviation (n = 3). No statistical significance was found between remediated soils or between original soils according to the Kruskal–Wallis test ($p < 0.05$). LOQ below the level of quantification.

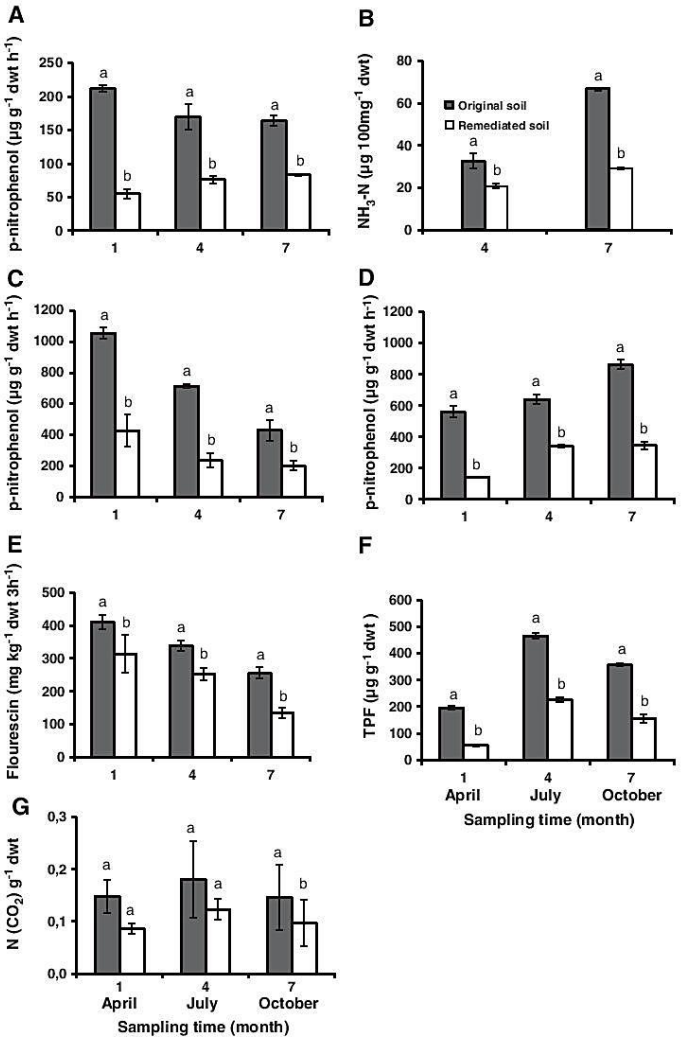


Fig. 5. β-Glucosidase (A), urease (B), acid and alkaline phosphatase (C, D), hydrolysis of fluorescein diacetate (E), dehydrogenase activity (F) and glucose-induced respiration of soil organisms (G) in original and remediated soils 1, 4 and 7 months after the beginning of the experiment, except for urease activity which we started measuring only in 4 month. Error bars represent standard deviation (n = 3–5). The letters "a" and "b" denote statistically different treatments according to the Student t-test ($p < 0.05$).

months of environmental exposure of the remediated soil, no changes in bio-accessibility for the stomach or intestinal phases were measured (Table 3).

4.3. Microbial and enzyme activity and soil functioning

Soil microbial characteristics through enzyme activity are being increasingly employed as indicators of soil quality owing to their rapid response, sensitivity and capacity to provide information that integrates many environmental factors (Mijangos et al., 2006). The origin of enzyme activity has not been identified, although it is generally agreed that the microbial component is the main source of enzymes in soils.

The index of active microbial biomass and a variety of enzyme tests were deployed to provide a thorough overview of both soils' biological properties. SIR measurements provide an approximation of soil microbial biomass. β-Glucosidases catalyze the hydrolysis of cellulose, being the rate-limiting enzymes in the microbial degradation of cellulose to glucose, with a critical role in C cycling (Baker et al., 2011). Phosphatases catalyze the hydrolysis of phosphate esters to release orthophosphate and play an important role in plant P nutrition. Ureases catalyze the hydrolysis of urea to CO_2 and NH_4^+

and play an important role in the N cycle. Hydrolysis of fluorescein diacetate includes the activity of several enzymes: lipases, esterases and proteases. Dehydrogenases are cell bound enzymes and are used as a measure of overall microbial activity (Alef and Nannipieri, 1995).

SIR and enzyme activities in both soils, remediated and original, have varied according to different seasons (Bastida et al., 2008), different plant covers and other environmental factors (Bastida et al., 2008; Wang et al., 2012).

Many studies of heavy metals and soil enzymes have been performed, all indicating the toxic effect of the measured metals on soil biology and biochemical processes and, consequently, inhibiting potential enzyme activities (Zhang et al., 2010b). However, in our study, remediated soil – soil with a reduced burden of potentially toxic metals – demonstrated lower potential enzyme activities (Fig. 5). The remediation process had obviously deprived the soil of certain metabolic activities to the extent that even after 7 months of plant cultivation and added fertilizers, the remediated soil could not restore itself.

One of the reasons may lie in the chemical nature of the non-specific chelating agent EDTA. EDTA is known to act as a non-specific chelating agent and is therefore capable of extracting a variety of metal ions from the soil matrix (Zhang et al., 2010a), some of them being macro- and micronutrients essential for microbial nutrition (Brock et al., 2003; Nannipieri et al., 2011). For example, micronutrient Zn plays a structural role in many enzymes (Brock et al., 2003) and, as TCLP and sequential analysis indicated (Fig. 3, Table 2), EDTA washing reduced its bioavailable forms, rendering it unavailable for some biochemical soil processes.

We also observed approximately four times lower concentrations of Mn (Part 2) in remediated soil. Mn(II)-oxidizing microorganisms are ubiquitous in soil and play an essential part in the biogeochemical cycling of manganese, iron, nitrogen, carbon, sulfur and several nutrients and trace metals (Tebo et al., 2005). Mn is used as a terminal electron acceptor for bacterial respiration; it is required as a trace nutrient for a large number of cellular functions; it can serve as an antioxidant and some bacteria even use it for protection from UV radiation, viral attack, predation or heavy metal toxicity (Archibald and Fridovich, 1981; Tebo et al., 2005).

Conflicting results were obtained by Udovic and Lestan (2012), since they observed a significant increase in some enzyme activities and SIR after relieving the soil of potentially toxic metals with 30 mM kg^{-1} EDTA. Epelde et al. (2008) observed that the application of EDTA on non-polluted soil significantly diminished dehydrogenase activity but had no effect on β-glucosidase or acid phosphatase potential activity. Furthermore EDTA had no effect on any of the enzyme activities when chelate was added to Pb-polluted soil. However, the relatively low concentrations of EDTA in the latter two studies were probably too small to extract the various metal cations needed for microbial activity. Additionally, the washing procedure had a negative impact on soil water holding capacity. Water availability is certainly one of the major factors affecting microbial activity in soil.

Hence, lacking nutrients and water, microorganism activity measured through enzyme activity was reduced despite the lowered burden of Pb, Zn and Cd and showed no trends of recovering even after 7 months of agricultural use.

The results of our study indicate that soil washing with 60 mM kg^{-1} EDTA efficiently removed Pb and Cd. Soil properties, except soil pH, organic matter and water holding capacity, remained unimpaired by the washing process. The toxicity status of all PTMs was significantly reduced and no shifts in relation to the human or environmental hazard of remediated soil were observed over 7 months. However, the remediation process did deprive the soil of some biological assets, presumably due to microelement shortage caused by the nature of the nonspecific chelating agent EDTA. The ultimate goal of any soil remediation process should be not only to remove contaminants and toxicity hazards from the polluted site but also to restore soil quality, i.e., the continued capacity of soil to perform or function according to its potential (Epelde et al., 2008). Some aspects of using remediated soil as a plant substrate have been studied and are presented in a follow-up paper.

Acknowledgments

This work was supported by the Slovenian Research Agency, Grant J4-3609.

References

Alef K, Nannipieri P. Methods in applied soil microbiology and biochemistry. 1st ed. London: Academic Press; 1995.
Archibald FS, Fridovich I. Manganese and defenses against oxygen toxicity in *Lactobacillus plantarum*. J Bacteriol 1981;145:442–51.
Baker LR, White PM, Pierzynski GM. Changes in microbial properties after manure, lime, and bentonite application to a heavy metal-contaminated mine waste. Appl Soil Ecol 2011;48:1–10. http://dx.doi.org/10.1016/j.apsoil.2011.02.007.
Barona A, Aranguiz I, Elías A. Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. Environ Pollut 2001;113:79–85. http://dx.doi.org/10.1016/S0269-7491(00)00158-5.
Bastida F, Barberá GG, García C, Hernández T. Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions. Appl Soil Ecol 2008;38:62–70. http://dx.doi.org/10.1016/j.apsoil.2007.09.002.
Brock TD, Madigan MT, Martinko JM, Parker J. Biology of microorganisms. 10th ed. London: Pearson Prentice Hall; 2003.
Dermont G, Bergeron M, Mercier G, Richer-Lafleche M. Metal-contaminated soils: remediation practices and treatment technologies. Pract Period Hazard Toxic Radioact Waste Manage 2008;12:188–209. http://dx.doi.org/10.1061/(ASCE)1090-025X(2008) 12:3(188).
R Development Core Team. A language and environment for statistical computing. Vienna, Austria: R foundation for statistical computing3-900051-07-0; 2010 [http://www.R-project.org].
Eivazi F, Tabatabai M. Glucosidases and galactosidases in soils. Soil Biol Biochem 1988;20:601–6. http://dx.doi.org/10.1016/0038-0717(88)90141-1.
Epelde L, Hernández-Allica J, Becerril JM, et al. Effects of chelates on plants and soil microbial community: comparison of EDTA and EDDS for lead phytoextraction. Sci Total Environ 2008;401:21–8. http://dx.doi.org/10.1016/j.scitotenv.2008.03.024.
European Commission. State of soil in Europe. Outlook 2012;80. http://dx.doi.org/10.2788/77361.
European Environment Agency. State of the environment No 1/2007 chapter 2. Office for official publications of the European communities; 2007.
Finzgar N, Lestan D. Multi-step leaching of Pb and Zn contaminated soils with EDTA. Chemosphere 2007;66:824–32. http://dx.doi.org/10.1016/j.chemosphere.2006.06.029.
García-Delgado RA, García-Herruzo F, Rodríguez-Maroto JM, Vereda C. Influence of soil carbonates in lead fixation. J Environ Sci Health A 1996;31:2099–109.
Gibbons S, Mourato S, Resende G. The amenity value of English nature: a hedonic price approach. London, UK: Spatial economics research centre (SERC), London School of Economics and Political Sciences; 2011.
Green VS, Stott DE, Diack M. Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. Soil Biol Biochem 2006;38:693–701. http://dx.doi.org/10.1016/j.soilbio.2005.06.020.
Hoffmann E, Teicher K. Ein kolorimetrisches verfahren zur bestimmung der ureaseaktivitat in boden. Z Pflanzenernaehr Dung und Bodenkd 1961;95:55–63.
ISO10390. Soil quality — determination of pH. Genève, Switzerland: International Organization for Standardization; 2005.
ISO11277. Soil quality — determination of particle size distribution in mineral soil material — method by sieving and sedimentation. Genève, Switzerland: International Organization for Standardization; 2009.
ISO11464. Soil quality — pretreatment of samples for physico-chemical analysis. Genève, Switzerland: International Organization for Standardization; 2006.
ISO13878. Soil quality — determination of total nitrogen content by dry combustion ("elemental analysis"). Genève, Switzerland: International Organization for Standardization; 1998.
ISO14235. Soil quality — determination of organic carbon by sulfochromic oxidation. Genève, Switzerland: International Organization for Standardization; 1998.
Jelusic M, Grcman H, Vodnik D, SuhadolKarc M, Lestan D. Functioning of metal contaminated garden soil after remediation. Environ Pollut 2013;174:63–70. http://dx.doi.org/10.1016/j.envpol.2012.10.027.
Kalra YP, Maynard DG. Methods manual for forest soil and plant analysis. Edmonton: Canadian forest service, Northern Forestry Centre; 1991.
Lestan D, Grcman H, Zupan M, Bacac N. Relationship of soil properties to fractionation of Pb and Zn in soil and their uptake into *Plantago lanceolata*. Soil Sediment Contam 2003;12:37–41. http://dx.doi.org/10.1080/713610986.
Martell AE, Smith RM. NIST critically selected stability constants of metal complexes7th ed. ; 2003 [Gaithersburg].
Mijangos I, Perez R, Albizu I, Garbisu C. Effects of fertilization and tillage on soil biological parameters. Enzyme Microb Technol 2006;40:100–6. http://dx.doi.org/10.1016/j.enzmictec.2005.10.043.

Mulligan CN, Yong RN, Gibbs BF. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng Geol 2001;60:193–207. http://dx.doi.org/10.1016/S0013-7952(00)00101-0.
Nannipieri P, Giagnoni L, Landi L, Renella G. Phosphorus in action. Soil Biol 2011;100:215–43. http://dx.doi.org/10.1007/978-3-642-15271-9. [chapter 9: role of phosphatase enzymes in soil].
Neale C, Bricka R, Chao A. Evaluating acids and chelating agents for removing heavy metals from contaminated soils. Environ Prog 1997;16:274–80. http://dx.doi.org/10.1002/ep.3300160416.
Nowack B, Sigg L. Dissolution of Fe(III) (hydr) oxides by metal–EDTA complexes. Geochim Cosmochim Acta 1997;61:951–63. http://dx.doi.org/10.1016/S0016-7037(96)00391-2.
Pociecha M, Lestan D. Recycling of EDTA solution after soil washing of Pb, Zn, Cd and As contaminated soil. Chemosphere 2012;86:843–6. http://dx.doi.org/10.1016/j.chemosphere.2011.11.004.
Ruby MV, Davis A, Schoof R, Eberle S, Sellstone CM, et al. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environ Sci Technol 1996;30:422–30. http://dx.doi.org/10.1021/es950057z.
Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1969;1:301–7. http://dx.doi.org/10.1016/0038-0717(69)90012-1.
Tebo BM, Johnson HA, McCarthy JK, Templeton AS. Geomicrobiology of manganese(II) oxidation. Trends Microbiol 2005;13:421–8. http://dx.doi.org/10.1016/j.tim.2005.07.009.
Thalmann A. Zur methodik der bestimmung der dehydrogenaseaktivität im boden mittels triphenyltetrazoliumchlorid (TTC). Landwirtsch Forsch 1968;21:249–59.
Tsang DCW, Zhang W, Lo IMC. Copper extraction effectiveness and soil dissolution issues of EDTA-flushing of artificially contaminated soils. Chemosphere 2007;68:234–43. http://dx.doi.org/10.1016/j.chemosphere.2007.01.022.
Udovic M, Lestan D. Pb, Zn and Cd mobility, availability and fractionation in aged soil remediated by EDTA leaching. Chemosphere 2009;74:1367–73. http://dx.doi.org/10.1016/j.chemosphere.2008.11.013.
Udovic M, Lestan D. Fractionation and bioavailability of Cu in soil remediated by EDTA leaching and processed by earthworms (*Lumbricus terrestris* L.). Environ Sci Pollut Res 2010;17:561–70. http://dx.doi.org/10.1007/s11356-009-0262-x.
Udovic M, Lestan D. EDTA and HCl leaching of calcareous and acidic soils polluted with potentially toxic metals: remediation efficiency and soil impact. Chemosphere 2012;88:718–24. http://dx.doi.org/10.1016/j.chemosphere.2012.04.040.
US Environmental Protection Agency. Test methods for evaluation of solid waste. Laboratory manual physical/chemical methods, 3rd ed, vol. IA. Washington, DC: Office of Solid Waste and Emergency Response (OSWER); 1995.
Voglar D, Lestan D. Pilot-scale washing of metal contaminated garden soil using EDTA. J Hazard Mater 2012;215–216:32–9. http://dx.doi.org/10.1016/j.jhazmat.2012.02.022.
Voglar D, Lestan D. Pilot-scale washing of Pb, Zn and Cd contaminated soil using EDTA and process water recycling. Chemosphere 2013;91:76–82. http://dx.doi.org/10.1016/j.chemosphere.2012.12.016.
Vulava VM, Seaman JC. Mobilization of lead from highly weathered porous material by extracting agents. Environ Sci Technol 2000;34:4828–34. http://dx.doi.org/10.1021/es001295j.
Wang B, Xue S, Liu GB, Zhang GH, Li G, Ren ZP, et al. Changes in soil nutrient and enzyme activities under different vegetations in the Loess Plateau area, Northwest China. Catena 2012;92:186–95. http://dx.doi.org/10.1016/j.catena.2011.12.004.
Wragg J, Cave M, Basta N, Brandon E, Casteel S, Deys S, et al. An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil. Sci Total Environ 2011;409:4016–30. http://dx.doi.org/10.1016/j.scitotenv.2011.05.019.
Zhang W, Huang H, Tan F, Wang H, Qui R. Influence of EDTA washing on the species and mobility of heavy metals residual in soils. J Hazard Mater 2010a;173:369–76. http://dx.doi.org/10.1016/j.jhazmat.2009.08.087.
Zhang FP, Li CF, Tong LG, Yue LX, Li P, Ciren YJ, et al. Response of microbial characteristics to heavy metal pollution of mining soils in central Tibet, China. Appl Soil Ecol 2010b;45:144–51. http://dx.doi.org/10.1016/j.apsoil.2010.03.006.
Zimakowska-Gnoinska D, Bech J. Estimation of the soil respiration using the constant pressure volumetric respirometer and the flow-through respirometer UNI-RES10. Environ Monit Assess 1999;64:583–90. http://dx.doi.org/10.1023/A:1006335812073.

2.1.3 Vpliv pranja z EDTA na onesnažena vrtna tla. Del II: ali remediirana tla lahko uporabimo kot rastlinski substrat?

JELUŠIČ Maša, VODNIK Dominik, MAČEK Irena, LEŠTAN Domen
Effect of EDTA washing of metal polluted garden soils. Part II: Can remediated soil be used as a plant substrate?
Science of the Total Environment, 2014, 475: 142-152

V poljskem poskusu smo na onesnaženih in z EDTA remediiranih tleh proučevali stanje rastlin, mikorizne združbe in možnosti ponovne uporabe remediiranih tal za rastlinski substrat. Dve eksperimentalni gredi velikosti 4 × 1 × 0,3 m smo napolnili z remediiranimi in originalnimi tlemi. Izbrane vrtnine smo kolobarili skozi obdobje 16 mesecev. Analizirali smo koncentracije Pb, Zn, Cd in mikronutrientov v rastlinah ter njihovo dostopnost z metodo DTPA. Splošno stanje rastlin smo ocenili s fluorescenco ter izmenjavo plinov in ocenili kolonizacijo korenin z mikoriznimi glivami. Remediacija je pri večini rastlin znižala koncentracije Pb in Cd v koreninah, zelenih delih in plodovih. Fitoakumulacija Zn se je znižala le v polovici analiziranih rastlin. Pri nekaterih rastlinah smo ugotovili pomakanje Mn. Koncentracije celokupnega Mn v remediiranih tleh so bile zmanjšane za 75 % prav tako pa tudi dostopnost za rastline naslednjih mikohranil: Cu za 54 %, Fe za 26 % in Mn za 79 %. Rastlinska biomasa na remediiranih tleh je bila manjša. Fotosintezni kazalci rastlin, ki so rasle na originalnih in remediiranih tleh, niso pokazali sprememb, razen poslabšanja pri špinači (*Spinacia oleracea* L.). Frekvenca mikorizne kolonizacije v koreninah graha (*Pisum sativum* L.) je bila petkrat manjša na remediiranih tleh. Pri koreninah čebule (*Allium cepa* L.) ni bilo opaženih nobenih sprememb. Vrtnine na remediiranih tleh so vsebovale koncentracije Pb pod zakonodajno mejo EU. Potrebni so še ukrepi za zmanjšanje koncentracij strupenih kovin v rastlinah in popolno revitalizacijo remediiranih tal.

Science of the Total Environment 475 (2014) 142–152

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effect of EDTA washing of metal polluted garden soils. Part II: Can remediated soil be used as a plant substrate?

Masa Jelusic [a], Dominik Vodnik [a], Irena Macek [a,b], Domen Lestan [a,*]

[a] Agronomy Department, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[b] FAMNIT, University of Primorska, Glagoljaška 8, 6000 Koper, Slovenia

HIGHLIGHTS

- Plots with Pb, Zn and Cd contaminated and EDTA remediated soils were constructed.
- Remediation reduced plant uptake of toxic metals from soil.
- The loss of bio-available micronutrients by remediation reduced plant biomass.
- Photosynthetic parameters indicated similar fitness of plants from remediated soil.
- The frequency of mycorrhizal fungal root colonization was reduced in remediated soil.

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 18 November 2013
Accepted 22 November 2013
Available online 14 December 2013

Keywords:
Remediated soil
Soil health
Metal plant uptake
Plant fitness
Mycorrhizae

ABSTRACT

In a field experiment on metal contaminated and EDTA-remediated soil we studied plant performance, mycorrhizal associations and prospects of potential re-use of remediated soil as a garden substrate. Two experimental plots of 4 × 1 × 0.3 m were filled, one with remediated and the other with original contaminated soil. Selected cultivars were rotated over the course of 16 months. Pb, Zn, Cd and micronutrient plant uptake was measured and their phytoaccessibility was analyzed by the DTPA method. Plant fitness was assessed by chlorophyll fluorescence and gas exchange measurements and evaluation of root colonization were analyzed with mycorrhizal fungi. Remediation reduced Pb and Cd concentrations in roots, green parts and fruits in most of the plants. Phytoaccumulation of Zn was reduced in one half of the cultivars. Some plants suffered from Mn deficiency as total soil Mn was reduced 4-fold and phytoaccessibility of micronutrients Cu, Fe and Mn for 54, 26 and 79%, respectively. Plant biomass was reduced. Photosynthetic parameters of plants grown in original and remediated soil were similar, except for the reduction in *Spinacia oleracea*. The frequency of mycorrhizal colonization in the roots of *Pisum sativum* was reduced five-fold and no significant changes were found in *Allium cepa* roots. Remediation reduced plant uptake of Pb below the concentration stipulated by legislation. Measures to reduce plant accumulation of other toxic metals and to revitalize remediated soil are needed.

1. Introduction

The consequences of living with harmful and potentially toxic metals (PTMs) in our living environment are not easy to comprehend, because they take an extended period to manifest themselves. Urban allotment gardens have recently been offered by local governments to encourage low income senior citizens to produce their own food (Tei et al., 2010) but many cities have inherited a long industrial history and an associated legacy of urban soil contamination (Wong et al., 2006). For example, 52% of crop samples from Berlin (Germany) inner city vegetable gardens exceeded EU standards (EC, 1881/2006) for Pb concentration in food crops (Säumel et al., 2012). Efficient soil remediation technologies are urgently needed.

In the preceding paper (Jelusic and Lestan, 2014), we applied our novel EDTA-based method (Pociecha and Lestan, 2012; Voglar and Lestan, 2012) on soil from a Pb, Zn and Cd contaminated vegetable garden in the Meža Valley, Slovenia. We demonstrated significant removal of all PTMs and effective bio-availability stripping of pollutants, which remained stable in non-labile forms throughout the duration of the field experiment. While we successfully reduced the hazard of contaminated soil to human health, the potential use of remediated soil remains to be investigated.

There is an extensive body of literature on EDTA and other chemical soil washing of PTMs; however, these remediation techniques all too

* Corresponding author at: Centre for Soil and Environmental Science, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia. Tel.: +386 1 320 31 62; fax: +386 1 256 57 82.
E-mail address: domen.lestan@bf.uni-lj.si (D. Lestan).

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.11.111

often concentrate only on metal removal efficiency or on residual metal bioavailability, and overlook the treated soil's overall health, functioning and potential use after remediation. Examples of efficient remediation technologies and scale-ups are also scarce. Furthermore, to the best of our knowledge, there are no papers in the scientific literature on using such remediated soil as a plant substrate in field conditions.

Along with the toxic metal remediation function, EDTA-extraction is also used as a method of assessing soil metal bioavailability; pollutants as well as micronutrients (Haq et al., 1980; Feng et al., 2005). We can therefore presume that, in EDTA-washed soil, most of the available forms of metals are extracted—removed from the soil matrix. Even though EDTA-remediated soil effectively lowers the human health risk and leaching hazard, could soil remediated in such a way – soil deprived of micronutrients and most of the microbial life (Jelusic and Lestan, 2014) – be suitable for plant cultivation or agricultural use? What measures, if any, are needed for revitalization?

For EDTA-treated soil to evolve into a substrate suitable for plant cultivation missing micronutrients need to be added. However, soil is a complex system and once unbalanced is hard to restore. Furthermore, plants with their roots system (rhizosphere zone) create a special microenvironment with physical and chemical properties distinct from bulk soil (Feng et al., 2005). The rhizosphere extends beyond the root systems through plant's symbiotic relationship with arbuscular mycorrhizal fungi, obligatory root endosymbionts and an ubiquitous functional group in soils that are estimated to colonize around two thirds of plant species (Fitter and Moyersoen, 1996), including most crop plants. Through acidification, chelating, oxidation–reduction reactions (Feng et al., 2005) and other processes roots and mycorrhizal fungi (Vodnik et al., 2008; Wang et al., 2012) can affect the speciation and transportation of metals into the plant and are thereby constantly changing the newly established soil equilibrium. The loss of bio-available micronutrients by the remediation process could thus be reduced but the toxic metal uptake risk could be greater (Sterckeman et al., 2005) than bulk soil availability tests indicate (Jelusic and Lestan, 2014).

This second part of the study focused on plant cultivation and the prospects of remediated soil being employed as an agricultural or garden substrate. In a four-crop rotation experiment, several plant species were cultivated and their metal and nutrient uptake, yield and general fitness through photosynthesis measurements and ability to form mychorrhizal associations, were analyzed.

2. Materials and methods

2.1. Soil remediation and field experiment

The contaminated soil used in this experiment was collected from the upper 30 cm layer of a managed vegetable garden near the abandoned lead smelter in the Meža Valley, Slovenia (x = 489,300 m and y = 152,300 m, Gauβ–Krüger coordinate system). The original contaminated soil was remediated by chelant washing as reported by (Voglar and Lestan, 2012), using 2 h extraction of 65 kg (air dried) soil batches with 60 mmol EDTA per kg of soil in a rotary mixer. The soil to washing solution weight ratio was 1:1. Extracted soil was separated from the soil washing solution in a filter chamber press and in a press rinsed with fresh water to remove EDTA-mobilized metallic species. Used washing and rinsing solutions were treated by an electrochemical advanced oxidation process using a graphite anode to degrade EDTA oxidatively and to electro-precipitate PTMs onto a stainless-steel cathode before cleansed waste water was discharged.

Two experimental plots on raised garden beds 4 m × 1 m × 0.3 m were filled with EDTA-remediated and original soil up to 0.15 m in height (Fig. 1). The garden beds were exposed to environmental conditions from April 2011 to September 2012. The ten year average temperature for the location of the field experiment with the continental climate was 10.9 °C, with a peak in July (21 °C) and a minimum in January (0.8 °C) and total year precipitation of 1350 mm.

2.2. Soil sampling and properties

Original and remediated soils were sampled for analyses immediately after garden beds were filled-up, before fertilization and cultivation, in April 2011 (0 months). Further samples were taken 4 and 7 months after the first sampling. Each soil sampling was conducted in three replicates. One replicate consisted of thoroughly mixed ten subsamples collected from randomly selected points (depth 5–10 cm).

For metal determination air-dried samples of the 2 mm soil fraction (1 g) were ground in an agate mill, sieved to 250 μm and digested in *aqua regia* solution, consisting of HCl and HNO_3 in a 3:1 ratio (v/v). Samples were then filtered through Whatman no. 4 filter paper and diluted with deionized water to a total 50 mL volume. Reference material used in inter-laboratory comparisons was WEPAL 2003 and 2004 (Wageningen University, Wageningen, Netherlands) Flame (acetylene/air) atomic absorption spectrometry (AAS, Varian AA240FS) was used for the metal analysis.

For soil analyses, samples were air-dried and sieved to 2 mm (ISO11464 2006). Soil pH was measured in a 1/2.5 (w/v) ratio of soil and 0.01 M $CaCl_2$ suspension (ISO10390 2006). Soil samples were analyzed for organic matter and organic carbon by modified Walkley–Black titrations (ISO14235 1998), total nitrogen was determined after dry combustion (ISO13878 1998) and C/N was calculated from the organic carbon and total nitrogen ratio. Cation exchange capacity (CEC), as the sum of base cations, was measured after soil extraction with ammonium acetate (pH 7) and soil texture was analyzed by the pipette method (ISO11277 2009). Easily extractable P (P_2O_5) and K (K_2O) were measured colorimetrically according to the Kalra and Maynard, 1991.

Original and remediated soils demonstrated the following properties: pH 7.1 and 7.4, 400 and 330 mg kg^{-1} P_2O_5, 348 and 53 mg kg^{-1} K_2O, 21.4 and 20.4 CEC ($mmol_c$ 100 g^{-1}), 6.5% and 4.9% of organic matter and 9.7 and 9.7 C/N respectively. Average metal concentrations for Pb, Zn, Cd, Mn, Cu and Fe were 1585, 525, 8.8, 195, 21.2 and 7949 for original soil and 313, 378, 2.5, 58, 17.3 and 8226 for remediated soil mg kg^{-1} of dry soil, respectively.

2.3. Crop rotation, sampling and fertilization

The first crop rotation (April 2011) was represented by onion (*Allium cepa* L.), pea (*Pisum sativum* L.), spinach (*Spinacia oleracea* L.) and cauliflower (*Brassica oleracea* L., Botrytis), the second rotation (August 2011) by spinach and Chinese cabbage (*Brassica rapa* L., Pekinensis), the third rotation (April 2012) by carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.) and spinach and the fourth rotation (August 2012) by basil (*Ocimum basilicum* L.) and bell pepper (*Capsicum annuum* L.) (Table 1). Selected plant species were planted in randomly placed rows throughout the two garden beds (Fig. 1) where each vegetable was represented by three to five rows. Seeds and seedlings (treated in Klassman Deilman substrates) were planted directly in remediated and original soils. All the plants harvested from a single row represented one replicate for metal analysis and yield mass analysis where values were re-calculated for 1 m^2 (Table 1).

Both treatments were fertilized in April and in August 2011, 2012 with 300 kg ha^{-1} K as KNO_3 and 120 kg ha^{-1} N as NH_4NO_3 (Acros Organics, New Jersey, USA) and in April 2012 additionally with 50 t/ha of five year old dry bovine farm-yard manure from a near farm (pH 6.0, 279 mg kg^{-1} K_2O, 718 mg kg^{-1} P_2O_5, C/N 12.5 and 30 mg kg^{-1} Pb, 198 mg kg^{-1} Zn, 912 mg kg^{-1} Mn, 12580 mg kg^{-1} Fe, 47 mg kg^{-1} Cu and below the level of quantification of Cd).

Due to underdeveloped first rotation spinach plants, grown in remediated soil, rows with this vegetable were additionally fertilized three times with 50 kg ha^{-1} Mg as MgO (Merck, Germany). Since the amendment did not have any effect, additional fertilizer in the form of 15 kg ha^{-1} Mn as $MnSO_4$ (Merck, Germany) was added just a week before harvesting. The garden beds were mixed afterwards to ensure

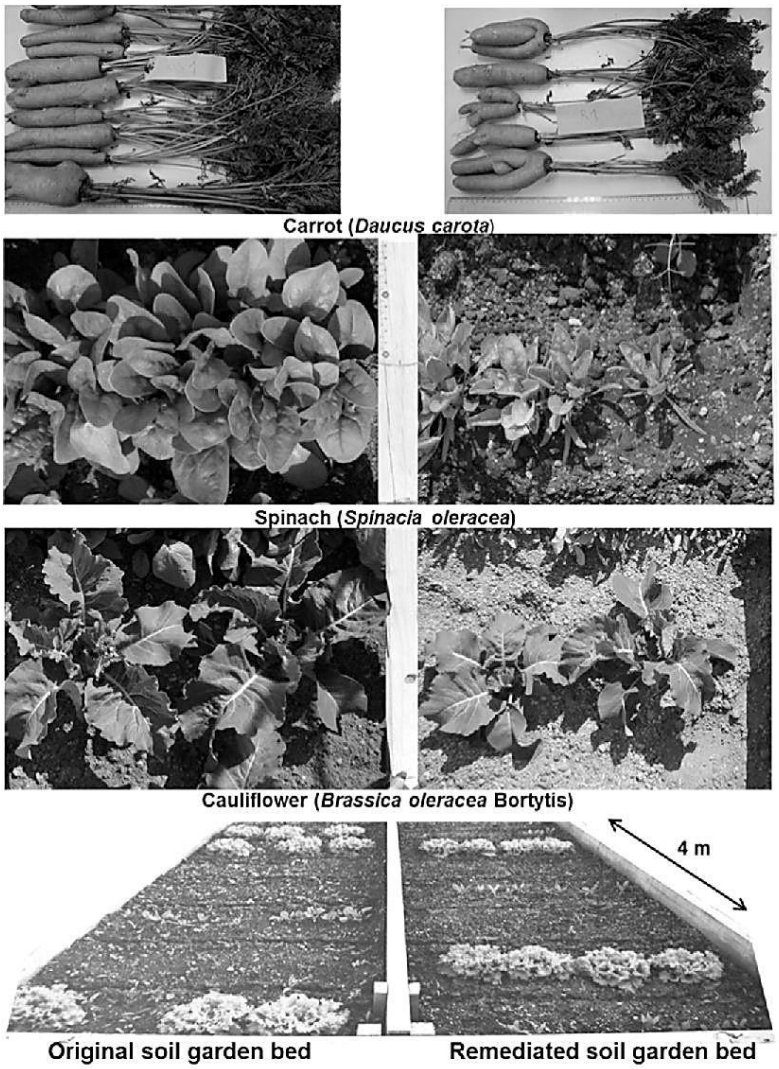


Fig. 1. Original and remediated garden bed. Shown on the left side of the figure are the plants grown on original soil and on the right side plants grown on remediated soil. Up to down: carrot (*Daucus carota* L.), spinach (*Spinacia oleracea* L.), cauliflower (*Brassica oleracea* L., Botrytis) and the beginning third rotation with lettuce (*Lactuca sativa* L.).

equal Mg and Mn distributions in all rows for the next cultivars. As symptoms were later recognized as possible Mn deficiency in August 2011, for the second crop rotation of spinach and Chinese cabbage, one half of the remediated garden bed was, additionally fertilized with 20 kg ha^{-1} Mn before planting. Three spinach rows and three Chinese cabbage rows were thus additionally fertilized with Mn and three spinach and Chinese cabbage rows were not fertilized with Mn. In April 2012, for the third crop rotation the whole remediated garden bed was again thoroughly mixed and fertilized whole with 20 kg ha^{-1} Mn.

