FACULTY OF AGRICULTURE
UNIVERSITY OF ZAGREB

M.Sc. Željka Zgorelec, dipl. chem. ing.

Phytoaccumulation of Metals and Metalloids from Soil Polluted by Coal Ash

DISSERTATION

Zagreb, 2009

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Phytoaccumulation of Metals and Metalloids from Soil Polluted by Coal Ash

Fitoakumulacija metala i metaloida iz tla onečišćenog ugljenim pepelom

DISSERTATION

Mentor: Prof. Ferdo Bašić

Zagreb, 2009

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Željka Zgorelec

CONTENTS

ABSTRACT

Key words: *coal ash, metalloids, metals, pollution, soil*

Around the city of Tuzla, BiH, soil covered coal ash disposal sites have been used for food and fodder production since 1992. To the best of our knowledge this is the first report on the cultivation of coal ash disposal sites for agriculture purposes. During the war, in the nineties of the past century, the chronic lack of agricultural land in the area of thermo electric power plant, Tuzla (TEP) and in the surrounding area has forced refugees and local farmers to cultivate the surface of dried ponds in order to get arable land and to use it as pastures, meadows or for food production. Nowadays, 63 ha of soil covered disposal sites (Drežnik and Plane) are still used for agricultural purposes.

High concentrations of As, B, Cr, Cu and Ni in alkaline (pH = 8.77 in 1M KCl, as 1:2.5 (*w/v*)) coal ash (analysed in aqua regia extract, HCl/HNO_3 = 3:1 (*v/v*) on ICP-MS (ELAN 9000 DRC-e, Parkin Elmer) as 1:12 (*w/v*) were observed and their mass ratio were 112 mg As/kg, 204 mg B/kg, 281 mg Cr/kg, 85.9 mg Cu/kg and 593 mg Ni/kg, respectively. According to soil classification damage criteria the ash from Jezero disposal site belongs to the 5th class or waste soil in respect to As, Cr and Ni, further to the 4th class or polluted soil in respect to B and Cu, next to the 3rd class or high polluted soil in respect to Zn, Sr, Fe, Co and Cd, to the 2nd class or low polluted soil in respect to Cs, Pb, Rb and U and to the 1st class or clean soil in respect to Mo, P and Se, keeping in mind that this is ash not soil, but still this ash is used for food and fodder production.

This study investigated metals (Cr, Cu, Ni, Cd, Mo, Cs, Co, Pb, Fe, Zn, Rb and Sr), metalloids (As and B), non-metals (Se and P) and actinide U transport and accumulation in winter barley (*Hordeum vulgare* L. cv. Rex) and soybean (*Glycine max* L. cv. Dora) grown on substrates made up of different soil and ash ratios, and six treatments were as follows: (1) soil / ash = 100 % mass / 0 % mass, (2) soil / ash = 0 % mass / 100 % mass, (3) soil / ash = 75 % mass / 25 % mass, (4) soil / ash = 50 % mass / 50 % mass, (5) soil / ash = 47.5 % mass / 47.5 % mass + 5 % mass saw dust amendment of beech tree and (6) soil / ash = 25 % mass / 75 % mass.

After three vegetation seasons: two of winter barley (*Hordeum vulgare* L. cv. Rex) grown from 15.11.05. until 26.06.06. - barley (1) and from 09.11.06. until 18.06.07. - barley (2) and one of soybean (*Glycine max* L. cv. Dora) grown from 03.07.06. until 20.10.06., on the same substrate and in controlled conditions: yield components, total elements of nitrogen,

sulphur, carbon and hydrogen in grain (by dry combustion method, Vario Macro CHNS analyzer, Elementar, 2006) and total elements (Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Rb, Sr, U, Se, Zn, Cs, As, B and P) content in grain (in HNO_3/H_2O_2 = 5:1 (*v/v)* extract, on ICP-MS at 1:30 (w/v) ratio) were determined for barley and soybean. Quality management, internal and external QC were conducted. The analyses of data observed in the experiment were performed by statistical Software *SAS 9.1 (SAS Inst. Inc.)*. The level of significance was set at 5 % in all statistical tests.

Soybean grain grown on pure alkaline coal ash or on its different soil mixtures was high accumulator, in at least one treatment, of B, Cd, P, Mo, Rb and Se, moderately accumulator of Cr, Cs, Cu, Ni and Zn, low accumulator of Pb and Sr and non accumulator of As, Co, Fe and U and barley grain grown on pure alkaline coal ash or on its different soil mixtures was high accumulator, in at least one treatment, of B, P, Mo, Rb and Se, moderately accumulator of Cs, Cu, Cd and Zn, low accumulator of Pb, Cr and Sr and non accumulator of As, Co, Fe, Ni and U.

The observed data showed that barley and soybean were sensible and in their grains were high accumulators (EC > 1) of: P in all treatments of soybean and all treatments of barley; Rb only in pure ash treatments of soybean and of barley; Mo in all treatments of soybean and in some treatments (No. 1, 2 and 3) of barley; Se in all treatments of soybean and all treatments of barley; B in some treatments (No. 3, 4, 5 and 6) of soybean and in one treatment (No. 3) of barley; Cd only in pure ash treatment of soybean.

The content of As, Co, Cu, Fe, Pb and Zn in soybean and barleys grains was below normal values according to the available literature in all treatments, although a considerably high concentration of As and Cu was noted in the ash.

These findings support the conclusion that very poor yields and visible plants damage in some treatments, especially in treatment with pure ash compared to treatment with pure soil of both species soybean and barley, were probably caused by toxicity (B, Ni, U, Mo) or deficiency (N, Cu, Zn, Fe). Non-essential trace elements (As, Co and Pb) were within normal concentration ranges and significantly below critical (1 mg As/kg, 1 mg Co/kg and 6 mg Pb/kg) levels in all treatments for both species.

PROŠIRENI SAŽETAK

Fitoakumulacija metala i metaloida iz tla onečišćenog ugljenim pepelom

Ključne riječi: *metali, metaloidi, onečišćenje, tlo, ugljeni pepeo*

UVOD

U okolišu Termo-elektrane Tuzla (TEP) u Bosni i Hercegovini (BiH) oko 1 % površine prekriveno je otpadom ugljenog pepela. TEP opskrbljuje više od 20 000 potrošača i proizvodi 50 % ukupne godišnje državne proizvodnje električne energije. U zadnjih je 50 godina na 5 odlagališta (Drežnik, Plane, Jezero, Divkovići I i II) u okolici Tuzle skladišteno više od 40 mil. m^3 šljake i pepela. Ukupna prekrivena površina otpadnim pepelom iznosi više od 300 ha. Kronični nedostatak poljoprivrednog zemljišta u okolici TEP-a nagnalo je lokalno stanovništvo, posebno izbjeglice i poljoprivrednike da počnu kultivirati površine suhih bazena odlagališta s ciljem dobivanja obradivih i/ili plodnih površina zemljišta odnosno korištenje za pašnjake, livade i uzgoj hrane. Trenutno se oko 63 ha tlom prekrivenog odlagališta (Drežnik i Plane) koriste u poljoprivredne svrhe. Navezeno pokrovno tlo je pseudoglej koji dominira u okolici Tuzle. Do danas na ovim prostorima nije provedeno niti jedno istraživanje stupnja rizika od korištenja odlagališta kao poljoprivrednog tla u kojem bi se razmatrala opasnost i škodljivost onečišćivača (metala i metaloida) i njihova fitoakumulacija u biljkama kao prvog koraka ulaska u hranidbeni lanac konzumacijom hrane izrasle na odlagalištu direktno od strane ljudi ili indirektno preko životinja (zbog ispaše). Najzastupljenije uzgajane kulture na odlagalištima su kukuruz (*Zea mays* L.), pšenica (*Triticum aestivum* L.), ječam (*Hordeum vulgare* L.) i krumpir (*Solanum tuberosum L.*), te stočne kulture; lucerna (*Medicago sativa* L.) i crvena djetelina (*Trifolium pretense* L.), manje zastupljene kulture su soja (*Glycine max* L.) i grah (*Phaseolus vulgaris* L.). Svaka od tih kultura može biti tolerantna, umjereno senzibilna ili senzibilna na određene metale i metaloide koji se nalaze u tlu, a što ovisi o mnogim faktorima. Biljka može biti akumulator, umjereni akumulator i ne akumulator određenog metala ili metaloida.

Ugljeni pepeo može pak biti i jako lužnat i jako kiseo i može sadržavati značajne količine elemenata u tragovima. Nekoliko studija bavilo se istraživanjem sudbine elemenata u tragovima u okolišu tvornica kojima je pogonsko gorivo spaljivanje ugljena. Analiza ulaznog ugljena i produkata koji nastaju nakon spaljivanja pokazala je da su Ba, Ce, Co, Cs, Cu, Dy, Ga, Ge, La, Lu, Mn, Ni, Rb, Sr, Tb, Th, Y, Yb, Zn i Zr zadržani u krutom otpadu (Sandhu i sur., 1993); da su As, B, Be, Cd, Cr, Li, Mo, Pb, Sb, Sn, Ta, Tl, U, V i W samo djelomično zadržani u krutom otpadu (Sandelin i Beckman, 2001) i da su Hg i Se primarno emitirani u atmosferu (Lorens i sur., 2001; Sandelin i Beckman, 2001). U poljoprivredi pepeo kao nusprodukt procesa spaljivanja ugljena ima potencijal mijenjanja teksture tla i povećanja kapaciteta zadržavanja vode pjeskovitih tala zbog visokog sadržaja čestica praha i gline (Ghodrati i sur., 1995) također može i smanjiti potencijal otapanja P i njegovog ispiranja iz tla (Stout i sur., 2000). Međutim, mora se uzeti u obzir da su ukupno 25 potencijalno toksičnih elemenata u dosadašnjim istraživanjima bili pronađeni u znatnim količinama u pepelu koji je nusprodukt procesa spaljivanja ugljena. Neki od tih elemenata (Ba, B, Mo, Se, S, Sr, As i Al) bili su nađeni u povišenim koncentracijama u biljkama izraslim na tlima kojima se kao poboljšivač dodavao pepeo (Adriano i sur., 1980). Prema istraživanjima (Keefer, 1993) te manje doze nisu prouzročile fitotoksikacijske probleme u biljkama (izuzev B u nekim biljkama), no mogle bi biti potencijalno štetne životinjama koje se hrane tim biljkama kao i ljudima kao konzumentima poljoprivrednih proizvoda izraslima na takvim tlima. Granične vrijednosti mobilnih elemenata u tragovima (ionski oblici) utvrđeni su za poljoprivredna tla, pri čemu su Cu, Pb, Cd i Tl metali koji imaju najveći utjecaj vezanja na biljke koje se koriste kao stočna hrana (Prüess, 1994). Menon i sur., 1993 navode da pristupačnost toksičnih elemenata biljkama varira u ovisnosti o teksturi pojedinih vrsta pepela. Weinstein i sur., 1993 zabilježili su da se selen (Se) akumulira u količinama i do 50 puta većim u biljkama izraslim na odlagalištima ugljenog pepela nego u biljkama izraslim na kontrolnim varijantama (u čistom tlu). Također je zabilježeno da dodavanje i primjena gipsa s pepelom smanjuje unos i pristupačnost selena u travama (Woodbury i sur., 1999). Kukuruz i soja pokazali su snažne simptome toksikacije borom, kada su izrastali na tlima u kojima je pepeo kao nusprodukt procesa spaljivanja ugljena bio dodavan kao poboljšivač i u pokusima gdje se pepeo miješao sa 5% stajskog gnoja (Kukier i Summer, 1996; Schwab, 1993). Dodatno uz toksikaciju borom u soji su nađene i visoke koncentracije Zn, Mo i Cd (Schwab, 1993). Unatoč tomu što je identificirano nekoliko korisnih alternativnih načina upotrebe pepela kao nusprodukta u procesu spaljivanja ugljena umjesto odlaganja u bazene, već su velike površine u Jugo-Istočnoj Europi (JIE) i u svijetu pokrivene odlagalištima ugljenog pepela i u budućnosti će još biti potrebe za njegovim odlaganjem. Stoga je strategija za optimalnu reintegraciju i produktivnu upotrebu takvog zemljišta hitna i neophodna, posebno u područjima gdje je aktualan nedostatak poljoprivrednog tla. Neke studije (Jala i Goyal, 2006) su pokazale da upotreba ugljenog pepela u poljoprivredi ima potencijala i da uzgoj zdrave hrane na odlagalištima pepela može biti moguć kada je odlagalište prekriveno dovoljnom količinom tla. Međutim, ne postoji još dovoljno dostupnih informacija o najbolje odabranim vrstama u smislu bezopasnih koncentracija elemenata u tragovima iako su provedena brojna istraživanja o fitoakumulaciji određenih elemenata i prinosu, te naravno njihovoj ovisnosti o optimalnim debljinama i tipovima primijenjenog pokrovnog tla, te o optimalnim omjerima pokrovnog tla i pepela kao supstrata koji bi se koristio u uspješnoj biljnoj proizvodnji (Kabata-Pendias i Mukherjee, 2007; Huang i Gobran, 2005; Adriano, 2001; Dos Santos Utmazian i Wenzel, 2007; Puschenreiter i sur., 2005; Fitz i Wenzel, 2002; Unterbrunner i sur., 2007; Wenzel, 2009; Wenzel i sur., 1999; Wenzel i sur., 2004; Latkoczy i sur., 2000; Vego i sur., 2008; Kisic i sur., 2005; Custovic, 2005; Dellantonio i sur., 2008).

Pretpostavla se da će testirane kulture (soja i ozimi ječam) koje izrastu na supstratu s različitim udjelima pepela različito reagirati na kvantitetu i kvalitetu zrna u smislu određenih sastavnica prinosa i kemijskog sastava zrna te da će različito akumulirati određene metale (Cr, Cu, Ni, Cd, Mo, Cs, Co, Pb, Fe, Zn, Rb i Sr) i metaloide (As i B) u svojim jestivim dijelovima. U suglasju s navedenom hipotezom definirani su i slijedeći ciljevi:

- utvrditi utjecaj različitih udjela pepela na sastavnice prinosa soje i ječma
- istražiti utjecaj udjela pepela na akumulaciju metala i metaloida u zrnu testiranih kultura
- usporediti akumulaciju metala i metaloida u zrnu soje i ječma uzgajanim na istim udjelima pepela
- utvrditi utjecaj vegetacijske sezone na akumulaciju metala i metaloida u zrnu ječma

MATERIJALI I METODE

Lokacija i uvjeti istraživanja

Eksperiment je bio postavljen u kontroliranim uvjetima u stakleniku na Agronomskom fakultetu Sveučilišta u Zagrebu. Istraživanjem su obuhvaćene sljedeće vrste i sorte u vegetacijskom periodu sjetva-žetva:

- ozimi ječam (*Hordeum vulgare* L. cv. Rex) uzgajan tijekom dvije vegetacijske sezone od 15.11.05. do 26.06.06. kao ječam (1) i od 09.11.06. do 18.06.07. kao ječam (2) na istom supstratu
- soja (*Glycine max* L. cv. Dora) uzgajana tijekom jedne vegetacijske sezone od 03.07.06. do 20.10.06.

Osnovnu parcelu (OP) činila je plastična posuda promjera (ϕ = 27 cm) sa 5 kg supstrata, u koju je bilo posijano po 100 zrna ozimog ječma, odnosno 15 zrna soje. Gnojidba je bila uključena i ukupne doze primijenjenog gnojiva iznosile su:

- za ozimi ječam: 0,72 g N/OP; 0,72 g P/OP i 0,48 g K/OP
- za soju: 0,72 g N/OP; 0,72 g P/OP i 1,08 g K/OP

Na dno svake OP stavljeno je približno 2 cm oblutaka zbog bolje vodo propusnosti.

Pepeo korišten u eksperimentu uzet je prema metodici za uzorkovanje prosječnih uzoraka kao čista homogena smjesa sa odlagališta pepela Jezero.

Osnovni supstrat je pseudoglejno tlo, uzeto u Šašinovcu, Hrvatska, koje prema analiziranim fizikalnim i kemijskim karakteristikama odgovara tlu iz okolice Tuzle, a koje se koristi na odlagalištima kao pokrovno tlo.

Eksperimentalni dizajn

Pokusi s ozimim ječmom i sojom postavljeni su po slučajnom bloknom rasporedu s 4 ponavljanja. Tretiranja u pokusima predstavljaju različiti maseni udjeli pepela i to od 0 do 100 %, odnosno:

1. Tlo/ pepeo = 100 % / 0 % (*m/m*)
2. Tlo / pepeo = 0 % / 100 % (*m/m*)
3. Tlo / pepeo = 75 % / 25 % (*m/m*)
4. Tlo / pepeo = 50 % / 50 % (*m/m*)
5. Tlo / pepeo = 47,5 % / 47.5 % (*m/m*) + 5 % (*m*) poboljšivača (piljevina bukovine)
6. Tlo / pepeo = 25 % / 75 % (*m/m*)

Piljevina bukovine uzeta je iz obližnje tvornice drvenih proizvoda “Manico”, Mala Brijesnica, BiH.

Uzorkovanje i analiza supstrata

Neposredno prije prve sjetve sa svake OP, uzeti su uzorci supstrata, i u njima je određeno:

- ukupni maseni udio Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Rb, Sr, U, Se, Zn, Cs, As, B i P
- biljci pristupačni maseni udio Cd, Co, Cr, Cu, Mo, Ni, Pb, Rb, Sr, U, Se, Zn, Cs, As, B i P
- standardni kemijski parametri: pH, EC, pristupačni P i K i ukupni C, H, N i S
- kationi: Li^+, Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+} i anioni: F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-} i PO_4^{3-}

Uzorkovanje i analiza biljnog materijala

Nakon žetve ozimog ječma (26.06.06. i 18.06.07.) i soje (20.10.06.) sa svake OP, uzeti su uzorci biljnog materijala (cjelokupna nadzemna biomasa), u njima su utvrđene sastavnice prinosa, a jestivi dio biljke (zrno) analizirano je na dolje navedene promatrane varijable.

