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CELKOVÉ OBSAHY A SPECIACE ARSENU A SELENU V ROSTLINÁCH ROSTOUCÍCH NA PŮDÁCH S RŮZNÝMI FYZIKÁLNĚ-CHEMICKÝMI VLASTNOSTMI

Disertační práce

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Praha 2016

Prohlášení

Prohlašuji, že jsem předloženou disertační práci na téma "**Celkové obsahy a speciace arsenu a selenu v rostlinách rostoucích na půdách s různými fyzikálně-chemickými vlastnostmi**" vypracovala samostatně, s využitím citovaných literárních pramenů a pod odborným vedením mé školitelky prof. Ing. Jiřiny Szákové, CSc..

v Praze dne: _____ _____

Ing. Jana Tremlová

Poděkování

Chtěla bych velmi poděkovat své vedoucí doktorské disertační práce prof. Ing. Jiřině Szákové, CSc. a odbornému konzultantovi prof. Ing. Pavlu Tlustošovi, CSc. za jejich pomoc a odborné vedení v průběhu celého doktorského studia a tvorbě publikací.

Také bych ráda poděkovala Ústavu analytické chemie VŠCHT v Praze, Ústavu chemie Univerzity Karl-Franzens v Grazu a členům Katedry agroenvironmentální chemie a výživy rostlin České zemědělské univerzity v Praze za veškerou poskytnutou pomoc a spolupráci.

Zvláště pak děkuji své rodině a blízkým přátelům, kteří pro mě v průběhu doktorského studia byli velkou oporou.

Obsah

1 ÚVOD

Problematika rizikových prvků a jejich působení v životním prostředí je široce zkoumané téma, které bylo popsáno již v mnoha publikacích, např. Adriano (2001), Kabata-Pendias et Pendias (2001), Kabata-Pendias et Mukherjee (2007), Hooda (2010) aj. Během posledních desetiletí bylo zejména díky technickému pokroku vyvinuto mnoho nových metod stanovení rizikových prvků v nejrůznějších matricích a v širokém rozpětí koncentrací, často přesahujících několik řádů. Proto se i samotný výzkum mohl posunout ke studiu velice nízkých (až ultrastopových) koncentrací ve vzorcích, ve kterých by dříve koncentrace některých prvků byly pod detekčními limity analytických technik. Navíc se nemusíme omezovat pouze na celkové obsahy, jejichž vypovídací hodnota často není dostačující, ale je možné využít i speciační techniky, které stanoví, jaké sloučeniny daného prvku jsou ve vzorku přítomny (Meers et al., 2005; Buffle et Tercier-Waeber, 2005; Citak et al. 2010).

I přesto, že jsou na emise polutantů do životního prostředí kladeny přísné legislativní limity, ani tak nelze zcela zamezit jejich vstupu do biosféry a interakce s živými organismy. Ať už proto, že jsou součástí starých ekologických zátěží nebo proto, že rizikové prvky, na rozdíl od mnoha organických polutantů, které jsou často schopny degradace, se v životním prostředí kumulují a mohou tak dále vstupovat do potravních řetězců (Peralta-Videa et al., 2009). Závažnost problému kontaminace životního prostředí arsenem dokládají i oficiální dokumenty měst jako např. Kutná Hora (viz kapitola 9.2), která se snaží svými doporučeními např. o tom jaké plodiny pěstovat nebo jak se chovat v okolí hald, snížit škodlivé působení tohoto prvku na své občany.

Na druhé straně jsou zde prvky jako selen, molybden, bor aj., které jsou v půdách na našem území deficitní (Poláková, 2010), ať už to je z důvodu nízkého obsahu v mateční hornině, intenzivního hospodaření na orné půdě bez přihnojování mikroprvky, nebo díky místním půdně-klimatickým podmínkám (Bell et Dell, 2008). Tato skutečnost může mít za následek nedostatek některých prvků v potravě na různých trofických úrovních (Singh, 2009; Chenery et al., 2012).

Významným přechodem mezi půdou a vyššími trofickými úrovněmi jsou rostlinná společenstva, která tak tvoří základ pro tok mikroprvků, makroprvků a současně i pro tok rizikových prvků (Chenery et al., 2012; Ehrenfeld, 2013). Podle výsledků dřívějších studií

(Jung, 2008; Song et al., 2009; Poláková et al., 2013) předpokládáme, že fyzikálně-chemické vlastnosti půd budou i v našem případě jedním z hlavních faktorů určujících celkové obsahy a specie arsenu respektive selenu v biomase rostlin.

Schopnost příjmu a akumulace prvků z půd rostlinami může být využívána buď k žádoucímu obohacení (zejména plodin a pícnin) o esenciální prvky, nebo naopak k akumulaci rizikových prvků v biomase rostlin tzv. fytoremediaci, a tím odstranění škodlivého působení prvků nacházejících se v půdě (Cameselle, 2013). Schopnost příjmu, akumulace a transformace na jednotlivé sloučeniny se mezi rostlinnými druhy významně liší (Kuehnelt et al., 2000; Schmidt et al., 2001; Jana et al., 2012). Předpokládáme, že budeme schopni tento fakt také potvrdit a najít rostlinné druhy běžně se vyskytující na vybraných stanovištích v rámci České republiky, které jsou schopné významné akumulace jak arsenu a jeho nejběžnějších sloučenin, tak druhy, schopné akumulace selenu. Podobné studie zabývající se obsahem arsenu v rostlinných společenstvech volně rostoucích rostlin byly provedeny např. na území Španělska (Larios et al., 2012) a Polska (Jedynak et al., 2009). Zatím nám však nejsou známy žádné, které by se zabývaly územím České republiky.

Studie hodnotící obsah selenu v rostlinných společenstvech volně rostoucích rostlin jsou pak celosvětově velmi ojedinělé (Miladinović et al., 1998; Sasmaz et al. 2015), a podobně jako u arsenu, studie zabývající se obsahem selenu v rostlinných společenstvech volně rostoucích rostlin na našem území zatím nebyly provedeny. Zajímavé je také sledovat to, v jakých sloučeninách se arsen či selen v rostlinách vyskytuje a jsou-li rostliny z vybraných stanovišť schopné transformace anorganických sloučenin, které se v půdě vyskytují nejčastěji, do sloučenin organických. Zjištění výskytu a rozložení jednotlivých specií v různých částech rostlin by mohlo pomoci k pochopení biochemických procesů probíhajících v rostlinách a mechanismu příjmu těchto prvků kořenovým systémem. Dále by tato data mohla napomoci k odhadu možného toxického vlivu zvýšených obsahů arsenu/selenu v rostlinné biomase, protože ne všechny sloučeniny arsenu a selenu vykazují pro člověka stejnou míru toxicity. Naopak výskyt některých sloučenin selenu, zejména těch s protirakovinovým účinkem, by byl pozitivním zjištěním, které by se dalo dále využít k ochraně lidského zdraví.

2 LITERÁRNÍ PŘEHLED

2.1 ARSEN

2.1.1 Základní charakteristika arsenu

Arsen spolu s antimonem, bismutem, dusíkem a fosforem tvoří v periodické tabulce skupinu 15 a jeho relativní atomová hmotnost je 74,92 g mol^{-1}. Je to polokov vyskytující se ve třech alotropických modifikacích: černá, žlutá a šedá neboli kovová, která se vyskytuje nejčastěji (Greenwood et Earnshaw, 1993). Chemicky je velmi podobný fosforu a může ho v některých biochemických reakcích i nahrazovat (Kabata-Pendias et Pendias, 2001; Dani, 2011). Ve sloučeninách je arsen stálý v oxidačních stavech -3, +3, +5 (Wang et Mulligan, 2006; Charlet et al., 2011). Většina jeho sloučenin je bez barvy a bez zápachu, snadno rozpustná ve vodě, což zvyšuje jeho potenciální riziko působení v životním prostředí (Wang et Mulligan, 2006).

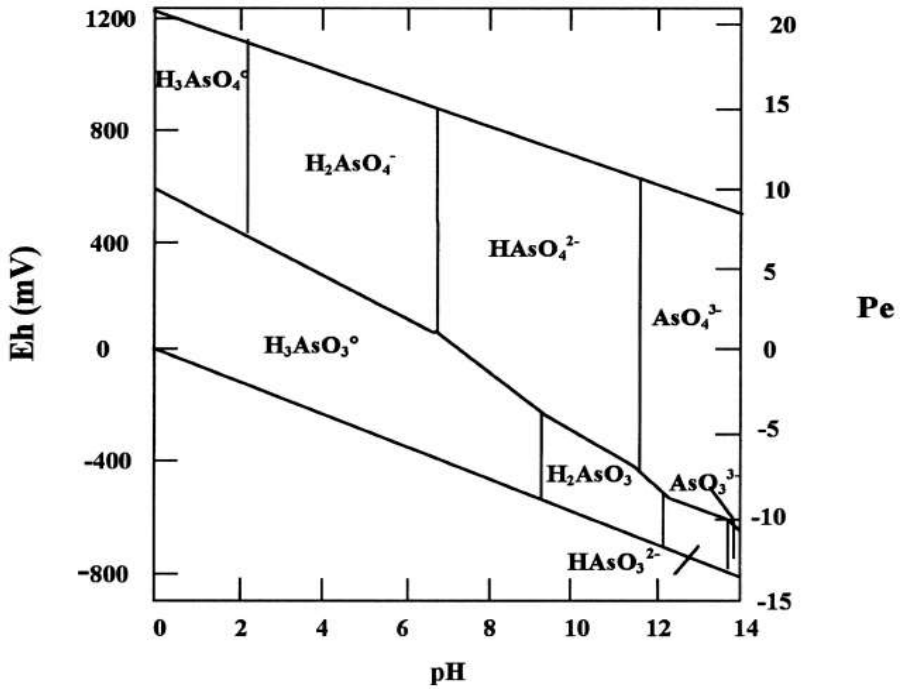
2.1.2 Výskyt arsenu v životním prostředí

Arsen je prvek, který se v životním prostředí vyskytuje zcela běžně. Je součástí hornin, půd, přírodních vod a ve stopových množstvích se vyskytuje ve všech živých organismech (Wang et Mulligan, 2006). Arsen je přirozenou součástí několika stovek různých minerálů, v 60 % z nich se pak vyskytuje jako arseničnan (Kabata-Pendias et Pendias, 2001). Takovými příklady jsou farmakosiderit, pitticit, skorodit a jiné (Filippi et al., 2004), nejznámějším minerálem obsahujícím arsen je však arsenopyrit (FeAsS) (Jiang et al., 2008). Průměrné obsahy arsenu v horninách se pohybují od 0,5 do 2,5 mg As kg^{-1}, v sedimentárních horninách mohou dosahovat až 13 mg As kg^{-1}.

V nekontaminovaných půdách mohou obsahy arsenu v závislosti na typu půd, podloží a místních podmínkách dosahovat až 93 mg As kg^{-1} sušiny (U.S.) (Kabata-Pendias et Pendias, 2001). Ačkoliv minerály a sloučeniny arsenu jsou snadno rozpustné ve vodě, jeho mobilita v půdě a půdním roztoku je z velké části limitována jeho silnou sorpcí na hydratované oxidy železa, kde může koncentrace arsenu dosáhnout až několika hmotnostních procent (Manning et Goldberg, 1996), dále na hydratované oxidy hliníku a manganu, jílové minerály i organickou hmotu. Jeho mobilita v půdě je tak ve srovnání s jinými prvky jako je např. kadmium nebo zinek

poměrně nižší (Kabata-Pendias et Pendias, 2001; De Brouwere et al., 2003). Al-Abed et al. (2007) ve studii vlivu hodnoty pH na mobilitu celkového arsenu uvádějí nejnižší mobilitu arsenu při pH 5 a nejvyšší při pH 11, přičemž celkové obsahy arsenu v rozmezí pH 3-11 kopírují tvar křivky V, která odráží chování různých sloučenin arsenu a precipitaci arsenu na oxidy a hydroxidy železa při různém pH. Nejčastěji vyskytující se formy arsenu v závislosti na pH a Eh půdy ukazuje obrázek č. 1. Za běžných podmínek v orné půdě (pH mezi 4-8, Eh okolo 500 mV) se nejčastěji vyskytuje As^{+5}, v menší míře pak As^{+3}. Směrem k anoxickým či anaerobním podmínkám v půdě se tento poměr obrací. Tento poměr je důležitý zejména proto, že arsenitan je pro rostliny přibližně 5x toxičtější než arseničnan. Je také více vodorozpustný a tudíž rostlinám snadněji přístupný. (Smedley et Kinniburgh, 2002)

Obrázek č.1: Eh-pH diagram pro vodorozpustné specie arsenu v půdě v závislosti na pH a Eh při teplotě 25 °C, za běžného atmosférického tlaku (Smedley a Kinniburgh, 2002)

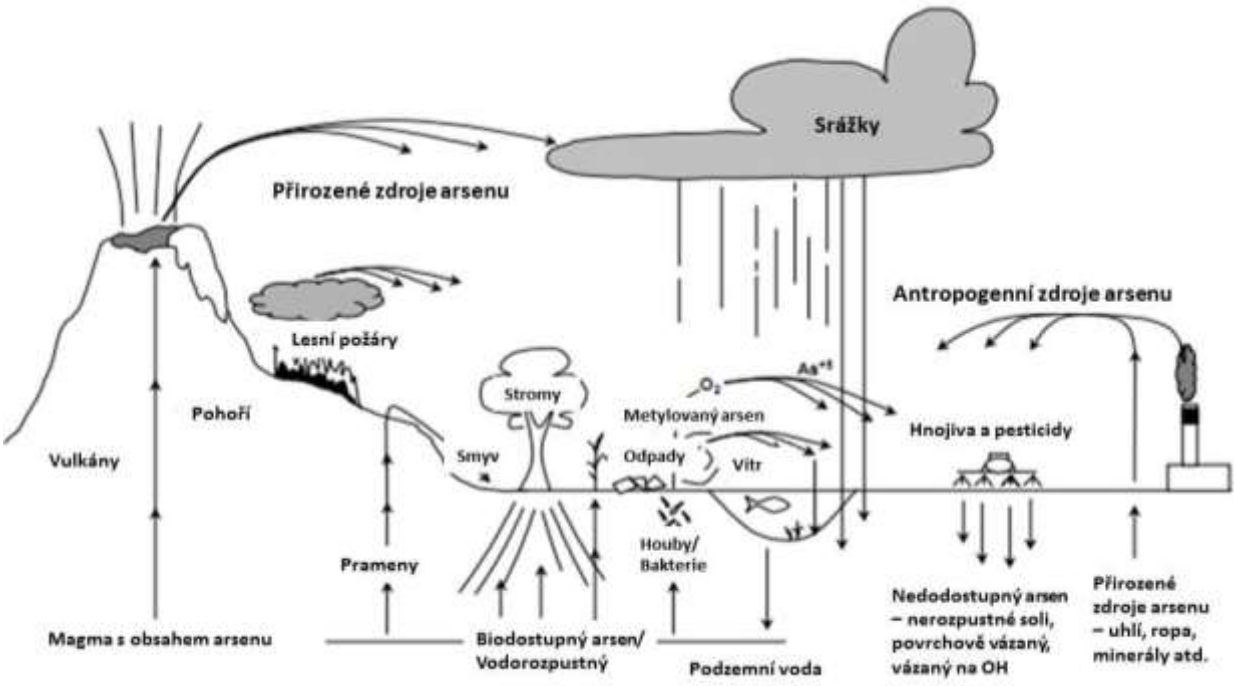


Z půdy, ale i z hornin, může arsen díky působení sorpčních a desorpčních sil přecházet z pevné fáze do fáze kapalné a tím způsobovat kontaminaci povrchové i podzemní vody (Welch et al., 2000; Li et al.[b], 2013; Sorg et al., 2014). Je zaznamenáno několik případů otravy několika desítek až několika tisíc lidí v důsledku kontaminace pitné vody arsenem, jehož koncentrace významně přesahovala doporučený limit WHO (2012) pro pitné vody, který je stanoven

na 10 μg As l^{-1}. Nejznámější případy takovýchto otrav jsou z okolí Bengálské zátoky v Indii, v Bangladéši (Chakraborty et al., 2010; McArthur et al., 2012; Freikowski et al., 2013), a v Jižní Americe např. v Chile, Argentině a Mexiku (Bundschuh et al., 2012; Alarcón-Herrera et al., 2013).

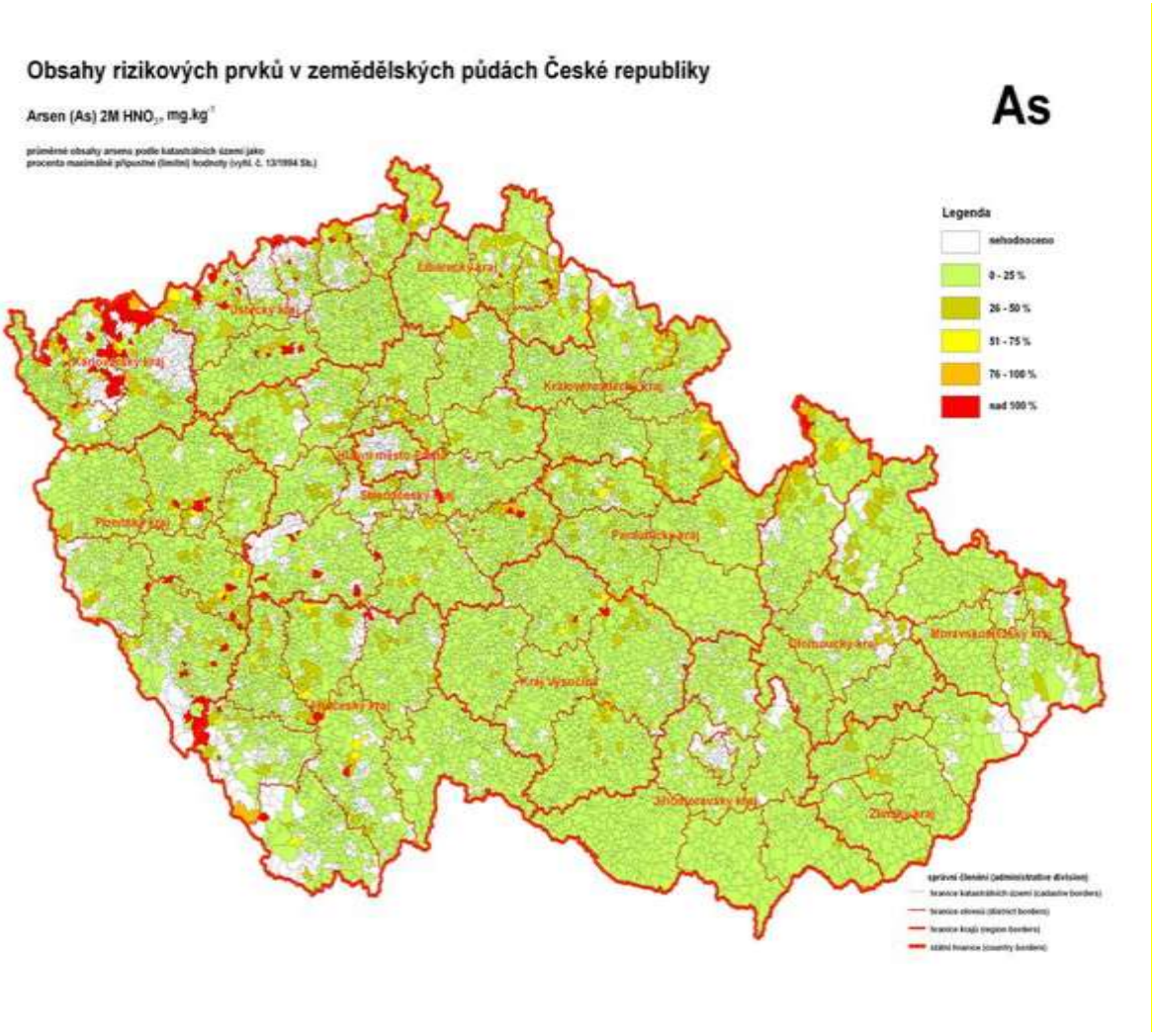
Zvýšené obsahy arsenu se vyskytují jak v důsledku geogenních, tak antropogenních vlivů (Villaescusa et Bollinger, 2008; Bundschuh et al., 2012) (obrázek č. 2). Autoři uvádějí zvýšení koncentrace arsenu v životním prostředí díky přírodním vlivům jako je zvětrávání hornin bohatých na arsenové minerály, biologická aktivita, vulkanická činnost aj. Z antropogenních vlivů zvyšuje obsahy arsenu v prostředí zejména důlní činnost, kde významně zvýšené koncentrace arsenu se vyskytují nejčastěji v blízkosti zlatých dolů a spaloven uhlí (Villaescusa et Bollinger, 2008), a používání některých zvířecích kokcidiostatik, herbicidů, fungicidů a pesticidů obsahující v účinné látce atom arsenu (Smedley et Kinniburgh, 2002). Mezi nejznámější patří např. kokcidiostatikum Roxarsone, tj. kyselina 3-nitro-4-hydroxyfenylarsonová (Bednar et al., 2003), pesticid a fungicid arseničnan měďnatý (CCA) používaný k ochraně dřeva (Nico et al., 2006), nebo natrium methylarsonát (MSMA), herbicid který se používá k ošetření trávníků na golfových hřištích (Pichler et al., 2008) nebo k ošetření plantáží bavlny a rýže (Hua et al., 2013).

Obrázek č. 2: Cyklus arsenu v životním prostředí a jeho nejdůležitější zdroje v životním prostředí. Převzato a upraveno z Anonym [a] [online].



Na území České republiky je známo několik míst, která se vyznačují zvýšenými obsahy arsenu v půdě (obrázek č. 3). Jde zejména o místa v okolí bývalých zlatých a stříbrných dolů, kde arsen a jeho minerály přirozeně doprovází zlatou a stříbrnou rudu. Nalžovské Hory (Tremlová et al., 2011), Kutná Hora, Mokrsko, Roudný, Kašperské hory jsou vybranými příklady míst s bývalou těžbou rud, kde se nyní vyskytuje vysoký obsah arsenu v půdě (Filippi, 2004), který může dosahovat i více než 1000 mg As kg^{-1} sušiny (Mokrsko) (Filippi et al., 2007).

Obrázek č. 3: Celkové obsahy arsenu v zemědělských půdách vyjádřené jako procenta maximální přípustné hodnoty dle Vyhlášky č. 13/1994 Sb (tj. 30 mg As kg^{-1}). Převzato a upraveno z ÚKZÚZ [online].



2.1.3 Vliv arsenu na organismy

Arsen je prvek toxický jak pro člověka, tak pro rostliny i zvířata. Zdá se, že pro zvířata je tento prvek v určitých velmi nízkých koncentracích prvkem esenciálním, i když jeho biochemická role ve fyziologii zvířat ještě není objasněna, proto je třeba uvažovat spíše o hormezním efektu arsenu (Pergantis et al., 1997; Li et Chen, 2005). Toxický efekt arsenu na rostliny je popsán v mnoha publikacích jako např. Kabata-Pendias et Pendias (2001), Caporale et al. (2013), i když např. Gulz et al. (2005) se zmiňují, že jeho velice nízké koncentrace v půdním roztoku mohou podporovat klíčení a růst rostlin. To autoři vysvětlují tím, že i) to může být způsobeno podobným mechanismem jako při stimulaci rostlinného růstu subletálními dávkami některých pesticidů, ii) nahrazením fosfátových iontů v půdě arseničnanovými a tím následně lepší dostupnosti fosforu rostlinám. Mezi příznaky toxického působení arsenu na rostliny patří snížení proliferace kořenových buněk, celkové snížení růstu, ztráta fertility a výnosu, a při vysokých koncetracích nebo dlouhodobém působení až odumření celé rostliny (Garg et Singla, 2011). Kabata-Pendias et Pendias (2001) navíc uvádějí žloutnutí listů, fialové skvrny na listech, vybělení kořenů a na buněčné úrovni pak plasmolýzu buněk. Arsen může také negativně ovlivňovat fotochemickou část fotosyntézy, kde zasahuje do elektrotransportního řetězce. Výsledkem může být změna ve formování redukujícího NADPH a ATP, zvýšení fluorescence, či podpoření uvolňování energie ve formě tepla (Gusman et al. 2013).

Arsen se řadí mezi potencionální karcinogeny a chronická expozice člověka tomuto prvku může způsobovat zhoubné kožní léze, rakovinu močového měchýře a plic, dýchací, zažívací a nervové potíže, poruchy funkce jater, krvetvorby a imunitního systému. Může se také podílet na vzniku cukrovky a kardiovaskulárních onemocnění (Santra et al., 2013; Hu et al., 2013). Dříve používaný limit PTWI (provisional tolerable weekly intake = dočasně tolerovatelný týdenní příjem), který byl stanoven na 15 µg As kg^{-1} tělesné hmotnosti již není nadále považován za dostatečný a došlo k jeho úplnému zrušení (JECFA, 2011). Zejména proto, že i významně nižší hodnoty přijatého arsenu mohou mít na člověka negativní dopad.

Arsen však nemusí být pro člověka jen škodlivý. Je součástí např. některých léků používaných při léčbě vzácných typů leukémie (Yang et al., 2013) a některé výzkumy poukazují i na jeho imunosupresivní účinky (Srivastava et al., 2013). V určitých okrajových případech a v přesném farmakologickém dávkování může tedy mít i pozitivní vliv na lidské zdraví. Toto využití je však velice specifické, proto se arsen běžně uvádí jako prvek pro člověka toxický a podle toho se s ním také zachází.

2.1.4 Arsen a rostliny

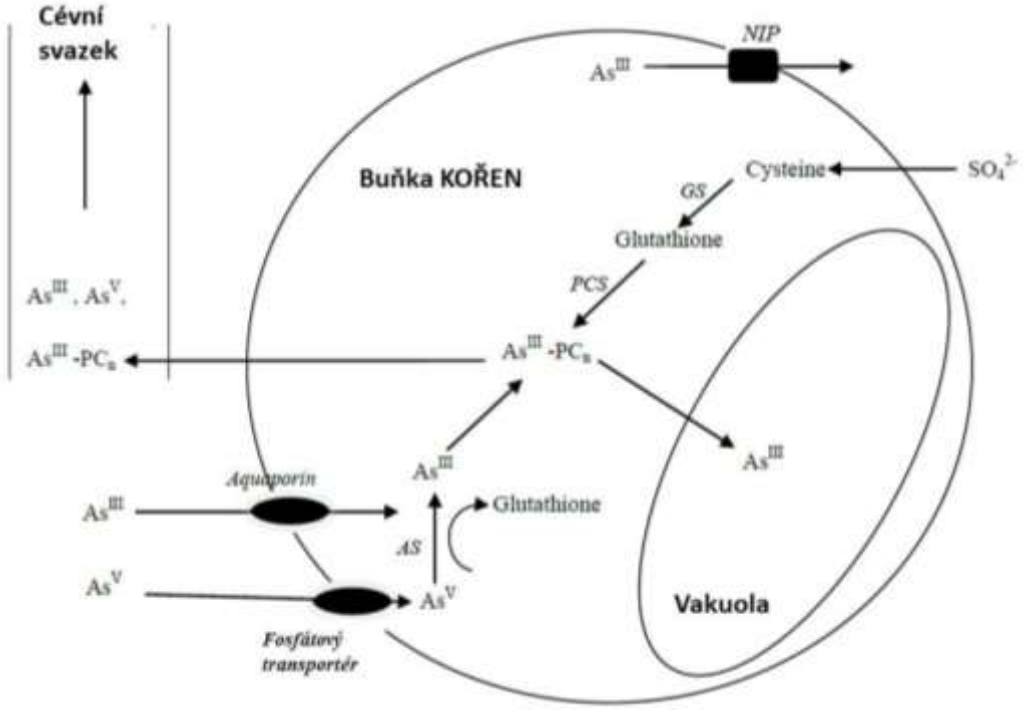
2.1.4.1 Příjem arsenu rostlinami

Rostliny přijímají arsen v největší míře z půdního roztoku (Ma et al., 2010), ale mohou ho přijímat i přes kutikulu listů (Schreck et al., 2012). Příjem prvků suchozemskými rostlinami je obecně ovlivněn koncentrací prvku v půdě, jeho dostupnými sloučeninami, fyzikálně-chemickými vlastnostmi půd, rostlinným druhem a celkovým výživovým stavem rostliny (Jedynak et al., 2009; Vithanage et al., 2012). V této souvislosti je to zejména fosfor, který významně ovlivňuje příjem a transformaci arsenu rostlinou, protože arsen využívá velice podobné biochemické dráhy jako fosfor (Vela et al., 2001; Quaghebeur et Rengel 2003). Dalším prvkem, který může ovlivňovat příjem a přeměnu arsenu v rostlině je vápník. Experimenty s tkáňovými kulturami brukve sítinovité (*Brassica juncea* L.) ukazují, že přidání vápníku může mít za následek nejen zvýšení celkového obsahu arsenu v rostlině, ale také podíl anorganického arsenu, zejména arsenitanu (Rai et al., 2012). Autoři si to vysvětlují signální funkcí vápníku a včasným vnímáním signálu stresu, který může ovlivnit další obranné mechanismy v rostlině. Posledním prvkem, který se zmiňuje v souvislosti s ovlivněním příjmu arsenu rostlinou, je železo, v tomto případě jeho nedostatek. U trav nedostatek železa způsobuje tvorbu a sekreci specifických chelátorů fytosiredoforů (Vaněk et al., 2007), které následně mohou zvyšovat možnost příjmu těžkých kovů z půdy (Tlustoš et al., 2006).

Arsen vstupuje do rostliny přes kořenový apoplast. Bravin et al. (2008) uvádějí, že obsah arsenu v apoplastu může být až tak významný, že např. u rýže (*Oryza sativa L.*) pěstované v redukčních podmínkách může tvořit až 60 % celkového obsahu arsenu v kořeni. Dle Chen et al. (2005) u *Pteris vittata* L. se může až 1/6 z celkového obsahu arsenu v rostlině nacházet právě v apoplastu. Z apopalstu může arsen přestupovat do rostlinného cytosolu. Meharg et Jardine (2003) uvádějí, že arseničnan k prostupu přes buněčnou membránu používá transportéry fosforu, arsenitan, dimethylarsenitan (DMA) a methylarseničnan (MA) pak aquaroporiny (obrázek č. 4).

Obrázek č. 4: Příjem, detoxifikace a transport arsenu buňkou kořene a cevním svazkem. As^{III} = arsenitan, As^{V} = arseničnan. Cystein je syntetizován z přijatých síranů a následně je transformován na glutathion pomocí glutathion syntetázy (GS). Fytochelatinsyntetáza (PCS) vytváří z glutathionu fytochealtiny. Arsenitan se váže na fytochelatiny (As^{III} –PC_n) a je transportován do vakuol nebo dále do cévního svazku.

Další možností je, že je arsenitan z buňky odstraněn pryč pomocí NIP aquaporinů. Arseničnan reduktása (AS) za přitomnosti glutathionu a redukčního činidla redukuje arseničnany na arsenitany. Převzato a upraveno z Tripathi et al. (2007).



Kontaminace půd arsenem obecně ovlivňuje nakládání s některými makroprvky a mikroprvky v rostlinném organismu. Dokládají to např. Liu et al. (2008), kteří uvádějí, že příjem arsenu snižuje v rostlině koncentraci prvků jako je draslík, železo, měď, zinek, dusík a částečně i fosfor, vápník a hořčík. Lu et al. (2010) uvádějí, že toxický účinek arsenu na rostliny rýže (*Oryza sativa* L.) lze úspěšně eliminovat právě přidáváním fosforu do půdy, kde pak tento prvek díky podobným drahám příjmu působí jako kompetitor arsenu.

Ačkoliv je arsen rostlinám málo dostupný (Pickering et al., 2000), jeho dostupnost mohou rostliny zvýšit prostřednictvím kořenových exudátů. Jsou to kořenové výměšky různého chemického složení, nejčastěji na bázi slabých organických kyselin (citrónová, jantarová, šťavelová,…), sacharidů, mastných kyselin, enzymů apod., které výrazně pomáhají a urychlují přestupu prvků vázaných v půdě do půdního roztoku (Moreno-Jimenéz, 2012; Bergqvist et al., 2014). Silva-Gonzaga et al. (2012) tento mechanismus mobilizace arsenu z půdy sledovali např. u kapradin *Pteris vittata* L. a *Pteris biaurita* L..

Dalším důležitým faktorem, který ovlivňuje dostupnost arsenu rostlinám je mikrobiální činnost v půdě. Mikroorganismy svými biochemickými pochody významně

působí na zvětrávání hornin, ze kterých získávají živiny jako např. železo, síra, molybden, měď nebo zinek. Společně s nimi však do prostředí uvolňují i další prvky, které jsou v horninách přítomny, často tedy i rizikové prvky včetně arsenu (Drewniak et Sklodowska, 2013). Svými procesy se také podílejí na tvorbě těkavých sloučenin arsenu (arsanu, methylarsanu, dimethylarsanu a trimethylarsanu), které pak mohou unikat do ovzduší (Michalke et al., 2000). Mohou se podílet i na celkové změně dostupnosti arsenu v půdě, přeměnou jeho sloučenin ve sloučeniny jiné s odlišnými fyzikálně-chemickými a biologickými vlastnostmi. Bylo prokázáno, že vlivem mikrobiální aktivity dochází k tvorbě methylovaných sloučenin MA a DMA, a ke změně poměru As^{III} a As^{V} (Dopp et al., 2004; Jia et al. 2013; Xu et al., 2016).

2.1.4.2 Hyperakumulace arsenu a ochranné mechanismy rostlin

Některé rostliny jsou bez problémů schopny přijímat a akumulovat vyšší koncentrace arsenu z půdního roztoku než jiné. Tato variabilita v příjmu se vyskytuje nejen u volně rostoucích rostlin, ale i u zemědělských plodin (Kabata-Pendias et Pendias, 2001). Kromě toho některé druhy rostlin můžeme označit jako hyperakumulátory arsenu, což jsou rostliny schopné akumulovat ve své biomase více než 1000 mg As kg^{-1} sušiny (Jedynak et al., 2009). Mezi hyperakumulátory arsenu patří např. kapradiny *Pteris vittata* L., *Pteris cretica*, L., *Adiantum capillus-veneris* L. a *Nephrolepis exaltata* L. (Wan et al., 2013; Singh et al., 2010).

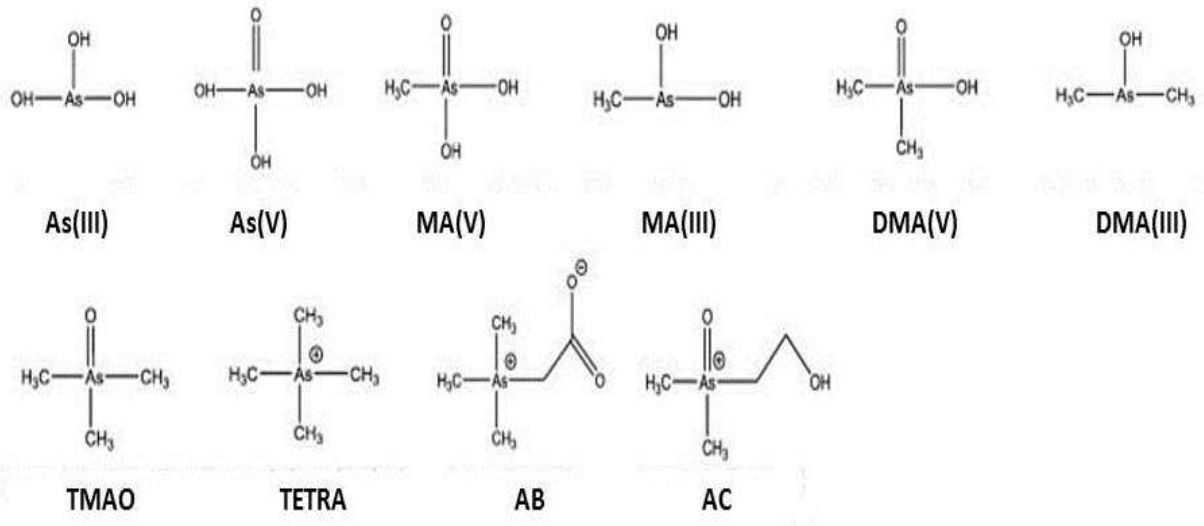
Jedním z detoxifikačních mechanismů u suchozemských rostlin, který je chrání před poškozením, je snižování toxicity tím, že rostliny vytváří s toxickými prvky komplexní sloučeniny. Nejznámější je tvorba chelátových komplexů s glutathiony, fytochelatiny nebo metalothioneiny. Glutathiony jsou neenzymatické antioxidanty, které mají nízkou molekulovou hmotnost. Fytochelatiny jsou deriváty peptidů (oligomery glutathionů), které jsou schopny vázat atomy toxického prvku a tím snižovat jeho toxicitu pro rostliny (Hall, 2002; Vatamaniuk et al., 2001; Jedynak et al., 2009; Gupta et al., 2011). Někteří autoři (Schmoger et al., 2000) uvádějí, že jejich stabilita, zvláště jsou-li vázány s arsenem je velmi nízká. Metalothioneiny jsou peptidy s nízkou molekulovou hmotností bohaté na obsah aminokyseliny cysteinu. Stejně jako fytochelatiny, metalothioneiny také vytvářejí vazby s některými rizikovými prvky a tím pomáhají ke snížení jejich toxického působení v rostlinném organismu. Navazujícím mechanismem může být uložení do nevitálních struktur, jako jsou např. vakuoly (Vatamaniuk et al., 2001; Zhao et al., 2010). Kromě tvorby výše uvedených sloučenin může docházet k detoxifikaci pomocí enzymatických reakcí se

superoxid dismutásou, katalásou, glutathion reduktásou, peroxidásou, dále pomocí neenzymatických reakcí s kyselinou askorbovou, α-tokoferolem, flavonoidy, anthokyany, karotenoidy, organickými kyselinami jako je kyselina citrónová, šťavelová nebo jablečná a také s již výše zmíněnými glutathiony. Tyto reakce pomáhají snižovat vliv volných kyslíkových radikálů, které jsou v organismu v přítomnosti toxických prvků produkovány (Sytar et al., 2013).

2.1.4.3 Sloučeniny arsenu

Pro celkové hodnocení toxického působení arsenu na organismus však není důležitý jen celkový obsah arsenu, ale i sloučenina (tzv. specie), ve které se arsen vyskytuje. Základní dělení arsenových specií je na aniontové (anorganické např. arsenitan – As^{III}, arseničnan – As^{V}, organické např. methylarseničnan – MA^{V}, dimethylarsenitan – DMA^{III}) a kationtové sloučeniny (např. arsenobetain – AB, arsenocholin – AC, tetramethylarsoniový ion – TETRA, trimethylarsenoxid – TMAO) (Fattorini et al., 2006) (obrázek č. 5).

Obrázek č. 5: Strukturní vzorce nejběžnějších sloučenin arsenu vyskytujících se v půdě a suchozemských rostlinách. Převzato a upraveno z Caumette et al. (2012).



Každá z těchto sloučenin vykazuje jinou míru toxicity pro různé organismy. Ruiz-Chancho et al. (2008) a další autoři jako Geiszinger et al.[a] (2002), Dembitsky et Řezanka (2003), Kuehnelt et Goessler (2003), Fattorini et al. (2006) shrnuli nejčastěji se

v rostlinách vyskytující sloučeniny arsenu a jejich toxicitu pro člověka následovně: As^{III} » As^{V} > DMA ~ MA ~ TETRA ~ TMAO > AB ~ AC ~ AsS (arsenocukry), přičemž posledně zmiňované sloučeniny (AB, AC, AsS) jsou považovány za více méně netoxické.

Výskyt jednotlivých sloučenin arsenu ve vyšších rostlinách a jejich distribuce do nadzemních částí těchto rostlin je jednoznačně ovlivněn druhem rostliny (Kuehnelt et al., 2000; Schmidt et al., 2004). Kuehnelt et al. (2000) stanovili ve 12 druzích rostlin rostoucích v oblasti kontaminované arsenem široké spektrum sloučenin arsenu, které sestávalo z As^{III}, As^{V}, DMA, MA, TMAO, TETRA a jednoho typu oxidu dimetyl- ribosyl arseničného. Vliv druhu rostliny na výskyt sloučenin arsenu a jejich koncentraci demonstrovali i Geiszinger et al.[a] (2002). Zatímco v extraktech nadzemní biomasy srhy laločnaté (*Dactylis glomerata* L.) a jitrocelu kopinatého (*Plantago lanceolata* L.) rostoucích v oblasti kontaminované arsenem byly stanoveny převážně anorganické sloučeniny arsenu, v extraktech nadzemní biomasy jetele lučního (*Trifolium pratense* L.) rostoucího na téže lokalitě byly stanoveny převážně organické sloučeniny arsenu, kdy dominantní sloučeninou byla MA. Sledování metylace arsenu v rostlinách psinečku tenkého (*Agrostis tenuis* Sibth.) ukázalo, že As^{V}, který byl přidán do kultivačního média, byl přijat kořeny rostlin, přeměněn na As^{III}, a později došlo k jeho metylaci v listech, kde byla zaznamenána zvýšená aktivita enzymu metyltransferasy (Wu et al., 2002). Tlustoš et al. (2002) nalezli v kořenech ředkvičky (*Raphanus sativus* L.) jako dominantní sloučeninu As^{III}, zatímco As^{V} byl více zastoupen v listech. Vysoké podíly DMA v rostlinách (17 % v kořenech a 18 % v listech) ve srovnání s půdou, kde bylo více než 90 % arsenu přítomno ve formě As^{V}, rovněž naznačují schopnost rostlin ředkvičky metylovat sloučeniny arsenu. Obdobné výsledky byly zaznamenány i v rostlinách paprik (*Capsicum annum* L.) pěstovaných v substrátu kontaminovaném sloučeninami arsenu (Száková et al. 2007).

Výskyt komplexních arsenových sloučenin jako je např. AB, AC nebo různé AsS je zcela běžný u mořských rostlin a živočichů (Ruiz-Chancho, 2008), přesto Mattusch et al. (2000) dokládají, že se tyto sloučeniny mohou v menších množstvích vyskytovat i u suchozemských rostlin, což bylo dále potvrzeno i v této disertační práci.

2.2 SELEN

2.2.1 Základní charakteristika selenu

Selen spolu s kyslíkem, sírou, tellurem a poloniem tvoří v periodické tabulce skupinu 16 a vyznačuje se relativní atomovou hmotností 78,96 g mol^{-1}. Je to polokov vyskytující se v několika alotropických modifikacích, z nichž nejběžnější je šedá hexagonální forma. Další, často se vyskytující, je forma červená (monoklinická) a amorfní, která se vyskytuje v černé nebo červené formě. Selen má podobné chemické vlastnosti a chování jako síra, proto se velice často vyskytuje v podobných formách a sloučeninách. Stejně jako síra patří mezi chalkogeny (Hoffmann et King, 1997) a často se vyskytuje jako doprovodný prvek v nejrůznějších rudách (Zhang et al., 2014).

Selen se nejčastěji vyskytuje v oxidačních stavech +6, +4, 0 a -2, ve kterých je stabilní (Barceloux, 1999). Podle dalších autorů se však může vyskytovat i v dalších oxidačních stavech jako např. -1 (Ashworth et Shaw, 2006) nebo +2 (Kabata-Pendias et Pendias, 2001). Toxicita, rozpustnost a biodostupnost sloučenin tohoto prvku silně závisí právě na oxidačním stavu, ve kterém se prvek nachází (Puranen et al., 2010).

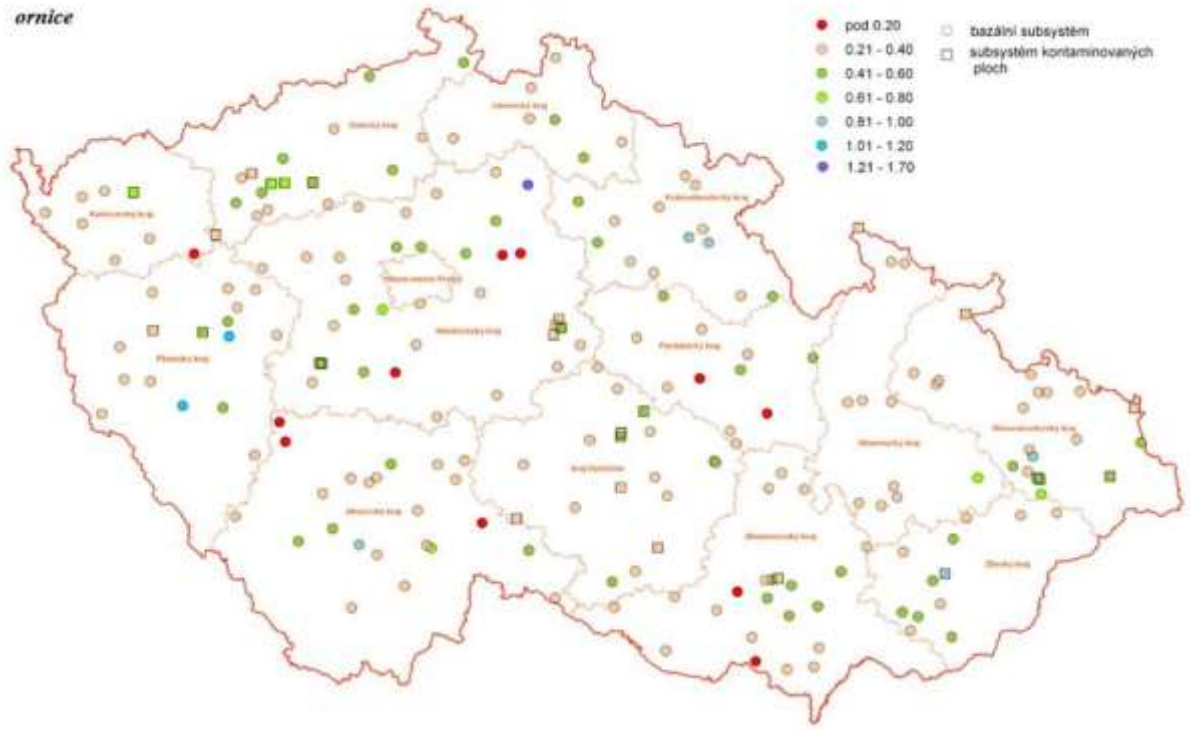
2.2.2 Výskyt selenu v životním prostředí

Selen se vyskytuje ve všech složkách životního prostředí (Lemly, 2004; Zhang et al., 2014), i když jeho přirozené obsahy v pedosféře se napříč světadíly velice liší. Plant et al. (2004) uvádějí v zemské kůře průměrný obsah selenu 0,05 mg Se kg^{-1}, dle Lemly (2004) je to 0,2 mg kg^{-1}. Jeho obsah v horninách závisí na typu horniny, jejích vlastnostech a zejména geologickém původu. Selen je jednou z nejtěkavějších složek magmatu, proto jeho obsah v horninách záleží na tom, zda se jedná o horniny vyvřelé či výlevné. U sedimentárních a přeměněných pak záleží na tom, z jakých původních hornin vznikly (Sharma et al., 2015).

Některé oblasti jako např. Finsko, Čína, Nový Zéland jsou na obsah selenu v půdách velice chudé (Cuvardic, 2003; Li et al., 2007). Naopak některé regiony v USA, zvláště pak ty s aridním klimatem jako je Kalifornie, v Evropě pak oblasti Francie a Německa jsou na obsahy selenu bohaté (Kabata-Pendias et Pendias, 2001), a tento prvek zde může dosahovat až koncentrací, které mohou být toxické pro rostliny i živočichy (Bañuelos et al., 2013). Za nízké obsahy v půdách Sigrist et al. (2012) považují obsahy menší než 5 mg Se kg^{-1}, za vysoké pak pokládají koncentrace větší než 5 mg Se kg^{-1}. Obsah selenu v půdách se běžně pohybuje v rozmezí 0,01 – 2 mg Se kg^{-1}, avšak existují i půdy s extrémně nízkým obsahem selenu, které vznikly např. zvětráváním pískovců nebo vápenců. Na druhé straně ale

existují i půdy s extrémně vysokým obsahem selenu přesahujícím 1200 mg kg^{-1}, které vznikly zvětráváním černých břidlic (Plant et al., 2004). Obsahy celkového selenu v ornici na území České republiky ukazuje obrázek č. 6.

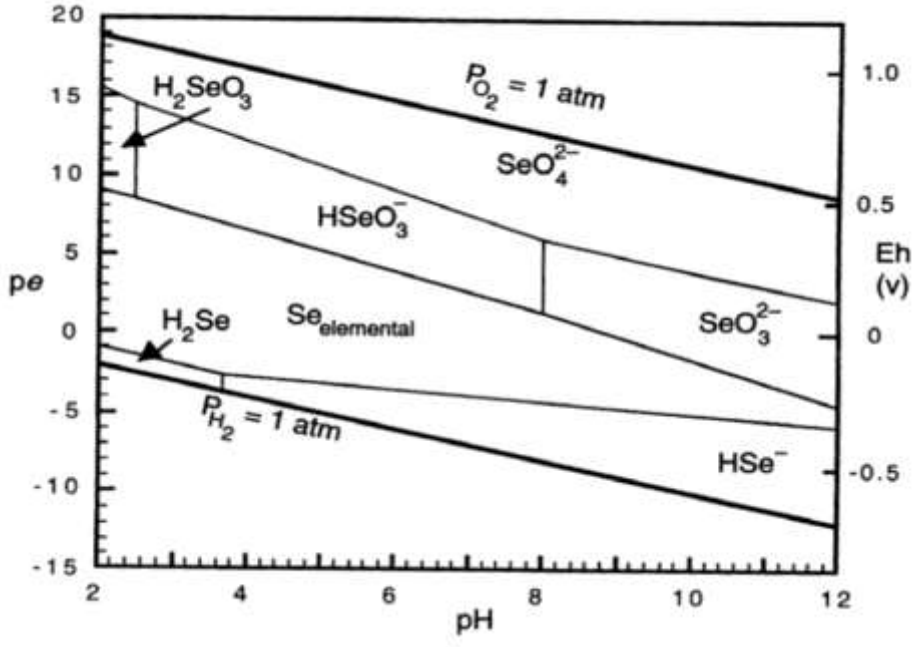
Obrázek č. 6: Celkové obsahy selenu ve vzorcích ornice Bázálního monitoringu půd (obsahy uvedené v mg Se kg^{-1}). Převzato a upraveno z Poláková (2010).