2.4. Plant metal determination

Plants were harvested and weighed for fresh weight after the roots were separated. Except for carrot where taproot was not detached for yield weight. Leaves and fruits were then separated and thoroughly washed with deionized water. Samples were dried at 60 °C to a constant weight and ground in a titanium centrifugal mill. Ground plant tissues (250–300 mg dry weight) were then submitted to acid-digestion (65% HNO_3) with microwave heating and left to cool. Diluted with deionized water to a volume of 10 or 25 ml, they were stored at 4 °C until metal

Table 1
Cultivation manner and mean ± standard deviation of yield mass of cultivated plants: pea (*Pisum sativum* L.), cauliflower (*Brassica oleracea* L., Botrytis), onion (*Allium cepa* L.), spinach I, II and III (*Spinacia oleracea* L.), Chinese cabbage (*Brassica rapa* L., Pekinensis), lettuce (*Lactuca sativa* L.), carrot (*Daucus carota* L.), basil (*Ocimum basilicum* L.) and bell pepper (*Capsicum annuum* L.).

	Planted (m^2)	Crop rotation	Days grown	Original soil fresh weight (g/m^2)	Remediated soil fresh weight (g/m^2)
Pea	75 seeds	I	72	860 ± 116[a]	680 ± 132[b]
Cauliflower	6–9 seedlings	I	64	9428 ± 1361[a]	5844 ± 1669[b]
Onion	36 seedlings	I	94	1403 ± 233[a]	1111 ± 235[b]
Spinach	2.7 g seed	I	64	1742 ± 229[a]	189 ± 9[a]
Spinach	2.7 g seed	II	46	334 ± 40[a]	158 ± 5[a]
Spinach[Mn]	2.7 g seed	II	46	NA	242 ± 31
Chinese cabbage	3–9 seedlings	II	46	9731 ± 1820[a]	8622 ± 1919[a]
Chinese cabbage[Mn]	3–9 seedlings	II	46	NA	9296 ± 219
Spinach	2.7 g seed	III	64	1877 ± 83[a]	282 ± 193[b]
Letucce	9–12 seedlings	III	75	4313 ± 553[a]	4151 ± 863[a]
Carrot	45 seeds	III	159	8305 ± 2130[a]	3762 ± 1514[b]
Bazil	12 seedlings	IV	102	2000 ± 140[a]	1562 ± 148[b]
Pepper	12 seedlings	IV	102	3685 ± 461[a]	2330 ± 405[b]

[a,b] Denote statistically different treatments (original and remediated) according to the Student *t*-test.
NA not applicable.
[Mn] Data for spinach and Chinese cabbage grown on remediated soil's additional treatment with added Mn fertilizer.

analysis. The metals Pb, Zn, Cd and also Mn, Fe and Cu were analyzed by flame (acetylene/air) atomic absorption spectrometry (AAS, Varian AA240FS). Reference material used in inter-laboratory comparisons Alva 2009/3 (Pflanzen und Futtermittelenquete 2009, Raumberg-Gumpenstein) was used in digestion and analysis. The limits of quantification (LOQ) as given by the manufacturer were 0.1, 0.01, 0.02, 0.03, 0.06 and 0.02 mg L^{-1} for Zn, Cd, Pb, Cu, Fe and Mn respectively. All samples were measured in triplicate (one row, one replicate), reagent blank and analytical duplicates were used to ensure the accuracy and precision of the analysis.

2.5. Plant metal accessibility

Metal accessibility for plants was assessed with diethylenetriamine pentaacetic acid (DTPA) extraction of remediated and original soil. The test was originally designed to assess plant accessible Zn, Fe, Mn and Cu in near-neutral and calcareous soils (Lindsay and Norvell, 1978), and later adopted to assess metal phyto-accessibility and ecotoxicity (Conder et al., 2001; Cheng and Wong, 2002). The DTPA extraction solution was prepared by mixing 0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M (triethanolamine) TEA and the solution adjusted to pH 7.30 ± 0.05. Samples of soil (5 g) were sieved through a 2 mm mesh, 10 mL of DTPA solution was poured over and the mixture shaken for 2 h on a horizontal shaker at about 120 cycles min^{-1}. The samples were filtered through Whatman no. 4 filter paper and analyzed for Pb, Zn, Cd. Fe, Cu and Mn contents. The extractions were conducted in triplicate.

2.6. Calculation of metal transfer factors

Three transfer factors were calculated for each element and each vegetable crop: TF_{RS}—from soil to roots, TF_{ES}—from soil to edible parts, and TF_{GR}—from roots to green parts such as stem and leaves. Transfer factors were calculated as follows:

$$TF_{RS} = \frac{C_{roots}}{C_{soil}}$$

$$TF_{ES} = \frac{C_{edible\ parts}}{C_{soil}}$$

$$TF_{GR} = \frac{C_{green\ parts}}{C_{soil}}$$

C_{roots}, $C_{edible\ parts}$ and $C_{green\ parts}$ represent total metal concentrations in roots, edible parts and green parts of plants in mg of metal per dry plant weight, respectively. Edible parts were: peas—seedpod of the pod fruit, onion—underground bulb, cauliflower—white curd, spinach, Chinese cabbage and lettuce—leaves, carrot—orange taproot, basil—leaves and bell pepper–pepper fruits. $C_{edible\ parts}$ for each species was categorized as follows: roots for carrot, stem/leaves for spinach, Chinese cabbage and lettuce; fruits for peas, onion, cauliflower, basil leaves and pepper (Table 2). C_{soil} represents the average total metal concentrations from soil samples taken on the 0, 4 and 7 months of the experiment, since the total metal concentrations did not significantly change during experiment.

2.7. Gas exchange and fluorescence measurements

Gas exchange measurements were made on pea, spinach and cauliflower 50 days after planting by using an LI-6400xt measuring system combined with a 6400-40 leaf chamber fluorometer equipped with a LED light source and CO_2 mixer (Li-Cor, Lincoln, NE, USA). Ten plants per treatment were included in the measurements and the first fully developed leaf was measured on each. Chamber conditions were maintained at 380 μmol CO_2 mol^{-1}, ambient humidity (ca. 70%) and saturating PAR intensity of 1500 μmol m^{-2} s^{-1}. Chamber temperature was regulated by average ambient temperature during the measurement (air temperature of 27 °C). Gas exchange data were registered when steady-state conditions were achieved, at the same as time steady-state fluorescence was captured (F_s). Immediately thereafter, a saturating light flash was applied over the same, light adapted leaf area and maximum fluorescence (F_m') was recorded. Minimum fluorescence (F_0') was captured after the leaf had been momentarily darkened. Photochemical efficiency, i.e., the efficiency of energy harvesting by oxidized (open) PSII reaction centers in the light, was calculated as $F_v'/F_m' = F_m' - F_0'/F_m'$. The electron transport rate (*ETR*) was calculated as $ETR = ((F_m' - F_s)/F_m') \cdot f \cdot I \cdot \alpha_{leaf}$, where f is the fraction of absorbed quanta used by PSII, I is the incident photon flux density and α_{leaf} is the leaf absorbance.

2.8. Root colonization with arbuscular mycorrhizal fungi

Sixty-three days after planting, the roots of five plants of onion and pea (first rotation), for original and remediated soils, were washed and stored in 70% ethanol. The roots were cleaned with hot 10% KOH and acidified with 1N HCl. The arbuscular mycorrhizal fungal tissue inside the roots was stained with 0.05% trypan blue in lactoglycerol. The arbuscular mycorrhizal fungal root colonization was assessed following Trouvelot et al. (1986), using an Olympus Provis AX70 microscope (n = 30, one cm long root fragments for each collected root sample). The computer program Mycocalc (Mycocalc, 2013) was used to calculate

Table 2
Concentrations of Pb, Zn and Cd in roots, shoots and fruits of pea (*Pisum sativum* L.), cauliflower (*Brassica oleracea* L., Botrytis), onion (*Allium cepa* L.), spinach I, II and III (*Spinacia oleracea* L.), Chinese cabbage (*Brassica rapa* L., Pekinensis), lettuce (*Lactuca sativa* L.), carrot (*Daucus carota* L.), basil (*Ocimum basilicum* L.) and bell pepper (*Capsicum annuum* L.) cultivated on EDTA remediated and original soil. Data printed on the gray background represent edible parts of each vegetable and printed bold within are the concentrations that exceed the values set by the EU union.

	Roots (mg kg^{-1})		Stem/leaves (mg kg^{-1})		Fruits (mg kg^{-1})	
Lead	Originial	Remediated	Originial	Remediated	Originial	Remediated
Peas I	^{a}151±21	^{b}15.6±1.6	5.3±0.6	LOQ	LOQ	LOQ
Onion I	^{a}231±30	^{b}26.3±4.0	3.8±1.3	LOQ	LOQ	LOQ
cauliflower I	^{a}45.0±4.7	^{b}5.8±1.0	LOQ	LOQ	LOQ	LOQ
spinach I	^{a}57.8±4.0	^{b}13.1±0.5	LOQ	1.5±0.3	NA	NA
spinach II	^{a}152±19	^{b}24.0±1.5	**13.5±1.4**	LOQ	NA	NA
Cabbage II	^{a}79.3±16.0	^{b}7.1±2.1	**6.6±1.1**	LOQ	NA	NA
Spinach III	^{a}54.8±3.6	^{b}13.3±2.3	**^{a}4.4±1.2**	^{a}2.6±1.5	NA	NA
Lettuce III	^{a}54.1±3.4	^{b}4.5±1.2	**7.8±2.5**	LOQ	NA	NA
Carrot III	**5.5±2.1**	LOQ	6.7±1.5	LOQ	NA	NA
Basil IV	^{a}133±29	^{b}14.8±11.1	6.5±0.5	LOQ	1.5±0.2	LOQ
Pepper IV	^{a}76.8±19.5	^{b}7.2±1.0	^{a}12.9±4.3	^{b}2.0±0.7	LOQ	LOQ
Zinc						
Peas I	^{a}247±32	^{b}181±3	^{a}109±8	^{b}71.8±5.3	^{a}56.7±3.4	^{a}61.5±2.9
Onion I	^{a}287±37	^{b}113±8	^{a}27.6±6.9	^{b}10.9±0.8	^{a}42.7±1.9	^{b}16.5±2.3
cauliflower I	^{a}104±6	^{b}44.9±4.6	^{a}71.2±5.7	^{b}24.9±3.2	^{a}40.8±5.8	^{a}28.8±6.8
spinach I	^{a}95.1±4.4	^{a}92.0±2.3	^{a}264.4±2.3	^{b}143.4±1.8	NA	NA
spinach II	^{a}77.4±12.0	^{a}77.5±14	^{a}111.4±6.4	^{b}74.3±0.6	NA	NA
Cabbage II	^{a}79.0±8.6	^{b}48.8±8.9	^{a}65.9±5.7	^{b}31.5±4.4	NA	NA
Spinach III	^{a}82.6±9.4	^{a}66.5±0.1	^{a}137±20	^{a}89.2±16.7	NA	NA
Letucce III	^{a}46.5±1.8	^{a}46.4±4.1	^{a}83.8±3.2	^{b}61.7±3.3	NA	NA
Carrot III	^{a}37.0±4.3	^{a}32.7±0.1	^{a}64.0±4.9	^{a}71.7±20.3	NA	NA
Basil IV	^{a}68.6±8.4	^{a}50.1±12.7	^{a}48.7±6.8	^{a}38.3±10.0	^{a}122±22	^{a}100±15
Pepper IV	^{a}136±36	^{b}51.3±13.2	^{a}219±42	^{a}196±24	^{a}34.5±2.3	^{a}31.7±1.3
Cadmium						
Peas I	^{a}8.4±2.3	^{b}2.9±0.7	1.1±0.1	LOQ	**0.7±0.0**	LOQ
Onion I	14.6±3.2	2.5*	2.1±0.7	LOQ	LOQ	**1.3±0.0**
Cauliflower I	1.4±0.1	LOQ	LOQ	LOQ	**0.6±0.0**	LOQ
spinach I	6.8*	*	^{a}1.4±0.1	**^{b}5.2±0.4**	NA	NA
spinach II	^{a}6.1±1.3	^{b}2.8±0.6	**^{a}9.3±2.8**	**^{b}2.8±0.4**	NA	NA
Cabbage II	1.8±0.2	1*	^{a}1.4±0.2	^{b}0.7±0.1	NA	NA
Spinach III	^{a}2.9±0.3	^{b}1.6±0.2	**^{a}4.7±0.4**	^{b}1.6±0.2	NA	NA
Letucce III	^{a}2.1±0.2	^{b}0.6±0.1	^{a}3.4±0.4	^{b}1.2±0.1	NA	NA
Carrot III	**^{a}1.6±0.4**	^{b}0.6±0.0	^{a}1.5±0.2	^{b}0.7±0.0	NA	NA
Basil IV	^{a}3.5±0.7	^{b}0.8±0.1	^{a}2.2±0.6	^{b}0.4±0.0	^{a}1.4±0.2	^{b}0.3±0.0
Pepper IV	^{a}10.4±2.9	^{b}1.7±0.1	^{a}13.4±3.3	^{b}4.7±0.6	**^{a}1.9±0.1**	**^{b}0.7±0.1**

Mean ± standard deviation (n = 3).
* Asterisk denotes data for which only one or no repetitions were analyzed, due to lack of plant material.
^{a,b} Denote statistically different treatments (original and remediated) according to the Student *t*-test.
LOQ denotes values below the level of quantification.
NA not applicable.

the root mycorrhization parameters (F—frequency of mycorrhiza in the root system, M—intensity of the mycorrhizal colonization in the root system, and a—arbuscule abundance in mycorrhizal parts of root fragments).

2.9. Statistical analysis

Student's *t*-test was applied to assess the differences between plant metal concentrations, their transfer factors, yields and photosynthetic

parameters of plant parts grown on remediated and original soil. *t*-Test was also applied and to compare the means of arbuscular mycorrhizal fungal root colonization parameters between the different soil types and for different plant species. The non-parametric Kruskal–Wallis test was applied to test the differences between bioavailable metal concentrations between time periods in remediated soils for DTPA. The non-parametric test was used since data transformation was not able to display equal variances between samples for calculating the ANOVA (analysis of variance) and possible further multiple range tests. Statistical analysis was done with R program (R Development Core Team, 2010). Differences between treatments were considered significant at $P < 0.05$.

3. Results

Vegetables grown in original soil exhibited greater and statistically different yields than vegetables grown in remediated soils (Table 1) (Fig. 1). The most evident difference was observed with spinach crops, in which the first rotation plants in remediated soil demonstrated symptoms of chlorosis and diminished growth and, consequently, a 10-fold lower yield than spinach plants in original soil.

3.1. Toxic metal uptake and translocation

Concentrations of Pb, Zn and Cd measured in plants grown in original polluted soil were in most cases statistically significantly higher than in plants grown in remediated soil (Table 2). Exceptions were observed in the first rotation in underdeveloped spinach leaves, in which the concentrations of Pb and Cd in plants grown in remediated soil significantly exceeded those found in plants grown in original soil. Higher values for Cd in plants grown in remediated soil were also found in onion fruits. The essential element Zn was distributed more uniformly, with no significant differences between remediated and original soils in the roots of spinach I, II, III, lettuce, carrot and basil; and in the green parts of spinach III, carrot, basil and pepper and in the fruits of peas, cauliflower, basil and pepper (Table 2).

Concentrations of Pb, Zn and Cd were generally higher in roots than in fruits and green parts, with some exceptions. Carrot plants grown in original and remediated soils for example demonstrated similar values for Pb and Cd in roots and green parts and higher values in green parts compared to roots for Zn in both treatments. Lettuce and pepper from both treatments also accumulated more Cd and Zn in their upper parts than in roots. The same favorable transition from roots to green parts was observed for Zn in all spinach rotations and treatments, and for Cd in the first and second spinach rotations grown in original soil. Spinach I and II grown in remediated soil demonstrated similar concentrations of Cd in roots and green parts of plants.

To examine the discussed metal transition from soil to roots, green parts and edible parts, three transfer factors were calculated for each element and each vegetable crop (Table 3). Pb had the lowest transfer factors of all analyzed metals, ranging from 0.001 to 0.008 for TF_{ED}, 0.004–0.15 for TF_{RS} and from 0.02 to 0.28 in TF_{GR}. Pb transfer factors calculated for plants grown in remediated soil were generally statistically significantly lower than those from original soil, although the differences between treatments were small. No measurable Pb concentrations were detected in edible parts of vegetables grown in remediated soil, so the TF_{ES} for remediated soil was zero, except for the first and the third spinach crops.

The highest transfer factors were observed with Zn TF_{GR}. As mentioned above, several plants accumulated higher concentrations of Zn in their green parts than in their roots. Otherwise, no specific trend for Zn factors was observed. Also high were the transfer factors from soil to plant tissue for Cd, up to 1.98. Cd TF_{GR} tended to decrease after remediation and Cd TF_{ES} tended to increase after remediation although the differences were not always statistically significant (Table 3).

Table 3
Pb, Zn and Cd transfer factors calculated for pea (*Pisum sativum* L.), cauliflower (*Brassica oleracea* L., Botrytis), onion (*Allium cepa* L.), spinach I, II and III (*Spinacia oleracea* L.), Chinese cabbage (*Brassica rapa* L., Pekinensis), lettuce (*Lactuca sativa* L.), carrot (*Daucus carota* L.), basil (*Ocimum basilicum* L.) and bell pepper (*Capsicum annuum* L.) cultivated on EDTA remediated and original soil.

	TF_{RS} (roots/soil)		TF_{ES} (edible parts/soil)		TF_{GR} (green parts/roots)	
Lead	Original	Remediated	Original	remediated	Original	remediated
Peas I	0.10^a	0.05^b	0	0	0.04	0
Onion I	0.15^a	0.09^b	0	0	0.02	0
Cauliflower I	0.03^a	0.01^b	0	0	0	0
Spinach I	0.04^a	0.04^b	0	0.01	0	0.12
Spinach II	0.1^a	0.08^a	0.01^a	0	0.09	0
Cabbage II	0.05^a	0.02^b	0.002^a	0	0.05	0
Spinach III	0.04^a	0.04^a	0.003^a	0.008^a	0.09^a	0.21^b
Lettuce III	0.04^a	0.01^b	0.005^a	0	0.14	0
Carrot III	0.004^a	0	0.003^a	0	1.37	–
Bazil IV	0.09^a	0.07^a	0.004^a	0	0.01	0
Pepper IV	0.05^a	0.02^b	0	0	0.17^a	0.28^a
Zinc						
Peas I	0.47^a	0.49^a	0.11^a	0.16^b	0.44^a	0.40^a
Onion I	0.54^a	0.30^b	0.08^a	0.04^b	0.1^a	0.1^a
Cauliflower I	0.20^a	0.12^a	0.08^a	0.08^a	0.68^a	0.55^a
Spinach I	0.18^a	0.25^b	0.50^a	0.38^b	2.78^a	1.56^b
Spinach II	0.15^a	0.21^b	0.21^a	0.20^a	1.44^a	0.96^b
Cabbage II	0.16^a	0.13^a	0.12^a	0.08^b	0.84^a	0.65^a
Spinach III	0.16^a	0.18^a	0.26^a	0.24^a	1.65^a	1.34^a
Letucce III	0.09^a	0.12^b	0.16^a	0.17^a	1.8^a	1.35^a
Carrot III	0.07^a	0.09^a	0.07^a	0.09^a	1.73^a	2.26^a
Bazil IV	0.13^a	0.13^a	0.23^a	0.27^a	0.71^a	0.79^a
Pepper IV	0.26^a	0.14^a	0.07^a	0.09^b	1.70^a	3.9^b
Cadmium						
Peas I	0.96^a	1.12^a	0.08^a	0	0.14^a	0
Onion I	1.67^a	0.96^a	0	0.51	0.16^a	0
Cauliflower I	0.16^a	0	0.07^a	0	0	0
Spinach I	0.77	–	0.16^a	$^b1.98$	0.21^a	–
Spinach II	0.70^a	1.14^b	1.06^a	$^a1.07$	1.53^a	0.99^b
Cabbage II	0.20^a	0.38^b	0.16^a	$^b0.28$	0.76^a	0.75^a
Spinach III	0.33^a	0.64^b	0.53^a	$^a0.63$	1.62^a	1.03^b
Letucce III	0.24^a	0.23^a	0.39^a	$^a0.45$	1.58^a	2.00^b
Carrot III	0.18^a	0.25^a	0.18^a	$^a0.25$	1.04^a	1.02^a
Bazil IV	0.40^a	0.32^a	0.15^a	$^a0.13$	0.62^a	0.51^a
Pepper IV	1.18^a	0.67^a	0.22^a	$^a0.27$	1.34^a	2.68^b

^{a,b} Denote statistically different treatments (original and remediated) according to the Student *t*-test.

3.2. Disturbance of plant nutrient uptake and plant fitness

The extraction of metals with DTPA test confirmed the high value of plant accessible fractions of Pb, Zn and Cd in original soil, which remediation successfully lowered (Fig. 2). Metals accessible for plant uptake were, based on the results, reduced for 83, 96 and 93% by Pb, Zn and Cd, respectively. There was also lower phyto-accessibility for three essential elements: Fe, Cu and Mn. The reduced phyto-accessibility of the measured elements for remediated soil remained unaltered during the 7 months of performing the DTPA test and was thus consistent with other availability tests presented in Part I (Jelusic and Lestan, 2014) of this study.

For selected plants in the first crop rotation, essential metal (Mn, Cu and Fe) concentrations were measured. Manganese concentrations in the leaves, roots and pods of peas were significantly greater in plants grown in original soil than in those cultivated in remediated soil (Fig. 3). In spinach plants, the values were reversed; higher concentrations of Mn were found in recently Mn-fertilized remediated spinach plants (Fig. 4). Concentrations of Fe in spinach leaves and pea roots and concentrations of Cu in spinach leaves and pea green parts were also greater for remediated soil grown plants. No significant differences between treatments were found in Fe concentrations in green parts and pods of pea plants or for Cu in pea roots and pods.

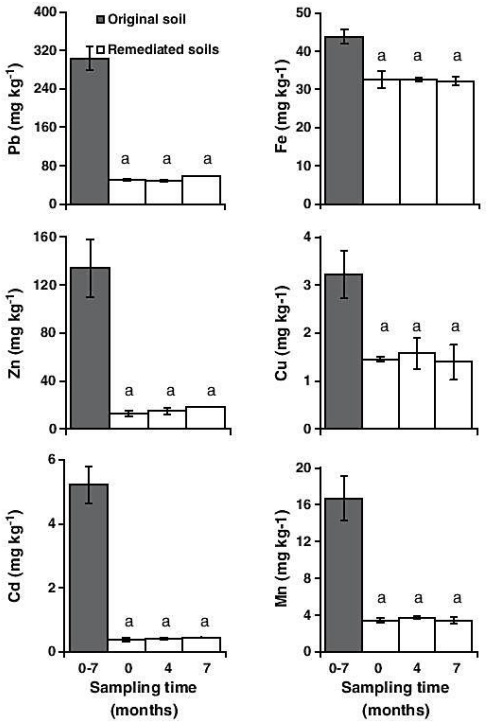


Fig. 2. Pb, Zn, Cd, Fe, Cu and Mn plant availability of original and remediated soils assessed by DTPA extraction. For the original soil, the average is calculated from 0, 4 and 7 month samples. Error bars represent standard deviation (n = 9 for original soil, n = 3 for remediated soils). The letter "a" denotes statistical significance between remediated soils according to Kruskal–Wallis test ($p < 0.05$).

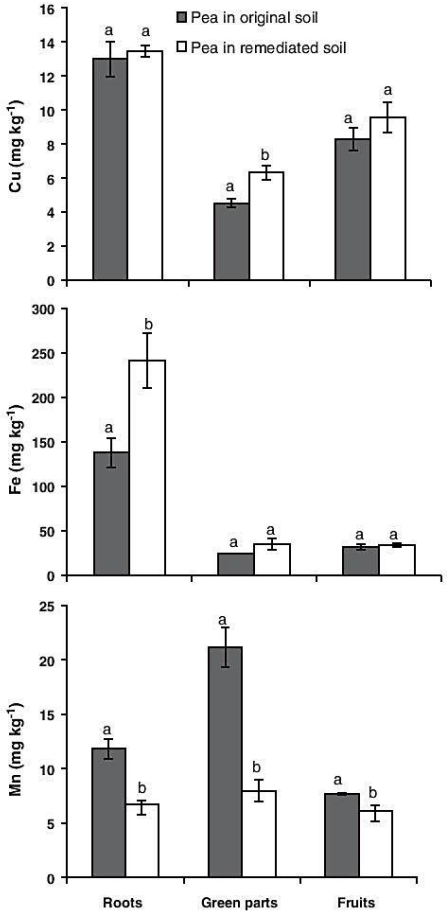


Fig. 3. Concentrations of micronutrients Cu, Fe and Mn (mg kg^{-1} per dry plant weight) measured in roots, green parts and fruits of peas (*Pisum sativum* L.). Error bars represent standard deviation (n = 3). The letters "a" and "b" denote significant differences between treatments according to the Student *t*-test ($p < 0.05$).

Three tested plant species (pea, spinach and cauliflower) differed in net-photosynthetic rates (P_n). P_n was higher in cauliflower (means 25.9 and 22.5 μmol CO_2 m^{-2} s^{-1} for control and remediated soil, respectively) than in pea and spinach (P_n around 15 μmol CO_2 m^{-2} s^{-1}) (Fig. 5). There were also species-specific differences in stomatal conductivity (g_s) and transpiration for plants from control soil which were both lower in pea. In the same, control treatment fluorescence measurements revealed lower photochemical efficiency and ETR in spinach and pea than in cauliflower. Photosynthetic and fluorescence parameters of cauliflower from remediated, EDTA washed soils were very similar to those found in control plants. The same was true for pea, in which a decrease was found only for ETR (154.6 in control soil, 108.1 in remediated soil). In spinach, however, dramatic reductions in all photosynthetic parameters were found in plants grown in remediated soil. P_n and g_s were reduced to less than 10% and F_V'/F_m' and ETR values reached 58 and 24% of the values in control plants.

3.3. Ability of plants to form arbuscular mycorrhiza

In the original, non-treated soil, the frequency of arbuscular mycorrhizal fungal colonization in roots (F) was on average 22 and 18% in pea and onion, respectively. With remediation soil mycorrhizal potential significantly decreased in pea, with the measured frequency of arbuscular mycorrhizal fungal colonization 4.9%. No significant differences between different soil treatments were observed in the frequency of fungal colonization in onion (Fig. 6). The low intensity of mycorrhizae (M) (below 3% for both plant species) is indicating low colonization in roots in the original soil and hardly detectable in the remediated soil, with less than 1% of roots colonized by arbuscular mycorrhizal fungi. Additionally, there was low (<3%) abundance of arbuscules in the root cortex, found only in onion grown in the original soil with complete absence of arbuscules in onion grown on remediated soil and in pea, in both original and remediated soil.

3.4. Legislative aspects

Concentrations of Pb and Cd in edible plant tissues were compared with the European Union commission regulation—setting maximum levels for certain contaminants in foodstuffs (EC, 1881/2006). Zn, being an essential nutrient, is not restricted by European legislation.

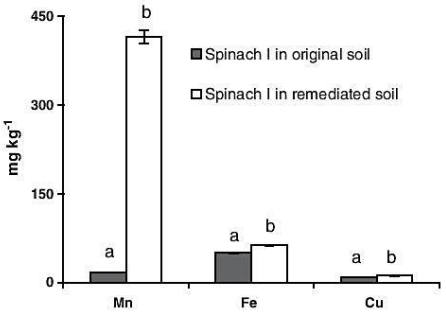


Fig. 4. Concentrations of micronutrients Cu, Fe and Mn measured in green parts of spinach (*Spinacia oleracea* L.). Error bars represent standard deviation (n = 3). The letters "a" and "b" denote significant differences between treatments according to the Student *t*-test ($p < 0.05$).

Maximum levels of Pb in EU vary according to plant species: onion, basil, pepper and carrot 0.1, cauliflower, spinach, lettuce and Chinese cabbage 0.3 and peas 0.2 mg Pb per kg^{-1} fresh vegetable weight. The same applies for Cd, where the maximum values are: onion and carrot 0.1, pepper, cauliflower and Chinese cabbage 0.05, basil, spinach and lettuce 0.2 mg Cd kg^{-1} wet vegetable weight.

In the original soil, the levels of Pb in edible parts of spinach, cabbage, lettuce and carrot all exceeded the European regulation (Table 2). Vegetables grown on remediated soil, on the other hand, did not exceed Pb limits. Pb was not transported to edible parts of any vegetable grown on remediated soil, except for sparse amounts found in spinach. Cadmium was found in the edible parts of all vegetables grown in the original soil, except in onion. The element exceeded the levels set by the European Union (EC, 1881/2006) for peas, cauliflower, spinach, carrot and pepper. After remediation, cadmium concentrations in plants were reduced but were still higher than the maximum allowed EU levels for onion, pepper and spinach.

4. Discussion

Although the original soil bared elevated Pb, Zn and Cd levels plants grown in polluted soil reached the mature phase without any difficulties or visible deficiencies, while plants grown in the remediated soil, in the contrary, demonstrated lower yield and, in the case of spinach, chlorosis and severe growth reduction (Fig. 1). One of the reasons could lie in insufficient nutrient availability. While the original soil contained 195 mg kg^{-1} Mn, remediated soil retained only 58 mg kg^{-1} of Mn. Concentrations of Fe and Cu remained unchanged. In the second rotation, in which the Mn-treatment was added in the remediated soil, progress in the health and growth of spinach plants was observed (Table 1), although it did not reach the original soil's yield.

4.1. Toxic metal uptake and translocation

Metal concentrations absorbed by plants can vary according to soil metal concentrations (Hardiman et al., 1984), soil properties (Cieśliński et al., 1996), plant species (Zhang et al., 1998), plant organs and growing stages (Liu et al., 2003) and various environmental factors (Twining et al., 2004).

Pb and Cd are believed to be non-essential elements for fauna and flora, elements not needed for the assembly of plant tissues or their functioning. Nevertheless, plants often do absorb these contaminants, for example instead of their biologically antagonistic micronutrients (Pb and Ca, Cd and Zn) with a similar ionic radius (Adriano, 1986). Most of the absorbed Pb in both treatments was accumulated in the

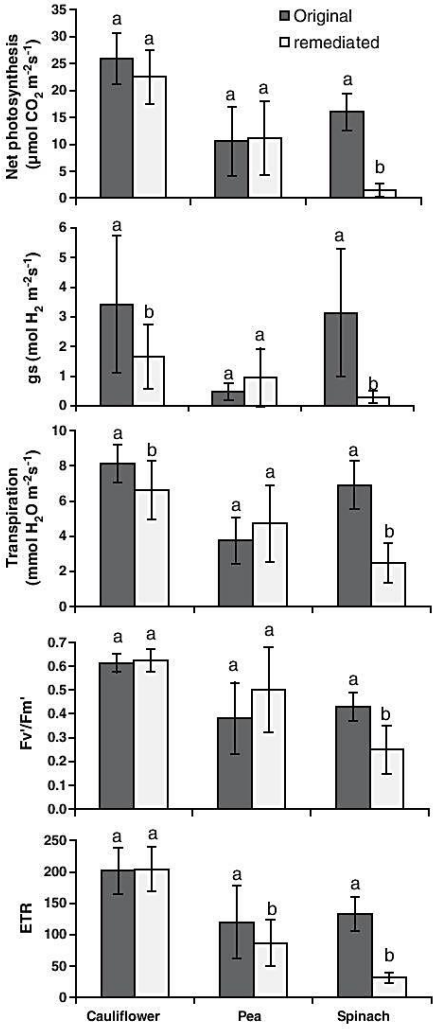


Fig. 5. Gas exchange (net-photosynthesis, stomatal conductance, transpiration) and fluorescence parameters (F_v'/F_m' = photochemical efficiency; ETR = electron transport rate) of different vegetable species grown in original and in remediated soil. Error bars represent standard deviation (n = 10). The letters "a" and "b" denote statistically different treatments according to the Student *t*-test ($p < 0.05$).

roots. Previous studies have also demonstrated that most plant species (excluding hyper-accumulators) accumulate absorbed lead in the roots and only a small fraction is transferred to stems and leaves (Piechalak et al., 2002; Pourrut et al., 2011). Consequently, the transfer factors of lead in our study were low (Table 3). However, previous reports have demonstrated that in the presence of EDTA in soil, Pb can be transported in the form of Pb-chelator complexes to the leaves and stems (Piechalak et al., 2003), thus decreasing the Pb concentration in the roots and increasing it in the aboveground plant organs. The latter effect was observed by Jelusic et al. (2013), with 60 mmol EDTA kg^{-1}

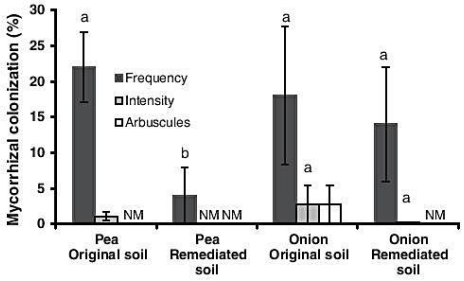


Fig. 6. Root colonization with arbuscular mycorrhizal fungi in roots of onion and pea, sampled in original and remediated soil. The frequency and intensity of mycorrhiza in roots, and the density of arbuscules in the colonized root cortex were calculated. Error bars represent standard deviation. The letters "a" and "b" denote significant differences between treatments according to the Student *t*-test ($p < 0.05$). NM denotes non mycorrhizal roots.

remediated soil, whereby plants of Chinese cabbage managed to absorb high concentrations of toxic metals in their upper parts, due to residual EDTA-metal complexes left in the soil after remediation. No such effect was observed in the current study in vegetables grown in remediated soil, even though a significant part of the initial 60 mmol kg^{-1} EDTA remained in the soil after washing (Voglar and Lestan, 2012), presumably bound to Fe sesquioxides (Nowack, 2002).