Određene sastavnice prinosa:

- ozimi ječam: broj biljaka s klasom i bez klasa, ukupan broj biljaka, duljina stabljike i klasa, broj i masa zrna i količina vlage u zrnu
- soja: ukupan broj biljaka, broj mahuna, masa zrna i količina vlage u zrnu

Analiza zrna (ozimi ječam i soja):

- ukupni maseni udio Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Rb, Sr, U, Se, Zn, Cs, As, B i P
- ukupni maseni udio C, H, N i S

Metode

Tablica 1 Popis metoda korištenih u istraživanju

Parametar	Metoda	Protokol / Norma
sušenje/mljevenje/ sijanje/homogeniziranje	Priprema uzoraka tla za fizikalne i kemijske analize	HRN ISO 11464:2006
w(ST), w(H_2O) [%]	Određivanje suhe tvari i sadržaja vlage - gravimetrijska metoda	HRN ISO 11465:2004
pH	Kakvoća tla - Određivanje pH vrijednosti u 0,01 M $CaCl_2$, 1 M KCl i H_2O u omjeru 1:2.5 (*m/v*)	HRN ISO 10390:2004
EC [μScm^{-1}]	Kakvoća tla - Određivanje specifične električne vodljivosti u H_2O u omjeru 1:5 i 1:10 (*m/v*)	HRN ISO 11265:2004
P_2O_5 i K_2O [mg/100g; g/kg]	Pristupačni fosfor i kalij u AL ekstraktu u omjeru 1:20 (*m/v*)	AL metoda (ekstrakcija sa amonij laktat octenom kiselinom)
TS [% ST; g/kg]	Kakvoća tla - Određivanje ukupnog sumpora metodom suhog spaljivanja	HRN ISO 15178:2005
TC [% ST; g/kg]	Kakvoća tla - Određivanje organskog i ukupnog ugljika metodom suhog spaljivanja («elementarna analiza»)	HRN ISO 10694:2004
TN [% ST; g/kg]	Kakvoća tla - Određivanje ukupnog dušika metodom suhog spaljivanja («elementarna analiza»)	HRN ISO 13878:2004
anioni i kationi u tlu	vodeni ekstrakt tla u omjeru 1:10 (*m/v*)	ONORM L 1092-93
F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-} i PO_4^{3-} [mg/kg]	Kakvoća vode - Određivanje otopljenih fluorida, klorida, nitrita, ortofosfata, bromida, nitrata i sulfata pomoću ionske tekućinske kromatografije - 1. dio. Metoda za slabo zagađene vode	HRN ISO 10304-1:1998
Li^+, Na^+, NH_4^+, K^+, Ca^{2+} i Mg^{2+} [mg/kg]	Kakvoća vode - Određivanje otopljenih Li^+, Na^+, NH_4^+, K^+, Mn^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+} ionskom kromatografijom - Metoda za vode i otpadne vode	HRN ISO 14911:2001
ekstrakcija (digestija) ukupnih metala i metaloida u tlu	Kakvoća tla - Ekstrakcija elemenata topljivih u zlatotopci (HCl/HNO_3 = 3:1 (*v/v*) digestija) u omjeru 1:12 (*m/v*)	ONORM L 1085 HRN ISO 11466:2004
ekstrakcija (digestija) ukupnih metala i metaloida u biljci	HNO_3/H_2O_2 = 5:1 (*v/v*) digestija u omjeru 1:30 (*m/v*)	
ekstrakcija pristupačnih metala i metaloida u tlu	Kakvoća tla - Ekstrakcija elemenata u tragovima s 1 M otopinom amonij nitrata (NH_4NO_3) u omjeru 1:2,5 (*m/v*)	ISO/DIS 19730:2006 DIN ISO 19730
ICP-MS analiza, ukupni i pristupačni metali (Cr, Cu, Ni, Cd, Mo, Cs, Co, Pb, Fe, Zn, Rb i Sr) i metaloidi (As i B), ne-metali (Se i P) i U [mg/kg]	Kakvoća tla - Određivanje elemenata u tragovima u ekstraktu tla metodom induktivno vezane plazme - atomske emisijske spektroskopije (ICP-AES) Kakvoća vode - Određivanje 33 elementa metodom atomske emisijske spektroskopije induktivno vezane plazme	ISO/DIS 22036:2006 HRN EN ISO 11885:1998

Statistička analiza

Za statističku obradu dobivenih podataka korišten je računalni program *SAS 9.1 (SAS Inst. Inc.)*. Razlike između tretiranja (maseni udjeli pepela) za sve promatrane parametre u pokusima s ozimim ječmom i sojom analizirane su analizom varijance (One-Way-ANOVA) i testirani, ako je bilo potrebno, *post-hoc* testom (Fishers LSD). Za sve statističke testove prag značajnosti iznosio je 5 %.

REZULTATI, RASPRAVA I ZAKLJUČCI

Karakteristike pepela

Alkalni ugljeni pepeo (8,77 u KCl; 8,75 u $CaCl_2$; 8,86 u H_2O; n=4) uzorkovan na odlagalištu Jezero ima sljedeće karakteristike:

Električna vodljivost izmjerena u vodenom ekstraktu, u omjeru 1:5 (*m/v*) iznosila je 1 591 µS/cm, a u omjeru 1:10 (*m/v*) 2 380 µS/cm (n=4), maseni udio vlage u pepelu iznosio je 0,26 %, pepeo je slabo opskrbljen s biljci pristupačnim P_2O_5 (6 mg/100g; n=4) i dobro opskrbljen s biljci pristupačnim K_2O (44 mg/100 g, n=4), dobro opskrbljen ukupnim dušikom, TN (0,110 % u ST, n=4), ukupna masena koncentracija ugljika iznosila je 2,772 % u ST, ukupna masena koncentracija sumpora bila je relativno niska 0,349 % u ST, a ukupna masena koncentracija vodika 0,433 % u ST (n=4). Omjer ugljika i dušika tj. C/N odnos iznosio je 25. Prema dobivenim rezultatima dominantna sol u ispitivanom pepelu je kalcij sulfat ($CaSO_4$; n=4).

Neke vrijednosti biljci pristupačnih elemenata izmjerenih u pepelu (**As**, **B**, **Cr**, **Mo**, **Ni**, **Sr**, **U** i **Zn**) a koji su ekstrahirani u 1M NH_4NO_3 u omjeru 1:2,5 (*m/v*) (n=4) bile su veće od vrijednosti koja upućuje na oprez (referentna vrijednost), a vrijednosti su im redom iznosile, 100 µg As/kg, 630 µg Ni/kg, 50 µg U/kg i 250 µg Zn/kg, neke pak izmjerene vrijednosti bile su veće od vrijednosti kod kojih je potrebna reakcija (remedijacijska vrijednost) kao 122,5 mg Mo/kg i 1 mg Cr/kg. Ostale su pak izmjerene vrijednosti bile veće od prirodnih (u 1M NH_4Ac) kao 39,3 mg B/kg (>>0,97 mg/kg) i 10,7 mg Sr/kg (>8 mg/kg).

Pepeo je bogat **As, B, Cr, Cu** i **Ni**, a izmjerene vrijednosti ukupnih masenih koncentracija iznosile su 112 mg As/kg, 204 mg B/kg, 281 mg Cr/kg, 85,9 mg Cu/kg i 593 mg Ni/kg (n=4). Prema Klasifikaciji oštećenja tala, pepeo sa odlagališta Jezero pripada 5. stupnju onečišćenja (So) ili zagađenom tlu s obzirom na As, Cr i Ni; 4. So ili onečišćenom tlu s obzirom na B i Cu; 3. So ili tlu velike onečišćenosti s obzirom na Zn, Sr, Fe, Co i Cd; 2. So ili tlu povećane onečišćenosti s obzirom na Cs, Pb, Rb i U te pripada 1. So ili čistom tlu s obzirom na Mo, P i Se, imajući stalno na umu kako to nije tlo već pepeo, ali ono koje se koristi kao supstrat za proizvodnju ljudske i stočne hrane.

Čisto tlo korišteno u eksperimentu bilo je također obogaćeno s As (27,7 mg/kg) i Ni (96,4 mg/kg) i prema kriterijima Klasifikacije, pripada 4. So ili onečišćenom tlu za oba elementa.

Rezultati kemijskih i fizikalnih analiza pepela napravljene početkom 2005. godine tijekom preliminarnih istraživanja pokazuju da pepeo s odlagališta Jezero pripada teksturnoj klasi na granici između ilovastog pijeska (IP) i praškaste ilovače (PI) [n=4; sa 75,1 % pijeska (20-200 μm); 19,1 % praha (2-20 μm) i 5,7 % gline (< 2 μm)], volumna gustoća (0,7 g/cm^3), gustoća čvrstih čestica (1,9 g/cm^3), poljski kapacitet (60,9 vol. %), točka venuća (8,6 vol. %), dostupna voda (52,3 vol. %), volumni udio zraka (7,7 vol. %) i porozitet (68,5 vol. %)(n=2), kapacitet zamjene kationa (CEC) iznosio je 318 mmol$_c$/kg, ukupna masena koncentracija karbonata ($CaCO_3$) iznosila je 49 g/kg (ili 4,9 % u ST) i ukupna masena koncentracija organskog ugljika (C_{org}) iznosila je 26,4 g/kg (ili 2,6 % u ST) (n=3).

Sastavnice prinosa ozimog ječma

Kod ječma (1) najlošiji rezultat zabilježen je u tretmanu br. 5 (tretiranje koje uključuje piljevinu bukovine kao poboljšivač) gdje je izmjeren najmanji broj biljaka s klasom po OP (23), najmanja srednja vrijednost duljine stabljike (18,6 cm), najmanja srednja vrijednost duljine klasa (1,4 cm), najmanji broj zrna (175), najmanja masa zrna (3,4 g) i najveći broj biljaka bez klasa po OP (83 sterilne biljke), a u usporedbi s rezultatima izmjerenim na svim ostalim tretmanima.

Godinu dana kasnije, a za ječam (2) najlošiji rezultat zabilježen je u tretmanu s čistim pepelom (br. 2) na kojem je zabilježen najmanji broj biljaka s klasom po OP (28), najmanji ukupni broj biljaka (32), najmanja srednja vrijednost duljine klasa (2.3 cm), najmanji broj zrna (51) i najmanja masa zrna (1.1 g) po OP, a u usporedbi s rezultatima izmjerenim na svim ostalim tretiranjima. Značajno manji u usporedbi s tretmanom na čistom tlu bio je broj biljaka s klasom (28) i srednja vrijednost duljine klasa (2,3 cm) na tretiranju s čistim pepelom.

Najbolji rezultat kod ječma (1) zabilježen je na tretmanu s čistim tlom gdje je izmjerena najveća srednja vrijednost duljine stabljike (36,9 cm), najveća srednja vrijednost duljine klasa (3,5 cm), najveća masa zrna (15,5 g) i najmanji broj biljaka bez klasa po OP (6 sterilnih biljaka) dok je kod ječma (2) najbolji rezultat zabilježen na tretmanu s 75 % tla i 25 % pepela gdje je izmjerena najveća srednja vrijednost duljine klasa (3,8 cm) i najveća masa zrna po OP (6,3 g).

Sastavnice prinosa soje

Najlošiji rezultat zabilježen je u tretmanu s čistim pepelom gdje je u usporedbi s tretmanom s čistim tlom izmjeren značajno manji broj mahuna po OP (11), broj mahuna po stabljici (1) i masa zrna (0,5 g). Najbolji rezultat postignut je na tretmanu s čistim tlom gdje je zabilježen najveći broj mahuna po OP (33), najveći broj mahuna po stabljici (4) i najveća masa zrna po OP (11,9 g).

Zrna soje izrasla na tretmanu s čistim ugljenim pepelom ili na njegovim različitim mješavinama s tlom bila su jak akumulator, u barem jednom tretmanu, B, Cd, P, Mo, Rb i Se, umjeren akumulator Cr, Cs, Cu, Ni i Zn, slab akumulator Pb i Sr i ne akumulator As, Co, Fe i U dok su zrna ječma izrasla na tretmanu s čistim pepelom ili na njegovim različitim mješavinama s tlom bila jak akumulator, u barem jednom tretmanu, B, P, Mo, Rb i Se, umjeren akumulator Cs, Cu, Cd i Zn, slab akumulator Pb, Cr i Sr i ne akumulator As, Co, Fe, Ni i U.

Dobiveni podaci pokazuju da su ozimi ječam i soja bili senzibilni, te u zrnu bili jaki akumulatori (EC > 1): fosfora (P) u svim tretmanima soje i svim tretmanima ječma; Rb samo u tretmanu s čistim pepelom, a za obje kulture i soju i ječam; Mo za sve tretmane za soju i neke tretmane za ječam (br. 1, 2 i 3); Se u svim tretmanima soje i u svim tretmanima ječma; B u nekim tretmanima soje (br. 3, 4, 5 and 6) i u jednom tretmanu (br. 3) ječma; Cd samo u tretmanu s čistim pepelom za soju.

Ukupne masene koncentracije As, Co, Cu, Fe, Pb i Zn u zrnu soje i ozimog ječma bile su u rasponu vrijednosti koje su označene kao prirodne, a prema dostupnoj literature, iako su povišene koncentracije As i Cu zabilježene u čistom pepelu.

Na osnovi dobivenih rezultata zaključujemo da su u nekim tretmanima vrlo siromašan prinos i vidljiva oštećenja biljaka, a posebno u tretmanu s čistim pepelom u usporedbi s tretmanom s čistim tlom, a za obje istraživane kulture najvjerojatniji uzrokovani zbog toksičnosti (B, Ni, U i Mo) ili deficita (N, Cu, Zn i Fe). Ne-esencijalni elementi u tragovima (As, Co i Pb) bili su u granicama normalnih, prirodnih koncentracija i značajno ispod kritičnih koncentracija od redom 1 mg As/kg, 1 mg Co/kg and 6 mg Pb/kg u svim tretmanima i za obje promatrane kulture.

Usporedba dviju vegetacijskih sezona ozimog ječma, prve od 15.11.05. do 26.06.06. - ječam (1) i druge od 09.11.06. do 18.06.07. - ječam (2), a koji su rasli na istom supstratu u kontroliranim uvjetima, pokazuje značajne razlike između nekih promatranih parametara:

U tretmanu s čistim tlom zabilježeno je značajno povećanje ukupne masene koncentracije N, S, B, Cs, Fe, P, Pb, Rb, Sr i Zn u zrnu izraslom u 2007. nego u onom izraslom u 2005., a značajno smanjenje duljine stabljike, broja zrna, mase zrna i ukupne masene koncentracije As, Cr i Mo.

U tretmanu s čistim pepelom zabilježeno je značajno povećanje duljine stabljike, duljine klasa, te ukupne masene koncentracije N, S, B, Cu, Fe, P, Sr i Zn u zrnu izraslom u 2007. u odnosu na onom izraslom u 2005., dok je zabilježeno značajno smanjenje broja biljaka bez klasa, ukupnog broja biljaka, broj zrna, masa zrna, te ukupna masena koncentracija C i Cr.

U tretmanu br. 3 sa 75 % tla i 25 % pepela utvrđeno je značajno povećanje u 2007. u odnosu na 2005. i to duljine klasa, te ukupne masene koncentracije N, S, H, Cu, Fe, Mo, P, Rb i Zn u zrnu, i značajno smanjenje broja biljaka sa klasom, ukupnog broja biljaka, broja i mase zrna i ukupne masene koncentracije C, As, Cd, Co, Se i Sr.

U tretmanu s jednakim masenim udjelom tla i pepela (50 %) zabilježeno je značajno povećanje u 2007. u odnosu na 2005. i to ukupne masene koncentracije H, Fe, Mo, P i Zn u zrnu, te značajno smanjenje broja biljaka sa klasom, ukupnog broja biljaka, broj i masa zrna, te ukupne masene koncentracije N, C, S, Cd, Se i Sr.

U tretmanu s poboljšivačem (piljevina bukovine) i s jednakim masenim udjelom tla i pepela (47.5 %) utvrđeno je značajno povećanje broja biljaka s klasom, duljine stabljike, duljine klasa, i ukupne masene koncentracije N, H, Cs, Cu, Fe, Mo, P, Rb i Zn u zrnu izraslom u 2007. u usporedbi s onim izraslim u 2005., te značajno smanjenje ukupnog broja biljaka, broja zrna, te ukupne masene koncentracije C, As, Cd, Co, Ni, Se i Sr.

U tretmanu br. 6 sa 25 % tla i 75 % pepela zabilježeno je značajno povećanje u 2007. u odnosu na 2005. i to duljine klasa, te ukupne masene koncentracije N, H, Cu, Fe, Mo, P i Zn u zrnu, a značajno smanjenje broja biljaka sa klasom, broja biljaka bez klasa, ukupnog broja biljaka, broja i mase zrna, te ukupne masene koncentracije C, Cd, Co, Cr, Se i Sr.

1 INTRODUCTION

The widespread black coal mining in South-East Europe (SEE) has been caused by the construction of several thermal power plants. The combustion of enormous quantities of coal used for energy production is closely related to the production of large amounts of waste materials, mostly ash and cinder that is usually pumped into large disposal sites. Disposal sites cover a large area of land in some regions of SEE. In the environment of Thermoelectric Power Plant Tuzla (TEP) in Bosnia and Herzegovina (BiH), around 0.06 % of total Canton land is covered with waste coal ash (3.33 km^2 or 333 ha), or around 1 % of the Canton area, account for mineral extraction sites and dump sites. The energy capacity of TEP is 715 MW which makes it the largest thermal power plant in BiH. Average annual production of electric power is 3 100 GWh (50 % of total annual state production) and annual coal consumption is around 3.5 to 4 million tons (Figure 1-1).