Krejčová et al. (2013) sledovali koncentrace selenu v půdách a horninách v obci Suchomasty v chráněné krajinné oblasti Český Kras ve Středočeském kraji a zjistili, že hodnoty selenu v horninách, jmenovitě bioklastickém vápenci, vápenitém jílovci a diabasu byly nadprůměrné oproti obsahům selenu v obdobných typech hornin ve světě. V bioklastickém vápenci pocházejících ze Středočeského kraje byla koncentrace selenu 4,61 mg kg^{-1} (celosvětově < 0,05 mg kg^{-1}), ve vápenitém jílovci 2,90 mg kg^{-1} (celosvětově 0,1 – 1500 mg Se kg^{-1}), v diabasu 0,3 mg kg^{-1} (celosvětově 0,05 mg Se $kg^{-1)}$. Průměrné obsahy selenu v půdě dosahovaly koncentrací 0,02 – 9,83 mg kg^{-1} (celosvětově 0,23 mg kg^{1}), přičemž vyšší koncentrace byly s největší pravděpodobností ovlivněny kontaminací půd ze staré skládky v blízkosti odběrových míst, kde byly prováděny sondáže. Tito autoři také poukazují na to, jak velikost částic, pH a obsah půdního vzduchu mohou ovlivňovat celkové obsahy selenu v jednotlivých vrstvách. Vysoký obsah půdního vzduchu ve vrchních vrstvách

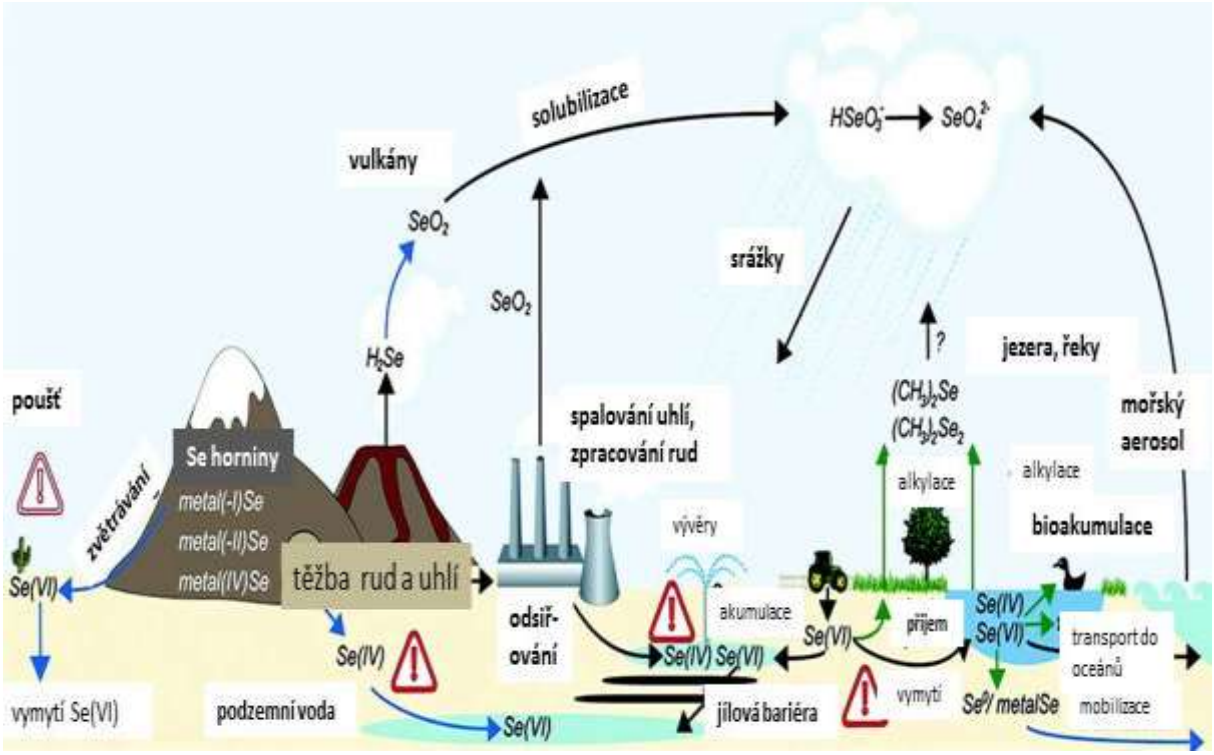
půdního profilu pravděpodobně způsobuje oxidaci Se^{IV} na Se^{VI}, který se vyznačuje větší mobilitou a tím pádem i migrací do nižších vrstev půdního profilu popř. infiltrací do povrchových a podzemních vod. Vliv velikosti půdních částic vysvětlují autoři tak, že čím jemnější jsou částice, tím větší je specifický povrch částic a schopnost adsorpce, tudíž jsou i vyšší obsahy selenu v těchto jemných frakcích. Tuto teorii podporují i výsledky studie Chan et al. (2009). V této studii byla provedena zkouška vyluhovatelnosti při různých hodnotách pH_{H_2O}, jmenovitě při pH roztoku 5, 7, 11. Zde se ukázalo, že selen byl nejmobilnější při neutrálním pH, což pravděpodobně souvisí s povrchovým nábojem půd. Podobnou závislost mobility a biodostupnosti selenu na pH uvádí i Johnsson (1991), který pěstoval řepku (*Brassica napus* L., var. Emil) a pšenici (*Triticum aestivum L.*, var. Drabant) v nádobových pokusech v půdách o pH_{H_2O} 5 a 7. Vedle těchto faktorů ovlivňujících mobilitu a biopřístupnost selenu pro rostliny někteří autoři (Chang et Randle, 2006; Spadoni et al., 2007) uvádějí ještě teplotu, jakožto pedo-klimatický faktor, který spolu se srážkami může měnit koncentraci půdního roztoku, obsah organické hmoty a redoxní podmínky, které mohou dostupnost selenu také ovlivnit. Závislost výskytu jednotlivých specií selenu v půdě na pH a Eh ukazuje obrázek č. 7.

Obrázek č. 7: Eh-pH diagram pro vodorozpustné specie selenu v půdě v závislosti na pH a Eh při teplotě 25 °C, za běžného atmosférického tlaku (Drever, 1997)



Přírodním zdrojem selenu v životním prostředí (obrázek č. 8) jsou zejména sulfidické minerály, vulkanická činnost, uhlí, vypařování sloučenin selenu z půd, sedimentů a bioevaporace selenu mikrorganismy (Sposito, 2008; Stolz et Oremland, 1999). Mezi nejvýznamnější zdroje antropogenní kontaminace prostředí selenem (obrázek č. 8) patří hlavně těžba, zpracování a spalování fosilních paliv (uhlí, ropa a jejich vedlejší produkty), těžba rud (zlato, stříbro, nikl) a zpracování kovů, skla a keramiky. Nezanedbatelným antropogenním zdrojem selenu v životním prostředí jsou také skládky elektrozařízení a nesprávné nakládání s nimi, zejména pak s fotokopírkami, expozimetry a fotoelektrickými články, které selen obsahují z důvodu jeho fotovoltaických vlastností. Selen se může do prostředí dostávat také jako příměs ve fosforečných hnojivech, z elektrárenského popílku, nebo v menších množstvích z cigaret (ať už z tabáku, ale i z cigaretového papíru) a různých kosmetických přípravků jako jsou např. šampony proti lupům, kde je součástí jejich účinné látky (Greenwood et Earnshaw, 1993; Wang et al., 2007; Anonym, 2009; Berra et Rizzo, 2009; Eich-Greatorex et al., 2010).

Obrázek č. 8: Cyklus selenu v životním prostředí a jeho nejdůležitější zdroje v životním prostředí. Převzato a upraveno z Sharma et al. (2015).



2.2.3 Vliv selenu na organismy

Selen je pro člověka i ostatní savce esenciálním prvkem s širokou škálou biologických funkcí (Rayman, 2000; Maseko et al., 2013). Nejdůležitější a nejvíce prozkoumaná je funkce některých selenoproteinů jako antioxidantů chránících tělo před oxidativním stresem (Navarro-Alarcon et Lopez-Martinez, 2000). Lobanov et al. (2009) konstatují, že se v těle savců vyskytuje až 30 různých selenoproteinů, jejichž hlavní funkcí je mimo jiné i antioxidační působení. Jedním z příkladů antioxidačně působící selenové sloučeniny je selenocystein, který tvoří aktivní centrum v glutathion peroxidázového enzymu a pomáhá eliminovat vliv působení volných radikálových iontů v těle (Copeland, 2003). Selen je součástí dalších enzymatických procesů a mimo jiné se podílí i na tvorbě hormonů štítné žlázy, zlepšuje plodnost a mobilitu spermií, podporuje funkci imunitního systému a pomáhá organismu bojovat proti závažným chorobám a částečně pomáhá zlepšovat stav pacientů s HIV nebo rakovinou (Rayman, 2000; Kellen et al., 2006). Denní doporučená dávka selenu pro dospělého člověka činí dle Nařízení EU č. 100/2008 Sb. [online] 0,55 µg den^{-1} a maximální dávka, která neznamená pro dospělého člověka riziko je 300 µg den^{-1}. Dlouhodobý nedostatek selenu v potravě může přispívat k rozvoji chronických onemocnění, jako je hypertenze, srdečně-cévní onemocnění, rakovina, astma, diabetes mellitus a k dalším patologickým symptomům (Brown et Arthur, 2001; Faure, 2003; Khadadah et Dashti, 2005; Rayman, 2005; Kellen et al., 2006). Velice známým onemocněním z nedostatku selenu, zejména v Číně a Severní Korei, kde lidé v některých oblastech trpí dlouhodobým nedostatkem jeho příjmu, je tzv. Keshanská nemoc (typ dětské kardiomyopatie – defekty srdečního svalu) (Li et al.[a], 2013) nebo Kashin-Beckova nemoc (typ chronické osteoartritidy – degenerativní onemocnění kloubů) (Yao et al., 2011).

Selen je, jak již bylo řečeno, prvek esenciální pro člověka i ostatní savce, na druhou stranu je to ale prvek s velice úzkou hranicí mezi koncentracemi, při kterých působí na organismus esenciálně a při kterých již toxicky. Nejen celková dávka, ale i sloučenina, ve které se selen vyskytuje, určuje jeho biodostupnost, toxicitu a funkci v organismu (Qin et al., 2013). Lze shrnout, že anorganické sloučeniny tohoto prvku jsou toxičtější než ty organické (Uglietta et al., 2008). Toxické působení selenu v organismu se může projevit ztrátou vlasů, nehtů, tvorbou lézí na kůži, nervovými poruchami nebo dokonce paralýzou až smrtí (Qin et al., 2013; Lemire et al., 2012). Toxické působení selenu se začíná projevovat od dávky 6300 µg selenu den^{-1} (Anonym, 2000).

Problém nedostatku selenu v potravě je však celosvětově palčivější než možné riziko jeho toxicity. Proto se mnoho studií zabývá tím, jak tímto prvkem obohatit zemědělské plodiny jako např. čočku (Rahman et al., 2013), zelí, salát, mangold, petržel (Funes-Collado et al., 2013), nebo kukuřici (Chilimba et al., 2012). Zatím asi nejkomplexnější výzkum napříč plodinami provedli De Temmerman et al. (2014), kteří studovali přechod selenu do celkem 36 druhů různých plodin včetně pšenice, brambor, mrkve, různých druhů listové zeleniny, paprik, rajčat, zelených fazolí, cibule, česneku atd. Přitom zjistili, že plodiny obsahující hodně sirných sloučenin, jako jsou plodiny z rodu *Allium* nebo *Brassica*, jsou schopné zvýšeného příjmu selenu oproti ostatním plodinám. Autoři tuto skutečnost vysvětlují možností nahrazení atomu síry v proteinových sloučeninách naakumulovaným selenem.

Nemá-li rostlinný organismus dostatek účinných mechanismů k omezení toxického působení selenu, může se to projevit redukcí růstu až odumřením rostliny (Fu et al., 2002). Většina volně žijících rostlin je schopna se se zvýšenými obsahy selenu v půdě bez větších potíží vyrovnat, toxické účinky se však někdy objeví u zemědělských plodin, jak dokládají Fiskesjo et al. (1979) kteří prokázali toxické působení selenu na kořeny cibule kuchyňské (*Allium cepa* L.). Kořeny prokazovaly vyšší procento deformací a snížení růstu od obsahu 1 mg Se L^{-1} v médiu, ale při snížení koncentrace na 0,5 mg Se L^{-1} se již neprojevily žádné známky toxicity.

2.2.4 Selen a rostliny

2.2.4.1 Příjem selenu rostlinami

Tak jako u ostatních prvků je nejvýznamnějším vstupem selenu do rostliny příjem tohoto prvku z půdního roztoku. Transportní mechanismus selenu je úzce spojen s transportním mechanismem síry a je zajišťován stejnými transportéry, které zajišťují příjem síry do rostliny (Severi, 2001; White et al., 2007). To dokládají i Sors et al.[a] (2005), kteří prokázali stejný princip přenosu selenanů i síranů pomocí ATPázy přes rhizodermální buňky proti biochemickému gradientu tj. cestou symplastickou. Gen kódující schopnost přenosu síranů byl poprvé objeven u kvasinek rodu *Saccharomyces* (Smith et al., 1995) později pak i u vyšších rostlin např. rodu *Arabidopsis* (Shibagaki et al., 2002). Uplatnění sulfát transportního genu závisí na aktuální koncentraci síry a glutathionu v rostlině a ne vždy se proto jeho vliv projeví (Hirai et al., 2003). Naproti tomu seleničitany využívají pro svůj vstup do rostliny výhradně pasivní přechod pomocí difúze po směru biochemického gradientu, tedy cestu apoplastickou.

Li et al. (2016) se ve své studii zaměřují na to, jaké prvky mohou ovlivňovat příjem a distribuci selenu v rostlinách rýže (*Oryza sativa* L.). Statistické analýzy jejich dat ukazují, že významný vliv má draslík, jehož přítomnost může potlačovat příjem selenu kořeny; přítomnost bóru, mědi a molybdenu v kořenech může bránit distribuci selenu z kořene do stonku; a síra, chrom, fosfor a hořčík nacházející se ve stonku mohou mít negativní vliv na akumulaci selenu v obilkách.

Mikrobiální aktivita v půdě hraje i u selenu důležitou roli v biopřístupnosti tohoto prvku rostlinám. Ovlivňuje oxidačně – redukční procesy a je zodpovědná zejména za volatilizaci anorganického selenu v půdě. Bylo zdokumentováno, že fakultativně anaerobní bakterie *Enterobacter cloacae* SLD1-a1, která je schopná redukovat selenany na seleničitany a dále na elementární selen, je zodpovědná za jeho volatilizaci v podobě dimethyselenu do ovzduší (Losi et Frankenberger, 1997).

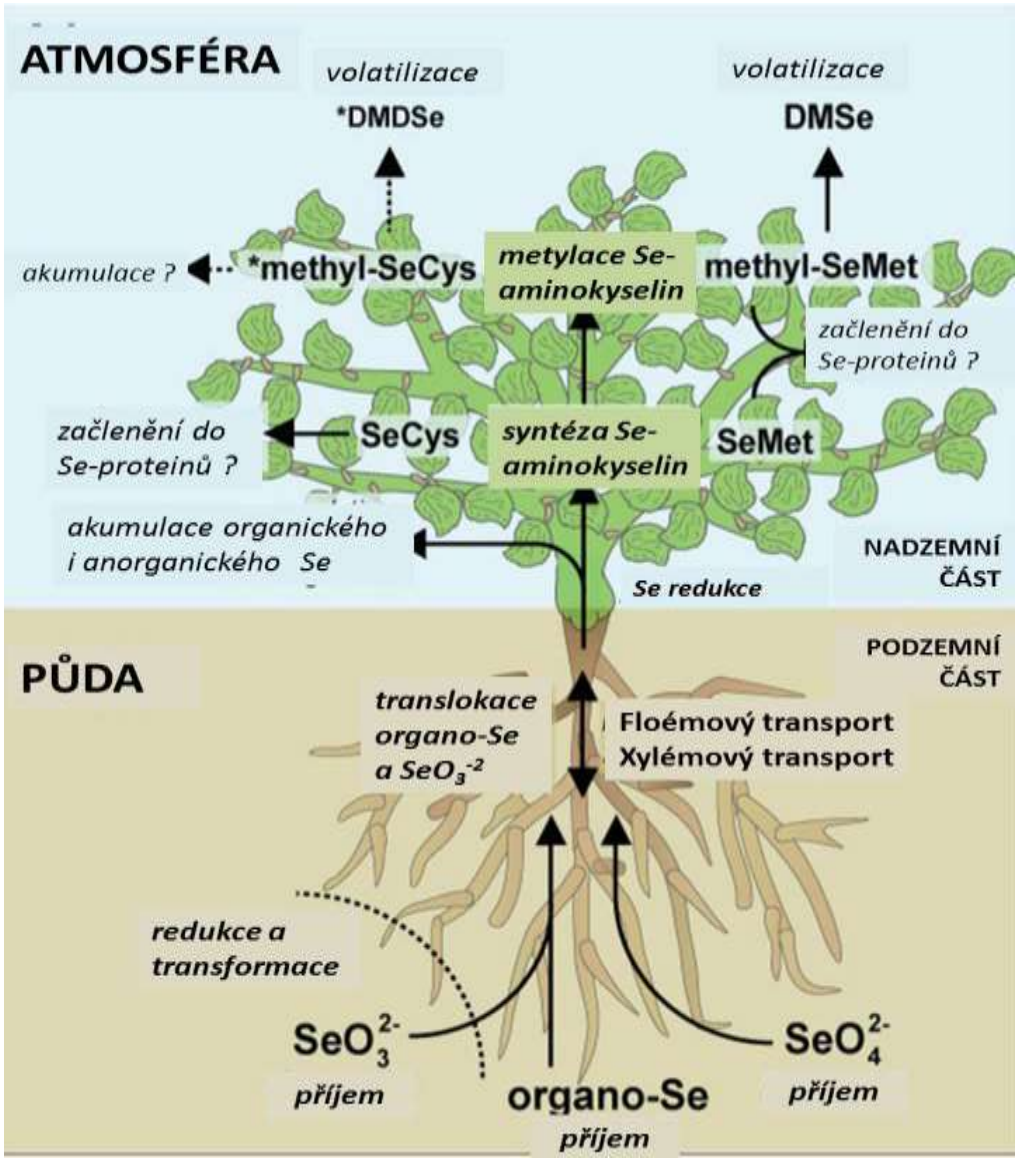
2.2.4.2 Hyperakumulace selenu a ochranné mechanismy rostlin

U selen hyperakumulujících rostlin někteří autoři jako např. White et al. (2004) poukazují na to, že rostliny schopné hyperakumulace selenu upřednostňují příjem selenu nad příjmem síry. Dalším specifikem selenových hyperakumulátorů je to, že oproti ostatním rostlinám netransformují selen jen do Se-methyl-methioninu, ale i do Se-methyl-cysteinu (Frankenberger et Engberg 1998; Freeman et al., 2006), který jak se zdá hraje důležitou metabolickou roli při akumulaci tohoto prvku. Dalším obranným mechanismem, který rostliny využívají, může být zapojení ATP sulfurylázy a APS reduktázy, které pomáhají transformovat toxické anorganické sloučeniny selenu na sloučeniny organické a méně toxické (Raspor et al., 2003; Sors et al.[b], 2005). Jako příklady hyperakumulátorů selenu mohou být uvedeny rostliny z rodů *Astragalus* či *Stanleya* (Freeman et al., 2006; Lindblom et al., 2013), které jsou v této souvislosti často zkoumány, nebo rostlinný akumulátor *Brassica juncea* L., který je schopný akumulovat 100 – 1000 mg Se kg^{-1} sušiny (Prins et al., 2011).

Vysoké obsahy selenu přijímané půdním roztokem ovlivňují rostlinný organismus nepřímo také tím, že potlačují akumulaci dusíku, fosforu, síry a některých aminokyselin v rostlinných pletivech. Na druhou stranu zvýšené obsahy selenu inhibují příjem některých těžkých kovů, zejména manganu, zinku, mědi, železa a kadmia. Tato funkce však závisí na vzájemných poměrech jednotlivých prvků a dalších faktorech. Pro snížení toxického vlivu selenu na rostliny se často používá aplikace hnojiv na bázi dusíku, fosforu a síry, kde pravděpodobně dochází k inhibici příjmu selenu kořeny, nebo k potlačení toxického vlivu selenu díky lepšímu vyživovému stavu rostliny (Kabata-Penidas et Pendias, 2001).

V praxi je pak aplikace síry významným remediačním opatřením při kontaminaci selenem (Stroud et al., 2010), i když Johnson (1975) uvádí, že je efektivní pouze na půdách s nízkým pozaďovým obsahem síry. Příjem a transformaci selenu ve vyšších rostlinách shrnuje obrázek č. 9.

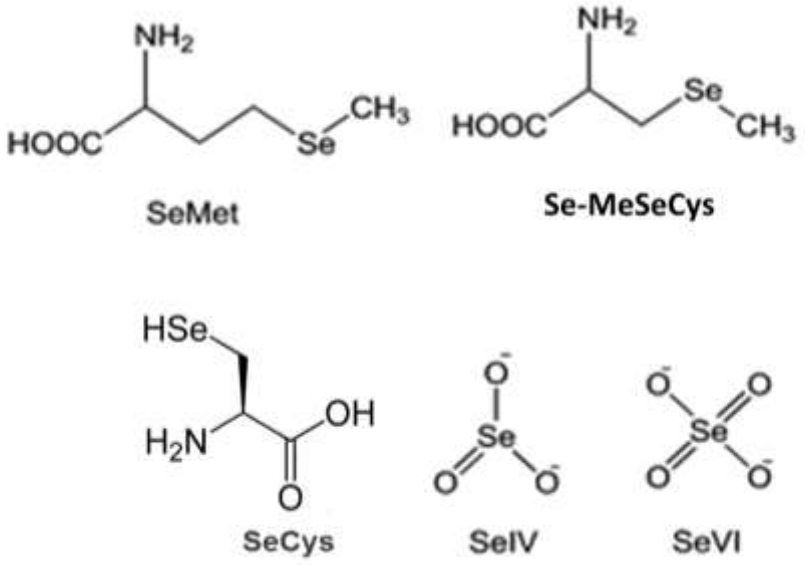
Obrázek č. 9: Přehled hlavních transportních a transformačních mechanismů v systému půda – rostlina – atmosféra. SeO_4^{2-} – selenan; SeO_3^{2-} – seleničitan; organo-Se – organicky vázaný selen; SeMet – selenomethionin; SeCys – selenocystein; methyl-SeMet – methylselenomethionin; methyl-SeCys – methylselenocystein; DMSe – dimethylselenid; DMDSe – dimethyldiselenid; sloučeniny označené hvězdičkou se vyskytují pouze u hyperakumulátorů. Převzato a upraveno z Winkel et al. (2015).



2.2.4.3 Sloučeniny selenu

Za nejběžněji se vyskytující sloučeniny selenu v suchozemských rostlinách se považují anorganický selenan (Se^{VI}) a seleničitan (Se^{IV}), dále pak organické sloučeniny selenocystein (SeCys), Se-methylselenocystein (Se-MeSeCys) a selenomethionin (SeMet) (Thosaikham et al., 2014), jejich vzorce prezentuje obrázek č. 10. Aureli et al. (2012) zmínili v rostlinách také výskyt některých selenových mono- a di-sacharidů, které se velice často nacházejí jako detoxifikační produkty v moči savců (Juresa et al., 2007; Jackson et al., 2013), ale jejichž výskyt v rostlinách do této doby zatím nebyl zmíněn. Další, méně časté sloučeniny selenu, ve své studii zmínil Whanger (2002), který uvádí v obilkách pšenice (*Triticum aestivum* L.) výskyt selenohomocysteinu, γ-glutamyl-selenocystathioninu, γ-glutamyl-Se-methyselenocysteinu, selenocysteinselenové kyseliny, Se methylselenomethioninu a dalších. Tyto sloučeniny se však podle tohoto autora vyskytují jen v nepatrných množstvích, a až 56 – 83 % z celkového obsahu selenových sloučenin tvoří právě SeMet, následovaný Se^{VI} (12 – 19 %), SeCys (4 – 12 %), Se-metylselenocysteinem (1 – 4 %). Podobné zastoupení sloučenin selenu uvádí i u rýže, sojových bobů, kukuřice a potravinářských kvasnic. Funes-Collado et al. (2013) ve své studii uvádějí, že pěstovali-li vybrané plodiny (zelí, salát, mangold a petržel) v médiu s přídavkem Se^{IV} a Se^{VI}, rostliny jej následně metabolizovaly výhradně na organický SeMet. Zdá se tedy, že SeMet je jedním z nejvýznamnějších metabolitů selenu u suchozemských rostlin.

Obrázek č. 10: Strukturální vzorce nejběžnějších selenových sloučenin vyskytujících se v půdě a suchozemských rostlinách. Převzato a upraveno z Jaeger et al. (2013).



2.3 ANALYTICKÉ METODY STANOVENÍ ARSENU A SELENU

2.3.1 Úvod do analytických metod

Arsen i selen jsou z pohledu analytického stanovení prvky poměrně problematickými. Ať už díky potřebě vysoké citlivosti přístroje (zejména kvůli nízkým koncentracím selenu v biologických vzorcích) nebo díky množství interferencí, které se při měření těchto prvků objevují a které mohou významně zkreslovat výsledky měření (Tanner et al., 2002). Během posledních desetiletí však bylo vyvinuto a zdokonaleno množství analytických metod, které chyby měření redukují a přináší zpřesnění výsledků a snížení detekčních limitů (Yin et al., 2013).

V průběhu vývoje instrumentálních analytických technik samozřejmě vzniklo mnoho modifikací jednotlivých přístrojů, které se mohou v některých součástech lišit. Základní principy, na kterých jednotlivé techniky pracují, však zůstávají stejné. V následujícím výčtu bude uvedeno několik základních analytických technik a jejich uspořádání, kterými se dají celkové obsahy arsenu, selenu a jejich specie kvantitativně i kvalitativně stanovit.

2.3.2 Stanovení celkových obsahů arsenu a selenu

Mezi nejběžnější a nejpoužívanější techniky, kterými se celkové obsahy arsenu a selenu stanovují, se řadí atomová absorpční spektrometrie s generováním těkavých sloučenin (HG-AAS), atomová fluorescenční spektrometrie s generováním těkavých sloučenin (HG-AFS), instrumentální neutronová aktivační analýza (INAA), optická emisní spektrometrie s indukčně vázaným plasmatem (ICP-OES) a námi používaná hmotnostní spektrometrie s indukčně vázaným plasmatem (ICP-MS) (Xiong et al., 2008; Tyburska et al., 2011; Srivastava et al., 2011).

2.3.2.1 Hmotnostní spektrometrie s indukčně vázaným plazmatem

ICP-MS (obrázek č. 11) je vysoce citlivá a selektivní metoda, kterou je možno použít pro měření většiny prvků periodické tabulky s vyjímkou prvků obsažených v pracovních plynech jako jsou např. argon, helium, vodík, kyslík a prvků s vyšší ionizační energií než kterou je argonové plasma schopné poskytnout. Příkladem takového prvku může být fluór nebo chlór, jejichž první ionizační potenciál je blízký (v případě chlóru 12,97 eV) nebo vyšší (v případě fluóru 17,42 eV) než první ionizační potenciál argonu (15,76 eV).

Argonové plazma o teplotě 6000 - 10000 K je indukováno střídavým vysokofrekvenčním magnetickým polem v cívce. Do tohoto plazmatu přichází

ze zmlžovače vzorek ve formě aerosolu, z něj se v plazmatu odpaří veškeré rozpouštědlo, zaniknou chemické vazby a díky energii plazmatu vzniknou volné ionty, které přes soustavu konusů a iontové optiky, které mají za cíl centralizovat paprsek iontů a odstranit nenabité částice, pokračují do hmotnostního separátoru (nejčastěji kvadrupólu). To je soustava čtyř kovových tyčí o délce 30 – 40 cm a průměru asi 10 mm, na které je postupně vkládáno stejnoměrné, vysokofrekvenční elektrické napětí tak, aby dvě protilehlé tyče měly vždy stejnou polaritu. Působením takto vytvořeného elektromagnetického pole se ionty, které vstupují do kvadrupólu rozkmitají a docílí se toho, že kvadrupólem proletí pouze částice o definovanén poměru m/z (hmota/náboj) a ty poté dopadnou na detektor (Thomas, 2001).

V některých přístrojích může kvadrupólu předcházet kolizní/reakční cela s multipólem (nejčastěji hexa- či oktapólem), která slouží ke snížení nebo odstranění případných interferencí. V závislosti na použitém plynu hovoříme buď o kolizní cele (v případě, že použijeme k odstranění interferencí inertní plyny jako helium, xenon, argon, neon), nebo reakční cele (tehdy, když použijeme k odstranění interferencí např. vodík). Rozdíl je v reakcích probíhajících v přítomnosti těchto plynů. Při použití interních plynů jako je např. helium, dochází pouze ke srážkám nabitých částic s molekulami helia a tím snížení jejich energie na mez, kdy již nejsou schopny vstoupit do detektoru, nebo dochází k rozpadu polyatomických částic (např. $ArNa^+$, ArO^+) na jednotlivé části, které pro další stanovení již nepředstavují problém. Při použití reakčního plynu dochází k chemickým reakcím mezi interferovanými částicemi a molekulami vodíku, kdy se nejčastěji uplatňují přenos náboje, přenos protonu, či přenos vodíkového atomu (Feldmann et al., 1999; Sloth et Larsen, 2000; Tanner et al., 2002).

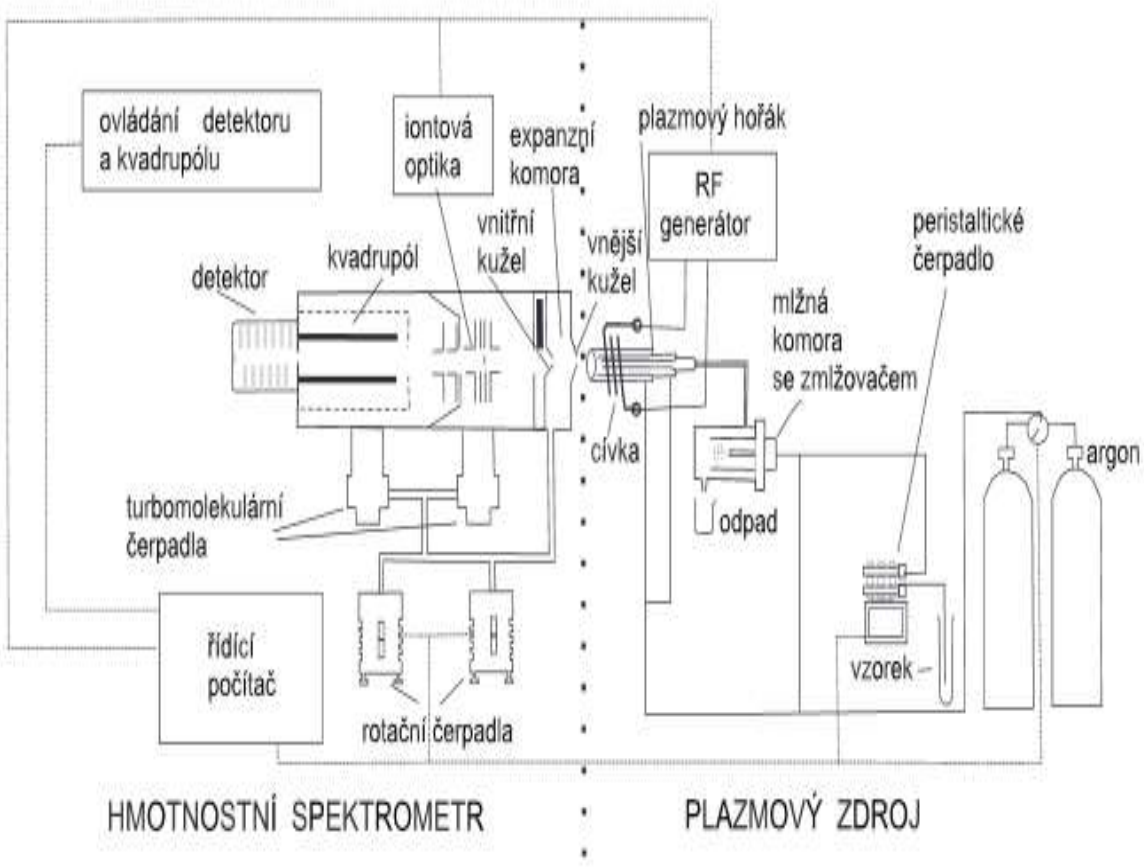
Samotný detektor ICP-MS je tvořen vícekanálovým elektronásobičem, jehož princip spočívá v mnohonásobném zesílení elektrického signálu, který vznikne po dopadu měřeného iontu na plochu detektoru, až na úroveň měřitelného elektrického proudu.

Při měření arsenu a selenu pomocí ICP-MS je výhodné měřit ty izotopy těchto prvků, které mají největší zastoupení v přírodě a zároveň málo interferencí, popř. interference takové, které jdou použitím reakční/kolizní cely snadno odstranit. U arsenu se nejčastěji používá izotop As^{75}, který však může být zatížen např. polyatomickou interferencí $ArCl^+$, proto je nutné při jeho měření použít reakční/kolizní cely, aby došlo k jejímu potlačení. U selenu se nejčastěji používá izotop Se^{78} popř. Se^{80}, u kterých je však také nutné použít reakční/kolizní celu z důvodu interferenčního překryvu, který tvoří např. $ArAr^+$ (Darrouzes et al., 2007).

Montaser et Golightly (1992) uvádějí dva různé druhy interferencí: 1) interference spektrální vyvolané překryvem izobarických iontů ve spektru, jejich zástupci mohou být výše uvedené interference u selenu a arsenu, a 2) interference nespektrální, které jsou způsobené zejména složením matrice. Mihaljevič et al. (2004) doplňují, že tyto nespektrální interference způsobují matriční prvky tím, že ovlivňují energetické poměry v plazmatu a tak způsobují potlačení signálu. Dále uvádějí, že tento typ interferencí se dá potlačit použitím metody přídavku standardu, zařazením interního standardu nebo využitím metody izotopového ředění.

ICP-MS je analytická technika vhodná i pro použití při ultrastopové analýze, protože její detekční limity jsou velice nízké. Darrouzes et al. (2007) uvádějí detekční limity standardů arsenu a selenu v 1g NaCl L^{-1} matrici 25 ng L^{-1} pro As^{75} a 35 ng L^{-1} pro Se^{80}, respektive 45 ng L^{-1} pro Se^{78} při použití reakční/kolizní cely a plynné směsi 3,8 mL H_2 min^{-1} s 0,5 mL He min^{-1}.

Obrázek č. 11: Základní schéma ICP-MS (Mihaljevič et al., 2004).



2.3.3 Stanovení sloučenin arsenu a selenu

S postupným vývojem metod a poznání v oblasti působení a toxických účinků nejrůznějších elementů se objevil názor, že není důležitý jen celkový obsah daného prvku, ale také sloučenina, ve které se prvek nachází (Jones-Lepp et Momplaisir, 2005). Týká se to zejména prvků jako je kadmium, rtuť, olovo, antimon, cín, germanium, ale i arsen a selen, jejichž jednotlivé specie vykazují různou míru toxicity pro různé druhy organismů (Jain et Ali, 2000; Maher et al., 2012; Chakraborty et al., 2012).

Jednotlivé specie mohou tvořit: i) různé oxidační stavy prvku (Se^{IV}, Se^{VI} ; As^{III}, As^{V}; Cr^{III}, Cr^{VI}; Mn^{II}, Mn^{VII}), ii) organokovové sloučeniny charakterizované silnou kovalentní vazbou kov−uhlík, kdy vazba nepodléhá disociaci a zajišťuje přijatelnou stabilitu během úpravy vzorku (selenoaminokyseliny; organosloučeniny arsenu; arsenocukry), iii) komplexy kovů charakterizované koordinační vazbou prvku a ligandu – a) malé organické ligandy (citrát, vínan, šťavelan, aminokyseliny, oligopeptidy), b) makrocyklické chelatační molekuly a makromolekuly (proteiny, DNA fragmenty, polysacharidy, metalopeptidy jako fytochelatiny, metalothioneiny) (Cornelis, 2005; Rychlovský, 2008).

Ke kvantitativnímu a kvalitativnímu měření těchto sloučenin je třeba konvenční techniky používané pro stanovení celkových obsahů prvků doplnit ještě o separační krok, který je schopen jednotlivé sloučeniny před vlastním stanovením obsahu prvku od sebe oddělit. Tato uspořádání, kdy se spojují dva a více přístrojů, se nazývají spřažené techniky.

V následujícím výčtu je uvedeno několik technik a základních principů, které se pro měření specií arsenu a selenu používají nejčastěji, ale literatura jich uvádí samozřejmě mnohem více. V tomto přehledu je zaměřena pozornost pouze na metody spektrometrické. Příkladem může být HG-AAS s použitím L-cysteinu jako redukčního činidla (Wieteska et al., 2003), kapilární elektroforéza (Lopez-Sanchez et al., 1994), kapilární elektroforéza ve spojení s ICP-MS (CE-ICP-MS) (Liu et al., 2013), nebo vymrazovací kolekce ve spojení s ICP-MS (CT-ICP-MS) (Geng et al., 2009). Mezi nejčastěji používané se řadí spojení vysokoúčinné kapalinové chromatografie (HPLC) s AAS, ICP-OES nebo ICP-MS a spojení plynové chromatografie (GC) s AAS, ICP-OES nebo ICP-MS.

Postup speciační analýzy lze obecně rozdělit do následujících kroků:

- příprava vzorku;
- separace jednotlivých sloučenin prvků nebo alespoň frakcí obsahujících skupinu specií pomocí vybrané separační techniky;

- detekce a stanovení hledaného prvku v izolované frakci pomocí vybrané prvkově selektivní detekční techniky;
- identifikace struktury vazebného partnera prvku pomocí vhodné molekulově specifické detekční techniky (hmotnostní spektrometrie, nukleární magnetická resonance, aj.) (Rychlovský, 2008).

2.3.3.1 Spojení spektrometrických a chromatografických metod

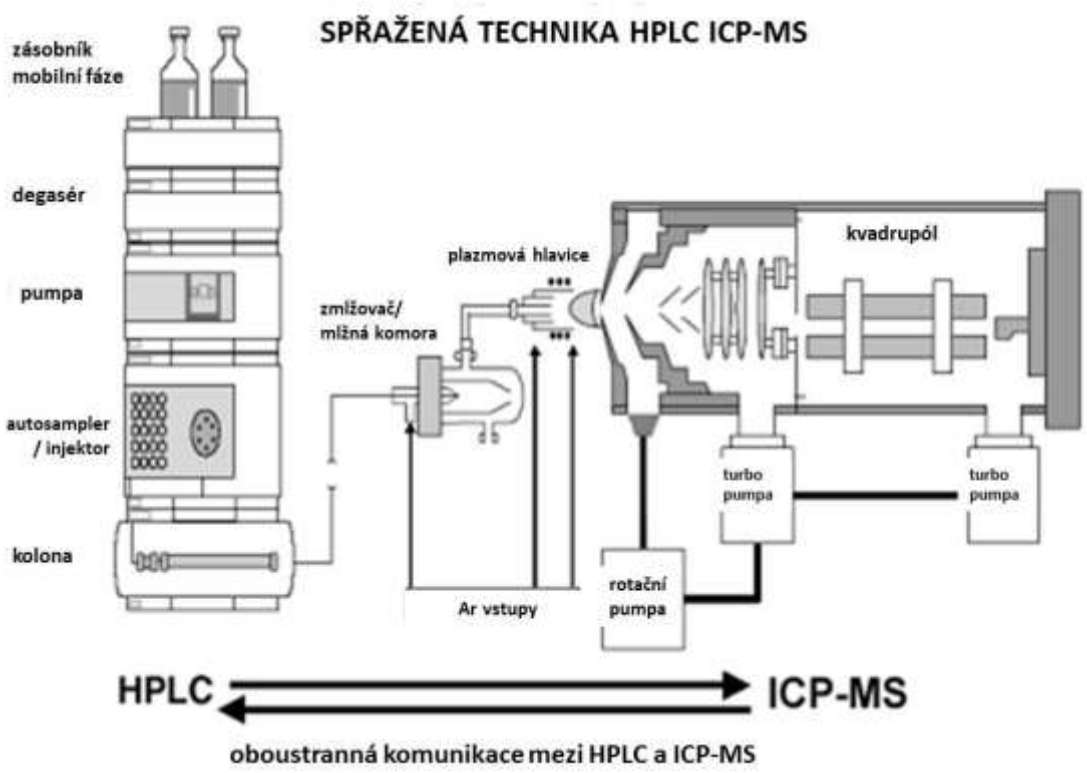
Chromatografické metody nabízejí široké možnosti pro separaci všech známých arsenových a selenových specií. Spojení ICP-MS s HPLC (obrázek č. 12) je pravděpodobně nejrozšířenější metodou speciací a má široké uplatnění. Základními součástmi systému HPLC je vysokotlaké čerpadlo, které umožňuje průtok mobilní fáze přes kolonu, kde díky specifickým vlastnostem sorbentu dochází k separci a následně eluci sloučenin v různých retenčních časech. Tyto sloučeniny, postupně dle svých retenčních časů, pokračují spolu s mobilní fází do vlastního analyzátoru, kde jsou stanovovány (Kupiec, 2004).

Pro potřeby speciační analýzy se z technik kapalinové chromatografie využívá zejména iontově výměnná chromatografie (IEC) a chromatografie s reverzními fázemi (RP-HPLC). Iontově výměnná chromatografie je založená na interakci mezi kationty analytu v mobilní fázi s negativně nabitými funkčními skupinami stacionární fáze (katex) nebo anionty analytu s pozitivně nabitými funkčními skupinami stacionární fáze (anex). Katexy i anexy, jsou široce užívané pro separaci specií kovů a polokovů. Kolony se silným anexem jsou např. používány při speciačních analýzách sloučenin arsenu a selenu. Dále je technika IEC aplikovatelná např. na analýzu metalothioneinů. Separace musí vždy probíhat za použití vodných mobilních fází s pufry s přesně danými hodnotami pH a s poměrně vysokými koncentracemi solí, což může působit problémy jako je ucpávání zmlžovačů a konusů, je-li pro následnou detekci použita technika ICP-MS.

V případě chromatografie s reverzními fázemi je analyt dávkován do polární mobilní fáze (nejčastěji směs voda-methanol, voda-acetonitril) a je separován na nepolární stacionární fázi (např. silikagel s chemicky navázaným uhlíkatým řetězcem, zpravidla o délce C4-C18). Výhodou této techniky, oproti IEC, je fakt, že plnící materiál kolony neobsahuje žádné ligandy umožňující konkurenční vazbu kovů. Typickými představiteli látek, které lze touto metodou separovat jsou polární sloučeniny bez náboje s molární hmotností menší než 3 000 g mol^{-1}. Jednou z negativních stránek používání této techniky ve spojení s ICP-MS je zvýšené usazování uhlíku na plazmové hlavici a kónusech. To je možno částečně potlačit přidáním

velmi malého množství kyslíku do proudu argonu pro účinnější rozložení organické fáze (Cornelis, 2003; Cornelis, 2005; Rychlovský, 2008).

Obrázek č. 12: Základní schéma spřažení HPLC s ICP-MS. Převzato a upraveno z Anonym [b] [online].



Plynová chromatografie tvoří alternativu ke kapalinové a může být použita pro separaci derivatizovaných sloučenin před vstupem do vlastního analyzátoru. Vzorky jsou nejčastěji derivatizovány pomocí tetraethylboritanu sodného a následně plynnou mobilní fází vedeny do kolony a po separaci následně do analyzátoru (Maher et al., 2012). Toto spojení s plynovou chromatografií však není tak časté, jako spojení s HPLC, zejména kvůli problematické derivatizaci a eluci vzorků v systému GC (McSheeny et al., 2013).

3 HYPOTÉZA A CÍLE PRÁCE

Příjem, akumulace a přeměna arsenu a selenu rostlinami jsou závislé na různých biotických a abiotických faktorech. Významný vliv na příjem, akumulaci a přeměnu těchto prvků má rostlinný druh. Dalším důležitým faktorem jsou fyzikálně-chemické charakteristiky půd, zejména celkové obsahy těchto prvků v půdách, na kterých rostliny rostou.

Mezi vybranými zemědělskými plodinami a společenstvy rostlin volně rostoucích na území ČR se vyskytují rostlinné druhy schopné zvýšené akumulace arsenu/ selenu a transformace těchto sloučenin do sloučenin pro člověka netoxických (v případě arsenu) nebo dokonce zdraví prospěšných (v případě selenu).

K potvrzení výše uvedených hypotéz byly stanoveny následující cíle:

Cíle práce:

- Posoudit mají-li vybrané fyzikálně-chemické vlastnosti půd, zejména rozdílný celkový obsah arsenu/ selenu, vliv na příjem arsenu/ selenu rostlinami, a jaká je míra tohoto vlivu ve srovnání s dalšími faktory.
- Posoudit zda a do jaké míry má rostlinný druh vliv na příjem arsenu/ selenu rostlinami.
- Sledovat a zhodnotit příjem, akumulaci a přeměny arsenu/selenu a jejich sloučenin ve vzorcích volně rostoucích rostlinných společenstev v rámci vybraných lokalit na území ČR s ohledem na možnost vstupu těchto prvků do organismu volně žijících živočichů.
- Sledování a zhodnocení příjmu, akumulace a přeměny arsenu/ selenu ve vybraných zemědělských plodinách pěstovaných za různých koncentrací arsenu/ selenu v prostředí s cílem posoudit jejich možný dopad na zdraví člověka.

4 MATERIÁL A METODY

V této kapitole jsou uvedeny základní informace k použitým materiálům, jejich odběru a přípravě k měření. Dále jsou zde uvedeny základní informace k použitým analytickým metodám a statistickému zpracování výsledků. Podrobnější informace lze najít v jednotlivých publikovaných výstupech uvedených v podkapitolách 5.1 až 5.6.

4.1 Materiál

4.1.1 Vzorkování v terénu

4.1.1.1 Volně rostoucí rostlinná společenstva I

Odebírání vzorků v terénu má za cíl zmapovat zastoupení sloučenin arsenu a selenu v široké škále rostlinných druhů rostoucích na lokalitách s různými fyzikálně chemickými vlastnostmi půd, včetně různých úrovní celkového obsahu zkoumaných prvků. Je známo, že fyzikálně-chemické vlastnosti půd mají vliv na výskyt jednotlivých sloučenin prvků v půdě (Kabata-Pendias et Pendias, 2001).

Mezi lokality, ve kterých byly rostliny sbírány za účelem stanovení arsenu, patří Roudný, Kutná Hora a Nalžovské Hory. Tyto lokality jsou místy bývalé těžby drahých kovů, která je často doprovázena kontaminací arsenem a dalšími prvky. Na každé lokalitě bylo vybráno několik jednotlivých stanovišť (do 5 stanovišť na lokalitu), která byla svými vlastnostmi a polohou vhodná pro tento typ výzkumu. Na těchto vytyčených stanovištích o rozměrech 1 x 1 m byly odebrány vzorky nadzemní biomasy všech rostlinných druhů, které na dané parcelce rostly.

4.1.1.2 Volně rostoucí rostlinná společenstva II

V případě selenu byla vybrána lokalita Nalžovské Hory, kde byla sledována především případná interakce mobility a příjmu selenu rostlinami s mobilitou a příjmem rizikových prvků, zejména kadmiem, olovem a arsenem. Jako druhá lokalita byl zvolen Humpolec. Půdy v okolí Humpolce jsou na rozdíl od půd v Nalžovských Horách mírně kyselé, můžeme se tedy domnívat, že selen je zde přítomen spíše v méně dostupných formách. Pokus na nekultivovaných loukách probíhal tak, že na každé lokalitě bylo vytyčeno 6 vzorkovacích stanovišť (1 x 1 m) a z těchto stanovišť byly odebrány vzorky nadzemní biomasy jednotlivých rostlinných druhů, které se na daném stanovišti nacházely.

4.1.1.3 Volně rostoucí rostlinná společenstva III

Při posledním vzorkování v terénu byly na nekultivovaných loukách lokality Humpolec vytyčeny plochy o rozměrech 25 m^2, které byly ošetřeny postřikem roztoku selenanu sodného (Na_2SeO_4) o koncentraci odpovídající: i) kontrolní varianta Se0 – 0 g Se ha^{-1}; ii) Se25 – 25 g Se ha^{-1}; iii) Se50 – 50 g Se ha^{-1}. Cca po 4 týdnech byly odebrány vzorky nadzemní biomasy jednotlivých druhů rostlin.

Ve všech případech sběru volně rostoucích rostlin sloužilo několik rostlin od každého druhu k přesnému určení rostlinného druhu. Zbývající rostliny byly sušeny v sušárně Venticell (BMT, a.s., ČR) při teplotě 60 °C, následně rozemlety, zhomogenizovány a připraveny k měření.

4.1.2 Nádobové pokusy

4.1.2.1 Nádobové pokusy I

V zástupcích čeledí jitrocelovité (*Plantaginaceae)* a šáchorovité (*Cyperaceae*) jsme dle našich předchozích výsledků, a výsledků, které publikovali Geiszinger et al.[b] (2002) očekávali zvýšená množství organických kationtových sloučenin, zejména arsenobetainu. Z toho důvodu byly vybrány následující druhy rostlin: jitrocel kopinatý (*Plantago lanceolata* L.), ostřice časná (*Carex praecox* L.), ostřice měchýřkatá (*Carex vesicaria* L.) a skřípina lesní (*Scirpus sylvaticus* L.) pocházející z lokalit Roudný, Mokrsko, Malín a Kutná Hora. Ty byly následně pěstovány v modelových nádobových pokusech s cílem popsat příjem, přeměnu a akumulaci arsenu v těchto rostlinách v průběhu vegetačního období.

Postup vlastního nádobového pokusu byl takový, že do jednotlivých nádob bylo naváženo 5 kg kontaminované zeminy odebrané v arsenem kontaminované oblasti Mokrsko. Tato zemina byla předem zhomogenizována, vysušena a byly z ní odstraněny hrubé nečistoty. Po navážení bylo do zeminy přidáno NPK hnojivo ve fromě vodných roztoků NH_4NO_3 a K_2HPO_4 a to v dávkách 0,5 g N, 0,16 g P, 0,4 g K. Poté bylo hnojivo se zeminou řádně promícháno. Následně byly do nádob vysázeny vzrostlé rostliny odebrané předešlé léto z kontaminovaných lokalit, které přezimovaly v nádobách s nekontaminovanou půdou. Takto bylo připraveno 6 opakování od každého rostlinného druhu po 10 rostlinách v každé nádobě. Nádoby byly umístěny ve venkovní vegetační hale a denně zalévány deionizovanou vodou na úroveň 60 % maximální vodní kapacity pro zástupce z čeledi jitrocelovitých, případně 80 % maximální vodní kapacity pro zástupce z čeledi šáchorovitých.