Cadmium is reported to be more plant accessible and mobile than lead and less than Zn (Chakravarty and Srivastava, 1997; Seregin and Ivanov, 2001; Tsonev et al., 2012) an essential element with which Cd shares a transport path into the plant (Pence et al., 2000). Due to lowered Zn availability (Fig. 2) (Jelusic and Lestan, 2014), and also due to residual EDTA in the soil, as discussed above, Cd could also have been more easily transferred into the plants grown in remediated soils (Ozturk et al., 2003). No such undesirable effect was observed, except in onion grown in remediated soil, in which Cd levels were greater than with the original soil (Table 2).

Previous studies have shown that Cd concentrations are often (but not always) greater in roots than in shoots (Gallego et al., 2012; Lux et al., 2011), which was consistent with our measurements. Abe et al. (2008), however, observed higher levels of Cd in shoots compared to roots in the Asteraceae family and Broadley et al. (2001) concluded that shoot Cd concentrations are generally higher in species from the Caryophyllale order. These studies explain the relatively high concentrations of Cd in lettuce (Asteraceae family) and spinach plants (order Caryophyllales) (Table 2).

Zn lies between pollutant and essential mineral. Due to active transport (Jelusic et al., 2013), Zn was still absorbed by plants grown in remediated soil, in concentrations close to those found in plants grown in the original soil (Table 2). Even though not all differences between treatments were statistically significant, we still observe reduced concentrations in all remediated plants, suggesting a trend of reduced nutrient accessibility, which may have contributed to lower yield (Alloway, 2009). Differences were the highest in leaves and stems, which contain most of the green mass. In fruits, however, no statistically significant differences between treatments were observed, except in onion, in which the excess of absorbed Cd in plants grown in remediated soil presumably replaced the essential Zn, since the elements share the transport path (Pence et al., 2000).

Studies show that bovine manure can greatly affect the metal behavior in soils by decreasing their bioavailability and also increase shoot growth (Walker et al., 2004). Nevertheless no measurable effect of added manure was observed in third and fourth cropping.

Lowered phytoavailability of Pb, Zn and Cd and the four essential elements, Fe, Cu, Mn after remediation procedure was measured with the DTPA test (Fig. 2). This implies the possibility of nutrient deficiency in cultivated plants, which might partly explain the lower yield and severely depressed growth of the spinach plants (Table 1). EDTA is, after all, capable of extracting a variety of metal ions from the soil matrix (Zhang et al., 2010), including the aforementioned essential micronutrients.

4.2. Disturbance of plant nutrient uptake and plant fitness

In order to study plant fitness and functioning in remediated soil further, and to examine more thoroughly the observed micronutrient deficiency induced by the soil remediation procedure, we tested selected plants of the first crop rotation. Pea, the most prospective, and spinach, the most hindered, were analyzed with micronutrient measurements and only onion was excluded from photosynthetic measurements for plant fitness, since the shape of its leaf does not allow simple gas exchange measurements.

As expected from the severe growth inhibition of spinach, its photosynthetic activity and fluorescence parameters were drastically reduced (Fig. 5). It can be assumed that this response results from an EDTA-induced imbalance in plant mineral nutrient availability. Micronutrient analysis of selected plants revealed a significant decrease in Zn and Mn but not in Cu and Fe in leaf tissues (Figs. 3 and 4, Table 2). Manganese deficiency in spinach of the first rotation could not be detected due to the fertilization that had been performed only a week before the measurements. On the other hand, a decrease of Mn in spinach grown in remediated soil was seen from analysis of the second rotation (data not shown). A deficiency of Mn and Zn can severely affect growth, since both elements are involved in important metabolic pathways (Marschner, 1995). They are associated with photosynthetic reactions, Mn with the oxygen evolving complex of photosystem II (Williams and Pittman, 2010) and Zn as a component of carbonic anhydrase (Clemens, 2010). Direct effects of Mn and Zn deficiency on C-assimilation cannot therefore be excluded. To what extent the EDTA-induced deficiency of Mn and Zn affects plant functions, however, must be tested in further experiments. The disturbance of plant mineral nutrition is complex and it may involve several elements and also changed cation/anion ratios in remediated soil.

Chlorophyll fluorescence parameters, which have been proved to be sensitive indicators of chelate induced stress (Ruley et al., 2004, 2006), also revealed in the present experiment relative insensitivity of cauliflower and pea to soil washing (Fig. 5). In the same plant species, instantaneous net-photosynthesis remained unaffected by soil treatment, but stomatal conductance was found to decrease in cauliflower grown in remediated soil. This response may be related to the fact that the EDTA treatment changed the soil water holding characteristics, i.e., it reduced water availability, as revealed by soil water desorption curves (Jelusic and Lestan, 2014). In the long term, stomatal limitations of photosynthesis may contribute to the changed C-balance on the whole plant level, which would explain the growth reduction. Interestingly, this reduction was the least in pea, which exhibited the most comparable photosynthetic performance and unaffected transpiration and stomatal conductance in the two soils (Fig. 5). The species specific response in remediated soil may therefore be related to water absorption and in regulation of the plant water balance, which can differ between species.

4.3. Ability of plants to form arbuscular mycorrhiza

As mentioned above, plants actively establish their own soil microenvironment rhizosphere, from which they absorb both nutrients and PTMs into their roots (Sterckeman et al., 2005). A plant's ability to form symbiotic mycorrhizal associations with ubiquitous arbuscular mycorrhizal fungi significantly affects rhizosphere functioning (Smith and Read, 2008). It was shown in part I (Jelusic and Lestan, 2014) of this study that soil enzymatic activity, presumably reflecting the

activity of the soil microbial community, as well as microbial soil respiration, was permanently hampered in remediated soil. A similar effect of the soil remediation procedure on arbuscular mycorrhizal soil fungi was thus expected. In order to test this hypothesis, the two mycorrrhizae forming plants of the first crop rotation, pea and onion, were selected for evaluation of different parameters of root colonization with arbuscular mycorrhizal fungi, indicative of the mycorrhizal potential in soils.

Plants, grown in PTMs contaminated soils usually have relatively high levels of mycorrhizal colonization in the roots and arbuscular mycorrhizal fungi have been reported to enhance the stress tolerance of plants, exposed to toxic metals in growth media (Andrade et al., 2009, 2010; Seguel et al., 2013; Wang et al., 2012).

The data on the frequency of arbuscular mycorrhizal fungal colonization in roots of both investigated plant species indicate that there is some mycorrhizal potential (infective mycorrhizal propagules such as fungal mycelium and viable spores) present in the original soil and that soil remediation decreases this potential. The low values of both additional parameters calculated, the intensity of mycorrhizae and the arbuscules abundance in the root cortex, indicate that functional mycorrhizae did not develop well over the course of the experiment and that additional inoculation of the soil either by natural (roots and soil collected in the field) or a commercial inoculum would probably be necessary, for both original and remediated soil in order to establish functional mycorrhizal symbiosis in this system.

4.4. Legislative aspects and practical implications

Having established the benefits and shortcomings of remediated soil, plant PTM concentrations needed to be validated in terms of legislative limits in order to assess the potential for vegetable cultivation. In terms of European legislation (EC, 1881/2006), the original soil thus produced vegetables of which only onion and basil contained levels permitting inclusion in the food list allowed by legislation. The situation was improved with remediated soil but onion, spinach and pepper still exceeded the Cd maximum limits.

In the current stage of development, our technology promises safe use of only Pb contaminated and remediated soil as a substrate for food production. Pb is major metallic contaminant in urban areas with past intense traffic and with soils suffering Pb from house paint yet soil is seldom mono-contaminated. For multi-metal contaminated soils, additional measures need to be introduced into the remediation scheme; we are, for example, testing further immobilization of residual PTMs into the solid matrix of remediated soil. In addition, revitalization of remediated soil by providing the missing structure, nutrients and microbial activity is essential and is the subject of our on-going research activities.

Conflict of interest

The authors declare they have no conflict of interest.

Acknowledgments

This work was supported by the Slovenian Research Agency, Grant J4-3609.

References

Abe T, Fukami M, Ogasawara M. Cadmium accumulation in the shoots and roots of 93 weed species. Soil Sci Plant Nutr 2008;54:566–73. http://dx.doi.org/10.1111/j.1747-0765.2008.00288.x.
Adriano DC. Trace elements in the terrestrial environment. New York: Springer-Verlag; 1986.
Alloway BJ. Soil factors associated with zinc deficiency in crops and humans. Environ Geochem Health 2009;31:537–48. http://dx.doi.org/10.1007/s10653-009-9255-4.
Andrade SA, Gratao PL, Schiavinato MA, Silveira AP, Azevedo RA, Mazzafera P. Zn uptake, physiological response and stress attenuation in mycorrhizal jack bean growing in soil with increasing Zn concentrations. Chemosphere 2009;75:1363–70. http://dx.doi.org/10.1016/j.chemosphere.2009.02.008.
Andrade SA, Silveira AD, Mazzafera P. Arbuscular mycorrhiza alters metal uptake and the physiological response of *Coffea arabica* seedlings to increasing Zn and Cu concentrations in soil. Sci Total Environ 2010;408:5381–91. http://dx.doi.org/10.1016/j.scitotenv.2010.07.064.
Broadley MR, Willey NJ, Wilkins JC, Baker AJM, Mead A, White PJ. Phylogenetic variation in heavy metal accumulation in angiosperms. New Phytol 2001;152:9–27. http://dx.doi.org/10.1046/j.0028-646x.2001.00238.x.
Chakravarty B, Srivastava S. Effect of cadmium and zinc interaction on metal uptake and regeneration of tolerant plants in linseed. Agr Ecosyst Environ 1997;61:45–50. http://dx.doi.org/10.1016/S0167-8809(96)01078-X.
Cheng J, Wong MH. Effect of earthworms of Zn fractionation in soils. Biol Fertil Soils 2002;36:72–8. http://dx.doi.org/10.1007/s00374-002-0507-z.
Cieśliński G, Van Rees KCJ, Huang PM, Kozak LM, Rostad HPW, Knott DR. Cadmium uptake and bioaccumulation in selected cultivars of durum wheat and flax as affected by soil type. Plant Soil 1996;182:115–24.
Clemens S. Zn—a versatile player in plant cell biology. In: Hell R, Mendel RR, editors. Cell biology of metals and nutrients. Berlin, Heidelberg: Springer; 2010. p. 281–98.
Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
Conder JM, Lanno RP, Basta NT. Assessment of metal availability in smelter soil using earthworms and chemical extractions. J Environ Qual 2001;30:1231–7. http://dx.doi.org/10.2134/jeq2001.3041231x.
Feng MH, Shan XQ, Zhang S, Wen B. A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl2, and NaNO3 extraction methods for prediction of bioavailability of metals in soil to barley. Environ Pollut 2005;137:231–40. http://dx.doi.org/10.1016/j.envpol.2005.02.003.
Fitter AH, Moyersoen B. Evolutionary trends in root-microbe symbioses. Philos Trans R Soc 1996;351:1367–75.
Gallego SM, Pena LB, Barcia R, Azpilicueta CE, Iannone MF, Rosales EP, et al. Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. Environ Exp Bot 2012;83:33–46. http://dx.doi.org/10.1016/j.envexpbot.2012.04.006.
Haq AU, Bates TE, Soon YK. Comparison of extractants for plant-available zinc, cadmium, nickel, and copper in contaminated soils. Soil Sci Soc Am J 1980;44:772–7.
Hardiman RT, Banin A, Jacoby B. The effect of soil type and degree of metal contamination upon uptake of Cd, Pb and Cu in bush beans (*Phaseolus vulgaris* L.). Plant Soil 1984;81:3–15.
Jelusic M, Lestan D. Effect of EDTA washing of metal polluted garden soils. Part I: toxicity hazards and impact on soil properties. Sci Total Environ 2014;475:132–41. http://dx.doi.org/10.1016/j.scitotenv.2013.11.049.
Jelusic M, Grcman H, Vodnik D, Suhadolc M, Lestan D. Functioning of metal contaminated garden soil after remediation. Environ Pollut 2013;174:63–70. http://dx.doi.org/10.1016/j.envpol.2012.10.027.
Kalra YP, Maynard DG. Methods manual for forest soil and plant analysis. Northern Forestry Centre, Edmonton: Canadian Forest Service; 1991.
Lindsay WL, Norvell WA. Development of a DTPA test for zinc, iron, manganese and copper. Soil Sci Soc Am J 1978;42:421–8.
Liu J, Li K, Xu J, Zhang Z, Ma T, Lu X, et al. Lead toxicity, uptake, and translocation in different rice cultivars. Plant Sci 2003;165:793–802. http://dx.doi.org/10.1016/S0168-9452(03)00273-5.
Lux A, Martinka M, Vaculik M, White PJ. Root responses to cadmium in the rhizosphere: a review. J Exp Bot 2011;62:21–37. http://dx.doi.org/10.1093/jxb/erq281.
Marschner H. Mineral nutrition of higher plants. 2nd ed. London: Academic Press; 1995.
Mycocalc. Program for estimating the level of mycorrhization; 2013 [http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html].
Nowack B. Environmental chemistry of aminopolycarboxylate chelating agents. Environ Sci Technol 2002;36:4009–16.
Ozturk L, Karanlik S, Ozkutlu F, Cakmak I, Kochian LV. Shoot biomass and zinc/cadmium uptake for hyperaccumulator and non-accumulator Thlaspi species in response to growth on a zinc-deficient calcareous soil. Plant Sci 2003;164:1095–101. http://dx.doi.org/10.1016/S0168-9452(03)00118-3.
Pence NS, Larsen PB, Ebbs SD, Letham DL, Lasat M, Garvin D, et al. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. Proc Natl Acad Sci U S A 2000;97:4956–60.
Piechalak A, Tomaszewska B, Baralkiewicz D, Malecka A. Accumulation and detoxification of lead ions in legumes. Phytochemistry 2002;60:153–62. http://dx.doi.org/10.1016/S0031-9422(02)00067-5.
Piechalak A, Tomaszewska B, Baralkiewicz D. Enhancing phytoremediative ability of *Pisum sativum* by EDTA application. Phytochemistry 2003;64:1239–51. http://dx.doi.org/10.1016/S0031-9422(03)00515-6.
Pociecha M, Lestan D. Recycling of EDTA solution after soil washing of Pb, Zn, Cd and As contaminated soil. Chemosphere 2012;86:843–6. http://dx.doi.org/10.1016/j.chemosphere.2011.11.004.
Pourrut B, Shahid M, Dumat C, Winterton P, Pinelli E. Lead uptake, toxicity, and detoxification in plants. Rev Environ Contam Toxicol 2011;213:113–36. http://dx.doi.org/10.1007/978-1-4419-9860-6_4.
R Development Core Team. A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria3-900051-07-0; 2010 [http://www.R-project.org].
Ruley AT, Sharma NC, Sahi SV. Antioxidant defense in a lead accumulating plant, *Sesbania drummondii*. Plant Physiol Biochem 2004;42:899–906. http://dx.doi.org/10.1016/j.plaphy.2004.12.001.
Ruley AT, Sharma NC, Sahi SV, Singh SR, Sajwan KS. Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in *Sesbania drummondii* grown in soil. Environ Pollut 2006;144:1–18. http://dx.doi.org/10.1016/j.envpol.2006.01.016.

Säumel I, Kotsyuk I, Hölscher M, Lenkereit C, Weber F, Kowarik I. How healthy is urban horticulture in high traffic areas? Trace metal concentrations in vegetable crops from plantings within inner city neighbourhoods in Berlin, Germany. Environ Pollut 2012;165:124–32. http://dx.doi.org/10.1016/j.envpol.2012.02.019.
Seguel A, Cumming JR, Klugh-Stewart K, Cornejo P, Borie F. The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review. Mycorrhiza 2013;23:167–83. http://dx.doi.org/10.1007/s00572-013-0479-x.
Seregin IV, Ivanov VB. Physiological aspects of cadmium and lead toxic effects on higher plants. Russ J Plant Physiol 2001;48:523–44.
Smith SE, Read DJ. Mycorrhizal symbiosis. 3rd ed. London, United Kingdom: Academic Press; 2008.
Sterckeman T, Duquène L, Perriguey J, Morel JL. Quantifying the effect of rhizosphere processes on the availability of soil cadmium and zinc. Plant Soil 2005;276:335–45. http://dx.doi.org/10.1007/s11104-005-5087-x.
Tei F, Benincasa P, Farneselli M, Caprai M. Allotment gardens for senior citizens in Italy: current status and technical proposals. Acta Hortic 2010;881:91–6.
Trouvelot A, Kough JL, Gianinazzi-Pearson V. Mesure du taux de mycorhization VA d'un syste'me radiculaire. Recherche de me'thodes d'estimation ayant une signification fonctionnelle. Physiological and genetical aspects of mycorrhizae. Paris, France: INRA Press; 1986.
Tsonev T, Jose F, Lidon C. Zinc in plants—an overview. Plant Sci 2012;24:322–33.
Twining JR, Payne TE, Itakura T. Soil–water distribution coefficients and plant transfer factors for (134)Cs, (85)Sr and (65)Zn under field conditions in tropical Australia. J Environ Radioact 2004;71:71–87. http://dx.doi.org/10.1016/S0265-931X(03)00142-5.
Vodnik D, Grcman H, Macek I, van Elteren JT, Kovacevic M. The contribution of glomalin-related soil protein to Pb and Zn sequestration in polluted soil. Sci Total Environ 2008;392:130–6.
Voglar D, Lestan D. Pilot-scale washing of metal contaminated garden soil using EDTA. J Hazard Mater 2012;215–216:32–9. http://dx.doi.org/10.1016/j.jhazmat.2012.02.022.
Walker DJ, Clemente R, Bernal MP. Contrasting effects of compost and manure on the growth and uptake of heavy metals by *Chenopodium album* L. in a soil contaminated by pyritic mine waste. Chemosphere 2004;57:215–24.
Wang YP, Huang J, Gao YZ. Arbuscular mycorrhizal colonization alters subcellular distribution and chemical forms of cadmium in *Medicago sativa* L. and resists cadmium toxicity. PLoS One 2012;7:1–7. http://dx.doi.org/10.1371/journal.pone.0048669.
Williams LE, Pittman JK. Dissecting pathways involved in manganese homeostasis and stress in higher plant cells. In: Hell R, Mendel RR, editors. Cell biology of metals and nutrients. Berlin, Heidelberg: Springer; 2010. p. 95–118.
Wong CSC, Li X, Thornton I. Urban environmental geochemistry of trace metals, review. Environ Pollut 2006;142:1–16. http://dx.doi.org/10.1016/j.envpol.2005.09.004.
Zhang ZW, Watanabe T, Shimbo S, Higashikawa K, Ikeda M. Lead and cadmium contents in cereals and pulses in north-eastern China. Sci Total Environ 1998;220:137–45. http://dx.doi.org/10.1016/S0048-9697(98)00252-6.
Zhang W, Huang H, Tan F, Wang H, Qui R. Influence of EDTA washing on the species and mobility of heavy metals residual in soils. J Hazard Mater 2010;173:369–76. http://dx.doi.org/10.1016/j.jhazmat.2009.08.087.

2.2 OSTALO POVEZOVALNO ZNANSTVENO DELO

2.2.1 Revitalizacija z EDTA remediiranih tal z gnojenjem in talnimi dodatki

JELUŠIČ Maša, VODNIK Dominik, LEŠTAN Domen
Revitalization of EDTA-remediated soil by fertilization and soil amendments

Remediacija tal z ekstrakcijo poškoduje talne lastnosti in ustvari potrebo za revitalizacijo tal. Onesnažena vrtna tla so bila oprana z visoko koncentracijo liganda EDTA, ki je odstranil 69 % Pb, 15 % Zn in 57 % Cd. Koncentracija Mn se je zmanjšala za 53 % in koncentracije ostalih mikrohranil (Cu, Fe, Mg in Ca) so ostale nespremenjene. Analizirali smo učinek remediacije na frakcionacijo kovin v tleh in tlom dodali gnojilo, gnoj, hidrogel, vermikulit, apatit in Slovakit ter zasadili špinačo. Remediacija je povzročila manjšo rast rastlin na remediiranih tleh, manjšo fotosintezo in zmanjšala vsebnost Pb, Zn in Cd v zelenih delih rastlin za 2,4, 1,8 in 6,4-krat. Gnojilo je delno popravilo fotosintezo, prevodnost listnih rež in transpiracijo ter pridelek testnih rastlin. Slabšo rast rastlin na remediiranih tleh je delno izboljšal tudi hidrogel (do 53 %). Ostali dodatki niso signifikantno vplivali na lastnosti remediiranih tal in stanje rastlin.

Revitalization of EDTA-remediated soil by fertilization and soil amendments

Masa Jelusic, Dominik Vodnik, Domen Lestan,
Agronomy Department, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana; Slovenia

Correspondence:
Domen Lestan
Centre for Soil and Environmental Science
Biotechnical Faculty, University of Ljubljana
Jamnikarjeva 101
1000 Ljubljana, Slovenia

tel.: +386 1 3203 162
fax.: +386 01 256 57 82
E. mail.: domen.lestan@bf.uni-lj.si

Abstract

Soil remediation using extractions deteriorates soil's properties and creates the need for soil revitalization. Contaminated garden soil was washed with a high EDTA dose to remove 69, 15 and 57% of Pb, Zn, and Cd, respectively. The concentration of Mn decreased by 53%, and concentrations of other essential metals: Cu, Fe, Mg, and Ca remained unchanged. The effect of remediation on the fractionation of soil metals was assessed and the soil was amended with fertilizer, manure, hydrogel, vermiculite, apatite, and absorbent and subjected to spinach (*Spinacia oleracea*) cultivation. Remediation reduced plant growth, photosynthesis and plant uptake of Pb, Zn, and Cd 2.4, 1.8, and 6.4 times respectively. Fertilization partly restored net photosynthesis, stomatal conductance, transpiration, and yield of the tested plants. Growth depression was found to be mitigated further by hydrogel (up to 53%). The other tested amendments did not significantly affect the properties of remediated soil and plant performance.

Keywords: remediated soil, soil revitalization, metal plant uptake, nutrient plant uptake, hydrogel.

1 Introduction

Soil contamination with potentially toxic metals (PTMs) commonly referred to as "heavy metals", is a worldwide concern. Washing with aminopolycarboxylate chelants such as EDTA (ethylenediaminetetraacetate), is one of the few enduring soil remedial options with a potentially less significant effect on soil properties and quality. EDTA is able to interact with most of PTMs and substantially decrease their concentration in various soils. Soil washing is carried out by dissolving and transferring PTMs from the soil's solid phase into an aqueous solution.

We recently demonstrated the technical and economic feasibility of EDTA-based soil washing technology for remediation of PTMs contaminated soils (Voglar and Lestan, 2014). The question remains, however, whether EDTA soil washing could qualify as a green and sustainable remediation method (US EPA, 2010). The concept of green and sustainable remediation focuses on minimizing the environmental impacts of remediation activities and covers a wide range of sustainability impacts and benefits, such as sustainable land use and soil management. EDTA successfully removed PTMs and reduced the human and environmental hazard of remediated soil but impaired the soil's water potential and microbial activity (Jelusic and Lestan, 2014). Remediation also reduced PTMs concentrations in roots, green parts, and fruits in plant cultivars grown in experimental plots, but the biomass of most tested plants significantly diminished (Jelusic et al., 2014). Presumably, alkaline-earth cations and micro-nutrients were removed along with PTMs because of the non-selective nature of EDTA chelation. Among the evaluated physical soil characteristics, poorer water-retention characteristics and the destruction of the natural soil structure were found to influence plant growth (Zupanc et al., 2014). Tsang et al. (2007) reported that the EDTA washing solution appreciably alters the soil's physical properties by dissolving indigenous oxides, carbonates and organic matter. EDTA is poorly biodegradable and highly persistent in the soil, which may lead to post-use emissions (Rahman et al., 2010). Jelusic et al. (2013) reported that 75% of Pb and Cd, and 30% of Zn was removed by EDTA soil washing, however, up to 0.4%, 0.8% and 0.3% of the initial Pb, Cd, and Zn soil concentration, respectively, was lost with the leachate. Barona et al. (2001) and Lei et al. (2008) found that the metals remaining in the soil after chelant-based washing become more mobile and more weakly associated with soil components. Enhanced mobility of residual metals presumably occurs because of the metal detachment, chelant attack, soil dissolution, or the cation exchange between the chelant complexes and the soil particles.

The results of the above studies indicate that effective means of revitalization are needed to restore health and reclaim EDTA-remediated soil as a fertile and safe plant substrate. In this study, we applied novel EDTA-based technology (Voglar and Lestan, 2014) to remedy garden soil highly contaminated with Pb, Zn, and Cd from a former Pb-smelting site in the Meza Valley, Slovenia. We assessed the removal and fractionation of PTMs and essential metallic micro-nutrients in soil using a modified Tessier sequential extraction procedure (Lestan et al., 2003). We conducted further tests with fertilization (solution of N, K, Mn and Mg) and several soil amendments, each performing a specific function, in an attempt to improve the properties of remediated soil. Bovine manure was applied to the soil to restore the nutrient pool. Hydrogel was applied to improve the soil's water-retention

characteristic (Chirino et al., 2011). We used vermiculite to improve the soil's structure and to contribute its numerous exchanging sites to retain the soil-added nutrients (Seaborn and Jameson, 1976). We also assessed apatite and Slovakite (a commercial mixture of absorbents) amendments for their ability to reduce leachability and plant availability of PTMs (Tica et al., 2013; Venäläinen, 2011) remaining in the soil after remediation. In our previous study, spinach (*Spinacia oleracea* L) was found to be particularly sensitive for growth on remediated soil (Jelusic et al., 2014). It was used here as a bio-indicator to evaluate the effects of soil remediation and revitalization measures by assessing plant biomass, photosynthetic functions, and the accumulation of PTMs.

2 Materials and methods

2.1 Soil properties

The contaminated soil used in this experiment originated from a managed vegetable garden near the abandoned lead smelter in the Meža Valley, Slovenia, polluted primarily with Pb and also with Zn and Cd. Soil samples were air-dried and sieved to 2 mm (ISO 11464, 2006). The soil's pH was measured in a 1/2.5 (w/v) ratio of soil and 0.01 M $CaCl_2$ suspension (ISO 10390, 2005). Cation exchange capacity (CEC) was determined after soil extraction with ammonium acetate (pH 7), organic matter was determined by modified Walkley-Black titrations (ISO 14235, 1998), and the soil's texture was analyzed by the pipette method (ISO 11277, 2009). Easily extractable P (P_2O_5) and K (K_2O) were measured colorimetrically using the method described by Kalra and Maynard (1991). The following properties were obtained for the original and remediated soils: pH 6.7 and 7.1, 800 and 800 mg kg^{-1} P_2O_5, 17.3 and 18.2 mg kg^{-1} K_2O, 29.3 and 36.6 CEC ($mmol_c$ $100g^{-1}$), 7% and 7% of organic matter respectively. The soil's water-retention capacity was determined by inserting soil samples into retaining rings placed on a ceramic plate and irrigated with deionized water for 48 hours. The ceramic plate with the soil sample was then placed in an extractor pressure vessel for another 48hours. Negative pressures of 0.33, 1, 2, 5, and 15 bar were applied. The samples were weighed, and then dried for 24 hours at 105°C and weighed again to determine the mass percentage of water sorbed.

2.2 Soil remediation

Soil was chelant-washed as reported by Voglar and Lestan (2014), using 120 mmol EDTA kg^{-1} of dry soil. Briefly, 60 kg of soil per batch were extracted with 60 L of recycled EDTA solution (20% of fresh Na2EDTA was added to each batch to replace process losses) in a concrete mixer for 2 hours. After extraction, the soil suspension was separated from the spent soil washing solution in a chamber filter press, and the soil was rinsed within the press with pressured-cleansed process water and tap water to remove all EDTA-mobilized toxic metal species. The EDTA in the used washing solution was first recycled as Ca-EDTA after the substitution of PTMs in the EDTA complex with Ca in alkaline conditions (pH > 12) created by the addition of lime ($Ca(OH)_2$), and by acidic precipitation (pH 1.8-2.2) created by the addition of H_2SO_4. The precipitation of insoluble $CaSO_4$ prevented the build-up of the added reagents after multiple remediation batches. For soil rinsing, process solution was cleansed in an electrolytic step for complete recycling of the process water.

Five batches in soil-washing facility were needed to prepare a sufficient quantity (264 kg) of remediated soil for a column experiment. No waste-water was generated and a total of 6.6 kg of solid waste composed of precipitated PTMs, lime and electro-corroded graphite. Before disposal, solid waste was bitumen stabilized to prevent the leaching of PTMs.

2.3 Soil column experiment

Thirteen different soil treatments were conducted in this experiment, each treatment with three replicates/columns filled with 8000 g (dry weight) of soil. Treatments of remediated soil were amended with hydrogel, vermiculite, manure (pH 6.0, 279 mg kg^{-1} K_2O, 718 mg kg^{-1} P_2O_5, C/N 12.5 and 30 mg kg^{-1} Pb, 198 mg kg^{-1} Zn, 912 mg $kg^{-}1$ Mn, 12580 mg kg^{-1} Fe, and 47 mg kg^{-1} Cu), apatite, Slovakite (commercial sorbent mixture of inorganic natural raw materials: dolomite, smectite, diatomite, basaltictuff, bentonite, alginite, and zeolite), and some treatments were fertilized with 200 kg ha^{-1} N as NH_4NO_3, 300 kg ha^{-1} K as KNO_3, 30 kg ha^{-1} Mg as $Mg(NO_3)_2$ and 40 kg ha^{-1} Mn as $MnSO_4$. The design of the experiment design and the abbreviations of the treatments are shown in Table 1. For control treatments, original soil (O) and original soil plus fertilizer (OF) treatments were set up (Table 1). Hydrogel and vermiculite treatments were prepared with two different fertilizer applications (Table 1). In the RHF and RVF treatments, the fertilizer solution was added to the soil and left to stabilize for one day. The next day, the vermiculite and hydrogel amendments were added. In the RHF* and RVF* treatments the fertilizer solution was first mixed with the amendments, left for one day for the nutrients to adsorb on free hydrogel and vermiculite sites, and then mixed with the soil.

All thirty-nine pots were assembled in a random order, planted with 20 seeds of *S. oleracea* each, and placed in a greenhouse for a period of 45 days. The pots were equipped with trapping devices for the collection of leachate and with a plastic mesh (D=0.2 mm) placed on the bottom of the columns to retain the soil. The columns were irrigated weekly with approximately 1800 mL of tap water. The leachates were collected three times during the experiment period and stored for metal analysis.

2.4. Soil metal fractionation and plant and soil metal determination

We used a modified Tessier sequential extraction procedure (Lestan et al., 2003) to assess the association of metals with different fractions in the original and remediated soils. The final metal fractional recovery was calculated by comparing the sum of the metals' concentrations in all six fractions with their total soil concentration. Metal concentration in soil and plant samples, extracts, and leachates was analyzed by flame (acetylene-air) atomic absorption spectrometry (AAS, Varian AA240FS), using sample preparation and quality control protocols as described previously (Jelusic et al., 2014).

2.5 Photosynthetic functions: gas exchange and fluorescence measurements

Gas exchange measurements were made on June 8, 15, and 22 (i.e., 30, 37, and 44 days after sowing) by using an LI-6400xt measuring system combined with a 6400-40 leaf chamber fluorometer equipped with a LED light source and CO_2 mixer (Li-Cor, Lincoln, NE, USA). Non-senescent, fully developed leaves from the inner part of the rosette of at

least one plant per column were measured. Chamber conditions were maintained at 400 μmol CO_2 mol^{-1}, ambient humidity (mean RH was 43.5, 33.5, and 49.23 for three consecutive samplings, respectively), and saturating PAR intensity of 1500 μmol m^{-2} s^{-1}. Chamber temperature was regulated by average ambient temperature during the measurement (mean air temperature was 23.9, 24.7, and 25.6°C). Gas exchange data (net-photosynthesis, Pn; stomatal conductance, gs and transpiration, E) were registered when steady-state conditions were achieved, at the same as time steady-state fluorescence was captured (Fs). Immediately thereafter, a saturating light flash was applied over the same, light-adapted leaf area and maximum fluorescence (Fm') was recorded. Minimum fluorescence (F0') was captured after the leaf had been momentarily darkened. Photochemical efficiency, (i.e., the efficiency of energy harvesting by oxidized (open) PSII reaction centers in the light) was calculated as Fv'/Fm' = Fm' – F0' / Fm'. The electron transport rate (ETR) was calculated as ETR = ((Fm'-Fs)/Fm')•f•I•αleaf, where f is the fraction of absorbed quanta used by PSII, I is the incident photon flux density and αleaf is the leaf absorbance.

2.6 Statistical analysis

The data measured in the original and remediated soils were statistically compared using student t-tests. The same test was applied to assess the significance of soil fertilization (between O and OF, R and RF, RC and RCF, and RH and RHF) and the effect of soil amendments (between RF and RCF, RF and RHF, RF and RHF*, RF and RVF*, RF and RAF, and RF and RSF). As two columns of fertilized remediated soil amended with vermiculite (RVF) were contaminated, no statistical comparison with only the one remaining column was eligible. Statistical analysis was carried out with the R program (R Development Core Team, 2010). Differences between treatments were considered significant at $P < 0.05$.

3 Results and discussion

3.1 Soil remediation, metal removal and fractionation

High levels of soil contaminants 4037, 2527, and 26 mg kg^{-1} of Pb, Zn, and Cd, respectively (Table 2) necessitate the use of high EDTA dose and stringent conditions of soil remediation. The soil was remediated in an experimental pilot-scale soil-washing facility; alkaline-to-acid pH gradient and an electrochemical advanced oxidation process were applied for EDTA and process water recycle in a closed process loop (Voglar and Lestan, 2014). As shown in Table 2, Pb and Cd concentrations were reduced by 69% and 57%, respectively and Zn by only 15% due to the lower affinity of EDTA for forming coordinative bonds with Zn (Martell and Smith, 2003) and because Zn was mostly associated with the residual soil fraction (explained below).