Figure 1-1 TEP Tuzla, (left, I Kisić, April 2005) and (right, Google earth, 2009)

TEP Tuzla supplies more than 20 000 consumers with heating energy. TEP was built in 1959 (Elektroprivreda BiH, 2009), and during the last 50 years more than 40 mil m^3 of ash and cinder have been disposed in Tuzla surroundings, at 5 disposal sites (Drežnik, Plane, Jezero, Divkovici I and II, Figure 1-2). Permanent lack of agricultural land in the area of TEP and surroundings has forced refugees and local farmers to cultivate the surface of dried ponds in order to get arable land and to use it for food production or as pastures and meadows. Nowadays, 63 ha of soil covered disposal sites (Drežnik and Plane) are used for agricultural purposes. Cover soil is Glaysols (Pseudogley) which is a predominant soil type in Tuzla region.

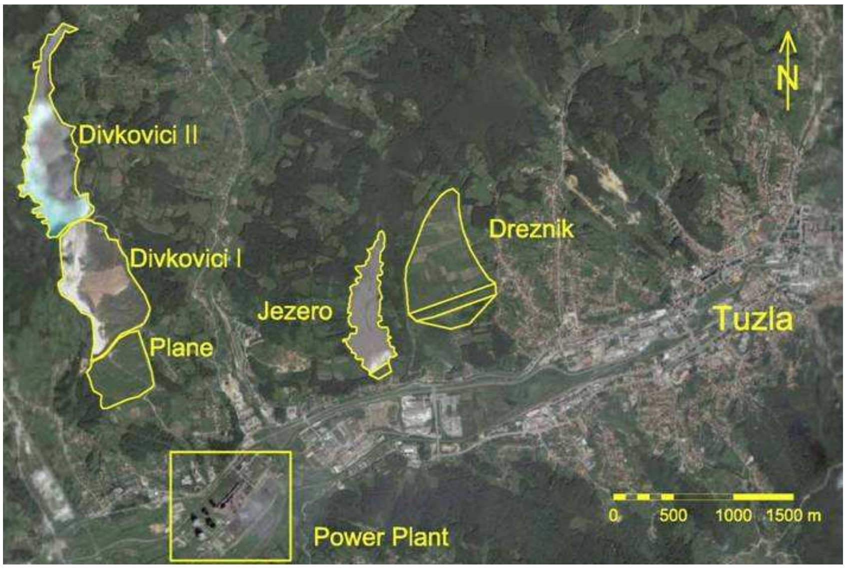

Figure 1-2 Disposal sites in Tuzla Region (RECOAL, Handbook, 2008)

So far, no investigations have been conducted in this part of the World regarding determination of risk assessment due to the usage of these dumping sites as agricultural land, that would consider unsafety and harmfulness of pollutants (metals and metalloids) and their phytoaccumulation in crops as the first stage of penetration into the food chain with regard to the intake of plants grown on disposal sites, directly or indirectly through animals (due to grazing). In this unusual and specific situation, during and after the war, new borders prevented migration of people and transport of goods, and the need to achieve a stable agriculture and therewith to ensure sufficient supply of food has become a very important issue in SEE area. Nowadays, major cultivating crops at disposal sites are maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), potato (*Solanum tuberosum L.*) and fodder crops; alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pretense* L.). Crops that are also cultivated in this area are soybean (*Glycine max*. L.) and bean (*Phaseolus vulgaris* L.) (Figure 1-3). All of these crops can be tolerant, moderately sensitive or sensitive to certain metals and metalloids that are present in the soil, and this characteristic depends on various factors. Plants can also be accumulators, moderately accumulators or non-accumulators of certain metals or metalloids.

Figure 1-3 Crops at disposal sites: alfalfa, wheat, potato, barley (M Marković, 2005)

2 LITERATURE REVIEW

Soil is essentially a non-renewable resource and a very dynamic system which delivers functions and services vital for human activities and ecosystems survival. Available information suggests that soil degradation processes have been increasing over the recent decades, and there is evidence that these processes will further increase if no action is taken (EU Directive, 2006).

Main **soil functions** are: (1) Food and other biomass production; (2) Environmental interaction: storage, filtering and transformation; (3) Biological habitat and gene pool; (4) Source of raw materials; (5) Physical and cultural heritage and (6) Platform for man-made structures: buildings or highways (Bašić, 2009).

Soil degradation is a process associated with the loss of equilibrium of a stable soil and occurs where human activities (either directly or indirectly) make it less vigorous or less healthy. Land/soil degradation can occur either as a result of natural hazards or due to unsuitable land use and inappropriate land management practices (FAO-ESCAP, 2000). Acidification, salinity, organic depletion, compaction, nutrient depletion, **chemical contamination**, landslides and erosions are all forms of soil degradation that can be caused by inappropriate land use practice (Cummings, 1999). Soil contamination is caused by presence of man-made chemicals or other alterations in natural soil environment. The most common chemicals involved in this process are petroleum hydrocarbons, solvents, pesticides and **heavy metals** (HM) (EUROSTAT, 1999).

Heavy metals occur naturally in the ecosystem with large variations in concentration. Soil is the main source of trace elements for plants, both as micronutrients and as pollutants. The soil-plant transfer (phytoaccumulation) of trace elements is a part of chemical element cycling in the nature (Kabata-Pendias and Mukherjee, 2007). In modern times, anthropogenic sources of heavy metals, i.e. pollution, have been introduced to the ecosystem. Heavy metal emissions are caused by mining and industrial activities. Transport and spreading of fertilizers and sewage sludge also discharge heavy metals into the environment (EUROSTAT, 1999).

Soil damage process with heavy metals contamination, according to the soil degradation classification (Bašić, 1994), belongs to the 2nd damage state (moderate level - hardly restorable, conditionally reversible). This kind of degradation results in unusable food, depression in plant growth and phytotoxic effects and also puts other ecosystems in jeopardy, due to mutagenic, cancerogenic or teratogenic effects.

According to Adriano, 2001, the term **trace elements** refers to elements that occur in natural and perturbed environments in small amounts (As, Ag, B, Ba, Be, Cd, Co, Cr, Cu, F, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Tl, V and Zn) and, when present in sufficient bioavailable concentrations, are toxic to living organisms. Other terms that are used and for all practical purposes considered synonymous with the term trace elements are: **trace metals**, **heavy metals**, **micronutrients** and **minor elements**. The term micronutrients is usually used to refer to elements that are **required** and **essential** by higher plants in small concentrations (Zn, Mn, Cu, Fe, Mo, B and Cl), whilst term heavy metals is used for elements with densities greater than 5 g/cm^3 and it denotes metals and metalloids that are associated with **pollution** and **toxicity** (Cd, Pb and Hg). It also refers to elements that are required by organisms (Cu, Zn and Mn) at relatively low concentrations.

Alloway, 1995, explains the difference between soil pollution and contamination. Although there are several interpretations of terms "pollution" and "contamination", a definition given by Holdgate, 1979, is widely accepted: "pollution is the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structures or amenity, or interference with legitimate uses of the environment". The term "contamination" is used when the anthropogenic inputs do not appear to cause obvious harmful effects and ‘‘pollution’’ is used only if toxicity occurs. However, in Holdgate's definition, ‘‘pollution’’ refers to any concentration of potentially harmful substances, whether or not adverse effects are observed. In practice, the terms contamination and pollution are frequently used interchangeably, although the term pollution is more pejorative. Fairly widely adopted convention for soils is to use the term contamination for any situation in which elevated concentrations of harmful substances occur.

One of the anthropogenic HM sources which are a global environmental concern is fossil fuel combustion (ash) (Keefer and Sajwan, 2000).

Coal ash can become highly alkaline or acidic and it can contain considerable amounts of trace elements. Several studies have investigated the environmental fate of trace elements in coal-fired power plants. Analysis of feed coal and combustion by-products has shown that Ba, Ce, Co, Cs, Cu, Dy, Ga, Ge, La, Lu, Mn, Ni, Rb, Sr, Tb, Th, Y, Yb, Zn and Zr are retained in solid wastes (Sandhu et al, 1993), As, B, Be, Cd, Cr, Li, Mo, Pb, Sb, Sn, Ta, Tl, U, V and W are only partly retained in solid wastes (Sandelin and Beckman, 2001) and Hg and Se are primarily emitted into the atmosphere (Lorens et al, 2001; Sandelin and Beckman, 2001).

In agriculture, fly ash has the potential of changing soil texture and increasing the water holding capacity of sandy soils due to high content of silt and clay-sized particles (Ghodrati et al, 1995), and it can also decrease the potential of soils to release dissolved P as well as its export from soils (Stout et al, 2000). However, a total of 25 potentially toxic elements were found in fly ash in considerable amounts. Some of these elements (Ba, B, Mo, Se, S, Sr, As and Al) were found in elevated concentrations in plants grown on fly ash amended soils (Adriano et al, 1980). According to Keefer, 1993, this does not pose a phytotoxicity threat to plants (except B in certain plants), but may be potentially harmful for animals which are eating these plants as well as for humans as consumers of agricultural products grown on these soils. Threshold values for mobile trace elements have been established for agricultural soils, where Cu, Pb, Cd and Tl are the metals of most concern, associated with fodder-plants for animals (Prüess, 1994).

Menon et al, 1993, found that the availability of toxic elements to plants varies with the texture of various types of ashes. Weinstein et al, 1993, reported that selenium (Se) is accumulated in amounts > 50 times higher in plants grown on coal ash landfill sites than in control plants. Application of gypsum to ash reduces Se uptake by grasses (Woodbury et al, 1999). Corn and soybean show severe symptoms of B toxicity, when grown on fly ash amended soils or fly ash mixed with 5% of manure (Kukier and Summer, 1996; Schwab, 1993). In addition to B toxicity, Schwab, 1993, also found Zn, Mo, and Cd at high concentrations in soybean.

Although several alternative uses of fly ash have been identified to replace ponds disposal, there are large areas throughout SEE countries and over the world that are already covered with coal ash landfill sites and more land for ash disposal will still be needed in future. Thus, strategies for an optimized reintegration and a productive use of this land are urgently required, especially in areas that lack agricultural land. Jala and Goyal, 2006, have shown that the use of fly ash in agriculture has a development potential and growing healthy crops on landfill sites might be possible when sufficient soil cover is applied. However, there is still not enough available information on the best performing plants relative to specific trace element concentrations, although some phytoaccumulate studies have been done (Kabata-Pendias and Mukherjee, 2007; Huang and Gobran, 2005; Adriano, 2001; Dos Santos Utmazian and Wenzel, 2007; Puschenreiter et al, 2005; Fitz and Wenzel, 2002; Unterbrunner et al, 2007; Wenzel, 2009; Wenzel et al, 1999; Wenzel et al, 2004; Latkoczy et al, 2000; Vego et al, 2008; Kisic et al, 2005; Custovic, 2005; Dellantonio et al, 2008) in respect of yield and of optimal thickness and types of soil covers, as well as the optimal ratio of soil cover and ash as substrate for useful and safe plant growth.

3 HYPOTHESIS AND GOALS OF INVESTIGATION

Hypothesis:

Investigated plants (soybean and winter barley) grown on substrate with different ash ratios will produce different quantity and quality of grain (Figure 3-1), regarding yield components and chemical composition of grain and they will differ in accumulation of investigated metals (Cr, Cu, Ni, Cd, Mo, Cs, Co, Pb, Fe, Zn, Rb and Sr), metalloids (As, B), non-metal Se and actinide U in their edible parts.

In accordance with this hypothesis, following **goals** were defined:

- determine the influence of different ash ratios on yield components of soybean and barley
- investigate the influence of ash ratio on metals and metalloids accumulation in grains of tested plants
- compare metals and metalloids accumulation in grains of soybean and barley grown on same ash ratios
- determine the influence of vegetation season on metals and metalloids accumulation in grains of barley

Figure 3- 1 *Visible influence of substrate on plant quality, glasshouse experiment*

4 MATERIALS AND METHODS

4.1 Location and conditions of investigation

The experiment was conducted under controlled conditions in a greenhouse at Faculty of Agriculture, University of Zagreb. Investigations included the following species and cultivars in vegetation period seeding time – harvest time (Figure 4-1):

- winter barley (*Hordeum vulgare* L. cv. Rex) grown on the same substrate during two vegetation seasons, from 15.11.05. to 26.06.06. barley (1) and from 09.11.06. to 18.06.07. barley (2)
- soybean (*Glycine max* L. cv. Dora) grown during one vegetation season, from 03.07.06. to 20.10.06.

Figure 4-1 Barley (1), soybean and barley (2) from 2005 to 2007, through three vegetation years

Base parcel (BP) was a plastic pot with 27 cm diameter (ϕ = 27 cm) filled with 5 kg of substrate, in which 100 seeds of winter barley and 15 grains of soybean were seeded. Fertilization was done and total applied doses of fertilizer were:

- winter barley fertilization: 0.72 g N/BP; 0.72 g P/BP and 0.48 g K/BP
- soybean fertilization: 0.72 g N/BP; 0.72 g P/BP and 1.08 g K/BP

Plants were watered at intervals, and approximately 2 cm of pebbles were placed at the bottom of the pots to improve water permeability.

The coal ash used in the experiment was taken as pure homogenous component from disposal site Jezero in TEP surrounding (Figure 4-2), according to methodology for sampling composite sample.

Figure 4-2 Disposal site Jezero, near Tuzla, BiH, ash used in experiment

Basic substrate was Gleysols (FAO-WRB, 2006) (or Pseudogley) soil taken as composite sample from Sasinovec in Croatia, which was according to preliminary analysis of physical and chemical characteristic analogous to soli cover from Tuzla surroundings (Figure 4-3).

Figure 4-3 Sasinovec, Croatia, Glaysols (Pseudogley) used in experiment

Saw dust of beech tree, taken from “Manico” saw mill, Mala Brijesnica in BiH, was used as amendment in treatment No. 5.

4.2 Experimental design

Experiments with soybean and barley were set up as randomized complete block design with four replications (Figure 4-4).

Different treatments varied in mass ratio of ash from 0 % to 100 %, as follows:

1. soil / ash = 100 % mass / 0 % mass
2. soil / ash = 0 % mass / 100 % mass
3. soil / ash = 75 % mass / 25 % mass
4. soil / ash = 50 % mass / 50 % mass
5. soil / ash = 47.5 % mass / 47.5 % mass + 5 % mass saw dust amendment of beech tree
6. soil / ash = 25 % mass / 75 % mass

Figure 4-4 Experimental design

4.3 Substrate sampling and analysis

Before the first seeding substrate samples were taken from each BP, and following parameters were determined:

- total content of Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Rb, Sr, U, Se, Zn, Cs, As, B and P

- plant available content of Cd, Co, Cr, Cu, Mo, Ni, Pb, Rb, Sr, U, Se, Zn, Cs, As, B and P

- chemical parameters: pH, EC, plant available P and K, total C, H, N and S

- cations: Li^+, Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}; anions: F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-} and PO_4^{3-}

4.4 Plant sampling and analysis

After the winter barley harvest (26.06.06. and 18.06.07.) and the soybean harvest (20.10.06.), samples of plants (whole above ground biomass) were taken from each BP (Figure 4-5), yield components were determined and edible parts of plants (grain) were analysed on investigated parameters.

Yield components:

- winter barley: number of plants with ear per pot, number of plants without ear per pot, total number of plants per pot, length of stems, length of ears, number and weight of grains and water content in grains
- soybean: total number of plants per pot, number of pods, weight of grains and water content in grains

Grain analysis (winter barley and soybean):

- total content of Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Rb, Sr, U, Se, Zn, Cs, As, B and P
- total content of C, H, N and S

Figure 4-5 Harvest time, 26 June 2006

4.5 Chronology of field work

This is the chronology of filed work that was done for the experiment (Figure 4-6).

15.11.2005. - winter barley seeding + base fertilization + taking samples of substrate - approximately 200 g

22.02.2006. additional fertilization- approximately 5 grains (0.2 g) of NPK per BP

06.04.2006. additional fertilization- approximately 5 grains (0.2 g) of NPK per BP

26.06.2006. winter barley harvest + taking samples of plants

03.07.2006. soybean seeding + fertilization

20.10.2006. soybean harvest + taking samples of plants

09.11.2006. winter barley seeding + fertilization

26.02.2007. additional fertilization - approximately 10 grains (0.4 g) of NPK per BP

18.06.2007. winter barley harvest + taking samples of plants

Figure 4-6 Field work during the experiment

4.6 Methods

Table 4-1 shows the list of methods for each investigation parameter used in the experiment.