V průběhu vegetace byla průběžně sledována speciace sloučenin v jednotlivých fyziologických částech rostlin (stonek, list, květ, na konci vegetační doby i kořen) s cílem zjistit, zda sledována druhy opravdu vykazují schopnost kationtové, ale i další arsenové sloučeniny syntetizovat, popř. v jakých fyziologických částech je nejčastěji ukládají. U jitrocele kopinatého byly během vegetační doby provedeny 3 odběry, u ostatních rostlin díky delší době potřebné k tvorbě generativních orgánu byly provedeny odběry 4.

Odebrané vzorky rostlin byly lyofilizovány (Lyovac GT-2, Německo), rozemlety, zhomogenizovány a připraveny k měření.

4.1.2.2 Nádobové pokusy II

Šest různých druhů zeleniny: tuřín (*Brassica napus* var. *napobrassica* L.), kadeřávek (*Brassica oleracea* convar. *acephela* L.), černá ředkev (*Raphanus sativus* var. *nigra* L.), černý kořen (*Scorzonera hispanica* L.), pastinák (*Pastinaca sativa* L.) a salát (*Lactuca sativa* L.) bylo pěstováno v nádobových pokusech na půdách s odlišnými fyzikálně-chemickými půdními vlastnostmi (Kutná Hora, Příbram). Rostliny, 4 opakování od každé varianty, byly pěstovány v 6 L nádobách naplněných 5 kg zeminy smíchané s NPK hnojivem (stejně jako viz výše). Nádoby byly umístěny ve venkovní vegetační hale a denně zalévány deionizovanou vodou na úroveň 60 % maximální vodní kapacity.

Jedlé části rostlin byly sklizeny v různých termínech v závislosti na vegetačním cyklu jednotlivých plodin. Rostliny byly jemně omyty deionizovanou vodou, byly odstraněny odumřelé části, a následně byly vzorky lyofilizovány (Lyovac GT-2, Německo), rozemlety, zhomogenizovány a připraveny k měření.

4.1.3 Parcelkové pokusy

4.1.3.1 Parcelkové pokusy I

Pro tento polní pokus s pěstováním selenem obohacených brokolic (*Brassica oleracea* var. *italica* L.) byl použit pozemek univerzitního políčka v lokalitě Praha Suchdol, jehož půda je charakterizovaná jako černozem s jílovitě-hlinitou strukturou. Před výsadbou byla do půdy zapravena minerální hnojiva v dávce odpovídající 500 kg NPK ha^{-1}. Rostliny byly pěstovány na přesně vyznačené parcele o velikosti 8 x 10 m, rozdělené na 12 menších parcelek (variant).

Na takto připravených a vyznačených pracelkách byly pěstovány 4 odrůdy brokolice: Heraklion F1, Marathon F1, Parthenon F1 a Naxos F1. Sazeničky byly předpěstovány v substrátu na bázi rašeliny, zalévány deionizovanou vodou a umístěny ve skleníku při teplotě pohybující se mezi 18 a 21 ° C. Pět týdnů po vyklíčení byly sazenice přesazeny do polních podmínek. Fungicidy a insekticidy byly aplikovány v souladu s požadavky rostlin během celé doby vegetace.

Vodný roztok Na_2SeO_4 byl aplikován postřikem na list na každou variantu v období začátku tvorby hlávky následujícím způsobem: i) Se0 – kontrolní varianta; ii) Se25 – odpovídající dávce 25 g Se ha^{-1}; iii) Se50 – odpovídající dávce 50 g Se ha^{-1}. Tři náhodně vybrané rostliny z každé varianty byly sklizeny v době, kdy byly hlávky připravené ke konzumaci (tj. asi 4 týdny po aplikaci selenu). Sklizené rostliny byly rozděleny na hlávku, stonky, listy a kořeny. Jednotlivé části byly zváženy a získaná biomasa byla opatrně omyta deionizovanou vodou, lyofilizována, jemně rozemleta za použití laboratorního mlýnku (Retsch SM 100, Německo), zhomogenizována a připravena k analýze.

4.2 Analytické metody

4.2.1 Analýzy rostlinného materiálu

4.2.1.1 Speciační analýzy – arsen

Pro získání obsahů jednotlivých sloučenin arsenu ve vzorcích byla použita vysoce sofistikovaná metoda spojení HPLC s ICP-MS. Při analýzách bylo částečně využito přístrojové vybavení a zkušenosti výzkumného týmu Ústavu chemie Karl-Franzens Univerzity v Grazu (Rakousko).

Pro extrakci sloučenin arsenu byla použita již dříve publikovaná metoda (Száková et al. 2011): jemně namleté a zhomogenizované vzorky byly extrahovány 0,02 M dihydrogenfosforečnanem amonným ($NH_4H_2PO_4$) (pH 6) v poměru 1+9 nebo 1+25 (w/v) po dobu 14 hod, upevněné do rotační třepačky (Biosan MultiRS 60, Litva) při otáčkách 45 rpm. Poté byly vzorky zcentrifugovány (Boeco C28A, Německo) při 3000 otáčkách po dobu 10 minut a nakonec přefiltrovány přes stříkačkové nitrátovo-celulosové filtry o velikosti porů 0,45 μm a průměru filtrů 0,25 mm (Roth, Německo) do vialek. Vzorky byly skladovány v lednici při 5 °C a během několik dní v nich byly proměřeny obsahy jednotlivých sloučenin.

Měření probíhalo pomocí spřažení HPLC (HPLC 1100, případně 1260 Infinity, Agilent Technologies Inc., USA) s ICP-MS (ICP-MS 7500ce, případně 7700x, Agilent Technologies Inc., U.S.), kde byla pro odstranění případných interferencí použita kolizní cela plněná heliem o průtoku 8 ml min^{-1}.

Analýza aniontových sloučenin probíhala za následujících podmínek: i) jako mobilní fáze (MF) byl použit 0,02 mol L^{-1} roztok $NH_4H_2PO_4$ o pH 6, který byl přiváděn na aniontově výměnnou kolonu PRPX100 (4,6 x 150 mm, částice o velikosti 5 µm, Hamilton, U. S.) průtokem 1,5 ml min^{-1} a objemu vzorku 10 µL; ii) jako MF byl použit roztok 2 mM dihydrogenfosforečnanu sodného (NaH_2PO_4) s přídavkem 20 mol L^{-1} disodné soli ethylendiamintetraoctové kyseliny (EDTA-2Na) o pH 6. Tato MF byla spolu se vzorkem přiváděna do kolony Agilent Technologies Inc. (U.S.) 4,6 mm x 150 mm i.d., s částicemi o velikosti 5 µm, a náplní tvořenou hydrofilní polymetakrylátovou pryskyřicí. Průtok mobilní fáze byl optimalizován na 1mL min^{-1} a dávkovaný objem vzorku na 10 µL.

Analýza kationtových sloučenin arsenu probíhala za podmínek: jako MF byl použit 10 mol L^{-1} roztok pyridinu o pH 2,3, který byl spolu se vzorkem přiváděn do kationtově výměnné kolony Zorbax 300-SCX (Agilent Technologies Inc., U.S.), 250 mm x 4.6 mm i.d., s částicemi o velikosti 5 µm a náplní tvořenou porózními silikátovými mikrosféramy o velikosti 300 Å. Průtok MF byl optimalizován na 1,5 mL min^{-1} a dávkovaný objem vzorku na 20 µL.

4.2.1.2 Speciační analýzy – selen

Analýzy byly provedeny s využitím instrumentace Ústavu analytické chemie na VŠCHT v Praze s využitím jejich již dříve publikovaných metod (Balan et al, 2014, Klognerova et al, 2015). Lyofilizované a zhomogenizované vzorky rostlinné biomasy byly extrahovány enzymatickou hydrolýzou následovně: ~0,5 g vzorku bylo naváženo do polyfluorovaných zkumavek a bylo k němu přidáno 25 mg proteázy XIV (Sigma-Aldrich, Japonsko) a 10 ml 0,02 mol L^{-1} tris-(hydroxymethyl)-amino-methanu (pH 7,5) (Fluka, Švýcarsko). Směs byla soustavně míchána po dobu 23 hodin a udržována při teplotě 37 °C. Následně byla zcentrifugována při 15000 rpm a 5 °C (centrifuga Sigma 2-16 K, Sigma, Německo), přefiltrována přes nylonový stříkačkový filtr s póry o velikosti 0,45 µm (Whatman,Velká Británie) a proměřena na obsah jednotlivých selenových sloučenin.

K měření byla použita spřažená technika HPLC s ICP-MS. Systém HPLC se skládal z těchto částí: vysokotlaké čerpadlo (Série 200, Perkin Elmer, U.S.), odplyňovač, vzorkovací ventil (Rheodyne 9010, IDEX Health and Science, U.S.) doplněný 50 µL PEEK

vzorkovací smyčkou a analytickou kolonou PR-C8 (Purosphere STAR-C8e, 4,6 x 250 mm, velikost částic 5 μm, Merck, U.S.). ICP-MS (Elan DRC-e, Perkin Elmer, U.S.) bylo vybaveno koncentrickým PTFE zmlžovačem a cyklonickou mlžnou komorou a vysoce účinnou plazmovou hlavicí. K potlačení případných interferencí byla použita reakční cela s methanem o průtoku 0,6 ml min^{-1}. Jako MF byla použita směs: 0,8 g l^{-1} butan-1-sulfonát sodný, 2,9 g l^{-1} hydroxid tetramethylamonný, 0,42 g l^{-1} malonové kyseliny a 1 % (v/v) methanolu. pH směsi bylo upraveno chlorovodíkovou kyselinou na hodnotu 5,0, a její průtok byl optimalizován na 1 ml min^{-1}.

4.2.1.3 Celkové obsahy

Celkové obsahy arsenu a selenu v biomase rostlin byly stanoveny za pomocí ICP-MS po předchozím rozkladu na mokré cestě v uzavřeném systému s fokusovaným mikrovlnným ohřevem Discover SPD-Plus (CEM Inc., U.S.) podle metodiky, kterou publikovali Kelly et al. (2013): 0,5 g vzorku bylo naváženo do křemených zkumavek, bylo přidáno 10 mL koncentrované HNO_3 (67 %, Analytika, čistota Analpure) a za teploty 200 °C, maximálního příkonu 300 W a maximálním tlaku 28 bar rozloženo. Následně byly vzorky kvantitativně převedeny do 50 mL polyethylenových zkumavek a uloženy při laboratorní teplotě do doby měření. Alternativně byly některé vzorky rozloženy na suché cestě ve směsi oxidačních plynů v mineralozátoru Apion dle Miholové et al. (1993).

4.2.2 Analýzy půd

4.2.2.1 Vybrané fyzikálně-chemické analýzy

Půdy odebráné z jednotlivých stanovišť byly usušeny při laboratorní teplotě, přesáty přes síto o velikosti ok 2 mm tak, aby vznikl reprezentativní homogenní vzorek vhodný k analýzám. V půdách byly podle potřeb jednotlivých pokusů stanoveny tyto fyzikálně-chemické parametry: pseudocelkové obsahy prvků (Száková et al., 2016), výměnná půdní reakce (Novozamsky et al., 1993), obsah oxidovatelného uhlíku (Sims et Haby, 1971), kationtová výměnná kapacita (ISO, 1994), dostupné živiny extrakčním činidlem Mehlich III (Mehlich, 1984), mobilní obsahy prvků (Quevauviller et al., 1993), maximální vodní kapacita (Gardner, 1986).

4.2.2.2 Celkové obsahy

Celkové obsahy prvků v půdách byly stanoveny z mineralizátů získaných po totálním rozkladu: ~0,5 g půdního vzorku bylo rozloženo na mokré cestě v uzavřeném systému s mikrovlnným ohřevem v zařízení Ethos 1 (MLS GmbH, Německo) po dobu 33 min při teplotě 210 °C ve směsi 8 ml HNO_3, 5 mL HCl a 2 ml HF. Po ochlazení byla reakční směs kvantitativně převedena do 50 mL Teflon® nádobky a odpařována do sucha při 160 °C. Odparek byl poté rozpuštěn ve 3 mL směsi HNO_3 a HCl (1+3), převeden do 25 mL skleněné zkumavky, doplněn deionizovanou vodou a uschován při laboratorní teplotě do doby měření (Száková et al., 2010).

Obsah prvků v připravených mineralizátech byl stanoven pomocí ICP-OES s axiální orientací plazmové hlavice na přístroji Varian VistaPro (Varian, Austrálie).

4.3 Zpracování a hodnocení naměřených dat

Výsledky a grafické výstupy byly zpracovány za použití různých programů v závislosti na vhodnosti jejich užití, jejich dostupnosti na pracovištích a především nároků kladených na statistickou analýzu a grafické zpracování jednotlivých pokusů.

Pro zpracování statistických analýz a tvorbu grafických výstupů byly použity následující programy: Microsoft Office Excel™ 2007, Microsoft Office Excel™ 2010 (Microsoft, U.S.), Statistica™ 10CZ, Statistica™ 12CZ (StatSoft, U.S.), SigmaPlot™ 11.0 (SyStat Software, U.S). Mezi použité statistické testy se řadí jednofaktorová analýza rozptylu ANOVA, s následným použitím Tukeyho nebo Scheffého post hoc testu, a korelační analýza pro odhad závislosti mezi proměnnými za použití Pearsonova korelačního koeficientu (Meloun et Militký, 2004). Hladina významnosti u všech provedených testů byla stanovena jako $\alpha = 0,05$. Transfer faktor, tedy ukazatel, který charakterizuje přestup prvku z půdy do rostliny, byl definován jako poměr celkového obsahu prvku v rostlině a v půdě (Huang et al., 2005).

5 VÝSLEDKY A PUBLIKOVANÉ VÝSTUPY

Seznam publikovaných výstupů a výsledků:

Publikovaný výstup č. 1:

Název článku: Arsenic compounds occurring in ruderal plant communities growing in arsenic contaminated soils [Sloučeniny arsenu vyskytujících se v ruderálních druzích rostlin rostoucích na arsenem kontaminovaných půdách]

Autoři: Tremlová, J., Vašíčková, I., Száková, J., Goessler, W., Steiner, O., Najmanová, J., Horáková, T., Tlustoš, P.

Publikováno v: Environmental and Experimental Botany 123. 2016. 108-115.

Publikovaný výstup č. 2:

Název článku: Distribution of arsenic compounds in *Plantaginaceae* and *Cyperaceae* plants growing in contaminated soil [Zastoupení sloučenin arsenu v rostlinách z čeledi Jitrocelovité a Šáchorovité rostoucích na kontaminovaných půdách]

Autoři: Tremlová, J., Száková, J., Golka, V., Babková, R., Najmanová, J., Tlustoš, P.

Publikováno v: Chemistry and Ecology (přijato do tisku), online od 2.8.2016, DOI 10.1080/02757540.2016.1216105

Publikovaný výstup č. 3:

Název článku: A profile of arsenic species in different vegetables growing in arsenic contaminated soils [Zastoupení sloučenin arsenu v zeleninách rostoucích na arsenem kontaminovaných půdách]

Autoři: Tremlová, J., Sehnal, M., Száková, J., Goessler, W., Steiner, O., Najmanová, J., Horáková, T., Tlustoš, P.

Publikováno v: Archives of Agronomy and Soil Science (přijato do tisku), DOI 10.1080/03650340.2016.1242721.

Publikovaný výstup č. 4:

Název článku: Soil-to-plant transfer of native selenium for wild vegetation cover at selected locations of the Czech Republic [Příjem přirozených obsahů selenu z půdy do volně rostoucích rostlin rostoucích na vybraných lokalitách České republiky]

Autoři: Száková, J., Tremlová, J., Pegová, K., Najmanová, J., Tlustoš, P.

Publikováno v: Environmental Monitoring and Assessment 187. 2015. 358-366.

Publikovaný výstup č. 5:

Název článku: Selenium uptake, transformation and inter-element interactions by selected wildlife plant species after foliar selenate application [Příjem selenu, jeho přeměna a vliv na obsahy vybraných prvků v biomase volně rostoucích rostlin po aplikaci selenanu na list]

Autoři: Drahoňovský, J., Száková, J., Mestek, O., Tremlová, J., Kaňa, J., Najmanová, J., Tlustoš, P.

Publikováno v: Environmental and Experimental Botany 125. 2016. 12-19.

Publikovaný výstup č. 6:

Název článku: The response of broccoli (*Brassica oleracea* convar. *italica*) varieties on foliar application of selenium: uptake, translocation, and speciation [Odezva různých odrůd brokolice na aplikaci selenu na list: příjem, přeměna a speciace]

Autoři: Šindelářová, K., Száková, J., Tremlová, J., Mestek, O., Praus, L., Kaňa, J., Najmanová, J., Tlustoš, P.

Publikováno v: Food Additives and Contaminants, Part A – Chemistry, Analysis, Control, Exposure and Risk Assessment 32. 2015. 2027-2038.

5.1 Publikovaný výstup č. 1

Název článku: Arsenic compounds occurring in ruderal plant communities growing in arsenic contaminated soils

Autoři: Tremlová, J., Vašíčková, I., Száková, J., Goessler, W., Steiner, O., Najmanová, J., Horáková, T., Tlustoš, P.

Publikováno v: Environmental and Experimental Botany 123. 2016. 108-115.

Environmental and Experimental Botany 123 (2016) 108–115

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Arsenic compounds occurring in ruderal plant communities growing in arsenic contaminated soils

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ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form 27 November 2015
Accepted 29 November 2015
Available online 1 December 2015

Keywords:
Speciation
Terrestrial plant
Hyphenated technique
HPLC
ICPMS
Arsenobetaine
Arsenocholine
TETRA
TMAO

ABSTRACT

Wild growing phytocenosis from three different areas near former gold or silver mines—Kutná Hora, Roudný and Nalžovské Hory (Czech Republic) were investigated for total arsenic and arsenic species concentrations. The most abundant plant families that occurred were *Fabaceae*,*Lamiaceae*, *Asteraceae*, *Poaceae* and *Plantaginaceae*. Several plant species such as *Achillea millefolium* L. (*Asteraceae*), *Anthoxanthum odoratum* L. (*Poaceae*), *Plantago lanceolata* L. (*Plantaginaceae*) are widespread and could be found in all of the investigated areas. Total As concentrations in aboveground biomass of plants growing on those three sites were determined by an inductively coupled plasma mass spectrometry (ICPMS) and ranged from 0.02* mg As kg^{-1} (*Stellaria* spp.) to 39.30 ± 6.32 mg As kg^{-1} (*Daucus carota* L.). The concentrations seem to be dependent on both plant species and physico-chemical soil properties. For the arsenic speciation a high performance liquid chromatography (HPLC) online connected with the ICPMS was used. Results have shown that arsenite and arsenate are the prevalent arsenic compounds. Methylarsonic acid (MA), dimethylarsinic acid (DMA), arsenobetaine (AB), arsenocholine (AC), tetramethylarsonium ion (TETRA), and trimethylarsine oxide (TMAO) were identified as minor species. Arsenobetaine was found at significant concentrations in *Carex praecox* Schreb. (28% of the extractable As amount) and *P. lanceolata* L. (1.2% of the extractable As amount).

1. Introduction

Several areas in the Czech Republic are characterized by elevated levels of As in soils mostly connected with gold and silver deposits. The most important ones are located in the central Bohemia. Locations such as Kutná Hora, Mokrsko, Roudný, *etc.* are well known examples with former mining activities on which high As content in soil occurs (Filippi et al., 2004).

The toxic effects of arsenic on plants are described by several authors (Bencko et al., 1995; Kabata-Pendias and Pendias, 2001; Rahman et al., 2008). Dembitsky and Rezanka (2003) summarized that methylated As species show generally lower toxicity than inorganic ones. For toxicity evaluations not only the total content is important, but the abundance of individual arsenic compounds (speciation). Arsenobetaine (AB) and arsenocholine (AC) are considered as non-toxic or harmless compounds to man (Kuehnelt and Goessler, 2003).

Although As is an element with limited plant availability (Pickering et al., 2000), its content in plants in contaminated areas of the Czech Republic exceeds the maximum content in forage and thus may pose a health risk for wild or outside keeping and grazing herbivores and might follow-up the food chain (Tremlová et al., 2011). Among the identified arsenic compounds in terrestrial plants predominantly As(III) and As(V) can be found. Methylarsonic acid (MA), dimethylarsonic acid (DMA), arsenobetaine (AB), arsenocholine (AC), tetramethylarsonium ion (TETRA), trimethylarsine oxide (TMAO) and some arsenosugar analogs have been observed as well (Ruiz-Chancho et al., 2008). A study of Tlustoš et al. (2002) showed that in roots of radish (*Raphanus sativus* L.) the dominant As compound was As(III), whereas As(V) was more represented in leaves. High proportions of DMA in plants (about 17% of total As content in the roots and 18% in leaves) compared to

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http://dx.doi.org/10.1016/j.envexpbot.2015.11.012
0098-8472/

Table 1
List of individual plant species occuring on investigated locations and sites and total As content in their aboveground biomass.

No.	Latin name	Family	Total As content (mg kg^{-1})				
1	*Aegopodium podagraria* L. [P]	*Apiaceae*	0.87 ± 0.09 (NH 2)	0.93 ± 0.03 (NH 3)	0.73 ± 0.12 (NH 4)	0.50 ± 0.05 (NH 5)	
2	*Agrostis stolonifera* L. [P]	*Poaceae*	2.15 ± 0.15 (KH 1)	1.09 ± 0.08 (KH 3)	1.13 ± 0.06 (KH 5)		
3	*Achillea millefolium* L. [P]	*Asteraceae*	1.51* (KH 3)	14.4 ± 1.7 (R 1)	5.23 ± 0.62 (R 2)	1.67 ± 0.08 (R 4)	0.51 ± 0.03 (NH 1)
			0.08 ± 0.01 (NH 3)	0.88 ± 0.20 (NH 4)			
4	*Alchemilla vulgaris* L. [P]	*Rosaceae*	0.21* (NH 2)				
5	*Alopecurus pratensis* L. [P]	*Poaceae*	1.02 ± 0.40 (KH 3)	0.58 ± 0.08 (KH 4)			
6	*Anthoxanthum odoratum* L. [P]	*Poaceae*	0.68* (KH 3)	3.83 ± 1.00 (R 1)	0.49 ± 0.07 (NH 2)		
7	*Arrhenatherum elatius* (L.) Presl [P]	*Poaceae*	0.76* (KH 1)	0.66 ± 0.03 (KH 2)	1.24* (KH 3)	0.89 ± 0.08 (KH 4)	0.94* (KH 5)
			1.00 ± 0.05 (NH 1)	0.54 ± 0.03 (NH 2)			
8	*Artemisia vulgaris* L. [P]	*Asteraceae*	0.711 ± 0.04 (NH 3)	0.71 ± 0.04 (NH 4)			
9	*Ballota nigra* L. [P]	*Lamiaceae*	0.42 ± 0.05 (NH 3)				
10	*Brachypodium pinnatum* Beauv. [P]	*Poaceae*	0.60 ± 0.05 (KH 1)	0.69 ± 0.14 (KH 2)	0.59 ± 0.04 (KH 3)	0.45 ± 0.07 (KH 4)	
11	*Briza media* L. [P]	*Poaceae*	1.68 ± 0.55 (KH 4)				
12	*Calamagrostis epigejos* L. [P]	*Poaceae*	3.09 ± 0.21 (R 1)	1.54 ± 0.08 (R 2)	5.02 ± 0.51 (R 5)		
13	*Campanula rapunculoides* L. [P]	*Campanulaceae*	0.86 ± 0.12 (NH 3)				
14	*Campanula* spp. [P]	*Campanulaceae*	7.48* (NH 5)				
15	*Carex nigra* L. [P]	*Poaceae*	0.58 ± 0.05 (NH 2)				
16	*Carex praecox* Schreb. [P]	*Poaceae*	0.68 ± 0.07 (KH 2)	1.16 ± 0.21 (KH 4)			
17	*Centaurea jacea* L. [P]	*Asteraceae*	2.38 ± 0.41 (R 1)	1.09 ± 0.05 (R 4)			
18	*Cerastium holosteoides* Fr. [a/p]	*Caryophyllaceae*	0.85* (NH 2)				
19	*Cirsium arvense* (L.) Scop. [P]	*Asteraceae*	0.44 ± 0.10 (KH 5)	6.93 ± 0.87 (R 4)			
20	*Convallaria majalis* L. [P]	*Liliaceae*	0.53 ± 0.03 (NH 5)				
21	*Convolvulus arvensis* L. [P]	*Convolvulaceae*	1.84* (KH 2)	0.18 ± 0.03 (NH 3)			
22	*Crepis biennis* L. [a/p]	*Asteraceae*	1.10* (NH 5)				
23	*Cynosurus cristatus* L. [P]	*Poaceae*	0.73* (NH 2)	0.11* (NH 3)			
24	*Cytisus scoparius* L. [P]	*Fabaceae*	0.89 ± 0.11 (NH 1)				
25	*Dactylis glomerata* L. [P]	*Poaceae*	0.76 ± 0.23 (NH 2)	0.70 ± 0.03 (NH 3)	0.58 ± 0.06 (NH 4)	0.53 ± 0.05 (NH 5)	
26	*Daucus carota* L. [P]	*Apiaceae*	39.30 ± 6.32 (R1)	0.73 ± 0.08 (NH 3)			
27	*Deschampsia caespitosa* L. [P]	*Poaceae*	5.49 ± 0.29 (R 1)	2.00 ± 0.10 (R 3)	1.14 ± 0.12 (R 5)	0.82 ± 0.04 (NH 2)	0.84 ± 0.03 (NH 4)
28	*Dianthus* spp. [a/b/p]	*Caryophyllaceae*	0.49 ± 0.03 (NH 4)				
29	*Dryopteris filix-mas* L. [P]	*Dryopteridaceae*	0.46 ± 0.13 (NH 5)				
30	*Echium vulgare* L. [b/p]	*Boraginaceae*	0.92 ± 0.05 (NH 1)				
31	*Elytrigia repens* (L.) Desv. [P]	*Poaceae*	1.33 ± 0.07 (KH 1)				
32	*Epilobium montanum* Huds. [P]	*Onagraceae*	0.44 ± 0.12 (NH 5)				
33	*Epilobium* spp. [a/p]	*Onagraceae*	0.60* (NH 2)				
34	*Epipactis helleborine* L. [P]	*Orchidaceae*	5.45 ± 0.27 (R 1)				
35	*Equisetum arvense* L. [P]	*Equisetaceae*	34.75 ± 4.88 (R 1)	29.40 ± 2.74 (R 2)	14.90 ± 0.80 (R 3)		
36	*Erodium neuradifolium* L. [a/b]	*Geraniaceae*	0.95* (NH 5)				
37	*Festuca pallens* Host [P]	*Poaceae*	1.16 ± 0.19 (KH 1)	0.22 ± 0.01 (KH 2)	1.08 ± 0.40 (KH 3)	0.38 ± 0.03 (KH 4)	1.95* (KH 5)
38	*Fragaria vesca* L. [P]	*Rosaceae*	1.77 ± 0.34 (R 2)	1.08 ± 0.38 (R 5)			
39	*Galium mollugo* L. [P]	*Rubiaceae*	2.32 ± 0.24 (R 4)	0.72 ± 0.13 (NH 2)	0.52 ± 0.15 (NH 4)		
40	*Galium verum* L. [P]	*Rubiaceae*	2.79 ± 0.21 (KH 1)	0.86 ± 0.07 (KH 2)	1.25 ± 0.08 (KH 4)	0.74 ± 0.04 (KH 5)	
41	*Geum urbanum* L. [P]	*Rosaceae*	0.90* (NH 4)	0.18 ± 0.01 (NH 5)			
42	*Hieracium pilosella* L. [P]	*Asteraceae*	9.96 ± 1.15 (R 1)	6.57 ± 0.34 (R 2)	1.38 ± 0.28 (NH 1)		
43	*Hypericum perforatum* L. [P]	*Hypericaceae*	1.40 ± 0.07 (KH 4)	1.76* (KH 5)	0.30 ± 0.01 (NH 2)	0.83 ± 0.09 (NH 3)	0.78* (NH 4)
			0.27* (NH 5)				
44	*Chaerophyllum aromaticum* L. [P]	*Apiaceae*	8.45 ± 0.42 (R 4)				
45	*Chenopodium album* L. [a]	*Apiaceae*	0.46 ± 0.02 (NH 5)				
46	*Impatiens parviflora* L. [a]	*Balsaminaceae*	5.69* (R 5)				
47	*Impatiens* spp. [a]	*Balsaminaceae*	0.49 ± 0.02 (NH 5)				
48	*Lactuca serriola* L. [a/b]	*Asteraceae*	6.04 ± 1.50 (R 5)				
49	*Lactuca* spp. [a/b]	*Asteraceae*	0.79* (NH 3)				
50	*Lamium* spp. [a/p]	*Lamiaceae*	0.51 ± 0.06 (NH 5)				
51	*Lolium* spp. [a/b/p]	*Fabaceae*	0.20 ± 0.01 (NH3)	0.25 ± 0.04 (NH 4)	0.90 ± 0.12 (NH 5)		
52	*Lotus corniculatus* L. [P]	*Fabaceae*	4.97 ± 0.78 (R 1)	2.36 ± 0.42 (R 2)	1.69 ± 0.08 (R 3)	1.05 ± 0.05 (NH 1)	0.47 ± 0.02 (NH 3)
			0.11 ± 0.01 (NH 4)				
53	*Lysimachia vulgaris* L. [P]	*Primulaceae*	2.29 ± 0.25 (NH 5)				
54	*Medicago lupulina* L. [a/p]	*Fabaceae*	0.48* (NH 1)	0.16 ± 0.01 (NH 2)	1.02 ± 0.04 (NH 3)		
55	*Medicago* spp. [a/p]	*Fabaceae*	0.78 ± 0.02 (NH 5)				
56	*Melandrium album* L. [a/b]	*Caryophyllaceae*	0.61 ± 0.02 (NH 3)				
57	*Pastinaca sativa* L. [P]	*Apiaceae*	0.63 ± 0.11 (NH 2)				
58	*Phleum pratense* L. [P]	*Poaceae*	0.72 ± 0.04 (R 4)	0.42 ± 0.04 (NH 3)			
59	*Phragmites communis* L. [P]	*Poaceae*	1.08 ± 0.08 (R 2)	1.09 ± 0.06 (R 3)			
60	*Pimpinella major* L. [P]	*Apiaceae*	2.31* (NH 4)	2.31* (NH 4)			
61	*Pimpinella saxifraga* L. [P]	*Apiaceae*	5.96* (KH 4)				
62	*Pimpinella* spp. [P]	*Apiaceae*	0.80 ± 0.04 (NH 1)				
63	*Plantago lanceolata* L. [P]	*Plantaginaceae*	1.33 ± 0.98 (KH 3)	13.50 ± 0.88 (R 1)	3.23 ± 0.57 (R 4)	0.56 ± 0.03 (NH 1)	0.47 ± 0.05 (NH 2)
			0.77 ± 0.04 (NH 3)	1.75 ± 0.09 (NH 4)	0.83 ± 0.08 (NH 5)		
64	*Plantago major* L. [P]	*Plantaginaceae*	3.28 ± 1.04 (R 4)	0.81 ± 0.02 (NH 5)			
65	*Poa pratensis* L. [P]	*Poaceae*	0.56 ± 0.03 (NH 2)	0.36 ± 0.02 (NH 3)	1.19* (NH 5)		
66	*Polygala vulgaris* L. [P]	*Polygalaceae*	8.49* (R 1)				
67	*Potentilla anserina* L. [P]	*Rosaceae*	17.64* (R 1)	6.62 ± 0.33 (R 4)			
68	*Potentilla heptaphylla* L. [P]	*Rosaceae*	0.46 ± 0.06 (KH 4)				
69	*Prunella vulgaris* L. [P]	*Lamiaceae*	1.07 ± 0.04 (NH 3)				
70	*Ranunculus acris* L. [P]	*Ranunculaceae*	1.39* (KH 4)	0.31 ± 0.30 (NH 2)			
71	*Ranunculus repens* L. [P]	*Ranunculaceae*	1.25 ± 0.13 (R 4)				

Table 1 (*Continued*)

No.	Latin name	Family	Total As content (mg kg^{-1})			
72	*Rumex obtusifolium* L. [p]	*Polygonaceae*	0.78 ± 0.03 (NH 3)			
73	*Rumex* spp. [p]	*Polygonaceae*	0.71 ± 0.04 (NH 3)			
74	*Scabiosa ochroleuca* L. [b/p]	*Dipsacaceae*	0.93 ± 0.06 (KH 1)	0.65 ± 0.03 (KH 3)		
75	*Securigera varia* L. [p]	*Fabaceae*	1.67* (NH 1)	0.78 ± 0.13 (NH 2)	0.27 ± 0.04 (NH 3)	0.49 ± 0.08 (NH 4)
76	*Senecio vulgaris* L. [a]	*Asteraceae*	0.29 ± 0.01 (NH 3)			
77	*Silene* spp. [p]	*Caryophyllaceae*	0.92 ± 0.07 (NH 1)	0.38 ± 0.07 (NH 3)		
78	*Solidago virgaurea* L. [p]	*Asteraceae*	1.79 ± 0.22 (NH 2)			
79	*Sonchus arvensis* L. [p]	*Asteraceae*	0.84 ± 0.09 (NH 2)			
80	*Stellaria graminea* L. [p]	*Caryophyllaceae*	1.63 ± 0.10 (R 4)	0.58* (NH 2)		
81	*Stellaria* spp. [p]	*Caryophyllaceae*	0.02* (NH 4)			
82	*Tanacetum vulgare* L. [p]	*Asteraceae*	3.47 ± 0.18 (R 4)	0.88 ± 0.09 (NH 3)		
83	*Taraxacum officinale* L. [p]	*Asteraceae*	0.46 ± 0.02 (NH 3)			
84	*Tetragonolobus maritimus* Roth [p]	*Fabaceae*	0.20 ± 0.01 (KH 5)			
85	*Thymus serpyllum* L. [p]	*Lamiaceae*	2.13 ± 0.15 (KH 3)	1.46 ± 0.42 (NH 1)	0.30 ± 0.05 (NH 4)	
86	*Torilis arvensis* L. [a]	*Brassicaceae*	0.13* (NH 3)			
87	*Trifolium campestre* Schreb. [a]	*Fabaceae*	0.56 ± 0.03 (NH 4)			
88	*Trifolium pratense* L. [p]	*Fabaceae*	5.24 ± 0.44 (R 3)	1.37 ± 0.08 (NH 2)	0.45 ± 0.07 (NH 3)	0.41 ± 0.09 (NH 4)
89	*Trifolium repens* L. [p]	*Fabaceae*	0.47* (NH 1)	1.64* (NH 2)	0.44 ± 0.02 (NH 3)	0.47 ± 0.02 (NH 5)
90	*Trisetum flavescens* (L.) Beauv. [p]	*Poaceae*	0.73 ± 0.04 (KH 1)	0.74 ± 0.20 (KH 2)		
91	*Tussilago farfara* L. [p]	*Asteraceae*	31.20 ± 9.30 (R 3)	2.27 ± 0.13 (R 5)		
92	*Urtica dioica* L. [p]	*Urticaceae*	0.71 ± 0.03 (NH 5)			
93	*Verbascum nigrum* L. [b/p]	*Scrophulariaceae*	0.66 ± 0.07 (NH 3)	1.51 ± 0.08 (NH 4)		
94	*Veronica chamaedrys* L. [p]	*Scrophulariaceae*	2.73 ± 0.014 (KH 5)	0.55 ± 0.04 (NH 2)	0.55* (NH 4)	
95	*Veronica* spp. [p]	*Scrophulariaceae*	0.42 ± 0.05 (NH 3)			
96	*Vicia cracca* L. [p]	*Fabaceae*	1.05 ± 0.08 (NH 4)			
97	*Vicia villosa* L. [a/b]	*Fabaceae*	0.71 ± 0.03 (NH 3)			
98	*Vinca minor* L. [p]	*Fabaceae*	1.04* (NH 5)			
99	*Viola odorata* L. [p]	*Fabaceae*	1.12* (NH 3)			

Note: [p] perennial plants, [b] biennial plants, [a] annual plant, * the amount of dry biomass was not sufficient for a multiplication of total content analysis and for an accomplishment of speciation analysis, KH—Kutná Hora (site 1–5), R—Roudný (site 1–5), NH—Nalžovské Hory (site 1–5).

the soil, with more than 90% of As was present as As(V) indicates an ability of some plants to methylate a part of inorganic arsenic. The same trend was observed in tissues of *Vinca minor* L., grown in a medium supplemented with As(V), showing dominant contents of As(III), As(V) but also significant amounts of MA and DMA (Cullen and Reimer, 1989).

The distribution of individual As species in higher plants and their aboveground parts is clearly influenced by plant species (Kuehnelt et al., 2000; Schmidt et al., 2001). Kuehnelt et al. (2000) found a wide range of As compounds, such as As(III), As(V), DMA, MA, TMAO, TETRA and one type of dimethyl(ribosyl)-arsine oxide, in 12 plant species growing on As contaminated sites. The effect of plant species on the occurrence of As species and their concentrations was also demonstrated by Geiszinger et al. (2002). While in aboveground biomass extracts of *Dactylis glomerata* L. and *Plantago lanceolata* L. growing on As contaminated areas mainly inorganic compounds were determined in the extracts of aboveground biomass of *Trifolium pratense* L. growing on the same site primarily organic compounds with mainly MA were present. The observation of As methylation in *Agrostis tenuis* Sibth. shows that As(V) that was added to the growing medium, was adopted by plant roots, converted to As(III), and later was methylated in leaves, where an increased activity of the enzyme methyltransferase was observed (Wu et al., 2002).

This study is focused on wild growing terrestrial plants exposed to soil As in the vicinity of Kutná Hora, Roudný and Nalžovské Hory (Czech Republic). The objectives of this study are focused on (i) a screening of total As uptake by nearly 100 plants growing in soils significantly affected by former mining activities, (ii) a dependency of total As plant uptake on physico-chemical soil properties and individual plant species, and (iii) an assessment of the ability of terrestrial plant species to take up and transform different As compounds in their tissues.

2. Experimental

2.1. Sampling

During the field survey 5 representative habitats of each area of interest were delineated (1 m^2) on which phytocenosis monitoring was conducted. In each square, the number of individual plant species were identified and reported. Monitoring was carried out during the plant's most abundant phase of the growing season in the area of the Czech Republic (May–July). A total of 99 plant species belonging to 27 different families (Table 1) were found in the survey. The aboveground biomass was gently washed by deionised water, checked for fresh biomass, freeze-dried (Lyovac GT2, Germany), ground (ZM 200, Retsch, Germany) and analyzed. Soil samples were collected from individual squares from the depth 0–25 cm, air dried at 20 °C, ground in a porcellaneous mortar and passed through a 2 mm plastic sieve and analyzed.

2.2. Total arsenic contents in soils and plants

The pseudo-total concentrations of elements in the soils were determined in digests obtained by a following decomposition procedure: Aliquots (~0.5 g) of air-dried soil samples were decomposed in digestion vessels with 10 ml of Aqua regia (n = 3). The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave assisted wet digestion system for 33 min at 210 °C. After cooling, the digest was quantitatively transferred into a 25 ml glass tube, filled up by deionized water, and kept at laboratory temperature until measurement. A certified reference material RM 7001 Light Sandy Soil (Czech Metrology Institute, Czech Republic) containing 10.4 ± 1.0 mg As kg^{-1} was applied for the quality assurance of analytical data. In this sample, 10.8 ± 1.5 mg As kg^{-1} was determined. Total organic carbon (TOC) was

determined spectrophotometrically at 600 nm after the oxidation of organic matter by $K_2Cr_2O_7$ (Sims and Haby, 1971). The pH was determined using deionised water or 0.01 mol l^{-1} $CaCl_2$ (w/v = 1:2.5) (Novozamsky et al., 1993). Cation-exchange capacity (CEC) was calculated as a sum of Ca, Mg, K, Na and Al extractable with 0.1 mol l^{-1} $BaCl_2$ (w/v = 1:20 for 2 h) (ISO, 1994).

Plant samples were decomposed using a dry ashing procedure as follows: An aliquot (~1 g) of the dried and powdered aboveground biomass or roots was weighed to 1 mg (n = 3), placed into a borosilicate glass test tube and decomposed in a mixture of oxidizing gases ($O_2 + O_3 + NO_x$) at 400 °C for 10 h in a Dry Mode Mineralizer Apion (Tessek, Czech Republic). The ash was dissolved in 20 ml of 1.5% HNO_3 (electronic grade purity, Analytika, Czech Republic) and kept in glass tubes until analysis (Miholová et al., 1993). Aliquots of the certified reference material RM NCS DC 73350 Poplar leaves (purchased from Analytika, CZ) were mineralized under the same conditions for quality assurance of the total arsenic contents in experimental plants. This material was certified to contain 0.37 ± 0.06 mg As kg^{-1}, and it was determined 0.35 ± 0.04 mg As kg^{-1}. For the determination of mobile fractions of arsenic in soils, extraction with a 0.11 mol l^{-1} solution of CH_3COOH at a ratio of 1:20 (w/v) for 16 h (Quevauviller et al., 1993) was applied. Each extraction was carried out in three replicates. For the centrifugation of extracts, a Hettich Universal 30 RF (Germany) device was used. The reaction mixture was centrifuged at 3000 rpm for 10 min at the end of each extraction procedure, and the supernatants were kept at 6 °C prior to measurements. The total concentrations of As in soils and plant digests were determined by using ICP OES (Varian VistaPro, Varian, Australia) with axial plasma configuration, equipped with an autosampler SPS-5. External calibration solutions were prepared in corresponding extraction agents with concentrations of 100–1000 μg l^{-1} As. The operating measurement wavelength for ICP OES was 188.9 nm. Plasma conditions used for measurements were as follows: power 1.2 kW, plasma flow 15.0 l min^{-1}, auxillary flow 0.75 l min^{-1}, nebulizer flow 0.9 l min^{-1}. For the determination of low As concentrations in soil extracts and plant digests, hydride generation atomic absorption spectrometry (Varian AA280Z, Varian, Australia), equipped with a continuous hydride generator VGA-77, were used where a mixture of potassium iodide and ascorbic acid was used for pre-reduction of the sample and the extract was acidified with HCl before measurement (Brodie et al., 1983).

2.3. Determination of individual As species

For a determination of As species in aboveground biomass of the plants following procedure was applied: dried and powdered samples were weighed into 10 ml screwcapped polyethylene tubes (n = 2), extracted with 0.02 mol l^{-1} $NH_4H_2PO_4$ in the ratio 1 + 9 (w/v) (pH 6.0) and fastened to a crossshaped rotor and turned top over bottom at 45 rpm for 14 h. The mixtures were then centrifuged for 10 min at 3000 rpm, and filtered through 0.22 μm cellulose-nitrate ester filters (MillexGS, Millipore, Bedford, MA, U.S).

Anionic As compounds were separated with a high performance liquid chromatography (HPLC 1100 Series, Agilent Technologies, U.S.), using an anion-exchange column PRP-X100 150 × 4.6 mm with 5 μm particles (Hamilton, U.S), column temperature 30 °C and injection volume 10 μl. A good separation was achieved by using an isocratic elution with 0.02 mol l^{-1} $NH_4H_2PO_4$ (pH 6.0) as a mobile phase at a flow of 1.5 ml min^{-1}. Typical chromatogram of the anionic As species present in *Carex praecox* sample present in Fig. 1.

Cationic As compounds were determined with the same instruments while using Zorbax 300-SCX 250 × 4.6 mm with 5 μm particles column (Agilent Technologies Inc., U.S.), column temperature 30 °C, injection volume 20 μl, 0.01 mol l^{-1} pyridine buffer (pH 2.3) served as a mobile phase with a flow of 1.5 ml min^{-1} (Ruiz-Chancho et al., 2008). Typical chromatogram of the cationic As species present in *C. praecox* sample present in Fig. 2.

An inductively coupled plasma mass spectrometer (ICPMS, 7500ce, Agilent Technologies, U.S.) was used as arsenic-selective detector (Szaková et al., 2011). A PEEK capillary tubing (0.125 mm i. d.) was used to connect the column outlet with a micro-mist nebulizer of the ICPMS. The intensivity of As ions at m/z 75 and also potential argon chloride ($^{40}Ar^{37}Cl$) interferences at m/z 77 were monitored by using a time-resolved analysis software.

All standards and solutions were prepared with purified water (18.2 MΩ cm, Merck Millipore, U.S.), pyridine (p.a.) was purchased

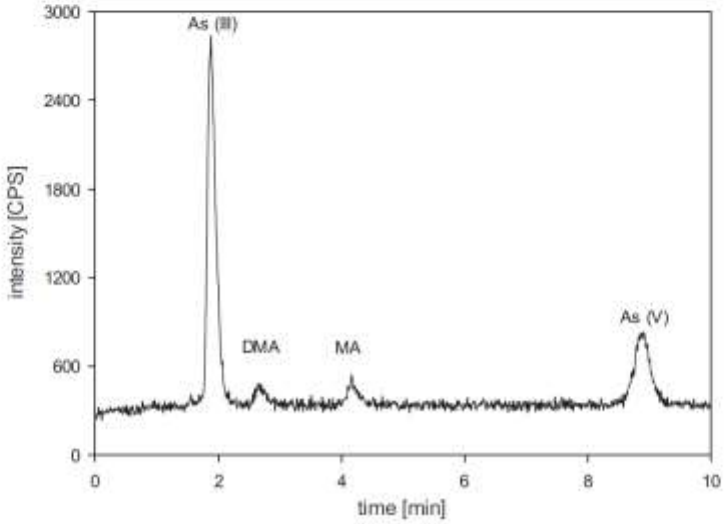

Fig. 1. Chromatogram of anionic As species in *Carex praecox* Schreb.
Note: an example chromatogram of anionic As species of *Carex praecox* Schreb., Kutná Hora, Czech Republic, signals of As species follow as As(III), DMA, MA, As(V), compounds were determined with HPLC ICPMS, Hamilton PRP-X100 150 × 4.6 mm column with 5 μm particles (Hamilton Inc., U.S.), isocratic elution with 0.02 mol l^{-1} NH4H2PO4 (pH 6.0) mobile phase, flow 1.5 ml min^{-1}.

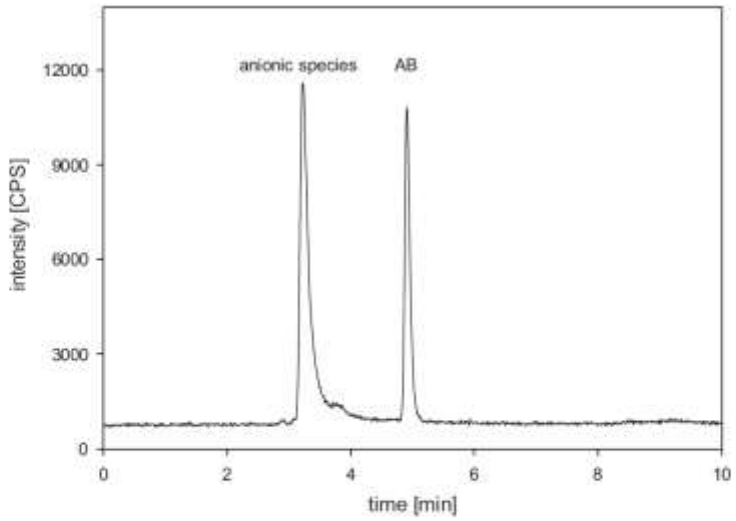

Fig. 2. Chromatogram of cationic As species in *Carex praecox* Schreb.
Note: an example chromatogram of cationic As species of *Carex praecox* Schreb., Kutná Hora area, signals of As species follow as sum of all anionic species, AB, no other cationic compounds were detected in this sample, compounds were determined with HPLC ICPMS (Agilent, Zorbax 300-SCX 150 × 4.6 mm column with 5 μm particles (Agilent Technologies Inc., U.S.), isocratic elution of 0.01 mol l^{-1} pyridin buffer (pH 2.3) mobile phase, flow 1.5 ml min^{-1}.

from Merck (Germany), methanol (p.a.), formic acid (p.a.), ammonium dihydrogen phosphate (p.a.), and an aqueous ammonia solution (25% suprapur) were purchased from Fluka (Switzerland). Standard solutions of arsenic species were prepared from $NaAsO_2$ (As(III)), $Na_2HAsO_4{\cdot}7H_2O$ (As(V)) both purchased from Merck (Germany), sodium dimethylarsinate trihydrate (DMA) purchased from Fluka (Switzerland). Methylarsonate, arsenobetaine, arsenocholine, trymethylarsine oxide and tetramethylarsonium cation were synthesized in-house. The detection limits (LODs) for the individual As compounds were as follows: 0.012 μg kg^{-1} for As(III), 0.008 μg kg^{-1} for DMA, 0.006 μg kg^{-1} for MA, 0.006 μg kg^{-1} for As(V), 0.022 μg kg^{-1} for AB, 0.011 μg kg^{-1} for TMAO, 0.011 μg kg^{-1} for AC, and 0.010 μg kg^{-1} for TETRA.

2.4. Statistical analysis

Statistical software STATISTICA™ version 12 (StatSoft®, Inc., USA), Microsoft Excel 2010™ software (Microsoft Corp.®, USA) and SigmaPlot™ 11.0 (SyStat Software Inc., USA) were used to carry out the statistical analysis and outputs. Statistical tests (one-way ANOVA) were carried out at a significance level $\alpha = 0.05$ for the total contents of analytes in the soil and plant samples. Scheffe's post-hoc test for carrying out differences in pairs of means was performed. Correlation analysis was used for the assessment of relationships between variables, where Pearson correlation coefficients were applied. The so-called transfer factors (TF) quantifying the element transfer from soil to plants were calculated as the ratio of the element content in plant dry matter to the pseudototal element content in soil.

3. Results and discussion

3.1. Phytocenosis

The total of 99 terrestrial plant species belonging to 27 different plant families (Table 1) were identified on experimental sites. *Poaceae* and *Fabaceae* family were the most abundant ones of all. The proportion of plant species and families were as follows—Kutná Hora: 23 species from 13 different families, with a predominance of *Poaceae*, Roudný: 27 species from 13 families, with a predominance of *Poaceae* and *Asteraceae*, Nalžovské Hory: 72 species from 25 families, predominance of *Poaceae* and *Fabaceae*, respectively. According to the other studies (Jana et al., 2012; Freitas et al., 2004), similar plant communities representing the families *Fabaceae*, *Lamiaceae*, *Asteraceae*, *Poaceae*, *Plantaginaceae* are widespread on sites with former mining activities across Europe. Among the plant species in our study, *Achillea millefolium* L. (*Asteraceae*), *Anthoxanthum odoratum* L. (*Poaceae*), *P. lanceolata* L. (*Plantaginaceae*) occurred on all 3 locations which may denote ability of these plants to grow on contaminated sites under different environmental conditions and also their widespread character across the Czech Republic.