The objective of remediation is to relieve the soil of PTMs with as little harm to the soil as possible. As also observed in our previous study with less contaminated soil and lower chelant dose (Jelusic and Lestan, 2014), remediation with EDTA did not have a notable effect on the soil's pedological properties (Table 2). EDTA, however, is a non-selective agent (Zhang et al., 2010) that binds with many soil cations, whether they be PTMs (Cd,

Pb), essential micronutrients (Mn, Mg, Ca, Fe), or both (Zn, Cu). For the purpose of this paper, and because of the nature of contamination, we will refer to Zn as a PTM and Cu as an essential micronutrient. Along with PTMs, remediation stripped the soil of 52% of Mn. Manganese was the only measured essential nutrient that was significantly reduced. Cu, Fe, Mg, and Ca remained largely unchanged after the remediation process (Table 2).

Sequential analysis was performed on eight metals (Pb, Zn, Cd, Mn, Cu, Fe, Mg, and Ca) to associate their distribution in the soil before and after remediation with six fractions (Figure 1). The metals associated with the first two fractions are generally considered bio-accessible and more labile than others (Alloway, 2013). As reported by Zhang et al. (2010) soil washing with EDTA can extract metals from all fractions, apart from the tightly crystalline-bound elements in the residual fraction.

The concentrations of metals in remediated soil, except for Mn, were higher in at least one of the first two (labile) soil fractions (Figure 1). This occurrence is probably the result of an incomplete soil rinsing during the remediation procedure (Jelusic et al., 2013), allowing the metals to rebind to opportune sites. A complete removal of metallic species mobilized during remediation is not feasible in a realistic remedial situation. The share of metals in the first two fractions was, however, negligible compared to the metals bound to non-labile soil fractions (Figure 1). High organic matter and carbonate content (Table 2) provided abundant binding sites for Pb and Cd in the original soil (Figure 1). EDTA washing substantially reduced concentrations associated with those fractions. Zn was primarily bound to the residual fraction, from which EDTA could not extract the element. Mn was primarily associated with the fourth and residual fractions (Figure 1). EDTA was unable to extract Mn from the residual fraction, but was able to wash a substantial part of Mn associated with the fourth fraction. Most of the Ca, Cu, and Mg were bound to organic matter, and most of the Fe was incorporated into the residual fraction. The concentrations of these metals did not decrease after EDTA washing, and the pattern of their distribution in the soil fractions was not considerably altered (Figure 1).

3.2 Effect of remediation on soil water potential and metal leaching

Water-retention capacities of the original soil and differently treated remediated soils are shown in Figure 2. The pressure point of −0.33 bar represents the field capacity of the soil where all the gravitational water is drained out of macropores and resides in micropores. The pressure point of −15 bar is the wilting coefficient where all the water resides only in the smallest of the micropores and is thus mostly inaccessible to plants. In between those two points lies the plant available water (PAW) (Brady and Weil, 2002). Overall, we achieved an increase in PAW in remediated soil compared to the original one (Figure 2). Remediation significantly increased the soil field capacity because of an added final step in the remediation procedure, where remediated soil was gently pressed through a 5 mm sieve, an action that created artificial structural aggregates capable of retaining more gravitational water. At the same time the conditions during remediation increased the soil's wilting coefficient (Figure 2), presumably by compacting larger soil pores into a structure of finer micropores. As expected, fertilization did not affect the plant available water.

The cumulative leaching of metals from soil columns is shown in Figure 3. The original soil was free of soluble metal species, and the leaching from non-fertilized remediated soil was statistically significantly higher for all the metals except for Mn (as explained above, a complete removal of all EDTA-mobilized metallic species was not feasible by remediation method). The trend of decreasing metal concentrations in leachates was evident for all treatments (supplemental material) except for Ca, which was added as a reagent in the remediation process. The amounts of leached metals were quite low, even for the most problematic PTMs. The total amount of any rinsed metal was, regardless of the treatment, on average, 0.05% and never higher than 0.7% of the total metal content in remediated soil (Table 2).

Mn leached from fertilized soils, an effect not observed with Mg, which was also an ingredient in the fertilizer solution (Figure 3). Fertilization promoted Pb and Cd leaching; statistically significant differences were observed between R and RF, and RC and RCF treatments (Figure 3). The added nutrients, especially Mg^{2+}, presumably replaced Pb and Cd in the exchangeable sites in the remediated soil matrix as explained above, labile fractions of Pb and Cd increased after remediation (Figure 1). Similarly, the Mg^{2+} displacement capacity is used in sequential extractions to determine the exchangeable metal fraction (Gleyzes at al., 2002).

3.3 Plant performance and metal uptake

In our previous study, we cultivated a selection of crops and vegetables in field plots with remediated soil (Jelusic et al., 2014). The growth of most plants was depressed: the least affected was lettuce (Lactuca sativa L.) with 4% lower biomass, while the biomass of *S. oleracea*, the most sensitive of the tested plants, was diminished for by up to 10 times. The poor growth of *S. oleracea* on remediated soil in the present study (9 times lower biomass compared to the original soil) (Figure 4), was therefore expected. Fertilization of the original soil increased the plant biomass 1.7 times and in remediated soil 4.9 times, indicating that the depletion of nutrients is a major contributing factor to poor plant growth. In particular, soil Mn concentration was reduced from 828 to 392 mg kg^{-1} (Table 2). This is close to the average concentration of Mn (382 mg kg^{-1}) in European soils (Alloway, 2013). However, plants absorb Mn mostly as a free Mn^{2+} ion, and its deficiency is very common in calcareous soils with high organic matter, such as the soil used in this study. Furthermore, remediation stripped the soil of the Mn-oxides fraction, leaving the remediated soil without the readily reducible Mn (Mn-oxides) necessary for plant growth (Figure 1). *S. oleracea* grown on the original soil absorbed more Mn (along with PTMs), still the concentration of Mn in plant tissue was below 20 mg kg^{-1} (Figure 5), which is the lower tissue limit for the normal development of most plants (Bergmann, 1992). In *S. oleracea* cultivated on remediated soils, the Mn concentration in plant tissue was below 3 mg kg^{-1} with no increase after fertilization. Presumably, the Mn in the fertilizer solution was subjected to microbial oxidation in the soil and made inaccessible to plants (Alloway, 2013). A pH increase in remediated soil (Table 2) could help sustain the shift of redox "equilibrium" in favor of the microbial oxidation of the element (Alloway, 2013).

The uptake of PTMs by *S. olercea* was substantially reduced after remediation (Figure 5), with the exception of Zn, an essential nutrient with actively regulated plant transport whose

uptake remained relatively high compared to Pb and Cd. Fertilization of both the original and remediated soils considerably reduced the plant Pb uptake (Figure 5), presumably by decreasing the relative share of Pb in the total cation concentration in the soil solution. Rather high concentrations of Cd were observed in *S. oleracea* plants grown on the original soil, more than 40 mg kg^{-1}. *S. oleracea* is Cd tolerant and can accumulate high concentrations of this element (Salaskar et al., 2011). After remediation, concentrations of Cd in the plant dropped substantially, by 85% (Figure 5); however the level in all treatments was still above the EU legislation limits for contaminants in foodstuffs (EC, 1881/2006). The Pb concentrations in *S. oleracea* were below the EU legislation limits in most treatments except for R, RC, and RVF.

Among essential micronutrients, a slight but statistically significant elevation in Fe concentrations in plants grown on remediated soils (Figure 5) could be attributed to Fe-EDTA complexes in the soil solution (leachate) after remediation (Figure 3). Chelators are known to increase the mobility of Fe by dissolving the hardly soluble Fe hydroxide species (Hasegawa et al., 2011; Lucena, 2006). Plants can then uptake the metal by a plasma-membrane-bound ferric reductase, which reduces the chelated Fe, which is then absorbed into the plant as a free Fe2+ ion. EDTA can also promote the uptake of other micronutrients and Cu levels in plants also increased after remediation (Figure 5). Plants cultivated on soils with different Cu levels usually absorb the same amounts of the element in their upper parts (Barker and Pilbeam, 2007). This is due to an internal, rather than external mechanism, which allows plants to exclude/adapt to toxic Cu concentrations in soil (Muller et al., 2000). High concentrations of phytoavailable Cu, achieved by chelator addition can disrupt the exclusion mechanism and enhance Cu up-take (Zeremski-Skoric et al., 2010).

Shifts in the content of essential mineral micronutrients and PTMs affected plant physiology. Net photosynthetic rates (Pn) of *S. oleracea* grown in the original soil exceeded 25 μmol CO_2 m^{-2} s^{-1} at the first measuring date (Table 3). In non-fertilized remediated soil, however, Pn only reached half of this value, because of reduced photochemical efficiency (Fv'/Fm', ETR) and conductivity of stomata (gs). Differences between photosynthetic characteristics of plants growing on O, OF, R, and RF soils persisted throughout the experiment, although, in general, lower Pn rates and Fv'/Fm' values were recorded 37 and 44 days after sowing when compared to the first measuring date. This can be related to the fact that the plants were grown in semi-controlled conditions in the greenhouse, and differences in relative humidity and temperature, soil water content, etc. could therefore influence daily gas-exchange and fluorescence values.

Fertilization did not influence the photosynthetic rate of plants growing in the original substrate but significantly repaired the Pn of plants in remediated substrate (ca. 75% of the OF) (Table 3, Figure 6). The results clearly showed that Mn is the main essential nutrient which availability is severely reduced after remediation process. Mn is a micronutrient essential for the function of thylakoid photosynthetic processes, such as O2 evolution in photosystem II (PSII; Marschner et al., 2012). Mn deficiency changes the ultrastructure of thylakoid membrane, reducing the number of PSII functional units in the stacked areas of thylakoid membranes (Husted et al., 2010). It is therefore not surprising that leaf chlorophyll fluorescence, which originates mainly form PSII (Schreiber et al., 1995), was

highly responsive in our experiment. Low Fv'/Fm' values (Table 3) reveal severely reduced photochemical efficiency in plants growing in remediated soil. However, fertilization treatment reveals that there are other factors contributing to the photosynthetic limitation. Despite the fact that fertilization did not improve foliar Mn content (Figure 5), it contributed to better photosynthetic performance (Pn, Fv'/Fm') and resulted in higher yield. The soil-plant-nutrient cycle is a complex system with many variables and unknowns. The remediation process disturbed the delicate soil nutrient balance that could not be fully restored by an addition of fertilizer.

3.4 Soil amendments as conditioners of remediated soil

With various amendments, we strived to restore the diminished capacity of remediated soil to function as a healthy and fertile plant substrate. Chosen for their characteristics and positive impact on soil properties were manure, hydrogel, vermiculite, apatite, and Slovakite.

Fluorescence data (photochemical efficiency of PSII, Fv'/ Fm'), which are very indicative of expressing the general fitness of a plant (Schreiber et al., 1995), and net photosynthesis rates (Pn) reveal a negligible effect of manure, hydrogel, and vermiculite on leaf photosynthetic function. Plants growing in RAF soil (apatite amended) were characterized by reduced photochemical efficiency, and their Pn decreased with respect to RF throughout the experiment (Figure 6). On the other hand, slightly higher Fv'/Fm' values were measured in plants growing in the remediated-fertilized soil amended with Slovakite (RSF).

3.4.1 Manure

Five-year-old, dry bovine manure was added to enrich the remediated soil with organic matter and micronutrients, and to strengthen the soil structure (Haynes and Naidu, 1998). *S. oleracea* yield, however, did not change after the addition of manure. The amendment did not have any effect on metal uptake or leaching, although studies show (Udovic and McBride, 2011) that addition of manure can reduce the availability of PTMs by forming non-soluble complexes with humic substances in manure. Regarding physical soil properties, manure lowered the plant available water of remediated soil by raising (statistically significantly) its wilting point (Figure 2).

3.4.2 Hydrogel

Hydrogel was selected to improve the soil's water characteristics and nutrient availability, and consequently, improve conditions for plant cultivation (El-Hady and El-Dewiny, 2006; El-Hady and Abo-Sedera, 2006). Whereas hydrogel is used in agriculture primarily in drought areas (Chirino et al., 2011), Orikiriza et al. (2009) showed that it improved the growth of tree species in different soil types, even in non-water stress conditions. Hydrogel application increases the soil's field water capacity and wilting point, and generally also PAW (El-Hady and Abo-Sedera, 2006). In our study, however, PAW decreased, since the wilting point increased considerably more than the field water capacity (Figure 2). According to Sullivan and Ball (1993) and Narjary et al. (2012), soil water retained by a

soil matrix with 0.1–1 bar should be considered as water readily available for plants (RPAW). In hydrogel treatments (RH and RHF*) the part of RPAW retained by 0.33–1 bar remained unchanged compared to RF (Figure 2).

All three hydrogel treatments (RH, RHF, and RHF*) resulted in the highest *S. oleracea* biomass on remediated soils (Figure 4). El-Hady and El-Dewiny (2006) also observed the positive effects of added hydrogel on plant (tomato) growth, absorption of nutrients, soil water characteristics, and biological properties. They concluded that hydrogel improves the soil's physical and chemical characteristics, and consequently enhances its microbial life. Soil microorganisms with various beneficial effects on the soil's properties can release essential nutrients from the soil matrix in a plant available form, and thus increase plant health and yield.

Among all soil amendments, only hydrogel demonstrated a statistical difference in the lower leaching of metals (Figure 3). Hydrogel treatments (RH, RHF, and RHF*) leached the smallest volume of water by reducing water drainage (data not shown) and thus indirectly preventing metals from leaching. The effect was observed by Kos and Lestan (2003), who intentionally used hydrogel for retaining chelate complexes in the soil to reduce leaching as an unwanted side effect of EDTA-induced phytoextraction.

To determine whether the manner of fertilization affects metal leaching and plant uptake, the fertilizer was applied separately into the soil before the hydrogel and vermiculite (RHF and RVF) additions, or in formulation with the hydrogel and vermiculite substrate (RHF* and RVF*) (Table 1). Leachates from the RHF columns contained fewer PTMs and a lower concentration of essential metals (Figure 3). Higher concentrations of cations in the solution impair gel swelling capacity, which presumably explains the poorer RHF* performance. No change in metal plant uptake was observed regardless of the fertilization method (Figure 5).

3.4.3 Vermiculite

Vermiculite is a natural expanding mineral commonly used in agriculture as a soil conditioner (Headlee et al., 2013). Although lacking essential elements, vermiculite has a high cation exchange capacity and a high specific surface area, which enable it to bind nutrients available for plant uptake. Despite these properties, *S. oleracea* yield on vermiculite-amended (and fertilized) soils (RVF and RVF*) was reduced compared to the RF treatment (Figure 4). Several studies have shown that vermiculite has a high sorption affinity for Mn (Malandrino et al., 2006; Abollino et al., 2008), which could reduce plant-availability of Mn in added fertilizer. Vermiculite was used in previous soil remediation studies for the immobilization of PTMs (Panuccio et al., 2009; Abollino et al., 2007). In our study, however, no changes were observed in PTMs or essential metal uptake by *S. oleracea* (Figure 5). There was also no statistically significant reduction in metal leaching (except for Ca in RVF*) (Figure 3) or water retention characteristics of vermiculite amended soils (Figure 2).

3.4.4 Apatite and Slovakite

Apatite and Slovakite (commercial absorbent) are commonly used for the stabilization of PTMs in contaminated soils (Raicevic et al., 2005; Madrid et al., 2008; Tica et al., 2013). Apatite (Ca5(PO4)3) can incorporate various cations: Ca^{2+}, Ba^{2+}, Sr^{2+}, Cd^{2+}, and Pb^{2+} in its crystalline structure (Srinivasan et al., 2006) through ionic substitution and replacement, and precipitation of pyromorphite-type minerals (Kumpiene et al., 2008). Slovakite has a high sorption affinity for Pb, Zn, Cu, and Ni ions and was developed specifically for the remediation of soils contaminated with PTMs. Slovakite and apatite increased the soil pH to 7.2 and 7.6, respectively.

As shown in Table 2 and Figure 1, EDTA soil washing was not able to remove PTMs from the soil completely, and we administered apatite and Slovakite to remediated soils in an attempt to reduce the hazards of these residual PTMs. Nevertheless, neither amendment (RAF and RSF) was effective in decreasing the leaching of residual PTMs. Slovakite even slightly increased the leaching of essential Mn, Ca, and Mg. Slovakite substantially raised the pH of the soil and consequently neutralized the positive charge of soil sesquioxides with its capacity to retain part of the negatively charged metal-EDTA complexes. These were presumably detached and released into the soil solution. Additionally no beneficial effect by either amendment was observed in restraining residual PTMs form plant uptake.

Apatite was the only amendment (RAF) capable of increasing the content of plant available water (Figure 2), by significantly raising the field capacity and only slightly the wilting coefficient. Apatite, (polysulphate) can act as a soil adhesive, presumably creating soil structures more capable of capturing gravitational water. On the other hand, RAF produced the lowest yield of *S. oleracea* (Figure 4), supposedly by further immobilizing micronutrients as insoluble phosphates. The yield of *S. oleracea* cultivated in columns amended with Slovakite (RSF) was the same as on fertilized remediated soil (Figure 4).

4 Conclusion

Soil washing with high doses of EDTA efficiently removed most Pb and Cd from heavily contaminated soil, and collaterally also the essential metal Mn. These metals and Zn diminished in all soil fractions except the residual one with non-bioacessible metal species. The results clearly showed that Mn was the main essential metal whose concentration and plant availability were significantly reduced; the concentrations and fractionation of Cu, Fe, Mg, and Ca remained largely unchanged after remediation. Fertilization significantly repaired the photosynthetic rate and biomass production of *S. oleracea* grown on remediated soil, and, somewhat surprisingly, further reduced plant uptake of toxic metals. After remediation, the Pb plant concentration in most treatments was below the legislative limits, unlike the Cd concentration. Except for hydrogel, the other tested soil amendments did not improve plant growth or further reduce toxic metal emissions.

The results of our study indicate that highly contaminated soil from the Meza Valley, Slovenia, can be revitalized to support plant growth after a stringent remediation process. Other potential amendments, such as the increasingly popular biochar, will be tested to help create more functional remediated soil. Experiments are under way on using

remediated soil as a substrate for industrial, non-food-producing plants, and in horticulture, i.e., as a substrate for grasses and ornamental plants and flowers, in an effort to establish EDTA soil washing as a viable option in the paradigm of green and sustainable soil remediation.

Table 1: Design of the column experiment with amendments of manure, hydrogel, vermiculite, apatite and Slovakite and fertilizer solution.

abbreviation	soil	amendment	amount of amendment	fertilizer
O	original	-		
OF	original	-		✓
R	remediated	-		
RF	remediated	-		✓
RC	remediated	manure	50t/ha	
RCF	remediated	manure	50t/ha	✓
RH	remediated	hydrogel	0,7% (w/w)	
RHF	remediated	hydrogel	0,7% (w/w)	✓
RHF*	remediated	hydrogel	0,7% (w/w)	✓
RVF	remediated	vermiculite	3% (w/w)	✓
RVF*	remediated	vermiculite	3% (w/w)	✓
RAF	remediated	apatite	2,5%	✓
RSF	remediated	Slovakite	2,5%	✓

***** Fertilizer was mixed with amendment and only than added to the soil

Table 2: Selected pedological properties and metal concentrations in original and remediated soil. Letters "a" and "b" denote statistical significance for metal content between original and remediated soil according to Student t-test ($P < 0.05$), mean ± standard deviation ($n = 3$).

Pedological analysis	Original soil	Remediated soil
pH ($CaCl_2$)	6,7	7,1
organic matter (%)	7	7
P_2O_5 (mg $100g^{-1}$)	106	106
K_2O (mg $100g^{-1}$)	17,3	18,2
CEC ($mmol_c$ $100g^{-1}$)	29,3	36,6
Carbonates	20,3	17,0
sand (%)	42,2	34,8
silt (%)	47,7	55,3
clay (%)	10,1	9,9
Metal content (mg kg^{-1})		
Pb	^{a}4037±56	^{b}1246±50
Zn	^{a}2527±42	^{b}2138±46
Cd	^{a}26±1	^{b}11±0
Mn	^{a}828±14	^{b}392±33
Cu	^{a}53±3	^{a}51±1
Fe	^{a}31388±689	^{a}30180±28
Mg	^{a}29150±997	^{b}33549±1122
Ca	^{a}52445±4143	^{a}54975±2042

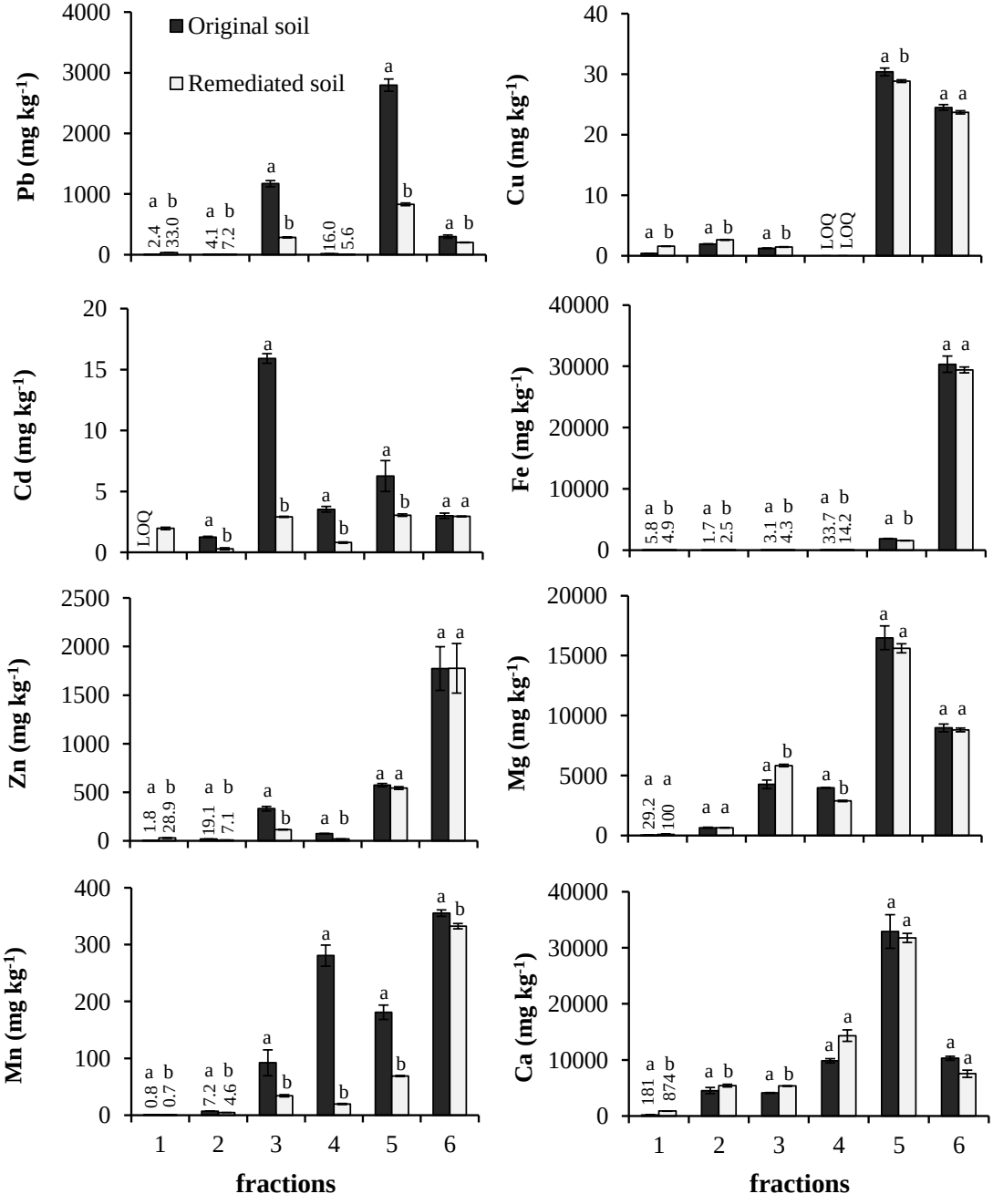


Figure 1: Sequential extraction indicated the concentrations of Pb, Zn and Cd in the soil washing solution (Fraction 1), exchangeably bound to soil colloids (Fraction 2), bound to carbonates (Fraction 3), bound to Fe- and Mn-oxides (Fraction 4), bound to soil organic matter (Fraction 5) and in the residual soil fraction (Fraction 6), for remediated soil and original soil. Error bars represent standard deviation (n=3). Letters a and b denote statistical significance between original and remediated soil according to Student t-test ($P < 0.05$). LOQ below the limit of quantification.

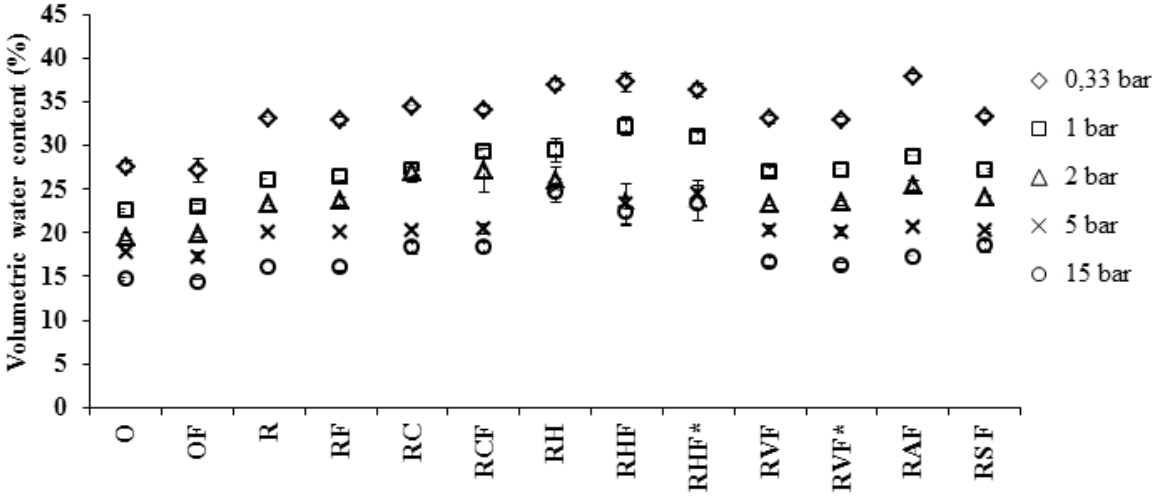


Figure 2: Volumetric water content in different soil treatments at different matric potentials. Error bars represent standard deviation (n=3).

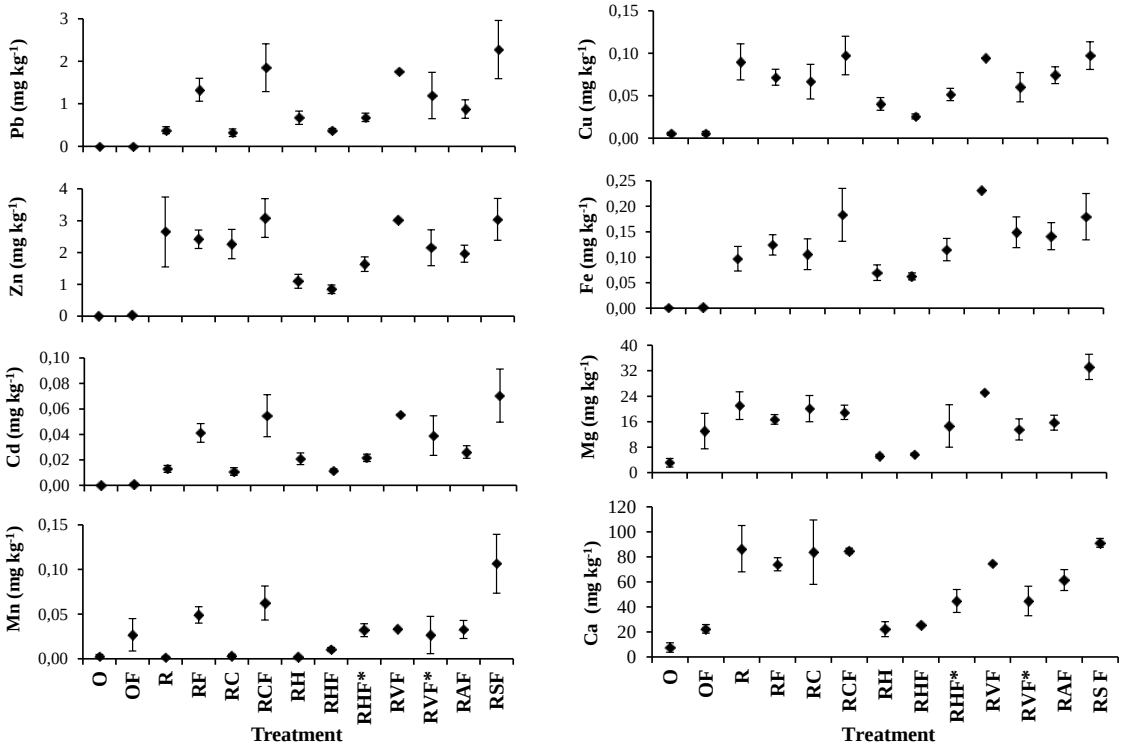


Figure 3: Concentrations of Pb, Zn, Cd, Mn, Cu, Fe, Mg, Ca in leachates set under the spinach columns with different treatments. Mean±standard deviation, n=3.

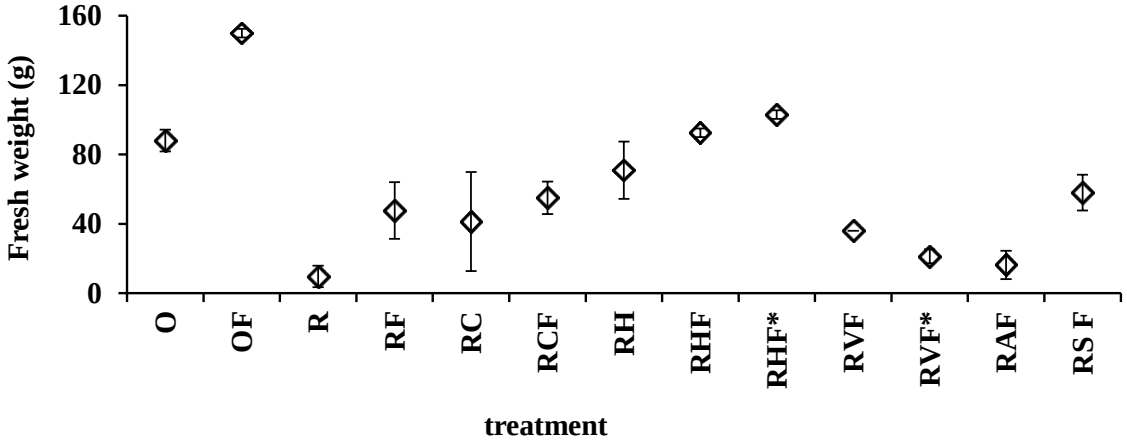


Figure 4: Fresh weight of spinach plants (*Spinacea oleracea* L.) grown on differently treated soils. Error bars represent standard deviation (n=3).

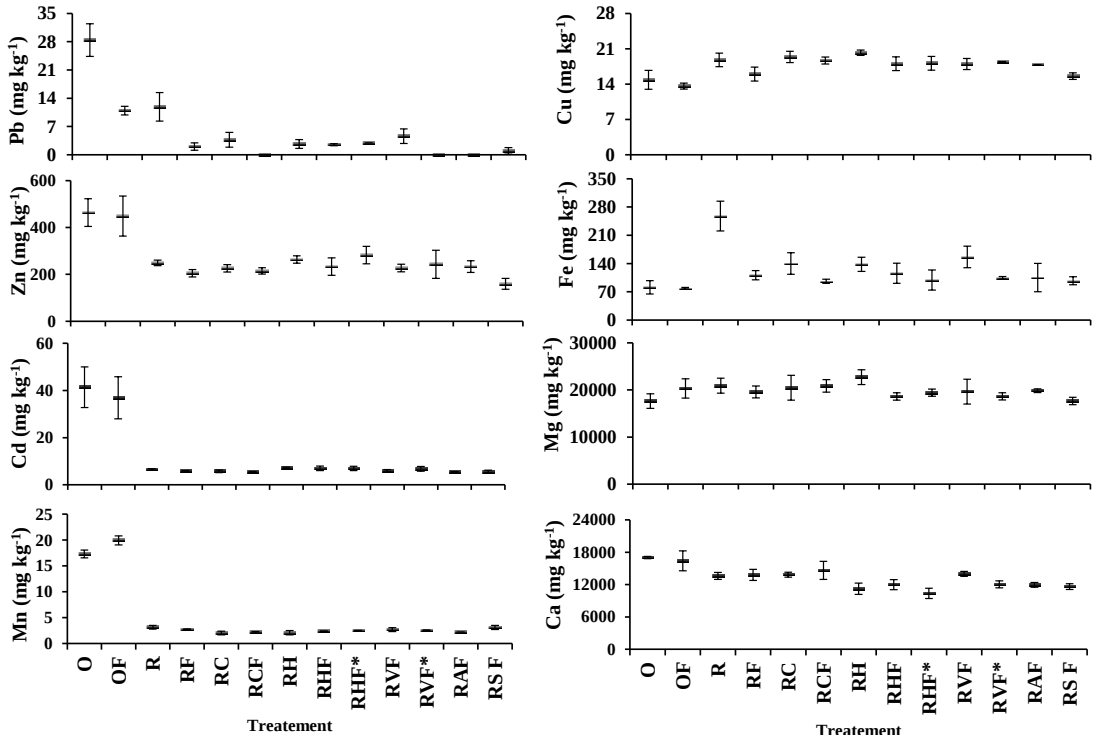
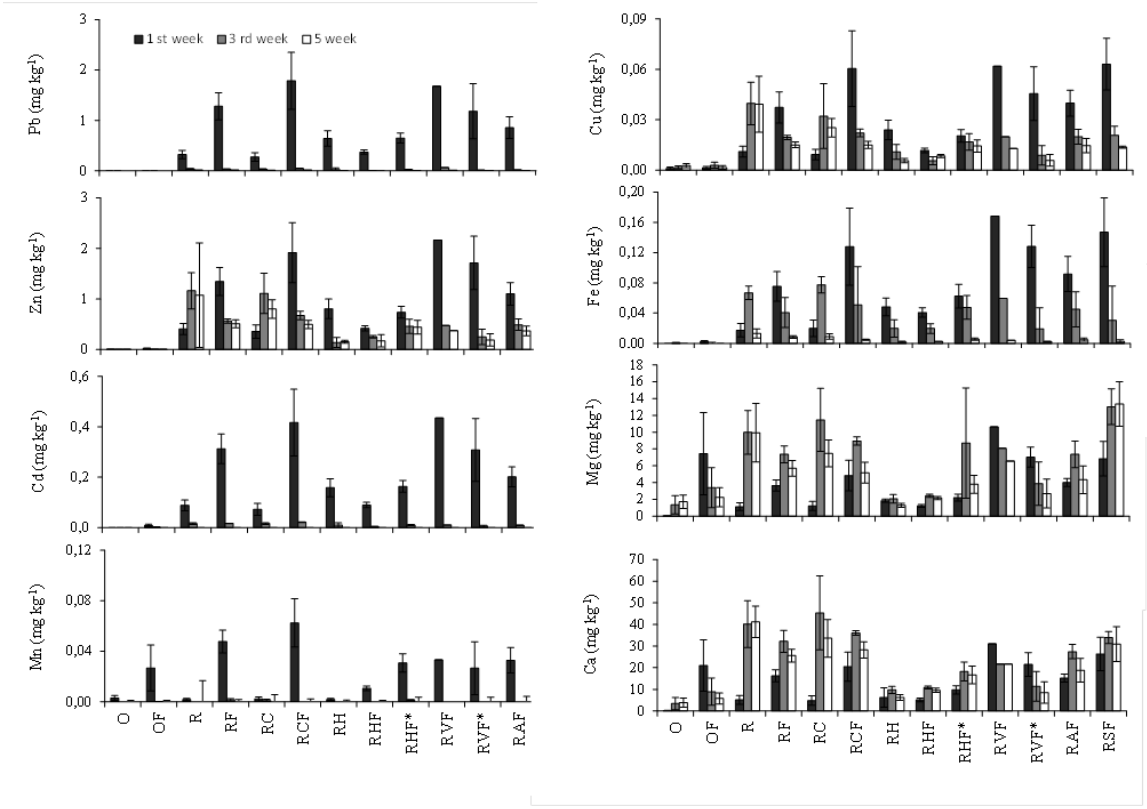


Figure 5: Concentrations of Pb, Zn, Cd, Mn, Cu, Fe, Mg, Ca in spinach (S*pinacia oleracea*) in aboveground tissue for different treatments. Mean±standard deviation, n=3.