Table 4-1 List of methods used in investigation

Parameter	**Method name**	**Protocol / Norm**
drying/grinding/ sieving/homogenizing	Soil samples preparation for physical and chemical analysis	HRN ISO 11464:2006
w(DM), w(H_2O) [%]	Determination of dry matter and water content - gravimetric method	HRN ISO 11465:2004
pH	Soil quality - Determination of pH in 0.01 M $CaCl_2$, 1 M KCl and H_2O at 1:2.5 (*w/v*) ratio	HRN ISO 10390:2004
EC [μScm^{-1}]	Soil quality - Determination of specific electric conductivity, in H_2O at 1:5 and 1:10 (*w/v*) ratio	HRN ISO 11265:2004
P_2O_5 and K_2O [mg/100g; g/kg]	Available phosphorus and potassium in AL extract at 1:20 (*w/v*) ratio	AL method (extraction with ammonium lactate acetic acid)
TS [% DM; g/kg]	Soil quality - Determination of total sulphur with dry combustion method	HRN ISO 15178:2005
TC [% DM; g/kg]	Soil quality - Determination of organic and total carbon with dry combustion method («elemental analysis»)	HRN ISO 10694:2004
TN [% DM; g/kg]	Soil quality - Determination of total nitrogen with dry combustion method («elemental analysis»)	HRN ISO 13878:2004
anions and cations soil extract	soil water extract at 1:10 (*w/v*) ratio	ONORM L 1092-93
F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-} and PO_4^{3-} [mg/kg]	Water quality - Determination of fluorides, chlorides, nitrites, orthophosphates, bromides, nitrates and sulfates with ion liquid chromatography - part 1. Method for water with low contamination	HRN ISO 10304-1:1998
Li^+, Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+} [mg/kg]	Water quality - Determination of Li^+, $Na^{+,}$ NH_4^+, K^+, Mn^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+} with ion liquid chromatography – Method for water and waste water	HRN ISO 14911:2001
extraction of total metals and metalloids in soil	Soil quality - Extraction of elements soluble in aqua regia (HCl/HNO_3 = 3:1 (*v/v*) digestion) at 1:12 (*w/v*) ratio	ONORM L 1085 HRN ISO 11466:2004
extraction of total metals and metalloids in plants	in HNO_3/H_2O_2 = 5:1 (*v/v*) digestion at 1:30 (*w/v*) ratio	
extraction of available metals and metalloids in soil	Soil quality - Extraction of trace elements using 1 M ammonium nitrate solution (NH_4NO_3) at 1:2.5 (*w/v*) ratio	ISO/DIS 19730:2006 DIN ISO 19730
ICP-MS analysis, total and available(labile) metals (Cr, Cu, Ni, Cd, Mo, Cs, Co, Pb, Fe, Zn, Rb and Sr), metalloids (As and B) and non-metals (Se and P) and U [mg/kg]	Soil quality - Determination of trace elements in extracts of soil by inductively coupled plasma - atomic emission spectrometry (ICP-AES) Water Quality - Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy	ISO/DIS 22036:2006 HRN EN ISO 11885:1998

4.6.1 Samples preparation for physical and chemical analysis

Substrate

Substrate samples were air-dried in a room with controlled temperature (T < 40°C). Samples were then grinded on knife mill (Euclid, MZ 1), sieved through < 2 mm sieve (Retsch) and homogenized. Each sample was subdivided by hand into two sub-samples and stored in plastic boxes. One sub-sample was prepared for laboratory analysis and the other for archive (Figure 4-7).

Figure 4-7 Substrate samples preparation

Plants

After the plant sampling (whole above ground biomass) and determination of yield components, grain parts of the plants were dried in the oven at 60°C. After drying, samples were grinded with a knife mill (IKA, M20) and sieved through a < 200 µm sieve (Retsch). As well as the substrate samples, plant samples (powder) were then homogenized and each was subdivided by hand into two sub-samples and stored in plastic boxes. One sub-sample was prepared for laboratory analysis and the other for archive (Figure 4-8).

Figure 4-8 Plant samples preparation

4.6.2 Determination of dry matter and water content

In substrate and plant samples water content and dry matter content were determined by gravimetric method. Samples (approximately 10 – 15 g) were dried in the oven at 105°C ± 5°C untill constant mass was reached (24 h). Samples were then cooled, first in a drying oven and then in a desiccator, until room temperature was reached. Water content and dry matter content were determined from differences in mass before and after drying (Figure 4-9).

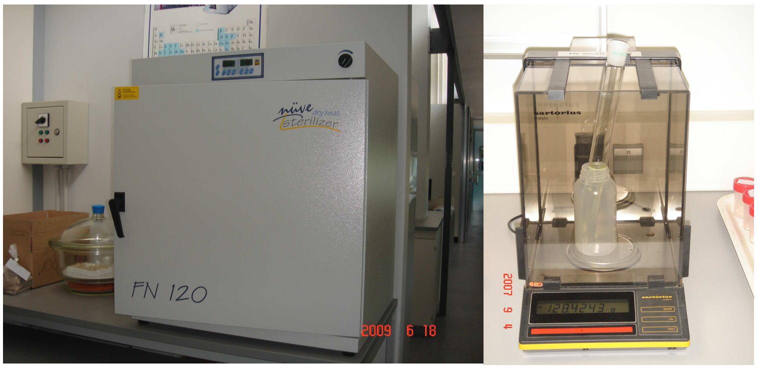

Figure 4-9 Drying oven and the balance for dry matter determination

4.6.3 Determination of substrate pH

The substrate pH was measured using the electrometric method with the Beckman pH-meter Φ^{TM} 72 (1990), with combined glass electrode (BlueLine 17 pH BNC, Schott instruments) in three different suspensions (Figure 4-10):

1. pH in ultra pure H_2O (ultra pure water electroconductivity < 0.055 μScm^{-1}) a ratio of 1:2.5 (*w/v*) (10 g substrate / 25 mL H_2O)
2. pH in 1 M (mol/L) KCl, a ratio of 1:2.5 (*w/v*) (10 g substrate / 25 mL KCl)
3. pH in 0.01 M $CaCl_2$, a ratio of 1:2.5 (*w/v*) (10 g substrate / 25 mL $CaCl_2$)

After substrate weighting, solutions of accurately known pH were added, suspensions were stirred (homogenized) for 1 h ± 10 min, and pH values were measured in suspension, until the value of pH was stabilized.

Buffer solutions were used for calibration (pH = 4.00 ± 0.05 and pH = 7.00 ± 0.05). Accuracy and repeatability were checked with buffer solution pH = 5.00 ± 0.05 and were satisfactory according to those regulated in norms.

In soil analyses pH measurements in KCl or $CaCl_2$ are more common than in H_2O. In soil H_2O extract only free H^+ ions can be measured (active acidity), while KCl or $CaCl_2$ extracts give information on free, as well as on H^+ ions that are bonded in adsorption complex (potential or substitute acidity).

4.6.4 Determination of substrate specific electric conductivity

The substrate specific electrical conductivity (EC) was measured using the electrometric method with the Conductometer Lab 960, SCHOTT Instruments (2006), with Pt electrode (measure cell, Schott LF413T) in H_2O suspension in different ratios (Figure 4-10):

1. EC in ultra pure H_2O, a ratio of 1:10 (*w/v*) (10 g substrate / 100 mL H_2O),
2. EC in ultra pure H_2O, a ratio of 1:5 (*w/v*) (20 g substrate / 100 mL H_2O),

Suspensions were shaken over a period of 30 ± 10 min in a horizontal shaking machine.

After the substrate weighting, liquid was added, and suspensions were filtered through filter paper. EC values were measured in filtrates. Conductometer had a temperature auto correction up to 25 °C. Standard KCl solution was used for calibration (0.01 mol/L, $\chi = 1.41$ mS/cm). Accuracy and repeatability were checked with KCl solution 0.001 mol/L and were satisfactory according to norms.

Figure 4-10 pH meter and Conductometer for pH and EC determination

4.6.5 Determination of available phosphorus and potassium in substrate

Available phosphorus (P) in substrate, expressed as P_2O_5, and available potassium (K) in substrate, expressed as K_2O, were determined by AL method. AL method consists of extraction with ammonium lactate acetic acid and spectrophotometric detection of phosphorus and flame photometric detection of potassium. Five grams of substrate were mixed with 100 mL AL solution in plastic bottles (1:20 *w/v* ratio). Bottles were shaken in rotating shaking machine for a period of 2 h ± 10 min. Suspensions were filtered through a filter paper. P_2O_5

and K_2O values in filtrates were measured with different detection methods (phosphorus with spectrophotometric and potassium with flame photometric) (Figure 4-11).

Figure 4-11 Flame photometer and spectrophotometer for K and P determination

Potassium, K_2O was measured in original filtrates using the Flamephotometer Jenway, (1999) (Figure 4-11). Samples were dispersed in nebuliser. Under the influence of heat of flame, caused by gas (fuel) activity, liquid takes the form of aerosol. In aerosol state, ions are reduced to atoms, which are then excited to higher energetic levels. When excited atoms return to ground state, they emit energy (hν) at a characteristic wavelength and the emission intensity, measured on detector, is proportional to concentration of this element in the sample. Atoms in gaseous form give line spectra and coloured glass filter can separate the lines of different elements (with specific wavelength). Potassium emission wavelength is λ = 766 nm and it has a specific violet flame colour.

During the calibration standard K_2O solutions were used (10, 20, 30 and 40 mg/100 g soil) as well as pure AL solution (0 mg/100 g soil). Accuracy and repeatability were checked with K_2O solution (40 mg/100 g soil) and were satisfactory.

Phosphorus, P_2O_5 was measured by colorimetric method using the spectrophotometer DR/2000 HACH, (1996) (Figure 4-11). In 100 mL Erlenmeyer flasks 15 mL of ultra pure water, 10 mL of sample, 2 mL of molybdenum-fotorex solution and 1 mL of $SnCl_2$ solution were added. Calibration standards and blanks were prepared at the same time and in the same way as samples. Calibration blanks were prepared with 10 mL of AL solution instead of 10 mL of sample, and standards were prepared with 10 mL of standard P_2O_5 solutions with different concentration (10, 20, 30 and 40 mg/100 g soil). Accuracy and repeatability were checked with P_2O_5 solution (20 mg/100 g soil) and were satisfactory.

Thus prepared probes were shaken by hand and left to react for a period of 1/2 h. Probes developed blue color, and after exactly 1/2 h colour intensity (optical density) was λ = 620 nm, measured by spectrophotometer. The amount of electromagnetic radiation of specific wavelength which was absorbed by determined compound was measured, and this absorption was proportional to the concentration of phosphorus in the sample (Lambert-Beer law).

4.6.6 Determination of total nitrogen, sulphur, carbon and hydrogen with dry combustion method in substrate and plants

Total nitrogen (TN), total sulphur (TS), total carbon (TC) and total hydrogen (TH) content in substrate and plants grain were simultaneously determined using the dry combustion method (elemental analysis) with the Vario Macro CHNS analyzer, Elementar (2006) (Figure 4-12). The content of TN, TC, TS and TH in substrate and plant was determined by heating the sample in oxidation column to a temperature of at least 900 °C for TN, TC and TH and 1150 °C for TS in the presence of oxygen gas (the temperature of oxidation column was 1150 °C, p (O_2) = 2.5 bar; $\dot{V}(O_2)=$from 10 to 75 mL/min depending on the chosen method) and in the presence of WO_3 as a catalyst for complete combustion of sample. Mineral and organic element compounds are oxidized and/or volatilized. Combustion products are a mixture of different oxides: NO_x (NO and NO_2), N_2, SO_3, SO_2, CO_2 and H_2O. In a reduction column (the temperature was 850 °C at which Cu^o was oxidised into Cu^{2+}) all nitrogen forms were transformed (reduced) into N_2, sulphur forms into SO_2, carbon forms into CO_2 and hydrogen forms into H_2O. All these gases were transported by the carrier gas helium (p (He) = 2.0 bar; $\dot{V}(He)=500 mL/\min$) to the thermal conductivity detector (TCD) and after adsorption and desorption in specific columns (CO_2 column at 230°C; H_2O column at 150 °C and SO_2 column at 220 °C) the content of TN, TC, TH and TS was measured. Pressure in the system was 1.24 bars.

Standard sulphanilamide was used for daily calibration and daily factor calculation (99 %, Sigma; 16.27 % TN; 41.85 % TC; 4.68 % TH and 18.62 % TS).

The amount of test sample for analysis depended on the expected TN, TS, TC and TH content. Approximately 200 mg ± 2 mg of oven-dried substrate samples and approximately 100 mg ± 2 mg of oven-dried plant samples were weighed in tin foils. In solid sample, capsules were formed by pressing and compressing samples in folded tin boats. Samples prepared in this way were placed in autosampler and were ready for analysis. Methods used in analysis were: method plant for plants (flow of O_2 up to 60 mL/min) and method soil 10 % for substrate (content of TC between 4-10 % and flow of O_2 up to 75 mL/min).

Analyses were carried out in accordance with the manufacturer's instructions for the apparatus.

Accuracy and repeatability were checked for each sample batch (sample feeder with 40 positions for samples) with RM and CRM and were satisfactory.

Figure 4-12 CHNS analyzer, Vario Macro, Elementar

4.6.7 Determination of anions and cations in soil (substrate) water extracts

Simultaneous determination of 13 ions, whereof seven anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-} and PO_4^{3-}) and six cations (Li^+, Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) in substrate was done using the ion chromatography method with two IC 1000 systems, Dionex (2004) (Figure 4-13). The separation and determination of ions were based on the principle of different ion exchange on stationary phase (column). Typical ion chromatographic system consists of: pump for eluens (mobile phase), pre column and column (stationary phase), injector, suppressor, detector and PC.

Ten grams of substrate were weighed and mixed with 100 mL of ultra pure water in 500 mL plastic bottles (1:10 *w/v* ratio). Bottles were left to settle over night and in the morning they were shaken in rotating shaking machine for 1 h ± 10 min. Suspensions were allowed to settle for 15 minutes and extracts were taken from clear filtrate by syringe (10 mL). Filtrates were filtered through a membrane filters (thickness 0.45 µm). Samples were placed in plastic vials in autosampler for analysis.

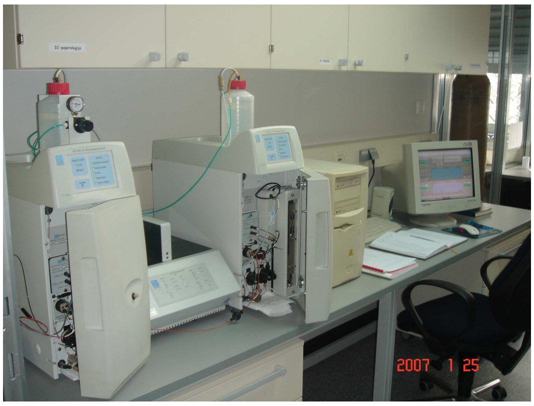

Figure 4-13 Ion chromatographic system, in OPBLab in Zagreb

Simultaneous analysis was enabled by a splitter, installed on autosampler AS40, which split the sample in two pipelines, one for anion system and other for cation system. For identification or qualitative determination of ion, retention time of specific ions on column was used (compared with retention time of standard). Quantification of specific ions in unknown sample was determined in relation to calibration curves derived from analysis of standards. The area below the peaks [μS×min] was calculated by Chromeleon® software (this area is proportional to mass concentration of ions [mg/L] in the sample). Eluent was degassed with inert helium (99,999 % and p (He) = 1 bar). Standard solutions (1000 ppm or mg/L) used to identify calibration curves were prepared from different salts of high purity.

For the purpose of verification of data collected in the Analytical laboratory of Department of General Agronomy, Faculty of Agriculture, University of Zagreb (OPBLab), comparative analytical measurements were done at University of Natural Resources and Applied Life Sciences, Vienna (BOKU), Austria (Figure 4-14).

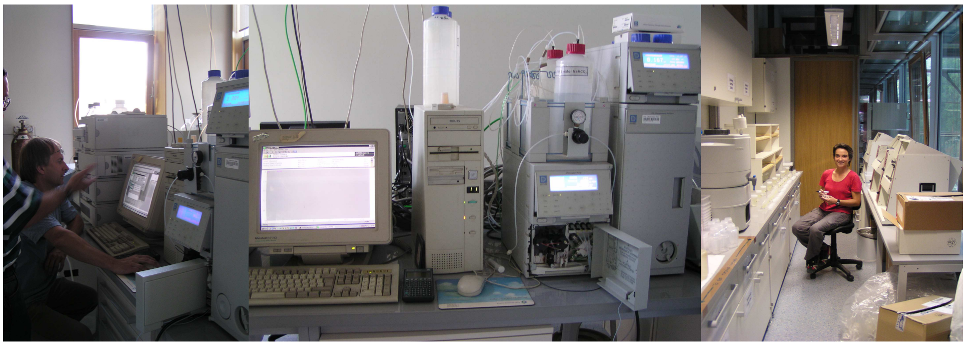

Figure 4-14 Ion chromatographic system in BOKU, Vienna

4.6.7.1 Determination of anions in soil water extracts

IC 1000 anion system, Dionex

Eluens reservoir for ultra pure water (EC < 0,055 μS/cm), polypropylene, capacity of 2 L.
Eluens reservoir for KOH (100%, EG Dionex), EGC-KOH, RFC-30, **eluens generator** module with on line **eluens preparation** and additional cleaning of entering ultra pure water
Eluens flow: 1.00 ml/min, constant during the whole analysis
Eluens concentration (KOH) was changed from 10-35-10 mmol/L during the analysis by eluens generator (EGC-KOH, RFC-30) module with on line eluens preparation. This module can change ratio of mobile phase (eluens (H_2O and KOH)). For the first 5.5 minutes of analysis eluens the concentration was 10 mmol/L and then it was increased to 35 mmol/L, which was reached in 16.5 minutes. Total time of anions analysis was 18 minutes. After 18 minutes all seven anions left the column and were detected on a detector, but due to

simultaneous analysis of cations, which takes 30 minutes, for the last 12 minutes the concentration of anion eluens was 10 mmol/L.

Pump, piston, two in serial with a very low effect of pulsation, microprocessor with electronic gradient control

Injector, 25µL (injection valve)

Guard column, IonPac AG 17 (4 × 50 mm), thermostated at 30°C

Separation column, IonPac AS 17 (4 × 250 mm), thermostated at 30°C

Suppressor, ASRS-ULTRA II (4 mm), self-regenerating anion suppression

Conductometer detector (ECD), microprocessor controlled with digital signal (conductometer cell temperature: 35°C)

Maximal pressure: 5000 psi

Minimal pressure: 0 psi

Current power in suppressor: 90 mA

Velocity of data collecting: defined with frequency of 5.0 Hz

Total time of analysis: 30 min.

4.6.7.2 Determination of cations in soil water extracts

IC 1000 cation system, Dionex

Eluens reservoir for MSA (methasulfonic acid, 99 %, Fluka), polypropylene, capacity of 2 L.