In this study, only herbaceous plants, most of them perennial (Table 1), were evaluated and no at first sight visible symptoms of phytotoxicity were observed. However, trees from *Pinaceae*, *Fagaceae*, *Betulaceae* families demonstrated a higher tolerance to high As contents and/or extreme soil conditions (852 mg total As kg^{-1}, 1% TOC, 2.4 < pH < 3.9) growing near mining areas in France (Jana et al., 2012). Authors referred to these plants as phytostabilizers, a group of plants adapted to the harsh environment. General attributes of these plant species are adaptability and tolerance to the contamination and poor accumulation capacities, particularly in the shoots. Among herbaceous plants in the same study, the most tolerant species belong to *Fabaceae*, *Juncaceae*, *Plantaginaceae* and *Poaceae* family similarly as in the case of our study. Last 3 named families which grown on our sites too, reached in that study the total As contents in dry biomass ranging from 7 mg As kg^{-1} in leaves of *Plantago major* L. (*Plantaginaceae*) to 225 mg As kg^{-1} in roots of *Bromus* spp. L. (*Poaceae*) in our case it was 0.47–13.5 mg As kg^{-1} in *Plantago major* L. aboveground biomass. As concentrations in roots/underground biomass are not available in our study.

3.2. Total As contents in soils and plant biomass

Table 2 describes the main physico-chemical soil properties and total and mobile As contents of each investigated site. According to the Czech Public Notice No. 13/1994 are the concentrations of As in

Table 2
Physico-chemical properties of soils from investigated areas.

Site/area	As_{tot} (mg kg^{-1})	As_{extr} (mg kg^{-1})	pH	CEC (mmol kg)	TOC (%)
Kutná Hora					
Site 1	101 ± 4	2.74 ± 0.30	5.19	138	3.34
Site 2	108 ± 2	5.14 ± 0.57	5.85	154	3.96
Site 3	444 ± 3	14.1 ± 0.2	4.59	121	3.31
Site 4	128 ± 2	3.35 ± 0.75	4.78	128	3.30
Site 5	153 ± 1	4.11 ± 0.46	5.50	138	2.80
Roudný					
Site 1	675 ± 62	7.81 ± 1.31	4.69	53.8	1.00
Site 2	740 ± 62	6.61 ± 1.30	4.57	52.8	0.61
Site 3	1120 ± 10	4.55 ± 0.64	5.16	106	1.39
Site 4	46.8 ± 6	0.072 ± 0.024	5.27	159	4.62
Site 5	1100 ± 42	6.42 ± 1.61	4.53	52.0	1.33
Nalžovské Hory					
Site 1	72.7 ± 2	0.94 ± 0.04	5.58	76.7	8.29
Site 2	76.0 ± 2	1.11 ± 0.01	6.56	95.1	5.70
Site 3	28.7 ± 1	0.64 ± 0.00	6.59	80.4	1.71
Site 4	39.5 ± 1	1.15 ± 0.01	5.74	70.8	1.96
Site 5	21.8 ± 1	1.65 ± 0.16	6.90	81.0	5.65

As_{tot}: As content extractable with Aqua regia; As_{extr}: As content extractable with 0.11 mol l^{-1} solution of CH_3COOH.

soils limited by a threshold limit 30 mg As kg^{-1} in dry weight (Anonymous, 1994). Only one site among fifteen (Nalžovské Hory, site 5) was within the Czech standard of permissible limits of As in soil. Although high variability of the As contents occurred within one location, the results showed significantly ($\alpha = 0.05$) higher As contents at the location Roudný. Whereas the TOC levels did not significantly differ (most probably due to high variability of this parameter at the individual locations), the pH levels at the Nalžovské Hory location were significantly ($\alpha = 0.05$) higher compared to the other locations and Kutná Hora soils were characterized by significantly ($\alpha = 0.05$) higher CEC value. However, the mobile As pool in the experimental soils showed different pattern (Table 2). The extractable pool of As at the location Roudný was not significantly differing from the location Kutná Hora. The extractable As proportions at the location Roudný varied between 0.15 and 1.1% of the total As content and was significantly ($\alpha = 0.05$) lower than at the location Kutná Hora (between 2.6 and 4.7%). The highest mobility of As was observed at the less contaminated area Nalžovské Hory, where the extractable proportions reached the levels between 1.3 and 5.9% of the total As content. These results confirm our previous results where no unambiguous relationships between mobile proportions of As and the main physicochemical parameters of the soils were observed (Száková et al., 2000; Száková et al., 2007) and the As mobility in the soils is affected by the whole complex of the soil characteristics. Thus determination of the main soil properties seems to be weakly predictable for estimation of As mobility and plant-availability in the soil and more detailed fractionation and/or speciation of the mobile As in soils will be necessary.

A variability of the physico-chemical soil properties especially the total As concentration was quite high the most probably due to a different history and different local conditions which could cause differences between the total As contents of the same plant growing on different sites and/or areas. A typical example was *A. millefolium* L. (*Asteraceae*) that showed differences between sites and as well as between areas (Kutná Hora site 3: 1.51* mg As kg^{-1}, Roudný site 1: 14.4 ± 1.7 mg As kg^{-1}, Roudný site 4: 1.67 ± 0.08 mg As kg^{-1}, Nalžovské Hory site 3: 0.08 ± 0.01 mg As kg^{-1}, Nalžovské Hory site 4: 0.88 ± 0.20 mg As kg^{-1}). It indicates that the physico-chemical soil properties and especially total contents of As and its variability at sites play an important role in a determination of As total contents in biomass. However, no significant correlations were found neither between total As in soil nor mobile As contents in the soils and the total contents of As in plants. Total contents of As in plant biomass at Kutná Hora fluctuated between 0.20 ± 0.01 mg As kg^{-1} (*Tetragonolobus maritimus* L., site 1) and 5.96 mg As kg^{-1} (*Pimpinela saxifraga* L., site 5), at Roudný between 0.72 ± 0.01 mg As kg^{-1} (*Phleum pratense* L., site 4) and 39.3 ± 6.3 mg As kg^{-1} (*Daucus carota*, site 1), Nalžovské Hory between 0.02 mg As kg^{-1} (*Stellaria* spp. site 4) and 7.47 mg As kg^{-1} (*Campanula* spp. site 5), respectively. According to Kabata-Pendias and Pendias (2001) As content in plants growing on uncontaminated soils commonly reaches between 0.01 and 1.5 mg As kg^{-1} in dry matter. Directive No. 2002/32/ES the maximum values of elements in raw feedstuffs (Anonymous, 2002) states the permissible level to 2 mg As kg^{-1} (calculating the average moisture of the raw feedstuff cca 12%, the maximum acceptable value of As recalculated to the dry matter is 2.24 mg kg^{-1}). Therefore, a potential transfer of the elements originating from plants growing on examined contaminated soils to the food chain especially *via* herbivorous livestock/wild animals should be taken into account. Differences in the total As content among the plant species reflected a contamination level and a variability in physico-chemical soil properties on individual sites and the As contents in plants at the Roudný location were significantly ($\alpha = 0.05$) higher compared to both Kutná Hora and Nalžovské Hory which also shows the highest As contents in soils and the highest variability of the results within sites was observed on this location, as well. Because of high variability of the results at the individual locations no significant differences were observed among individual plant species and/or families at the individual locations. Only one exception was *Equisetum arvense* L. demonstrating high As contents with low variability among the sites and, therefore, the As content was significantly ($\alpha = 0.05$) higher compared to the other species. However, all the *E. arvense* samples originated from the various sites of the Roudný location characterized by the highest As contents, as well. Although none of this plant belong to the group of metallophytes or hyperaccumulators all of them well prosper on the investigated highly polluted sites. This could indicate that they probably have developed appropriate mechanisms helping them to grow and avoid As toxicity. These mechanisms seem to be connected with a soil-plant-root system and with an uptake of the soil solution into the plant organism e.g., a barrier made of Casparian root strips (Grebe, 2011; Chen et al., 2011). The same good prosperity of plants on highly As polluted sites was also shown in the study by Jedynak et al. (2009) focusing on the plants *Calamagrostis arundinacea* L. (*Poaceae*) and *Athyrium filix-femina* L. (*Athyriaceae*) collected in the vicinity of a former mining area in Poland where soils contained between 96.4 ± 7.2 and 7960 ± 532 mg As kg^{-1} of dry matter.

3.3. Content of individual As species in plants

Extraction efficiency of As species in this case varied according to plants species. For some plant species the extraction efficiency was quite high e.g., *A. odoratum* L. (Kutná Hora, 78.3%), *Tussilago farfara* L. (Roudný, 71.5%) while for some other the extraction efficiency was very poor (*Hieracium pillowcase* L., Nalžovské Hory, 1%; *Sonchus arvensis* L., Nalžovské Hory, 1.1%). Similarly, very different and sometimes very low extraction efficiency in plant biomass was observed also by Geiszinger et al. (2002). Our previous experiments (Száková et al., 2005) showed different extractability of As even for individual parts of one plant. This could be related to poor penetration of individual plant cell components where the different composition of the tissues of the individual plant species will play an important role. Optimization of the extraction procedure for individual plant species could be helpful for the improvement of the extraction yield but it requires further research, especially the question whether the extractants

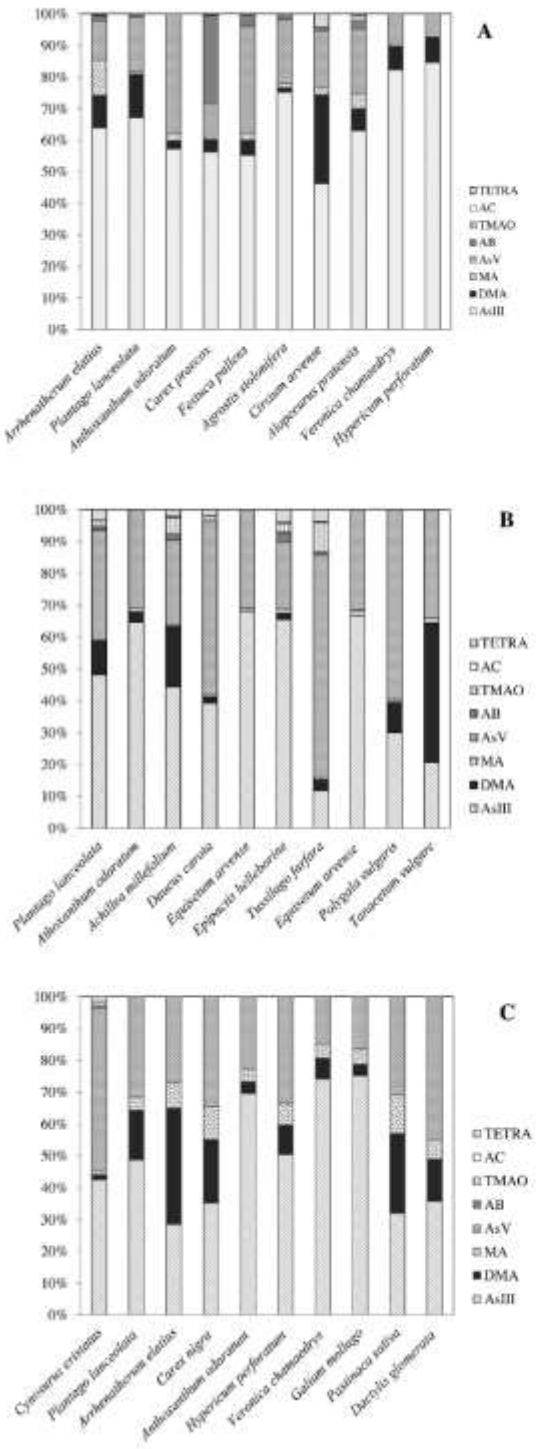

Fig. 3. The distribution of individual As species within dry plant biomass of the selected plant species; (A) Kutná Hora, (B) Roudný, (C) Nalžovské Hory.

do not change the As species distribution should be clearly answered.

Eight individual species, As(III), As(V), DMA, MA, AC, AB, TETRA and TMAO were detected in plant tissues where previously published analytical procedure (Geiszinger et al., 2002) was used for the resolution of the individual compounds. The most illustrative examples of the distribution of the As compounds in the individual species are presented in Fig. 3. Both As(III) and As(V) were dominant As species in all analysed plant biomass, reaching up to 13.3 mg kg^{-1} and 6.0 mg kg^{-1}, respectively, in *E. arvense* L. The same trend was obtained also in the study by Larios et al. (2012) which was focusing on As anionic species in different plants growing in northern Spain. Similarly, water mint plants (*Mentha aquatica* L.) growing in the vicinity of Kutná Hora showed a domination of As(V) in stems and leaves followed by As(III) which was found in aboveground biomass and roots as well (Száková et al., 2009a). At the Kutná Hora location, our previous results (Száková et al., 2009b) confirmed dominant position of As(V) in the aerated soil with presence of low amount of As (III) and minor contents of the organic As compounds such as DMA detectable only at the high total As contents in the soils. It can be also suggested that some ruderal plant species e.g., *Thymus serpyllum* L., *P. lanceolata* L., *Veronica chamaedrys* L. are capable to take up higher amounts of As under same conditions than some others (see Supplementary material). Fig. 3 of As species the most common occurring species while organic are minor and depending on plant species *i.e.*, plant metabolic system. The presence of small portions of organic As compounds indicate the ability of some terrestrial plants to methylate As as a possible way of detoxification.

Lomax et al. (2012) suggested that the plants were not able to synthesize the methylated As compounds such as DMA, but could only take up those produced by soil microorganisms. However, the spectrum of organic As compounds determined in some plant species indicate that the plant response on the As compounds present in the soil seems to be more complex. The results suggest that the transformation of organoarsenicals occur either in the plant rhizosphere or directly within plant cells and these processes need to be elucidated in the further research.transformation to the anionic As species seems to be easier performed by terrestrial plants nevertheless *P. lanceolata* L. and *C. praecox* Schreb. also showed in our case significant amounts of AB up to 0.12 mg As kg^{-1} and 0.02 mg As kg^{-1}, respectively. Although this ability is a domain of marine organisms (Kuehnelt and Goessler, 2003; Rahman et al., 2012) Šlejkovec et al. (1997) found a common occurence of AB in some representatives of *Fungi* kingdom; Jedynak et al. (2009) determined a small percentage of organic As compounds in stems and leaves of pepper plants (*Capsicum annum* L.). In our study the highest amounts of AB were found in *C. praecox* Schreb. (28% of the extractable amount) although the average AB concentrations in plant biomass were much lower, e.g., *P. lanceolata* L. (1.2% of the extractable As amount). In most of the other plants arsenobetaine was below the detection limit of 0.022 ng g^{-1} and the Figs. 1 and 2 document clearly the unambiguous resolution of the individual As compounds in this case.

4. Conclusions

Ruderal plant communities growing on 3 different As highly contaminated soils seems to be well adapted to this fact even though none of them belongs to neither metallophytes nor hyperaccumulators. Studied plant communities prosper well on these contaminated soils even though elevated levels of As occurred in some plants. It may refer to the ability of dealing with high arsenic concentrations occurring in the soil solution *via* As accumulation and/or As transformation. On the other hand non-elevated levels in some other plant species shows the ability of

avoiding the As uptake to the aboveground biomass which means that there probably is a soil-roots mechanism that protects them against such a high As contamination from environment.

The collected data showed the relationship between the total As contents in plants and different physico-chemical soil properties. They also showed that plant specie can differ in the total As contents in plant aboveground biomass e.g., different levels of As contents in the same plant specie growing on different soils. This can be due to a synergic effect of both plant specie and physico-chemical soil properties.

Speciation analysis confirm an occurrence of all investigated arsenic species even though AC, TETRA and TMAO were presented only in some samples and in trace amounts. As species most often occurring in plant aboveground biomass were As(III) and As(V); other compounds (DMA, MA, AB) occured in plant samples more rarely. AB commonly occurring in marine organisms but rarely in terrestrial plants was found to be produced in significant amounts by 2 plant species *C. praecox* Schreb. and *P. lanceolata* L.

Potential uptake of arsenic into a food chain *via* herbivores and subsequently to human might not be that great but surely should not be underestimated especially in context with crop, feedstuff and meat production in this area.

Acknowledgements

This work was supported by the grant project of Technological Agency of the Czech Republic No. TA01020366 and the Grant Agency of the Czech University of Life Sciences Prague No. 20131001.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.envexpbot.2015.11.012.

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5.2 Publikovaný výstup č. 2

Název článku: Distribution of arsenic compounds in *Plantaginaceae* and *Cyperaceae* plants growing in contaminated soil

Autoři: Tremlová, J., Száková, J., Golka, V., Babková, R., Najmanová, J., Tlustoš, P.

Publikováno v: Chemistry and Ecology (přijato do tisku), online od 2.8.2016, DOI 10.1080/02757540.2016.1216105

CHEMISTRY AND ECOLOGY, 2016
http://dx.doi.org/10.1080/02757540.2016.1216105

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Distribution of arsenic compounds in *Plantaginaceae* and *Cyperaceae* plants growing in contaminated soil

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ABSTRACT

The ability of plant species to accumulate arsenic (As) species in the biomass from As-contaminated soils is variable. Among the plants widely grown at the As-contaminated locations, *Plantaginaceae* and *Cyperaceae* families belong to the frequent ones. In this study, the ability of *Plantago lanceolata* (*Plantaginaceae*) and three wetland plant species representing the family *Cyperaceae* (*Carex praecox*, *Carex vesicaria*, and *Scirpus sylvaticus*) naturally occurring in the soils with an elevated As in the Czech Republic were investigated. The plants were cultivated under controlled conditions in an As-contaminated soil reaching 735 mg kg^{-1} of the total As. The total As in plants reached up to 8.3 mg kg^{-1} in leaves, and up to 155 mg kg^{-1} in roots of *C. praecox*. Dominant As compounds were arsenite and arsenate with a small abundance of dimethylarsinic acid (DMA) in all the plant species. In *Cyperaceae*, small percentages of arsenobetaine (AB) and arsenocholine (AC) were detected, suggesting the ability of these plants to transform As into less toxic compounds. Moreover, the important role of As(V) sequestration on iron plaque on the root surface of *Cyperaceae* was confirmed. In this context, root washing with oxalic acid partially disrupted the iron plaque for the better release of arsenate.

ARTICLE HISTORY
Received 6 February 2016
Accepted 19 July 2016

KEYWORDS
Arsenic; speciation; contaminated soil; wildlife plants

1. Introduction

The soils surrounding the former silver and gold mining areas are frequently characterised by the elevated arsenic (As) contents in soil. In the Czech Republic, there are some locations where mining activities were already terminated and numerous ruderal plant communities can be found at these sites.[1] The mobility and bioavailability of As in soil depends on soil redox conditions, pH, sorption capacity, and biological activity. Chemical and biological transformations result in various As compounds in soil.[2] The metabolic activity of the specific microbial populations plays an important role in the speciation of As compounds in soil solutions. Soil bacteria are able to reduce arsenates to arsenites and, subsequently, can methylate arsenites to volatile methylarsanes; various fungi species can also produce volatile methylarsanes.[3,4] Zhang et al. [5] suggested that

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arbuscular mycorrhizal fungi were involved in the methylation of inorganic As into less toxic organic dimethylarsinic acid (DMA) and in the reduction of arsenate to arsenite. However, in aerobic soil conditions, the fast microbial conversion of arsenite to arsenate was observed, whereas the reduction of arsenates was not reported.[6] Arsenate was the main species in rhizosphere and root exudates of *Lactuca sativa*.[7]

As is one of the most intensively investigated elements because of its toxicity to humans and animals. However, at elevated levels, this element can also be toxic to most of the higher plants.[8] As accumulation in plants depends on plant species and soil conditions, and the uptake by terrestrial plants is usually low.[9] Zandsalimi et al. [10] monitored the As concentration in wildlife plants growing in soil with total As ranging from 105.4 to 1500 mg kg^{-1}. In general, the As concentration in plants was low, especially in the most common wild species. The highest mean As concentration was found in the leaves of *Mentha longifolia*, reaching up to 79.4 mg kg^{-1}. In other plant species, the As concentrations in leaves at the same location varied between 2.3 mg kg^{-1} (*Achilleum millefolium*) and 29.6 mg kg^{-1} (*Medicago sativa*, *Astragalus* spp.). The effect of As mobility in soil on the As uptake of plants was demonstrated by Bergqvist et al. [7] where the As accumulation was higher in plants cultivated in soil with higher As mobility. To prevent the potentially toxic effects of trace metals in plant cells, the element inactivation mechanisms such as phytochelatin synthesis were involved in the plant biochemical processes.[11] Arsenites showed high affinity to the sulphhydryl groups occurring in the glutathione and phytochelatin complexes.[12–14] However, no evidence of the presence of arsenite–thiol complexes were found by Ye et al. [15] in the phloem and xylem extracts of *Ricinus communis*, suggesting that more investigations are necessary in this field.

Biomethylation also affects the mobility and toxicity of As in plants.[16] However, regardless of the many publications in this field, the mechanisms of the methylation processes are relatively poorly understood. Among the terrestrial plant species, rice (*Oryza sativa*) is one of the most intensively investigated crops: DMA and monomethylarsonic acid (MA) have been found in shoots and grain of rice.[16] Syu et al. [17] reported that the predominant As species found in *O. sativa* grains were DMA and arsenite, where the contents of DMA increased with total As grain concentrations and the arsenite remained almost unchanged. Garcia-Salgado and Quijano [18] reported a significant presence of arsenate followed by arsenite in both roots and aboveground biomass with a minor presence of MA and trimethylarsine oxide (TMAO) in the set of wildlife plants (collected in the vicinity of Mónica mine, Madrid, Spain); some plants growing in the soil contained up to 3500 mg kg^{-1} of As. On the contrary, Huang et al. [19] showed that arsenite was the predominant As form, followed by arsenate in turnip (*Brassica rapa*) and lettuce (*Lactuca sativa*) plants; a low content of DMA was detected only in lettuce roots if the plants were treated with chicken manure amended by the metabolites of roxarsone, that is (4-hydroxy-3-nitrophenyl) As acid. Predominant contents of the inorganic As species were determined in a set of medicinal plant species.[20] Similarly, Wu et al. [21] identified arsenite as the major As species detected in roots and shoots of rice (*O. sativa*), ranging from 34% to 78% of the total As. The effect of plant ecotype on the As uptake and speciation was demonstrated by Wan et al.,[22] where *Pteris vittata* plants, on a temporarily waterlogged riverside, preferred taking up As in the form of As

(III), whereas a population of *P. vittata* collected from dry land demonstrated a preference for As(V).

Wei et al. [23] determined the As compounds in the extracts of plants, including *Imperata cylindrica*, *Rumex patientia*, *Dryopteris erythrosora*, *Oplismenus undulatifolius*, *Erigeron annuus*, *Boehmeria nivea*, *Fagopyrum dibotrys*, and *P. vittata*, growing in soil contaminated by mining activity. As(V) was the dominant species in the roots, whereas in the stems and leaves, As(III) was prevalent and present in much higher proportions than As(V). DMA was detected in significant proportions in the roots, stems, and leaves of all plants, whereas MA was rarely detected, and was only found in small proportions. Conversely, Larios et al. [24] found that As(V) was always the predominant species in the presence of As(III), MA, and DMA. In addition, Márquez-García et al. [25] reported a predominant proportion of inorganic As with a small abundance of DMA in aboveground biomass of *Erica andevalensis* and *Erica australis*. However, *E. australis* accumulated mainly arsenite, while *E. andevalensis* accumulated arsenate, indicating different tolerance mechanisms of both species. Bergquist and Greger [9] investigated the As accumulation in 124 plant species collected from different habitats where water-submerged plants showed a higher As accumulation than emergent and terrestrial plants. They found inorganic As species, arsenate, and arsenite in plants from all habitats and occasionally found MA. Their results showed the strong influence of the habitat and the soil As content on the As accumulation in plants.

One of the important aspects affecting the bioavailability and speciation of As in plants represents the iron plaque covering the root surface, especially in wetland environments. As summarised by Tripathi et al.,[26] iron plaque formation is frequent in aquatic and wetland plant species and is responsible for the sequestration of various metals(loids) including As. The presence of iron plaque may act as a buffer or barrier and may, thus, enhance or reduce the uptake of potentially phytotoxic metals and metalloids by plants. For example, As was sequestered by iron plaque on rice (*O. sativa*) roots, resulting in decreased As accumulation in both roots and shoots.[21] Syu et al. [27] observed that 76–93% of As uptake from soils could be sequestered in iron plaque. Blute et al. [28] demonstrated that the plaque coating *Typha latifolia* roots was predominantly ferric iron and contained approximately 20% As(III) and 80% As(V). Both iron plaque formation and As deposition are strongly affected by spatial and temporal redox variations in the soil matrix,[29] as well as by the root anatomy, where the specific surface area of the roots [30] and the root porosity [31] (Yang et al.) will play important roles. The effect of the soil microbial community structure was also observed.[32]

Plantago lanceolata was mentioned as an example of plants able to survive (although some phytotoxicity symptoms occurred) in soil containing up to 21,000 mg kg^{-1} of the total As.[33] Geiszinger et al. [34] reported a wide spectrum of As compounds, including arsenite, arsenate, MA, DMA, arsenobetaine (AB), arsenocholine (AC), tetramethylarsonium ions (TETRA) and, TMAO, in terrestrial plants growing in the As-contaminated soil on the top of an ore vein. *P. lanceolata* was mentioned as one of the species containing detectable percentages of AC and AB. Therefore, *P. lanceolata* was chosen as a model plant organism for an evaluation of the changes in As speciation in our experiment. Our previous results [1] showed a relatively high percentage of AB in the *C. praecox* aboveground biomass. Thus, this plant species was chosen for the more detailed investigation within

this study. For a comparison, other plant species representing the *Cyperaceae* family were collected and investigated in this case.

The main objectives of the study were (i) to compare the uptake and translocation of As compounds into different parts of plant species during the vegetation period; (ii) to assess the effect of the individual plant species origin regarding their ability to accumulate and transform the As compounds; and (iii) to compare the role of iron plaque on the root surface of different wetland plant species with the accumulation and distribution of As compounds within the plants. Hypothesis, the uptake, translocation, and transformation of the As species in plants, is a result of the effects of plant species, soil As contents and physicochemical characteristics, and the origin of the plants. As apparent from the abovementioned previously published results, most of the experiments were based on the monitoring of the wild-growing plants. Therefore, a pot experiment was carried out in controlled conditions to describe the inter-species variation in As accumulation ability of these species and to contribute to the knowledge of the transformation of As compounds in plants.

2. Materials and methods

2.1. Experimental design of soil and plant sampling

The plant species were collected at three locations in the Czech Republic connected to gold and silver ore deposits. The locations were characterised by an elevated concentration of As in the soil. The locations are in the central part of Bohemia and have been described and characterised in detail in previous papers. Thus, the locations are mentioned here briefly with the corresponding references:

(1) The Roudný gold deposit has already been described from a geochemical and mineralogical point of view by Filippi et al.[35] Subsequently, the As concentrations in soil and plants at this location were published by Tremlová et al.[1] Accordingly, *P. lanceolata* plants were collected from the mine tailing called 'Danica', and *S. sylvaticus* plants from the wetland next to the 'Barbora adit'.
(2) In the mediaeval silver mining area surrounding Kutná Hora, Central Bohemia, the main source of soil contamination by risk elements (especially As, Cd, Pb, and Zn) is the weathering of ore residues, mine tailings, and old waste dumps.[36,37] At this location, two sampling points characterised by different soil characteristics and total As contents were chosen. The *C. vesicaria* plants were sampled from the suburb of the Malín municipality close to a mediaeval silver mine (so-called gallery of 14 assistants) at an unused site, close to a small streamlet, called Beránka.[38] *P. lanceolata* and *C. praecox* plants were collected at the wet meadow close to the Kaňk municipality.[1]
(3) In the Mokrsko district, the gold deposits have never been mined, but an elevated concentration of As in soil around the municipality has been reported.[39] Detailed descriptions of the geochemistry, mineralogy, As content, and mobility within soil profiles have been given by Drahota et al.[40] These authors found that As in soils is bonded mainly to secondary arseniosiderite, pharmacosiderite, and Fe oxyhydroxides and rarely to scorodite. At this location, *C. vesicaria* plants were sampled in the wetland.

The soil samples collected to be analysed were collected from the individual locations in triplicate (about 300 g each) allways in the location where the experimental plants were found, air dried at 20°C, ground in a mortar, passed through a 2-mm plastic sieve, and kept at the laboratory temperature until analysis. The main soil characteristics and risk element concentrations in the soils were determined (Table 1). The plants were sampled during the summer; whole plants, including roots, were collected. The plants were then potted in uncontaminated soil (the remaining soil from the roots was previously removed by thorough washing in deionised water) in a greenhouse and were given the winter to adapt. The next spring, the plants were re-potted in 6 L plastic pots with 5 kg of air-dried soil originating from the Mokrsko location; six replicates were prepared for each treatment where 10 plants were cultivated in each pot. NPK (0.5 g N, 0.16 g P, 0.4 g K per pot as aqueous NH_4NO_3 and K_2HPO_4 solutions) was added before planting. The soil from Mokrsko was chosen as the experimental soil with an elevated As content because of the low content of risk elements (except As) (Table 1). Soil moisture was regularly controlled and kept at 60% of the maximum water-holding capacity (MWHC) using deionised water for *P. lanceolata*, and at 80% of the MWHC for *Cyperaceae*. Pots were placed in the outdoor precipitation-controlled vegetation hall, and weed plants were regularly removed; other cultivation conditions, such as light and temperature, were not managed.

In the case of *P. lanceolata*, three samplings were provided during the vegetation to encourage the changes in As uptake by plants within the season, where leaves, stems, and inflorescences were separated. For *Cyperaceae*, leaves were sampled four times because of longer staying of the vegetation after harvest of the matured generative organs, whereas stems and inflorescens were sampled three times. The harvested aboveground biomass of plants from the pots was gently washed with deionised water, freeze-dried, and finely ground. At the end of the experiment, roots of *Cyperaceae* plants were sampled and separated into two parts. One part was washed with deionised water and the second was washed with a 0.1 mol L^{-1} water solution of oxalic acid where sonication for 10 min was used to speed up the cleaning procedure. The roots and the samples of the aboveground biomass were then freeze-dried and ground.

Table 1. The main characteristics of the experimental soils; the averages marked by the same letter did not significantly differ at $p < .05$ within individual rows; data are presented as mean ± standard deviation, $n = 3$.

Parameters	Roudný	Kaňk	Mokrsko	Malín
pH	4.63 ± 0.08^{a}	5.10 ± 0.56^{a}	5.36 ± 0.04^{a}	6.97 ± 0.03^{b}
C_{ox} (%)	1.08 ± 0.19^{a}	3.41 ± 0.39^{b}	0.78 ± 0.02^{a}	0.95 ± 0.00^{a}
CEC (mmol kg^{-1})	58.8 ± 3.3^{a}	135 ± 15^{b}	116 ± 11^{b}	148 ± 8^{b}
As (mg kg^{-1})	675 ± 62^{b}	443 ± 3^{a}	735 ± 96^{b}	$1{,}573 \pm 46^{c}$
Cd (mg kg^{-1})	1.19 ± 0.10^{b}	1.28 ± 0.08^{b}	0.145 ± 0.054^{a}	3.63 ± 0.64^{c}
Pb (mg kg^{-1})	23.1 ± 2.1^{a}	106 ± 2^{b}	21.4 ± 2.1^{a}	14.9 ± 5.5^{a}
Zn (mg kg^{-1})	57.3 ± 0.9^{a}	153 ± 2^{b}	34.0 ± 3.9^{a}	342 ± 19^{c}
Ca^{d} (mg kg^{-1})	250 ± 45^{a}	2210 ± 1^{b}	1715 ± 24^{b}	14060 ± 659^{c}
K^{d} (mg kg^{-1})	64.4 ± 11.9^{a}	179 ± 0^{c}	90.7 ± 16.9^{ab}	103 ± 7^{b}
Mg^{d} (mg kg^{-1})	247 ± 6^{a}	184 ± 0^{a}	196 ± 12^{a}	201 ± 6^{a}
P^{d} (mg kg^{-1})	4.4 ± 1.1^{b}	11.9 ± 0.2^{c}	33.8 ± 1.1^{d}	1.10 ± 0.12^{a}

Note: C_{ox} – oxidisable carbon, CEC – cation exchange capacity.
[d]Mobile contents of elements determined by Mehlich III extraction procedure [42].

2.2. Analytical methods

2.2.1. Concentrations of trace metals in soils and plants

The freeze-dried and homogenised plant samples were decomposed in a microwave-assisted wet digestion system with focused microwave heating (Discover SPD-Plus, CEM Inc., USA). An aliquot (~0.1 g of dry matter) of the plant sample was weighed in a quartz-glass digestion vessel (35 mL), and 5.0 mL of concentrated nitric acid (Analytika Ltd., Czech Republic) was added; the mixture was heated to a maximum temperature of 202°C, and pressure 17 bar for 8 min. After cooling, the solution was quantitatively transferred to plastic tubes, filled to 50 mL with deionised water and kept at laboratory temperature until measurements were taken.[41] Blank samples represented 10% of the total number of the samples. As concentration in the digests was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA). The auto-sampler ASX-500, a three-channel peristaltic pump, and a MicroMist nebuliser equipped the ICP-MS. The pseudo total concentrations of elements in the soil were determined in the digests obtained by the following decomposition procedure: Aliquots (~0.5 g) of air-dried soil samples were decomposed in digestion vessels with 10 mL of Aqua Regia (i.e. nitric and hydrochloric acid mixture in a ratio of 1:3). The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 33 min at 210°C. After cooling, the digest was quantitatively transferred into a 25 mL glass tube, topped with deionised water, and kept at laboratory temperature until measurements were taken. Similarly as for the plant samples, blank samples represented 10% of the total number of the samples. Inductively coupled plasma-optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) was used for the determination of As, Cd, Pb, Zn in soil extracts. Mehlich III extraction procedure (0.2 mol L^{-1} of CH_3COOH + 0.25 mol L^{-1} of NH_4NO_3 + 0.013 mol L^{-1} of HNO_3 + 0.015 mol L^{-1} of NH_4F + 0.001 mol L^{-1} of ethylenediaminetetraacetic acid (EDTA) [42] was applied for the determination of available Ca, K, Mg, and P in the soils where ICP-OES was applied for the determination of the elements in the extracts. For quality assurance of the analytical data, certified reference materials SRM NIST 1547 Peach leaves (NIST, USA), and RM 7001 Light Sandy Soil (Analytika Ltd., Czech Republic) were applied.

2.2.2. Determination of As compounds

For the determination of As species in aboveground biomass of the plants, the following procedure was applied: aliquots of 0.1 g of dried and powdered samples were weighed into 15 mL screw-capped polyethylene tubes ($n = 2$), extracted with 0.02 mol L^{-1} NH_4H_2PO4 in the ratio 1 + 25 (w/v) (pH 6.0) and fastened to a cross-shaped rotor and turned top over bottom at 45 rpm for 14 h (Biosan MultiRS 60, Latvia). The mixtures were then centrifuged for 10 min at 3000 rpm (Boeco C28A, Germany), and filtered through 0.22 µm cellulose-nitrate ester filters (MillexGS, Millipore, Bedford, MA, USA).

Anionic As compounds were separated with high performance liquid chromatography (HPLC 1260 Infinity, Agilent Technologies, USA) using an anion-exchange column PRP-X100 150 mm × 4.6 mm with 5 µm particles (Hamilton, USA), ambient column temperature, and an injection volume of 10 µL. Good separation was achieved using an isocratic elution with 0.02 mol L^{-1} $NH_4H_2PO_4$ (pH 6.0) as a mobile phase at a flow of 1.5 mL min^{-1}. Cationic As compounds were determined with the same instruments

using Zorbax 300-SCX 250 mm × 4.6 mm with a 5 µm particle column (Agilent Technologies Inc., USA), ambient column temperature, injection volume of 20 µL, and a 0.01 mol L^{-1} pyridine buffer (pH 2.3) served as a mobile phase with a flow of 1.5 mL min^{-1}. As an online detector, an ICP-MS (7700x, Agilent Technologies, USA) was used, whereas PEEK capillary tubing (0.125 mm i.d.) was used to connect the HPLC outlet with a MicroMist nebuliser of the ICP-MS.

2.3. Data processing

Statistical software STATISTICA™ version 12 (StatSoft®, Inc., USA), Microsoft Excel 2010™ software (Microsoft Corp.®, USA), and SigmaPlotTM 11.0 (SyStat Software Inc., USA) were used to carry out the statistical analysis and outputs. Statistical tests (one-way ANOVA) were carried out at a significance level of $\alpha = 0.05$ for the total contents of analytes in the soil and plant samples. Scheffe's post-hoc test for carrying out differencies in pairs of means was performed.

The so-called transfer factor quantifying the element transfer from soil to plants is frequently used as a parameter for evaluation of the uptake of soil elements by plants. In this experiment the ratio of the element content in plants to the pseudo total element content in soil was applied.[43]

3. Results and discussion

3.1. As concentrations in soils and plants

Table 1 documents the differences in soil characteristics and element contents where the soil pH at Malín location (most probably due to the highest content of soil carbon) was significantly higher compared to the remaining locations. The Kaňk soil is characterised by the significantly highest content of oxidisable carbon. The lowest sorption capacity compared to the other soil samples was reported for Roudný. Whereas the Mokrsko soil is characterised by the elevated As content only, the remaining soils contain elevated contents of at least one of the other risk elements (Cd, Pb, and Zn). According to the public notice of the Czech Ministry of the Environment,[44] the maximum acceptable contents of the risk elements in the agricultural soils are 30 mg kg^{-1} of As, 1.0 mg kg^{-1} of Cd, 140 mg kg^{-1} of Pb, and 200 mg kg^{-1} of Zn. Thus, the reason why the Mokrsko soil was chosen for the pot experiment was to keep the plants unaffected by potential interactions with risk elements other than As.

The total As concentration in *P. lanceolata* plants is summarised in Table 2. Although the As levels in the plants are relatively low regarding the soil contamination level (Table 1), the levels exceeded the maximum permissible limits of As (2 mg kg^{-1}) in forage (Directive No. 2002/32/ES,[45]), indicating the potential risk for herbivores in the contaminated areas. The total As concentration in the plants followed the order: leaves > inflorescens > stems. Higher As concentrations in leaves than in stems in the soil from Malín and Mokrsko were observed by Száková et al. [46] in the case of *Mentha aquatica*. On the contrary, Geiszinger et al. [34] reported higher concentrations of As in inflorescens (5.93 mg kg^{-1}) than in leaves (4.30 mg kg^{-1}) of *P. lanceolata* growing in soil on the top of the ore vein in Gasen, Austria. Comparing the plant origin, the plants from the more contaminated

Table 2. Total As concentrations (mg kg^{-1}) in the individual parts of *P. lanceolata* plants according to the individual samplings during the vegetation period and sample origin; data are presented as mean ± standard deviation, $n = 6$; the differences between the plants of different origin were not significant at $p < .05$.

	Sampling No.		
Sample origin	1	2	3
Leaves			
Roudný	2.68 ± 0.84	1.99 ± 0.78	5.13 ± 1.39
Kaňk	2.92 ± 0.81	1.15 ± 0.50	2.92 ± 0.24
Stems			
Roudný	0.49 ± 0.08	0.73 ± 0.32	0.65 ± 0.17
Kaňk	0.26 ± 0.05	0.54 ± 0.13	0.29 ± 0.12
Inflorescens			
Roudný	1.99 ± 0.41	1.32 ± 0.57	0.95 ± 0.29
Kaňk	1.38 ± 0.31	1.09 ± 0.31	0.53 ± 0.34

area (Roudný) showed a higher As concentration, although the differences were not significant at $p < .05$. Therefore, the plants originating from the more contaminated area indicated better accumulation ability, although the plants were kept in uncontaminated soil for several months before the start of the experiment. These speculations should be verified in a long-term experiment covering more than one vegetation period. Potential visible symptoms of phytotoxicity were not observed in the case of *P. lanceolata* plants. Among the factors affecting the relatively high tolerance of *P. lanceolata* to the high As level in soil, as well as the low uptake of As by this plant species, the potential role of arbuscular mycorrhizal fungi was discussed by Orlowska et al.,[33] in which inoculated plants were more able to survive in the extremely contaminated substrate (60–90%) compared to the non-inoculated ones (30%).

The total As concentration in *Cyperaceae* aboveground biomass and roots is summarised in Tables 3 and 4, where the As concentration followed the order: roots > leaves > stems + inflorescens. Compared to *P. lanceolata,* the As uptake, especially by *S. sylvaticus* and *C. praecox*, was higher, whereas, for *C. vesicaria*, the As levels were comparable. However, phytotoxicity symptoms manifested as the limited development of the

Table 3. Total As concentrations (mg kg^{-1}) in the aboveground biomass of *Cyperaceae* plants according to the individual samplings during the vegetation period and sample origin; data are presented as mean ± standard deviation, $n = 6$; the averages marked by the same letter did not significantly differ at $p < .05$ among the plant species.

		Sampling No.			
Plant species	Sample origin	1	2	3	4
Leaves					
S. sylvaticus	Roudný	4.96 ± 0.64^{b}	5.90 ± 1.69^{b}	4.87 ± 1.34^{a}	7.47 ± 1.52^{b}
C. praecox	Kaňk	2.98 ± 0.93^{b}	4.49 ± 0.10^{b}	6.88 ± 0.86^{a}	8.25 ± 0.82^{b}
C. vesicaria	Mokrsko	1.34 ± 0.44^{a}	3.2 ± 0.22^{a}	5.43 ± 1.04^{a}	5.63 ± 0.27^{ab}
C. vesicaria	Malín	0.94 ± 0.07^{a}	2.99 ± 0.93^{a}	4.64 ± 1.51^{a}	3.43 ± 0.15^{a}
Stems + Inflorescens					
S. sylvaticus	Roudný	0.43 ± 0.05^{a}	1.5 ± 0.17^{b}	c	d
C. praecox	Kaňk	1.3 ± 0.05^{a}	2.59 ± 0.07^{b}	4.43 ± 0.37	d
C. vesicaria	Mokrsko	3.18 ± 0.12^{b}	0.57 ± 0.06^{a}	c	d
C. vesicaria	Malín	d	d	d	d

[c]Data under detection limit.
[d]Inflorescens not developed.

Table 4. Total As concentrations and As compounds in roots of *Cyperaceae* plants according to the root cleaning procedure and sample origin; data are presented as mean ± standard deviation, $n = 6$; the averages marked by the same letter did not significantly differ at $p < .05$ among the plant species.

Plant species	Sample origin	Total (mg kg^{-1})	As(III) (mg kg^{-1})	As(V) (mg kg^{-1})	DMA (mg kg^{-1})
Washed in deionised water					
S. sylvaticus	Roudný	107 ± 3^{a}	15.9 ± 5.9^{b}	1.47 ± 0.62^{b}	0.484 ± 0.143^{b}
C. praecox	Kaňk	155 ± 11^{b}	7.24 ± 0.53^{b}	3.30 ± 0.94^{b}	0.100 ± 0.029^{a}
C. vesicaria	Mokrsko	147 ± 12^{b}	13.4 ± 7.2^{b}	1.94 ± 0.45^{b}	0.356 ± 0.079^{b}
C. vesicaria	Malín	136 ± 6^{b}	3.91 ± 0.79^{a}	0.780 ± 0.259^{a}	0.249 ± 0.027^{b}
Washed in 0.1 mol L^{-1} oxalic acid					
S. sylvaticus	Roudný	113 ± 14^{a}	2.78 ± 0.37^{b}	4.84 ± 1.80^{a}	0.107 ± 0.018^{a}
C. praecox	Kaňk	145 ± 13^{b}	2.08 ± 0.21^{b}	4.32 ± 0.30^{a}	0.052 ± 0.001^{a}
C. vesicaria	Mokrsko	139 ± 8^{b}	2.22 ± 0.52^{b}	6.44 ± 0.54^{a}	0.071 ± 0.015^{a}
C. vesicaria	Malín	129 ± 10^{b}	0.696 ± 0.268^{a}	3.55 ± 1.25^{a}	0.018 ± 0.018^{a}

inflorescens was observed in the case of *C. vesicaria*, especially in the plants originating from Malín. In ordinary plant species, phytotoxicity symptoms can occur at As levels from 3 to 10 mg kg^{-1}.[47] Tlustoš et al. [48] observed a limited yield of radish plants (both leaves and roots) at As level ≈ 5 mg kg^{-1}. The transfer factors, TFs, quantifying the element transfer from soil to plants showed negligible translocation of As from soil to the aerial parts of plants. The TF values varied between 0.001 and 0.004 for stems + inflorescens, and from 0.004 to 0.008 for leaves. The TFs for roots varied between 0.15 and 0.21, documenting that (i) As is predominantly accumulated in roots and is not translocated to the aboveground biomass, and (ii) the relatively low root TFs confirm low plant-availability of As for *Cyperaceae* plants. In all the cases, the maximum TF values were observed for *C. praecox*.

Root washing (using a 0.1 mol L^{-1} solution of oxalic acid) was used to release the As sequestered to the iron plaque at the root surface. However, only a minor portion of As was released by this procedure, and the differences between the roots washed in deionised water and in a 0.1 mol L^{-1} solution of oxalic acid did not significantly differ at $\alpha = 0.05$. Possible extraction agents for the dissolution of iron plaque from plant roots were tested by Rahman et al.,[49] where a sodium citrate + sodium bicarbonate + ethylenediaminetetraacetate mixture was compared with a dithionite–citrate–bicarbonate extraction: the former was able to dissolve 94% of Fe. In our case, a mild procedure was chosen to avoid changes in As compounds released from the root's surface. However, this procedure seems to be insufficient for dissolution of the iron plaque.

Martínez-Sánchez et al. [50] collected plant samples in As-contaminated soils containing between 40 and 3115 mg kg^{-1} of As. The samples included five species: *Limonium carthaginens*, *Arthrocnemum macrostachyum*, *Dittrichia viscosa*, *Glaucium flavum*, and *Zygophyllum fabago*. The average concentration of As in roots and leaves was 18.1 and 23.5 mg kg^{-1}, respectively. Baroni et al. [51] monitored As levels in soil and in 64 plant species in the area contaminated by former mining activity (the total As concentration in soil varied in range from 5.3 up to 1226 mg kg^{-1}) and observed low As concentrations in plants regardless of the contamination level at the particular site. Higher As concentrations in aboveground biomass (216 mg kg^{-1}) and in roots (540 mg kg^{-1}) were determined exclusively in the sample of *Mentha aquatica*. Hong et al. [52] investigated the effects of As species, such as As(III), As(V), and DMA, on the accumulation of As in

cucumbers (*Cucumis sativus*), where the plants accumulated As more in the roots than the shoots, and easily accumulated inorganic rather than organic As from the soil into its tissue.

In our case, the available As compounds in soil were not determined; however, in aerated soil, As is present mostly as arsenate, and small percentages of methylated As compounds were also reported [53]: a similar situation was found in our soil. Márquez-García et al. [25] determined a total As concentration varying between 1 and 24.4 mg kg^{-1} in As-tolerant plants (*E. andevalensis* and *E. australis)* growing in soil containing from 194 to 7924 mg kg^{-1} of the total As. Relatively high total As concentrations were determined by Larios et al. [24] in the set of plants growing in extremely contaminated soil (up to 25,900 mg kg^{-1} of the total As). The maximum As concentrations were determined in *Calluna vulgaris*, which had 359 mg kg^{-1} of As in its leaves, 150 mg kg^{-1} in its stalks, and 280 and 1217 mg kg^{-1} in its woody and radicle roots, respectively. Further, *Crupina vulgaris* had 167 and 791 mg kg^{-1} of As in its aerial part and roots, respectively, and *Dryopteris filix-mas* had 177 and 377 mg kg^{-1} of As in its aerial part and roots, respectively. Similarly, Vaculík et al. [54] investigated the As uptake by selected medicinal plants growing in the contaminated soil (between 146 and 540 mg kg^{-1}). They documented high variability of the results among the species and/or sampling sites, where the maximum As concentration (519 mg kg^{-1}) was found in shoots of *Fragaria vesca*. Mir et al. [55] determined the As concentrations in plants growing in the extremely contaminated soil (ranging from 335 to 100,000 mg kg^{-1}). Plant samples had concentrations ranging from 2.3 to 241 µg g^{-1} of As confirming the high differences among the plant species and the highest As concentration was determined in *Equisetum arvensis*. These levels were not reached in our experiment, but differences in As uptake and translocation, as well as different tolerances to high As contents in soil, even within one family, were confirmed.