Supplementary material: Concentrations of Pb, Zn, Cd, Mn, Cu, Fe, Mg, and Ca in the leachates collected after first week, third week and fifth week after the start of the experiment. Lysimeters were set under the spinach columns with 13 different treatments. Mean ± standard deviation, n = 3.

5 References

Abollino O., Giacomino A., Malandrino M., Mentasti E. 2007. The efficiency of vermiculite as natural sorbent for heavy metals. Application to a contaminated soil. Water, Air, and Soil Pollution, 181:149-160

Abollino O., Giacomino A., Malandrino M., Metasti E. 2008. Interaction of metal ions with montmorillonite and vermiculite. Applied Clay Science, 38: 227-236

Alloway B.J. 2013. Heavy metals in soils. Trace Metals and Metalloids in Soils and their Bioavailability. Third edition, Environmental pollution, Springer, London, 613 pp

Barker A.V., Pilbeam D.J. 2007. Handbook of plant nutrition. Taylor and Francis. 613 pp

Barona A., Aranguiz I., Elias A. 2001. Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean up procedures, Environmental Pollution, 113:79-85

Bergmann W. 1992. Nutritional disorders of Plants. Causes, development, and diagnosis of symptoms resulting from mineral element deficiency and exess. New York, Gustav Fisher, 741 pp

Brady N.C., Weil R.R. 2002. The nature and properties of soils. Chapter 5: Soil Water characteristics and behavior. Thirteenth edition. New Jersey, Pearson Education, 960 pp

Chirino E., Vilagrosa V., Vallejo V.R. 2011. Using hydrogel and clay to improve the water status of seedlings for dryland restoration. Plant and Soil, 344: 99-110

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

El-Hady O.A., El-Dewiny C.Y. 2006. The conditioning effect of composts (natural) or / and acrylamide hydrogels (synthesized) on a sandy calcareous soil 1. Growth response, nutrients uptake and water and fertilizers use efficiency by tomato plants. Journal of Applied Sciences Research, 2: 890-898

El-Hady O.A., Abo-Sedera S.A. 2006. Conditioning Effect of Composts and Acrylamide Hydrogels on a Sandy Calcareous Soil. II-Physico-bio-chemical Properties of the Soil. International Journal of agriculture and Biology, 876-884

Gleyzes C., Tellier S., Astruc M. 2002. Fractionation studies of trace elements in contaminated soils and sedments: a review of sequential extraction procedures. Trends in Analytical Chemistry, 21: 451-467

Hasegawa H., Rahman M., Saitou K., Kobayashi M., Okumura C. 2011. Influence of chelating ligands on bioavailability and mobility of iron in plant growth media and their effect on radish growth. Environmental and Experimental Botany, 71: 345-351

Haynes R.J., Naidu R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions - A review. Nutrient Cycling in Agroecosystems, 51: 123-137

Headlee W.L. Brewer C.E., Hall R.B. 2013. Biochar as a substitute for vermiculite in potting mix for hybrid poplar. Bio Eenergy research. Doi: 10.1007/s12155-013-9355-y

Husted S., Laursen K. H., Hebbern C. A., Schmidt S. B., Pedas P., Haldrup A., Jensen P. E. 2009. Manganese deficiency leads to genotype-specific changes in fluorescence induction kinetics and state transitions. Plant Physiology, 150: 825-833.

ISO 10390 (2005) Soil quality-Determination of pH. International Organization for Standardization, Genève, Switzerland

ISO 11277 (2009) Soil quality- Determination of particle size distribution in mineral soil material-Method by sieving and sedimentation. International Organization for Standardization, Genève, Switzerland

ISO 11464 (2006) Soil quality-Pretreatment of samples for physico-chemical analysis. International Organization for Standardization, Genève, Switzerland

ISO 14235 (1998) Soil Quality- Determination of Organic Carbon by Sulfochromic Oxidation. International Organization for Standardization, Genève, Switzerland

Jelusic M., Lestan D. 2014. Effect of EDTA washing of metal polluted garden soils. Part I: Toxicity hazards and impact on soil properties. Science of the Total Environment, 475: 132-141

Jelusic M., Vodnik D., Macek I., Lestan D. 2014. Effect of EDTA washing of metal polluted garden soils. Part II: Can remediated soil be used as a plant substrate? Science of the Total Environment, 475: 142-152

Jelusic M., Grcman H., Vodnik D., Suhadolc M., Lestan D. 2013. Functioning of metal contaminated garden soil after remediation. Environmental Pollution, 174: 63-70

Kalra Y.P., Maynard D.G. 1991. Methods manual for forest soil and plant analysis. Northern Forestry Centre, Edmonton: Canadian Forest Service; 1991.

Kos B., Lestan D. 2003. Influence of a biodegradable ([S,S]-EDDS) and nondegradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextraction and leaching. Plant and Soil, 253: 403-411

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

Lei M., Liao B.H., Zeng Q.R., Qin P.F., Khan S. 2008. Fraction distributions of lead, cadmium, copper, and zinc in metal-contaminated soil before and after extraction with disodium ethylenediaminetetraacetic acid. Communications in Soil Science and Plant analysis, 39: 1963–1978

Lestan D., Grcman H., Zupan M., Bacac N. 2003. Relationship of soil properties to fractionation of Pb and Zn in soil and their uptake into plantago lanceolata. Soil and Sediment Contamination, 12: 37-41

Lucena J., 2006. Iron nutrition in plants and rhizospheric microorganisms. Chapter: Synthetic iron chelates to correct iron deficiency in plants. Netherlands, Springer, 103-128 pp

Madrid F., Díaz-Barrientos E., Florido M.C. 2008. Inorganic amendments to decrease metal availability in soils of recreational urban areas: limitations to their efficiency and possible drawbacks. Water Air and Soil Pollution, 192: 117-125

Malandrino M., Abollino O., Giacomino A., Aceto M., Mentasti E. 2006. Adsorption of heavy metals on vermiculite: Influence of pH and organic ligands. Journal of Colloid and Interface Science, 299: 537-546

Martell A.E., Smith R.M. 2003. NIST Critically selected stability constants of metal complexes, 7th ed. Gaithersburg

Narjary B., Aggarwal P., Singh A., Chakraborty D., Singh R. 2012. Water availability in different soils in relation to hydrogel application. Geoderma, 187-188: 94-101

Orikiriza L.J.B., Agaba H., Tweheyo M., Eilu G., Kabasa J.D., Huttermann A. 2009. Amending soils with hydrogels increases the biomass of nine tree species under non-water stress conditions. Clean, 37: 615-620

Marschner P. 2012. Marschner's mineral nutrition of higher plants. Third Edition, Elsevier, Amsterdam, 651 pp.

Muller D.H., Oort F., Gelie B., Balabane M. 2000. Strategies of heavy metal uptake by three plant species growing near metal smelter. Environmetal Pollution, 109: 231-238

Panuccio M.R., Sorgona A., Rizzo M., Cacco G. 2009. Cadmium adsorption on vermiculite, zeolite and pumice: Batch experimental studies. Journal of Environmental Management, 90: 364-374

R Development Core Team. 2010. A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria, ISBN 3- 900051-07-0. http://www.R-project.org

Rahman I.M.M., Hossain M.M., Begum Z.A., Rahman M.A., Hasegawa H. 2010. Eco-environmental consequences associated with chelant-assisted phytoremediation of metal-

contaminated soil. Handbook of Phytoremediation. Nova Science Publishers, New York, pp., 709–722

Raicevic S., Kaludjerovic-Radoicic T., Zouboulis A.I. 2005. In situ stabilization of toxic metals in polluted soils using phosphates: theoretical prediction and experimental verification. Journal of Hazardous Materials, B117: 41-53

Salaskar D., Shrivastava M., Kale S.P. 2011. Bioremediation potential of spinach (*Spinacia oleracea* L.) for decontamination of cadmium in soil. Current Science, 101: 1359-1363

Seaborn D.J., Jameson G.J. 1976. Some ion exchange properties of exfoliated vermiculite. Hydrometallurgy, 2: 141-155

Schreiber U., Bilger W., Neubauer C. 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In Schulze E.-D., Caldwell (Eds.) Ecophysiology of Photosynthesis. Springer, Berlin, Heidelberg, New York, 49-70.

Srinivasan M., Ferraris C., White T. 2006. Cadmium and lead ion capture with three dimensionally ordered macroporous hydroxyapatite. Environmental Science and Technology, 40: 7054-7059

Sullivan M.F.O., Ball B.C. 1993. The shape of the water release characteristic as affected by tillage, compaction and soil type. Soil and Tillage Research, 25: 339-349

Tica D., Udovic M., Lestan D. 2013. Long-term efficiency of soil stabilization with apatite and Slovakite: the impact of two earthworm species (Lumbricus terrestris and Dendrobaena veneta) on lead bioaccessibility and soil functioning. Chemosphere, 91: 1-6

Tsang D.C.W., Zhang W.H., Lo I.M.C. 2007. Copper extraction effectiveness and soil dissolution issues of EDTA-flushing of contaminated soils. Chemosphere, 68: 234–243

Udovic M., McBride M.B. 2011. Influence of compost addition on lead and arsenic bioavailability in reclaimed orchard soil assessed using Porcellio scaber bioaccumulation test. Journal of Hazardous Materials, 205-206: 122-149

US Environmental Protection Agency (US EPA). 2010. Archive of July 12, 2010 seminar: US and EU perspectives on green and sustainable remediation. http://www.cluin.org/consoil/.

Venäläinen S.H. 2011. Apatite ore mine tailings as an amendment for remediation of a lead-contaminated shooting range soil. Science of The Total Environment, 409: 4628-4634

Voglar D., Lestan D. 2014. Chelant soil-washing technology for metal-contaminated soil. Environmental Technology, 35: 1389-1400

Zeremski-Skoric T.M., Sekulic P.D, Maksimovic I.V., Seremesic S.I., Ninkov J.M., Milic S.B., Vasin J.R. 2010. Chelate-assisted phytoextraction: effect of EDTA and EDDS on copper uptake by Brassica napus L. Journal of Serbian Chemical Society, 75: 1279-1289

Zhang W., Huang H., Tan F., Wang H., Qui R. 2010. Influence of EDTA washing on the species and mobility of heavy metals residual in soils. Journal of Hazardous Materials, 173: 369-76

Zupanc V., Kastelec D., Lestan D., Grcman H. 2014. Soil physical characteristics after EDTA washing and amendment with inorganic and organic additives. Environmental Pollution, 186: 56-62

2.2.2 Remediacija in obnovitev s težkimi kovinami močno onesnaženih tal kot substrat za ozelenitev z okrasnimi rastlinami in travami

JELUŠIČ Maša, LEŠTAN Domen

Remediation and reclamation of soils, heavily contaminated with toxic metals, as a substrate for greening with ornamental plants and grasses

Tla močno onesnažena s strupenimi kovinami se trenutno obravnavajo kot nevarni odpadek, navkljub njihovi potencialni rodovitnosti. Tla onesnažena s 4037 Pb mg kg^{-1}, 2527 Zn mg kg^{-1} in 26 Cd mg kg^{-1} so bila očiščena s tehnologijo pranja tal z EDTA z recikliranjem vode in liganda. Z visokim odmerkom EDTA, 120 mmol kg^{-1} tal, so se zmanjšale koncentracije strupenih kovin za 70 % Pb, 15 % Zn in 58 % Cd. Zmanjšala se je tudi biodosegljivost Pb pod nivo kvantifikacije ter Zn in Cd za 3.2-krat. Onesnažena in remediirana tla so bila v poljskem poskusu položena v dve vrtni gredici (4 x 1 x 0,3 m), opremljeni z lizimetri, in podvržena kultivaciji okrasnih rastlin *Impatiens walleriana*, *Tagetes erecta*, *Pelargonium* × *peltatum* in *Verbena* × *hybrida* ter trav: *Dactylis glomerata*, *Lolium multiflorum* in *Festuca pratensis*. Rastline gojene na remediiranih tleh so pokazale večji ali enak pridelek in manjšo vsebnost potencialno strupenih kovin v primerjavi z rastlinami gojenimi na originalnih tleh. Rezultati nakazujejo, da z EDTA remediiacijo pridobimo tla primerna za revegetacijo onesnaženih območij z okrasnimi rastlinami in travami.

Remediation and reclamation of soils heavily contaminated with toxic metals as a substrate for greening with ornamental plants and grasses

Masa Jelusic, Domen Lestan
Agronomy Department, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana; Slovenia

Correspondence:
Domen Lestan
Centre for Soil and Environmental Science
Biotechnical Faculty, University of Ljubljana
Jamnikarjeva 101
1000 Ljubljana, Slovenia

tel.: +386 1 3203 162
fax.: +386 01 256 57 82
E. mail.: domen.lestan@bf.uni-lj.si

Abstract

Soils highly contaminated with toxic metals are currently treated as waste despite their potential inherent fertility. We applied EDTA washing technology featuring chelant and process water recovery for remediation of soil with 4037, 2527, and 26 mg kg^{-1} of Pb, Zn and Cd, respectively in a pilot scale. A high EDTA dose (120 mmol kg^{-1} of soil) removed 70%, 15%, and 58% of Pb, Zn, and Cd, respectively, and reduced human oral bioaccessibility of Pb below the limit of quantification and that of Zn and Cd 3.2 times. In a field experiment, the contaminated and remediated soils were laid into two garden beds (4 x 1 x 0.3 m) equipped with lysimeters, and subjected to cultivation of ornamental plants: *Impatiens walleriana*, *Tagetes erecta*, *Pelargonium* × *peltatum,* and *Verbena* × *hybrida* and grasses: *Dactylis glomerata*, *Lolium multiflorum*, and *Festuca pratensis*. Plants grown on remediated soil demonstrated the same or greater biomass yield and reduced the uptake of Pb and, Zn up to 10, 2.5 and 9.5 times, respectively, compared to plants cultivated on the original soil. The results suggest that EDTA remediation produced soil suitable for greening.

Keywords: soil remediation, potentially toxic metals, plant uptake, reclamation of polluted areas, horticulture.

1 Introduction

Healthy and fertile soil is a limited non-renewable resource, vanishing ever more quickly because of numerous human degrading activities. Urbanization, industrialization, traffic, and improper agricultural practices have left the soils of the world vulnerable and unprotected. One of the aspects of soil degradation is contamination and the most prevalent pollutants in Europe are mineral oils and potentially toxic trace metals (PTMs), contributing 60% of soil contamination. As of today, more than 2.5 million sites in Europe are potentially contaminated [1] and numbers are expected to increase by 50% by 2025 [1].The problem is exacerbated by the slow soil-forming process where a mere 10 cm of fertile soil is formed in as much as 2000 years [2]. The rate of soil deterioration is faster than soil-forming processes and the resolution of the problem with various soil remediation techniques is also lagging behind with only about 80 000 polluted sites having been remediated thus far [1].

Therefore, efficient, economic, and non-soil-destructive remediation techniques are needed to remedy and reclaim the polluted areas. Soil remediation needs not only to relieve the soil of potential hazards/pollutants, but also to re-establish a healthy ecosystem capable of supporting flora, fauna, and microbial life. EDTA soil washing has the prospect of becoming a green and sustainable soil remediation technique, as it is able to substantially decrease PTMs concentrations in various soils without much interference into the soil's pedological properties [3]. We have thus recently demonstrated the technical and economic feasibility of EDTA-based soil washing technology for remediation of PTMs contaminated soils [4] and, in a field experiment, tested the remediated soil for agricultural use [5]. In medium contaminated soil with 1585 mg kg^{-1} of Pb as the main pollutant, the levels of PTMs in the crops dropped substantially after remediation and human hazard tests demonstrated metal concentrations below the limit of quantification.

However, when working with highly contaminated soil, even high-dosage EDTA soil washing still leaves to high residual contamination for the remediated soil to be permissible for food crop cultivation. In the past, a simple soil "dig and dump" was seen as an effective solution, but is now considered unsustainable and increasingly more difficult because of to the high cost and rigorous landfilling conditions. Is there potential to reclaim even highly polluted areas (with inherently fertile soils) by applying remediation and alternative uses of soil in horticulture, such as for ornamental plant cultivation and greening?

To the best of our knowledge, this is the first report on the reclamation of highly contaminated soil by chemical extraction for potential use in horticulture. Different grass species are otherwise commonly used for revitalizing of degraded areas. Meeinkuirt et al. [6], for instance, demonstrated the potential of grasses for phytostabilization in soils

contaminated with Pb tailings. Recently, ornamental flowers were tested as an option for phytoremediation of polluted areas, since many of them were found to have hyperaccumulator properties [7,8].

In the present study, soil highly contaminated with Pb, Zn, and Cd from the vicinity of a former Pb smelting plant was washed with a high EDTA dose in a pilot-scale remediation plant. The remediated soil was fertilized and amended with hydrogel to repair its physical properties damaged in the process, and subjected to a selection of ornamental plants and grass coverage in experimental plots. Potential human hazards of PTMs remaining in the soil after remediation, plants yields, and the uptake of PTMs were assessed. The consequences of PTMs presence in grass and flower cuttings for composting (an integral part in horticultural practice) were discussed.

2 Materials and methods

2.1 Soil properties

Soil was collected from the upper 40 cm of an abandoned garden in the vicinity of a former mine and Pb smelting plant in the city of Zerjav in Meza Valley, Slovenia, EU (x=489300, y=152300, Gauss-Kruger coordinate system). Soil samples were air-dried and sieved to 2 mm [9]. The soil's pH was measured in a 1/2.5 (w/v) ratio of soil and 0.01 M $CaCl_2$ suspension [10]. Cation exchange capacity (CEC) was determined after soil extraction with ammonium acetate (pH 7), organic matter with modified Walkley-Black titrations [11], and soil texture was analyzed with the pipette method [12]. Easily extractable P (P_2O_5) and K (K_2O) were measured colorimetrically according to the Kalra and Maynard method [13]. The following properties were obtained for the original and remediated soils: pH 6.7 and 7.1, 800 and 800 mg kg^{-1} P_2O_5, 17.3 and 18.2 mg kg^{-1} K_2O, 29.3 and 36.6 CEC ($mmol_c$ $100g^{-1}$), 7% and 7% of organic matter, respectively.

2.2 Pilot-scale soil remediation

Soil was chelant-washed as reported by Voglar and Lestan (2014), using 120 mmol EDTA kg^{-1} of dry soil. Briefly, 60 kg of soil per batch were extracted with 60 L of recycled EDTA solution (20% of fresh Na_2EDTA was added to each batch to replace process losses) in a concrete mixer for 2 hours. After extraction, the soil suspension was separated from the spent soil-washing solution in a chamber filter press, and the soil was rinsed within the press with pressure-cleansed process water and tap water to remove all EDTA-mobilized toxic metal species. The EDTA in the used washing solution was first recycled as Ca-EDTA after the substitution of PTMs in the EDTA complex with Ca in alkaline conditions (pH > 12) created by the addition of lime ($Ca(OH)_2$), and by acidic precipitation (pH 1.8-2.2) created by the addition of H_2SO_4. The precipitation of insoluble $CaSO_4$ prevented the

build-up of the added reagents after multiple remediation batches. For soil rinsing, the process solution was cleansed in an electrolytic step for a complete recycling of the process water.

Twenty batches in the soil-washing facility were needed to prepare a sufficient quantity (1100 kg) of remediated soil for a field experiment. No waste-water was generated along with a total of 30 kg of solid waste composed of precipitated PTMs, lime, and electro-corroded graphite. Before disposal, solid waste was bitumen stabilized to prevent the leaching of PTMs.

2.3 Field experiment and soil sampling

In a field experiment, two garden beds (4 x 1 x 0.3 m) were assembled and filled, with remediated and original soils, respectively. Both beds were fertilized with 50 kg ha^{-1} of N as NH_4NO_3 and 3 t ha^{-1} of complex organic substrate-pellets (pH 11.9, organic matter 735 g kg^{-1}, organic C 312 g kg^{-1}, total N 21.2 g kg^{-1}, K 16.5 g kg^{-1}, and P 10 g kg^{-1}). On the remediated garden bed only, 100 g m^{-2} of conditioner hydrogel (acrylamide sodium acrylate copolymer) and 20 kg ha^{-1} of Mn as $MnSO_4$ were also added. In April 2013, four ornamental plants (*Impatiens walleriana*, *Tagetes erecta*, *Pelargonium* × *peltatum* and *Verbena* × *hybrid*) were planted and cultivated for a period of 11 weeks (Supplementary material). In July 2013, both garden beds were thoroughly shuffled and fertilized with 100 kg ha^{-1} of K as KNO_3 and 100 kg ha^{-1} of N as NH_4NO_3. Afterwards, three different grass cultivars (*Dactylis glomerata*, *Lolium multiflorum* and *Festuca pratensis*) were seeded and cultivated for 8 weeks (Supplementary material). The ten-year average temperature for the location of the field experiment with the continental climate was 10.9 ◦C, with a peak in July and August (21 ◦C), a minimum in January (0.8 ◦C), and a total annual precipitation of 1350 mm.

One lysimeter was placed under the original soil and three under the remediated soil (Supplementary material). Samples from each of the four lysimeters were taken after each substantial precipitation during the initial phase of cultivation. The volume of lysimeter water was measured after the leachates had been collected. Samples were then filtered through Whatman no. 4 filter paper and stored at 5 °C until PTMs concentrations in the leachates were determined.

Soil samples for total metal analysis and pedological analysis were collected at the beginning of the experiment. For the UBM analysis, samples were collected after the harvest of ornamental flowers and before grass seeding. Each soil sampling was conducted in three replicates. One replicate consisted of ten thoroughly mixed subsamples collected from randomly selected points (depth 5-10 cm).

2.4 Soil and plant metal determination

For metal determination, air-dried soil samples (2 mm fraction) were ground in an agate mill, sieved to 250 µm, and digested in *aqua regia* solution, consisting of HCl and HNO_3 in a 3:1 ratio (v/v). The samples were then filtered through Whatman no. 4 filter paper and diluted with deionized water to a total volume of 25 mL. The reference material used in inter-laboratory comparisons was WEPAL 2003 and 2004 (Wageningen University, Wageningen, Netherlands). The average metal concentrations for Pb, Zn, Cd, Mn, Cu, Fe, Mg and Ca were 4037, 2527, 26, 828, 53, 31388, 29150, 52445, and 7949 for the original soil and 1246, 2138, 11, 392, 51, 30180, 33549, and 54975 for the remediated soil, mg kg^{-1} of dry soil, respectively.

The plants were harvested, the roots and green parts were separated and then thoroughly washed. Grass roots were too fragile and intertwined to efficiently rinse the dirt and only the aboveground parts were thus analyzed. The plant samples were dried at 60 °C to a constant weight and ground in a titanium centrifugal mill. The ground plant tissues (250-300 mg dry weight) were then submitted to acid-digestion (65% HNO_3) with microwave heating and left to cool. Diluted with deionized water to a volume of 10 or 25 mL, they were stored at 4 °C until metal analysis. The reference material used in inter-laboratory comparisons Alva 2009/1 (Pflanzen und Futtermittelenquete 2009, Raumberg-Gumpenstein) was used in digestion and analysis.

Flame (acetylene/air) atomic absorption spectrometry (AAS, Varian AA240FS) was used for the soil and plant metal analysis. The metals Pb, Zn, Cd, Mn, Cu, Fe, Mg, and Ca were analyzed by flame (acetylene/air) atomic absorption spectrometry (AAS, Varian AA240FS). The limits of quantification (LOQ) as given by the manufacturer were 0.1, 0.01, 0.02, 0.02, 0.03, 0.06, 0.003, and 0.01 mg L^{-1} for Pb, Zn, Cd, Mn, Cu, Fe, Mg, and Ca, respectively. All samples were measured in triplicate (one row, one replicate), reagent blank and analytical duplicates were used to ensure the accuracy and precision of the analysis.

2.5 Transfer factors

To analyze the transport of metals form the soil to the roots, from the roots to aboveground tissue, and their translocation from thr soil to aboveground tissue, three transfer factors were calculated.

$$TF_{RS} = \frac{C(roots)}{C(soil)}$$

$$TF_{GR} = \frac{C(green\ parts)}{C(roots)}$$

$$TF_{GS} = \frac{C(green\ parts)}{C(soil)}$$

C (roots) and G (green parts) represent the concentration of PTMs in different plant parts, roots and aboveground parts (stem, leaves and buds). S (soil) represents the soil total metal concentration of PTMs.

2.6 Oral bioaccessibility of PTMs

For evaluating the human hazard, a modified unified bioaccessibility method (UBM) was applied according to Wragg et al. [14] and the bioaccessibility research group of Europe (BARGE). Four digestive fluids: saliva, gastric, duodenal, and bile were made for the test. Each solution was a combination of organic and inorganic solutions and specific enzymes. Approximately 0.6 g of soil was weighed directly into polycarbonate tubes, to which 9 mL of simulated saliva (pH 6.5 ± 0.5) was added. The suspension was manually shaken and adjusted with HCl or NaOH to pH 1.20 ± 0.05 before a simulated gastric solution (13.5 mL) of pH 0.9-1.0 was added. The extraction vessel was then placed in an end-over-end shaker in a thermostatically controlled water bath at 37 °C, thus simulating the stomach (pH 1.2-1.7). After 1 hour it was checked whether the pH<1.50, if not, the procedure was restarted from the beginning with special insistence on pH stability of 1.20 ± 0.05 every 15 min. Next, to simulate the intestinal phase (pH 6.3 ± 0.5), 27 mL of duodenal (pH 7.4 ± 0.2) and 9 mL of bile (pH 8.0 ± 0.2) solutions were added and the tubes were returned to the water bath for a further 4 hours. The vessels were then centrifuged for 20 min at 4500 g and supernatant was collected by careful pipetting and stored at 4 °C until metal analysis.

2.7 Statistical analysis

To analyze the differences between the remediated and original soils for various parameters, student t-test was employed. T-test was thus applied to assess the difference between the biomass of the tested plants, PTMs uptake in the roots and green parts, transfer factors, and bioavailability assessed with a UBM test. Statistical analysis was carried out with the R program [15]. Differences between treatments were considered significant at $P < 0.05$.

3 Results and discussion

3.1 Remediation and soil toxicity hazard

The original soil remediated in this study was heavily contaminated with PTMs: 4037, 2527, and 26 mg kg^{-1} of Pb, Zn, and Cd, respectively. Washing with EDTA removed 70%, 15%, and 58% of Pb, Zn, and Cd, respectively, but also 58% of essential micronutrient Mn. The other essential elements measured (Cu, Fe, Mg, and Ca) were not significantly affected. PTMs which remain in the soil after EDTA washing mostly reside in inaccessible forms and soil fractions; however, a small pool of accessible PTMs can still persist in the matrix of the remediated soil [3,16]. The amount of these PTMs that were orally bioaccessible to humans or potentially leachable was assessed with an *in vitro* simulation of the human digestive tract using a UBM test [14] and measurements of PTMs concentrated in waters of the three lyzimeters set under the remediated garden bed (Supplementary material) .

In the original soil, 0.5 and 3.7% of the total Pb and Zn content, respectively, was orally bioaccessible (Figure 1). The oral accessibility of Cd was notably higher with 38% of the total soil metal being potentially hazardous to human health by ingestion. EDTA remediation efficiently lowered the pool of bioaccessible PTMs, with Pb even under the limit of quantification (LOQ) of the instrument (Figure 1).

Figure 2 shows the concentrations of Pb, Zn, and Cd measured in the leachates from the lysimeters set under the remediated garden beds. Even though the values of approx. 140 mg L^{-1} of Zn and Pb, and 2.1 mg L^{-1} of Cd in the first measurement seem high, it has to be noted that the total emissions of metals leached were small; approx. 1.1%, 1.0%, and 3.9% of soil for Pb, Zn, and Cd, respectively. Nevertheless, more efficient rinsing of the remediated soil with cleansed recycled process water at the end of the remediating process [17] could further reduce the unnecessary leaching of PTMs. As expected, no PTMs were leached from the original soil, since it had been permanently exposed to rain and other environmental factors in its previous environment.

Despite the reduced concentration and oral bioaccessibility of PTMs in the remediated soil (and, consequently, toxicity for humans), the residual contamination (1246, 2138, and 11 of Pb, Zn, and Cd, respectively) exceeded or was close to the critical concentration for Pb, Zn, and Cd in soils (critical concentrations: 530, 720, and 12 mg kg^{-1} respectively) stipulated by Slovenian legislature [18] and was too high to permit cultivation of edible crops.

3.2 Greening with ornamental plants

Four different ornamental plant seedlings were planted in the original and remediated soils (Supplementary material): *Verbena× hybrida* and *Impatiens walleriana*, *Tagetes erecta* and *Pelargonium* × *peltatum*, which were recently identified as effective metal

accumulators [8,19]. The plants cultivated on the remediated soil demonstrated higher than or the same yield as the plants grown on the original soils (Table 1). The largest difference, 3.5 times higher biomass, was observed in *V. × hybrida*.

One of the reasons for the higher yield presumably lies in the enrichment of the remediated soil with hydrogel and Mn fertilizer, which both improve soil properties, and, consequently plant growth [20,21,22]. Zupanc et al. [23] found that hydrogel increased the plant available water in remediated soils by 50%. Although the concentration of soil Mn in the original soil was 828 mg kg^{-1} and only 392 mg kg^{-1} in the remediated soil, the Mn added as fertilizer (20 kg Mn ha^{-1} as $MnSO_4$) was readily available to plants and could thus stimulate plant growth. Additionally, EDTA remediated soil was less toxic and harmful to plant growth; presumably free of most of the plant available PTMs [5]. The toxic effects of PTMs may thus have been the reason for depressed growth of *V. × hybrida* grown on the original soil. The plants grown in the original soil accumulated/translocated the highest concentrations of Pb in the green parts and the highest concentration of Cd in the roots (Figure 3), indicating an impaired exclusion mechanism and little tolerance to these metals. The *V. × hybrida* grown on the remediated soil absorbed significantly lower concentrations of Pb, Cd, as well as Zn in all plant parts (Figure 3) and, consequently, demonstrated higher yield (Table 1).

While plants non-tolerant to PTMs can bear various symptoms, including depressed growth [24], hyperaccumulators can successfully thrive in soils rich in PTMs. There seem to be two definitive characteristics for hyperaccumulator plants: the ability to accumulate metals in the shoots at least 100-fold (metal depending) the amount of common non-accumulator plants [25] and a transfer factor from roots to shoot (TF_{RS}) greater than 1 [19]. The hyperaccumulating ability of a specific plant differs according to various soil and environmental factors [19]. Thus, *I. waleriana*, *T. erecta* and *P. peltatum*, which were all recognized as hyperaccumulators in previous studies, did not express their full accumulator nature in the present study, not even on the original contaminated soil. The total amounts of Pb, Zn, and Cd did not exceed the concentrations needed for hyperaccumulator status (1000, 10000 and 100 mg kg^{-1}respectively) but TF_{GR} for *I. walleriana* and *T. erecta* did exceed value 1 for Zn and Cd (Table 2).

All the plants cultivated on the original soil accumulated high concentrations of Pb in their roots, but very small amounts of the metal were translocated to the stems and leaves (Figure 2). Although some *Pelargonium* cultivars are categorized with Pb hyperaccumulator characteristics [26], the cultivar used in this study was not, since both Pb and Cd concentrations in the plant tissue were the lowest measured. Arshad et al. [14] observed differences in *Pelargonium* Pb uptake in relation to various cultivars and soil contamination. In highly contaminated soils (pH 6, 39250 Pb mg kg^{-1}), shoot concentration

varied from 4000-7000 mg kg^{-1}, and in less contaminated soil (pH 8, 1830 mg kg^{-1}) from 468-1467 mg kg^{-1}.