Eluens flow: 1.50 ml/min, constant during analysis

Eluens concentration (KOH): 30 mmol/L, constant during analysis

Pump, piston, two in serial with a very low effect of pulsation, microprocessor with electronic gradient control

Injector, 25µL (injection valve)

Guard column, IonPac CG 16 (5 × 50 mm), thermostated at 40°C

Separation column, IonPac CS 16 (5 × 250 mm), thermostated at 40°C

Suppressor, CSRS 300 (4 mm), self regenerating anion suppression

Conductometer detector (ECD), microprocessor controlled with digital signal (conductometer cell temperature: 35°C)

Maximal pressure: 5000 psi

Minimal pressure: 0 psi

Current power in suppressor: 88 mA

Velocity of data collecting: defined with frequency of 5.0 Hz

Total analysis time: 30 min.

4.6.8 Determination of total and available metals and metalloids in substrate and total metals and metalloids in plants

All extracts were analysed using inductively coupled plasma mass spectrometry, ICP-MS (ELAN 9000 DRC-e, Parkin Elmer) at BOKU, Vienna (Figure 4-15).

Sample digestion (extraction) for the analysis of total (Figure 4-16) and available (Figure 4-17) metals and metalloids in substrate was done at BOKU, while digestion (extraction) for the analysis of total metals and metalloids in plants was done at FAZ (Figure 4-18). Both digestions were done in open Heating digester (DK, Velp, Scientifica).

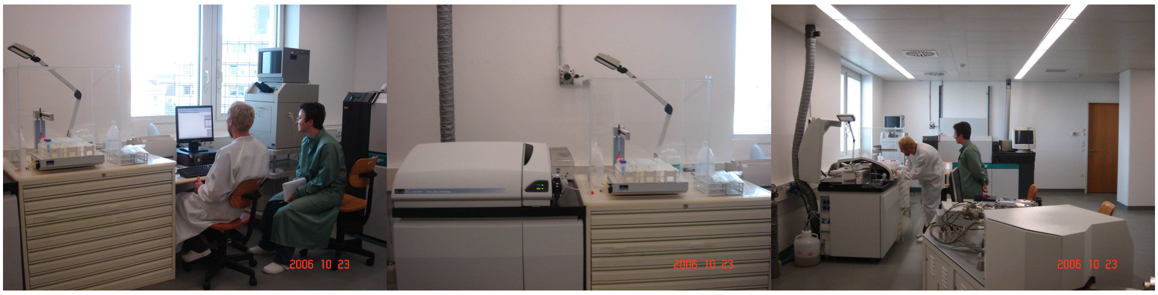

Figure 4-15 Analysis of all extracts on ICP-MS at BOKU, Vienna

4.6.8.1 Extraction of total metals and metalloids in substrate

Extraction (digestion) of the substrate for determination of total metals and metalloids content was done in aqua regia, $HCl:HNO_3 = 3:1$ (*v/v*). All laboratory dishes used in digestion were initially cleaned in a washing machine, afterwards in 5 % HNO_3 acid bath (minimum cleaning time was 3 h) and finally in ultra pure water. All substances used in digestion were high purity chemicals (p.a.).

Substrates were dried, grounded, sieved and homogenized, and 0.500 g (± 0.010 g) was weighed on analytical balance (Sartorius, 1991) into tubes that were placed in the fume hood. Acids were then added from dispensers: first 4.5 ml of HCl and then 1.5 ml of HNO_3 (1:12 w/v ratio). One drop of octanol was added to inhibit foaming. When the whole sample came in contact with the acid, the coolers were put on tubes and samples were left to react over night. In the morning the digestion block was turned on (Velp, 2003) the soil-digestion programme 2 (1 h at 65°C and 3 h at 150 °C) (Figure 4-16). After digestion the inside of the cooler was rinsed with ultra pure water, which was collected into the tube and more ultra pure water was added to approximately 50 ml. The sample was then mixed using the vortex-shaker and the exact weight of the tube was measured (including: tube + sample + acid + water). The sample was filtered into an appropriate vial. Samples prepared in this way were ready for analysis.

Figure 4-16 Determination the total metals and metalloids in substrate at BOKU, Vienna

4.6.8.2 Extraction of available metals and metalloids in substrate

The extraction of substrate for available (labile) metals and metalloids content was done in 1 M NH_4NO_3 solution. All laboratory dishes were cleaned in accordance with the procedure for total metals and metalloids (explained in chapter 4.6.8.1).

Twenty grams of substrate were weighed and mixed with 50 mL of 1M NH_4NO_3 solution in 100 mL plastic bottles (1:2.5 *w/v* ratio). Bottles were shaken in rotating shaking machine for a period of 2 h ± 10 min. Suspensions were left to settle for 5 minutes and then filtered using filter papers. Clear filtrate extracts were placed in plastic vials and acidified (by adding 1 % of concentrated HNO_3 of extract volume) and were ready for analysis (Figure 4-17).

Figure 4-17 Determinating labile metals and metalloids at BOKU, Vienna

4.6.8.3 Extraction of total metals and metalloids in plants

The extraction of plants for total metals and metalloids content was done in HNO_3 and H_2O_2 mixture, HNO_3:H_2O_2 = 5:1 (*v/v*). All laboratory dishes used in digestion were cleaned in Kemex®A, Kemika and in ultra pure water. All substances used in digestion were high purity chemicals (p.a.).

Substrates were dried, grounded, sieved and homogenized, and 0.2000 g (± 0.0100 g) was weighed on analytical balance (Sartorius, 1991) into tubes that were placed in the fume hood. After that, acids were added using the dispensers: first 5 ml of HNO_3 and then 1 ml of H_2O_2 using Pasteur-pipette (1:30 w/v ratio). When the whole sample came in contact with the acid, the coolers were put on tubes and samples were left to react over night.

In the morning the digestion block was turned on (Velp, 2007) the soil-digestion programme 3 (1h at 65°C and 3h at 150 °C).

After digestion the inside of the cooler was rinsed with ultra pure water, which was collected into the tube and more ultra pure water was added to approximately 50 ml. The sample was then mixed using the vortex-shaker and the exact weight of the tube was measured (including: tube + sample + acid + water). The sample was then filtered into an appropriate vial. Samples prepared in this way were ready for analysis (Figure 4-18).

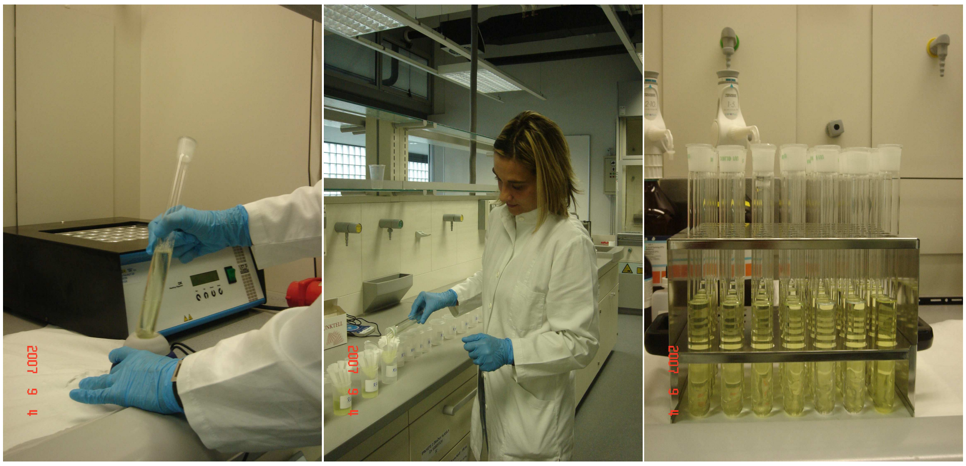

Figure 4-18 Extraction of total metals and metalloids in plants at FAZ, Zagreb

4.6.9 Quality management and quality control

In 2005 Faculty of Agriculture University of Zagreb (FAZ) has established quality management (QM) system in compliance with HRN EN ISO 9001:2000.
Analytical laboratory of General Agronomy is establishing (QM) system in line with the standard HRN EN ISO 17 025.

In 2006 Croatian Ministry of Environmental Protection, Physical Planning and Construction granted licences to Department of General Agronomy (cro.OPB) for conducting studies in (1) influence on environment, (2) monitoring environment conditions and (3) elaborates in environment protection.

The analytical laboratory of Department of General Agronomy (cro.OPBLab) integrates Internal and External quality control (QC).

4.6.9.1 Internal quality control

During their daily proceedings in laboratory analysts are working according to standard operating procedures (SOPs) respecting the good laboratory practice (GLP).
Internal QC involves checking the accuracy and repeatability of measurements by analysing the reference material (RM) and doing a minimum of three consecutive measurements. Standard solutions with known concentrations are sometimes prepared in the laboratoy, for the same purpose.

RM samples from different interlaboratory trials were used in the laboratory: ISE (**I**nternational **S**oil analytical **E**xchange Programme organized by WEPAL [**W**ageningen **E**valuating **P**rograms for **A**nalytical **L**aboratories, organized by Soil Chemistry and Chemical Soil Quality group, Department of Soil Quality of Wageningen University (WU), Netherlands]), and MOEL (Soil ring test organized by National Office for Agriculture [germ. Thüringer Landesanstalt für Landwirtschaft] from Jena, Germany) for **soil**, IPE (**I**nternational **P**lant analytical **E**xchange Programme organized by WEPAL) for **plant,** IFA (Water Proficiency Testing scheme organized by BOKU, Department for Agrobiotechnology, **IFA**-Tulln, Canter for Analytical Chemistry) for **water,** **CRM** (Certified reference material) **Eurosoil** for **soil** (Joint research Centre - Institute for Reference Materials and Measurements, JRC – IRMM).

4.6.9.2 External quality control

OPBLab is annually participating in different proficiency testing (PT) programs in plants, soil and water fields.

Samples with unknown concentrations of specific parameters are brought into the laboratory, and after analyses the data is sent to a data collection and evaluation center. After statistical calculations each participating laboratory is informed of accuracy and precision of their results. If there are any unallowed deviations caused by define error it is necessary to take adequate corrective actions.

OPBLab participates in following proficiency tests:

Water

Since 2006 OPBLab participates in water ring tests IFA (Test Systems PT) Scheme organized by University of Natural Resources and Applied Life Sciences, Department for Agrobiotechnology, IFA-Tullin, Center for Analytical Chemistry, Vienna, Austria.

The purpose of IFA ring test is testing the methods for determination of quality of ground and surface water for numerous inorganic and organic parameters.

http://www.ifatest.at/

Soil

In soil ring test MOEL, organized by National Office for Agriculture (Thüringer Landesanstalt für Landwirtschaft, from Jena, Germany) OPBLab participated in 2007.

MOEL test which included pH, P, K and Mg analyses.

http://www.tll.de/

Since 2009 OPBLab participates in soil ring tests in ISE (International Soil-analytical Exchange) Programme organized by WEPAL (Wageningen Evaluating Programs for Analytical Laboratories), Soil Chemistry and Chemical Soil Quality group, Department of Soil Quality of Wageningen University (WU), Netherlands.

http://www.wepal.nl/

Plant

Since 2009 OPBLab participates in plant ring tests in IPE (International Plant-analytical Exchange) Programme organized by WEPAL (Wageningen Evaluating Programs for Analytical Laboratories), Soil Chemistry and Chemical Soil Quality group, Department of Soil Quality of Wageningen University (WU), Netherlands.

http://www.wepal.nl/

5 RESULTS

All data observed in this experiment were analysed and performed using statistical Software *SAS 9.1 (SAS Inst. Inc.)*. Variability between treatments (mass ratios of ash) for all monitored parameters in experiments with winter barley and soybean were evaluated with analysis of variance (One-Way ANOVA) and tested, if it were necessary, with adequate *post-hoc* (Fishers LSD) t tests. In all statistical tests significance level was 5 %.

All tables of results show mean values (superscript capital letters), which were the results of statistical data analysis performed using SAS *9.1 (SAS Inst. Inc.)*. Mean values of certain parameter that have the same superscript for certain treatments of that parameter were not significantly different with 95 % confidence ($p < 0.05$).

All tables of results also show the relative standard deviation (RSD in [%]) calculated from four measurements, and it was noted as ± number, which was the measure of spread in the observed data.

If the concentration of investigated element was below the limit of detection (LOD) of the instrument on which certain parameter was determined, a not detected (n.d.) abbreviation was used in tables.

5.1 Initial content of elements in growing substrate

Mean values of pH (at 1:2.5 (*w/v*) ratio of KCl, $CaCl_2$ and H_2O), electrical conductivity (EC in 1:5 and 1:10 (*w/v*) ratio of water extract), moisture content in %, plant available potassium and phosphorus in mass ratios and total mass content of carbon, sulphur, hydrogen and nitrogen in percent of dry matter and C/N ratio measured in substrate, are shown in Table 5-1.

Table 5-1 *Mean values of pH, EC, moisture content, available P and K and total nitrogen, carbon, sulphur and hydrogen in substrate*

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
pH, Mean ± RSD						
1 M KCl, 1:2.5 (*w/v*)	$7.35^{D} \pm 0.71$	$8.77^{C} \pm 1.41$	$8.87^{B} \pm 0.37$	$8.93^{AB} \pm 0.80$	$8.94^{AB} \pm 0.18$	$9.01^{A} \pm 0.42$
0.01 M $CaCl_2$, 1:2.5 (*w/v*)	$7.37^{C} \pm 1.47$	$8.75^{B} \pm 0.69$	$8.71^{B} \pm 0.38$	$8.74^{B} \pm 0.71$	$8.74^{B} \pm 0.34$	$8.87^{A} \pm 0.22$
H_2O, 1:2.5 (*w/v*)	$8.11^{C} \pm 0.69$	$8.86^{B} \pm 0.71$	$8.92^{AB} \pm 0.66$	$8.90^{AB} \pm 0.23$	$8.87^{AB} \pm 0.14$	$8.93^{A} \pm 0.33$
EC, Mean ± RSD						
H_2O 1:5 (*w/v*) µS/cm	$172.7^{E} \pm 2.0$	$2380^{A} \pm 3$	$987^{D} \pm 6$	$1559^{C} \pm 12$	$1517^{C} \pm 7$	$1947^{B} \pm 6$
H_2O 1:10 (*w/v*) µS/cm	$123.0^{E} \pm 1.2$	$1591^{A} \pm 5$	$628^{D} \pm 6$	$949^{C} \pm 6$	$944^{C} \pm 8$	$1234^{B} \pm 4$
Moisture, Mean ± RSD						
w (H_2O), %	$0.21^{C} \pm 21.6$	$0.26^{ABC} \pm 18.7$	$0.25^{BC} \pm 6.4$	$0.27^{AB} \pm 6.4$	$0.30^{A} \pm 5.6$	$0.27^{AB} \pm 10.0$
Available P and K, Mean ± RSD						
g P_2O_5/ kg substrate	$0.40^{A} \pm 2.3$	$0.06^{E} \pm 14.6$	$0.27^{B} \pm 5.4$	$0.20^{C} \pm 12.9$	$0.20^{C} \pm 15.3$	$0.10^{D} \pm 22.9$
g K_2O/ kg substrate	$0.22^{E} \pm 1.52$	$0.44^{A} \pm 1.52$	$0.27^{D} \pm 3.25$	$0.36^{C} \pm 6.66$	$0.38^{C} \pm 5.40$	$0.40^{B} \pm 1.31$
Total NCSH, Mean ± RSD						
TN, % in DM	$0.155^{A} \pm 4$	$0.110^{D} \pm 5$	$0.145^{AB} \pm 2$	$0.136^{B} \pm 5$	$0.124^{C} \pm 5$	$0.110^{D} \pm 11$
TC, % in DM	$1.989^{D} \pm 2$	$2.772^{B} \pm 9$	$2.323^{CD} \pm 10$	$2.523^{CB} \pm 5$	$4.936^{A} \pm 7$	$2.512^{CB} \pm 12$
TS, % in DM	$0.598^{B} \pm 8$	$0.349^{D} \pm 8$	$0.519^{C} \pm 5$	$0.487^{C} \pm 5$	$0.789^{A} \pm 6$	$0.397^{D} \pm 7$
TH, % in DM	$0.067^{C} \pm 67$	$0.433^{A} \pm 25$	$0.151^{CB} \pm 20$	$0.244^{B} \pm 18$	$0.454^{A} \pm 39$	$0.422^{A} \pm 12$
C/N ratio, Mean ± RSD						
C/N	$13^{D} \pm 2$	$25^{B} \pm 9$	$16^{CD} \pm 10$	$19^{C} \pm 11$	$40^{A} \pm 9$	$23^{B} \pm 10$

Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

Seven anions: fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-), bromide (Br^-), nitrate (NO_3^-), sulfate (SO_4^{2-}) and phosphate (PO_4^{3-}) and six cations: lithium (Li^+), sodium (Na^+), ammonium (NH_4^+), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) mass ratios measured in 1:10 *(w/v)* water extract are shown in table 5-2.