3.2. As compounds in plants

In the *P. lanceolata* plants, only As(III), As(V), and DMA were identified and quantified (Figure 1). The distribution of As compounds differed according to plant origin, sampling time, and what part of the plant was analysed. The plants from Roudný were characterised by lower percentages of DMA in leaves and stems, but slightly higher percentages in the inflorescens compared to the plants from Kaňk. In stems, the predominant As compound was arsenite. In leaves, the distribution of the inorganic species changed with changing sampling times where, at the beginning of the vegetation period, both inorganic species were covered equally; at the end of the vegetation period, arsenite was the dominant species (up to 80%). In the inflorescens, the dominant species was arsenate and the differences among the individual samples were the lowest among the investigated plant parts. Schmidt et al. [56] investigated uptake and transformation of As species in *Silene vulgaris* and *Plantago major*. The results showed that *S. vulgaris* had a higher tolerance compared to *P. major*, and a dominant percentage of arsenite followed by arsenate with traces of DMA and other organic As compounds were found in *S. vulgaris*. These findings seem to be in agreement with our results, whereas *P. major* had equal percentages of arsenite and arsenate in both stems and leaves. In addition, Geiszinger et al. [34] and Mir et al. [55] documented a dominant role of inorganic As species in the aboveground biomass of terrestrial plants. Carbonell-Barrachina et al. [57] discussed possible tolerance mechanisms of

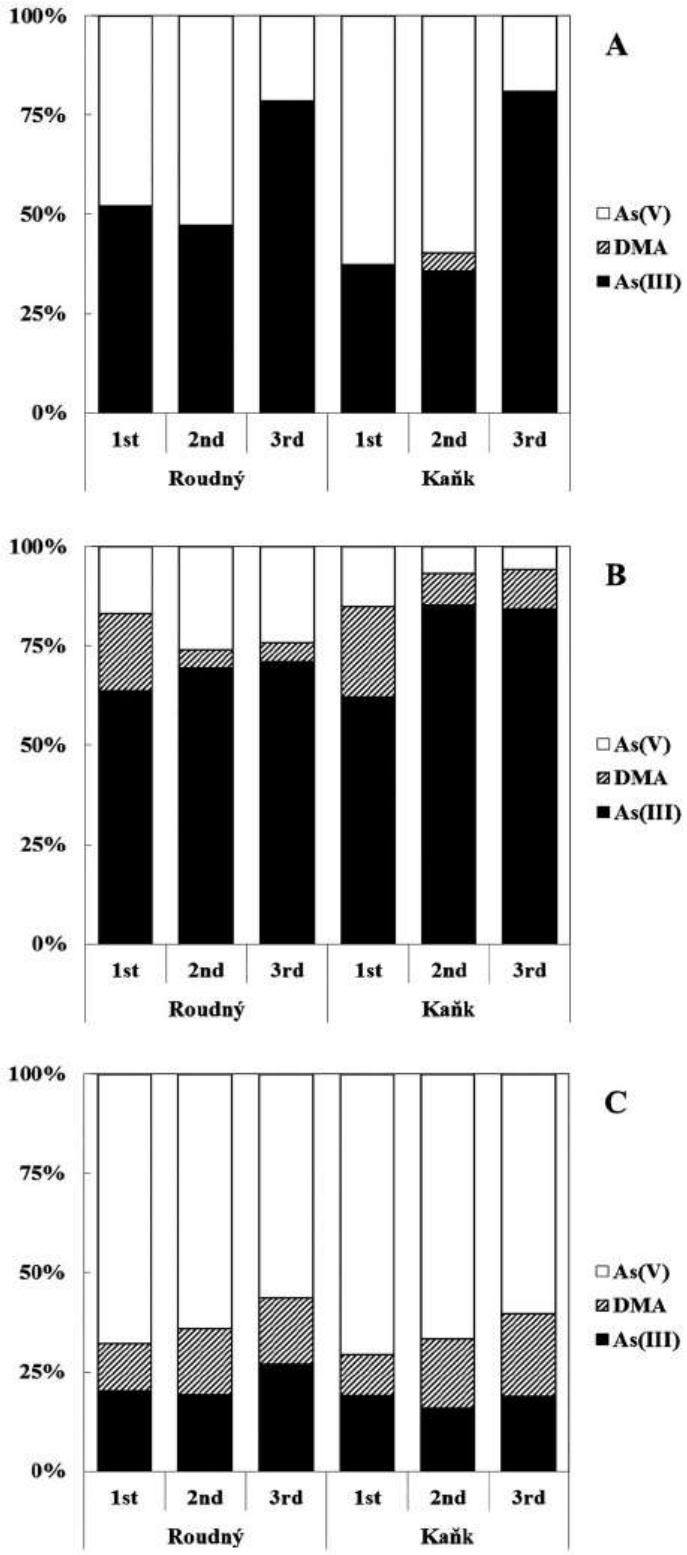

Figure 1. The distribution of As compounds within *P. lanceolata* plants according to the plant origin in Sampling No. 1, 2, 3; A – leaves, B – stems, C – inflorescens.

plants to the elevated As contents in soil, and highlighted three possible mechanisms: (i) limited uptake of As and/or its limited translocation to the aboveground biomass, (ii) detoxification via As binding in cell walls or compartments, and (iii) specific biochemical mechanisms of tolerance. Regarding the last point, the As–phytochelatin complexes are frequently discussed.[58,59] Our results suggested the limited uptake of As by *P. lanceolata* as a possible mechanism of tolerance. The analytical techniques used in our experiment do not allow us to determine the potential As–phytochelatin complexes in the plants.

In the *Cyperaceae* plants, As(III), As(V), DMA, AB, and AC were identified, whereas other As compounds investigated in the samples (MA, TETRA, and TMAO) were under detection limits of the analytical technique (Tables 4–6). In all cases, arsenite was the predominant As compound, followed by arsenate with an occasional abundance of methylated As compounds. Compared to the aboveground biomass, the roots showed poor extractability of As compounds most probably due to a strong binding of As to the poorly soluble iron plaque. In the roots washed with deionised water, the As compounds followed the order As(III) > As(V) > DMA (Figure 2), which was also observed by Kuehnelt et al. [60] in other terrestrial plant species and Száková et al. [61] in *Phaseolus vulgaris*. However, the root washing with 0.1 mol L^{-1} of oxalic acid significantly changed the distribution of the released As compounds (Figure 2), where arsenate was the predominant species and the percentage of DMA decreased compared to the water-washed samples. Our available analytical techniques do not allow us to determine the abundance of As compounds in roots undissolved by the applied extraction agents, and we can only speculate that the root washing with oxalic acid removed, predominantly, the soluble proportion of arsenite and, simultaneously, partially disrupted the iron plaque for the better release of arsenate. The decreasing proportion of DMA seems to support the theory that microbially induced

Table 5. As compounds in leaves of *Cyperaceae* plants according to the individual samplings during the vegetation period and sample origin; data are presented as mean ± standard deviation, $n = 6$; the differences among the plant species were not significant at $p < .05$.

Plant species	Sample origin	As(III) (mg kg^{-1})	As(V) (mg kg^{-1})	DMA (mg kg^{-1})	AC (mg kg^{-1})	AB (mg kg^{-1})
Sampling No. 1						
S. sylvaticus	Roudný	0.720 ± 0.158	0.014 ± 0.002	a	a	a
C. praecox	Kaňk	0.704 ± 0.094	0.018 ± 0.009	a	0.003 ± 0.001	0.001 ± 0.000
C. vesicaria	Mokrsko	0.605 ± 0.038	0.025 ± 0.014	a	0.002 ± 0.001	0.023 ± 0.001
C. vesicaria	Malín	0.536 ± 0.016	0.005 ± 0.001	a	a	a
Sampling No. 2						
S. sylvaticus	Roudný	5.12 ± 0.89	0.022 ± 0.004	a	a	a
C. praecox	Kaňk	2.53 ± 0.79	0.019 ± 0.004	a	0.012 ± 0.001	0.021 ± 0.002
C. vesicaria	Mokrsko	2.04 ± 0.32	0.006 ± 0.002	a	a	a
C. vesicaria	Malín	3.02 ± 0.53	0.007 ± 0.002	a	a	a
Sampling No. 3						
S. sylvaticus	Roudný	4.92 ± 0.72	0.019 ± 0.005	a	a	a
C. praecox	Kaňk	4.40 ± 0.42	0.071 ± 0.009	a	a	a
C. vesicaria	Mokrsko	4.08 ± 0.72	0.013 ± 0.003	a	a	a
C. vesicaria	Malín	3.54 ± 0.54	0.010 ± 0.001	a	a	a
Sampling No. 4						
S. sylvaticus	Roudný	5.56 ± 1.14	0.019 ± 0.007	0.009 ± 0.001	a	a
C. praecox	Kaňk	5.00 ± 1.08	0.090 ± 0.006	a	a	a
C. vesicaria	Mokrsko	5.16 ± 0.71	0.013 ± 0.003	a	a	a
C. vesicaria	Malín	5.80 ± 0.64	0.012 ± 0.002	a	a	a

[a]Data under detection limit.

Table 6. As compounds in stems + inflorescens of *Cyperaceae* plants according to the individual samplings during the vegetation period and sample origin; data are presented as mean ± standard deviation, $n = 3$; the averages marked by the same letter did not significantly differ at $p < .05$ among the plant species.

Plant species	Sample origin	As(III) (mg kg^{-1})	As(V) (mg kg^{-1})	DMA (mg kg^{-1})	AC (mg kg^{-1})	AB (mg kg^{-1})
Sampling No. 1						
S. sylvaticus	Roudný	1.21 ± 0.41[b]	0.057 ± 0.015[b]	0.089 ± 0.008	0.001 ± 0.000	0.008 ± 0.002
C. praecox	Kaňk	0.463 ± 0.059[a]	0.021 ± 0.002[a]	0.021 ± 0.004	0.001 ± 0.000	0.037 ± 0.001
C. vesicaria	Mokrsko	d	d	d	d	d
C. vesicaria	Malín	d	d	d	d	d
Sampling No. 2						
S. sylvaticus	Roudný	1.90 ± 0.48[b]	0.050 ± 0.004[b]	c	c	c
C. praecox	Kaňk	1.36 ± 0.19[b]	0.025 ± 0.005[b]	c	0.004 ± 0.001[a]	0.054 ± 0.001[a]
C. vesicaria	Mokrsko	0.285 ± 0.035[a]	0.008 ± 0.002[a]	c	0.004 ± 0.001[a]	0.003 ± 0.001[a]
C. vesicaria	Malín	d	d	d	d	d
Sampling No. 3						
S. sylvaticus	Roudný	d	d	d	d	d
C. praecox	Kaňk	2.97 ± 0.37	0.005 ± 0.001	c	0.007 ± 0.002	0.141 ± 0.019
C. vesicaria	Mokrsko	d	d	d	d	d
C. vesicaria	Malín	d	d	d	d	d

[c]Data under detection limit.
[d]Inflorescens not developed.

DMA is taken up by plants from the soil and cannot be synthetised.[62] Thus, the soil-bearing DMA can be sequestered in the iron plaque as well as the inorganic As species. A low percentage of DMA in aboveground biomass of *Cyperaceae* (Tables 5 and 6) compared to *P. lanceolata* (Figure 1) seems to support this theory.

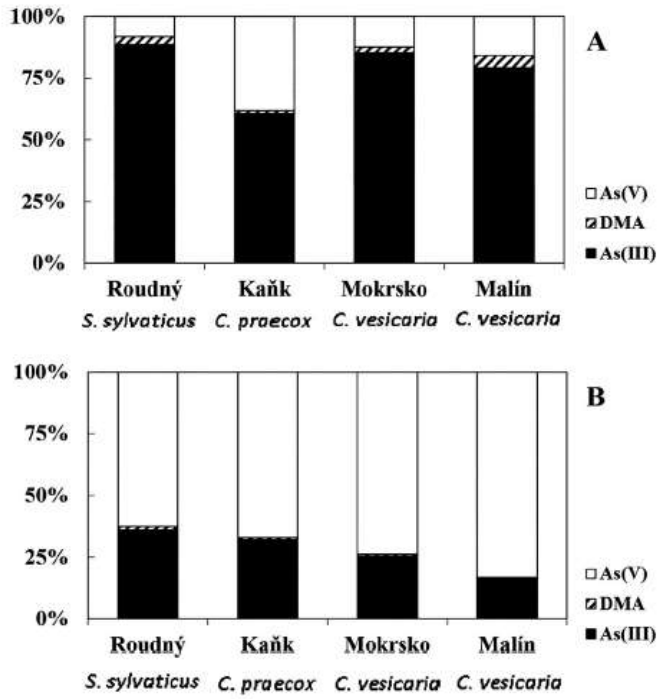

Figure 2. The distribution of As compounds within individual *Cyperaceae* plant roots according to the plant origin and sample cleaning procedure; A – samples washed by deionised water, B – samples washed by 0.1 mol L^{-1} oxalic acid.

In the plant leaves (Table 5), the As(III) concentrations increased with increasing plant age, whereas As(V) was relatively stable, and AC and AB were detected only at the beginning of the vegetation and only in the *C. praecox* and (to a lesser extent) in the *C. vesicaria* originating from Mokrsko. These plants showed lower phytotoxicity symptoms compared with the *C. vesicaria* plants originating from Malín, where no stems and inflorescens were developed. We can speculate the plant synthesis of AB and AC was a plant detoxification mechanism. In stems and inflorescens (Table 6), the distribution of As compounds was similar to the leaves with a higher abundance of organic As compounds; the individual As compounds followed the order: As(III) > As(V) ≈ DMA ≫ AB > AC. Therefore, the results indicated the ability of *Cyperaceae* (and especially *C. praecox*) to synthetise AB and AC, the compounds suggested as typical for marine organisms.[63]

Ruiz-Chancho et al. [64] determined the As compounds in various terrestrial plants, such as *Dryopteris filix-max*, *Quercus pubescens*, *Dipsacus fullonum*, *Alnus glutinosa*, *Buxus sempervirens*, and *Brachythecium* cf. *Reflexum,* where the As contents decreased in the order of roots > branches > leaves. Higher percentages of organo-As compounds were recorded in branches and leaves (up to 35%) than in roots (up to 5.2%). The low translocation of As from roots to the aboveground biomass was observed also in *O. sativa*.[65] Increasing the bioavailability of As in reducing conditions of *O. sativa* cultures resulted in increasing As(V) content compared to other As species.[66] However, the role of soil microorganisms in the As methylation was highlighted by Lomax et al.,[62] who compared As speciation in plants cultivated in a hydroponic culture amended with DMA and MA in either axenic or non-sterile conditions. They found that the axenically grown plants were able to take up MA or DMA from the cultivation medium, and reduce MA (V) and MA(III), but were not able to produce DMA. They determined the methylated As compounds in soil-grown plants and plants from non-sterile hydroponic cultures, indicating that the plants were not able to synthetise the methylated As compounds, but could only take up those produced by microorganisms. Moreover, the presence of the microbial As methyltransferase gene *arsM* was shown in the soil. In our case, a higher percentage of DMA in the stems than in the leaves of *P. lanceolata* (Figure 1) seems to support this theory. However, the presence of AB and AC in the plants remains unexplained. The potential role of plant or plant–rhizosphere interactions needs to be elucidated in the further research.

4. Conclusions

To summarise our results, similar As concentrations were determined in *P. lanceolata* and *Cyperaceae* plants, but the individual species differed in abundance and distribution of the As compounds. While *P. lanceolata* seemed to be more tolerant to the elevated As concentration in the soil (and inorganic As species, with a small percentage of DMA, were identified), *Cyperaceae* showed a lower tolerance to soil As (demonstrated as an absence of the generative organs). Where AB and AC were identified, especially in *C. praecox*, the species were less harmed by the high soil As concentration. Thus, the plants showed one possible way to eliminate the phytotoxic effects of the elevated As concentration. However, the mechanisms of the potential transformations/detoxifications of the As compounds remain for further research, which should include the role of rhizosphere processes.

Acknowledgements

Correction and improvement of language was provided by Proof-Reading-Service.com Ltd., Devonshire Business Centre, Works Road, Letchworth Garden City SG6 1GJ, United Kingdom.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Authors thank for financial support from the National Agency of the Agricultural Research (NAZV) project no. Q31210211.

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5.3 Publikovaný výstup č. 3

Název článku: A profile of arsenic species in different vegetables growing in arsenic contaminated soils

Autoři: Tremlová, J., Sehnal, M., Száková, J., Goessler, W., Steiner, O., Najmanová, J., Horáková, T., Tlustoš, P.

Publikováno v: Archives of Agronomy and Soil Science (přijato do tisku), DOI 10.1080/03650340.2016.1242721.

A Profile of Arsenic Species in Different Vegetables Growing in Arsenic-Contaminated Soils

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Abstract

Six different vegetables (black radish, black salsify, lettuce, parsnip, Savoy cabbage and Swede turnip) were cultivated in model pot experiments. The soils used in the experiments originated from two mining and smelting sites in the Czech Republic – Příbram and Kutná Hora, respectively. These soils showed differences in physicochemical properties and/or total contents of arsenic, reaching 36.0± 1.0 mg As kg^{-1} and 473 ± 10 mg As kg^{-1}, respectively. The four most common anionic arsenic compounds (arsenite As[III], arsenate As[V], dimethylarsinate [DMA], methylarsonate [MA]) were determined by high-performance liquid chromatography (HPLC) coupled to an inductively coupled plasma mass spectrometer (ICPMS). The concentration of arsenic species determined in edible plant parts decreased in the following order: As(V) ~ As(III) >> DMA ~ MA. Higher proportions of both DMA and MA were found in the aboveground edible parts (leaves) compared to the underground parts (tubers). The results indicate that the distribution of arsenic compounds differed predominantly according to individual plant

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species whereas almost no effect was observed due to the different soil properties. However, a higher arsenic concentration in soils resulted in more arsenic in the plant independently of the aboveground biomass (leaves) or the underground plant parts (tubers).

Keywords

Arsenic speciation; Contaminated soil; Hyphenated technique; Mining area; Vegetable

1. Introduction

Arsenic (As) anthropogenic pollution caused by the mining and smelting industry and/or agriculture may lead to a significant increase in As background concentrations in soils and subsequent soil contamination. In the Czech Republic, most of the As pollution originate from the industrial and mining activity that is often connected with former or present gold, silver, and brown-coal mining, which occurs around the areas of Kutná Hora, Příbram (metals), Chomutov, Most and Ostrava (coal).

Terrestrial plants are able to accumulate various amounts of As depending on the plant species, soil characteristics – including the amount of available As species – and climatic and dietary conditions, *etc.* The concentration of As in plants growing in uncontaminated soils with an As concentration of below 30 mg As kg^{-1} (dry weight) (Anonymous 1994) commonly reaches 0.01–1.5 mg As kg^{-1} in the dry weight (Kabata-Pendias and Pendias 2001; Chaney 1985). However, contaminated soils may result in much higher As concentrations in the plant biomass. Although plants have several mechanisms to cope with high As concentrations in their environment – *e.g.* restricted uptake by plant roots, limited translocation of As from roots to shoots, reduction of As(V) to As(III) and subsequent complexation with phytochelatins, sequestration of As complexes in vacuoles (Wang et al. 2002) via volatilization of methylated As compounds (Jia et al. 2012), *etc.* – it

50 is still imperative to know not only the total As concentration in the plant biomass but also
51 the distribution of individual As compounds (Nolan et al. 2003). Each individual As
52 compound exhibits different toxicities in humans: arsine > inorganic arsenites > organic
53 trivalent compounds (arsenoxides) > inorganic arsenates > organic pentavalent compounds
54 > arsonium compounds > elemental As (Dembitsky and Rezanka 2003). However, it has
55 also been suggested that some methylated metabolites may be more toxic than previously
56 thought (NRC 1999, 2001; Rossman 2003) and that the organic trivalent compound
57 dimethylarsinate (DMA^{-III}) demonstrates greater toxicity than arsenite in some bioassays.
58 It is widely accepted that in soil solutions, the most frequent forms are the inorganic
59 As species, especially As(III) and As(V) (Castaldi et al. 2014; Georgiadis et al. 2006;
60 Moreno-Jimenéz 2013). The distribution of As species in the soil solution depends highly
61 on the redox system, as well as the microbiochemistry near the rhizosphere (Dick et al.
62 2013). Many researchers (Dembitsky and Rezanka 2003; Francesconi and Kuehnelt 2004;
63 Reyes et al. 2008; Száková et al. 2007) state that arsenite As(III), arsenate As(V),
64 dimethylarsinate (DMA) and methylarsonate (MA) are usually the most common detectable
65 compounds in terrestrial plants. Occurring less often are arsenocholine (AC) and
66 arsenobetaine (AB). These cationic As compounds are more likely to appear in marine
67 organisms (Rahman et al. 2012; Soudek et al. 2006). Falk and Emons (2000) and Ruiz-
68 Chancho et al. (2008) reported significant concentrations of other cationic compounds in
69 terrestrial plants, such as trimethylarsine oxide (TMAO) and the tetramethylarsonium ion
70 (TETRA).
71 The present investigation focuses on the distribution of As compounds in traditional
72 vegetables that are widely grown for own consumption. The ability of various crops to take
73 up and transform As into different forms was shown in several previous studies (Tlustoš et
74 al. 1998; Warren et al. 2003; Huang et al. 2006). Therefore, providing information about

75 the total amount of As and the distribution of As compounds in traditional Czech
76 vegetables grown in As-contaminated soils is important in order to estimate and prevent the
77 possible health risk for consumers. This is of particular importance since a former
78 provisional tolerable weekly intake (PTWI) value of 15 μg As kg^{-1} bw was withdrawn due
79 to no longer being considered as health-protective, and since epidemiological studies have
80 reported that As causes various carcinogenic effects even at lower exposures (JECFA
81 2011).
82

83 **2. Experimental**

84 ***2.1 Pot Experiment and Materials***

85 Six different vegetables that represent crops often grown for consumption in the
86 area of the Czech Republic and Central Europe – turnip (*Brassica napus var. napobrassica*,
87 L.), Savoy cabbage (*Brassica oleracea convar. Acephela*, L.), black radish (*Raphanus*
88 *sativus* var. *nigra*, L.), black salsify (*Scorzonera hispanica*, L.), parsnips (*Pastinaca sativa*,
89 L.) and lettuce (*Lactuca sativa* L.) – were grown in pots.

90 Experimental soil from Příbram (site coordinates 49°42´41,6´´ N, 13°59´12,9´´ E;
91 for physicochemical soil properties, see TABLE 1) was taken from a field that was polluted
92 by nearby lead mining and the smelting industry. Mining and metallurgical activities in this
93 area led to an enhancement of As, Cd and Zn contents in soil due to the high content of the
94 risk elements in the parent rock and emissions from the lead smelter (Šichorová et al.
95 2004).

96 The other experimental soil from Kutná Hora (site coordinates 49°58´07,9´´ N,
97 15°17´55,7´´ E) originates from an area that is contaminated by As, Cd and Zn, mainly
98 from tailings of silver mining in the middle-ages. Furthermore, the background

99 concentrations of Cd and Zn are substantially elevated due to the composition of the parent
100 rock (Malec and Pauliš 1995; Králová at al. 2010).

101 Before planting, laboratory soil samples were air-dried at 20°C, ground in a
102 porcelain mortar and passed through a 2-mm plastic sieve, and subsequently analysed for
103 total and plant-available As contents as described in Section *2.2*. Plants were cultivated in
104 6-L plastic pots filled with 5 kg of dry soil (for each variant, n=4). Nutrients NPK
105 (equivalent to 0.5 g N, 0.16 g P and 0.4 g K per pot) were added before sowing. Pots were
106 placed in an outdoor weather-controlled vegetation hall. Soil moisture was kept at 60% of
107 the water-holding capacity (WHC) by watering daily with deionised water, and leachate
108 was collected and re-circulated to the system.

109 The edible parts of the plants were harvested at different dates when ready for
110 consumption. Individual plant parts were gently washed with deionised water, checked for
111 fresh biomass, freeze-dried (Lyovac GT-2, Germany), ground in a mill and analysed.

112

113 ***2.2 Analytical Methods***

114 Pseudo-total concentrations of elements in soils were determined in digests obtained
115 by the following decomposition procedure: an aliquot (~0.5 g) of dry soil sample was
116 decomposed in a digestion vessel with 10 mL of *Aqua Regia* (*i.e.* nitric and hydrochloric
117 acid mixture in a ratio 1:3; n=3). The mixture was heated in an Ethos 1 (MLS GmbH,
118 Germany) microwave-assisted wet-digestion system for 33 minutes at 210°C. After
119 cooling, the soil extract was quantitatively transferred into a 25-mL glass tube that was
120 filled with deionised water and kept at laboratory temperature until measurement. A
121 certified reference material (CRM 7001 Light Sandy Soil; Analytika Co. Ltd., Czech
122 Republic) was applied for quality assurance of the analytical data. The certified value of As
123 in the CRM is declared as being 12.3 ± 1.1 mg As kg^{-1}, and 12.5 ± 1.4 mg As kg^{-1} was

124 obtained. The pH was determined using 0.2 mol L^{-1} KCl (w/v = 1:2.5) (Fiala et al. 1999).
125 Plant-available As was determined using 0.01 M aqueous $CaCl_2$ solution (w/v = 1:10, for 6
126 hours) (Novozamsky et al. 1993). Cation-exchange capacity (CEC) was calculated as the
127 sum of Ca, Mg, K, Na and Al that was extractable in 0.1 mol L^{-1} $BaCl_2$ (w/v = 1:20, for 2
128 hours) (ISO 1994). Total organic carbon (TOC) was determined spectrophotometrically at
129 600 nm after oxidation of the organic matter by $K_2Cr_2O_7$ (Sims and Haby 1971). Available
130 nutrients were determined by a Mehlich III soil-extraction procedure (Mehlich 1984) using
131 flame atomic absorption spectroscopy (FAAS, Varian 280FS, Australia) for Ca, K and Mg
132 and optical emission spectrometry with inductively coupled plasma for P.
133 Plant samples were decomposed by using a dry-ashing procedure as follows: an
134 aliquot (~1 g) of dry and powdered aboveground biomass or roots was weighed to 1 mg
135 (n=3), placed into a borosilicate glass test-tube and decomposed in a mixture of oxidising
136 gases (O_2 + O_3 + NO_x) at 400°C for 10 hours in a Dry Mode Mineralizer Apion (Tessek,
137 Czech Republic). The ash was dissolved in 20 mL of 1.5% HNO_3 (electronic-grade purity,
138 Analytika Co. Ltd.) and kept in a glass tube until analysis (Miholová et al. 1993).
139 The concentrations of elements in the soil and plant digests were determined by
140 optical emission spectroscopy with inductively coupled plasma (ICPOES) with axial
141 plasma configuration (Varian VistaPro, Varian, Australia), equipped with an SPS–5
142 autosampler. External calibration solutions (Analytika Co. Ltd.) were prepared in
143 corresponding extraction agents with concentrations of 100–1000 µg L^{-1} As. Measurement
144 conditions were as follows: power 1.2 kW, plasma flow 15.0 L min^{-1}, auxiliary flow 0.75 L
145 min^{-1}, nebulizer flow 0.9 L min^{-1}. For the determination of low As concentrations in soil
146 extracts and plant digests, a hydride-generation atomic absorption spectrometer (Varian
147 AA280Z, Varian) equipped with a VGA–77 continuous hydride generator was used. A

148 mixture of potassium iodide and ascorbic acid was used for pre-reduction of the sample and
149 the extract was acidified with HCl before measurement (Brodie et al. 1983).

150 For the determination of As species in the edible vegetable parts, 1 g of dried and
151 powdered samples were weighed to ±0.1 mg into 15-mL screw-capped polypropylene
152 tubes, and 10 mL of 0.02 mol L^{-1} $NH_4H_2PO_4$ (pH 6.0) was added. The tubes were fastened
153 to a cross-shaped rotor and turned top-over-bottom at 45 rpm for 14 hours. The mixtures
154 were then centrifuged for 10 min at 3000 rpm and filtered through a 0.22-µm cellulose-
155 nitrate ester filter (Millex-GS, Millipore, Bedford, MA, US).

156 Individual As compounds were determined by high-performance liquid
157 chromatography (HPLC 1100 series, Agilent Technologies, US) equipped with an anion-
158 exchange column (PRP-X100, 150 x 4.6 mm with 5-µm particles, Hamilton, US). The
159 column was operated at room temperature and 10 µL of each extract was injected. For a
160 good separation of species, an isocratic elution with 0.02 mol L^{-1} $NH_4H_2PO_4$ (pH 6.0) as the
161 mobile phase at a flow of 1.5 mL min^{-1} was used. An Agilent ICPMS 7500ce equipped with
162 a helium collision cell and a quadrupole was used as an element-selective detector. The
163 outlet of the HPLC column was connected with a PEEK capillary (0.125 mm i.d.) to the
164 nebulizer of the ICPMS system. The intensity of ions at *m/z* 75 (^{75}As and $^{40}Ar^{35}Cl$) and also
165 potential argon chloride ($^{40}Ar^{37}Cl$) interferences at *m/z* 77 were monitored.

166 All standards and solutions were prepared with purified water (18.2 MΩ cm, Merck
167 Millipore, US). The ammonium dihydrogen phosphate (p.a.) and aqueous ammonia
168 solution (25% suprapur) that was used for adjusting the pH value of the extractant, as well
169 as the mobile phase, were purchased from Fluka (Switzerland). Standard solutions of As
170 species were prepared from $NaAsO_2$ (As[III]) and $Na_2HAsO_4 \cdot 7H_2O$ (As[V]) (Merck), and
171 from sodium dimethylarsinate trihydrate (DMA) (Fluka). Methylarsonate (MA) was

synthesised in-house. The standard reference material (Pine needles; National Institute of Chemistry, US) was used for the quality control.

2.3 Statistical Analysis

Statistical software STATISTICA™ version 12 (StatSoft, Inc., USA) and Microsoft Excel 2010™ software (Microsoft Corp., US) were used to carry out the statistical analysis and outputs. One-way analysis of variance (ANOVA) at a significance level of α=0.05 followed by the Scheffé test was applied to the data.

3. Results and Discussion

TABLE 1 shows that there was a high level of soil As contamination at the Kutná Hora location, reaching 473 ± 10 mg As kg^{-1} which is in accordance with other authors (Hyršl and Kaden 1992; Králová et al. 2010). According to the public notice No. 13/1994 (Anonymous 1994), the permissible element content must not exceed 30 mg As kg^{-1} in soils used for agricultural purposes. In this case, however, the total As concentrations exceeded the legislation threshold limit (Anonymous 1994) in both experimental soils. Nevertheless, the mobile portions of As in these soils are relatively low, as was documented in our previous experiments (Száková et al. 2009). The water-extractable As portions in the soil of Kutná Hora are characterised by a high total organic matter content and a high sorption capacity that do not exceed 0.1% of the total soil As content. In contrast, a higher mobile proportion of As was found in Příbram soil that was characterised by lower pH, organic matter content and sorption capacity. The abundance of As compounds in water extracts of the soils was investigated in a previous study (Száková et al. 2009) and the results were similar to those of oxidising soil conditions (Marin et al. 1993; Reynolds and Naylor 1999),

196 where As(V) is the dominant As compound with a small percentage of As(III). Small
197 amounts of DMA were also present in the extracts of the highly contaminated soil from
198 Kutná Hora, whereas, in the less-contaminated soil from Příbram, this compound was not
199 detected (Száková et al. 2009). We can speculate that the higher microbial activity in the
200 organic matter-rich soil of Kutná Hora results in a higher As methylation rate but this
201 aspect needs to be verified in further research.

202 A relatively low plant-availability of As from the Kutná Hora location is evident
203 from the relatively low As concentrations in the aboveground biomass of wild-growing
204 plants occurring on these soils (Králová et al. 2010). Transfer factors, given as a ratio of the
205 total As content in the plant and its pseudo-total (*Aqua regia* soluble) content in soil were
206 as follows: 0.005 (Savoy cabbage, Kutná Hora), 0.045 (black salsify, Příbram), 0.14
207 (parsnip, Kutná Hora) and 0.18 (parsnip, Příbram) (TABLE 2). Huang et al. (2005)
208 calculated an average transfer factor for As from artificially As-contaminated soil (total
209 concentrations 4806 mg As kg^{-1}) into lettuce leaves, which reached up to 0.018 and also
210 supports the fact that As is less available to the agricultural plants tested. Although the low
211 mobile proportion of As in the soil from Kutná Hora probably decreased the uptake of As
212 from highly contaminated soils, the concentration is still too high and poses a potential risk
213 to humans.

214 Total contents of As in dried plant edible tissues ranged from 1.6 ± 0.8 mg kg^{-1}
215 (black salsify) to 64 ± 2.1 mg kg^{-1} (parsnip). Accumulated amounts of As decreased in the
216 studied crops as follows: parsnip >> black radish > black salsify > Swede turnip > Savoy
217 cabbage > lettuce for soil from Kutná Hora, parsnip > Swede turnip > black radish > Savoy
218 cabbage > lettuce > black salsify for soil from Příbram (TABLE 2). Except for black
219 salsify, the results confirmed the predominant accumulation of As in roots and limited
220 translocation of this element into the aboveground biomass where the differences between

221 the As concentrations in leaves and tubers tended to increase in the highly contaminated
222 soil from Hutná Hora compared to the moderately contaminated soil from Příbram. Higher
223 concentrations of the total As in underground parts and declining total As concentrations in
224 the aboveground parts of the plants were also observed in other studies (Smith et al. 2009).
225 Even though the total As content in soil from Kutná Hora was much higher than in
226 the soil from Příbram, the higher total organic carbon level and sorption capacity compared
227 to the Příbram soil (TABLE 1) most probably resulted in relatively low mobility and plant-
228 availability of As. This assumption is supported by Kabata-Pendias and Pendias (2001), Fu
229 et al. (2011) and Fitamo et al. (2011).
230 For rice, the European Union (EU) has set a threshold limit 0.2 mg As kg^{-1}
231 (Anonymous 2015). Te maximum permissible concentrations of risk elements in fresh leafy
232 vegetables given by Public Notice No. 53/2002 applied in the Czech Republic (Anonymous
233 2002) states that the threshold limit of As is 0.5 mg As kg^{-1} of fresh matter. By estimating
234 approximately 10% of the dry matter of the surveyed vegetables, the As levels in the
235 parsnip, black salsify and black radish (all from Kutná Hora) exceeded this threshold. Much
236 lower As levels in leafy vegetables (lettuce, Savoy cabbage) suggest that the right choice of
237 crop growing in the As-contaminated soils can reduce the potential health risk. This idea is
238 supported by a study of Rapant et al. (2006) in which high As concentrations that exceeded
239 the maximum permissible limits for vegetables (kohlrabi, carrot, parsley and beetroot) were
240 also found in an abandoned mining area where the total As contents in soil reached up to
241 890 mg As kg^{-1} but low plant-availability was confirmed by the low levels of plant-soil
242 transfer factors. These results also confirmed higher As accumulation in the roots and bulbs
243 of the investigated plants.
244 Speciation analyses show that both As(III) and As(V) are the dominant As forms in
245 analysed vegetables, whereas DMA and MA occur rarely. As(III) concentrations ranged

246 from 0.25 mg kg^{-1} (Savoy cabbage) to 5.97 mg kg^{-1} (black radish), and As(V)
247 concentrations from 0.36 mg kg^{-1} (lettuce) to 2.87 mg kg^{-1} (black salsify). The dominating
248 As species in most of the studied vegetables was As(V) (FIGURE 1) as was also found by
249 Jedynak et al. (2009). The distribution of As species in the individual vegetables was
250 strongly species-dependent while the potential effect of the soil characteristics and/or As
251 content was less apparent. The only exception was black radish, which contained higher
252 amounts of As(III) than As(V). Predominant contents of inorganic As species compared to
253 organic species were observed by many authors although different proportions of As(III)
254 and As(V) have been reported in plants of different species and/or originating from
255 different locations and cultivation conditions (Wei et al. 2015; Larios et al. 2012; Bergquist
256 and Greger 2012). Differences in the proportions of inorganic As compounds were reported
257 even for different plant species within one genus (Márquez-García et al. 2012)
258 Tlustoš et al. (2002) determined that As(III) was the dominant compound in roots of
259 radish while As(V) was mostly translocated to the leaves. A higher percentage of DMA in
260 plants (17% in roots and 18% in leaves) compared to soil suggests that radish plants are
261 able to methylate available As compounds, as was described by Cullen and Reimer (1989)
262 in the case of *Vinca minor* L. However, more recent research suggests that microbially
263 induced DMA is taken up by plants from the soil and cannot be synthetised (Lomax et al.,
264 2012). Moreover, arbuscular mycorrhizal fungi are able to increase the ability of plants to
265 survive in extremely As-polluted substrates (Orlowska et al. 2012). Thus, the rhizosphere
266 processes are important for As uptake and speciation in plants. Smith et al. (2009)
267 confirmed that As was present in the roots of radish, chard and lettuce as the As(V) form
268 and comprised between 77% and 92% of the total As concentration, whereas in mung
269 beans, the As(III) form comprised around 90% of the total As concentration. In aerial
270 portions of the vegetables, As was distributed equally between As(V) and As(III) in radish

and chard but was present mainly as As(V) in the case of lettuce. Methylated As compounds were not detected. The dominant role of inorganic As compounds in silverbeet shoot and amaranth was confirmed by Rahman et al. (2009). The samples contained mostly inorganic As, especially As(III) (~90%), in the edible part of the vegetables.

In this case, slight differences in the As distribution in individual species between experimental soils (physicochemical soil properties) were observed. However, a higher percentage of organic As compounds occurred in the samples from less As-contaminated soil (Příbram) and a higher portion of As(III) was observed in the more contaminated soil (Kutná Hora). Bioavailability of As for plants is dominated by the plant species, as was also confirmed for wild-growing plant species (Kuehnelt et al. 2000; Schmidt et al. 2001).

4. Conclusions

We conclude that the traditional Czech vegetables studied are able to take up, accumulate and even transform As compounds in their tissues. The traditional vegetables widely planted in local private gardens showed a similar As uptake ability as common crops and wild plants. The underground plant parts (tubers, bulbs) showed a greater ability to accumulate As in their tissue than the aboveground parts (leaves) and so it seems that a good choice of crop might be sufficient for decreasing the potential As exposure of inhabitants living around and growing their crops in the highly As-contaminated areas.

In this study, the inorganic forms prevailed over the organic forms. The determination of As species confirmed a dominant portion of inorganic As compounds as well as the presence of methylated compounds, especially in the aboveground plant biomass. The distribution of As compounds in plants is determined predominantly by the plant species regardless of the soil As level and/or other physicochemical soil properties, although the latter can affect the total amount of As in the plant biomass.

296 **Acknowledgements**

297 This work was supported by the grant project of Technological Agency of the Czech
298 Republic No. TA01020366; the Internal Grant Agency of the Czech University of Life Sciences
299 Prague No. 20131001; and the Internal Grant Agency of the Czech University of Life Sciences
300 Prague FAPPZ No. SV12-21-21140.

301

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444 Wei C, Ge Z, Chu W, Feng R. 2015: Speciation of antimony and arsenic in the soils and
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446 TABLE 1: Total and available As content and main physicochemical parameters of experimental soils. The averages marked by the same letter
447 did not significantly differ at $P < 0.05$ within individual columns. Data are presented as the mean ± standard deviation
448

soil	Total As mg kg^{-1}	Available As mg kg^{-1}	Ca* mg kg^{-1}	Mg* mg kg^{-1}	K* mg kg^{-1}	P* mg kg^{-1}	CEC mmol+ kg^{-1}	TOC %	pH
Kutná Hora	473 ± 10^{b}	0.299 ± 0.024^{b}	6877 ± 41^{b}	513 ± 8^{b}	610 ± 11^{b}	56.2 ± 1.1^{a}	295 ± 25^{b}	4.0 ± 0.3^{b}	7.4 ± 0.3^{b}
Příbram	36.0 ± 1.0^{a}	0.067 ± 0.04^{a}	951 ± 15^{a}	76.7 ± 2.1^{a}	160 ± 2^{a}	283 ± 5^{b}	123 ± 12^{a}	1.9 ± 0.00^{a}	4.5 ± 0.1^{a}

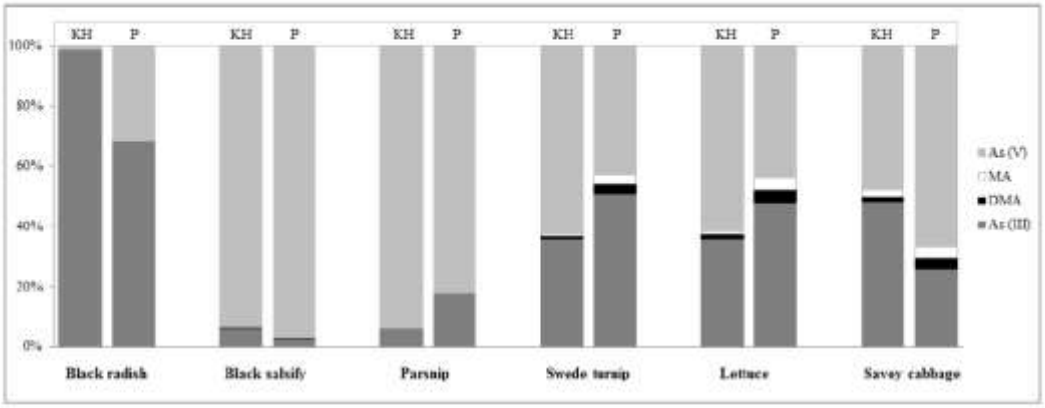
449 Notes: * – available element contents determined according to Mehlich III soil-extraction procedure (Mehlich, 1984); CEC – cation-exchange capacity; TOC – total
450 organic carbon content; pH – pH/KCl.

451 TABLE 2: Total As contents and transfer factors in edible vegetable parts. The averages
452 marked by the same letter did not significantly differ at $P < 0.05$ within individual columns.
453 Data are presented as the mean ± standard deviation.

	soil Kutná Hora		soil Příbram	
vegetable	As [mg kg^{-1}]	TF	As [mg kg^{-1}]	TF
Lettuce leaves	4.5 ± 1.5^{a}	0.01	1.9 ± 0.50^{a}	0.052
Black radish tuber	13 ± 3.1^{a}	0.03	3.5 ± 1.5^{abc}	0.097
Parsnip tuber	64 ± 2.1^{b}	0.14	6.4 ± 2.3^{c}	0.18
Black salsify tuber	11 ± 5.5^{a}	0.023	1.6 ± 0.80^{ab}	0.045
Swede turnip tuber	6.7 ± 1.3^{a}	0.014	4.9 ± 2.7^{bc}	0.14
Savoy cabbage leaves	2.4 ± 0.71^{a}	0.005	2.4 ± 0.51^{ab}	0.066

454 Notes: As [mg kg^{-1}] – total As contents in dry matter of edible vegetable parts; TF – transfer factor.

455 FIGURE 1: Distribution of individual As species in edible parts of surveyed vegetables.



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457 Notes: KH – soil from Kutná Hora, P – soil from Příbram; As(III) – arsenite, DMA – dimethylarsinate, MA – methylarsonate, As(V) – arsenate.

5.4 Publikovaný výstup č. 4

Název článku: Soil-to-plant transfer of native selenium for wild vegetation cover at selected locations of the Czech Republic

Autoři: Száková, J., Tremlová, J., Pegová, K., Najmanová, J., Tlustoš, P.

Publikováno v: Environmental Monitoring and Assessment 187. 2015. 358-366.

Environ Monit Assess (2015) 187: 358
DOI 10.1007/s10661-015-4588-1

Soil-to-plant transfer of native selenium for wild vegetation cover at selected locations of the Czech Republic

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Received: 14 October 2014 / Accepted: 5 May 2015 / Published online: 16 May 2015

Abstract Total selenium (Se) contents were determined in aboveground biomass of wild plant species growing in two uncultivated meadows at two different locations. The soils in these locations had pseudototal (*Aqua Regia* soluble) Se in concentration ranges of between 0.2 and 0.3 mg kg^{-1} at the first location, and between 0.7 and 1.4 mg kg^{-1} at the second location. The plant species represented 29 plant families where the most numerous ones were *Poaceae*, *Rosaceae*, *Fabaceae*, and *Asteraceae*. The selenium contents in the plants varied between undetectable levels (*Aegopodium podagraria*, *Achillea millefolium*, *Lotus corniculatus*) and 0.158 mg kg^{-1} (*Veronica arvensis*, *Veronicaceae*). The Se levels were roughly one order of magnitude lower compared to other elements with similar soil content, such as cadmium and molybdenum. The transfer factors of Se, quantifying the element transfer from soil to plants, varied between <0.001 and 0.146 with no significant differences between the locations, confirming the limited soil-plant selenium transfer regardless of location, soil Se level, and plant species. Among the plant families, no unambiguous trend to potential elevated Se uptake was observed. Low Se content in the soil and its plant availability was comparable to other Se-deficient areas within Europe.

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Keywords Selenium · Plant communities · Se plant uptake · Transfer factor

Introduction

Selenium (Se) is a trace element essential to human health. The main sources of Se for humans are food and drinking water. Se enters the food chain via plant biomass, and the soil's Se content and bioavailability affect the Se content in plants. Therefore, the content of Se in food depends on the Se content of the soil where plants are grown or animals are raised. Bitterli et al. (2010) reviewed common soil Se concentrations, ranging from between 0.1 and 5 mg kg^{-1} Se, with an average of around 0.4 mg kg^{-1} Se of soil dry matter. However, low soil Se levels characterize some regions in Europe. For example, soil Se concentrations in Scandinavia were found in the range of 0.42 to 0.57 mg kg^{-1} Se (Gupta and Gupta 2000), while soils in Denmark are reported to contain 0.14–0.52 g kg^{-1} Se. For comparison, the total Se contents in soils in Mediterranean area of Spain vary between 0.06 and 1.51 mg kg^{-1} where significant correlations between Se content with soil organic matter content were observed (Roca-Perez et al. 2010). The role of soil physicochemical parameters, and especially of soil organic matter, on Se plant availability was described (Coppin et al. 2006; Eich-Greatorex et al. 2007; Gawalko et al. 2002).

However, relevant data are lacking on the spatial and temporal distribution of Se in soil, and factors governing its bioavailability are usually lacking or insufficient for

an assessment of Se transfer from the soil to the plants. Soil characteristics and soil processes need to be studied in much greater detail in order to understand the Se behavior in the soil, as well in the plants (Bitterli et al. 2010). The soils containing less than 0.6 mg kg^{-1} of Se are considered insufficient for optimum intake of Se by grazing animals (Gupta and Winter 1975). In our previous experiments, soil Se content (*Aqua regia* soluble portions) varied between 0.21 and 0.31 mg kg^{-1} Se. Low Se levels were also confirmed in the Czech Republic soils (Pegová et al. 2011). Low selenium contents indicating insufficient soil-plant-animal transport of this element were determined in skeletal muscles of wildlife animals, such as red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*) (Kursa et al. 2010).

In livestock, low Se levels in whole blood were confirmed for horses (Ludvíková et al. 2005) and cattle (Pavlata et al. 2002) in the Czech Republic.

For plants, the beneficial effect of Se on growth and/or stress resistance was already reported where Se essentiality was not unambiguously verified (Hartikainen 2005; Seppaenen et al. 2010). Moreover, Se interactions with both essential and toxic elements were reported. Malik et al. (2012) described the protective effect of Se against arsenic toxicity where both As content and oxidative damages due to As decreased with increasing Se concentration in the growth medium of *Phaseolus aureus*. A similar role of Se was observed in the case of cadmium uptake and toxicity in *Allium sativum*, *Brassica rapa*, *Lactuca sativa*, and *Oryza sativa* (Feng et al. 2013; He et al. 2004; Sun et al. 2010). In contrast, increasing macronutrient and micronutrient (Fe, Mn, Cu, Ca, K, Mg) contents in plants with increasing Se content in both growth medium and soil were observed (Matraszek and Hawrylak-Nowak 2009). Similarly, de Souza et al. (2013) observed enhanced Fe, Zn, S, Mo, Mg, Ca, and Mn content in wheat seedlings after selenate was supplied, whereas selenite supplementation reduced Zn, S, Mo, Mg, Ca, and Mn levels in the plants.

The main objectives of our experiment were (i) to determine Se contents in wildlife plants at two locations differing in the soil selenium content in order to estimate the potential risk of Se deficiency in plants for wildlife grazing animals and (ii) to compare the Se uptake ability of the wide spectrum of plant species as affected by individual plant species and/or families, and soil selenium levels.

Materials and methods

Experimental sites and sampling

Two locations, differing in their soil physicochemical parameters as well as both nutrient and risk elements, were chosen and the samples of wildlife plant species were collected in uncultivated meadows (i) in vicinity of Humpolec (Czech Republic), the city in eastern part of Bohemia characterized by low levels of smokestack industries, and (ii) the former silver mining area in the vicinity of Nalžovské Hory (Czech Republic), the city in the southwestern part of Bohemia where elevated contents of risk elements such as Cd, Pb, and Zn were previously found. At the Humpolec location, an uncultivated meadow was selected, where the area was randomly divided into six sampling areas. One sampling point occurred in each sampling area in order to encourage the variability of element contents in the soil. The individual sampling points are found between 49° 33.42′ N, 15° 21.06′ E and 49° 33.46′ N, 15° 21.02′ E. The bedrock is based predominantly on paragleiss, the soil type is gleyic Cambisol, the texture is loam. Similarly, six sampling areas, with one soil sampling point in each sampling area, were selected in the uncultivated meadow in the vicinity of the former silver mine in Nalžovské Hory. The individual sampling points are found between 49° 19.71′ N, 13° 32.80′ E and 49° 19.78′ N, 13° 32.71′ E. The silver (and also tin and lead) mining is dated between 1521 and 1896 and resulted in significant increase of risk element (especially Pb, Cd, and Zn) in the soils (Table 1).

At each sampling point, representative samples of the whole aboveground biomass of the individual plant species were taken during the flowering stage. The plant samples were dried at 60 °C to a constant mass and the whole plants were then ground into a fine powder using a laboratory mill. Soil samples for the determination of total and mobile concentrations of the elements were collected for each sampling point from a depth of 0–25 cm, dried at 20 °C, ground in a mortar, and passed through a 2-mm plastic sieve.

Analytical methods

The pseudototal concentrations of elements in the soils were determined in the digests obtained by the following decomposition procedure: Aliquots (~0.5 g) of air-dried soil samples were decomposed in a digestion

Table 1 The pseudototal contents of elements in the soils expressed in mg kg^{-1} of dry matter; $n=6$

	Humpolec							
	As	Cd	Cu	Mo	Ni	Pb	Se	Zn
Minimum	4.36	0.232	14.5	0.143	12.7	15.3	0.206	42.7
Maximum	7.71	0.375	19.2	0.425	18.2	22.9	0.305	66.1
Average	6.43	0.274	16.2	0.295	14.7	18.8	0.248	52.8
Standard deviation	0.94	0.041	1.76	0.075	1.70	3.18	0.031	7.44
Median	6.73	0.258	15.3	0.298	14.7	18.3	0.246	50.8
MAD	0.508	0.013	0.771	0.030	1.46	3.26	0.026	5.44
	Nalžovské Hory							
	As	Cd	Cu	Mo	Ni	Pb	Se	Zn
Minimum	23.4	3.99	34.5	0.503	10.4	1328	0.722	650
Maximum	46.3	6.18	51.3	1.338	18.1	3818	1.408	1033
Average	32.7	5.19	42.6	0.759	13.7	2079	1.104	816
Standard deviation	9.86	0.950	6.08	0.316	3.02	1012	0.306	143
Median	27.9	5.39	41.9	0.668	12.7	1593	1.201	835
MAD	2.96	0.74	3.9	0.158	1.6	249	0.174	106
Limit[a]	30	1	100	5	80	140	[b]	200

MAD median of absolute deviations

[a] Maximum permissible limits for risk element contents in arable soils in the Czech Republic (Czech Ministry of the Environment 1994)

[b] Not regulated

vessel with 10 ml of *Aqua Regia* (i.e., nitric and hydrochloric acid mixture in ratio 1+3). The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 33 min at 210 °C. After cooling, the digest was quantitatively transferred into a 25 ml glass tube, topped up with deionized water, and kept at laboratory temperature until measurement. A certified reference material RM 7003 Silty Clay Loam (Analytika, Czech Republic) was applied for the quality assurance of the analytical data.