After remediation, the root Pb concentrations significantly dropped in all plants (Figure 3), likewise the translocation factors from the soil to the roots (TF_{RS}), but TF_{GR} was elevated or remained the same (Table 2). This slightly greater translocation of Pb from the roots to the green parts in remediated soil was presumably an effect of residual EDTA chelates (complexes with Pb) left in the soil after remediation. An addition of EDTA to the soil can help promote Pb plant uptake and also stimulate the metal translocation from the roots to the shoots, as was observed in *T. ereceta* phytoextraction study by Sinhal et al. [27]. Similar chelate uptake effects, although to a greater extent, were observed in EDTA remediated soil with Chinese cabbage by Jelusic et al. [28].

The highest TF_{GR} were calculated for Cd, specifically for translocation in *I. walleriana* and *T. erecta* (Table 2). This is in accord with the results of other groups. *I. walleriana* was categorized as a hyperaccumulator of Cd with a high root-to-shoot translocation capacity and *T. erecta* as a plant with a high potential for Cd phytoremediation [8,29,30]. In all plants, the concentrations of Cd in the roots and aboveground parts significantly dropped after remediation (Figure 3). The highest TF_{RS} (0.72) was observed in *V. × hybrida.*

In the case of Zn, concentrations in the green parts of *I. walleriana*, *T.erecta*, and *V.× hybrida* and in the roots of *I. walleriana* remained unchanged after remediation. This is presumably the consequence of Zn being an essential micronutrient with active plant transport.

In all plants, the transfer factors from the soil to the green parts (TF_{GS}) did not significantly change after remediation (Table 2), although the absolute values of PTMs in the green parts (except for Zn) decreased significantly which would allow for safer disposal of plant material (discussed below).

3.3 Greening with grasses

Grasses are tolerant and adaptable plants [31] often used for revegetation of contaminated areas [32,33]. Thus, after the ornamental plant coverage and harvest, we seeded three common grass cultivars: (supplementary material) *Dactylis glomerata*, *Lolium miltiflorum* and *Festuca pratensis.*

The biomass of the cultivars did not significantly differ between the two soils, except for *L. multiflorum*, where the plants grown on the remediated soil demonstrated a twice-larger yield than the ones grown on the original soil (Table 1). Interestingly, Gorlach et al. [34] observed no such decrease in the dry mass yield of *L. multiflorum* after soil spiking with

Cd, Cu, Ni, Pb and Zn. As explained above, the yield difference presumably originated not only from the toxic effects of PTMs in the original soil, but also from more favorable conditions in the remediated soil due to the addition of Mn fertilizer and hydrogel. Other than the lower yield in *L. multiflorum*, no other visible differences in the grasses grown on the original or remediated soils were observed.

As previously reported, grasses can thrive in soils contaminated with PTMs [34,35], although (as with ornamental plants) the uptake of PTMs varies according to the soil properties and grass species. In our study, Pb concentration in all species grown on the original soil was approximately 25 mg kg^{-1} and, after remediation, dropped to 5 mg kg^{-1} (Figure 4). Zn concentration in the green parts was reduced for up to 1.9 times and, for Cd, up to 3.3 times (Figure 4). The highest Cd concentration in the grasses grown on the original soil was observed in *D. glomerata* and the lowest in *F. pratensis* (Figure 4). This was rather unexpected, since Soleimani et al. [35] had observed shoot concentrations of 100 mg kg^{-1} Cd (hyperaccumulator criteria) in *F. pratensis* grown on artificially contaminated soil. Results comparable to ours (26, 75, and 1.3 mg kg^{-1} of Pb, Zn, and Cd in shoots, respectively) with no effect on dry mass yield were for *D. glomerata* reported by Ortiz and Alcaniz [36]. They were, however, using significantly less contaminated soil (220, 674, and 2 mg kg^{-1} of Pb, Zn, and Cd, respectively). After remediation, the transfer factors remained the same or were reduced for all grasses and PTMs (Table 2).

PTMs plant translocation and accumulation is important when considering the disposal of contaminated plant material, for example by composting, which is an efficient way of reducing green waste by transforming it into stabilized material, which can be used as a soil conditioner. Several studies showed that during composting PTMs are concentrated, because of organic matter decomposition and the release of CO_2 and water [37,38,39. Sing and Kalamdhad [40] composted water hyacinths (green waste) in combination with cattle manure and sawdust, which resulted in approximately 84%, 93%, and 25% higher concentrations of Zn, Cd, and Pb, respectively, in the compost dry weight. The national (Slovenian) regulation standard for compost quality stipulates 120, 400, and 1.5 mg of Pb, Zn, and Cd, respectively, per kg of dry matter for first-class compost for non-restricted use. By approximate calculation, *V. × hybrida* grown on the original soil would exceed Pb values for composting and all the plants, except *P. × peltatum*, would exceed Cd values. In terms of the Pb and Zn concentrations in the plants grown on the remediated soils, compost made from such material would be classified in first-class. However the high Cd tissue concentrations in two hyperaccumulating ornamental plants, *I. walleriana* (9.9 mg kg^{-1}) and *T. erecta* (8.3 mg kg^{-1}), makes these two plant species unsuitable for composting even after cultivation on remediated soils.

4 Conclusion

After EDTA-based remediation of highly contaminated garden soil PTMs partly remained anchored into the soil matrix with significantly reduced availability and human toxicity hazard. Nevertheless, the residual contamination was above critical values as stipulated by legislation and the soils were therefore, not suitable for food production. An alternative coverage in the form of ornamental plants and grasses proved to be feasible. Higher or similar plant biomass yields were observed in soils remediated with EDTA washing, along with a significant reduction in the plant uptake of PTMs, even though some plants had been identified as hyperaccumulators in previous studies. We therefore suggest that EDTA soil washing has significant potential for reclaiming highly contaminated soils as a substrate for use in horticulture.

Table 1: Cultivation manner and mean ± standard deviation of biomass of cultivated ornamental plants and grasses grown on original and remediated soils.

	Days grown	Planted (m^2)	Fresh weight (g)	
Ornamental plants			original	remediated
Impatiens walleriana	73	12 seedlings	[a]943±234	[a]1285±411
Tagetes erecta	78	12 seedlings	[a]1502±147	[a]1523±34
Pelargonium x *peltatum*	77	9 seedlings	[a]903±42	[b]1048±75
Verbena x *hybrida*	72	12 seedlings	[a]233±37	[b]827±118
Grasses				
Dactylis glomerata	51	30 g of seeds	[a]636±208	[a]585±163
Lolium multiflorum	51	30 g of seeds	[a]1136±254	[b]2223±439
Festuca pratensis	51	30 g of seeds	[a]521±161	[a]638±176

[a,b] denote statistically different treatments (original and remediated) according to the Student t-test

Table 2: Pb, Zn, and Cd transfer factors calculated for ornamental plants (*Impatiens walleriana, Tagetes erecta, Pelargonium x peltatum, Verbena x hybrida)* and grasses *(Dactylis glomerata, Lolium multiflorum, Festuca pratensis)* cultivated on original and remediated soils.

	TF_{RS} (roots/soil)		TF_{GR} (green parts/roots)		TF_{GS} (green parts/soil)	
	original	remediated	original	remediated	original	remediated
Pb						
I. walleriana	[a]0,03	[a]0,03	[a]0,17	[a]0,19	[a]0,01	[a]0,01
T. erecta	[a]0,06	[b]0,02	[a]0,15	[b]0,46	[a]0,01	[a]0,01
P. peltatum	[a]0,02	[b]0,01	[a]0,25	[a]0,65	[a]0,01	[a]0,00
V. hybrida	[a]0,03	[a]0,02	[a]0,8	[a]0,5	[a]0,02	[a]0,01
D. glomerata	-	-	-	-	[a]0,01	[b]0,00
L. multiflorum	-	-	-	-	[a]0,01	[b]0,00
F. pratensis	-	-	-	-	[a]0,01	[a]0,00
Zn						
I. walleriana	[a]0,05	[b]0,07	[a]1,41	[a]1,16	[a]0,07	[a]0,08
T. erecta	[a]0,05	[b]0,04	[a]0,63	[b]1,04	[a]0,03	[a]0,04
P. peltatum	[a]0,06	[b]0,03	[a]1,0	[a]1,36	[a]0,06	[b]0,04
V. hybrida	[a]0,07	[b]0,04	[a]0,73	[a]0,97	[a]0,05	[a]0,04
D. glomerata	-	-	-	-	[a]0,07	[b]0,04
L. multiflorum	-	-	-	-	[a]0,07	[b]0,05
F. pratensis	-	-	-	-	[a]0,11	[a]0,08
Cd						
I. walleriana	[a]0,46	[b]0,8	[a]1,97	[a]1,26	[a]0,01	[a]0,96
T. erecta	[a]0,42	[a]0,46	[a]1,46	[a]1,75	[a]0,61	[b]0,81
P. peltatum	[a]0,25	[b]0,06	[a]0,07	[b]0,3	[a]0,02	[a]0,02
V. hybrida	[a]0,72	[a]0,6	[a]0,19	[a]0,24	[a]0,12	[a]0,14
D. glomerata	-	-	-	-	[a]0,21	[b]0,15
L. multiflorum	-	-	-	-	[a]0,09	[a]0,08
F. pratensis	-	-	-	-	[a]0,07	[a]0,07

[a,b] denote statistically different treatments (original and remediated) according to the Student t-test.

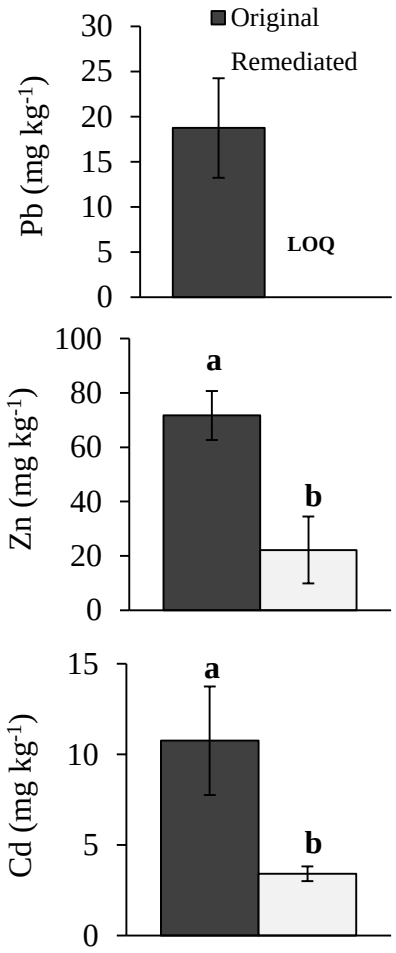


Figure1: Concentrations of Pb, Zn, and Cd in the intestinal phase of the UBM test in original and remediated soils. Mean ± standard deviation (n = 3). The letters *a* and *b* represent the statistical difference between original and remediated soils assessed with Student t-test. LOQ represents below the limit of quantification measurement of AAS (AAS_{LOQPb} 0.1 mg L^{-1}), which when recalculated for Pb, amounted for 9.8 mg kg^{-1} dry soil.

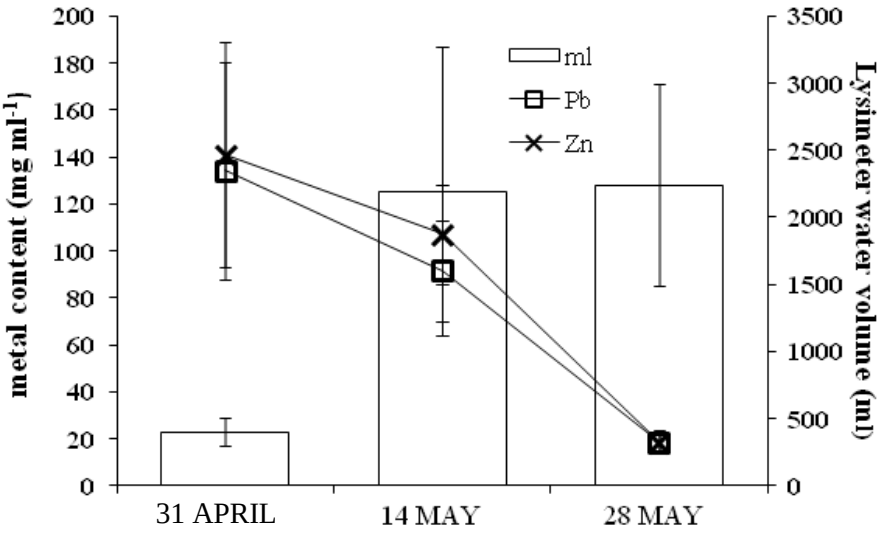


Figure 2: Concentrations of Pb, Zn, and Cd (mg kg^{-1}) and amount of water in leachates (mL) from lysimeters set under the remediated garden bed during the field experiment. The error bars represent standard deviation (n = 3).

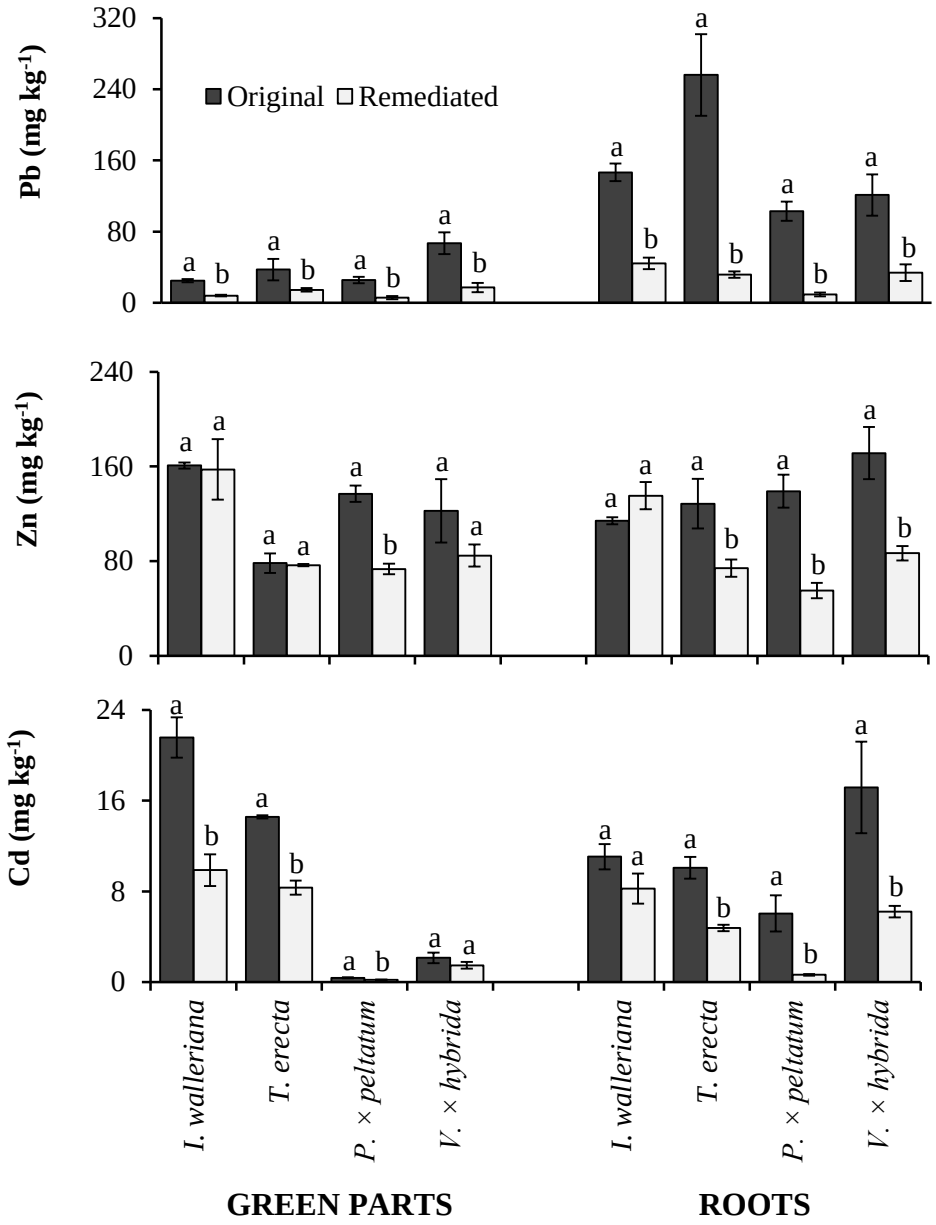


Figure 3: Concentrations of Pb, Zn, and Cd (mg kg^{-1} per dry plant weight) measured in roots and green parts of ornamental plants (I*mpatiens walleriana, Tagetes erecta, Pelargonium × peltatum, verbena × hybrida*). The error bars represent standard deviation (n = 3). The letters *a* and *b* denote significant differences between treatments according to the Student t-test ($p < 0.05$).

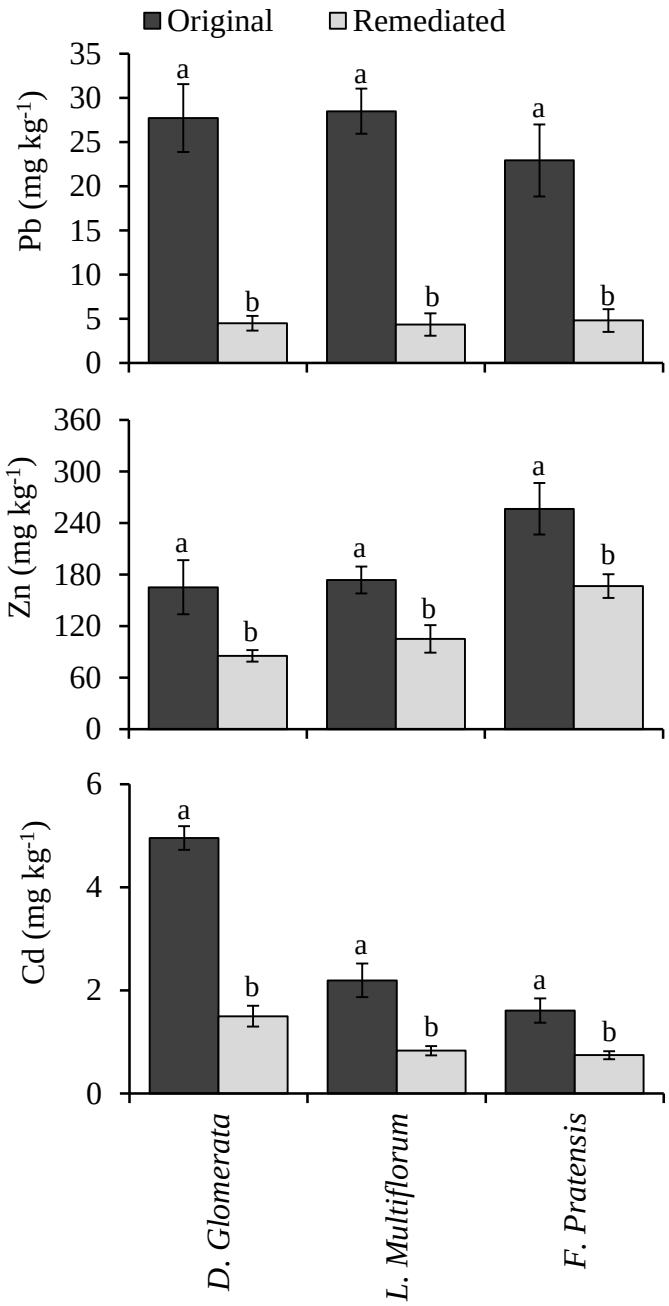
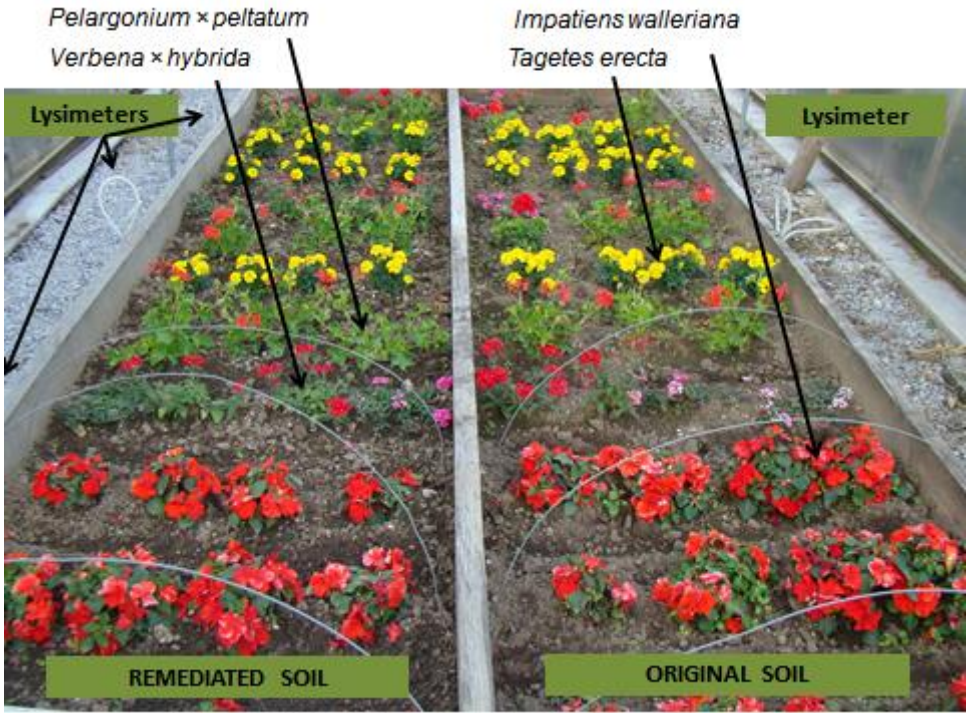
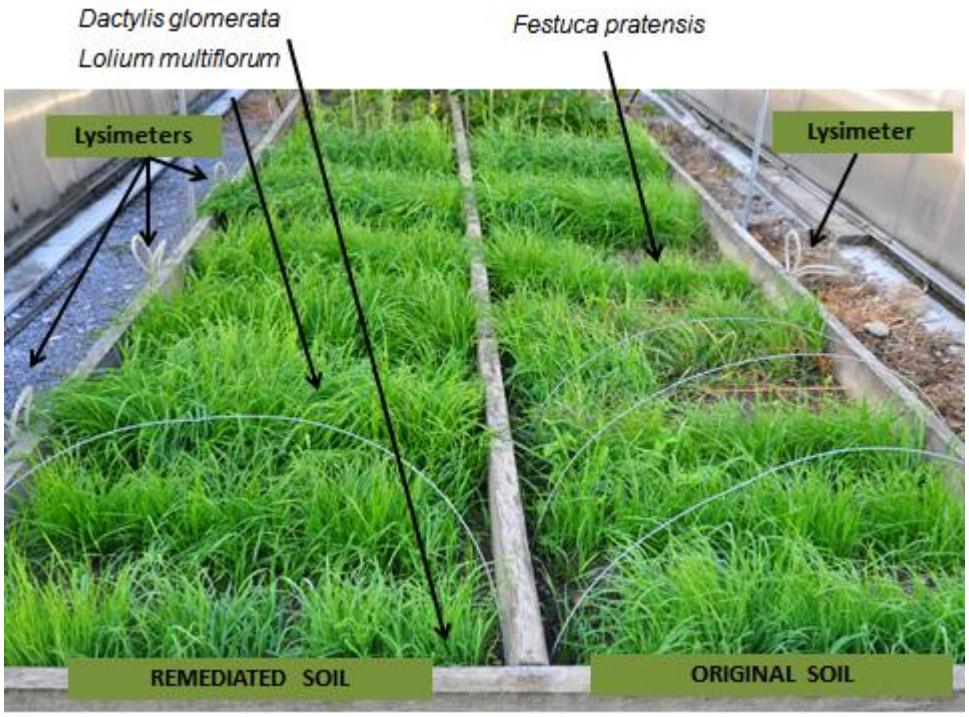


Figure 4: Concentrations of Pb, Zn, and Cd measured in green parts of grasses (*Dactylis glomerata, Lolium multiflorum*, and *Festuca pratensis*). The error bars represent standard deviation (n=3). The letters a and b denote significant differences between treatments according to the Student t-test ($p < 0.05$)

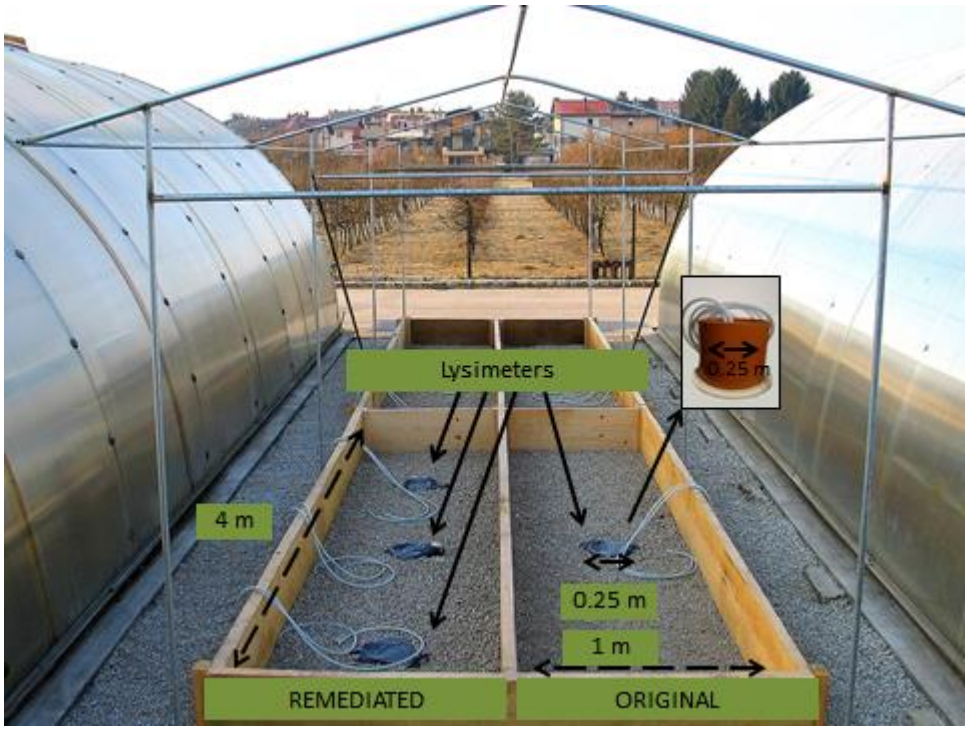
A


Pelargonium × peltatum
Verbena × hybrida
Impatiens walleriana
Tagetes erecta
Lysimeters
Lysimeter
REMEDIATED SOIL
ORIGINAL SOIL

B


Dactylis glomerata
Lolium multiflorum
Festuca pratensis
Lysimeters
Lysimeter
REMEDIATED SOIL
ORIGINAL SOIL

C



Supplementary material: original and remediated garden bed: A ornamental flowers, B grasses, C position of lysimeters.

5 References

[1] EEA (2007), European Environment Agency, 2007. Progress in management of contaminated sites (CSI 015) Copenhagen: European Environment Agency. Available at: http://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites/progress-in-management-of-contaminated-1

[2] IAAS. 2013. Global soil forum. Fertile soils: Fundamental in the struggle against hunger and climate change! Institute for advanced sustainability studies (IASS) Postdams. Available at: http://globalsoilweek.org/wp-content/uploads/2013/10/GSW_factsheet_Fertile-Soils_en.pdf 19.12.2013

[3] M. Jelusic, D. Lestan, Effect of EDTA washing of metal polluted garden soils. Part I: Toxicity hazards and impact on soil properties, Sci. Total Environ. 475 (2014) 132-141.

[4] D. Voglar, D. Lestan, Pilot-scale washing of metal contaminated garden soil using EDTA J. Hazard. Mater. 215-216 (2012) 32-39.

[5] M. Jelusic, D. Vodnik, I. Macek, D. Lestan, Effect of EDTA washing of metal polluted garden soils. Part II: Can remediated soil be used as a plant substrate? Sci. Total Environ. 475 (2014) 142-152.

[6] W. Meeinkuirt, M. Kruatrachue, P Tanhan, R. Chaiyarat, P. Pokethitiyook, Phytostabilization potential of Pb mine tailings by two grass species, *Thysanolaena maxima* and *Vetiveria zizanioides*, Water Air Soil Pollut. 224 (2013) 1-12.

[7] M. Bosiacki, Phytoextraction of cadmium and lead by selected cultivars of *Tagetes erecta* L. Part II contents of Cd and Pb in plants, Acta Sci. Pol-Hortoru. 8 (2009) 15-26.

[8] C.C. Lin, H.Y. Lai, Z.S. Chen, Bioavailability assessment and accumulation by five garden flower species grown in artificially cadmium-contaminated soils, Int. J. Phytoremediat. 12 (2010) 454-467.

[9] ISO 11464 (2006), Soil quality-Pretreatment of samples for physico-chemical analysis. International Organization for Standardization, Genève, Switzerland.

[10] ISO 10390 (2005), Soil quality-Determination of pH. International Organization for Standardization, Genève, Switzerland.

[11] ISO 14235 (1998), Soil Quality- Determination of Organic Carbon by Sulfochromic Oxidation. International Organization for Standardization, Genève, Switzerland.

[12] ISO 11277 (2009), Soil quality- Determination of particle size distribution in mineral soil material-Method by sieving and sedimentation. International Organization for Standardization, Genève, Switzerland.

[13] Y.P. Kalra, D.G. Maynard, Methods manual for forest soil and plant analysis. Northern Forestry Centre, Edmonton: Canadian Forest Service, 1991.

[14] J. Wragg, M. Cave, N. Basta, E. Brandon, S. Casteel, S. Denys, C. Gron, A. Oomen, K. Reimer, K. Tack, T.V.D. Wiele, An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil, Sci. Total Environ. 409 (2011) 4016-30.

[15] R Development Core Team, A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria, ISBN 3- 900051-07-0. http://www.R-project.org 2009.

[16] W. Zhang, H. Huang, F. Tan, H. Wang, R. Qiu, Influence of EDTA washing on the species and mobility of heavy metals residual in soils, J. Hazard. Mater. 173 (2010) 369-76.

[17] D. Voglar, D. Leštan, Chelant soil-washing technology for metal-contaminated soil. Environ. Technol. 35 (2014) 1389-1400.

[18] Decree on limit values, alert thresholds and critical levels of dangerous substances into the soil, 1996, Uradni list RS, št. 68/96 in 41/04 - ZVO-1.
http://www.pisrs.si/Pis.web/pregledPredpisa?id=URED114

[19] M. Arshad, J. Silvestre, E. Pinelli, J. Kallerhoff, M. Kaemmerer, A. Tarigo, M. Shahid, M. Guiresse, P. Pradere, C. Dumat, A field study of lead phytoextraction by various scented Pelargonium cultivars, Chemosphere. 71 (2008) 2187-2192.

[20] O.A. El-Hady, C.Y. El-Dewiny, The conditioning effect of composts (natural) or / and acrylamide hydrogels (synthesized) on a sandy calcareous soil 1. Growth response, nutrients uptake and water and fertilizers use efficiency by tomato plants, J. Appl. Sci. Res. 2 (2006) 890-898.

[21] O.A. El-Hady, S.A. Abo-Sedera, Conditioning Effect of Composts and Acrylamide Hydrogels on a Sandy Calcareous Soil. II-Physico-bio-chemical Properties of the Soil, Int. J. Agr. Biol. 2 (2006) 876-884.

[22] M. Laszlo, Manganese requirement of sunflower (Helianthus annuus L.), tobacco (Nicotiana tabacum L.) and triticale (x Triticosecale W.) at early stage of growth, Eur. J. Agron. 28 (2008) 586-596.

[23] V. Zupanc, D. Kastelec, D. Lestan, H. Grcman, Soil physical characteristics after EDTA washing and amendment with inorganic and organic additives, Environ. Pollut. 186 (2014) 56-62.

[24] M. Ovecka, T. Takac, Managing heavy metal toxicity stress in plants: Biological and biotechnological tools, Biotechnol. Adv. 32 (2014) 73-86.

[25] D.K. Gupta, Plant-based remediation processes. Soil Biology, Springer. 35 (2013) 1-299.

[26] M. Mahdieh, M. Yazdani, S. Mahdieh, The high potential of Pelargonium roseum plant for phytoremediation of heavy metals, Environ. Monit. Assess. 185 (2013) 7877-881.

[27] V.K. Sinhal, A. Srivastava, V.P. Singh, EDTA and citric acid mediated phytoextraction of Zn, Cu, Pb and Cd through marigold (Tagetes erecta), J. Environ. Biol. 31 (2010) 255-259.

[28] M. Jelusic, H. Grcman, D. Vodnik, M. Suhadolc, D. Lestan, Functioning of metal contaminated garden soil after remediation, Environ. Pollut. 174 (2013) 63-70.

[29] Q. Qin, X. Li, H Wu, Y Zhang, Q. Feng, P. Tai, Characterization of cadmium (^{108}Cd) distribution and accumulation in *Tagetes erecta* L. seedlings: Effect of split-root and of remove-xylem/phloem, Chemosphere. 93 (2013) 2284-2288.

[30] K. Lal, P.S. Minhas, S.R.K. Chaturvedi, R. Yadav, Extraction of cadmium and tolerance of three annual cut flowers on Cd-contaminated soils, Bioresource Tehnol. 99 (2008) 1006-1011.

[31] R.N. Malik, S.Z. Husain, A.I. Nazir, Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan, Pak. J. Bot. 42 (2010) 291-301.

[32] US EPA, Environmental protection agency, 2006, Revegetating land fields and waste containment areas fact sheet.

[33] J. Vangronsveld, F.V. Assche, H, Clijsters, Reclamation of a bare industrial area contaminated by non-ferrous metals: In situ metal immobilization and revegetation, Environ. Pollut. 87 (1995) 51-59.

[34] E. Gorlach, F. Gambus, A. Michniak, The effect of pH on the uptake of heavy metals by Italian ryegrass (Lolium multiflorum) in the conditions of their differentiated contents in soil, Polish Journal of Soil Science. 23 (1990) 17-23.

[35] M. Soleimani, M.A. Hajabbasi, M. Afyuni, A. Mirlohi, O.K. Borggaard, P.E. Holm, Effect of endophytic fungi on cadmium tolerance and bioaccumulation by Festuca arundinacea and Festuca pratensis, Int. J. Phytoremediat. 12 (2010) 535-549.