Table 5-2 Mean values of anions and cations in substrate measured in 1:10 (w/v) water extract

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Anions, mg/kg, Mean ± RSD						
F^-	4.59^{CB} ± 3	8.53^{B} ± 18	2.20^{C} ± 8	7.94^{B} ± 5	44.3^{A} ± 15	6.92^{B} ± 20
Cl^-	18.3^{AB} ± 27	10.4^{BC} ± 50	n.d.^{C}	10.8^{B} ± 7	22.0^{A} ± 70	14.9^{AB} ± 31
NO_2^-	4.14^{B} ± 5	7.91^{A} ± 22	2.44^{C} ± 16	6.98^{A} ± 10	7.30^{A} ± 17	7.84^{A} ± 3
Br^-	0.21^{A} ± 13	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
NO_3^-	25.7^{C} ± 5	24.7^{C} ± 4	13.3^{D} ± 11	39.3^{A} ± 5	34.9^{B} ± 4	32.3^{B} ± 9
SO_4^{2-}	27.1^{E} ± 42	11 843^{A} ± 1	863^{D} ± 9	5 920^{C} ± 14	6 015^{C} ± 11	8 800^{B} ± 8
PO_4^{3-}	10.2^{A} ± 3	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Cations, mg/kg, Mean ± RSD						
Li^+	n.d.^{D}	1.16^{A} ± 3	n.d.^{D}	0.49^{C} ± 10	0.51^{C} ± 10	0.77^{B} ± 13
Na^+	5.44^{C} ± 25	34.2^{AB} ± 8	5.59^{C} ± 12	23.0^{B} ± 5	35.9^{A} ± 39	41.3^{A} ± 27
NH_4^+	8.31^{A} ± 10	2.42^{B} ± 41	1.34^{B} ± 103	6.06^{A} ± 13	6.96^{A} ± 50	3.14^{B} ± 72
K^+	42.4^{C} ± 15	64.7^{B} ± 6	8.71^{D} ± 20	58.7^{B} ± 3	82.1^{A} ± 5	60.4^{B} ± 7
Mg^{2+}	8.60^{D} ± 8	118^{A} ± 6	8.90^{D} ± 13	51.2^{C} ± 15	60.2^{C} ± 15	80.7^{B} ± 11
Ca^{2+}	224^{D} ± 3	4 660^{A} ± 2	403^{D} ± 8	2 502^{C} ± 13	2 587^{C} ± 9	3 546^{B} ± 7

n.d. – not detected; Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

Table 5-3 shows mean values of twelve total metals: cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), molybdenum (Mo), nickel (Ni), lead (Pb), rubidium (Rb), strontium (Sr), zinc (Zn) and caesium (Cs); two metalloids: boron (B) and arsenic (As); non-metals phosphorus (P) and selenium (Se) and actinoid uranium (U), content in substrate analysed in aqua regia (HCl/HNO_3 = 3:1, *v/v*) extract. In table 5-3, and all following tables, elements (metals, metalloids and non-metals) are given in alphabetical order.

Table 5-3 *Mean values of total metals, metalloids and non-metals in substrates analysed in aqua regia extract, at HCl/HNO_3 = 3:1 (v/v) ratio*

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Total element content, mg/kg, Mean ± RSD						
As, 75	27.7^{E} ± 1	112^{A} ± 11	48.6^{D}	69.6^{C}	67.7^{C} ± 8	90.6^{B}
B, 10	n.d.^{F}	204^{A} ± 6	51.1^{E}	102^{C}	89.0^{D} ± 17	153^{B}
Cd, 111	0.29^{E} ± 2	0.52^{A} ± 16	0.35^{D}	0.40^{C}	0.37^{CD} ± 4	0.46^{B}
Co, 59	16.2^{F} ± 1	34.1^{A} ± 2	20.7^{E}	25.1^{C}	22.9^{D} ± 5	29.6^{B}
Cr, 53	59.7^{F} ± 2	281^{A} ± 2	115^{E}	170^{C}	153^{D} ± 10	225^{B}
Cs, 133	2.77^{F} ± 2	13.6^{A} ± 1	5.47^{E}	8.18^{C}	6.59^{D} ± 12	10.9^{B}
Cu, 63	24.2^{F} ± 1	85.9^{A} ± 2	39.6^{E}	55.0^{C}	49.2^{D} ± 9	70.5^{B}
Fe, 57	22 699^{E} ± 2	33 480^{A} ± 2	25 394^{D}	28 089^{C}	25 571^{D} ± 2	30 785^{B}
Mo, 95	0.43^{E} ± 2	1.68^{A} ± 8	0.74^{D}	1.06^{C}	1.05^{C} ± 9	1.37^{B}
Ni, 62	96.4^{F} ± 2	593^{A} ± 1	220^{E}	344^{C}	308^{D} ± 11	469^{B}
P, 31	752^{A} ± 3	217^{E} ± 1	618^{B}	485^{C}	454^{C} ± 24	351^{D}
Pb, 208	28.9^{A} ± 2	29.5^{A} ± 11	29.0^{A}	29.2^{A}	26.7^{B} ± 2	29.3^{A}
Rb, 85	21.8^{E} ± 2	48.7^{A} ± 1	28.5^{D}	35.3^{C}	29.5^{D} ± 6	42.0^{B}
Se, 82	0.06^{E} ± 13	0.23^{A} ± 14	0.10^{D}	0.14^{C}	0.10^{D} ± 44	0.18^{B}
Sr, 88	87.9^{F} ± 2	182.2^{A} ± 1	111.5^{E}	135.0^{C}	119.8^{D} ± 4	158.6^{B}
U, 238	1.67^{F} ± 13	5.07^{A} ± 2	2.52^{E}	3.37^{C}	2.94^{D} ± 7	4.22^{B}
Zn, 68	60.9^{E} ± 1	101.6^{A} ± 9	71.1^{D}	81.3^{C}	74.7^{D} ± 2	91.4^{B}

n.d. – not detected; Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

Mean values of eleven plant available (labile) metals: cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), molybdenum (Mo), nickel (Ni), lead (Pb), rubidium (Rb), strontium (Sr), zinc (Zn) and caesium (Cs), than two metalloids: boron (B) and arsenic (As), non-metals phosphorus (P) and selenium (Se) and actinoid uranium (U), content in substrate analysed in 1 M ammonium nitrate (NH_4NO_3, 1:2.5, *w/v*) extract, are shown in table 5-4.

Table 5-4 Mean values of labile metals, metalloids and non-metals in substrate measured in 1 M ammonium nitrate (NH_4NO_3) extract, at 1:2.5 (w/v)ratio

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Labile element content, mg/kg, Mean ± RSD						
As, 75	0.01^{D}	$0.10^{A} \pm 15$	$0.07^{C} \pm 14$	$0.08^{BC} \pm 10$	$0.10^{A} \pm 6$	$0.09^{AB} \pm 5$
B, 10	0.02^{E}	$39.3^{A} \pm 12$	$11.7^{D} \pm 14$	$18.1^{C} \pm 13$	$17.4^{C} \pm 10$	$25.2^{B} \pm 7$
Cd, 111	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}
Co, 59	0.01^{B}	0.01^{B}	0.01^{B}	0.01^{B}	0.01^{B}	$0.02^{A} \pm 29$
Cr, 52	0.01^{E}	$1.00^{A} \pm 4$	$0.22^{D} \pm 16$	$0.39^{C} \pm 21$	$0.37^{C} \pm 10$	$0.74^{B} \pm 13$
Cs, 133	0.01^{E}	$0.69^{A} \pm 10$	$0.09^{D} \pm 29$	$0.19^{C} \pm 24$	$0.18^{C} \pm 20$	$0.45^{B} \pm 19$
Cu, 63	0.07^{B}	$0.14^{A} \pm 22$	$0.08^{B} \pm 8$	$0.08^{B} \pm 8$	$0.08^{B} \pm 6$	$0.12^{A} \pm 22$
Mo, 95	10.0^{E}	$122.5^{A} \pm 10$	$50.0^{D} \pm 16$	$75.0^{C} \pm 13$	$87.5^{C} \pm 11$	$105.0^{B} \pm 10$
Ni, 62	$0.14^{D} \pm 10$	$0.63^{A} \pm 7$	$0.28^{C} \pm 9$	$0.39^{B} \pm 11$	$0.38^{B} \pm 4$	$0.59^{A} \pm 11$
P, 31	$1.20^{A} \pm 3$	$0.36^{D} \pm 6$	$0.68^{B} \pm 7$	$0.49^{C} \pm 11$	$0.49^{C} \pm 6$	$0.31^{D} \pm 7$
Pb, 208	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}
Rb, 85	$0.09^{E} \pm 10$	$2.27^{A} \pm 5$	$0.39^{D} \pm 15$	$0.72^{C} \pm 20$	$0.70^{C} \pm 17$	$1.52^{B} \pm 15$
Se, 78	n.d.^{D}	$0.03^{A} \pm 23$	0.01^{C}	0.02^{B}	0.02^{B}	$0.03^{A} \pm 18$
Sr, 88	$9.91^{D} \pm 2$	$10.7^{C} \pm 4$	$11.2^{B} \pm 2$	$11.3^{B} \pm 2$	$11.1^{BC} \pm 1$	$11.7^{A} \pm 0.4$
U, 238	n.d.^{D}	$0.05^{C} \pm 11$	$0.08^{A} \pm 6$	$0.08^{A} \pm 10$	$0.08^{A} \pm 6$	$0.07^{B} \pm 14$
Zn, 68	$0.76^{A} \pm 3$	$0.25^{D} \pm 13$	$0.49^{B} \pm 21$	$0.34^{C} \pm 15$	$0.38^{C} \pm 9$	$0.36^{C} \pm 6$

n.d. – not detected; Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

According to the available literature (Scheffer and Schachtschabel, 1992; Essington, 2004; Kabata-Pendias and Mukherjee, 2007; Mengel and Kirkby, 1987), table 5-5 shows normal range of total content of investigated elements in soils and threshold or maximal acceptable content (MAC) values according to the Croatian legislation for agricultural soils (HR NN 15, 1992). In Croatian regulations MAC values for soils are defined according to soil texture, and are different for light and heavy soils.

Table 5-5 Normal ranges and threshold values of total concentration of trace elements in soils according to the available literature

	Total metal content extracted in aqua regia, mg/kg					
	Maximal acceptable content (MAC) in Croatian Agricultural soils, HR NN 15/1992		Normal ranges for soil, Scheffer and Schachtschabel, 1992	Normal ranges for soil, Essington, 2004	Normal ranges for soil, Kabata-Pendias and Mukherjee, 2007	Normal ranges for soil, Mengel and Kirkby, 1987
	light soils	heavy soils				
As	20	30	2-20		4.4 - 30	
B					22-134	
Cd	1	2	5-80		0.37 - 2.7	
Co	50	50	2-40		5.5 - 65	
Cr	60	100	5-100			
Cs					0.8 - 30	
Cu	60	100	2-40		1 - 140	
Fe					300 - 35 000	
Mo	10	15			0.6 - 3.4	
Ni	50	60	5-50		13 - 110	
P						200 – 15 000
Pb	100	150	2-60		22 - 70	
Rb					10 - 140	
Se				0.1 -2	0.25 -1.9	
Sr					87-210	
U			< 0.5		1.2- 11	
Zn	200	300	10-80		31 - 100	

Table 5-6 shows normal range (Prüess, 1994; Khan et al., 2009; Reimann et al., 2003 and Vago, 2006) of labile content of investigated elements in soil extracted in ammonium nitrate (1 M NH_4NO_3, at 1:2.5 (*w/v*) ratio) and in ammonium acetate (1 M NH_4Ac (or according to nomenclature of (IUPAC, 2009) NH_4OOCCH_3 or $NH_4O_2C_2H_3$), 3:50 (*w/v*)), as quoted in the available literature.

Table 5-6 Normal values of labile concentration of trace elements in soil as quoted in the available literature

	Labile metal content extracted in 1 M NH_4NO_3 (1:2.5 *w/v*), **µg/kg**		Medians In North Europe Agricultural soils, in 1 M NH_4Ac (3g /50 mL), mg/kg Reimann et al., 2003	Range for 50 Hungarian soils, in 1 M NH_4Ac , µg/g ≡ mg/kg Vago, 2006
	In soil, Prüess, 1994	In soil, Khan et al., 2009		
As	45-50* 100**		< 0.05 (< 50 µg/kg)	
B				0.08-0.97
Cd	3* 20** 30-90***	134	0.22 (220 µg/kg)	
Co	20* 150**		0.18 (180 µg/kg)	
Cr	15* 50**		0.13 (130 µg/kg)	
Cs			n.d.	
Cu	350-400* 800-2 000** 2500***	1 230	0.5 (500 µg/kg)	
Fe			45 (45 000 µg/kg)	
Mo	70-110* 500**		< 0.05 (< 50 µg/kg)	
Ni	200* 1 000**	58	< 0.2 (< 200 µg/kg)	
P			35 (35 000 µg/kg)	
Pb	3-4* 300** 10 000***		0.7 (700 µg/kg)	
Rb			n.d.	
Se			n.d.	
Sr			8 (8 000 µg/kg)	
U	4-5*		n.d.	
Zn	100-130* 5 000**	2 970	1.5 (1 500 µg/kg)	

*precautionary value; **trigger value; ***action value; n.d. – not detected;

5.2 Plant uptake in barley grain grown from 15.11.05. to 26.06.06.

Table 5-7 shows yield components of barley grown from 15.11.05. to 26.06.06. - barley (1). Yield components include: number of plants with and without ears per pot, total number of plants per pot, average length of stem and ear, number and weight of barley grains per pot.

Table 5-7 Yield components of barley (1) grown from 15.11.05. to 26.06.06.

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Yield components, Mean ± RSD						
Number of plants with ear per pot	62^{B} ± 67	32^{C} ± 31	90^{A} ± 10	96^{A} ± 7	23^{C} ± 29	76^{AB} ± 14
Number of plants without ear per pot	6^{C} ± 80	75^{A} ± 7	8^{C} ± 27	8^{C} ± 26	83^{A} ± 23	36^{B} ± 35
Total number of plants per pot	68^{B} ± 67	107^{B} ± 11	98^{AB} ± 8	103^{A} ± 5	105^{A} ± 14	111^{A} ± 5
Length of stem, cm (average of 10 stems)	36.9^{A} ± 9	18.7^{D} ± 17	33.3^{A} ± 15	28.4^{B} ± 4	18.6^{D} ± 12	24.0^{C} ± 4
Length of ear, cm (average of 10 ears)	3.5^{A} ± 13	1.4^{D} ± 6	2.6^{B} ± 5	1.9^{C} ± 3	1.4^{D} ± 7	1.7^{CD} ± 9
Number of grains per pot	406^{AB} ± 37	221^{C} ± 15	489^{A} ± 10	391^{AB} ± 6	175^{C} ± 11	333^{B} ± 10
Weight of barley grains per pot, g	15.5^{A} ± 29	5.3^{CD} ± 21	13.1^{A} ± 15	9.2^{B} ± 9	3.4^{D} ± 12	8.4^{BC} ± 10

Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

Total mass ratios of nitrogen, carbon, sulphur and hydrogen in barley (1) grain are shown in table 5-8.

Table 5-8 Total nitrogen, carbon, sulphur and hydrogen content in barley (1) grain

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Total NCSH content (%) in DM of grain, Mean ± RSD						
TN	1.16^{D} ± 11	2.04^{A} ± 5	1.20^{D} ± 3	1.40^{C} ± 3	1.35^{C} ± 5	1.55^{B} ± 5
TC	42.9^{B} ± 0.4	43.7^{A} ± 0.6	42.6^{C} ± 0.3	42.6^{C} ± 0.2	42.9^{B} ± 0.4	42.8^{BC} ± 0.1
TS	0.137^{D} ± 5	0.195^{BC} ± 31	0.167^{CD} ± 18	0.216^{AB} ± 1	0.219^{AB} ± 8	0.248^{A} ± 2
TH	6.59^{A} ± 0.4	6.53^{B} ± 0.4	6.47^{C} ± 0.2	6.46^{CD} ± 0.2	6.43^{D} ± 0.2	6.47^{C} ± 0.1

Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

Mean values of twelve total metals: cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), molybdenum (Mo), nickel (Ni), lead (Pb), rubidium (Rb), strontium (Sr), zinc (Zn) and caesium (Cs), than two metalloids: boron (B) and arsenic (As), non-metals phosphorus (P) and selenium (Se) and actinoid uranium (U), content in barley (1) grain analysed in mixture of nitric acid and hydrogen peroxide (HNO_3/H_2O_2 = 5:1 *v/v*) extract are shown in table 5-9.

Table 5-9 Mean values of total metals, metalloids and non-metals in barley(1) grain analysed in mixture of nitric acid and hydrogen peroxide extract, HNO_3/H_2O_2=5:1 (v/v)

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Total element content, mg/kg, Mean ± RSD						
As, 75	$0.11^{B} \pm 39$	$0.53^{A} \pm 57$	0.09^{B}	$0.14^{B} \pm 11$	$0.47^{A} \pm 20$	$0.23^{B} \pm 17$
B, 10	$14.9^{B} \pm 156$	$15.6^{B} \pm 122$	$55.6^{AB} \pm 86$	$23.9^{AB} \pm 117$	$50.1^{AB} \pm 166$	$90.5^{A} \pm 65$
Cd, 111	$0.03^{A} \pm 47$	0.01^{C}	0.03^{A}	$0.03^{AB} \pm 18$	$0.01^{C} \pm 40$	$0.02^{BC} \pm 29$
Co, 59	$0.025^{B} \pm 52$	$0.030^{AB} \pm 27$	$0.013^{C} \pm 40$	$0.023^{BC} \pm 22$	0.040^{A}	$0.030^{AB} \pm 27$
Cr, 53	$3.73^{A} \pm 60$	$1.85^{B} \pm 33$	$0.73^{B} \pm 15$	$1.12^{B} \pm 50$	$1.06^{B} \pm 120$	$1.00^{B} \pm 21$
Cs, 133	$0.01^{B} \pm 115$	$0.91^{A} \pm 32$	0.01^{B}	$0.02^{B} \pm 41$	$0.01^{B} \pm 40$	$0.09^{B} \pm 48$
Cu, 63	$3.26^{A} \pm 18$	$1.08^{C} \pm 11$	$2.21^{B} \pm 5$	$2.05^{B} \pm 11$	$1.26^{C} \pm 8$	$2.31^{B} \pm 14$
Fe, 57	$21.6^{AB} \pm 15$	$16.5^{B} \pm 18$	$20.9^{AB} \pm 7$	$19.0^{B} \pm 6$	$24.8^{A} \pm 16$	$20.2^{AB} \pm 31$
Mo, 95	$0.83^{B} \pm 28$	$1.95^{A} \pm 19$	$0.12^{D} \pm 31$	$0.24^{D} \pm 36$	$0.32^{CD} \pm 60$	$0.55^{CB} \pm 15$
Ni, 62	0.91^{A}	$1.04^{A} \pm 38$	0.03^{B}	$0.26^{B} \pm 142$	$0.94^{A} \pm 59$	$0.30^{B} \pm 57$
P, 31	$2\,059^{BC} \pm 5$	$722^{D} \pm 11$	$2\,021^{BC} \pm 5$	$2\,324^{A} \pm 6$	$1\,941^{C} \pm 4$	$2\,139^{B} \pm 3$
Pb, 208	n.d.^{A}	n.d.^{A}	$0.01^{A} \pm 200$	$0.08^{A} \pm 200$	n.d.^{A}	n.d.^{A}
Rb, 85	$1.47^{E} \pm 38$	$54.0^{A} \pm 14$	$3.78^{DE} \pm 6$	$9.49^{C} \pm 5$	$6.33^{CD} \pm 11$	$19.8^{B} \pm 9$
Se, 82	$0.01^{D} \pm 82$	$0.79^{A} \pm 32$	$0.18^{CD} \pm 37$	$0.58^{B} \pm 16$	$0.21^{C} \pm 12$	$0.60^{B} \pm 11$
Sr, 88	$1.35^{D} \pm 16$	$1.05^{D} \pm 20$	$1.89^{BC} \pm 9$	$2.14^{B} \pm 13$	$2.71^{A} \pm 6$	$1.73^{C} \pm 23$
U, 238	$0.003^{A} \pm 200$	$0.003^{A} \pm 200$	$0.003^{A} \pm 200$	$0.005^{A} \pm 200$	$0.005^{A} \pm 115$	$0.010^{A} \pm 82$
Zn, 68	$10.5^{C} \pm 27$	$9.93^{C} \pm 11$	$9.07^{C} \pm 7$	$12.8^{B} \pm 13$	$16.2^{A} \pm 9$	$13.4^{B} \pm 3$

n.d. – not detected; Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

According to the available literature (Scheffer and Schachtschabel, 1992; Grbeša, 2004; Kabata-Pendias and Mukherjee, 2007; Mengel and Kirkby, 1987; HR NN 16, 2005), table 5-10 shows normal range of total content of elements in plants (in general, in barley or in soybean grain).