For the determination of the element contents in the aboveground biomass, an aliquot (~0.5 g of dry matter) of the plant sample was weighed in a digestion vessel. Concentrated nitric acid (8.0 ml) (Analytika Ltd., Czech Republic) and 30 % H_2O_2 (2.0 ml) (Analytika Ltd., Czech Republic) were added, and the mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 30 min at 220 °C. After cooling, the digest was quantitatively transferred into a 20-ml glass tube filled up with deionized water.

The soil pH was determined using deionized water or 0.01 M $CaCl_2$ (w/v=1+10) (Novozamsky et al. 1993). Cation-exchange capacity (CEC) was calculated as the sum of Ca, Mg, K, Na, Fe, Mn, and Al, extractable in 0.1 M $BaCl_2$ (w/v=1+20 for 2 h) (ISO 1994). Total organic carbon (TOC) was determined spectrophotometrically after the oxidation of organic matter by $K_2Cr_2O_7$ (Sims and Haby 1971). Al, As, Cd, Cu, Mo, Ni, Pb, Na, Fe, Mn, and Zn concentrations in the digests and extracts were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian, VistaPro, Australia), whereas flame atomic absorption spectrometry (F-AAS, Varian 280FS, Varian, Australia) was used for Ca, Mg, and K determination in the extracts. For the determination of Se, hydride generation atomic absorption spectrometry (VARIAN AA280Z, Varian, Australia), equipped with a continuous hydride generator VGA-77 and inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA), equipped with an auto-sampler ASX-500, a three channel peristaltic pump and a MicroMist nebulizer were used.

The so-called transfer factor quantifying the element transfer from soil to plants is frequently used as a parameter for the evaluation of the uptake of soil elements by plants. There are different mathematical approaches that can be used to calculate this parameter; in our

experiment, the ratio of the element content in plant dry matter to the pseudototal element content in soil was applied (Sauerbeck 1985).

Statistics

The analytical data were processed using Statistica 10 Cz statistical software. A correlation analysis was used for the assessment of the relationships between the variables, where Pearson's correlation coefficients were applied (Meloun and Militký 2004).

Results

Low pH levels varying between 4.7 and 5.9 characterize the soils from the Humpolec location. The CEC level between 45 and 76 mmol+kg^{-1} demonstrated low sorption capacity of the soil. The TOC values varied between 1.8 and 2.0 %. Slightly higher pH levels varying between 5.7 and 6.9 were observed at the Nalžovské Hory location. The CEC levels were comparable to the Humpolec location, falling between 71 and 81 mmol+kg^{-1} whereas TIC values varied in wide range between 1.0 and 2.1 %. The pseudototal element contents in the soils are summarized in Table 1. The maximum permissible limits of the elements in the soils of the Czech Republic are given by public notice (Czech Ministry of the Environment 1994), see Table 1. Whereas Humpolec soils were under these limits, the Nalžovské Hory soils reflected the former mining activities by the elevated contents of As, and especially Cd, Pb, and Zn. The soil Se contents are not regulated by the mentioned public notice, but the levels found in the Humpolec soils are low, indicating a deficiency of this element for plant, and subsequently, animal nutrition (Gupta and Winter 1975). However, slightly higher Se levels in the Nalžovské Hory soils are within the normal Se values in the European soils, i.e., between 0.1 and 5 mg kg^{-1} (Bitterli et al. 2010).

The element contents in the aboveground biomass of plants for the two studied sites are summarized in Table 2 and main statistical characteristics of the selenium and other determined elements are presented in Table 3. In Fig. 1, the selenium contents in plants were clustered according to the individual plant families of the two studied sites. At the Humpolec location, a total of 43 plant species representing 18 families were identified and sampled, where the most numerous ones were *Poaceae* and *Rosaceae*. The Se contents varied between 0.008 mg kg^{-1} (*Geum urbanum*, *Rosaceae*) and 0.036 mg kg^{-1} (*Stellaria graminea*, *Silenaceae*) [see Fig. 1a]. The results suggested slightly higher Se uptake ability by the species representing *Asteraceae*, *Silenaceae*, and especially *Cyperaceae*. As expected, higher Se levels in the plants at the Nalžovské Hory location were found in agreement with the higher soil Se content (Fig. 1, Table 1). A total of 40 plant species representing 20 families were identified, with the most numerous ones being *Fabaceae* and *Asteraceae*. At this location, the Se content varied from undetectable levels (*Lotus corniculatus*, *Fabaceae*) and 0.158 mg kg^{-1} (*Veronica arvensis*, *Veronicaceae*). Higher levels of variability of the results at this location also reflect the higher variability of soil Se. Figure 1b shows no similar trend in Se uptake by individual plant families, most likely because of different plant species representing the individual families occurring at the specific locations. Only nine plant families were present at both locations, and the presence of the same species representing these families at both locations was very rare. For example, the family *Veronicaceae* was represented by *Veronica chamaedrys* at the Humpolec location, and *Veronica arvensis* at the Nalžovské Hory location.

The elevated contents of Cd, Pb, and Zn in soils from the mining area Nalžovské Hory resulted in increased levels of these elements in plants. According to the Directive No. 2002/32/ES (European Parliament and Council of Europe 2002), the maximum values of the elements in raw feedstuffs (which were calculated in 12 % moisture) are 2 mg kg^{-1} of As and 1 mg kg^{-1} of Cd. A maximum permissible limit of 30 mg kg^{-1} in fodder was defined in the case of Pb. In our situation, these values of Pb were exceeded in *Verbascum nigrum* and *Vicia cracca*. For Cd, 40 % of the results exceeded the limit, with a maximum of 12.1 mg kg^{-1} for *Chenopodium album*. According to Alloway (1990), the maximum Zn content found in *Vicia cracca* can be considered to be elevated as well. Higher soil Mo and Cu levels resulted in higher contents of these elements in the plants at the Nalžovské Hory location compared to Humpolec, whereas the opposite pattern was observed in the case of As. Surprisingly, although the Ni content in soil was comparable at both locations, higher contents of Ni in plant biomass were found at the Nalžovské Hory location.

Table 2 Selenium contents in the aboveground biomass of the individual plant species

Species Humpolec	Family	Se (mg kg^{-1})	Species Nalžovské Hory	Family	Se (mg kg^{-1})
Geum urbanum L.	*Rosaceae*	0.008	*Melandrium album* L.	*Caryophyllaceae*	<DL
Lolium perenne L.	*Poaceae*	0.008	*Hypericum perforatum* L.	*Hypericaceae*	<DL
Campanula patula L.	*Campanulaceae*	0.009	*Trifolium repens* L.	*Fabaceae*	0.005
Trifolium repens L.	*Fabaceae*	0.009	*Convolvulvus arvensis* L.	*Convolvulaceae*	0.005
Veronica chamaedrys L.	*Veronicaceae*	0.010	*Poa pratensis* L.	*Poaceae*	0.006
Arrhenatherum elatius (L.) PRESL.	*Poaceae*	0.011	*Campanula rapunculoides* L.	*Campanulaceae*	0.007
Urtica dioica L.	*Urticaceae*	0.011	*Rumex obtusifolius* L.	*Polygonaceae*	0.007
Lysimachia vulgaris L.	*Primulaceae*	0.011	*Daucus carota* L.	*Apiaceae*	0.007
Alopecurus pratensis L.	*Poaceae*	0.012	*Achillea millefolium* L.	*Asteraceae*	0.007
Holcus lanatus L.	*Poaceae*	0.012	*Taraxacum officinale* L.	*Asteraceae*	0.008
Rumex obtusifolius L.	*Polygonaceae*	0.012	*Trifolium campestre* Schreb.	*Fabaceae*	0.009
Heracleum sphondylium L.	*Apiaceae*	0.013	*Lotus corniculatus* L.	*Fabaceae*	0.011
Vicia cracca L.	*Fabaceae*	0.013	*Silene* spp.	*Caryophyllaceae*	0.012
Thlaspi arvence L.	*Brassicaceae*	0.013	*Phleum pratense* L.	*Poaceae*	0.013
Dactylis glomerata L.	*Poaceae*	0.013	*Vicia villosa* L.	*Fabaceae*	0.014
Galium aparine L.	*Rubiaceae*	0.014	*Ballota nigra* L.	*Lamiaceae*	0.014
Holcus lanatus L.	*Poaceae*	0.014	*Dianthus* spp.	*Caryophyllaceae*	0.015
Artemisia absinthum L.	*Asteraceae*	0.015	*Artemisia vulgaris* L.	*Asteraceae*	0.017
Juncus conglomeratus L.	*Juncaceae*	0.015	*Vinca minor* L.	*Apocynaceae*	0.017
Trisetum flavescens (L.) P. BEAUV.	*Poaceae*	0.015	*Convallaria majalis* L.	*Asparagaceae*	0.017
Potentilla anserina L.	*Rosaceae*	0.015	*Geum urbanum* L.	*Rosaceae*	0.018
Anthriscus sylvestris (L.) Hoffm.	*Apiaceae*	0.017	*Senecio vulgaris* L.	*Asteraceae*	0.02
Taraxacum officinale WEB.	*Asteraceae*	0.017	*Epilobium montanum* Huds.	*Onagraceae*	0.02
Geranium pratense L.	*Geraniaceae*	0.017	*Aegopodium podagraria* L.	*Apiaceae*	0.026
Lysimachia nummularia L.	*Primulaceae*	0.017	Pimpinella major L.	*Apiaceae*	0.026
Filipendula ulmaria MAX.	*Rosaceae*	0.017	*Tanacetum vulgare* L.	*Asteraceae*	0.032
Lychnis flos-cuculi L.	*Silenaceae*	0.017	*Medicago lupulina* L.	*Fabaceae*	0.032
Capsella bursa pastoris (L.) MED.	*Brassicaceae*	0.018	*Dactylis glomerata* L.	*Poaceae*	0.033
Caltha palustris L.	*Ranunculaceae*	0.018	*Rumex* spp.	*Polygonaceae*	0.034
Equisetum arvense L.	*Equisetaceae*	0.020	*Trifolium pratense* L.	*Fabaceae*	0.035
Trifolium hybridum L.	*Fabaceae*	0.020	*Lolium* spp.	*Poaceae*	0.035
Poa pratensis L.	*Poaceae*	0.021	*Chenopodium album* L.	*Chenopodiaceae*	0.036
Galium mollugo L.	*Rubiaceae*	0.021	*Urtica dioica* L.	*Urticaceae*	0.039
Holcus lanatus L.	*Poaceae*	0.022	*Prunella vulgaris* L.	*Lamiaceae*	0.049
Ranunculus repens L.	*Ranunculaceae*	0.023	*Securigera varia* L.	*Fabaceae*	0.051
Scirpus sylvaticus L.	*Cyperaceae*	0.024	*Plantago lanceolata* L.	*Plantaginaceae*	0.053
Alchemilla vulgaris L.	*Rosaceae*	0.024	*Galium mollugo* L.	*Rubiaceae*	0.054
Sanguisorba officinalis L.	*Rosaceae*	0.024	*Verbascum nigrum* L.	*Scrophulariaceae*	0.067
Trifolium pratense L.	*Fabaceae*	0.027	*Vicia cracca* L.	*Fabaceae*	0.08
Cirsium arvense (L.) SCOP.	*Asteraceae*	0.028	*Veronica arvensis* L.	*Veronicaceae*	0.158
Carex hirta L.	*Cyperaceae*	0.028			
Carduus acanthoides L.	*Asteraceae*	0.031			
Stellaria graminea L.	*Silenaceae*	0.036			

The data are presented in ascending order of the Se contents

Table 3 The main statistical characteristics of the element contents in dry aboveground plant biomass expressed in mg kg^{-1}

	Humpolec (n=43)							
	As	Cd	Cu	Mo	Ni	Pb	Se	Zn
Minimum	0.296	0.011	2.13	0.060	0.236	0.016	0.008	4.96
Maximum	1.58	1.04	12.1	0.713	3.237	1.405	0.036	54.5
Average	0.634	0.269	5.31	0.221	1.105	0.479	0.017	22.0
Standard deviation	0.272	0.275	2.07	0.129	0.638	0.298	0.007	10.1
Median	0.569	0.146	5.10	0.182	0.925	0.468	0.016	21.3
MAD	0.140	0.094	1.50	0.074	0.417	0.163	0.004	3.72
	Nalžovské Hory (n=40)							
	As	Cd	Cu	Mo	Ni	Pb	Se	Zn
Minimum	0.053	0.035	3.25	0.370	0.439	0.276	<DL	22.0
Maximum	0.454	12.1	37.3	8.32	17.7	56.1	0.158	334
Average	0.203	1.94	11.4	2.71	4.01	5.68	0.025	103
Standard deviation	0.105	2.70	6.41	1.62	4.2	10.1	0.029	72.2
Median	0.187	0.774	9.71	2.29	2.42	1.55	0.015	80.3
MAD	0.069	1.41	2.73	0.847	0.761	0.967	0.010	29.7

MAD median of absolute deviations

Discussion

Obviously, the Se content in plants growing in the uncontaminated soils does not exceed 1 mg kg^{-1}, as also found by Pilon-Smits et al. (2009), corresponding with our results (Table 2). Although some plant species with a higher Se accumulation ability were described, such as *Brassica napus* (*Brassicacceae*) or Se-

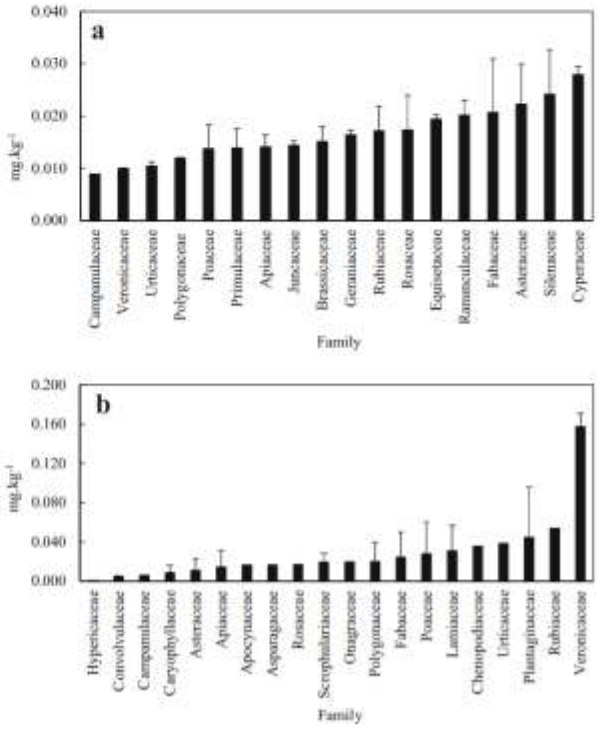

Fig. 1 Average selenium contents in the plants according to the individual families. **a** Humpolec, **b** Nalžovské Hory; the *error bars* indicate standard deviation of the data

Table 4 The main statistical characteristics of the transfer factor levels for the individual elements

	Humpolec (n=43)							
	As	Cd	Cu	Mo	Ni	Pb	Se	Zn
Minimum	0.046	0.040	0.131	0.203	0.016	0.001	0.029	0.094
Maximum	0.245	3.786	0.747	2.416	0.220	0.075	0.146	1.032
Average	0.099	0.983	0.328	0.749	0.075	0.025	0.070	0.416
Standard deviation	0.042	1.005	0.128	0.439	0.043	0.016	0.027	0.192
Median	0.088	0.534	0.315	0.618	0.063	0.025	0.064	0.403
MAD	0.022	0.343	0.092	0.250	0.028	0.009	0.016	0.070
	Nalžovské Hory (n=40)							
	As	Cd	Cu	Mo	Ni	Pb	Se	Zn
Minimum	0.002	0.007	0.076	0.488	0.032	<0.001	<0.001	<0.001
Maximum	0.014	2.331	0.876	10.967	1.291	0.027	0.143	0.113
Average	0.006	0.374	0.269	3.573	0.298	0.003	0.024	0.017
Standard deviation	0.003	0.521	0.150	2.132	0.307	0.005	0.027	0.024
Median	0.006	0.149	0.228	3.022	0.177	0.001	0.015	0.006
MAD	0.002	0.131	0.064	1.115	0.056	<0.001	0.011	0.005

MAD median of absolute deviations

hyperaccumulating *Astragalus* spp. (*Fabaceae*) (Bañuelos and Mayland 2000; Pilon-Smits et al. 2009), no species representing this group of plants were identified at our experimental sites. At the Humpolec location, only two species representing the *Brassiccaceae* family were found (*Capsella bursa pastoris* and *Thlaspi arvense*), and no plant representing *Brassica* genus was present for which a tendency toward better Se uptake can be expected (Seppaenen et al. 2010). At the Nalžovské Hory location, no species representing the *Brassiccaceae* family was identified. Among the *Fabaceae* family, only *Trifolium* spp. and *Vicia cracca* were identified at the Humpolec location. At the Nalžovské Hory location, the *Fabaceae* family was more numerous, representing *Trifolium* spp., *Vicia cracca*, *V. villosa*, *Lotus corniculatus*, *Medicago lupulina*, and *Securigera varia*. The findings did not suggest any trend towards increased Se uptake at either location.

Munier-Lamy et al. (2007) assumed that the interspecies differences are affected by the specific rhizosphere physical, chemical, and biological characteristics. This was not possible to verify in our case because only bulk soil was sampled for the determination of basic soil characteristics, and no apparent differences in the soil sorption capacity was observed among the analyzed soil samples. The predominant Se forms in the alkaline soils are selenates, while selenites are present in the acidic soils (Mikkelsen et al. 1987). Therefore, the presence of fewer plant-available selenites could be expected, especially at the Humpolec location.

The low levels of transfer factors (TF) of Se, varying between <0.001 and 0.146 (*Veronica arvensis*, *Veronicaceae*), regardless of the location, suggest low plant availability of this element at the experimental sites (Table 4). Therefore, the role of different pH levels was not the main soil parameter determining the Se uptake by plants. These data were in the range reviewed by Bitterli et al. (2010) where the TF values in the wildlife plants varied between 0.01 (*Trifolium repens*, *Fabaceae*) and 0.23 (*Lotus corniculatus*, *Fabaceae*). However, Bitterli et al. (2010) calculated the TF values based on the fresh matter Se content, and even higher values could be expected if the plant dry matter representing ~25 % of the fresh plant matter are calculated. The TF values determined in our experiment did not reach the values that had been reviewed and evaluated by Bitterli et al. (2010), confirming low plant availability of soil Se in the Czech Republic.

Low Se uptake by plants can be documented by comparing the levels with the uptake of other elements, using similar pseudototal content in soil of Cd and Mo at the Humpolec location (Table 1). The Cd content in the plants (Table 3) varied between 0.011 mg kg^{-1} (*Trifolium repens*, *Fabaceae*) and 1.04 mg kg^{-1}

(*Alchemilla vulgaris*, *Rosaceae*), documenting at least one order of magnitude higher Cd content compared to Se. A similar situation was reported for molybdenum. The TF values (Table 4) for the individual elements decreased in the order of Mo > Cd > Zn > Cu > As > Ni ~ Se > Pb at the Humpolec location, and Mo > Ni > Cu > Cd > Se > Zn ~ As > Pb at the Nalžovské Hory location. At this location, low levels of SE availability were confirmed, as compared to the other micronutrients. Lower TF values for risk elements (As, Cd, Pb, Zn) at the Nalžovské Hory location, as compared to Humpolec, documents the ability of plant species to eliminate the risk element uptake in the soils with increased levels of these elements. Králová et al. (2010) found similar TF values varying from 0.0003 to 0.003 for As, from 0.001 to 0.174 for Cd, and from 0.016 to 0.169 for Zn in the wildlife plants growing on other former silver mining areas characterized by similar Cd and Zn content, and even higher value of As.

The regression analysis indicated significantly increasing Zn and Cu content (correlation coefficients varied between r=0.27 and r=0.54), regardless of the location, as well as slightly decreasing As levels (r=−0.22). Therefore, possible Se interactions with other essential and/or toxic elements, as mentioned by other authors (de Souza et al. 2013; Malik et al. 2012; Matraszek and Hawrylak-Nowak 2009) should be taken into account. For instance, selenate supply can enhance the macronutrient and micronutrient contents (including zinc) in wheat seedlings. Malik et al. (2012) reported an antagonistic interaction between Se and As, where the As uptake was significantly reduced at a Se concentration level of 5 μmol L^{-1} in the hydroponic culture of *Phaseolus aureus* (*Fabaceae*) plants. These interactions were indicated by our results, but further research is necessary for more precise verification of these observations. In our case, the potential interactions were hampered by the low Se uptake by the analyzed plants. For other elements determined in our experiment, i.e., Cd, Mo, Ni, and Pb, no significant interrelationships were indicated by the linear regression analysis.

Summarizing the results, we can conclude that low Se content in soils, together with their low plant availability, resulted in low Se content in the aboveground biomass of plants, regardless of location, soil parameters, and Se level in the soils. The Se low content in the plants and low Se uptake ability of these plants resulting in the potential risk of Se deficiency in the wildlife food chain should be taken into account, in these and in the other Se-deficient areas within Europe and worldwide (Gupta and Gupta 2000; Lee et al. 1999).

Acknowledgments Authors thank for financial support of the Czech Science Foundation (GACR) project No. 13-04580S; correction and improvement of language was provided by Proof-Reading-Service.com Ltd., Devonshire Business Centre, Works Road, Letchworth Garden City SG6 1GJ, UK.

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5.5 Publikovaný výstup č. 5

Název článku: Selenium uptake, transformation and inter-element interactions by selected wildlife plant species after foliar selenate application

Autoři: Drahoňovský, J., Száková, J., Mestek, O., Tremlová, J., Kaňa, J., Najmanová, J., Tlustoš, P.

Publikováno v: Environmental and Experimental Botany 125. 2016. 12-19.

Environmental and Experimental Botany 125 (2016) 12–19

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Selenium uptake, transformation and inter-element interactions by selected wildlife plant species after foliar selenate application

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ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form 4 December 2015
Accepted 24 January 2016
Available online 27 January 2016

Keywords:
Selenium
Selenate
Essential elements
Foliar application
Speciation
Grassland

ABSTRACT

Plants are characterized by differing capabilities to accumulate selenium. A model small-scale field experiment was set up to investigate the selenium (Se) uptake by twelve different plant species growing at an uncultivated meadow, as well as the effect of Se foliar application on the uptake of essential elements for plants calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), and zinc (Zn). Foliar application of sodium selenate (Na_2SeO_4) was carried out in two rates (25 and 50 g Se/ha), and an untreated control variant was included and the element contents in the aboveground biomass were determined. The results showed that selenium levels actually increased due to application of selenium where confirmed the hypothesis, that foliar application of selenium will lead to an increase of this element content, depending on the plant species. The highest Se contents were determined in *Veronica chamaedrys* (1.052 ± 0.024 mg Se/kg), *Stellaria holostea* (0.775 ± 0.064 mg Se/kg), *Gallium aparine* (0.745 ± 0.027 mg Se/kg) and *Urtica dioica* (0.720 ± 0.011 mg Se/kg) biomass whereas *Cirsium arvense* and *Carex vesicaria* showed the lowest Se uptake. No symptoms of potential Se phytotoxicity were observed at these concentration levels. Among the selenium compounds, selenate and selenomethionine (SeMet) were the predominant ones regardless of the plant species documenting relative low ability of plants to transform the applied selenate to the organoselenium compounds. Regarding the minor organoselenium compounds such as selenocystine (SeCys2) and Se-methylselenocysteine (Se-MeSeCys) the results suggested differences in Se transformation between monocotyledoneous and dicotyledoneous plants where Se-MeSeCys exceeded SeCys2 in monocotyledoneous and opposite pattern was observed in dicotyledoneous plants. These findings as well as the ambiguous changes in other essential element contents in the plant biomass needs to be investigated in further research.

1. Introduction

Selenium belongs to the most important essential elements for animals and various beneficial effects were reported for plants, as well. For example, Hawrylak-Nowak et al. (2010) observed increasing tolerance of *Cucumis sativus* plants against the cold-induced stress after Se application. Similarly, selenium decreases the negative effect of high temperature on *Sorghum bicolor* (Djanaguiraman et al., 2010). Among other beneficial effects of Se improvement of *Solanum tuberosum* growth (Turakainen et al., 2004) or increasing number and weight of seeds of *Brasicca rapa* (Lyons et al., 2008). However, Landberg and Greger (1994) showed that selenium does not reduce the toxicity of cadmium and copper to plants. They observed that selenite increased cadmium contents in *Pisum sativum* roots up to 300% and selenate increased cadmium of *Triticum aestivum* shoots up to 50%. As obvious for most of the essential elements, the effect of Se on plants is dose-dependent. Hartikainen et al. (2000) documented induction of antioxidative effect and enhanced growth of *Lolium multiflorum* at low Se levels and enhanced oxidative stress at the high Se levels. The plants are able to take up selenite, selenate, and organic Se compounds such as selenocysteine (SeCys), and selenomethionine (SeMet) via their root system. On the contrary, selenides and elemental Se are not plant-available (Abrams et al., 1990; White and Broadley, 2009). Soilless cultivation of *Triticum turgidum* and *Brassica napus* showed better uptake of SeMet compared to the inorganic Se compounds (Kikkert and Berkelaar 2013). Opposite pattern was observed in soil-cultured *Festuca arundinacea* and *Brassica napus* plants where

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http://dx.doi.org/10.1016/j.envexpbot.2016.01.006
0098-8472/

the soil was treated either with selenate or with seleniferous organic materials (Ajwa et al., 1998). In this case, more than 80% of the Se added as the Se-enriched organic matter remained in soils. Arvy (1993) documented at *Phaseolus vulgaris* plants that selenate is easily translocated from roots to the aboveground biomass whereas translocation of selenite was very limited. The enhancement of Se accumulation in *Zea mays* and *Medicago sativa* plants due to mycorrhizal accumulation was observed by Yu et al. (2011).

Plants vary substantially in their Se uptake and physiological response to selenium (Terry et al., 2000). Most of the common plants are not able to accumulate more than 25 mg Se/kg of dry matter in the aboveground biomass. These plant species belong to so-called non-accumulators usually intolerant to the elevated Se contents in the environment (White et al., 2007). Although *Portulaca oleracea* belongs to the plant species relatively tolerant to the elevated Se contents in soil, the increase in soil Se contents resulted in the decrease of plant growth (Prabha et al., 2015). Similarly, Hermosillo-Cereceres et al. (2013) reported reduced biomass yield of *P. vulgaris* grown in solution containing more than 20 μM solution of selenite. Selenium in plants is bound into various compounds where sulfur is replaced by selenium, such as SeCys and SeMet (Ng and Anderson, 1978; Ellis and Salt, 2003). As an example of the complexity of Se metabolism in plants following biochemical process can be presented: SeMet can be synthesized from SeCys *via* three-step reaction catalyzed by three enzymes. The first one is cystathionine-γ-synthase binding SeCys to *o*-phosphohomoserine resulting in Se-cystathionine. In the second step, Se-cystathionine is transformed to Se-homocysteine with help of cystathionine-b-lyase. In the end, Met-synthase is responsible for transformation of Se-homocysteine to SeMet (Pilon-Smits and Quinn, 2010). Other frequently occurring organic compound, Se-methylselenocysteine (Se-MeSeCys) is synthesized from SeCys in the presence of selenocysteine methyltransferase. This compound is typically present in Se-fortified selenium accumulating plants whereas in non-accumulating plants treated by selenate the predominant Se compound in plants is again selenate (de Souza et al., 1999; Freeman et al., 2007).

Selenium is characterized by narrow range between essential and toxic levels in animals. Therefore, the cases documenting either selenium deficit or overdose of animals are relatively frequent (Terry et al., 2000). The regular consumption or the diet containing more than 1 mg Se/kg of the dry matter can result in chronic poisoning of animals; the diet containing 1000 mg Se/kg of dry matter can be lethal (Rosenfeld and Beath, 1964; Wilber, 1980). The response of Se application on the contents of this element in plants was widely investigated in crops suitable for human consumption (Rahman et al., 2015; Golob et al., 2015; Hermosillo-Cereceres et al., 2013; Mechora et al., 2011; Mahmud et al., 2010) or animal feeding (Bañuelos and Mayland, 2000; Seppaenen et al., 2010). However, the information concerning the fate of Se in wildlife plants potentially available for wildlife herbivores are limited. According to Žáková (2014) the pasture for horses collected at different places of the Czech Republic was Se-deficient and the animals reached the physiological Se levels in blood only *due* to Se-fortified commercial feed additives. The information concerning interactions of selenium with other essential macro- and microelements in plants (except sulfur) are limited, as well. On the contrary to the essential elements, the interactions between Se and risk elements such as As, Cd, and Pb were more intensively investigated (Duan et al., 2013; Hu et al., 2014; Yathavakilla and Caruso, 2007). Therefore, the main objectives of this study were (i) to assess the response of selected grassland plant species on foliar application of Se as affected by Se dose and plant species; in this context, relatively wide range of Se contents in different plant species growing at one location were observed by Sasmaz et al. (2015) and the differences among the individual plant species were expected on our study, as well; (ii) to compare Se transformation ability of the individual plant species; (iii) to estimate potential effect of increasing Se uptake by plants on the uptake of other essential elements by these plant species.

2. Material and methods

2.1. Experimental design and sampling

At the Humpolec location, an uncultivated meadow was selected, where three subplots (25 m^2 each) were marked out between 49°33.42′N, 15°21.06′E, and 49°33.46′N, 15°21.02′E. The bedrock is based predominantly on paragleyis, the soil type is gleyic Cambisol, the texture is loam. The natural pseudototal (*Aqua regia* soluble) Se contents in the soil at this area were monitored in our previous study (Száková et al., 2015). Water solution of sodium selenate (Na_2SeO_4) of the analytical grade purity was applied to each subplot at the beginning of the stem elongation as follows: (i) C—untreated variant (control); (ii) Se 1—the Se amount corresponding to the rate 25 g Se/ha; (iii) Se 2—the Se amount corresponding to the rate 50 g Se/ha. Representative samples (≈25 g) of the aboveground biomass of the individual plant species occurring at all the tree subplots from each subplot were randomly harvested in the flowering stage (*i.e.* ≈four weeks after Se application). The harvested biomass of plants was gently washed with deionized water, freeze-dried and finely ground by using of the laboratory mortar (Retsch SM 100, Germany) and kept at the dry place until the laboratory analyses. The plant species sampled were: *Holcus lanatus* L. and *Alopecurus pratensis* L. (*Poaceae*), *Carex vesicaria* L. (*Cyperaceae*), *Galium mollugo* (L) Scop. and *Galium aparine* L. (*Rubiaceae*), *Juncus effusus* L. (*Juncaceae*), *Chaerophyllum temulum* L. (*Apiaceae*), *Cirsium arvense* (L) Scop. (*Asteraceae*), *Ranunculus repens* L. (*Ranunculaceae*), *Veronica chamaedrys* L. (*Veronicaceae*), *Stellaria holostea* L. (*Silenaceae*), *Urtica dioica* L. (*Urticaceae*).

The composite samples of soil (depth 0–25 cm) were collected at each subplot together with the plant samples. Soil samples were dried at 20 °C, ground in a mortar, and passed through a 2-mm plastic sieve. The pseudototal (*i.e. Aqua regia* soluble) contents of investigated elements in the soil are summarized in Table 1.

2.2. Analytical methods

2.2.1. Determination of total element contents in plants and soils

For determination of element contents in freeze-dried and homogenized aboveground biomass of plants, an aliquot (~500 mg of dry matter) of the plant sample was weighed in a digestion vessel. Concentrated nitric acid (8.0 mL) (Analytika Ltd., Czech Republic), and 30% H_2O_2 (2.0 mL) (Analytika Ltd., Czech Republic) were added. The mixture was heated in an Ethos 1 (MLS GmbH,

Table 1
The pseudototal contents of investigated elements in soil (mg/kg) according to the individual subplots determined after harvest of the plants; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; data are presented as mean ± standard deviation, $n = 3$.

Treatment	Se	Ca	Cu	Fe	K
C	0.578^a	2178^a	19.8^b	22224^a	5978^a
Se 1	0.476^a	2138^a	17.1^a	22906^a	5592^a
Se 2	0.485^a	2104^a	17.0^a	23715^a	4719^a
	Mg	Mn	P	S	Zn
C	4458^a	233^a	418^a	453^b	58.5^a
Se 1	4335^a	248^a	380^b	321^a	55.2^a
Se 2	4319^a	316^b	409^a	337^a	57.1^a

Germany) microwave assisted wet digestion system for 30 min at 220 °C. The pseudototal concentrations of elements in the soils were determined in the digests obtained by the following decomposition procedure: Aliquots (~0.5 g) of air-dried soil samples were decomposed in a digestion vessel with 10 ml of *Aqua Regia* (*i.e.* nitric and hydrochloric acid mixture in a ratio of 1:3). The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 33 min at 210 °C. After cooling, the soil and plant digests were quantitatively transferred into a 25 mL glass tube, topped up by deionized water, and kept at laboratory temperature until measurements were taken.

Se content in the plant and soil digests was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA). The auto-sampler ASX-500, a three channel peristaltic pump, and MicroMist nebulizer equipped the ICP-MS. Inductively coupled plasma-atomic emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) equipped with a two channel peristaltic pump, a Sturman-Masters spray chamber and a V-groove pneumatic nebulizer made of inert material was applied for the determination of Cu, Fe, Mn, Zn, P, and S in the digests. The experimental conditions are described in detail by Šindelářová et al. (2015). Flame atomic absorption spectrometry (F-AAS, Varian 280FS, Varian, Australia) was used for Ca, Mg, and K determination in the digests.

For quality assurance of the data SRM NIST 1547 Peach leaves was applied. This material contains 0.120 ± 0.009 mg kg^{-1} Se, 15600 ± 2000 mg kg^{-1} Ca, 3.7 ± 0.4 mg kg^{-1} Cu, 218 ± 14 mg kg^{-1} Fe, 98 ± 3 mg kg^{-1} Mn, 24300 mg kg^{-1} K, 4320 mg kg^{-1} Mg, 1370 mg kg^{-1} P, 2000 mg kg^{-1} S, and 17.9 ± 0.4 mg kg^{-1} Zn. The obtained results were 0.125 ± 0.008 mg kg^{-1} Se, 15324 ± 528 mg kg^{-1} Ca, 3.6 ± 0.1 mg kg^{-1} Cu, 215 ± 11 mg kg^{-1} Fe, 93 ± 7 mg kg^{-1} Mn, 24285 ± 544 mg kg^{-1} K, 4338 ± 29 mg kg^{-1} Mg, 1363 ± 24 mg kg^{-1} P, 1962 ± 48 mg kg^{-1} S, and 17.2 ± 0.8 mg kg^{-1} Zn.

2.3. Selenium speciation in plants

For speciation analysis only Se2 treatments were analyzed and eight plant species were chosen for this analysis: four monocotyledoneous species (*J. effusus, H. lanatus, C. vesicaria,* and *A. pratensis*) and four dicotyledoneous species (*V. chamaedrys, U. dioica, S. holostea,* and *R. repens*). The samples were extracted by enzymatic hydrolysis as follows: ~0.5 g of the sample was exposed to 25 mg of protease XIV (Sigma–Aldrich, Japan) and 10 mL 20 mmol/L tris-(hydroxymetyl)-aminomethan (Fluka, Buchs, Switzerland) solution buffered (pH = 7.5) by HCl (Suprapur®, Merck, Darmstad, Germany) in polyfluor tube for 23 h at 37 °C under continual shaking. The reaction mixture was than centrifuged at 15000 rpm and 5 °C (Sigma 2–16 K centrifuge, Sigma Laborzentrifugen, Osterode, Germany), filtered through 0.45 μm syringe Nylon filter (Whatman, United Kingdom) and analyzed.

The chromatographic system consisted of a high pressure pump Series 200 (PerkinElmer, Shelton, USA), a degasser, a Rheodyne 9010 sampling valve (IDEX Health & Science LLC, Rohnert Park, CA, USA), and an analytical column PR-C8 (Purospher STAR-C8e, 250 × 4.6 mm, 5 μm, Merck). ICP-MS detection was performed with an ELAN DRC-e (Perkin Elmer Concord, Canada) equipped with a concentric PTFE nebulizer, a cyclonic glass spray chamber, and a high efficiency quartz torch. Measurement conditions were described in detail in our previous study (Šindelářová et al., 2015). The standards of the selenium species selenate (SeVI), selenite (SeIV), SeMet, selenocystine (SeCys2) and Se-MeSeCys were obtained from Sigma Aldrich (Steinheim, Germany). Selenoethionine (SeEt) was purchased from Santa Cruz Biotechnology (Dallas, USA).

2.4. Statistics

The analytical data were processed using Microsoft Office Excel 2007 and Statistica 12CZ statistical software. One-way analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$ followed by the Tukey's test were applied to the data.

3. Results and discussion

3.1. Uptake of selenium by individual plant species

Table 1 summarizes the pseudototal element contents in the soils varying between 0.476 and 0.578 mg/kg and confirming low soil Se levels comparable to low selenium levels in various European countries. For example, soil Se concentrations in Se-deficient North European countries were found in the range of 0.42–0.57 mg/kg Se (Gupta and Gupta, 2000). The results also indicate that the Se application did not affect the soil Se contents but the high variability of the results do not allow to assess the impact of selenization on the soil Se levels. However, the results also indicated relatively high variability of the element contents such as Cu, Mn, P, and S among the plots, as well. These differences should be taken into account in the case of evaluation of the differences these element contents in plants.

The Se contents in plants confirmed (i) differences among the selenization response of the individual species, (ii) increasing plant Se content with increasing Se rate, and low Se levels in untreated species confirming that no potential Se accumulating plant species occurred at the experimental plot (Table 2). The Se contents in the untreated plants were close to the detection limit 0.007 mg/kg in most of the species where only *C. vesicaria* and *G. mollugo* showed detectable Se contents, *i.e.* 0.016 and 0.31 mg/kg, respectively (Table 2). These values are comparable to the native Se contents in plants growing in the uncontaminated soil within the Europe. Lacatusu et al. (2012) monitored the Se levels in plants growing in the Se-deficient soil with an average value of 0.268 mg/kg. The average selenium content in the fodder plants at the investigated location was 0.019 mg/kg. De Temmerman et al. (2014) surveyed hundreds of soil and crop samples in Belgium for total Se contents where the Se contents in the soils were low (range 0.14–0.70 mg/kg). The Se contents in edible parts were dependent on plant species (for example, the vegetables within the genera *Allium* and *Brassica* tended to slightly higher Se accumulation ability) and varied in relatively wide range (the Se contents in more than 180 samples of *T. aestivum* varied between 0.004 and 0.303 mg/kg). In our set of samples, however, no species representing *Amaryllidaceae* and/or

Table 2
The total contents of selenium in aboveground biomass of investigated plant species (mg/kg) according to the individual subplots; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual rows; data are presented as mean ± standard deviation, $n = 3$.

Plant species	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)
Holcus lanatus	<0.007^a	0.377^b	0.399^b
Carex vesicaria	0.016^a	0.302^b	0.633^c
Gallium mollugo	0.031^a	0.270^b	0.608^c
Chaerophyllum temulum	<0.007^a	0.147^b	0.468^c
Cirsium arvense	<0.007^a	0.094^b	0.256^c
Alopecurus pratensis	<0.007^a	0.183^b	0.512^c
Ranunculus repens	<0.007^a	0.146^b	0.553^c
Veronica chamaedrys	<0.007^a	0.447^b	1.052^c
Stellaria holostea	<0.007^a	0.297^b	0.775^c
Urtica dioica	<0.007^a	0.720^b	0.712^b
Juncus effusus	<0.007^a	0.102^b	0.273^c
Gallium aparine	<0.007^a	0.620^b	0.745^c

Brassicaceae families occurred at the experimental site. Low Se uptake was also documented by Anjum et al. (2015) in the specific case of the Se contents in *Lolium perenne* growing at the extremely contaminated soil (total soil Se content 28.5 ± 3.7 mg/kg) reached up to 4.5 mg/kg in shoots.

Among the selenized plants, most of the species except *H. lanatus* and *U. dioica* (Table 2) showed stepwise increase of Se content with increasing Se dose. In the mentioned two species, the selenization resulted in the significant increase of Se content compared to control but the higher dose (50 g Se/ha) did not lead to significant increase of plant Se content compared to the dose 25 g Se/ha. For instance, in *H. lanatus* the Se contents increased from <0.007 mg/kg at the C subplot to 0.377 mg/kg at the lower Se dose and than reached only 0.399 at the higher dose although the applied Se content increased twice. Rahman et al. (2015) investigated the response of *Lens culinaris* on foliar application of Se. They applied total of 40 g/ha of Se as potassium selenate and seed Se concentration increased from 0.201 to 2.77 mg/kg, without any effect on seed weight and seed yield. Any of the plants tested in this study did not reach such high Se levels and proved that these species are not Se accumulators. The highest Se content exceeding slightly 1 mg/kg was determined in *V. chamaedrys* (Table 2). The results showed different Se uptake rate by the individual plant species even within one genus as can be documented by a comparison of Se uptake by *G. mollugo* (0.031, 0.270, and 0.608 mg/kg at C, Se1, and Se2 subplots, respectively) and *G. aparine* (<0.007, 0.620, and 0.745 mg/kg at C, Se1, and Se2 subplots, respectively). Similarly, apparent differences were observed for plants belonging both into *Poaceae* family, *H. lanatus* and *A. pratensis*, where the Se contents at the Se2 subplot were 0.399, and 0.512 mg/kg, respectively. Sors et al. (2009) explained the differences in Se accumulation ability between Se-hyperaccumulator *Astragalus bisulcatus* and the non-accumulator *A. drummondii* by the different biochemical processes of both plants demonstrated as the different activity of selenocysteine methyltransferase *in vitro*. These biochemical differences result in different abundance of Se compounds in plants. In our study, no contrast accumulating *vs.* non-accumulating plants occurred. However, detailed study of the biochemical processes in the individual selenized plant species could be helpful for the interpretation of the results as well as the determination of the

Table 3
The total contents of macro-and microelements in aboveground biomass of investigated plant species (mg/kg) according to the individual subplots; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual rows and elements; data are presented as mean ± standard deviation, n = 3.

Plant species	Ca			Cu			Fe		
	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)
Holcus lanatus	2699^{a}	1949^{a}	2665^{a}	2.99^{a}	2.97^{a}	2.64^{a}	39.7^{a}	30.4^{a}	33.8^{a}
Carex vesicaria	3422^{a}	1980^{b}	3351^{a}	5.37^{a}	4.11^{a}	5.19^{a}	51.3^{a}	19.6^{a}	42.4^{a}
Gallium mollugo	8385^{a}	6638^{a}	6216^{a}	5.72^{a}	3.24^{a}	3.90^{a}	49.4^{a}	32.2^{a}	22.8^{a}
Chaerophyllum temulum	22147^{a}	13794^{b}	19094^{c}	2.83^{a}	3.80^{b}	3.52^{c}	57.0^{a}	62.4^{a}	80.5^{a}
Cirsium arvense	18512^{a}	18114^{a}	21012^{a}	12.5^{a}	12.5^{a}	11.2^{b}	39.8^{a}	44.4^{a}	57.2a
Alopecurus pratensis	1556^{a}	1683^{a}	1120^{a}	4.38^{a}	3.75^{a}	3.77^{b}	56.7^{a}	44.5^{a}	28.9^{a}
Ranunculus repens	9984^{a}	8471^{a}	8424^{a}	9.77^{a}	8.57^{a}	6.74^{a}	27.6^{a}	36.5^{a}	41.5^{a}
Veronica chamaedrys	8094^{a}	8853^{b}	8445^{ab}	8.95^{a}	7.67^{a}	5.95^{b}	68.4^{a}	81.3^{a}	76.7^{a}
Stellaria holostea	4304^{a}	2471^{a}	3518^{a}	3.10^{a}	2.07^{a}	3.52^{a}	44.3^{a}	58.5^{a}	47.3^{a}
Urtica dioica	19904^{a}	21519^{a}	17380^{b}	7.51^{a}	3.33^{b}	6.91^{a}	52.0^{ab}	41.6^{a}	64.6^{b}
Juncus effusus	1551^{a}	1099^{a}	1359^{a}	5.29^{a}	6.23^{a}	5.02^{a}	25.1^{a}	21.4^{a}	15.3^{a}
Gallium aparine	7125^{a}	8874^{a}	9942^{a}	3.35^{a}	2.04^{a}	3.39a	23.2^{a}	21.9^{a}	59.9^{b}

Plant species	K			Mg			Mn		
	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)
Holcus lanatus	10522^{a}	9971^{a}	14247^{b}	1328^{a}	1009^{b}	1157^{ab}	212^{b}	155^{a}	169^{a}
Carex vesicaria	17964^{b}	22838^{a}	23074^{a}	2171^{a}	1185^{b}	1385^{c}	144^{b}	72.7^{a}	88.7^{a}
Gallium mollugo	16534^{a}	9191^{b}	16863^{a}	1993^{a}	1507^{a}	1417^{a}	46.0^{a}	43.1^{a}	46.9^{a}
Chaerophyllum temulum	11546^{b}	21408^{a}	22124^{a}	4461^{a}	3889^{b}	4202^{c}	81.6^{a}	41.3^{b}	90.8^{c}
Cirsium arvense	21201^{a}	26405^{b}	17307^{c}	5026^{a}	4271^{b}	5975^{c}	41.5^{a}	41.3^{a}	28.8^{b}
Alopecurus pratensis	12256^{a}	13093^{b}	13249^{c}	1344^{a}	985^{b}	630^{c}	80.6^{a}	76.8^{a}	36.6^{b}
Ranunculus repens	18772^{a}	13506^{ab}	10768^{b}	3983^{a}	2663^{a}	2347^{a}	125^{a}	84.5^{ab}	56.8^{b}
Veronica chamaedrys	17262^{a}	22790^{b}	18347^{a}	3158^{a}	3261^{a}	3274^{a}	45.6^{a}	52.3^{a}	51.2^{a}
Stellaria holostea	10389^{ab}	9756^{a}	18928^{b}	1811^{a}	1402^{a}	1957^{a}	90.1^{a}	80.5^{a}	172^{b}
Urtica dioica	18610^{a}	9067^{b}	18861^{a}	4266^{ab}	4555^{a}	4101^{b}	54.7^{a}	71.2^{a}	124^{b}
Juncus effusus	8573^{a}	8947^{a}	7500^{a}	850^{a}	741^{a}	726^{a}	146^{a}	121^{a}	135^{a}
Gallium aparine	19885^{a}	20109^{a}	19565^{a}	1392^{a}	1976^{ab}	2647^{b}	89.2^{a}	65.6^{a}	150^{b}

Plant species	P			S			Zn		
	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)	C (mg/kg)	Se 1 (mg/kg)	Se 2 (mg/kg)
Holcus lanatus	2023^{b}	1511^{a}	1707^{a}	2120^{a}	1325^{b}	2008^{a}	23.8^{a}	13.5^{b}	17.0^{c}
Carex vesicaria	1259^{b}	966^{a}	969^{a}	4598^{b}	3162^{a}	3006^{a}	35.6^{a}	18.9^{b}	24.9^{ab}
Gallium mollugo	2164^{a}	1704^{a}	1529^{a}	1865^{a}	1433^{a}	1338^{a}	29.7^{b}	12.7^{a}	15.1^{a}
Chaerophyllum temulum	1397^{b}	2321^{a}	2283^{a}	3558^{a}	4767^{b}	3758^{a}	7.71^{a}	9.68^{b}	10.6^{c}
Cirsium arvense	1747^{a}	1892^{a}	1787^{a}	7892^{a}	7425^{a}	6198^{b}	29.6^{a}	31.3^{a}	26.7^{a}
Alopecurus pratensis	1414^{a}	998^{a}	935^{a}	1419^{a}	1160^{a}	716^{b}	20.2^{a}	14.3^{a}	9.23^{a}
Ranunculus repens	2057^{a}	1982^{a}	1576^{a}	2102^{a}	1680^{a}	1495^{a}	33.1^{a}	23.9^{ab}	15.1^{b}
Veronica chamaedrys	1712^{a}	2367^{b}	1596^{a}	2457^{a}	3637^{b}	2321^{a}	29.2^{a}	31.6^{a}	26.7^{a}
Stellaria holostea	1409^{a}	1246^{a}	2161^{a}	1134^{a}	2083^{b}	1783^{c}	35.4^{a}	19.8^{b}	44.8^{c}
Urtica dioica	2263^{a}	1370^{b}	2447^{a}	3246^{a}	3147^{a}	5090^{a}	16.5^{a}	12.4^{a}	27.5^{b}
Juncus effusus	929^{a}	1056^{a}	858^{a}	1388^{a}	1547^{a}	1139^{a}	27.0^{a}	35.7^{a}	25.4^{a}
Gallium aparine	1557^{ab}	1116^{a}	1756^{b}	1458^{ab}	1100^{a}	1915^{b}	9.63^{a}	9.38^{a}	35.0^{b}

individual Se compounds in these plants. Filek et al. (2010) observed higher Se uptake ability of the dicotyledoneous plants compared to the monocotyledoneous because of higher permeability of membranes. In our case, however, the set of sampled species (among them, 4 species belong to the monocotyledoneous and remaining 8 species to the dicotyledoneous) is insufficient for reasonable statement in this case. Although the plant demonstrating the highest Se accumulation ability, *V. chamaedrys* reaching 1.05 mg/kg of Se (Table 2), belong into dicotyledoneous, for the other species no unambiguous trend was observed. White et al. (2007) documented that most of the common plants are characterized by the low tolerance to increased Se levels and are not able to accumulate more than 25 mg Se per kg of dry matter. Moreover, no visible symptoms of the potential Se phytotoxicity were observed.

3.2. The effect of selenization on the uptake of other essential elements

The Table 3 show various changes in the contents of other investigated elements in the plants indicating possible interrelationships between Se and other essential elements. The only exception is *C. vesicaria* without any significant changes in essential element contents with increasing Se dose. Among the elements, the relationships between selenium and sulfur were most frequently studied because of chemical similarity of these elements. Relatively complex interrelationships of Se and S in the rhizosphere of *Arabidopsis thaliana* was described by White et al. (2004). They observed that rhizosphere sulphate inhibits selenate uptake whereas rhizosphere selenate promotes sulphate. Thus, the potential Se–S mobility and plant-availability in soil are affected by the element forms and/or Se–S ratio in the soils. Moreover, interactions of these elements are expected within the plant biomass, as well. Se and S showed competitive relationships for a biochemical process, such as assimilation into amino acids of essential proteins. Antagonistic interrelationships of these elements in plants were described also by Dhillon and Dhillon (2000). In our case, no significant changes in plant S contents were observed in *G. mollugo*, *R. repens*, *U. dioica*, and *C. vesicaria*. Although the S contents in the remaining plant species varied (increased or decreased) significantly among the individual Se treatments, they did not reflect clearly the Se application rate. However, the mobile Se concentrations as well as the plant-available Se compounds in soil were not determined being under detection limit of the analytical technique. Moreover, variability of soil S at the experimental site (although the total S is not clearly related to the plant-availability of this element) should be also taken into account.