[36] O. Ortiz, J.M. Alcaniz, Bioaccumulation of heavy metals in Dactylis glomerata L. growing in a calcareous soil amended with sewage sludge, Bioresource Tehnol. 97 (2006) 545-552.

[37] C. Ciavatta, M. Govi, A. Sequi, P. Simoni, Evaluation of heavy metals during stabilization of organic matter in compost produced with municipal solid wastes. Bioresource Tehnol. 43 (1993) 147-153.

[38] Y. Liu, L. Ma, Y. Li, L. Zheng, Evolution of heavy metal speciation during the aerobic composting process of sewage sludge, Chemosphere. 67 (2007) 1025-1032.

[39] J. Singh, A.S. Kalamdhad, Concentration and speciation of heavy metals during water hyacinth composting, Bioresource Tehnol. 124 (2012) 169-179.

[40] J. Singh, A.S. Kalamdhad, Effects of lime on bioavailability and leachability of heavy metals during agitated pile composting of water hyacinth, Bioresource Tehnol. 138 (2013) 148-155.

3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

V doktorski nalogi smo analizirali vplive pranja tal z ligandom EDTA na kakovost s Pb, Zn in Cd onesnaženih vrtnih tal iz Mežiške doline. Remediirana tla smo pridobili z metodo pranja tal, ki je predstavljena v uvodu in podrobneje opisana v doktorski nalogi »Uporaba elektrokemijskih procesov pri remediaciji tal, onesnaženih s kovinami« (Voglar, 2013). Vsebnost PSK v tleh, remediiranih po postopku, se zmanjša tudi za 80 % (Voglar, 2013). Dosedanje raziskave čiščenja onesnaženih tal so se osredotočile na zmanjšanje celokupne in dostopne koncentracije kovin v očiščenih tleh, a zanemarile vpliv liganda in postopka čiščenja na kakovost in funkcionalnost tal (Zhang in sod., 2010a). V raziskavi smo v poljskem poskusu remediirana tla izpostavili živim (rastline, mikro in makro favna, mikroorganizmi) in neživim okolijskim dejavnikom (temperatura, osončenost, letni časi, padavine) ter proučili njihovo potencialno rabo kot vrtni substrat.

3.1.1 Optimalna koncentracija EDTA

Vrtna tla iz Mežiške doline (x = 489.300 m in y = 152.300 m, Gaub-Krüger koordinatni sistem), onesnažena s Pb, Zn in Cd, smo očistili z različnimi koncentracijami EDTA po metodi, ki sta jo razvila Voglar in Leštan (2012). V kolonskem poskusu s kitajskim kapusom smo preverili vpliv pranja tal z različnimi koncentracijami liganda EDTA 0, 10, 30 in 60 mmol EDTA kg^{-1} tal na frakcionacijo PSK v tleh, biodostopnost PSK za rastline in človeka ter vpliv na mikroorganizme in splošno stanje rastlin (Jelušič in sod., 2013).

Skladno z dosedanjimi raziskavami smo ugotovili, da višje koncentracije EDTA iz tal odstranijo večji delež PSK. Ligand EDTA lahko izpere kovine iz vseh frakcij. V tleh tvori komplekse s kovinami in tako iz tal neposredno odstrani rahlo vezane elemente (Martell in Smith, 2003). Hkrati tvorba koordinacijskih spojin med večveznimi kationi in EDTA lahko povzroči razpad organo-mineralnih kompleksov v tleh ter s tem izpiranje majhnih vodotopnih molekul humusa (DOM, dissolved organic matter) iz tal (Nowack in Sigg 1997; Tsang in sod., 2007; Vulava in Seaman, 2000). Razpad strukturnih kompleksov dodatno vpliva na večjo ekstrakcijo kovin v tleh in zmanjšanje celokupne vsebnosti PSK v remediiranih tleh, v našem primeru do 75 % Pb in Cd ter do 29 % Zn. Razlog za slabšo učinkovitost pri odstranitvi Zn lahko pripišemo nižji konstanti stabilnosti kompleksa Zn-EDTA (Martell in Smith, 2003), poleg tega pa je sekvenčna ekstrakcija modificirana po Tessier-ju in sod. (1979), pokazala, da je bila večina Zn (64 %) v tleh trdno vezanega na nedostopno silikatno-sulfatno frakcijo. Pb in Cd sta v tleh pogosto asociirana s karbonatno in organsko frakcijo in ker so tla iz Mežiške doline precej karbonatna (do 71 %) ter bogata z organsko snovjo, smo posledično v omenjenih frakcijah izmerili večji del Cd in Pb. Skladno z dosedanjimi raziskavami smo ugotovili, da EDTA tvori kelatne komplekse s

kovinami iz vseh talnih frakcij, še posebej iz prvih treh. Precejšnje zmanjšanje pa smo izmerili tudi v frakciji organske snovi (Jelušič in sod., 2014).

Nadalje smo preverili varnost remediiranih tal za človeka in v ta namen uporabili *in vitro* test biodostopnosti PBET (Ruby in sod., 1996), s katerim ugotavljamo biodostopnost PSK v želodcu in tankem črevesju ter ocenimo koncentracije PSK, ki so potencialno dosegljive človeškemu organizmu. Ker večina absorpcije hranil poteka v črevesju (Ruby in sod., 1996), je podatek črevesne biodostopnosti bolj reprezentativen. Odkrili smo, da tla, remediirana z večjo koncentracijo EDTA, zadržijo manj biodostopnih oblik PSK kot tla, remediirana z manjšo koncentracijo EDTA, ali onesnažena tla.

Zaradi sposobnosti akumulacije PSK smo za testno rastlino izbrali kitajski kapus (*Brassica napa* L.) (Grčman in sod., 2001). Rastline na remediiranih tleh so v korenine absorbirale manj PSK kot rastline v kolonah z onesnaženimi tlemi. Nismo pa opazili razlike v koncentracijah PSK v listih kitajskega zelja, ne glede na koncentracijo liganda EDTA. Razlog verjetno leži v EDTA-kovinskih kompleksih, ki so ostali v remediiranih tleh (se v procesu remediacije niso sprali) in ki lahko povzročijo premeščanje PSK iz korenin v zelene dele rastlin (Piechalak in sod., 2003). EDTA, ki po remediaciji ostane v tleh, ima lahko negativen učinek tudi na fotosintezni proces v rastlinah. Ruley in sod. (2004, 2006) so namreč odkrili značilno zmanjšanje fotosintezne učinkovitosti rastline *Sesbania drummondi*, ko so neonesnaženim tlom dodajali liganda EDTA in HEDTA. V naši raziskavi pri kitajskem kapusu nismo opazili negativnih učinkov na fotosintezni aparat, ne glede na dodano koncentracijo EDTA.

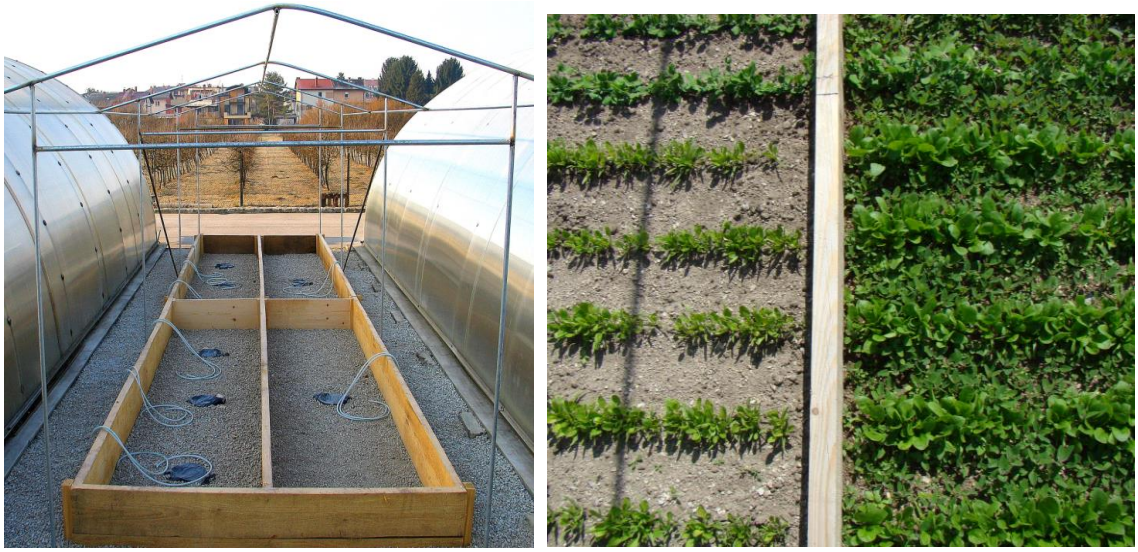
Pri analizi talne mikrobne biomase smo opazili večjo aktivnost v onesnaženih tleh v primerjavi z remediiranimi tlemi. Padec je verjetno povezan z za mikroorganizme težkimi pogoji med postopkom remediacije tal. Poleg tega ima EDTA negativne učinke na mikroorganizme, kar so že poročali Grčman in sod. (2001) in Mühlbachova (2011). Po sedmih tednih kolonskega poskusa so si mikroorganizmi v remediiranih tleh opomogli, še posebej v obravnavanjih 30 in 60 mmol EDTA kg^{-1}.

3.1.2 Ali so remediirana tla primerna za vrtno uporabo?

Rezultati pranja tal z različnimi koncentracijami EDTA so nakazali, da so remediirana tla potencialno primerna za vrtno uporabo tal. Remediacija tal z EDTA je odstranila dostopni del PSK in še nekaj močneje vezanih PSK ter s tem zmanjšala potencialno nevarnost tal za zdravje človeka. Nadalje smo ugotovili, da na remediiranih tleh brez stresa in poškodb fotosinteznega aparata uspeva kitajski kapus, a vsebnost PSK v zelenih delih rastline se kljub remediaciji ni zmanjšala. Začetni padec mikrobnega življenja se je po sedmih tednih vrnil na prvotno raven in tako naznanil ponovno mikrobiološko ravnotežje remediiranih

tal. Za najučinkovitejšo koncentracijo se je pri vseh parametrih izkazala 60 mmol EDTA, ki smo jo uporabili pri nadaljnjem poskusu.

S poljskim poskusom smo raziskali, ali se pridobljene lastnosti remediiranih tal s časom spreminjajo in ali tla lahko vzdržujejo tudi rastline drugih rodov ter kljubujejo okolijskim dejavnikom (Jelušič in Leštan, 2014; Jelušič in sod., 2014). Dve eksperimentalni gredi velikosti 4 × 1 × 0,3 m smo napolnili z onesnaženimi vrtnimi tlemi iz Mežiške doline in z remediiranimi tlemi ter zasadili raznovrstne vrtnine, ki so se v dveh sezonah zvrstile v štirih kolobarjih: grah (*Pisum sativum* L.), cvetača (*Brassica oleracea* L., Bortytis), čebula (*Allium cepa* L.), kitajski kapus (*Brassica rapa* L., Pekinensis), vrtna solata (*Lactuca sativa* L.), korenje (*Daucus carota* L.), bazilika (*Ocimum basilicum* L.), paprika (*Capsicum annuum* L.) in štirje kolobarji špinače (*Spinacia oleracea* L.). Dno gredic smo opremili z lizimetri, v katere smo lovili izprano vodo, in nad gredice namestili protitočno mrežo (Slika 4).



Slika 4: Eksperimentalne gredice z lizimetri (na levi) in mlada špinača (na desni), kjer je viden manjši pridelek na remediiranih tleh in večji na originalnih tleh.

Figure 4: Experimental plots with lyzimeters (left) and spinach plant (right), where the lower yield of spinach growth on remediated soil is clearly visible.

Tla Mežiške doline so bila onesnažena s 1585 mg kg^{-1} Pb, 525 mg kg^{-1} Zn in 8,8 mg kg^{-1} Cd, z remediacijo pa so se koncentracije PSK znižale na 313 mg kg^{-1} Pb, 378 mg kg^{-1} Zn in 2,5 mg kg^{-1} Cd. Analizirali smo glavne pedološke značilnost obeh obravnavanj in pri remediiranih tleh zabeležili zmanjšanje organske snovi in rahel dvig pH. Izmerili smo tudi manjšo vsebnost K v remediiranih tleh, kar je verjetno posledica uporabe Na-EDTA pri čiščenju tal. Na ioni so tako izpodrinili K ione, ki so se nato izprali iz talne raztopine.

Problemu bi se lahko izognili z uporabo Ca-EDTA ali EDTA v obliki čiste kisline. Pedološke lastnosti remediiranih tal so po sedmih mesecih ostale nespremenjene, razen povečanja vsebnosti K, ki smo ga pred sajenjem vrtnin dodali v obliki KNO_3.

Vodnozadrževalne lastnosti tal so se z remediacijo poslabšale, predvsem zaradi sprememb v strukturi tal, do katerih je prišlo med postopkom remediacije.

Analiza sekvenčne ekstrakcije tal takoj po remediaciji je pokazala zmanjšanje PSK v vseh frakcijah, predvsem v dostopnih. Sekvenčno ekstrakcijo remediiranih tal smo ponovno izvedli po štirih in sedmih mesecih, da bi ugotovili, ali je pod vplivom okolijskih dejavnikov prišlo do premeščanja trdno vezanih kovin v dostopnejše oblike. Ugotovili smo, da so preostale PSK v remediiranih tleh v kemijsko stabilnih oblikah, saj se frakcionacija PSK v remediiranih tleh ni spremenila. Stabilnost remediiranih tal se je pokazala tudi pri toksikoloških testih TCLP in UBM. Test UBM je novejši *in vitro* test, ki ga čedalje več študij uporablja kot indikator oralne dostopnosti PSK iz tal (Wragg in sod., 2011). Test posnema človeški prebavni trakt, pomembni pa so predvsem rezultati biodostopnosti v črevesju, kjer tudi poteka absorpcija večine hranil. S to metodo smo ugotovili, da v remediiranih tleh PSK niso več prisotne v dostopnih oblikah za razliko od onesnaženih tal. Tudi z ekstrakcijsko metodo TCPL, ki se uporablja za določanje mobilnosti PSK (nevarnost izpiranja PSK), smo ugotovili precejšnje zmanjšanje toksičnosti remediiranih tal v primerjavi z onesnaženimi tlemi, ki je po sedmih mesecih ostalo nespremenjeno.

Mikrobna aktivnost tal se lahko ocenjuje posredno prek aktivnosti talnih encimov in se pogosto uporablja kot indikator kakovosti tal, predvsem zaradi hitrega odziva mikroorganizmov na spremembe v talnem okolju in zaradi njihove povezanosti z biologijo tal (Mijangos in sod., 2006). Poskušali smo izbrati čim primernejše encimske teste, da bi lahko ocenili vpliv remediacije na mikroorganizme: *ß*-glukozidaza, kisla in bazična fosfataza, ureaza, dehidrogenaza in hidroliza fluorescin diacetata, ter poleg tega izvedli še test s substratom (glukozo) induciranega dihanja (SIR) za približno določitev mikrobne biomase v tleh. Ugotovili smo, da sta bili aktivnost talnih encimov in mikrobna biomasa kljub onesnaženosti s PSK višji v onesnaženih kot v remediiranih tleh. Aktivnost talnih encimov in SIR se lahko spreminja glede na letni čas (Bastida in sod., 2008), rastlinsko odejo in druge okolijske dejavnike (Bastida in sod., 2008; Wang in sod., 2012), kar smo zabeležili tudi pri naših meritvah. Kljub temu aktivnost encimov v remediiranih tleh tudi po sedmih mesecih ni dosegla aktivnosti encimov onesnaženih tal. EDTA je nespecifični ligand in iz tal poleg PSK izpere tudi za življenje esencialne kovine, vsaj njihove dostopne oblike. Tako smo izmerili petkrat manjšo koncentracijo Mn v remediiranih tleh v primerjavi z onesnaženimi tlemi, kar bi lahko bil razlog za slabšo aktivnost encimov v remediiranih tleh.

Pomanjkanje esencialnih kovin pa je verjetno vplivalo tudi na rast vrtnin, saj je bil pridelek vseh rastlin, gojenih na remediiranih tleh, manjši v primerjavi s pridelkom na onesnaženih tleh. Rastlina, ki je na remediiranih tleh najslabše uspevala, je bila špinača. Njen pridelek je bil tudi do 10-krat manjši od pridelka na onesnaženih tleh. Pridelek ostalih vrtnin je bil slabši za približno 20 %. Remediirana tla smo naknadno pognojili z Mn in opazili rahlo izboljšanje v pridelku vrtnin, gojenih na remediiranih tleh. Koncentracije Mn in Zn, izmerjene v zelenih delih špinače in graha, gojenih na remediiranih tleh, so bile manjše kot v rastlinah, zraslih na onesnaženih tleh. Koncentracije Fe in Cu pa so bile večje v rastlinah, zraslih na remediiranih tleh. Odnosi med hranili v tleh so zapleteni in ko je ravnovesje okrnjeno, ga ni več enostavno vzpostaviti (Marschner, 2012).

Poleg tega si mikronutrienti delijo transportne poti z nekaterimi PSK. Tako si Cd zaradi kemijske podobnosti z Zn, ki je hkrati tudi mikronutrient, z njim deli vstopno pot v rastlino. Ozturk in sod. (2003) so pri nizkih koncentracijah Zn izmerili škodljive učinke Cd in njegov povečan vstop v rastlino. Koncentracije Cd smo izmerili v nadzemnih delih vseh vrtnin, zraslih na remediiranih tleh, razen pri grahu in cvetači. Cd se večinoma koncentrira v koreninah (Gallego in sod., 2012; Lux in sod., 2011), vendar so za rastline iz družine nebinovk (Asteraceae) in reda klinčkovci (Caryophyllales) značilne višje koncentracije v zelenih delih. Tudi v našem poskusu se je večina Cd koncentrirala v koreninah, pri špinači (Caryophyllales) in solati (Asteraceae) pa smo izmerili večje ali enake koncentracije v nadzemnih delih rastlin tako na onesnaženih kot remediiranih tleh.

Pb v primerjavi s Cd ni tako mobilen in največje koncentracije Pb smo tako pri obeh obravnavanjih in vseh vrtninah izmerili v koreninah. V rastlinah, gojenih na onesnaženih tleh, smo Pb pri večini izmerili tudi v nadzemnih delih, v rastlinah, gojenih na remediiranih tleh pa smo Pb izmerili le v zelenih delih oslabele špinače.

Koncentracije Pb in Cd v užitnih delih rastlin, pridelanih na onesnaženih in remediiranih tleh, smo primerjali z Evropsko zakonodajo (ES 1881/2006). Na onesnaženih tleh so špinača, solata, kitajski kapus in korenje presegli mejne vrednosti Pb, določene z uredbo EU, medtem ko jih na remediiranih tleh ni presegla nobena izmed vrtnin. Večje vrednosti od predpisanih za Cd so presegli paprika, grah, cvetača in korenje, gojeni na onesnaženih tleh ter paprika, špinača in čebula, gojeni na remediiranih tleh. Skupno je torej kar sedem od devetih vrtnin gojenih na onesnaženih tleh preseglo predpisane meje za Pb oz. Cd in le tri od devetih na remediiranih tleh, vse tri za Cd.

Ugotovili smo, da je postopek pranja tal z odvzemom mikronutrientov ter spremembo strukture in vodnih lastnosti tal, spremenil talno okolje do te mere, da je porušil ravnovesje v onesnaženih tleh. Manjši pridelki in še vedno previsoke koncentracije Cd v užitnih delih nekaterih vrtnin so tako razlog, da remediirana tla brez nadaljnje obdelave/revitalizacije niso primerna za gojenje vrtnin.

3.1.3 Revitalizacija remediiranih tal z izbranimi dodatki

Rezultati omenjenih raziskav so pokazali na potrebo po učinkovitem načinu revitalizacije, ki bi povrnil sposobnost remediiranih tal, da funkcionirajo kot varen in ploden rastlinski substrat. Glede na pomanjkljivosti tal smo tako izbrali specifične aditive/dodatke, ki bi lahko izboljšali rodovitnost in varnost remediiranih tal.

Za kolonski poskus smo remediirali tla iz Mežiške doline, ki so imela približno trikrat večjo vsebnost PSK v primerjavi s tlemi, uporabljenimi za poljski poskus, zato smo za pralno raztopino uporabili večjo koncentracijo EDTA, in sicer 120 mmol kg^{-1} tal. Po remediaciji smo tla nadrobili v enakomerne agregate skozi 5-mm sito in jim s tem delno povrnili strukturo ter izboljšali vodnozadrževalne lastnosti. Razpoložljivost talne vode za rastline je bila celo večja kot v onesnaženih tleh.

Remediiranim tlom smo dodali uležan gnoj, hidrogel, vermikulit, Slovakit in apatit ter mešanico hranil N, K, Mn in Mg. V vsako kolono (8 kg tal) smo posadili semena špinače, ki je v prejšnjih poskusih izkazala največjo občutljivost na remediirana tla.

Po remediiaciji se je vsebnost Pb, Zn, Cd, Mn in Ca v rastlinskem tkivu znižala, vsebnost Cu in Fe pa rahlo zvišala. Gnojilna mešanica je pri obeh obravnavanjih vplivala na manjši vnos Pb in Cd v rastline, povečala pridelek in na remediiranih tleh izboljšala tudi splošno stanje rastlin, merjeno skozi fotosintezne parametre. Noben izmed izbranih dodatkov ni imel večjega vpliva na vnos PSK ali mikroelementov v rastlino. Prav tako dodatki niso imeli signifikantnega vpliva na fotosintezo in fotokemično učinkovitost.

Gnoj smo remediiranim tlom umešali predvsem zato, da bi tla obogatili z organsko snovjo in mikroelementi ter da bi jim izboljšali strukturo (Haynes in Naidu, 1997). Pridelek špinače se navkljub dodatku gnoja ni spremenil, prav tako ne vsebnost PSK v rastlinah in izpirkih, vodnozadrževalne lastnosti tal pa so se celo malce poslabšale.

Hidrogel smo izbrali zaradi številnih študij, ki kažejo, da izboljšuje vodne lastnosti tal in s tem posredno vpliva na boljše talne pogoje za rast rastlin (Orikiriza in sod., 2009; Chirino in sod., 2011). Pridelek špinače v kolonah s hidrogelom je bil največji od vseh obravnavanj remediiranih tal. V kolonah s hidrogelom smo izmerili tudi najmanjše koncentracije izpiranja PSK, predvsem zato, ker se je iz kolon s hidrogelom izpralo najmanj vode, kar lahko pripišemo njegovim močnim vodnozadrževalnim lastnostim. Podoben učinek sta opazila Kos in Leštan (2003), ko sta namenoma uporabila hidrogel za zadrževanje kelatnih kompleksov v tleh in s tem preprečila njihovo izpiranje.

Vermikulit se v kmetijstvu uporablja predvsem kot substrat za gojenje rastlin (Headlee in sod., 2013). Premore veliko kationsko izmenjevalno kapaciteto in specifično površino, kar

pomeni, da lahko veže tako hranila kot PSK (Seaborn in Jameson, 1976; Panuccio in sod., 2009). Navkljub omenjenim lastnostim nismo opazili sprememb pri izpiranju ali vnosu PSK in hranil v rastlino. Pridelek špinače pa je bil celo manjši od pridelka na netretiranih remediiranih tleh.

Ker smo v kolonskem poskusu uporabili bolj onesnažena tla, ki so tudi po remediaciji še vedno vsebovala relativno visoke vsebnosti PSK, smo tlom dodali tudi stabilizanta Slovakit in apatit, da bi preprečili izpiranje in vnos PSK v rastline. Apatit je fosfatni aditiv, ki povzroča izobarjanje dostopnih kovin kot netopnih fosfatov in se pogosto uporablja kot stabilizator PSK v tleh (Madrid in sod., 2008; Tica in sod., 2013). V naši raziskavi nismo opazili manjšega izpiranja ali vnosa PSK v rastline špinače, pridelek pa je bil najmanjši od vseh obravnavanj. Slovakit, komercialna mešanica absorbentov, je bil razvit prav za namen remediacije prek stabilizacije PSK v tleh. Aditiv je povišal pH v talni raztopini in posredno omogočil večje izpiranje PSK iz tal. Pridelek špinače in vnos PSK in hranil v rastline sta ostala nespremenjena.

Od vseh dodatkov smo najboljše rezultate dosegli s hidrogelom, a rezultati so bili pri skoraj vseh parametrih še vedno slabši od onesnaženih tal. Izbrani dodatki in gnojilo tako niso uspeli popolnoma revitalizirati remediiranih tal. Potrebne so dodatne raziskave, z dodatki kot je npr. vse popularnejše bio-oglje, ki bi lahko pripomogli k popolni revitalizaciji in vzpostavitvi funkcionalnosti remediiranih tal.

3.1.4 Revegetacija remediiranih tal z okrasnimi rastlinami in travami

Remediirana tla brez nadaljnjih raziskav še niso primerna za gojenje užitnih vrtnin, predvsem zaradi manjšega pridelka in absorpcije PSK v užitne dele rastlin. Še posebej to velja za tla, močno onesnažena s PSK, kjer tudi visoke koncentracije EDTA pri postopku remediacije v tleh pustijo previsoke vsebnosti PSK, da bi bila tla primerna za gojenje užitnih rastlin. Zatorej smo preizkusili alternativno uporabo remediiranih tal kot substrat za okrasne rastline in travnate površine.

Močno onesnažena tla s 4037 mg kg^{-1} Pb, 2527 mg kg^{-1} Zn in 26 mg kg^{-1} Cd smo remediirali (120 mmol EDTA kg^{-1} tal) do 1246 mg kg^{-1} Pb, 2138 mg kg^{-1} Zn in 11 mg kg^{-1} Cd. Poleg tega pa smo odstranili še 57 % esencialnega mikronutrienta Mn.

Onesnažena in remediirana tla (120 mmol EDTA kg^{-1} tal) smo položili v vrtne gredice in remediirana tla dodatno pognojili z Mn ter jim dodali hidrogel. Zasadili smo naslednje okrasne rastline: vodenko (*Impatiens walleriana)*, tagetes (*Tagetes erecta)*, pelargonijo (*Pelargonium × peltatum)* kot predstavnike nedavno ugotovljenih hiperakumulatorjev in verbeno (*Verbena × hybrida)* ter trave: pasjo travo (*Dactylis glomerata)*, mnogocvetno ljulko (*Lolium multiflorum)* in travniško bilnico (*Festuca pratensis).*

Pri pelargoniji, verbeni in ljulki smo izmerili večjo biomaso na remediiranih tleh kot na originalnih tleh. Pri verbeni kar 5-krat večjo. Razlog verjetno leži v boljših razmerah vzpostavljenih na remediiranih tleh zaradi dodatka hidrogela in gnojenja z Mn. Poleg tega so remediirana tla očiščena PSK, ki lahko negativno vplivajo na rast in razvoj rastlin (Ovecka in Takac, 2014). Verbena, gojena na originalnih tleh, je tako vsebovala največjo koncentracijo Pb v zelenih delih in največjo koncentracijo Cd v koreninah, kar skupaj z zmanjšano biomaso nakazuje na toksični učinek PSK.

Pri okrasnih rastlinah in travah smo izmerili manjše koncentracije PSK v koreninah in nadzemnih delih, z izjemo Zn, kjer so koncentracije pri nekaterih okrasnih rastlinah ostale nespremenjene.

Preverili smo tudi varnost onesnaženih in remediiranih tal za zdravje človeka. Z UBM testom smo ugotovili zmanjšanje dostopnosti za vse PSK, dostopnost Pb je celo padla pod mejo kvantifikacije.

Rezultati poskusa nakazujejo, da remediacija z EDTA učinkovito zmanjša dostopnost PSK in s tem nevarnost tal za zdravje ljudi tudi v s PSK bolj onesnaženih tleh. Poleg tega smo ugotovili, da so remediirana tla (ob dodatku hidrogela in Mn) ugodna in plodna podlaga za izbrane okrasne rastline in trave ter tako potencialno primerna izbira za revitalizacijo onesnaženih območij.

3.2 SKLEPI

V okviru doktorskega dela smo prišli do naslednjih zaključkov:

− Remediacija tal z EDTA učinkovito odstrani PSK iz tal, predvsem tisti del, ki je v tleh prisoten v kemijsko in biološko dostopnejših oblikah

− Pri staranju tal (7 mesecev) ne prihaja do prehajanja PSK iz manj dostopnih v kemijsko in biološko dostopnejše oblike – PSK ne prehajajo med različnimi talnimi frakcijami.

− Hkrati z odstranitvijo PSK EDTA iz tal izpere tudi nekatera nujno potrebna hranila, predvsem Mn.

− Spremenjeno razmerje med vsebnostjo hranil v remediiranih tleh se odraža pri zmanjšani mikrobni aktivnosti in biomasi ter zdravju rastlin.

− (Eko)toksikološki testi tal nakazujejo na zmanjšanje nevarnosti PSK za zdravje človeka in izpiranje v okolje.

− Vsebnosti Pb v užitnih delih rastlin, gojenih na remediiranih tleh, je pod zakonodajno mejo EU. Vsebnost Cd pri nekaterih rastlinah mejo še vedno presega.

− Testirani aditivi (gnoj, hidrogel, vermikulit, apatit in Slovakit), razen hidrogela, ki je ugodno vplival na rast rastlin, niso imeli statistično značilnega vpliva na mobilnost in prevzem/vnos PSK in hranil v rastlino.

− Remediacija povzroči spremembe v lastnostih in funkcioniranju tal. Pred končno uporabo jim je treba dodati hranila in izboljšati njihove strukturne lastnosti ter mikrobno aktivnost.

− Remediirana tla so se ob dodatku hidrogela, Mn in kompleksnega gnojila rastlinskih peletov izkazala kot ugodna in plodna podlaga za rast izbranih okrasnih rastlin in trav.

− Rezultati, pridobljeni v doktorskem delu, so vezani na specifične talne lastnosti tal iz Mežiške doline.

4 POVZETEK (SUMMARY)

4.1 POVZETEK

Onesnaženje tal s potencialno strupenimi kovinami (PSK) predstavlja vse večji globalni problem. PSK so biološko nerazgradljive, strupene že pri nizkih koncentracijah in potencialno mobilne ob spreminjanju fizikalno-kemijskih lastnosti tal. Zaradi degradacije tal in povečevanja svetovne populacije vse več ljudi živi na onesnaženih območjih (urbana območja ter območja nekdanjih rudnikov in topilnic) in na onesnaženih tleh prideluje kmetijske kulture in vrtnine. Zahteve po čiščenju tal, onesnaženih s PSK naraščajo in iskanje primerne remediacijske metode postaja pomemben del okoljevarstvenih raziskav. Remediirana tla morajo biti varna, kakovostna in primerna za kmetijsko ali vrtno obdelavo.

Metoda pranja tal z ligandom EDTA je učinkovita pri odstranjevanju PSK in stroškovno ugodna. V doktorski disertaciji smo raziskali vpliv remediacije z ligandom EDTA na s Pb, Zn in Cd onesnažena tla iz Mežiške doline in potencialno uporabo remediiranih tal za pridelovanje vrtnin. Onesnažena tla smo tretirali z EDTA in iz talne matrice sprali večino PSK, vendar tudi mikrohranilo Mn. Da bi ugotovili, ali so remediirana tla kakovostna, varna in primerna za pridelavo vrtnin, smo izvedli poljski poskus. Remediirana in onesnažena tla smo položili v dve vrtni gredici (4 × 1 × 0,3 m) in zasadili raznovrstne vrtnine.

Analiza frakcionacije PSK na različne talne komponente (sekvenčna analiza) je pokazala, da so v remediiranih tleh ostale predvsem nedostopne PSK. Biodostopnost Pb, Zn in Cd v remediiranih tleh za človeka (UBM test) in rastline (DTPA test) se je z remediacijo bistveno zmanjšala, prav tako pa tudi nevarnost izpiranja Pb, Zn in Cd (TCLP test). Pridobljene fizikalno-kemijske lastnosti remediiranih tal se skozi trajanje eksperimenta niso spreminjale.

Vrtnine, gojene na remediiranih tleh, so vsebovale manjše koncentracije Pb in Cd v vseh delih rastlin in imele, verjetno zaradi pomanjkanja mikrohranil, predvsem Mn, manjšo biomaso oz. pridelek v primerjavi z onesnaženimi tlemi. Čeprav zmanjšane, so bile koncentracije Cd v užitnih delih nekaterih rastlin na remediiranih tleh še vedno nad mejo, določeno z zakonodajo Evropske unije. Koncentracije Zn v rastlinah so se z remediacijo večinoma zmanjšale, ponekod pa ostale nespremenjene. Rastlina, ki je na remediiranih tleh najslabše uspevala, je bila špinača. Njen pridelek je bil tudi do 10-krat manjši od pridelka na onesnaženih tleh. Pridelek ostalih vrtnin je bil slabši za približno 20 %.

Rezultati aktivnosti talnih encimov so pokazali ugodnejše razmere za mikroorganizme na onesnaženih kot na remediiranih tleh.

Da bi izboljšali nekatere lastnosti remediiranih tal, smo jim v posebnem kolonskem poskusu dodali izbrane aditive: hlevski gnoj, hidrogel, vermikulit, apatit in Slovakit ter zasadili špinačo, ki je izkazala največjo občutljivost za rast na remediiranih tleh. Za najobetavnejši dodatek se je izkazal hidrogel, saj je omogočil največji pridelek in najmanjše izpiranje PSK, a vseeno v merjenih parametrih ni presegel onesnaženih tal.

V posebnem poljskem poskusu smo preizkusili še alternativno uporabo remediiranih tal (ob dodatku hidrogela, kompleksnega gnojila rastlinskih peletov in Mn) za okrasne rastline (tudi hiperakumulatorje) in trave. Izbrane rastline so izkazale boljšo ali enako uspešnost na remediiranih tleh v primerjavi z onesnaženimi tlemi in tako uspešno revitalizirale remediirana tla.

Remediacija tal z EDTA povzroči spremembe v lastnostih in funkcioniranju tal, ki lahko negativno vplivajo na rast in razvoj nekaterih rastlin. Pred končno uporabo jim je treba dodati hranila, izboljšati njihove mikrobiološke lastnosti in znova vzpostaviti ravnovesje med številnimi talnimi procesi.