Table 5-10 Normal ranges of total concentration of trace elements in plants according to the available literature

	Total metal content, mg/kg					
	Normal ranges for plants, Scheffer and Schachtschabel, 1992	Normal concentrations in barley grain, Grbeša, 2004	Normal concentrations in soybean grain, Grbeša, 2004	Normal ranges for plants, Mengel and Kirkby, 1987	Normal ranges for plants, Kabata-Pendias and Mukherjee, 2007	Maximal acceptable content (MAC) in foodstuffs, HR NN 16/2005
As	0.01 – 1				0.79 in soybean	0.5 in cereals
B	5 – 100				5-30 in plants 0.83 in barley grains	
Cd	0.05 – 0.4				13 – 22 μg/kg in barley grains 5.6 – 32 μg/kg in cereal grains	0.1 in cereals (barley) 0.2 in soybean
Co	0.04 - 1	0.30	0.20		4.4 – 44 μg/kg in barley grains 5– 270 μg/kg in cereal grains	
Cr	0.1 - 1				0.01 – 0.02 in barley grains	
Cs	0.02 – 0.5				< 0.1 - 3	
Cu	2 - 20	7	18		4 – 15 in barley grains 0.3 – 13 in cereal	
Fe		185	250		31 – 98 in grains (barley, wheat, rye and oats)	
Mo	0.05 – 1.5	0.8	5		0.2 – 1.2 in barley grains 0.9 – 1.6 in kidney bean seeds	
Ni	0.1 - 3				0.10 – 0.67 in barley grains	
P		3 800	6 700	4 000 – 5 000 in cereal grains		
Pb	0.1 - 6				0.1 – 0.4 in barley grains < 1 in cereal grains	0.2 in legume and in cereals
Rb					4.7 in barley grains 220 in soybean seeds	
Se		0.10	0.20		100 –800 μg/kg in cereal	
Sr					1.5 - 2.5 in grains	
U					0.2 μg/kg in barley grains	
Zn	15 - 100	32	45		20-30 in barley grains 18 – 33 in cereal grains	

5.3 Plant uptake in soybean grain grown from 03.07.06. to 20.10.06.

Yield components of soybean grown from 03.07.06. to 20.10.06. are shown in table 5-11. Yield components include: total number of plants in a pot, number of pods per pot, number of pods per stem and weight of soybean grains per pot.

Table 5-11 Yield components for soybean

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Yield components, Mean ± RSD						
Number of plants per pot	$8^{BC} \pm 17$	$9^{AB} \pm 7$	$8^{BC} \pm 17$	$7^{C} \pm 32$	$9^{A} \pm 5$	7^{BC}
Number of pods per pot	$33^{A} \pm 12$	$11^{C} \pm 13$	$32^{A} \pm 12$	$25^{B} \pm 21$	$23^{B} \pm 5$	$24^{B} \pm 7$
Number of pods per stem	$4^{A} \pm 21$	$1^{C} \pm 19$	$4^{A} \pm 23$	$4^{A} \pm 22$	$2^{B} \pm 5$	$3^{AB} \pm 7$
Weight of soybean grains per pot, g	$11.9^{A} \pm 6$	$0.5^{F} \pm 63$	$10.9^{B} \pm 6$	$7.7^{C} \pm 10$	$4.7^{E} \pm 9$	$5.7^{D} \pm 9$

Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

Total mass ratios of nitrogen, carbon, sulphur and hydrogen in soybean grain are shown in table 5-12.

Table 5-12 Total nitrogen, carbon, sulphur and hydrogen content in soybean grain

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Total NCSH content (%) in DM of the grain, Mean ± RSD						
TN	$6.73^{A} \pm 3$	$5.49^{E} \pm 4$	$6.50^{AB} \pm 1$	$6.37^{BC} \pm 2$	$6.09^{D} \pm 2$	$6.20^{CD} \pm 3$
TC	$51.0^{A} \pm 0.4$	$51.3^{A} \pm 1.5$	$50.8^{A} \pm 0.2$	$51.2^{A} \pm 0.4$	$51.3^{A} \pm 0.4$	$50.9^{A} \pm 0.3$
TS	$0.327^{B} \pm 5$	$0.338^{B} \pm 5$	$0.372^{A} \pm 1$	$0.344^{B} \pm 2$	$0.334^{B} \pm 7$	$0.338^{B} \pm 1$
TH	$7.74^{B} \pm 1.4$	$7.74^{B} \pm 1.4$	$7.75^{B} \pm 0.3$	$8.07^{A} \pm 0.9$	$8.09^{A} \pm 0.3$	$8.04^{A} \pm 0.4$

Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1)*

Mean values of twelve total metals content: cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), molybdenum (Mo), nickel (Ni), lead (Pb), rubidium (Rb), strontium (Sr), zinc (Zn) and caesium (Cs), than two metalloids: boron (B) and arsenic (As), non-metals phosphorus (P) and selenium (Se) and actinoid uranium (U) in soybean grain analysed in mixture of nitric acid and hydrogen peroxide (HNO_3/H_2O_2 = 5:1 *v/v*) extract are shown in table 5-13.

Table 5-13 Mean values of total metals, metalloids and non-metals in soybean grain analysed in mixture of nitric acid and hydrogen peroxide extract, HNO_3/H_2O_2=5:1 (v/v)

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Total element content, mg/kg, Mean ± RSD						
As, 75	0.03^{D} ± 79	0.47^{A} ± 8	0.03^{D} ± 6	0.03^{D} ± 9	0.19^{B} ± 21	0.13^{C} ± 15
B, 11	190^{B} ± 19	191^{B} ± 3	256^{A} ± 21	123^{C} ± 22	159^{BC} ± 26	175^{BC} ± 20
Cd, 112	n.d.^{B}	0.90^{A} ± 113	n.d.^{B}	0.01^{B} ± 200	0.25^{AB} ± 139	0.02^{B} ± 200
Co, 59	0.18^{A} ± 29	0.10^{B} ± 5	0.16^{A} ± 16	0.10^{B} ± 9	0.05^{C} ± 18	0.10^{B} ± 5
Cr, 52	17.8^{A} ± 140	1.39^{B} ± 6	0.96^{B} ± 5	1.87^{B} ± 111	0.97^{B} ± 14	1.03^{B} ± 18
Cs, 133	0.02^{C} ± 62	1.65^{A} ± 8	0.03^{C} ± 7	0.05^{C} ± 19	0.03^{C} ± 10	0.16^{B} ± 24
Cu, 65	7.46^{A} ± 8	4.08^{C} ± 5	6.39^{AB} ± 9	5.79^{B} ± 17	3.63^{C} ± 2	6.46^{AB} ± 30
Fe, 57	122^{A} ± 11	89.1^{BC} ± 34	99.7^{AB} ± 6	75.0^{C} ± 12	88.4^{BC} ± 13	87.9^{BC} ± 14
Mo, 98	26.1^{AB} ± 108	31.8^{AB} ± 11	17.4^{B} ± 7	21.1^{B} ± 14	40.9^{A} ± 6	19.7^{B} ± 8
Ni, 60	33.5^{B} ± 61	54.5^{A} ± 8	16.6^{C} ± 6	22.8^{CB} ± 41	22.4^{CB} ± 3	26.4^{CB} ± 10
P, 31	$4\,965^{C}$ ± 4	$5\,072^{C}$ ± 2	$6\,293^{A}$ ± 1	$6\,368^{A}$ ± 4	$3\,021^{D}$ ± 3	$5\,638^{B}$ ± 5
Pb, 208	0.14^{A} ± 200	0.31^{A} ± 115	0.48^{A} ± 156	0.52^{A} ± 184	0.27^{A} ± 200	0.14^{A} ± 117
Rb, 85	4.87^{D} ± 23	100^{A} ± 2	8.00^{D} ± 7	12.3^{C} ± 11	13.4^{C} ± 5	27.7^{B} ± 16
Se, 82	0.06^{E} ± 46	2.13^{A} ± 2	0.17^{D} ± 27	0.24^{C} ± 6	0.16^{D} ± 12	0.42^{B} ± 4
Sr, 88	2.77^{CD} ± 7	3.77^{A} ± 28	2.16^{D} ± 9	2.98^{BC} ± 5	2.21^{D} ± 1	3.58^{AB} ± 7
U, 238	0.003^{A} ± 200	0.009^{A} ± 84	0.007^{A} ± 23	0.006^{A} ± 32	0.006^{A} ± 77	0.004^{A} ± 24
Zn, 68	14.0^{B} ± 13	31.6^{A} ± 3	17.0^{B} ± 4	21.1^{B} ± 9	18.1^{B} ± 4	32.4^{A} ± 36

Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

5.4 Plant uptake in barley grain grown from 09.11.06. to 18.06.07.

Table 5-14 shows the yield components of barley grown from 09.11.06. to 18.06.07. barley (2). Yield components were analysed with the same parameters as barley (1), namely: number of plants with and without ears per pot, total number of plants per pot, average length of stem and ear, number and weight of barley grains per pot.

Table 5-14 Yield components for barley (2) grown from 09.11.06. to 18.06.07.

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Yield components, Mean ± RSD						
Number of plants with ear per pot	45^{A} ± 36	28^{B} ± 62	56^{A} ± 10	58^{A} ± 5	50^{A} ± 16	55^{A} ± 5
Number of plants without ear per pot	2^{B} ± 38	4^{B} ± 35	8^{A} ± 15	8^{A} ± 15	11^{A} ± 12	11^{A} ± 32
Total number of plants per pot	46^{BC} ± 35	32^{C} ± 50	64^{A} ± 7	67^{A} ± 5	61^{AB} ± 12	66^{A} ± 4
Length of stem, cm (average of 10 stems)	30.2^{A} ± 10	26.2^{BC} ± 9	27.0^{AB} ± 8	26.1^{BC} ± 15	22.3^{C} ± 5	24.0^{BC} ± 10
Length of ear, cm (average of 10 ears)	3.5^{A} ± 11	2.3^{B} ± 13	3.8^{A} ± 13	3.5^{A} ± 11	2.7^{B} ± 9	3.5^{A} ± 11
Number of grains per pot	71^{BC} ± 32	51^{C} ± 61	189^{A} ± 41	179^{A} ± 31	128^{AB} ± 23	190^{A} ± 19
Weight of barley grains per pot, g	1.9^{CD} ± 32	1.1^{D} ± 58	6.3^{A} ± 43	5.2^{AB} ± 28	3.9^{BC} ± 36	5.4^{AB} ± 26

Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

Total mass ratios of nitrogen, carbon, sulphur and hydrogen in barley (2) grain are shown in table 5-15.

Table 5-15 Total nitrogen, carbon, sulphur and hydrogen content in barley (2) grain

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Total NCSH content (%) in DM of the grain, Mean ± RSD						
TN	2.76^{A} ± 9	2.55^{A} ± 8	2.00^{B} ± 18	2.04^{B} ± 8	1.82^{B} ± 6	2.69^{A} ± 10
TC	42.7^{A} ± 1.1	41.7^{B} ± 0.5	41.4^{B} ± 0.8	41.5^{B} ± 1.5	41.4^{B} ± 0.6	41.9^{B} ± 0.2
TS	0.321^{A} ± 32	0.284^{AB} ± 9	0.214^{C} ± 9	0.205^{C} ± 3	0.197^{C} ± 3	0.239^{BC} ± 4
TH	6.52^{B} ± 3.1	6.63^{AB} ± 1.1	6.71^{A} ± 0.5	6.74^{A} ± 1.3	6.72^{A} ± 1.1	6.75^{A} ± 0.3

Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

Table 5-16 shows mean values of twelve total metals: cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), molybdenum (Mo), nickel (Ni), lead (Pb), rubidium (Rb), strontium (Sr), zinc (Zn) and caesium (Cs); three metalloids: boron (B), selenium (Se) and arsenic (As); non-metal phosphorus (P) and actinoid uranium (U), content in barley (2) grain analysed in mixture of nitric acid and hydrogen peroxide(HNO_3/H_2O_2 = 5:1 *v/v*) extract.

Table 5-16 Mean values of total metals, metalloids and non-metals content in barley(2) grain analysed in mixture of nitric acid and hydrogen peroxide extract, HNO_3/H_2O_2=5:1 (v/v)

Parameter	**Treatments**					
	1	**2**	**3**	**4**	**5**	**6**
Total element content, mg/kg, Mean ± RSD						
As, 75	0.03^{D} ± 23	0.57^{A} ± 27	0.04^{D} ± 48	0.16^{C} ± 40	0.09^{CD} ± 4	0.26^{B} ± 8
B, 11	120^{A} ± 41	160^{A} ± 19	43.9^{B} ± 60	36.6^{B} ± 29	56.6^{B} ± 34	26.6^{B} ± 75
Cd, 112	0.04^{A} ± 119	0.03^{A} ± 200	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}
Co, 59	0.025^{B} ± 24	0.044^{A} ± 40	0.005^{C} ± 50	0.012^{BC} ± 76	0.001^{C} ± 116	0.007^{C} ± 74
Cr, 52	0.59^{AB} ± 25	0.54^{AB} ± 31	0.73^{A} ± 57	0.69^{AB} ± 38	0.37^{B} ± 9	0.59^{AB} ± 9
Cs, 133	0.02^{B} ± 33	1.46^{A} ± 36	0.01^{B} ± 32	0.03^{B} ± 7	0.02^{B} ± 10	0.12^{B} ± 17
Cu, 65	2.54^{C} ± 27	4.40^{AB} ± 20	5.07^{A} ± 45	2.85^{BC} ± 28	2.81^{BC} ± 17	4.20^{ABC} ± 19
Fe, 57	81.4^{A} ± 44	72.9^{AB} ± 38	44.8^{BC} ± 18	39.3^{C} ± 17	32.0^{C} ± 12	36.7^{C} ± 23
Mo, 98	0.46^{C} ± 28	1.83^{A} ± 17	1.04^{B} ± 47	0.70^{BC} ± 12	0.81^{BC} ± 4	0.93^{B} ± 26
Ni, 60	0.78^{AB} ± 29	1.81^{A} ± 40	1.52^{AB} ± 161	0.84^{AB} ± 42	0.19^{B} ± 29	0.40^{AB} ± 19
P, 31	$3\,431^{C}$ ± 15	$3\,805^{BC}$ ± 7	$4\,811^{A}$ ± 11	$4\,745^{A}$ ± 6	$4\,409^{AB}$ ± 12	$4\,045^{BC}$ ± 10
Pb, 208	0.28^{A} ± 68	0.24^{A} ± 196	1.81^{A} ± 167	0.04^{A} ± 200	n.d.^{A}	n.d.^{A}
Rb, 85	4.15^{C} ± 11	70.4^{A} ± 21	6.91^{C} ± 11	9.92^{C} ± 2	10.4^{C} ± 9	20.7^{B} ± 9
Se, 82	0.13^{B} ± 115	0.87^{A} ± 24	0.09^{B} ± 25	0.13^{B} ± 10	0.02^{B} ± 49	0.17^{B} ± 19
Sr, 88	2.66^{A} ± 27	2.03^{B} ± 30	1.34^{C} ± 16	1.38^{C} ± 3	1.33^{C} ± 14	1.15^{C} ± 11
U, 238	0.008^{A} ± 46	0.007^{AB} ± 107	0.003^{AB} ± 196	0.002^{B} ± 135	0.002^{B} ± 56	0.002^{B} ± 113
Zn, 66	18.1^{C} ± 10	43.4^{A} ± 15	20.8^{C} ± 13	26.9^{B} ± 10	27.8^{B} ± 19	38.5^{A} ± 6

Means with the same letter are not significantly different ($p < 0.05$, *SAS 9.1*)

5.5 Enrichment Coefficients

In various literature enrichment coefficient is also called enrichment ratio (Chesworth, 2008), translocation coefficient (Fismes et al., 2005), transfer factor (Dobrin et al., 2004), soil-plant transfer coefficient (Adriano, 2001 and Alloway, 1995), accumulation factor (Khan, 2001), phytoaccumulation factor (Zurayk, 2002), bioaccumulation factor (Sanborn and Brodberg, 2006), biological adsorption coefficient (BAC) (Kabata-Pendias, 2000; Malayeri et al., 2008) and uptake coefficient (Sinha and Singh, 1976).