The behavior of other investigated essential elements was variable, as well. Among these elements, the less affected one seems to be iron. At the lower Se level no significant effect on Fe content was reported and at the higher Se value the significant increase of Fe was observed in *U. dioica* and *G. aparine* (Table 3). Ambiguous response of various essential elements on the Se application was reported by several other authors. Dhillon and Dhillon (2009) investigated the effect of elevated Se content in various crops on the uptake of other essential elements. The crops were cultivated in highly Se-contaminated soil containing from 2.8 to 4.3 mg Se per kg in the surface layer (0–15 cm). The Se contents in crops were crop-dependent and reached up to 200 mg/kg in leaves of *B. napus*. Except for S and P where significant positive correlations were calculated, contents of other nutrients (Zn, Cu, Mn and Fe) were not significantly affected by variations in the Se content of plants.

Presumably, the changes of the contents of individual essential elements are related to the particular biochemical processes of the individual plant species. The application of 25 g Se/ha resulted in significant decrease of Mg contents (compared to the C treatment) in 5 species whereas for the dose 50 g Se/ha in 3 species. For instance, the Mg contents in *C. vesicaria* dropped down from 2171 mg/kg at the C subplot to 1185 mg/kg at the Se1 subplot. Similarly, the Mg contents in *A. pratensis* varied between 1344 mg/kg at the C subplot and 630 mg/kg at the subplot Se2. These findings correspond to the results of Hartikainen and Xue (1999) and Hartikainen et al. (2000) documenting the adverse effects of Se, such as chlorosis, indicating supressed photosynthetic activity and therefore decreased Mg content in plants. However, in the cases of *C. temulum*,*C. vesicaria*, *C. arvense*, and *A. pratensis* (Table 3) the Mg contents decreased after application of 25 g Se/ha and again increased after application of 50 g Se/ha (in *C.vesicaria* from 1185 mg/kg at Se1 to 1385 mg/kg at Se2 subplots, respectively). These findings suggest that the plants mobilised their defence mechanisms against the potential Se toxicity. In this context, the changes in Fe, Cu, Mn, and Zn could be connected with the potential changes in the activity of antioxidative superoxid dismutases. These considerations are speculative because the activity of plant enzymes were not determined but these elements are connected with superoxid dismutases activity and increase of element levels can indicate the increase of the appropriate enzymatic activity (Fridovich 1995; Bannister et al., 1991; Alscher and Hess 1993). In our experiment, the Cu and Zn contents increased together in *C. temulum*, and *U. dioica* (Table 3). In other plant species either Cu or Zn increased or the both elements showed opposite pattern. For manganese, the plant contents tended more to decrease with increasing Se dose. The low alterations in plant Fe contents suggest that (i) the activity of Fe-superoxid dismutase remained unchanged because specific dismutases are related to the specific oxidative stress (Tsang et al. (1991), or (ii) chloroplasts were not subjected to the oxidative stress. Moreover, various enzymatic systems can be included into the biochemical response of plants on the oxidative stress. Hartikainen et al. (2000) demonstrated superoxid dismutase activity with increasing glutathione peroxidase (GSH-Px) activity in young plants of *L. multiflorum*. The authors assumed that Se-evoked decreased activity of superoxid dismutase indicate lower amounts of superoxide radicals *due* to the higher activity of GSH-Px. Xue and Hartikainen (2000) investigated the potential synergistic effect of Se supplementation and UV-irradiation in plants. This effect was apparent at lower Se levels where the activity of GSH-Px and catalase increased whereas ascorbate peroxidase decreased. However, the behavior of other enzymes such as glutathione-S-transferase and superoxide dismutase was plant-specific. The changes in the plant enzymatic activity (and subsequently the microelement contents in the plant tissues) can result from other antagonistic interrelationships of the elements, as well. For example, Van Camp et al. (1996) observed that increasing Fe-superoxid dismutase activity in chloroplasts can lead to decrease of Cu and Zn dismutases. The contents of remaining macronutrients (*i.e.* Ca, K, and P) in plants were altered in several cases, as well. However, the changes were not unambiguous. As documented by Filek et al. (2010), decreasing K contents in *B. napus* and *T. aestivum* plants can indicate the role of potassium in the regulation of osmotic pressure in the cells. Calcium can play the role in rebuilding of the cell membranes harmed due to potential Se toxicity (Filek et al., 2010; Guerrero et al., 2014). In our case, only in *C. temulum* and *V. chamaedrys* (Table 3) the Ca contents increased with increasing Se content indicating that the Se rates in our experiment did not lead to the damage of cell membranes. However, these speculations should be supported by more targeted further research. Phosphorus is involved in many biochemical processes in plants such as the transport of energy, and is bound into many essential molecules such as nucleic acids and phospholipids, but these processed are not directly connected with the uptake and transformation of selenium. In our case, the results did not indicate any changes connected with the changing

Se uptake by plants. Moreover, the substantial variability of the soil P contents at the investigated location (Table 1) can affect the plant P contents, as well.

3.3. Selenium compounds in the individual plant species

The results of the determination of individual Se species in aboveground biomass of plants are summarized in Table 4. The sample extraction *via* enzymatic hydrolysis released between 25 and 90% of the total Se content in plants. Total 10 different species were observed, but only 4 of them were identified, SeVI, SeCys2, Se-MeSeCys, and SeMet. The typical chromatograms of the Se species in two selected plants, *H. lanatus* and *V. chamaedrys*, are presented in Fig. 1. Concerning the minor unidentified species, their abundance in the total Se content in the extracts did not exceed 6%. Eiche et al. (2015) compared the Se uptake and speciation in *Triticum aestivum* and *Brassica juncea* growing in Se-rich soil. They found high proportion of selenate in *T. aestivum* and *B. juncea* leaves (47% and 70%, respectively) as the result of the inability of the plants to completely transform selenate to non-toxic organic forms under extremely high soil Se content. However, dimethylselenide and methylselenocysteine (MeSeCys) were identified in different parts of both plants, indicating an active detoxification response of plants to high Se uptake. Se species determined by using ion pair reversed phase HPLC-ICP-MS in biofortified grains (*i.e., T. aestivum* and *x Triticosecale*) were distributed into SeMet (76–85%) and selenomethionine selenoxide (51–60%), respectively (Kirby et al., 2008). In our case, selenate and SeMet were the predominant compounds in plants regardless of the plant species confirming only partial transformation of the applied selenate to the organoselenium compounds in the selenized plants.

Although only limited number of plant species were analyzed, the results suggested differences in the abundance of minor Se species, SeCys2 and Se-MeSeCys between monocotyledoneous and dicotyledoneous plants (Table 4). The occurence of various minor organoselenium compounds was documented by many previous investigations. Kápolna et al. (2009) applied HPLC-ICP-MS for determination of Se compounds in proteolytic extracts of the *Daucus carota* roots and leaves treated by foliar application of selenite and selenate. Besides of the inorganic Se present in both roots and leaves they identified SeMet in both roots and leaves and c-glutamyl-selenomethyl-selenocysteine (c-glu-MeSeCys) in roots regardless of the compound applied. Similarly, c-glu-MeSeCys, SeMet and Se-MeSeCys were identified in the proteolytic extracts of *Allium cepa* (Kápolna et al., 2012). However, the potential differences between Se transformation processes in monocotyledoneous and dicotyledoneous plants were not systematically investigated and compared. Our results indicate different response of various terrestrial plant species and the elucidation of the principles of the Se transformation on different plant species still remains for further research.

4. Conclusions

Both Se application rates resulted in significant increase of the Se content in plants where the response on Se treatment was affected by the plant species. The highest Se contents were determined in *V. chamaedrys* (1.052 ± 0.024 mg Se/kg) and *U. dioica* (0.720 ± 0.011 mg Se/kg) biomass whereas *C. arvense* and *C. vesicaria* showed the lowest Se uptake. Thus, the increase of the *V. chamaedrys* rate in the plant community of the selenized pasture areas can lead to the more effective biofortification of the roughage for ruminants but only in the mixture with the less accumulating plant species. The selenium speciation proved differences in the Se transformations among the individual plant species, especially in the case of SeCys2 and Se-MeSeCys. Although the results of the other essential macro-and microelements tended to change with increasing Se application rate, this study did not allow to express unambiguous conclusions concerning the potential inter-element interactions in the selenized plants. However, these aspects could be taken into account for further more detailed investigation.

The potential variability of Se uptake by the plant species growing in different climatic and soil conditions as well as the stability of the Se contents during several vegetation periods needs to be documented in further research. Moreover, the investigation should be targeted to the detailed description of the transformations of the Se compounds in future. The more detailed investigation of inter-element interactions in plants needs to emphasize their role in the plant biochemical processes. For better understanding of the Se metabolical processes in individual plant species the detailed determination of the antioxidative enzymes activities is necessary in further research to differentiate more clearly the Se proportion involved into the antioxidation activities. In this context, the relationships between Se transformation within the individual plants and related plant enzymatic activity should be investigated in further research. Moreover, other aspect remaining for the further research should be the metabolism of lipides where the saturation of lipides seems to be one of the plant defence mechanisms against the oxidative stress (Djebali et al., 2005). As observed by Filek et al. (2010), high Se doses caused saturation of lipides and subsequently in the increased membrane stiffness resulting in a smaller membrane permeability leading to the decrease in the accumulation of micro- and macroelements in plant biomass. Although no direct interrelationships between Se and other essential elements were observed in our case, the role of lipid metabolism could bring a new knowledge of Se role in the plant organisms.

Table 4
The contents of individual Se compounds in plants (μg/kg).

t_R (min) Species		1.98 SeVI	2.42 SeCys2	3.23 Se-MeSeCys	3.83	4.00	4.25	4.92 SeMet	5.77	6.18	10.20
J. effusus	M	29.0	2.1	22.6	*	*	*	17.3	*	*	*
H. lanatus	M	140.2	2.2	37.8	*	3.4	*	134.0	3.8	*	4.5
C. vesicaria	M	259.5	4.0	13.5	*	*	*	107.5	*	*	*
A. pratensis	M	16.3	10.5	14.8	*	4.6	*	121.9	*	*	5.5
V. chamaedrys	D	134.3	39.9	*	4.5	*	*	139.1	*	*	3.6
U. dioica	D	271.4	4.6	5.0	*	*	7.6	151.7	3.0	*	*
S. holostea	D	146.1	280.3	*	23.5	*	*	328.7	12.0	*	5.8
R. repens	D	43.9	109.8	3.5	19.2	*	*	176.1	*	*	*

*Data under detection limit (DL); DL = 0.1 μg/L Se in the extract, *i.e.* 2 μg/kg Se in sample.
M . . . monocotyledoneous plants, D . . . dicotyledoneous plants.

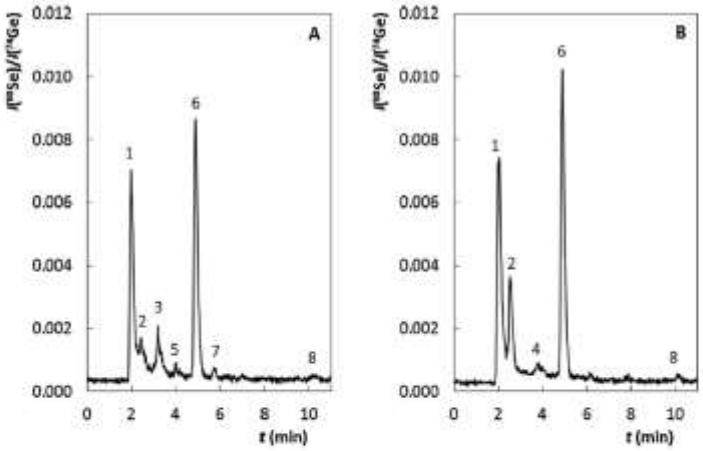

Fig. 1. An example of typical chromatogram of selenium species. A—*Holcus lanatus*, B—*Veronica chamaedrys*. 1—SeVI, 2—SeCys2, 3—Se-MeSeCys, 4, 5, 7, 8—unknown species, 6—SeMet.

Acknowledgements

Authors thank for financial support of the Czech Science Foundation (GACR) project No. 13-04580S; correction and improvement of language was provided by Proof-Reading-Service.com Ltd., Devonshire Business Centre, Works Road, Letchworth Garden City SG6 1GJ, United Kingdom.

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5.6 Publikovaný výstup č. 6

Název článku: The response of broccoli (*Brassica oleracea* convar. *italica*) varieties on foliar application of selenium: uptake, translocation, and speciation

Autoři: Šindelářová, K., Száková, J., Tremlová, J., Mestek, O., Praus, L., Kaňa, J., Najmanová, J., Tlustoš, P.

Publikováno v: Food Additives and Contaminants, Part A – Chemistry, Analysis, Control, Exposure and Risk Assessment 32. 2015. 2027-2038.

Food Additives & Contaminants: Part A, 2015
http://dx.doi.org/10.1080/19440049.2015.1099744

Taylor & Francis

The response of broccoli (*Brassica oleracea* convar. *italica*) varieties on foliar application of selenium: uptake, translocation, and speciation

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(*Received 7 July 2015; accepted 20 September 2015*)

A model small-scale field experiment was set up to investigate selenium (Se) uptake by four different varieties of broccoli plants, as well as the effect of Se foliar application on the uptake of essential elements for plants calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), and zinc (Zn). Foliar application of sodium selenate (Na_2SeO_4) was carried out at two rates (25 and 50 g Se/ha), and an untreated control variant was included. Analyses of individual parts of broccoli were performed, whereby it was found that Se in the plant accumulates mainly in the flower heads and slightly less in the leaves, stems, and roots, regardless of the Se rate and broccoli variety. In most cases, there was a statistically significant increase of Se content in all parts of the plant, while there was no confirmed systematic influence of the addition of Se on the changing intake of other monitored elements. Selenization of broccoli leads to an effective increase in the Se content at a rate of 25 g/ha, whereas the higher rate did not result in a substantial increase of Se content compared to the lower rate in all varieties. Therefore, the rate of 25 g/ha can be recommended as effective to produce broccoli with an increased Se content suitable for consumption. Moreover, Se application resulted in an adequate increase of the main organic compounds of Se, such as selenocystine (SeCys2), selenomethionine (SeMet), and Se-methylselenocysteine (Se-MeSeCys).

Keywords: selenium; broccoli varieties; supplementation; selenocystine; selenomethionine; Se-methylselenocysteine

Introduction

Selenium (Se) is an essential trace element that is important for humans and animals. Its main physiological function relates to the fact that Se is included in the enzyme glutathione peroxidase, which is responsible for removing excess peroxides and free radicals from the cells, which prevents the formation of malignant tumors. Another essential feature is, for example, Se's impact on the immune responses and maintenance of adequate levels of thyroid hormones. Thus, Se belongs to the elements with wide spectra of beneficial health effects, such as providing a decrease in the incidence of cancer, prophylaxis of cardiovascular diseases, therapy of particular muscle disorders, and delay of the onset of AIDS in HIV-positive patients (Hatfield 2001; Hatfield et al. 2014). Se belongs to the ubiquitous soil elements, but soil physicochemical parameters, the ability of the plant species to accumulate Se, and particular environmental conditions can affect its contents in crop production (Mehdi et al. 2013. The Se content of various diets differs significantly depending on the food items, and to a greater extent on the geographical origin and respective soil Se content (Kieliszek & Błażejak 2013). According to Dharmasena (2014), about 15% of the world's human population is hampered by insufficient uptake of Se. Although in the Czech Republic the Se status has increased due to human dietary supplements and/or Se supplementation of livestock feeding mixtures, the Se levels in blood serum indicate a deficit (Kvíčala 2003; Sager 2006).

The positive effect of Se in plants has also been described. Se supports the plant response to oxidative stress, slows down the ageing process, and improves the quality of crop production. For instance, Se prevents the decrease of tocoferol content during the ageing of lettuce (*Lactuca sativa*) leaves, increases the yield of potato tubers as well as the starch content, *etc*. (Xue et al. 2001; Turakainen et al. 2004). Plants are able to take up Se as selenite, selenate, and organic Se compounds as SeMet and selenocysteine (SeCys), whereas elemental Se as well as metal selenides are unavailable (White & Broadley 2009). While selenite is retained in roots, selenate is more easily transported to the aboveground biomass (Liu et al. 2009) and selenates are up to 20-fold more plant-available compared to selenite (Bitterli et al. 2010). Within the plants, selenates are metabolised predominantly to SeMet, the main Se compound in plants (Sunde 2001). SeMet can be further metabolised to Se-methylselenomethionine (Se-MeSeMet), which in turn is

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converted to Se-methylselenocysteine (Se-MeSeCys) and γ-glutamine-Se-methylselenocysteine (γ-glu-Se-MeSeCys) (Dumont et al. 2006). Among crops, fruit and vegetables rich in sugars and starch belong to the species with low ability to uptake Se. On the contrary, legumes, garlic, mushrooms, cabbage, and broccoli are characterised by higher Se uptake (Giri et al. 1990; Kadrabová et al. 1997; Sager 2006). The differences in Se contents in cereals in the different parts of the world document the role of soil Se content and/or mobility: the soil Se contents in Europe have varied between 0.009 and 0.034 mg/kg, in Turkey up to 0.072 mg/kg, and in the Se-rich areas of the USA up to 1.2 mg/kg (Kumpulainen 1993; Sager 2006).

As mentioned above, broccoli (*Brassica oleracea convar. italica, Brassicaceae*) belongs to the crops with a relatively high ability to take up Se, even in soils with a low content of this element. Moreover, a substantial portion of the accumulated Se is translocated to the flower heads (Pedrero et al. 2007). Broccoli itself contains bioactive compounds such as phenolic acids and sulphoraphanes, *i.e.* organosulfur compounds within the isothiocyanate group proven as agents inhibiting the risk of cancer incidence (Keck & Finley 2004). Vasanthi et al. (2009) explained that broccoli is an excellent source of bioactive compounds, including glucosinolates and their byproducts, phenolic compounds, antioxidant vitamins, and dietary minerals. Abdulah et al. (2009) and Liu et al. (2009) observed that Se-enriched broccoli sprouts could potentially be used as an alternative Se source for prostate cancer prevention and therapy. Bentley-Hewitt et al. (2014) proved that consumption of Se-enriched broccoli may increase immune responses toward a range of immune challenges. Therefore, the potential effect of Se supplementation on the quality and contents of the bioactive compounds in broccoli was widely investigated including the model animal experiments. Although enhanced Se content in broccoli has resulted in effective inhibition of colon cancer in rats, the production of phenolic acids and sulphoraphanes in broccoli was inhibited (even by 80%). Therefore, the enhancement of one of the bioactive components can result in an imbalance of other bioactive agents, and subsequently can result in unexpected metabolic interactions within the body (Finley et al. 2000; Finley 2005; Robbins et al. 2005). Matich et al. (2015) observed that fertilisation with Se slightly reduced (methylthio)glucosinolates and aglycons in the roots, but increased them in the flower heads, leaves, and stems of broccoli. Moreover, the contents of selenoglucosinolates exceeded those of their sulfur analogues in flower heads. In contrast to mature plants, no relationship between accumulation of Se and glucosinolates was observed in selenized broccoli sprouts (Ávila et al. 2014; Carvacho et al. 2014; Piekarska et al. 2014). Dietary intake of Se-fertilised broccoli can increase serum Se concentration by 25%, but did not affect the concentrations of glucosinolate metabolites in plasma and urine compared to regular broccoli (Hauder et al. 2011).

In contradiction to the interactions between organic bioactive compounds and Se in broccoli plants, information concerning the interactions of Se with other essential macro – and microelements in plants is limited. Mao et al. (2015) reported that Se, supplied either as selenate or selenite, may improve the germination and growth of broccoli growing in Zn-deficient soil. No substantial changes of Fe, Zn, Cu, or Mn contents in the selenized broccoli of different genotypes were observed by Ramos et al. (2011). Expectably, greater attention was given to the metabolism of sulfur, because selenate and sulfate share the initial assimilation route and Se fertilisation could interfere with sulfur metabolism and plant growth (Hsu et al. 2011). Hsu et al. (2011) observed increasing sulfate and total S contents in the shoots and decreasing contents of these analytes in the roots of young broccoli plants cultivated in sand culture where selenate was applied via the plant roots. Among the risk elements, the protective effect of Se supplementation against cadmium-induced oxidative stress in broccoli plants has been reported (Pedrero et al. 2008).

Among the available methods of Se content enhancement in crop production, foliar application is frequently used, although its effectivity can be influenced by various factors, such as the growth stage of the plant, climate, Se application technology and procedure, *etc.* (Aspila 2005). Whereas no differences were observed for organic compounds such as glucosinolates, tocopherols, and carotenoids in different broccoli varieties (Renaud et al. 2014), the Se contents in broccoli can be affected by the variety (Farnham et al. 2007). In our experiment, a small-scale field experiment was carried out to assess (i) the potential differences in plant response on foliar Se application among different varieties of broccoli and (ii) to evaluate and quantify the potential imbalance of the macro- and micronutrients in selenized broccoli plants.

Materials and methods

Field experiment

For the experiment, the experimental field of the University of Life Sciences was used (50°7'40"N, 14° 22'33"E, altitude 286 m a.s.l., average annual temperature 8.4°C). The soil is Chernozem with silt loam texture, a cation exchange capacity (CEC) of 230 mmol+/kg, pH (determined in 0.01 mol/L $CaCl_2$) 7.5, and an oxidisable carbon content (Cox) of 2.6%. Before planting, mineral fertiliser was applied in the rate of 500 kg NPK per hectare. The plants were cultivated in a precise field experiment (plot size of 8 × 10 m separated to 12 subplots). Four varieties of broccoli were cultivated – Heraklion F1, Marathon F1, Parthenon F1, and Naxos

F1 – where the plants were germinated in peat-based substrate moistened with deionised water in a greenhouse at a temperature varying between 18 and 21°C. Five weeks after germination, seedlings were transferred to field conditions. The plants of the individual varieties were put on three subplots (16 plants per subplot) where a completely randomised plot design was applied. Fungicides and insecticides were regularly applied during the vegetation according to the requirements. A water solution of sodium selenate (Na_2SeO_4) of the analytical grade purity was applied to each variety at the beginning of the head formation as follows: (i) Se0 – untreated variant; (ii) Se25 – the Se amount corresponding to the rate 25 g Se/ha; iii) Se50 – the Se amount corresponding to the rate 50 g Se/ha.

Three plants from each subplot were randomly harvested in consumable maturity (*i.e.* 4 weeks after Se application), separated to the flower heads, stems, leaves, and roots, and weighed. The harvested biomass of plants was gently washed with deionised water, freeze-dried, finely ground using a laboratory mortar (Retsch SM 100, Germany), and kept in a dry place until the laboratory analyses.

Analytical methods

Determination of total element contents in plants

The freeze-dried and homogenised plant samples were decomposed in a microwave-assisted wet digestion system with focused microwave heating (Discover SPD-Plus, CEM Inc., USA). An aliquot (~0.5 g of dry matter) of the plant sample was weighed in a quartz-glass digestion vessel (volume 35 mL) and 10.0 ml of concentrated nitric acid (Analytika Ltd., Czech Republic) was added; the mixture was heated at maximum power 300 W, temperature 202°C, and pressure 21 bar for 8 minutes. After cooling, the solution was quantitatively transferred to plastic tubes, filled to 50 mL with deionised water, and kept at laboratory temperature until measurement (Kelly et al. 2013). For quality assurance of the data SRM NIST 1547 Peach leaves was applied. This material contains 0.120 ± 0.009 mg.kg^{-1} Se, 15,600 ± 2000 mg.kg^{-1} Ca, 3.7 ± 0.4 mg.kg^{-1} Cu, 218 ± 14 mg.kg^{-1} Fe, 98 ± 3 mg.kg^{-1} Mn, 24,300 mg.kg^{-1} K, 4320 mg.kg^{-1} Mg, 1370 mg.kg^{-1} P, 2000 mg.kg^{-1} S, and 17.9 ± 0.4 mg.kg^{-1} Zn. The obtained results were 0.119 ± 0.006 mg.kg^{-1} Se, 15,237 ± 833 mg.kg^{-1} Ca, 3.5 ± 0.2 mg.kg^{-1} Cu, 212 ± 20 mg.kg^{-1} Fe, 95 ± 6 mg.kg^{-1} Mn, 24,184 ± 1606 mg.kg^{-1} K, 4320 ± 92 mg.kg^{-1} Mg, 1331 ± 34 mg.kg^{-1} P, 1821 ± 173 mg.kg^{-1} S, and 17.6 ± 1.1 mg.kg^{-1} Zn. Se content in the digests was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA) using a collision cell to reduce potential interferences (the experimental conditions were as follows: RF power of 1550 V, sample depth of 8 mm, plasma flow of 15.0 L min^{-1}, auxiliary flow of 0.9 L.min^{-1}, helium collision cell flow of 8 L.min^{-1}). The auto-sampler ASX-500, a three-channel peristaltic pump, and a MicroMist nebulizer equipped the ICP-MS. Inductively coupled plasma-atomic emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) equipped with a two-channel peristaltic pump, a Struman–Masters spray chamber, and a V-groove pneumatic nebulizer made of inert material was applied for the determination of Cu, Fe, Mn, Zn, P, and S in the digests (the experimental conditions were as follows: power of 1.2 kW, plasma flow of 15.0 L min^{-1}, auxiliary flow of 0.75 L.min^{-1}, nebulizer flow of 0.9 L.min^{-1}), whereas flame atomic absorption spectrometry (F-AAS, Varian 280FS, Varian, Australia; air flow of 13.5 L min^{-1}, acetylene flow of 2.2 L min^{-1}, burner height of 13.5 cm, nebulizer uptake rate of 5 mL min^{-1}) was used for Ca, Mg, and K determination in the digests.

Selenium speciation in plant samples

For speciation analysis, only flower heads were chosen where only Se0 and Se50 treatments were analysed. The freeze-dried and homogenised samples were extracted by enzymatic hydrolysis as follows: ~0.5 g of the sample was exposed to 25 mg of protease XIV (Sigma-Aldrich, Japan) and 10 mL of 20 mmol.L^{-1} tris-(hydroxymetyl)-aminomethan (Fluka, Buchs, Swiss) solution buffered (pH = 7.5) by HCl (Suprapur®, Merck, Darmstadt, Germany) in a polyfluor tube for 23 h at 37°C under continual shaking. The reaction mixture was than centrifuged at 15,000 rpm and 5°C (Sigma 2–16 K centrifuge, Sigma Laborzentrifugen, Osterode, Germany), filtered through a 0.45 µm syringe Nylon filter (Whatman, United Kingdom), and analysed.

The chromatographic system consisted of a high pressure pump Series 200 (Perkin Elmer, Shelton, USA), a degasser, a Rheodyne 9010 sampling valve (IDEX Health & Science LLC, Rohnert Park, CA, USA) equipped with a 50 µL PEEK sample loop, and an analytical column PR-C8 (Purospher STAR-C8e, 250 × 4.6 mm, 5 µm, Merck). ICP-MS detection was performed with an ELAN DRC-e (Perkin Elmer Concord, Canada) equipped with a concentric PTFE nebulizer, a cyclonic glass spray chamber, and a high-efficiency quartz torch. Measurement conditions are: r.f. power 1 400 W, nebulizer flow of 0.72 L/min, auxiliary gas flow of 1.0 L/min, plasma flow of 11.0 L/min. Measured isotopes were ^{80}Se (analyte) ^{74}Ge (internal standard). Methan (flow 0,60 mL/min) was used as DRC reaction gas for the removal of spectral interferences due to formation of polyatomic ions. The course of chromatograms were monitored in steps of 1 s duration.

The mobile phase for the chromatographic separation consisted of 0.8 g/L sodium butane-1-sulfonate, 2.9 g/L tetramethylammonium hydroxide (all purchased from Sigma Aldrich), 0.42 g/L malonic acid, and 1% (v/v) methanol. The pH of the solution was adjusted to 5.0 by adding small portions of hydrochloric acid (Suprapur, Merck). The mobile phase was spiked by 1 g/L stock Ge solution (CertiPur, Merck) to make a final concentration of 100 µg/·L Ge.

The mobile phase flow was 1 mL/min. The standards of the selenium species selenate (SeVI) (BioXtra grade), selenite (SeIV) (BioReagent grade), SeMet (min. 99%), SeCys2 (min. 98%) and Se-MeSeCys (min. 95%) were obtained from Sigma Aldrich (Steinheim, Germany). Selenoethionine (SeEt) (min. 99%) was purchased from Santa Cruz Biotechnology (Dallas, USA).

Basic performance characteristic of selenium species determination are: LOD 0.1 ng/L Se (corresponding with 2 ng/g Se in solid sample), LOQ 0,3 ng/L Se (corresponding with 6 ng/g Se in solid sample), repeatability 11%. For more detail see our previous papers (Balan et al. 2014; Klognerova et al. 2015). Linearity of determination of selenium species was proven in range minimaly 0–100 ng/L Se (corresponding with 2000 ng/g Se in solid sample).

Statistics

The analytical data were processed using Microsoft Office Excel 2007 and Statistica 12 CZ statistical software. One-way analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$ followed by the Tukey's test were applied to the data.

Results

Uptake of selenium and other macro- and microelements by plants

Table 1 summarises the yields of the individual parts of broccoli plants where the results documented that the yields were not significantly affected by the Se application. Although Se is a beneficial element for plants, toxicity symptoms, such as limited growth, chlorosis, decreased protein synthesis, and enhanced oxidative stress, can result from high Se contents in the plants (Hartikainen et al. 2000). In our case, the Se rates did not cause any visible toxicity symptoms or other adverse effects. Except for Marathon, the varieties tended toward an increased yield with increasing Se rate. More apparent differences in plant yield were observed among the varieties than among the treatments. The highest yield of flower heads was observed for Naxos and the lowest in the case of Heraklion. The optimisation of the foliar application of Se is an important measure for effective production of Se-enriched crops and will differ according to the individual plant species and cropping conditions. Rahman et al. (2015) applied 40 g Se/ha (as K_2SeO_4 solution) on leaves of lentil (*Lens culinaris*) without any adverse effects on the yield or size of seeds. Similarly, Broadley et al. (2010) and Lyons et al. (2005) reported no significant changes in yield parameters of winter wheat (*Triticum aestivum*) after foliar application of Na_2SeO_4 in rates of up to 100 g Se/ha and 120 g Se/ha, respectively.

Table 1. The yield of individual parts of broccoli plants (g of fresh matter per plant) according to the individual varieties; The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; if no letters within the columnn, no significant differences were observed; data are presented as mean ± standard deviation, n = 3.

Treatment	Flower heads (g)	Leaves (g)	Stems (g)	Roots (g)
Heraklion				
Se 0	570 ± 136	1130 ± 521	334 ± 90	90 ± 27
Se 25	562 ± 164	980 ± 263	365 ± 86	127 ± 8
Se 50	584 ± 116	1108 ± 128	431 ± 142	95 ± 32
Marathon				
Se 0	624 ± 83	1367 ± 269	435 ± 47	107 ± 7
Se 25	538 ± 89	1473 ± 246	448 ± 64	81 ± 29
Se 50	521 ± 75	1643 ± 375	565 ± 136	81 ± 21
Parthenon				
Se 0	617 ± 109	1455 ± 196	374 ± 8	88 ± 11
Se 25	818 ± 156	1795 ± 261	548 ± 72	120 ± 30
Se 50	732 ± 142	2085 ± 49	544 ± 49	94 ± 6
Naxos				
Se 0	883 ± 255	1545 ± 224	366 ± 69	101 ± 15
Se 25	885 ± 183	1483 ± 246	378 ± 103	119 ± 27
Se 50	955 ± 229	1690 ± 262	405 ± 69	147 ± 42

The contents of Se and other macro- and microelements in individual parts of plants are summarised in Tables 2–5. In flower heads, the Se contents increased in the selenized treatments, but the significant stepwise increase with increasing Se rate was observed only in Parthenon and Naxos. For the other varieties, the Se50 rate did not result in significant increase of Se contents in flower heads compared to Se25. A similar pattern was observed for leaves. In the case of stems and roots, the significant stepwise increase of Se content occurred only in Parthenon, whereas Se contents in Naxos tended to the increase as well, but the statistical evaluation was hampered by high variability of the results. The Se contents in untreated (Se0) flower heads varied between 0.034 and 0.056 mg/kg, with higher variability within one variety than among the varieties. Similar levels were documented by Farnham et al. (2007), where Se levels in flower heads of different varieties were in the range between 0.053 mg/kg and 0.085 mg/kg. The foliar application of Se resulted in a significant increase of the Se contents in all the plant parts, including the roots. However, the highest Se contents were observed in flower heads varying between

Table 2. The element contents in broccoli heads according to the individual varieties (mg/kg of dry matter); The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; if no letters within the columnn, no significant differences were observed; data are presented as mean ± standard deviation, n = 3.

	Se mg/kg	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg	S mg/kg	Zn mg/kg
Heraklion										
Se 0	0.056 ± 0.014a	2603 ± 308	2.86 ± 0.17	39.7 ± 3.4	28,653 ± 4640	975 ± 194	19.5 ± 0.8	4786 ± 431	6567 ± 115	26.8 ± 2.0
Se 25	0.670 ± 0.339b	2766 ± 183	3.01 ± 0.16	96.0 ± 83.4	31,177 ± 2364	1119 ± 82	20.4 ± 2.3	5108 ± 61	6755 ± 422	27.9 ± 2.4
Se 50	1.01 ± 0.15b	2690 ± 284	3.27 ± 0.17	63.7 ± 18.1	28,417 ± 1615	1162 ± 122	20.1 ± 0.6	5086 ± 287	6770 ± 994	30.7 ± 2.8
Marathon										
Se 0	0.038 ± 0.023a	2987 ± 323	3.30 ± 0.23	45.0 ± 9.4	31,412 ± 2601	1072 ± 64	19.9 ± 0.8b	5597 ± 164	7220 ± 214	29.3 ± 0.4
Se 25	0.335 ± 0.103b	3060 ± 169	3.07 ± 0.47	29.6 ± 1.8	29,844 ± 3235	1014 ± 156	16.9 ± 0.8a	5495 ± 424	7405 ± 544	29.0 ± 1.6
Se 50	0.577 ± 0.145b	2725 ± 277	3.00 ± 0.30	34.6 ± 4.9	29,077 ± 2605	1161 ± 29	16.9 ± 1.4a	5684 ± 159	6870 ± 442	29.4 ± 1.7
Parthenon										
Se 0	0.034 ± 0.007a	3260 ± 399	3.15 ± 0.36	38.2 ± 5.0	28,259 ± 2828	1122 ± 165	17.7 ± 2.4	5586 ± 454	7854 ± 718	28.2 ± 2.7
Se 25	0.336 ± 0.067b	3034 ± 290	3.22 ± 0.19	40.2 ± 4.1	26,471 ± 1039	1264 ± 71	19.1 ± 0.9	5932 ± 314	8238 ± 280	30.7 ± 3.0
Se 50	0.513 ± 0.097c	3097 ± 137	3.58 ± 0.45	43.9 ± 5.3	26,734 ± 870	1279 ± 102	19.4 ± 1.3	6028 ± 698	8166 ± 387	32.6 ± 4.7
Naxos										
Se 0	0.052 ± 0.054a	2508 ± 242	2.61 ± 0.44	50.3 ± 6.2	31,315 ± 2963	1111 ± 111	18.3 ± 1.7a	5353 ± 358	7356 ± 106	26.4 ± 2.8
Se 25	0.427 ± 0.068b	2396 ± 440	2.79 ± 0.27	42.9 ± 3.0	28,921 ± 3421	1152 ± 46	18.7 ± 1.2	5522 ± 196	7190 ± 122	29.7 ± 1.4
Se 50	0.931 ± 0.220c	2628 ± 317	2.81 ± 0.17a	46.7 ± 2.6	32,401 ± 1688	1156 ± 122	18.6 ± 0.5	5420 ± 327	7308 ± 208	28.0 ± 1.1

Table 3. The element contents in broccoli leaves according to the individual varieties (mg/kg of dry matter); The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; if no letters within the column, no significant differences were observed; data are presented as mean ± standard deviation, n = 3.

	Se mg/kg	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg	S mg/kg	Zn mg/kg
Heraklion										
Se 0	0.046 ± 0.019a	24,673 ± 3548	1.61 ± 0.46	54.0 ± 12.9	30,119 ± 3940	852 ± 213	45.4 ± 6.4	3146 ± 801	5967 ± 890	19.4 ± 2.0
Se 25	0.925 ± 0.457b	31,149 ± 4882	1.97 ± 0.38	85.6 ± 27.2	35,774 ± 5201	1100 ± 33	54.0 ± 2.0	3492 ± 463	8078 ± 1493	20.0 ± 5.6
Se 50	0.689 ± 0.054ab	36,073 ± 6847	2.03 ± 0.29	83.8 ± 31.0	30,238 ± 1897	1284 ± 223	55.6 ± 8.1	3406 ± 200	6944 ± 2173	19.1 ± 2.5
Marathon										
Se 0	0.029 ± 0.005a	25,902 ± 3684	2.45 ± 0.17	112 ± 15	33,977 ± 6859	1015 ± 53a	50.9 ± 5.9	4514 ± 233	7551 ± 478	19.8 ± 0.8b
Se 25	0.351 ± 0.173ab	28,272 ± 5472	2.48 ± 0.23	133 ± 18	30,561 ± 571	962 ± 16a	47.2 ± 1.4	3921 ± 389	8159 ± 554	17.4 ± 0.9a
Se 50	0.468 ± 0.165b	31,232 ± 6965	2.39 ± 0.03	124 ± 32	35,206 ± 6379	1242 ± 41b	60.4 ± 15.8	3982 ± 656	6978 ± 612	18.3 ± 0.5ab
Parthenon										
Se 0	0.024 ± 0.007a	28,828 ± 9026	1.82 ± 0.39	116 ± 10	27,727 ± 4611	1076 ± 144	48.0 ± 6.3a	3259 ± 943	6925 ± 1120	14.9 ± 2.7
Se 25	0.205 ± 0.042b	25,456 ± 1695	2.06 ± 0.31	71.6 ± 6.7	30,983 ± 2855	1141 ± 174	51.8 ± 5.0ab	3748 ± 185	7592 ± 331	15.6 ± 1.2
Se 50	0.383 ± 0.050c	28,284 ± 1020	2.21 ± 0.30	77.1 ± 8.3	29,165 ± 7370	1240 ± 131	61.7 ± 2.1b	3966 ± 354	7299 ± 92	17.5 ± 1.7
Naxos										
Se 0	0.026 ± 0.012a	24,872 ± 4676a	2.09 ± 0.26	75.8 ± 12.9	27,263 ± 512	1180 ± 43	47.1 ± 0.9	4254 ± 993	8067 ± 577	19.9 ± 3.0
Se 25	0.319 ± 0.077b	36,558 ± 4294b	2.23 ± 0.35	132 ± 14.6	30,487 ± 5343	1537 ± 252	59.6 ± 12.0	3560 ± 222	7282 ± 1186	16.6 ± 1.7
Se 50	0.847 ± 0.171c	33,531 ± 2187ab	2.46 ± 0.14	109 ± 17.2	27,084 ± 2669	1540 ± 250	60.1 ± 4.5	3902 ± 267	8594 ± 591	19.4 ± 1.3

Table 4. The element contents in broccoli stems according to the individual varieties (mg/kg of dry matter); The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; if no letters within the column, no significant differences were observed; data are presented as mean ± standard deviation, n = 3.

	Se mg/kg	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg	S mg/kg	Zn mg/kg
Heraklion										
Se 0	0.067 ± 0.028a	7070 ± 1236	3.46 ± 0.34	27.2 ± 8.3	65,463 ± 1538	1840 ± 636	14.3 ± 3.4	4374 ± 457	7421 ± 1585	20.9 ± 6.6
Se 25	0.800 ± 0.210b	8441 ± 1400	3.21 ± 0.47	41.0 ± 7.3	64,092 ± 9572	1970 ± 39	15.0 ± 1.1	4588 ± 629	7111 ± 967	20.8 ± 3.1
Se 50	0.777 ± 0.233b	8723 ± 678	3.77 ± 0.64	57.6 ± 34.2	68,306 ± 8505	2176 ± 242	15.6 ± 2.6	4839 ± 611	6924 ± 1435	22.1 ± 4.3
Marathon										
Se 0	0.035 ± 0.017a	8699 ± 790	4.96 ± 0.29	29.2 ± 1.0	76,564 ± 2386	2024 ± 39	15.9 ± 1.8	5166 ± 20	8336 ± 121	24.1 ± 1.5
Se 25	0.284 ± 0.090ab	10,265 ± 1141	3.78 ± 0.28	43.0 ± 4.9	73,310 ± 7773	2117 ± 65	17.3 ± 3.1	5098 ± 120	8156 ± 114	25.2 ± 0.9
Se 50	0.494 ± 0.178b	8755 ± 1382	4.91 ± 0.93	38.0 ± 12.9	67,413 ± 8317a	2083 ± 91	17.2 ± 3.5	4609 ± 486	8154 ± 1427	24.8 ± 5.1
Parthenon										
Se 0	0.042 ± 0.010a	9228 ± 462	3.88 ± 0.79a	37.7 ± 6.1	56,096 ± 6914	2105 ± 184	16.3 ± 3.5	4592 ± 299a	7947 ± 490	24.8 ± 1.4
Se 25	0.203 ± 0.026b	8501 ± 1600	4.77 ± 0.15a	36.6 ± 5.0	53,346 ± 4797	2325 ± 132	18.8 ± 4.0	5278 ± 295ab	8112 ± 235	27.1 ± 0.6
Se 50	0.382 ± 0.049c	7820 ± 817	6.90 ± 1.10b	35.2 ± 4.1	75,158 ± 8079	2272 ± 191	19.4 ± 2.2	5748 ± 321b	8788 ± 406	28.7 ± 4.2
Naxos										
Se 0	0.026 ± 0.007a	8137 ± 1734	5.10 ± 0.39	42.7 ± 6.7	78,452 ± 15,476	2544 ± 359ab	16.1 ± 1.8	5160 ± 776	8818 ± 1053	23.8 ± 1.8
Se 25	0.388 ± 0.104ab	9589 ± 846	3.94 ± 0.50	45.0 ± 6.8	61,985 ± 10,205	2234 ± 113a	14.3 ± 0.9	4934 ± 652	7596 ± 754	21.6 ± 1.3
Se 50	0.899 ± 0.475b	9109 ± 773	5.42 ± 1.29	39.6 ± 2.9	76,307 ± 6130	2892 ± 228b	14.0 ± 1.4	5556 ± 666	9494 ± 704	25.6 ± 2.3

Table 5. The element contents in broccoli roots according to the individual varieties (mg/kg of dry matter); The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns; if no letters within the column, no significant differences were observed; data are presented as mean ± standard deviation, n = 3.

	Se mg/kg	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg	S mg/kg	Zn mg/kg
Heraklion										
Se 0	0.034 ± 0.010a	5842 ± 861	4.25 ± 0.96	316 ± 65	33,580 ± 3539	1893 ± 227	33.5 ± 12.6	2964 ± 300	3660 ± 393	26.3 ± 4.7
Se 25	0.372 ± 0.068b	5891 ± 387	13.4 ± 3.7	393 ± 73	38,723 ± 9647	2088 ± 56	24.3 ± 7.6	2988 ± 245	3990 ± 571	24.9 ± 5.5
Se 50	0.294 ± 0.031b	6103 ± 830	9.87 ± 0.96	161 ± 13	33,806 ± 4650	2064 ± 417	29.0 ± 5.9	2444 ± 504	3251 ± 119	23.4 ± 6.3
Marathon										
Se 0	0.032 ± 0.030a	7740 ± 1523	4.57 ± 1.70	123 ± 56	42,570 ± 7219	2185 ± 285	27.7 ± 5.4	3515 ± 445	3653 ± 672	26.4 ± 5.4
Se 25	0.170 ± 0.067b	5136 ± 1045	2.50 ± 0.09	73.9 ± 26.3	28,674 ± 5932	1733 ± 290	22.2 ± 6.2	3125 ± 310	2949 ± 537	18.5 ± 4.1
Se 50	0.277 ± 0.021b	5599 ± 579	2.80 ± 0.22	101 ± 16	28,903 ± 2482	1875 ± 140	26.9 ± 7.1	2681 ± 413	3104 ± 414	21.8 ± 2.8
Parthenon										
Se 0	0.018 ± 0.001a	4618 ± 744	2.64 ± 0.27	75.3 ± 4.6	25,079 ± 4603	1879 ± 116	20.8 ± 6.1	3409 ± 610	3686 ± 268	19.8 ± 2.6
Se 25	0.135 ± 0.026b	5647 ± 514	2.53 ± 0.15	90.4 ± 8.4	27,716 ± 2922	2132 ± 241	29.0 ± 6.1	3963 ± 609	4153 ± 473	25.0 ± 2.4
Se 50	0.276 ± 0.039c	6420 ± 1269	5.22 ± 2.28	201 ± 93	28,146 ± 3268	2229 ± 45	37.4 ± 7.7	4034 ± 143	4082 ± 289	27.0 ± 4.2
Naxos										
Se 0	0.024 ± 0.008a	6512 ± 653	4.63 ± 1.49a	185 ± 33a	35,084 ± 3274	2463 ± 190	30.8 ± 6.2	3160 ± 673	4885 ± 732	23.7 ± 0.7
Se 25	0.237 ± 0.063b	7683 ± 1692	14.6 ± 4.3b	355 ± 26b	35,352 ± 4440	2310 ± 239	34.3 ± 4.6	3173 ± 226	4947 ± 307	24.9 ± 2.4
Se 50	0.385 ± 0.130b	6421 ± 896	13.1 ± 3.2b	232 ± 73a	34,923 ± 2283	2225 ± 71	24.2 ± 6.2	2753 ± 361	4375 ± 359	22.8 ± 2.8

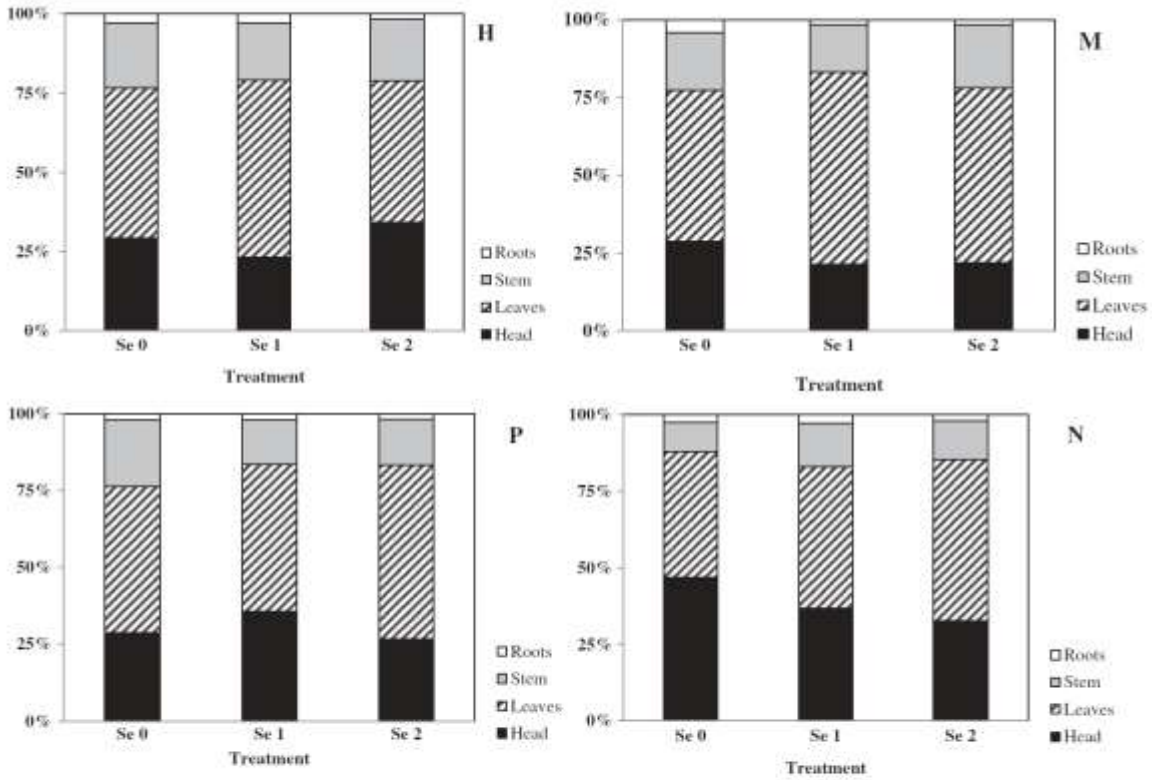

Figure 1. Selenium distribution within broccoli plants according to the selenium rate and variety; P – Parthenon, M – Marathon, H – Heraklion, N – Naxos.

0.513 (Parthenon) and 1.01 (Heraklion) mg/kg, respectively. Similarly, Tobiasz et al. (2014) observed higher Se contents in seedlings and leaves of wheat (*Triticum aestivum*) grown in soilless culture compared to roots, confirming that Se is preferably accumulated by the actively growing aboveground parts of plants. Comparable findings were published by Filek et al. (2010) for wheat (*Triticum aestivum*) and oilseed rape (*Brassica napus*) plants. Figure 1 presents the Se distribution within the broccoli plants as affected by Se application rate and variety. The predominant proportion of Se total amount was represented by leaves, followed by flower heads and stems. The lowest percentage of Se was found in roots, regardless of the variety and Se rate. Therefore, the foliar application of selenate did not result in significant imbalance in the Se distribution within the plant. Except for Heraklion, the Se proportion in the flower heads slightly decreased with the increasing Se rate, and consequently the se proportions in leaves increased.