4.2 SUMMARY

Soil contamination with potentially toxic metals (PTMs) is becoming a major worldwide problem. PTMs are non-degradable, toxic at low concentrations, and, due to physicochemical changes in the soil, potentially mobile. Soil degradation and growth of human population is resulting in increasingly more people living on contaminated sites (urban areas, and former mining and smelter sites) where they cultivate agricultural crops or manage urban gardens. Demand for soil treatment techniques is consequently growing and the development of efficient remediation technologies has become one of the key research activities in environmental science and technology. Remediated soil needs to be safe, fully functional, and suitable for agricultural use.

Soil washing with EDTA is economical and efficiently removes PTMs from the soil matrix. In the present doctoral dissertation, we have examined the effect of EDTA remediation on the soil from Mežica Valley contaminated primarily with Pb, but also with Zn and Cd, and examined the potential use of the remediated soil as a healthy garden substrate. Contaminated soil was treated with EDTA, which rinsed the majority of PTMs, but also the essential micronutrient Mn, from the soil matrix. To ensure that remediated soil be fully functional and safe for vegetable production, we set up a field experiment. Remediated and contaminated soils were laid into two experimental garden beds (4×1×0.3 m) and various vegetables were planted.

Analysis of metal association with different soil components (sequential analysis) revealed that after remediation, mainly the non-accessible share of PTMs remained in the soil. In remediated soil, human (UBM method) and plant (DTPA method) bioaccessibility for Pb, Zn, and Cd were reduced compared to contaminated soil, and the leaching hazard (TCLP method) was also reduced. Obtained physiochemical properties of the remediated soil did not change during the time of the experiment.

The vegetables cultivated on remediated soil absorbed lower concentrations of Pb and Cd in all plant parts compared to contaminated soil, but had inferior growth and, consequently yield, presumably due to lack of micronutrients, especially Mn. However the reduced Cd concentration in the edible parts of some plants, still exceeded the European Union legislation. Zn concentrations in plant tissue were reduced or remained the same compared to contaminated soil. The plant most sensitive to remediation changes was spinach (*Spinacia oleracea* L.) with a tenfold lower yield than the yield on contaminated soil. The yield of other plants was reduced by approximately 20%.

Enzyme activity tests revealed better conditions for microorganisms on original soil, than in remediated soil.

We strived to amend the impaired properties of the remediated soil and applied several amendments: manure, hydrogel, vermiculite, apatite and Slovakite. In a column experiment with amendments, we planted spinach, the plant most sensitive for EDTA- remediated soil. Hydrogel was the most prospective amendment, which enabled the largest yield and best prevented the leaching of PTMs, however the results obtained on contaminated soils were still better in most of the measured parameters.

Remediation with EDTA impairs the health and functioning of the soil and depresses the growth and development of certain plants. Before the soil can be used as a safe and fully functional plant substrate, nutrients must be added, microbiological properties need to be amended, and the lost balance between numerous soil processes needs to be reestablished.

5 VIRI

Abumaizar R., Khan L.I. 1996. Laboratory investigation of heavy metal removal by soil washing. Journal of the Air and Waste Management Association, 46, 8: 765-768

Adriano D.C. 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability and risk of metals. 2nd ed. New York, Springer: 867 str.

Alloway B.J. 2013. Heavy metals in soils. 3rd ed. New York, Springer: 613 str.

Autumn H., Pikai O. 2000. Rethinking urban poverty: a look at community gardens. Bulletin of Science, Technology and Society, 20: 207-2015

Baker A.J.M., Brooks R.R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements - review of their distribution, ecology and phytochemistry. Biorecovery, 1, 2: 81-126

Basta N. T., McGowen S. L. 2004. Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. Environmental Pollution, 127, 1: 73-82

Bastida F., Barberá G.G., García C., Hernández T. 2008. Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions. Applied Soil Ecology, 38, 1: 62-70

Barona A., Aranguiz I., Elias A. 2001. Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. Environmental Pollution, 113, 1: 79-85

Bešter K.P. 2013. Ocena tveganja vnosa kadmija z vrtninami na lokalno prebivalstvo mestne obične Celje. Doktorska dizertacija. Ljubljana, Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, 130 str.

Begum Z., Rahman I.M.M., Tate Y., Sawai H., Maki T., Hasegawa H. 2012. Remediation of toxic metal contaminated soil by washing with biodegradable aminopolycarboxylate chelants. Chemosphere, 87, 10: 1161-1170

Boisson J., Ruttens A., Mench M., Vangronsveld J.1999. Evaluation of hydroxyapatite as a metal immobilizing soil additive for the remediation of polluted soils. Part 1. Influence of hydroxyapatite on metal exchangeability in soil, plant growth and plant metal accumulation. Environmental Pollution, 104, 2: 225-233

Bradl H.B., Kim C., Kramar U., Stuben D. 2005. Interactions of heavy metals. V: Heavy metals in the environment. 1ed Neubrucke, Elsevier Academic press: 28-164 str.

Brady N.C., Weil R.R. 2002. The nature and properties of soils. 13th ed. New Jersey, Pearson Education: 960 str.

Brown S., Chaney R., Hallfrisch J., Ryan J. A., Berti W. R. 2004. In situ treatments to reduce the phyto- and bioavailability of lead, zinc and cadmium. Journal of Environmental Quality, 33: 522-531

Burton K.W., Morgan E., Roig A. 1984. The influence of heavy metals upon the growth of sitka-spruce in South Wales forests. Plant and Soil, 78, 3: 271-282

Chakravarty B., Srivastava S. 1997. Effect of cadmium and zinc interaction on metal uptake and regeneration of tolerant plants in linseed. Agriculture, Ecosystems and Environment, 61, 1: 45-50

Chirino E., Vilagrosa V., Vallejo V.R. 2011. Using hydrogel and clay to improve the water status of seedlings for dryland restoration. Plant and Soil, 344: 99-110

Chander K., Dyckmans J., Joergensen R.G., Meyer B., Raubuch M. 2001. Different sources of heavy metals and their long-term effects on soil microbial properties. Biology and Fertility of Soils, 34, 4: 241-247

Cieśliński G., Van Rees K.C.J., Huang P.M., Kozak L.M., Rostad H.P.W., Knott D.R. 1996. Cadmium uptake and bioaccumulation in selected cultivars of durum wheat and flax as affected by soil type. Plant and Soil, 182, 1: 115-124

COM (2006) 231. 2006. Sporočilo komisije svetu, evropskemu parlamentu, evropskemu ekonomsko-socialnemu odboru in odboru regij. Tematska strategija za varstvo tal. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2006:0231:FIN:SL:PDF 20.12.2013

Pankhurst BM., Doube B.M., Gupta V.V.S.R. 1997. Biological Indicators of Soil Health. Wallingford, CAB international: 437 str.

Dushenkov S. 2003. Trends in phytoremediation of radionuclides. Plant and Soil, 249, 1: 167-175

ES 1881/2006, Uredba komisije. 2006. Uredba komisije (ES) št. 1881/2006 o določitvi mejnih vrednosti nekaterih onesnaževal v živilih. Uradni list Evropske unije, I, 364: 6-24

EEA, European Environment Agency. 2007. State of the environment, No 1/2007 Chapter 2. Office for official publications of the European communities

Ebbs S.D., Kochian L.V. 1997. Toxicity of Zinc and Copper to Brassica Species: Implications for Phytoremediation. Journal of Environmental Quality, 26: 776-781

Elliot H. A., Brown G. A. 1989. Comparative evaluation of NTA and EDTA for extractive decontamination of Pb-polluted soils. Water, Air and Soil Pollution, 45, 3-4: 361-369

Gallego S.M., Pena L.B., Barcia R., Azpilicueta C.E., Iannone M.F., Rosales E.P., Zawoznik M.S., Groppa M.D., Benavides M.P. 2012. Unravelling cadmium toxicity and tolerance in plants: Insight into regulatory mechanisms. Environmental and Experimental Botany, 83: 33-46

Geebelen W., Adriano D.C., Lelie D. L., Mench M., Carleer R., Clijsters H., Vangronsveld J. 2003. Selected bioavailability assays to test the efficacy of amendment-induced immobilization of lead in soils. Plant and Soil, 249, 1: 217-228

Gibbons S., Mourato S., Resende G. 2011. The amenity value of English nature: A hedonic price approach. Spatial Economics Research Centre (SERC), London School: 31 str.

Gill S.S., Khan N.A., Tuteja N. 2012. Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.). Plant Science, 182: 112-120

Gosh M., Singh S.P. 2005. A review on phytoremediation of heavy metals and utilization of it's by products. Asian Journal on Energy and Environment, 6, 4: 214-231

Guo G., Zhou Q., Ma L. Q. 2006. Availability and assessment of fixing additives for the in situ remediation of heavy metal contaminated soils: a review. Environmental Monitoring and Assessment, 116, 1-3: 513-528

Gusiatin Z.M., Klimiuk E. 2012. Metal (Cu, Cd and Zn) removal and stabilization during multiple soil washing by saponin. Chemospgere, 86, 4: 383-391

Grčman H., Velikonja-Bolta S., Vodnik D., Kos B., Leštan D. 2001. EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant and Soil, 235, 1: 105-114

Hardiman R.T., Banin A., Jacoby B. 1984. The effect of soil type and degree of metal contamination upon uptake of Cd, Pb and Cu in bush beans (*Phaseolus vulgaris* L.). Plant and Soil, 81, 1: 3-15

Haynes R.J., Naidu R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions - a review. Nutrient Cycling in Agroecosystems, 51: 123-137

Headlee W.L. Brewer C.E., Hall R.B. 2013. Biochar as a substitute for vermiculite in potting mix for hybrid poplar. Bio Energy Research, 7: 120-131

IAAS. 2013. Global soil forum. Fertile soils: Fundamental in the struggle against hunger and climate change! Institute for advanced sustainability studies (IASS) Postdams. http://globalsoilweek.org/wp-content/uploads/2013/10/GSW_factsheet_Fertile-Soils_en.pdf 19.12.2013

Jamšek M., Šarc M. 2009. Diagnostika in zdravljenje zastrupitev s kovinami. Medicinski razgledi, 48: 101-113

Jamšek M., Šarc L., Krbavčič A. 2007. Nevarne kemikalije: simptomi in znaki, prva pomoč in zdravljenje zastrupitev. Ljubljana, Ministrstvo za zdravje, Urad RS za kemikalije: 176 str.

Jelušič M., Leštan D. 2014. Effect of EDTA washing of metal polluted garden soils. Part I: Toxicity hazards and impact on soil properties. Science of the Total Environment, 475: 132-141

Jelušič M., Vodnik D., Maček I., Leštan D. 2014. Effect of EDTA washing of metal polluted garden soils. Part II: Can remediated soil be used as a plant substrate? Science of the Total Environment, 475: 142-152

Jelušič M., Grčman H., Vodnik D., Suhadolc M., Leštan D. 2013. Functioning of metal contaminated garden soil after remediation. Environmental Pollution, 174: 63-70

Jiang X.J., Luo Y.M., Liu Q., Liu S.L., Zhao Q.G. 2004. Effects of cadmium on nutrient uptake and translocation by indian mustard. Environmental Geochemistry and Health, 26, 2: 319-324

Khan S., Hesham E.L.A., Qiao M., Rehman S., He J.Z. 2010. Effects of Cd and Pb on soil microbial community structure and activities. Environmental Science and Pollution Research, 17: 2: 288-296

Kim C., Lee Y., Ong S.K. 2003. Factors affecting EDTA extraction of lead from lead-contaminated soils. Chemosphere, 51, 9: 845-853

Kos B., Leštan D. 2003. Influence of a biodegradable ([S,S]-EDDS) and nondegradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextraction and leaching. Plant and Soil, 253: 403-411

Leštan D. 2002. Ekopedologija: Študijsko gradivo za študente opredelilnega izbirnega študija Ekopedologije. Ljubljana, Biotehniška fakulteta: 267 str.

Leštan D., Luo C., Li X. 2008. The use of chelating agents in the remediation of metal-contaminatd soils: a review. Environmental Pollution, 153, 1: 3-13

Lindsay W.L., Norvell W.A. 1978. Development of a DTPA test for zinc, iron, manganese and copper. Soil Science Society of America Journal, 42: 421-428

Liu J., Li K., Xu J., Zhang Z., Ma T., Lu X., Yang J., Zhu Q. 2003. Lead toxicity, uptake, and translocation in different rice cultivars. Plant Science, 165, 4: 793-802

Lombi E., Zhao F. J., Zhang G., Sun B., Fitz W., Zhang H., McGrath S. P. 2002. *In situ* fixation of metals in soils using bauxite residue: chemical assessment. Environmental Pollution, 118: 435-443

Lombi E., Zhao F.J., Dunham S.J., McGrath S.P. 2001. Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoex- traction. Journal of Environmental Quality, 30: 1919-1926

Lux A., Martinka M., Vaculík M., White P.J. 2011. Root responses to cadmium in the rhizosphere: a review. Journal of experimental botany, 62, 1: 21-37

Madrid F., Díaz-Barrientos E., Florido M.C. 2008. Inorganic amendments to decrease metal availability in soils of recreational urban areas: limitations to their efficiency and possible drawbacks. Water Air and Soil Pollution, 192: 117-12

Marschner P. 2012. Mineral nutrition of higher plants. 3rd ed. Elsevier: 651 str.

Martell A.E., Smith R.M. 2003. NIST Critically selected stability constants of metal complexes, 7th ed. Gaithersburg

Mijangos I., Perez R., Albizu I., Garbisu C. 2006. Effects of fertilization and tillage on soil biological parameters. Enzyme and Microbial Technology, 40: 100-106

Mobin M., Khan N. 2007. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. Journal of Plant Physiology, 164, 5: 601-10

Moreno J.L., Hernández T., García C. 1999. Effects of a cadmium-contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soils. Biology and Fertility of Soils, 28, 3: 230-237

Mühlbachova G. 2011. Soil microbial activities and heavy metal mobility in long- term contaminated soils after addition of EDTA and EDDS. Ecological Engineering, 37, 7: 1064-1071

Mulligan C.N., Yong R.N., Gibbs B.F. 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Engineering Geology, 60, 1-4: 193-207

Neale C., Bricka R., Chao A. 1997. Evaluating acids and chelating agents for removing heavy metals from contaminated soils. Environmental Progress, 16, 4: 274-280

Nowack B. VanBriesen J.M. 2005. Chelating agents in the environemnet. Biogeochemistry of Chelating Agents, 1: 1-18

Nowack B., Sigg L. 1997. Dissolution of Fe(III) (hydr) oxides by metal-EDTA complexes. Geochimica et Cosmochimica Acta, 61, 5: 951-963

Oliver M. A. 1997. Soil and human health: a review. European Journal of Soil Science 48, 4: 573-592

Oomen A. G., Rompelberg C. J. M., Bruil M. A., Dobbe C. J. G., Pereboom D. P. K. H., Sips A. J. A. M. 2003. Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. Archives of Environmental Contamination and Toxicology, 44: 281-287

Orikiriza L.J.B., Agaba H., Tweheyo M., Eilu G., Kabasa J.D., Huttermann A. 2009. Amending soils with hydrogels increases the biomass of nine tree species under non-water stress conditions. Clean, 37: 615-620

Ovecka M., Takac. T. 2014. Managing heavy metal toxicity stress in plants: Biological and biotechnological tools. Biotechnology advances, 32: 73-86

Ozturk L., Karanlik S., Ozkutlu F., Cakmak I., Kochian L.V. 2003. Shoot biomass and zinc/cadmium uptake for hyperaccumulator and non-accumulator Thlaspi species in response to growth on a zinc-deficient calcareous soil. Plant Science, 164, 6: 1095-1101

Panuccio M.R., Sorgona A., Rizzo M., Cacco G. 2009. Cadmium adsorption on vermiculite, zeolite and pumice: Batch experimental studies. Journal of Environmental Management, 90: 364-374

Patinha C., Reis A.P., Dias C., Cachada A., Adão R., Martins H., Ferreira da Silva E., Sousa A. J. 2012. Lead bioavailability in soils from Portugal's center region with special reference to bioaccessibility. Environmental Geochemistry and Health, 34: 213-227

Peer W.A., Baxter I.R., Richards E.L., Freeman J.L., Murphy A.S. 2005. Phytoremediation and hyperaccumulator plants. Molecular Biology of Metal Homeostasis and Detoxification, 14, 84: 299-341

Pelfrene A., Douay F., Richard A., Roussel H., Girondelot B. 2013. Assessment of potential health risk for inhabitants living near a former lead smelter. Part 2: site-specific human health risk assessment of Cd and Pb contamination in kitchen gardens. Environmental monitoring and assessment, 185, 4: 2999-3012

Peters R.W., Shem L. 1992. Use of chelating agents for remediation of heavy metal contaminated soil. Environmental Remediation, ACS Symposium Series, 509: 70-84

Peters R.W. 1999. Chelant extraction of heavy metals from contaminated soils. Journal of Hazardous Materials, 66, 1-2: 151-210

Piechalak A., Tomaszewska B., Baralkiewicz D. 2003. Enhancing phytoremediative ability of Pisum sativum by EDTA application. Phytochemistry, 64, 7: 1239-1251

Reese R.N., Roberts L.W. 1985. Effects of cadmium on whole cell and mitochondrial respiration in tobacco cell suspension cultures (*Nicotiana tabacum* L. var. xanthi). Journal of Plant Physiology, 120, 2: 123-130

Ruby M.V., Davis A., Schoof R., Eberle S., Sellstone C.M. 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environmental Science and Technology, 30: 422-430

Ruley A.T., Sharma N.C., Sahi S.V. 2004. Antioxidant defense in a lead accumulating plant, *Sesbania drummondii*. Plant Physiology Biochemistry, 42: 899-906

Ruley A.T., Sharma N.C., Sahi S.V., Singh S.R., Sajwan K.S. 2006. Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in *Sesbania drummondii* grown in soil. Environmental Pollution, 144, 1: 1-18.

Salt D.E., Smith R.D., Raskin I. 1998. Annual review of plant physiology and plant molecular biology, 49: 643-668

Sarma H. 2011. Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. Journal of Environmental Science and Technology, 4, 2: 118-138

Seaborn D.J., Jameson G.J. 1976. Some ion exchange properties of exfoliated vermiculite. Hydrometallurgy, 2:141-155

Sharma P., Dubey R.S. 2005. Lead toxicity in plants. Brazilian Journal of Plant Physiology, 17, 1: 35-52

Smejkalova M., Mikanova O., Boruvka L. 2003. Effects of heavy metal concentration on biological activity of soil micro-organisms. Plant Soil and Environment, 49, 7: 321-326

Steele M.C., Pichtel J. 1998. Ex-situ remediation of a metal-contaminated super- fund soil using selective extractants. Journal of Environmental Engineering, 124: 639-645

Tandy S., Bossart K., Mueller R., Ritschel J., Hauser L., Schulin R., and Nowack B. 2004 Extraction of heavy metals from soils using biodegradable chelating agents. Environmental science and technology, 38, 3: 937-944

Tessier A., Campbell P.G.C., Bisson M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry, 51: 844-851

Tica D. 2013. Dostopnost kovin v onesnaženih tleh po remediaciji z metodo stabilizacije**,** doktorska disertacija. (Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo), Ljubljana samozaložba: 101 str.

Tica D., Udovic M., Lestan D. 2013. Long-term efficiency of soil stabilization with apatite and Slovakite: the impact of two earthworm species (Lumbricus terrestris and Dendrobaena veneta) on lead bioaccessibility and soil functioning. Chemosphere, 91: 1-6

Tsang D.C.W., Zhang W., Lo I.M.C. 2007. Copper extraction effectiveness and soil dissolution issues of EDTA-flushing of artificially contaminated soils. Chemosphere, 68, 2: 234-43

Twining J.R., Payne T.E., Itakura T. 2004. Soil-water distribution coefficients and plant transfer factors for (134)Cs, (85)Sr and (65)Zn under field conditions in tropical Australia. Journal of Environmental Radioactivity, 71: 71-87

Udovič M., Leštan D. 2007. The effect of earthworms on the fractionation and bioavailability of heavy metals before and after soil remediation. Environmental Pollution, 148, 2: 663-668

Udovič M., Leštan D. 2009. Pb, Zn and Cd mobility, availability and fractionation in aged soil remediated by EDTA leaching. Chemosphere, 74, 10: 1367-1373

Udovič M., Leštan D. 2012. EDTA and HCl leaching of calcareous and acidic soils polluted with potentially toxic metals: remediation efficiency and soil impact. Chemosphere, 88, 6: 718-24

US Environmental Protection Agency. 1995. Test methods for evaluation of solid waste, vol. IA. Laboratory Manual Physical/Chemical Methods, 3rd ed. Office of Solid Waste and Emergency Response (OSWER), Washington, DC

Valcke R.L., Ciscato M., Heisel F., Miehe J.A., Sowinska M. 1999. Analysis of heavy-metal-stressed plants by fluorescence imaging. Laser Radar Technology and Applications, 3707: 82-90

Vulava V.M., Seaman J.C. 2000. Mobilization of lead from highly weathered porous material by extracting agents. Environmental Science and Technology, 34, 22: 4828-4834

Voglar D., Leštan D. 2012. Pilot-scale washing of metal contaminated garden soil using EDTA. Journal of Hazardous Materials, 215-216: 32-39

Voglar D. 2013. Uporaba elektrokemijskih procesov pri remediaciji tal, onesnaženih s kovinami. Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo: 115 str.

Voglar D., Leštan D. 2014. Chelant soil-washing technology for metal-contaminated soil. Environmental Technology, 35, 11: 1389-1400

Wang B., Xue S., Liu G. B., Zhang G.H., Li G., Ren Z.P. 2012. Changes in soil nutrient and enzyme activities under different vegetations in the Loess Plateau area, Northwest China. Catena, 92: 186-195

Warren G.P., Alloway B.J., Lepp N.W., Singh B., Bochereau F.J.M., Penny C. 2003. Field trials to assess the uptake of arsenic by vegetables from contaminated soils and soil remediation with iron oxides. Science of the Total Environment, 311, 1-3: 19-33

Wragg J., Cave M., Basta N., Brandon E., Casteel S., Denys S., Gron C., oomen A., Reimer K., Tack K., Wiele T.V.D. 2011. An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil. Science of the Total Environment, 409, 19: 4016-30

Wuana R.A., Okieimen F.E. 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology, 2011: 1-20

Xu Y., Zhao D., 2005. Removal of copper from contaminated soil by use of poly (amidoamine) dendrimers. Environmental Science and Technology, 39, 7: 2369-2375

Zhang Z.W., Watanabe T., Shimbo S., Higashikawa K., Ikeda M. 1998. Lead and cadmium contents in cereals and pulses in north-eastern China. Science of the Total Environment, 220, 2-3: 137-45

Zhang W., Huang H., Tan F, Wang H., Qiu R. 2010a. Influence of EDTA washing on the species and mobility of heavy metals residual in soils. Journal of Hazardous Materials, 173, 1-3: 369-76

Zhang W., Huang H., Tan F., Wang H., Qui R. 2010b. Influence of EDTA washing on the species and mobility of heavy metals residual in soils. Journal of Hazardous Materials, 173, 1-3: 369-76

Zhang X., Yang L., Li Y., Li H., Wang W., Ye B. 2012. Impacts of lead/zinc mining and smelting on the environment and human health in China. Environmental Monitoring and Assessment, 184, 4: 2261-2273

Zou Z., Rongliang Q., Weihua Z., Hanying D., Zhihao Z., Tao Z., Xiange W., Xinde C. 2009. The study of operating variables in soil washing with EDTA. Environmental Pollution, 157, 1: 229-36

Žukowska J., Biziuk M. 2008. Methodological evaluation of method for dietary heavy metal intake. Journal of Food Science, 73, 2: R21-R29

PRILOGA A

Licenčna pogodba za uporabo članka v tiskani in elektronski verziji disertacije 1

ELSEVIER LICENSE
TERMS AND CONDITIONS

This is a License Agreement between Masa Jelusic ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier Limited, The Boulevard, Langford Lane Kidlington, Oxford,OX5 1GB,UK
Registered Company Number	1982084
Customer name	Masa Jelusic
Customer address	Jamnikarjeva 101 Ljubljana, 1000
License number	3347460118447
License date	Mar 14, 2014
Licensed content publisher	Elsevier
Licensed content publication	Environmental Pollution
Licensed content title	Functioning of metal contaminated garden soil after remediation
Licensed content author	Masa Jelusic, Helena Grcman, Dominik Vodnik, Metka Suhadolc, Domen Lestan
Licensed content date	March 2013
Licensed content volume number	174
Licensed content issue number	None
Number of pages	8
Start Page	63
End Page	70
Type of Use	reuse in a thesis/dissertation
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Title of your thesis/dissertation	QUALITY AND FUNCTIONING OF CONTAMINATED GARDEN SOIL AFTER REMEDIATION
Expected completion date	May 2014
Estimated size (number of pages)	90
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 EUR
VAT/Local Sales Tax	0.00 EUR / 0.00 GBP
Total	0.00 EUR

PRILOGA B

Licenčna pogodba za uporabo članka v tiskani in elektronski verziji disertacije 2

ELSEVIER LICENSE
TERMS AND CONDITIONS

This is a License Agreement between Masa Jelusic ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier Limited, The Boulevard, Langford Lane Kidlington, Oxford,OX5 1GB,UK
Registered Company Number	1982084
Customer name	Masa Jelusic
Customer address	Jamnikarjeva 101 Ljubljana, 1000
License number	3347460517852
License date	Mar 14, 2014
Licensed content publisher	Elsevier
Licensed content publication	Science of The Total Environment
Licensed content title	Effect of EDTA washing of metal polluted garden soils. Part I: Toxicity hazards and impact on soil properties
Licensed content author	Masa Jelusic, Domen Lestan
Licensed content date	15 March 2014
Licensed content volume number	475
Licensed content issue number	None
Number of pages	10
Start Page	132
End Page	141
Type of Use	reuse in a thesis/dissertation
Intended publisher of new work	other
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Title of your thesis/dissertation	QUALITY AND FUNCTIONING OF CONTAMINATED GARDEN SOIL AFTER REMEDIATION
Expected completion date	May 2014
Estimated size (number of pages)	90
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 EUR
VAT/Local Sales Tax	0.00 EUR / 0.00 GBP
Total	0.00 EUR

PRILOGA C

Licenčna pogodba za uporabo članka v tiskani in elektronski verziji disertacije 3

ELSEVIER LICENSE
TERMS AND CONDITIONS

This is a License Agreement between Masa Jelusic ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier Limited, The Boulevard, Langford Lane Kidlington, Oxford,OX5 1GB,UK
Registered Company Number	1982084
Customer name	Masa Jelusic
Customer address	Jamnikarjeva 101 Ljubljana, 1000
License number	3347460818172
License date	Mar 14, 2014
Licensed content publisher	Elsevier
Licensed content publication	Science of The Total Environment
Licensed content title	Effect of EDTA washing of metal polluted garden soils. Part II: Can remediated soil be used as a plant substrate?
Licensed content author	Masa Jelusic, Dominik Vodnik, Irena Macek, Domen Lestan
Licensed content date	15 March 2014
Licensed content volume number	475
Licensed content issue number	None
Number of pages	11
Start Page	142
End Page	152
Type of Use	reuse in a thesis/dissertation
Intended publisher of new work	other
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Title of your thesis/dissertation	QUALITY AND FUNCTIONING OF CONTAMINATED GARDEN SOIL AFTER REMEDIATION
Expected completion date	May 2014
Estimated size (number of pages)	90
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 EUR
VAT/Local Sales Tax	0.00 EUR / 0.00 GBP
Total	0.00 EUR

ZAHVALA

Spodobi se, da se zahvalimo vsem, ki so nas česa naučili, pravijo. A včasih tega ne storimo zaradi spodobnosti, temveč zaradi druge šege – iskrene hvaležnosti. Morda je zahvala v disertaciji le izgovor in hkrati priložnost za nekaj misli o doktorski avanturi, ki naj bodo javne in zabeležene … da jih ne pozabim. Morda je najpomembnejša stran ravno pričujoča. Zakaj učeni doktorat ni le rezultat neštetih laboratorijskih in pisarniških ur, temveč so vanj nevidno vtkane misli in dejanja posameznikov, brez katerih se »misija doktorat« sploh ne bi začela, kaj šele dokončala. Ti nevidni, neobjavljeni rezultati spoznavanja in dozorevanja pomenijo veliko več kot črki d in r, ki v očeh družbe osvetljujeta pomembno ime. … Ker poleg vseh znanstvenih odkritij in čudes posameznik med študijem nehote odkrije tudi na prvi pogled izmikajoča dejstva današnje znanosti in akademije. Znanost ni več, kar je bila nekdaj, v zlatih časih. Velikokrat se ukvarja le sama s seboj ter kot larpurlartizem lebdi nad družbo in riše varljivo sliko pomembnosti, ki pa ob podrobnejšem pogledu hitro zbledi. Slika vsekakor ni lokalna in prav tako ne vseobsegajoča; odvisna je predvsem od posameznikov, znanstvenikov, ki so ne nazadnje ljudje, kot vsi mi: dobri in malo manj dobri. Vse prepogosto se tako ključna vprašanja pometejo pod preprogo koristoljubja, apatije ali preprosto nevednosti. Samopomembnost in komolčenje se skrivata za masko družbene koristi, ki se čedalje bolj odmika vsemogočnim faktorjem vpliva pomembnih znanstvenih revij. Po vsem svetu se prvotna ideja o znanosti, ki jo čuti le še peščica posameznikov, ruši pod koraki predvidljivega, pohlepnega človeštva. Večina vej na drevesu znanosti tako že zdavnaj ne proizvaja več zelenih idej, nekatere celo s prebujno rastjo ogrožajo ravnotežje drevesa, katerega korenine so že davno gnile in načete, in le redki majhni poganjki nekaterih posameznikov vlivajo upanje v novo pomlad. Kot politika in druge družbene strukture je znanost v rdečo nit pozabila vtkati osnove etike in medčloveškega spoštovanja, samokritičnost, otroško radovednost in drzne sanje o boljšem svetu. Kajti, ne nazadnje, tudi četudi znanost v današnjem stanju družbe odkrije zdravilo za vse vrste in podvrste rakov, razreši uganko o neusahljivem energetskem viru in nahrani vse lačne otroke tega sveta, se naše težave ne bodo končale … Dobili bomo nekaj novih Nobelovih posvečencev in zanimivih izumov, težave pa se bodo šele začele.

Morda je povedano le spontana, iskrena zahvala obdobju, ki mi je prineslo nove poglede in zanimive izzive, morda pa je le resnični in iskreni povzetek doktorske naloge.

V Zahvali se seveda spodobi zahvaliti tudi vsem, ki so se vključili v doktorsko delo, vsem, ki so pripisali kako pomembno številko, izmerili nek pomemben pojav ali delili kak dober nasvet. Torej, Hvala Domen za mentorstvo; bilo je poučno in predvsem nikoli dolgočasno. Hvala Simon in Hvala Erika za sproščeno delovno vzdušje zadnjega leta. Hvala Zalka in Hvala vsem posameznikom pedološke katedre. Ne nazadnje, Hvala tudi vsem, s katerimi smo sodelovali pri člankih, in Hvala celotni komisiji.

Spodobi se, in veliko več kot le to, zahvaliti tudi resničnim protagonistom, življenja izven službenih zidov. Večina pripetljajev doktorske ere je namreč izzvenela daleč stran od znanstvenih polj. Hvala, Urši, pa ne toliko za obiranje in štetje grahkov, kot za vse ostale reči. Hvala za odločnost, Hvala za sprejemanje, Hvala za vse besede in objeme, Hvala za tisti spontani nočni izlet in tišino na pomolu, Hvala za vse prebrane zgodbe in pesmi, Hvala za Indonezijo, službeno telefončkanje in vse wowo neumnosti, ki so se hote ali nehote pripetile. Aja, in hvala za piškotke. Hvala Gekko teamu za vsa prečudovita popotovanja in še prečudovitejše predpriprave. Hvala Tanja, Anja in Nina, I guess we will always have Paris, pa naj bo sredi mrzlega Vietnama, vroče ameriške puščave ali na vrhu starodavne piramide. Hvala vsem Ponijem, Samorogom in Pegazom, Hvala Alja, Anja in Urši. Hvala za Sigmunda, izkrivljene zorne kote in divje zabave (blink, blink).

Kati, Hvala za zabavno sobotno obiranje špinače ob štirih zjutraj in seveda za Perotov podpis! Prof. dr. Gregor Osterc ☺ Hvala ti za vse nasvete o okrasnih rožicah. Hvala, Marko, za arzenčkanje in klepete; hvala Anči kar tako in hvala Martina kar tako; hvala Tea za FB novičke in maile; hvala sošolcem z varstva okolja, še posebej Ireni in Špeli za zabavno družbo na dolgočasnih predavanjih; hvala agronomčkom in hvala še vsem, ki sem vas morda pozabila napisati; hvala.

Nataška, Hvala, ker zmoreš veliko več; ker pomagaš, ko niti ne veš; ker razumeš, tudi ko ne; ker si odločna, nepopustljiva, lojalna in včasih tako odkrito naivna, da svet deluje čisteje … Hvala, ker zaupaš vame in ker mi pomeniš veliko več, kot meniš. Janko, Hvala, ker sta skupaj. P.S. Hvala seveda tudi za košenje trave, špinače in sončnic ☺. Rada te imam, sestrica!

Tacko, Hvala za močno podporo iz ozadja, še posebej zadnje leto, Hvala. Hvala, da smo obdržali »Nova Leta«, Hvala za vse modrosti Ganeškota in predvsem Hvala, Hvala za novi dom.

Milko, Hvala za vse prevoze, nasvete ter košnjo trav in bazilike. Hvala za vse neumnosti, da je bilo bolj zanimivo, praviš :P, Hvala, ker me vedno znova pripelješ domov in Hvala za toliko reči, ki naj ostanejo nenapisane .. ..

Ker mame seveda morajo imeti zadnjo in najpomembnejšo besedo, bi na koncu **doktorsko nalogo** in, vse kar lahko pripnem zraven, **posvetila mami, Katjuši Jelušič Ziherl** ... ker boš ponosna ☺, ker si to najbolj zaslužiš, ker si moj nedosegljivi vzor v toliko čudovitih rečeh, ker si doživela preveč … ker nekje v sebi veš, da bo vse, kar spišem, rečem ali dam, še vedno premalo … Hvala! Hvala, ker me imaš čisto preveč rada, in enostavno Hvala, ker si moj največji fan ☺.