Selenium speciation in the flower heads of broccoli

The results of the determination of individual Se species in flower heads are summarised in Table 6. The mean extraction yield was 98%. As mentioned above, 17 different species were reported, but only four of them were identified. The typical chromatogram of the Se species is presented in Figure 2. Dominant Se species were eluted during the first 10 minutes of separation, *i.e.* SeVI, SeCys2, Se-MeSeCys, SeMet, and 2 unknown species. Further, 11 unknown species of negligible abundance were eluted during next 10 min. Among them, SeMet seemed to be the predominant species, followed by a relatively similar distribution of Se (VI), SeCys2, Se-MeSeCys, and one of the unknown species occurring at the t_R = 3.98 min, regardless of the Se application. Thosaikham et al. (2014) applied HPLC-ICP-MS based on ion-pairing reversed phase chromatography for the Se speciation using the mixture of 1-butanesulfonic acid (BA) and trifluoroacetic acid (TFA) as the mixed ion-pairing reagents to *Brassica chinensis var parachinensis* plants. They observed the presence of selenite, selenate,

Table 6. The contents of individual selenium species in broccoli heads according to the individual varieties (μg/kg of dry matter); P – Parthenon, M – Marathon, H – Heraklion, N – Naxos; the standard errors of the individual measurements were < 10 %.

Species	Se (VI)	SeCys2	U	U	Se-Me SeCys	U	SeMet	U	U	U	U	U	U	U	U	U	U
t_R (min)	2.08	2.53	2.98	3.32	3.40	3.98	4.95	5.82	7.12	7.80	8.92	9.87	13.27	14.25	16.95	19.17	20.10
H/Se0	15.8	10.5	*	*	5.64	10.9	22.5	*	*	*	*	*	*	*	*	*	*
M/Se0	8.75	11.6	*	*	10.7	8.53	15.5	*	*	*	*	*	*	*	*	*	*
P/Se0	7.48	9.77	*	*	6.58	5.31	10.9	*	*	*	*	*	*	*	*	*	*
N/Se0	7.48	9.06	*	*	2.33	4.37	7.24	*	*	*	*	*	*	*	*	*	*
H/Se50	162	126	21.9	42.5	120	166	413	36.9	4.85	11.5	15.3	13.2	10.4	16.6	9.96	5.04	4.09
M/Se50	24.6	41.5	19.0	12.7	54.6	101	238	7.59	5.47	6.96	*	8.16	*	8.94	2.35	*	*
P/Se50	20.8	50.5	10.4	*	52.0	80.3	177	15.6	*	4.29	*	2.97	*	6.15	*	7.32	7.32
N/Se50	116	171	35.3	18.8	93.6	112	287	26.3	4.82	7.09	8.75	7.73	2.55	6.50	4.90	*	*

U . . .unidentified; t_R. . .retention time; *values under detection limit

SeCys, Se-MeSeCys, SeMet, and several unknown species (limits of detection and quantitation of each Se species were lower than 5 and 16 μg Se/L, respectively). Similarly, predominant abundance of SeMet, Se-MeSeCys, and unknown species were determined in 0.1 mol/L HCl in 10% methanol extracts of *Brassica oleracea* var. *alboglabra* by ion-pair reversed-phase HPLC-ICP-MS (Maneetong et al. 2013). It should be pointed out that the occurrence of selenocysteine in a chromatographic column may positively distort the determined selenocystine signal (two selenocysteine units linked with a – Se-Se- covalent bond); Wróbel et al. (2004) insufficiently resolved SeCys and SeCys2 peaks during an ion-pair reversed-phase HPLC process. The results of the speciation analyses could be significantly affected by the sample pre-treatment and extraction procedure. Roberge et al. (2003) compared various extraction techniques for Se speciation regarding the potential changes of the Se compounds during the extraction and determination. They stated that the most suitable extractions are those matching the conditions present in the matrix. Therefore, enzymatic extraction seems to be a suitable technique in this case.

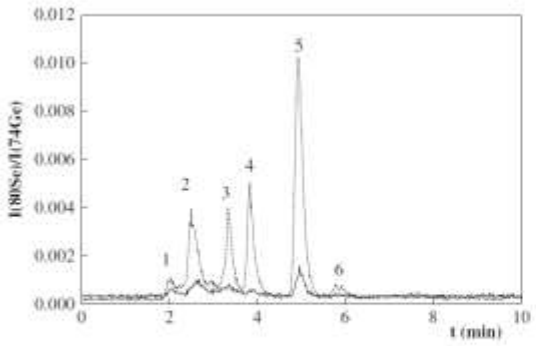

Figure 2. An example of typical chromatogram of selenium species. Full line – variety Parthenon without Se application, dashed line – Se treated variety Parthenon. 1 – SeVI, 2 – SeCys2, 3 – Se-MeSeCys, 4, 6 – unknown species, 5 – SeMet.

Discussion

Pedrero et al. (2007) cultivated broccoli plants in soilless culture enriched with sodium selenate in a concentration of 1 mg/L. The plants reached the Se levels in flower heads even up to 27 ± 3 mg/kg without any toxicity symptoms. Additionally, Hsu et al. (2011) observed the ability of broccoli to accumulate Se in levels exceeding the level recommended for human consumption without any changes in plant growth, and without any changes in contents of cysteine, glutathione, total glucosinolates, *etc*. These levels document a high tolerance of broccoli plants to extremely high Se contents, as well as their significant ability to accumulate this element in the aboveground biomass. However, a lower increase in Se contents can be expected in soil-growing broccoli because of the limited mobility and bioavailability of this element in the soil. In this context, different bioavailabilities of the various Se compounds should be mentioned, where better availability of selenate compared to selenite was previously documented after both soil application (Aspila 2005) and foliar application (Seppänen et al. 2010). Thus, the application of selenate will be more effective selenization measure leading to the higher Se contents in plants. Moreover, we did not make an effort to maximise Se contents in broccoli but to produce Se-enriched food with safe Se contents suitable for human consumption.

Similar Se contents in the plants selenized *via* foliar application of selenate solution have also been described for different crop species. Chen et al. (2002) investigated the effect of foliar application of Se in the rate of 20 g Se per ha on rice (*Oryza sativa*) culture. The average Se content of untreated rice was 0.025 ± 0.011 mg/kg, and in the selenized plants this content increased to levels between 0.471 and 0.640 mg/kg. Smrkolj et al. (2005)

studied the Se uptake and distribution by pea (*Pisum sativum*) plants after foliar application of 15 mg/L solution Na_2SeO_4. They found an average content of Se of 0.021 mg/kg in control seeds and of up to 0.743 mg/kg in selenized seeds. Hambuckers et al. (2008) monitored different species of meadow plants where the Se contents in the aboveground biomass of untreated plants varied between 0.018 and 0.121 mg/kg, where the highest levels were represented by *Brassicaceae* plant species. After application of selenate in the rate 9 g Se/ha, the Se contents increased to levels varying between 0.117 and 0.493 mg/kg. Thus, the Se accumulation ability of broccoli in our case did not differ from other plant species, including their response to foliar Se application.

As documented in Tables 2–5, the contents of other micro- and macroelements showed significant differences only occasionally. In flower heads, Mn content decreased significantly with increasing Se rate in the Marathon variety. In the opposite, Mn contents increased in leaves of Parthenon, whereas in other varieties they tended to increase without any statistical difference. Among the other elements, Ca increased in Naxos and Mg in Marathon. On the contrary, Zn tended to decrease in Marathon. An increase was reported of Cu and P (Parthenon) and Mg (Naxos) in stems and of Cu (Naxos) in roots. The changes in the uptake and translocation of micro- and macroelements can significantly affect the plant growth and physiological status (Cakmak 2000). Tobiasz et al. (2014) measured the micro- and macroelement contents during the growth of wheat (*Triticum aestivum*) in hydroponic solution containing 5 and 15 μM Na_2SeO_4, where the element contents were only weakly affected in the seedlings. However, they observed an increase of the Mo and S contents in the shoots and of the S and Cu contents in the roots in the flag-leaf stage, as well as a decrease in Ca and Fe in the roots in the generative phase with the increasing Se rate. Filek et al. (2010) studied in detail the response of essential elements (S, P, K, Fe, Mg, Ca, Mn, Cu, Zn, Mo) in wheat (*Triticum aestivum*) and oilseed rape (*Brassica napus*) on increasing Se concentration in soilless culture. They observed a significant decrease of Ca and K contents with an increasing Se rate, but the Se contents in the rape plants reached the highest Se level up to 2.5 mg/kg, *i.e.* at least a 2.5-fold higher Se content compared to our experiment. No significant changes were observed in the case of sulfur in accordance with Tobiasz et al. (2014), although a competition of Se for S transporters could be expected (Terry et al. 2000). Presumably, the reason could be in relatively low Se content compared to sulfur. Although broccoli is considered a crop characterised by a good ability to take up Se compared to other crops (Sager 2006), our results suggested that the selenized broccoli did not differ substantially from the other selenized crops presented in the literature. Moreover, an increasing Se rate did not result in the stepwise increase of the Se contents in plants.

Ávila et al. (2014) found that Se-biofortified sprouts of different vegetables representing the *Brassica* genus including broccoli were able to synthesise significant amounts of Se-MeSeCys. Pedrero et al. (2007) reported that MeSeCys was the major species in heads of broccoli after 40 days of exposure to selenate in a hydroponic culture. Although reduction was recognised as the rate-limiting step in plant selenate assimilation (de Souza et al. 1998), our selenized broccoli heads showed a remarkably lower portion of those anions than the controls. Apparently, absorbed selenate induced diverse enzymatic activities because the data indicated by several unknown species appearing exclusively after selenate application (Table 6). Surprisingly, Yuan et al. (2013) revealed SeCys2 (possibly SeCys2 + SeCys) as the predominant species in *Cardamine* sp. (a novel Se-hyperaccumulator) grown in a Se mining area, which contradicts the current conception of plant Se metabolism along with the defense mechanism against substantial SeCys build-up in a cell. Although the coupling between SeCys and SeCys2 via redox reaction is known (Bai et al. 2006), the physiological relevance of creating SeCys2 is not yet understood. In our case, we did not observe an increase in percentage of SeCys2 compared to untreated variants. Large Se-MeSeCys portions were determined in a broad spectrum of plant families (Sugihara et al. 2004), including broccoli (Pedrero et al. 2007; Ávila et al. 2014; Thosaikham et al. 2014), if subjected to a selenization procedure, regardless of cultivation technique. A metabolic pathway producing Se-MeSeCys (described in detail including underlying specific gene expression for broccoli by Lyi et al. 2005), and/or the ability of plants to keep absorbed selenate unmetabolized (Mazej et al. 2006), play a significant role in protecting plant proteins to be non-specifically biosynthesized from free seleno-amino acids. According to Table 6, Se-MeSeCys represents 9–12% of the total Se content after selenization. This abundance is considerably lower than if the Se_{tot} contents in tissues greatly exceeded 1 mg kg^{-1} as in the above-cited studies. In general, the eluted SeMet peaks seem to reflect rather respective hydrolysis products than free SeMet. With one observed exception, SeMet represents the main Se-binding form in both selenized and control broccoli heads (Table 6).

Conclusion

Summarizing the results, the foliar application of selenate confirmed an effective response of broccoli plants to increased Se intake *via* increased Se content in the above ground biomass of plants. Moreover, any substantial changes in uptake and distribution of the other essential macro- and microelements and/or Se speciation

in the flower heads were observed. Additionally, no substantial effect of the broccoli variety was observed. Speciation analysis confirmed that the cellular defense mechanism responsible for the specific production of desired organic Se-compounds in broccoli heads became activated to a significant extent at relatively low total Se contents (<1 mg kg^{-1}). Pezzarossa et al. (2014) showed that 100 g of tomato hydroponically grown with a nutrient solution supplemented with 1 mg/L Se provided a total of 58 µg Se. Thus, the daily consumption of 100 g of enriched tomato does not lead to Se toxicity, but can even provide a rational Se supplementation. Similar conclusions can be derived from our investigation. WHO (1996) recommended a maximum daily intake of Se 200 µg Se/day and a maximum Se content in the flower heads was 1.01 mg/kg of dry matter in the variety Heraklion. Calculating an average of 18% dry matter, 1 kg of fresh broccoli will contain cca 180 µg Se. Thus, selenized broccoli at these Se levels should not pose any toxicity risk for human consumption, but could in fact be a suitable source of Se supplementation. Moreover, selenization results in the substantial increase of beneficial organic Se compounds.

Acknowledgements

Authors thank Proof-Reading-Service.com Ltd., Devonshire Business Centre, Works Road, Letchworth Garden City, SG6 1GJ, United Kingdom, for the correction and improvement of language.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Czech Science Foundation (GACR) project No. 13-04580S.

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6 SUMÁRNÍ DISKUZE

6.1 Arsen a jeho sloučeniny

Byly provedeny analýzy téměř sta volně rostoucích rostlinných druhů pocházejících z arsenem kontaminovaných lokalit – Nalžovské Hory, Roudný a Kutná Hora. Výsledky poukazují na značnou odolnost ruderálních druhů rostlin vůči kontaminaci půd arsenem, která na odběrových stanovištích dosahovala celkových obsahů od 21.8±1 mg As kg^{-1} (Nalžovské Hory) až 1120±10 mg As kg^{-1} (Roudný). Hodnoty extrahovatelného arsenu dosahovaly hodnot od 0,072±0,024 mg As kg^{-1} (Roudný) do 14,1±0,2 mg As kg^{-1} (Kutná Hora). Je tedy zřejmé, že velká část celkového obsahu arsenu byla pevně vázána na půdní částice a nebyla rostlinám dostupná. Celkové obsahy naměřené v rostlinné biomase pak dosahovaly hodnot od 0,02* mg As kg^{-1} stanovené v ptačinci (*Stellaria* spp.) do 39.30±6,32 mg As kg^{-1} v mrkvi obecné (*Daucus carrota* L.). Pitten et al. (1999) uvádějí, že běžně se vyskytující celkové obsahy arsenu v rostlinné biomase rostoucí na nekontaminovaných půdách jsou nižší než 3,6 mg As kg^{-1}. Zdá se, že v našem případě je celkový naměřený obsah arsenu v biomase závislý jak na rostlinném druhu, tak na fyzikálně-chemických vlastnostech půd, zejména pak celkovém obsahu arsenu na jednotlivých lokalitách.

Výsledky speciačních analýz ukazují, že As^{III} a As^{V} jsou dominantními sloučeninami vyskytujícími se v rostlinné biomase námi sledovaných volně rostoucích rostlinných druhů. MA, DMA, TETRA a TMAO byly zastoupeny významně méně. Zajímavým objevem je výskyt AB v ostřici časné (*Carex praecox* Schreb.), který dosahoval až 28 % extrahovatelného obsahu arsenu v biomase, a v jitroceli kopinatém (*Plantago lanceolata* L.), kde dosahoval až 1,2 % extrahovatelého obsahu arsenu v rostlinné biomase. AB patří mezi složitější organické sloučeniny arsenu, o kterých se předpokládalo, že jsou tvořeny pouze v mořských organismech (Ruiz-Chancho, 2008) a jeho přítomnost v suchozemských rostlinách byla prokázána jen velmi sporadicky (Mattusch et al. 2000).

Druhá studie se zabývá chopnosti příjmu, přeměny a ukládání arsenu rostlinami jitrocele kopinatého (*Plantago laceolata* L.) z čeledi jitrocelovitých, a tří druhů mokřadních rostlin z čeledi šáchorovitých, jmenovitě skřípiny lesní (*Scirpus silvaticus* L.), ostřice časné (*Carex praecox* Schreb.) a ostřice měchýřkaté (*Carex vesicaria* L.), které se přirozeně vyskytují i na půdách s významně zvýšeným obsahem arsenu v okolí Kutné Hory a Roudného. Zvýšená odolnost vůči vysokým obsahům arsenu v půdě může být závislá nejen

na rostlinném druhu, ale i na mikrobiální aktivitě v oblasti rhizosféry, jak dokládá Orlowska et al. (2012), a jejíž mechanismus může vysvětlovat výskyt DMA v biomase (viz níže).

První studie prokázala přítomnost složitějších organických sloučenin arsenu, jako například AB, v nadzemní biomase některých druhů analyzovaných rostlin. Některé z těchto druhů byly tedy vybrány pro nádobový experiment s cílem popsat podrobněji příjem a transformaci sloučenin arsenu těmito rostlinami v průběhu vegetačního období. Rostliny byly pěstovány za kontrolovaných podmínek v nádobových pokusech na půdách s celkovým obsahem arsenu 735 mg As kg^{-1}. Celkové obsahy arsenu v takto pěstovaných rostlinách dosahovaly až 8,3 mg As kg^{-1} v listech, a až 155 mg As kg^{-1} v kořenech (skřípina lesní). Je zde patrné zvýšené ukládání arsenu v podzemních částech oproti nadzemním, které bude diskutováno dále. Kromě toho byla ještě potvrzena důležitá role vazby As^{V} na železitý povlak kořenového systému rostlin z čeledi šáchorovitých a také to, že k tvorbě DMA pravděpodobně nedochází v rostlině samotné, ale mikrobiální aktivitou nacházející se v oblasti rhizosféry a DMA je následně rostlinou pouze přijímána společně s půdním roztokem (Lomax et al., 2012).

Také v tomto pokusu byly dominantními sloučeninami v biomase rostlin As^{III} a As^{V}, navíc však s výskytem malého množství DMA nacházejícím se ve všech zkoumaných rostlinných druzích. U čeledi šáchorovitých pak byly nalezeny i malé obsahy AB a AC, pravděpodobně jako produkty detoxikačních mechanismů rostlin. Výsledky tedy potvrdily závěry naší první studie i starší poznatky známé z literatury (Mattusch et al. 2000).

Výsledky předchozích dvou studií prokázaly jednak zvýšené obsahy arsenu v kořenech rostlin, ale také vysoký obsah arsenu v nadzemní biomase mrkve obecné (*Daucus carota* L.). Přestože se jednalo o planý druh mrkve, lze uvažovat o případné akumulaci arsenu i v kulturních druzích rostlin, zejména v kořenové zelenině. V této studii jsme se tedy zaměřili na sledování příjmu arsenu a zastoupení jeho sloučenin v konzumních částech vybraných druhů zelenin. Šest různých druhů zeleniny: tuřín (*Brassica napus* var. *napobrassica* L.), kadeřávek (*Brassica oleracea* convar. *acephela* L.), černá ředkev (*Raphanus sativus* var. *nigra* L.), černý kořen (*Scorzonera hispanica* L.), pastinák (*Pastinaca sativa* L.) a salát (*Lactuca sativa* L.) bylo pěstováno v půdách pocházejících ze dvou arsenem kontaminovaných oblastí – Příbram a Kutná Hora. Tyto půdy s celkovým obsahem 36.0±1,0 mg As v kg^{-1}, respektive s 473±10 mg As v kg^{-1} překračují v obou případech maximální doporučený obsah arsenu v zemědělských půdách, který je Vyhláškou č. 13/1994 (Anonym,

1994) stanoven na 30 mg As kg^{-1}. Pokud jsou tyto půdy využívány pro zemědělské účely, zejména pak pro pěstování vlastních výpěstků, které je v těchto lokalitách velice oblíbené, představuje to pro občany konzumující plodiny z takto kontaminovaných oblastí zvýšené zdravotní riziko.

Pomocí spřažení HPLC s ICP-MS byly v jedlých částech pěstovaných zelenin stanoveny obsahy čtyř nejběžnějších aniontových sloučenin arsenu: As^{III}, As^{V}, DMA a MA. Obsahy jednotlivých sloučenin arsenu v jedlých částech se snižovaly v následujícím pořadí: As^{V} ~ As^{III} >> DMA ~ MA. Jasnou převahu As^{V} a As^{III} v biomase rostlin dokládají naše předchozí studie (viz kapitoly 5.1 a 5.2) i některé starší práce, např. Tlustoš et al. (2002).

Výsledky také naznačují, že distribuce sloučenin arsenu se liší hlavně na základě jednotlivých druhů rostlin a jejich jedlých částí. S výjimkou černého kořene výsledky potvrzují omezený přesun arsenu do nadzemních částí rostlin a jeho zvýšené ukládání zejména v podzemních částech, což je v souladu např. se studií Smith et al. (2009). I přes obecně nízké transfer faktory, které se pohybovaly v rozmezí od 0,005 u kadeřávku až po 0,18 u hlávkového salátu, byl u pastináku, černého kořene a černé ředkve (Kutná Hora) překročen maximální přípustný obsah arsenu v zelenině, který je dán Vyhláškou č. 53/2002 (Anonym 2002). Mnohem nižší obsahy arsenu se našly v listové zelenině (hlávkový salát, kadeřávek), což naznačuje, že správná volba plodin může potenciální zdravotní riziko významně snížit. Toto tvrzení podporuje i doporučení pro zemědělské využití půd města Kutná Hora uvedené v příloze této práce (kapitola 9.2).

Nebyl pozorován žádný statisticky průkazný rozdíl v důsledku odlišných fyzikálně-chemických vlastností, kromě celkového obsahu arsenu v půdě. Významně vyšší obsahy arsenu v půdě Kutná Hora měly za následek vyšší celkové obsahy arsenu v nadzemních i podzemních částech rostlin pěstovaných na této půdě.

6.2 Selen a jeho sloučeniny

Celkem 73 rostlinných druhů ze dvou vybraných lokalit bylo sesbíráno a následně zanalyzováno na celkový obsah selenu v jejich biomase. Rostliny pocházely z lokalit Humpolec a Nalžovské Hory, které mají přirozený obsah selenu v půdě 0,248±0,031 mg Se kg^{-1}, respektive 1,104±0,306 mg Se kg^{-1}. Půdy z těchto lokalit se tak řadí mezi půdy na selen relativně chudé. I tak však půdy z lokalit Humpolec a Nalžovské Hory odpovídají evropskému průměru, který se uvádí mezi 0,1 a 5 mg Se kg^{-1} (Bitterli et al., 2010).

Transfer faktory nalezených rostlinných druhů byly nízké a pohybovaly se mezi <0,001 a 0,146, přičemž nejvyšší hodnota byla zaznamenána u rozrazilu rolního (*Veronica arvensis* L.). Tyto hodnoty však nedosahují transfer faktorů, které uvádí ve své studii Bitterli et al. (2010) a naznačují tak nízkou schopnost příjmu selenu rostlinnými druhy rostoucími v podmínkách námi zkoumaných lokalit. Relativně malou schopnost příjmu selenu rostlinami dokládá i porovnání obsahu selenu v biomase vůči obsahu prvků s řádově podobným obsahem v půdě jako selen. V tomto případě obsahy kadmia v biomase dosahovaly o jeden řád vyšších obsahů než obsahy selenu. Obdobně pak vycházely i obsahy molybdenu.

Na nízkém obsahu selenu v rostlinách se může podílet i interakce s jinými prvky, jako je např. arsen, který vykazuje vůči příjmu selenu antagonistický efekt (Malik et al., 2012), a je ve zvýšené míře přítomen v půdách na lokalitě Nalžovské Hory.

Můžeme tedy shrnout, že přirozeně nízké obsahy selenu v půdách vybraných lokalit spolu s nízkou schopností příjmu selenu nalezenými druhy rostlin, vedou k nízkému obsahu selenu v biomase rostlin nezávisle na lokalitě. Nízký obsah selenu ve volně rostoucích rostlinných společenstvech tak může ve volné přírodě přispívat k deficitu selenu napříč celým potravním řetězcem.

Můžeme tedy předpokládat, že efektivní zvýšení obsahu selenu v lučních porostech by mohlo vést ke zlepšení obsahu selenu v organismu volně žijících býložravců. Například Kursa et al. (2010) zaznamenali deficitní obsahy selenu ve svalovině volně žijících sudokopytníků v České republice. Naše další studie se tedy zaměřuje na obohacení biomasy volně rostoucích rostlinných společenstvech selenem pomocí aplikace roztoku Na_2SeO_4 na list. Při jeho aplikaci byly použity dvě různé koncentrace roztoku, které odpovídají dávce 25 a 50 g Se ha^{-1}. 12 rostlinných druhů rostoucích na všech variantách, včetně kontrolní, byly analyzovány na celkové obsahy selenu, jeho jednotlivé sloučeniny a celkové obsahy vybraných esenciálních prvků.

Výsledky ukazují, že hnojení selenem na list zvyšuje jeho celkový obsah v biomase rostlin, a že v tomto procesu hraje důležitou roli rostlinný druh, což potvrzují například i De Temmerman et al. (2014). Celkové obsahy selenu v biomase se pohybovaly od obsahů pod detekčním limitem (0,007 mg Se kg^{-1}) u většiny druhů na neošetřené variantě až do 1,052±0,024 mg Se kg^{-1} u rozrazilu rezekvítku (*Veronica chamaedrys* L.) po aplikaci 50 g Se ha^{-1}. Ani u varianty s nejvyšší dávkou hnojení se u rostlin neprojevily žádné viditelné známky fytotoxicity.

Celkem bylo v rostlinné biomase nalezeno 10 různých sloučenin selenu, avšak pouze 4 z nich byly jednoznačně identifikovány. Se^{VI} a SeMet patřily mezi nejčastěji se vyskytující sloučeniny selenu nezávisle na rostlinném druhu, což naznačuje relativně nízkou schopnost rostlin přeměnit selen přijatý listy na složitější organické sloučeniny. V daleko menší míře se pak objevily sloučeniny SeCys a Se-MeSeCys, které však ukázaly na zajímavý rozdíl mezi jednoděložnými a dvouděložnými rostlinami. U jednoděložných rostlin převažovala tvorba Se-MeSeCys, zatímco u zástupců dvouděložných tvorba SeCys.

Dále byl zkoumán vliv selenu na příjem ostatních esenciálních prvků. Výsledky naznačují, že vliv selenu na příjem esenciálních prvků je z velké části závislý na rostlinném druhu a jeho biochemických procesech. Např. u hořčíku byl v obou hnojených variantách jeho obsah v biomase některých rostlinných druhů významně snížen, v určitých případech až o polovinu. Snížené obsahy lze pozorovat i u jiných prvků, nejméně ovlivněný se však zdá příjem železa. Snížení obsahů esenciálních prvků může, jak naznačují Bannister et al. (1991), souviset se změnami v aktivitě antioxidační superoxid dismutázy, která je produkována na ochranu rostlin, v tomto případě proti možnému oxidačnímu stresu způsobenému zvýšenými obsahy selenu.

Zástupci čeledi *Brassicaceae* jsou všeobecně považovány za rostliny se zvýšenou schopností příjmu a akumulace selenu a tvorby sloučenin selenu vyznačujících se protirakovinovým účinkem (Bañuelos et Mayland, 2000). V posledním pokusu jsme se tedy zaměřili na změny v příjmu a akumulaci selenu ve 4 různých odrůdách brokolice (*Brassica oleracea* L. convar. *italica*) v závislosti na různých dávkách hnojení selenu aplikací na list. Cílem pokusu bylo ověřit, zda je možno touto cestou zvýšit potenciální dietární příjem selenu.

Analýzami jednotlivých částí rostlin brokolice jsme zjistili, že se takto aplikovaný selen akumuluje nejvíce v hlávkách, a to do obsahu až 1,01±0,15 mg Se kg^{-1} suché biomasy, v listech, méně pak ve stoncích a nejméně v kořenech. Vliv odrůdy prokazatelný nebyl. Statistické analýzy u rostlin po aplikaci selenu prokázaly zvýšený příjem a zvýšenou akumulaci tohoto prvku ve všech nadzemních částech rostlin. Nepotvrdilo se, že by tento zvýšený příjem selenu následně jakkoli ovlivnil příjem a akumulaci dalších vybraných esenciálních prvků.

Jako nejefektivnější se ukázala aplikace roztoku Na_2SeO_4 odpovídající dávce 25 g Se ha^{-1}, jelikož další zvýšení dávky již nepřinášelo téměř žádné navýšení obsahu selenu

v biomase rostlin. Tuto dávku pak můžeme považovat za efektivní pro pěstování selenem obohacených brokolic určených ke konzumaci.

Celkově bylo nalezeno 17 různých sloučenin selenu, ale pouze 4 z nich byly jednoznačně identifikovány. Speciační analýzy naznačují schopnost brokolice tvořit z jednoduchých anorganických sloučenin selenu sloučeniny organické a daleko složitější, nejčastěji: SeMet, SeCys a Se-MeSeCys, které byly společně se Se^{VI} v biomase rostlin brokolice rovnoměrně zastoupené. Na rozdíl od pokusu s volně rostoucími rostlinami v podkapitole 5.4, nejvíce zastoupenou sloučeninou selenu u brokolice byla sloučenina SeMet. K podobným závěrům došli i Maneetong et al. (2013) u rostlin brukve čínské (*Brassica oleracea* var. *alboglabra* L.).

7 ZÁVĚRY

Analýzy širokého spektra volně rostoucích rostlinných druhů ukazují na značnou odolnost volně rostoucích ruderálních druhů rostlin vůči kontaminaci půd arsenem. Rostliny byly schopné prospívat bez viditelných známek toxicity i na půdách o obsahu arsenu až 1120±10 mg As kg^{-1} (Roudný). V tomto případě je tato odolnost pravděpodobně významně podpořena faktem, že většina arsenu je pevně vázaná na půdních částicích a obsahy extrahovatelného tj. rostlinám snadno dostupného arsenu dosahují hodnot řádově nižších. Nejvyšší extrahovatelný obsah byl naměřen v půdě Kutná Hora a to 14,1±0,2 mg As kg^{-1}. Zvýšené obsahy arsenu v některých rostlinách např. mrkev obecná (*Daucus carota* L.) 39,3±6,32 mg As kg^{-1} v porovnání s některými jinými např. ptačinec (*Stellaria* spp.) 0,02 mg As kg^{-1} naznačují, že existují druhově specifické mechanismy, které některé rostliny chrání od nadměrného příjmu arsenu z půdy. Na druhou stranu řada z analyzovaných rostlinných druhů přesahovala maximální limit celkového obsahu arsenu v biomase ve výši 2 mg As kg^{-1} daný Vyhláškou č. 52/2002 Sb. pro maximální obsahy prvků v krmivech. Rostliny využívající strategii akumulace arsenu proto teoreticky mohou zvyšovat riziko vstupu arsenu do potravního řetězce skrze pasoucí se skot a volně žijící divokou zvěř.

Zajímavým zjištěním je výskyt AB v ostřici časné (*Carex praecox* Schreb.) a jitroceli kopinatém (*Plantago lanceolata* L.), kde se jeho extrahovatelný obsah v biomase ostřice pohyboval až do 28 % z celkového obsahu arsenu v biomase, a 1,2 % v případě jitrocele. Tato skutečnost byla dále zkoumána v nádobových pokusech a schopnost tvorby AB některými suchozemskými rostlinami byla potvrzena. Naopak se zdá, že DMA, která se v biomase rostlin běžně nachází, není v rostlinách syntetizována, ale je pravděpodobně produkována mikrobiální činností v oblasti rhizosféry, následně uvolňována do půdního roztoku a pak rostlinami pouze přijímána. Role rhizosferních mikroorganismů ale nebyla v našich experimentech podrobně studována a zůstává jako téma pro další výzkum.

Příjem, akumulace a transformace arsenu byla zkoumaná nejen na rostlinách volně rostoucích, ale následně i na vybraných druzích zeleniny. Ty se v daných kontaminovaných lokalitách často pěstují pro vlastní spotřebu a mohou tak významně navyšovat vstup arsenu do potravního řetězce člověka. Půdy odebrané v daných lokalitách (Kutná Hora, Příbram) překračovaly maximální přípustné obsahy arsenu v zemědělských půdách, který je stanoven na 30 mg As kg^{-1}. Půda z okolí Příbrami tento limit překračovala mírně (36±1,0 mg As kg^{-1}), půda Kutná Hora více než 10x (473±10 mg As kg^{-1}). Výsledky naznačují,

že příjem a akumulace sloučenin arsenu se liší hlavně na základě jednotlivých druhů plodin a jednotlivých jedlých částí. Výsledky potvrzují omezený přesun arsenu z podzemních do nadzemních částí, což vede ke zvýšeným obsahům arsenu v bulvách pastináku (*Pastinaca sativa* L.), černého kořene (*Scorzonera hispanica* L.) a černé ředkve (*Raphanus sativus* var. *nigra* L.), jejichž obsahy arsenu překračují nejvyšší přípustný obsah arsenu v zelenině daný Vyhláškou č. 53/2002. Mnohem nižší obsahy byly nalezeny v listové zelenině - hlávkovém salátu (*Lactuca sativa* L.) a kadeřávku (*Brassica oleracea* convar. *acephela*), což naznačuje, že správná volba plodin může snížit potenciální zdravotní riziko plynoucí z konzumace plodin rostoucích v arsenem kontaminaných oblastech.

Nejvíce zastoupenými sloučeninami arsenu v námi zkoumaných rostlinách a plodinách jsou jednoznačně As^{V} a As^{III}. V daleko menší míře se pak v rostlinách nacházejí DMA, MA, AB, AC, TETRA a TMAO, u kterých můžeme předpokládat, že je jejich syntéza druhově specifická, protože ne všechny tyto sloučeniny se vyskytují ve všech druzích analyzovaných rostlin.

Obecně lze říci, že statistické analýzy potvrdily silnou závislost celkového obsahu arsenu v biomase a jeho jednotlivých sloučenin na rostlinném druhu. Závislost příjmu a akumulace arsenu rostlinami na vybraných fyzikálně-chemických vlastnostech nebyla, až na celkový obsah arsenu v půdě, dostatečně průkazně potvrzena.

V případě selenu se jeho relativně nízké přirozené obsahy v půdě (0,248±0,031 mg Se kg^{-1} v půdě Humpolec a 1,104±0,306 mg Se kg^{-1} v půdě Nalžovské Hory) projevily v celkově nízkém obsahu selenu v biomase rostlin. Avšak další důležitou roli pravděpodobně hraje malá schopnost příjmu selenu rostlinnými druhy rostoucími v podmínkách námi zkoumaných lokalit. To dokládají i nízké transfer faktory zkoumaných rostlinných druhů, které nepřekročily hodnotu 0,15. Relativně malou schopnost příjmu selenu rostlinami dokládá i porovnání obsahu selenu v biomase vůči obsahu prvků s řádově podobným obsahem v půdě jako selen. V tomto případě obsahy kadmia a molybdenu v biomase dosahovaly o jeden řád vyšších obsahů než obsahy selenu. Můžeme tedy shrnout, že přirozeně nízké obsahy selenu v půdách vybraných lokalit spolu s nízkou schopností příjmu selenu zkoumanými druhy rostlin, vedou k nízkému obsahu selenu v biomase rostlin nezávisle na lokalitě, což může ve volné přírodě přispívat k deficientu selenu napříč celým potravním řetězcem.

Z tohoto důvodu jsme se v další části zaměřili na přihnojování volně rostoucích rostlin selenem aplikací postřikem na list a to v dávce odpovídající 0, 25, 50 g Se ha^{-1}. Výsledky

naznačují, že tento typ hnojení zvyšuje celkový obsah selenu v biomase rostlin a také to, že v tomto procesu hraje důležitou roli rostlinný druh. Aplikace selenu vedla k významnému zvýšení obsahu tohoto prvku v biomase rostlin, přičemž celkové obsahy selenu po selenizaci jen vyjímečně přesáhly hodnotu 1 $mg.kg^{-1}$ (rozrazil rezekvítek, *Veronica chamaedrys*). Ani u varianty s nejvyšší dávkou hnojení se u rostlin neprojevily žádné vditelné známky fytotoxicity.

Výsledky dále naznačují, že vliv selenu na příjem esenciálních prvků je z velké části závislý na rostlinném druhu a jeho biochemických procesech. Např. u hořčíku byl v obou variantách jeho obsah v biomase některých rostlinných druhů významně snížen, v určitých případech až o polovinu. Snížené obsahy lze pozorovat i u jiných prvků, nejméně ovlivněný se však zdá příjem železa. Snížení obsahů esenciálních prvků může souviset s ochranou rostlin proti možnému oxidačnímu stresu způsobenému zvýšenými obsahy selenu.

Výše uvedené poznatky byly aplikovány na pěstování selenem obohacených brokolic (*Brassica oleracea* var. *italica* L.). Analýzami jednotlivých částí rostlin brokolice jsme zjistili, že se takto aplikovaný selen akumuluje nejvíce v hlávkách a to do obsahu až 1,1±0,15 g Se kg^{-1} a v listech, méně pak stoncích a kořenech, bez statisticky významných rozdílů mezi odrůdami. Statistické analýzy u rostlin po aplikaci selenu prokázaly zvýšený příjem a zvýšenou akumulaci tohoto prvku ve všech jejich nadzemních částech. Nepotvrdilo se, že by tento zvýšený příjem selenu následně nějak záporně ovlivnil příjem a akumulaci vybraných esenciálních prvků. Jako nejefektivnější se ukázala aplikace roztoku Na_2SeO_4 odpovídající dávce 25 g Se ha^{-1}, jelikož další navýšení této dávky již nepřinášelo téměř žádné navýšení obsahu selenu v biomase rostlin. Tuto dávku pak můžeme považovat za efektivní pro pěstování selenem obohacených brokolic určených ke konzumaci.

Se^{VI} a SeMet patřily mezi nejčastěji se vyskytující sloučeniny selenu nezávisle na rostlinném druhu, což naznačuje relativně nízkou schopnost rostlin přeměnit selen přijatý listy na složitější organické sloučeniny. V daleko menší míře se pak objevily sloučeniny SeCys a Se-MeSeCys, které však poukázaly na zajímavý rozdíl mezi jednoděložnými a dvouděložnými rostlinami. U jednoděložných rostlin převažovala tvorba Se-MeSeCys, zatímco u zástupců dvouděložných tvorba SeCys. V případě brokolice speciační analýzy naznačují schopnost brokolice tvořit z jednoduchých anorganických sloučenin selenu sloučeniny organické a daleko složitější, nejčastěji pak SeMet, SeCys a Se-MeSeCys, které byly společně se Se^{VI} v biomase rostlin brokolice zastoupeny nejčastěji. Příznivé zastoupení selenových sloučenin spolu se zvýšeným, ne však toxickým, obsahem selenu

v biomase brokolic naznačuje, že běžná konzumace selenem obohacené brokolice by mohla zvyšovat celkový denní příjem selenu a tím by následně měla celkově kladný vliv na lidské zdraví.

Byl prokázán vliv rostlinného druhu na příjem, akumulaci a transformaci selenu v rostlině, i když obsahy selenu přijatelné rostlinami z půdy byly všeobecně nízké. Nezdá se, že by vybrané fyzikálně-chemické půdní vlastnosti měly statisticky významný vliv na obsahy selenu v biomase.

8 SEZNAM LITERATURY

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9 SEZNAM PŘÍLOH

Příloha 1 – Fotografická dokumentace

Příloha 2 – Doporučení občanům žijícím v arsenem kontaminovaných oblastech

9.1 Příloha 1 – Fotografická dokumentace

9.1.1 Odběr šáchorovitých (*Cyperaceae*) pro nádobové pokusy

Lokalita Roudný, odběr rostlin poblíž pramene vyvěrajícího u štoly Barbora

Foto: Archiv Ing. Jana Najmanová

9.1.2 Foliární aplikace selenu na společenstva volně rostoucích rostlin

Lokalita Humpolec

Foto: Archiv Ing. Jana Najmanová

9.1.3 Parcelkový pokus s brokolicí (*Brassica oleracea* L. var. *italica*)

Vysazování sazenic brokolice a foliární aplikace roztoku Na_2SeO_4

Odrůda Heraklion F1

Odrůda Maraton F1

Odrůda Naxos F1

Odrůda Parthenon F1

Foto: Archiv Ing. Jana Najmanová

9.2 Příloha 2 – Doporučení občanům žijícím v arsenem kontaminovaných oblastech

9.2.1 Obecná doporučení občanům města Kutná Hora

Voda ve studni

Tomu, kdo užívá vodu z vlastní studny k vaření či pití, doporučujeme, aby si nechal provést rozbor vody na obsah těžkých kovů akreditovanou laboratoří. V případě výskytu nadlimitního množství těžkých kovů nedoporučujeme tuto vodu používat k pití ani k vaření ani k praní prádla (Vyhláška č. 252/2004 Sb., kterou se stanoví hygienické požadavky na pitnou a teplou vodu a rozsah kontroly pitné vody).

Zahrádkářské výpěstky

Tomu, kdo si ve větším množství pěstuje zeleninu a ovoce pro svou potřebu na svých zahrádkách doporučujeme, aby si nechal provést rozbor výpěstků na obsah těžkých kovů akreditovanou laboratoří. Ty druhy zeleniny a ovoce, u kterých bude zjištěn nadlimitní obsah těžkých kovů, nedoporučujeme opakovaně pěstovat (např. Vyhláška č. 157/2003 Sb., kterou se stanoví požadavky pro čerstvé ovoce a čerstvou zeleninu, zpracované ovoce a zpracovanou zeleninu, suché skořápkové plody, houby, brambory a výrobky z nich, jakož i další způsoby jejich označování).

Haldy a zeminy

Materiál z hlušinových a struskových hald obsahuje vždy nadlimitní množství těžkých kovů. Přestože se dříve tento materiál používal k různým terénním úpravám, zásadně nelze tyto haldy, zeminy jakkoli rozebírat a používat je k jiným účelům (např. k urovnání pozemku u nově budovaného rodinného domku atd.). Zvyšuje se tím nejen prašnost s následným zdravotním rizikem, ale i rozvlečení kontaminovaného materiálu do okolí a tím k dalšímu poškození půdy. Optimálním zajištěním haldy je její zalesnění nebo zatravnění (vyloučení eroze). Ostatní zeminu mimo haldy (skrývka kulturních vrstev půdy) nedoporučujeme převážet a ukládat mimo kutnohorský rudní revír.

Doporučení pro obyvatele dle studie Krajské hygienické stanice z roku 2016:

1. Zabránit vnášení půdy do domu.
2. Častý úklid vysavačem, nejlépe s praním vzduchu a vytření na vlhko. Utírání prachu na vlhko.
3. Snížit výměru nezpevněných, prašných ploch, které by mohly být zdrojem znečištění. Závažnou kontaminaci prostředí může způsobovat kromě výstavby objektu k bydlení i výstavba bazénů zabudovaných pod povrch terénu. Po provedení nutných terénních pracích co nejrychleji zajistit odhalenou zeminu proti roznosu.
4. Pokud analýza kontaminace půdy prokáže přítomnost těžkých kovů – nepěstovat zeleninu, jen květiny a pozemky zatravnit.
5. Nechovat slepice, pokud nebude vyloučena analýza kontaminace půdy, kde slepice hrabe.
6. Chovat mazlíčky jen doma, nebo pouze venku. Před pobytem v domácnosti zvířata umýt či otřít končetiny.
7. Nenarušovat kompaktní povrch hald a odvalů.
8. Po práci, sportu nebo hře v místech, kde dochází k přímému kontaktu se zeminou, dbát zvýšené hygieny a převlékat se do domácího oděvu.
9. Nepoužívat k hygieně, zalévání nebo do bazénu vodu z místních studní, pokud nebyla vyloučena přítomnost rozpustného arsenu a dalších prvků.
10. Vyloučit pohyb dětí na rozkrytém povrchu hald, nevnášet materiál z hald do domácnosti.

Zdroj: Převzato a upraveno z Anonym [c] [online]

9.2.2 Doporučení pro zemědělské využití půd v Kutné Hoře a okolí

Výzkumný ústav meliorací a ochrany půd Praha
oddělení hygieny půdy

Žabovřeská 250, 156 27 Praha 5 - Zbraslav

ARSEN V ZEMĚDĚLSKÝCH PŮDÁCH V OKRESE KUTNÁ HORA.

Zatížení půd rizikovými prvky, včetně arsenu, bylo Výzkumným ústavem meliorací a ochrany půd v Praze sledováno v roce 1999. V odběrové síti připadá 1 vzorek na plochu přibližně 20 km^2. Jak vyplynulo z výsledků tohoto sledování (Podlešáková a kol. 2000), které byly v minulém roce předány MZe ČR, obsahují orniční horizonty zemědělských půd nadlimitní obsahy arsenu.

Nejvyšší námi zjištěná hodnota, byla lokalizována v blízkosti obce Kaňk, kde celkový obsah arsenu v ornicním horizontu dosáhl hodnoty 246 mg/kg půdy.

Zvýšené obsahy arsenu v ornicních a drnových horizontech půd byly nalezeny v oblasti, kterou je možno zhruba vymezit obcemi (v závorkách jsou uvedeny celkové koncentrace arsenu v mg/kg a za rovnítkem je uveden údaj kolikrát byla překročena hodnota stanovená normou pro zemědělské půdy), Starý Kolín (56,2 = 1,87 x), Nové Dvory (63,8 = 2,13 x), Církvice (147 = 4,9 x), Čáslav sz. (35,8 = 1,2 x), Hořany (55,8 = 1,86 x), Červené Pečky (34,8 = 1,16 x), Šťáralka (51,6 = 1,72 x). Mimo tuto oblast dosahují koncentrace As v zemědělských půdách daného okresu podstatně nižší úrovně, průměrná hodnota, která zohledňuje i tuto zatíženou oblast, činí 12,98 mg/kg.
Maximální hodnota pro As v zemědělských půdách, která je uvedena ve vyhlášce 13/1994 Sb. (celkový obsah v lučavce královské) je 30 mg/kg.

Na lokalitě Kaňk byl následně proveden i profilový odběr vzorku půdy ze tří horizontů (z hloubek do 25 cm, 50 cm a 1 m). Vzhledem k tomu, že obsahy směrem do hloubky rostou (obsah v hloubce 1 m je 1150 mg/kg), lze usuzovat na zátěž, která byla v této lokalitě pravděpodobně historicky způsobena zapravením odpadového materiálu z těžby rud s vysokým obsahem As (arsenopyrit) do půdního profilu. Nelze však vyloučit i vliv imisních (ve vzduchu rozptýleného znečištění) spadů, vzhledem ke zvýšeným obsahům dalších rizikových prvků (kadminum Cd, olovo Pb). Přestože podíl spadů na vysokých obsazích As bude velmi pravděpodobně relativně nízký, může se podílet významněji na kontaminaci rostlin.

Transfer (přestup) rizikových prvků z půd s jejich geogenně zvýšenými obsahy (přirozeně zvýšené obsahy rizikových prvků v půdotvorném substrátu) do rostlin, je vzhledem ke stabilitě (stálosti) vazeb rizikových prvků v produktech zvětrávání substrátů (půdotvorný substrát-geologická zvětralina, na kterém vznikla půda a produkty jeho zvětrávání tvoří minerální podíl půdy) všeobecně nízký (Němeček, Podlešáková, Vácha 1996). Přesto je nutné počítat se zvýšeným transferem (přestupem) tam, kde celkové obsahy rizikových prvků dosahují extrémně (mimořádně) zvýšených koncentrací. Na lokalitě Kaňk jsme provedli odběr vojtěšky a obsah As v rostlinné hmotě byl stanoven na hodnotu 0,84 mg/kg. Tato hodnota se nepřibližuje ani polovině limitní hodnoty stanovené pro arsen v pícninách (2 mg/kg), která je stanovena vyhláškou č. 194/1996 Sb. V rámci okresu je však tato hodnota výrazně zvýšená. Geometrický průměr obsahu As v pícninách na okrese Kutná Hora je 0,08 mg/kg.

Při hodnocení nebezpečí transferu (přestupu) rizikových prvků z půdy do rostlin je třeba považovat za významné dva hlavní faktory, kterými jsou ***půda a rostlina***. Chování rizikových prvků v půdách závisí na celé řadě půdních vlastností, jakými jsou pH půdy, obsah organické hmoty, jílových minerálů, typ a zdroj kontaminace (znečištění) atd. Na základě našich výzkumů (Podlešáková a kol. 2000), závisí mobilita (pohyblivost) As v terénních podmínkách především na pH půdy. Zde je třeba připomenout pozitivní korelaci (přímo úměrné) chování As na pH, to znamená, že mobilita (pohyblivost) As roste se zvyšující se hodnotou pH půdy, na rozdíl od většiny ostatních rizikových prvků. Arsen lze hodnotit jako prvek s nižší, přesto však prokázanou mobilitou (pohyblivostí) a transferem (přestupem) do rostlin.

Jak vyplynulo z našich sledování, které jsou nově publikovány v citované zprávě, nehrozí nebezpečí kontaminace nadzemních částí většiny rostlin v geochemicky zatížených půdách až do hodnoty přes 1000 mg/kg celkového arsenu v půdě.

Je však nutné brát v úvahu faktor rostliny, který má na příjem rizikových prvků rostlinou podstatný vliv. **Všeobecně platí, že obsahy rizikových prvků v rostlinách klesají v posloupnosti kořeny, listy, stonky, zásobní orgány, semena (Sauberbeck a Lübben 1991).** Z toho vyplývá, že také koncentrace As se může podstatně lišit v kořenové zelenině a v zrnu obilnin. **Např. zmínění autoři řadí k rostlinám s vysokým příjmem rizikových prvků salát, špenát, celer, ředkvičku, o něco nižší transferové (přestupné) koeficienty uvádějí u mrkve, fazol a hrachu, k rostlinám s nejnižším příjmem patří pro většinu rizikových prvků kukuřice a pšenice.** Také v našich pokusech byly pozorovány mezi rostlinami značné rozdíly, např. obsah As v zelených orgánech hořčice byl řádově vyšší ve srovnání s obsahem as v zrnu žita a cca 5 x vyšší ve srovnání s obsahem As v zeleném ovsu (stádium pátého listu).

Transfer rizikových prvků z půdy do rostlin lze tedy ovlivnit výběrem vhodných plodin (minimální riziko lze předpokládat u ovoce ze stromů, ale i u zrna obilovin, vysoké naopak u listové a kořenové zeleniny) a dále úpravou půdních vlastností.

V případě As je potřebné nezvyšovat hodnotu pH půdy nad neutrální reakci.
Intentivním vápněním se může mobilita As v půdě významně zvýšit (Jones et al. 1997).
Předmětem našich výzkumů byla v posledních čtyřech letech i aplikace půdních aditiv, imobilizujících (omezující pohyb) rizikové prvky v půdě. Bohužel pro As nelze v této fázi žádné aditivum doporučit. Prozatím námi testované anorganické sorbenty (např. synt. zeolity) které se osvědčily pro některé rizikové prvky (Cd, Zn), jsou pro As nevhodné. V případě syntetického zeolitu jsme zjistili markantní (podstatný) vzrůst mobility As v půdě, ošetřené zapravením tohoto materiálu (Vácha et al. 2000). Lepších výsledků bylo dosaženo s cyprisovými jílovci (byly poskytnuty Výzkumným ústavem nerostných surovin v Kutné Hoře), které v omezené míře snížily mobilitu As v půdě, lze je však použít k imobilizaci některých rizikových prvků (Cd, Pb) v půdách, které jsou souběžně kontaminovány i arzenem. Problém As z tohoto pohledu bude řešen v následujícím období, budou testovány melioranty, vybrané na základě zkušeností zahraničních pracovišť.

Ing. Radim Vácha

Zdroj: Převzato a upraveno z Anonym [d] [online]