In his work on accumulation of Pb, Cu and Zn in native plant grown on contaminated sites Liu et al., 2008, quoted some authors saying that enrichment coefficient is an important factor when considering the phytoremediation potential of a plant species (Zhao et al., 2003). There are 4 rules for hyperaccumulator. One of the standards is enrichment coefficient, the enrichment coefficient > 1 shows a special ability of a plant to absorb and transport metals from soil and store them in their above-ground parts (Baker and Brooks, 1989; Brown et al., 1995). But at the same time some research indicates that some plants are heavy metal hyperaccumulator even though they have enrichment coefficients < 1, (Zu et al., 2005).

In this study the enrichment coefficient (EC) was calculated with this equation:

$$EC = C_{grain}/C_{soil} \qquad (1)$$

C_{grain} and C_{soil} represent the metal or metalloid concentration (mass ratio) in the grain parts of the plant and in the soil (substrate) on dry weight basis (mg/kg in DM), respectively.

Malayeri et al., 2008 grouped plant species according to their heavy metal uptake capacities and sensitivity to metal pollution:

High accumulator plants	EC between 1 - 10
Moderately accumulator plants	EC between 0.1 – 1.0
Low accumulator plants	EC between 0.01 – 0.1
Non accumulator plants	EC < 0.01

Since one of the goals of this experiment was to compare metals and metalloids accumulation in grains of soybean and barley grown on the same ash ratios, table 5-17 shows EC for As, B, Cd, Co, Cr, Cs, P, Pb, Cu and Fe and table 5-18 shows EC for Mo, Ni, Rb, Se, Sr, U and Zn calculated for winter barley (1) and (2) and for soybean.

Table 5-17 As, B, Cd, Co, Cr, Cs, P, Pb, Cu and Fe Enrichment coefficients (EC) calculated for winter barley and soybean

EC	**Arsenic (As)**			**Boron (B)**		
treatment	barley(1)	soybean	barley(2)	barley(1)	soybean	barley(2)
1	0.004	0.001	0.001	n.d.	n.d.	n.d.
2	0.005	0.004	0.005	0.08	0.93	0.78
3	0.002	0.001	0.001	1.09	5.01	0.86
4	0.002	< 0.001	0.002	0.23	1.21	0.36
5	0.007	0.003	0.001	0.56	1.78	0.64
6	0.002	0.001	0.003	0.59	1.14	0.17
EC	**Cadmium (Cd)**			**Cobalt (Co)**		
treatment	barley(1)	soybean	barley(2)	barley(1)	soybean	barley(2)
1	0.10	n.d.	0.13	0.002	0.011	0.002
2	0.02	1.75	0.05	0.001	0.003	0.001
3	0.09	n.d.	n.d.	0.001	0.008	< 0.001
4	0.07	0.03	n.d.	0.001	0.004	< 0.001
5	0.03	0.69	n.d.	0.002	0.002	< 0.001
6	0.04	0.05	n.d.	0.001	0.003	< 0.001
EC	**Chromium (Cr)**			**Caesium (Cs)**		
treatment	barley(1)	soybean	barley(2)	barley(1)	soybean	barley(2)
1	0.062	0.298	0.010	0.002	0.008	0.008
2	0.007	0.005	0.002	0.067	0.122	0.108
3	0.006	0.008	0.006	0.002	0.005	0.002
4	0.007	0.011	0.004	0.002	0.006	0.003
5	0.007	0.006	0.002	0.002	0.004	0.003
6	0.004	0.005	0.003	0.008	0.014	0.011
EC	**Phosphorus (P)**			**Lead (Pb)**		
treatment	barley(1)	soybean	barley(2)	barley(1)	soybean	barley(2)
1	2.74	6.6	4.56	n.d.	0.005	0.010
2	3.32	23.4	17.5	n.d.	0.010	0.008
3	3.27	10.2	7.78	< 0.001	0.017	0.062
4	4.80	13.1	9.79	0.003	0.018	0.001
5	4.27	6.6	9.70	n.d.	0.010	n.d.
6	6.10	16.1	11.5	n.d.	0.005	n.d.
EC	**Copper (Cu)**			**Iron (Fe)**		
treatment	barley(1)	soybean	barley(2)	barley(1)	soybean	barley(1)
1	0.13	0.31	0.13	0.001	0.005	0.004
2	0.01	0.05	0.01	< 0.001	0.003	0.002
3	0.06	0.16	0.06	0.001	0.004	0.002
4	0.04	0.11	0.04	0.001	0.003	0.001
5	0.03	0.07	0.03	0.001	0.003	0.001
6	0.03	0.09	0.03	0.001	0.003	0.001

Table 5-18 Mo, Ni, Rb, Se, Sr, U and Zn Enrichment coefficients (EC) calculated for winter barley and soybean

EC	**Molybdenum (Mo)**			**Nickel (Ni)**		
treatment	barley(1)	soybean	barley(2)	barley(1)	soybean	barley(2)
1	1.92	60.3	1.06	0.009	0.35	0.008
2	1.16	19.0	1.09	0.002	0.09	0.003
3	0.16	23.4	1.39	< 0.001	0.08	0.007
4	0.23	20.0	0.67	0.001	0.07	0.002
5	0.30	38.8	0.77	0.003	0.07	0.001
6	0.40	14.4	0.68	0.001	0.06	0.001
EC	**Rubidium (Rb)**			**Selenium (Se)**		
treatment	barley(1)	soybean	barley(2)	barley(1)	soybean	barley(2)
1	0.07	0.22	0.19	0.18	1.06	2.37
2	1.11	2.06	1.45	3.46	9.35	3.81
3	0.13	0.28	0.24	1.78	1.72	0.90
4	0.27	0.35	0.28	4.11	1.73	0.91
5	0.21	0.46	0.35	2.07	1.53	0.18
6	0.47	0.66	0.49	3.27	2.27	0.95
EC	**Strontium (Sr)**			**Uranium (U)**		
treatment	barley(1)	soybean	barley(2)	barley(1)	soybean	barley(2)
1	0,015	0,032	0,030	0.001	0.002	0.005
2	0,006	0,021	0,011	< 0.001	0.002	0.001
3	0,017	0,019	0,012	0.001	0.003	0.001
4	0,016	0,022	0,010	0.001	0.002	0.001
5	0,023	0,018	0,011	0.002	0.002	0.001
6	0,011	0,023	0,007	0.002	0.001	< 0.001
EC	**Zinc (Zn)**					
treatment	barley(1)	soybean	barley(2)			
1	0.17	0.23	0.30			
2	0.10	0.31	0.43			
3	0.13	0.24	0.29			
4	0.16	0.26	0.33			
5	0.22	0.24	0.37			
6	0.15	0.35	0.42			

5.6 Influence of vegetation season on monitored parameters of barley

Table 5-19 shows the influence of vegetation season on yield components of barley, total nitrogen, carbon, sulphur and hydrogen content for each treatment. Year 2005 (05) refers to barley (1) grown from 15.11.05. to 26.06.06. and year 2007 (07) refers to barley (2) grown from 09.11.06. to 18.06.07., on the same substrate and under controlled conditions.

Table 5-19 Influence of vegetation season on yield components and total nitrogen, carbon, sulphur and hydrogen content in barley

Parameter	**Treatment**											
Mean	**1**		**2**		**3**		**4**		**5**		**6**	
Year	**05**	**07**	**05**	**07**	**05**	**07**	**05**	**07**	**05**	**07**	**05**	**07**
Number of plants with ear per pot	62^{A}	45^{A}	32^{A}	28^{A}	90^{A}	56^{B}	96^{A}	58^{B}	23^{B}	50^{A}	76^{A}	55^{B}
Number of plants without ear per pot	6^{A}	2^{A}	75^{A}	4^{B}	8^{A}	8^{A}	8^{A}	8^{A}	83^{A}	11^{A}	36^{A}	11^{B}
Total number of plants per pot	68^{A}	46^{A}	107^{A}	32^{B}	98^{A}	64^{B}	103^{A}	67^{B}	105^{A}	61^{B}	111^{A}	66^{B}
Length of stem, cm	36.9^{A}	30.2^{B}	18.7^{B}	26.2^{A}	33.3^{A}	27.0^{A}	28.4^{A}	26.1^{A}	18.6^{B}	22.3^{A}	24.0^{A}	24.0^{A}
Length of ear, cm	3.5^{A}	3.5^{A}	1.4^{B}	2.3^{A}	2.6^{B}	3.8^{A}	1.9^{A}	3.5^{A}	1.4^{B}	2.7^{A}	1.7^{B}	3.5^{A}
Number of grains per pot	406^{A}	71^{B}	221^{A}	51^{B}	489^{A}	189^{B}	391^{A}	179^{B}	175^{A}	128^{B}	333^{A}	190^{B}
Weight of barley grains per pot, g	15.5^{A}	1.9^{B}	5.3^{A}	1.1^{B}	13.1^{A}	6.3^{B}	9.2^{A}	5.2^{B}	3.4^{A}	3.9^{A}	8.4^{A}	5.4^{B}
TN, % DM	1.16^{B}	2.76^{A}	2.04^{B}	2.55^{A}	1.20^{B}	2.00^{A}	1.40^{A}	2.04^{B}	1.35^{B}	1.82^{A}	1.55^{B}	2.69^{A}
TC, % DM	42.9^{A}	42.7^{A}	43.7^{A}	41.7^{B}	42.6^{A}	41.4^{B}	42.6^{A}	41.5^{B}	42.9^{A}	41.4^{B}	42.8^{A}	41.9^{B}
TS, % DM	0.137^{B}	0.321^{A}	0.195^{B}	0.284^{A}	0.167^{B}	0.214^{A}	0.216^{A}	0.205^{B}	0.219^{A}	0.197^{A}	0.248^{A}	0.239^{A}
TH, % DM	6.59^{A}	6.52^{A}	6.53^{A}	6.63^{A}	6.47^{B}	6.71^{A}	6.46^{B}	6.74^{A}	6.43^{B}	6.72^{A}	6.47^{B}	6.75^{A}

Means with the same letter in the same treatment are not significantly different ($p < 0.05$, *SAS 9.1*)

The influence of vegetation season on metals, metalloids and non-metals phytoaccumulation in grains of barley in different treatments is given in table 5-20.

Table 5-20 Influence of vegetation season on metals, metalloids and non-metals uptake in barley grain

Element	**Treatment**											
Mean	**1**		**2**		**3**		**4**		**5**		**6**	
Year	05	07	05	07	05	07	05	07	05	07	05	07
As	0.11^{A}	0.03^{B}	0.53^{A}	0.57^{A}	0.09^{A}	0.04^{B}	0.14^{A}	0.16^{A}	0.47^{A}	0.09^{B}	0.23^{A}	0.26^{A}
B	14.9^{B}	120^{A}	15.6^{B}	160^{A}	55.6^{A}	43.9^{A}	23.9^{A}	36.6^{A}	50.1^{A}	56.6^{A}	90.5^{A}	26.6^{A}
Cd	0.03^{A}	0.04^{A}	0.01^{A}	0.03^{A}	0.03^{A}	n.d.^{B}	0.03^{A}	n.d.^{B}	0.01^{A}	n.d.^{B}	0.02^{A}	n.d.^{B}
Co	0.025^{A}	0.025^{A}	0.030^{A}	0.044^{A}	0.013^{A}	0.005^{B}	0.023^{A}	0.012^{A}	0.040^{A}	0.001^{B}	0.030^{A}	0.007^{B}
Cr	3.73^{A}	0.59^{B}	1.85^{A}	0.54^{B}	0.73^{A}	0.73^{A}	1.12^{A}	0.69^{A}	1.06^{A}	0.37^{A}	1.00^{A}	0.59^{B}
Cs	0.01^{B}	0.02^{A}	0.91^{A}	1.46^{A}	0.01^{A}	0.01^{A}	0.02^{A}	0.03^{A}	0.01^{B}	0.02^{A}	0.09^{A}	0.12^{A}
Cu	3.26^{A}	2.54^{A}	1.08^{B}	4.40^{A}	2.21^{B}	5.07^{A}	2.05^{A}	2.85^{A}	1.26^{B}	2.81^{A}	2.31^{B}	4.20^{A}
Fe	21.6^{B}	81.4^{A}	16.5^{B}	72.9^{A}	20.9^{B}	44.8^{A}	19.0^{B}	39.3^{A}	24.8^{B}	32.0^{A}	20.2^{B}	36.7^{A}
Mo	0.83^{A}	0.46^{B}	1.95^{A}	1.83^{A}	0.12^{B}	1.04^{A}	0.24^{B}	0.70^{A}	0.32^{B}	0.81^{A}	0.55^{B}	0.93^{A}
Ni	0.91^{A}	0.78^{A}	1.04^{A}	1.81^{A}	0.03^{A}	1.52^{A}	0.26^{A}	0.84^{A}	0.94^{A}	0.19^{B}	0.30^{A}	0.40^{A}
P	2 059^{B}	3 431^{A}	722^{B}	3 805^{A}	2 021^{B}	4 811^{A}	2 324^{B}	4 745^{A}	1 941^{B}	4 409^{A}	2 139^{B}	4 045^{A}
Pb	n.d.^{B}	0.28^{A}	n.d.^{A}	0.24^{A}	0.01^{A}	1.81^{A}	0.08^{A}	0.04^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}
Rb	1.47^{B}	4.15^{A}	54.0^{A}	70.4^{A}	3.78^{B}	6.91^{A}	9.49^{A}	9.92^{A}	6.33^{B}	10.4^{A}	19.8^{A}	20.7^{A}
Se	0.01^{A}	0.13^{A}	0.79^{A}	0.87^{A}	0.18^{A}	0.09^{B}	0.58^{A}	0.13^{B}	0.21^{A}	0.02^{B}	0.60^{A}	0.17^{B}
Sr	1.35^{B}	2.66^{A}	1.05^{B}	2.03^{A}	1.89^{A}	1.34^{B}	2.14^{A}	1.38^{B}	2.71^{A}	1.33^{B}	1.73^{A}	1.15^{B}
U	0.003^{A}	0.008^{A}	0.003^{A}	0.007^{A}	0.003^{A}	0.003^{A}	0.005^{A}	0.002^{A}	0.005^{A}	0.002^{A}	0.010^{A}	0.002^{A}
Zn	10.5^{B}	18.1^{A}	9.93^{B}	43.4^{A}	9.07^{B}	20.8^{A}	12.8^{B}	26.9^{A}	16.2^{B}	27.8^{A}	13.4^{B}	38.5^{A}

n.d. – not detected; Means with the same letter in the same treatment are not significantly different ($p < 0.05$, *SAS 9.1*)

Correlation coefficients for substrate pH and EC values measured on three different media are shown in table 1 in Appendix. Table 2 in Appendix presents the substrate pollution levels (PL) of investigated elements according to soil damage classification (Bašić, 1994). Figures 1 to 17 in Appendix present the phytoaccumulation of As, B, Cd, Co, Cr, Cs, Cu, Fe, Mo, Ni, P, Pb, Rb, Se, Sr, U and Zn, in barley and soybean grains, respectively.

6 DISCUSSION

According to substrate mean pH values measurements, measured at 1:2.5 (*w/v*) ratio of KCl, as shown in table 5-1, the values varied from 7.35 (in pure soil) to 9.01 (in treatment with soil / ash ratio 25 % / 75 %) or from weak alkaline to alkaline (Škorić, 1982). At 1:2.5 (*w/v*) ratio of $CaCl_2$ the range of mean pH values was from 7.35 (in pure soil) to 8.87 (in treatment No. 6) and pH values measured at 1:2.5 (*w/v*) of H_2O varied from 8.11 (in pure soil) to 8.93 (in treatment with soil / ash ratio 25 % / 75 %). Table 1 in Appendix shows calculated correlation coefficients (CC) for substrate pH values measured on three different media and CC for EC measured in extracts with different ratios.

Correlation coefficients according to Roemer-Orphal scale (Vasilj, 2000) calculated for all three media varied from 0.9949 (among KCl and $CaCl_2$) to 0.9954 (among $CaCl_2$ and H_2O) which means that the strength of correlation between them was completely positive.

Electrical conductivity (EC) measured at 1:5 water extract (*w/v*) ratio varied from 172.7 μS/cm (in pure soil) to 2 380 μS/cm (in pure ash) and EC analysed at 1:10 water extract (*w/v*) ratio varied from 123.0 μS/cm (in pure soil) to 1 591 μS/cm (in pure ash) (table 5-1). Correlation between EC measured at 1:5 water extract (*w/v*) ratio and at 1:10 water extract (*w/v*) ratio was completely positive and CC was 0.9970 (Table 1 in Appendix).

After air drying, moisture content was determined in substrate samples and varied from 0.21 % (in pure soil) to 0.30 % (in treatment with saw dust amendment) (table 5-1).

Plant available phosphorus (P) expressed as P_2O_5 varied from low supplied (Škorić, 1982) 0.06 g/kg (6 mg/100 g) measured in pure ash, up to high supplied 0.40 g/kg (40 mg/100 g) measured in pure soil (table 5-1).

Mass ratio of plant available potassium (K) expressed as K_2O was in the range from minimum but still high supplied (Škorić, 1982) 0.22 g/kg (22 mg/100 g) measured in pure soil, up to high supplied 0.44 g/kg (44 mg/100 g) measured in pure ash (table 5-1). Bioavailability of potassium and its fixation is strongly affected by the soil texture (Vukadinovic, 2006).

Total content of nitrogen measured in substrate varied from 0.110 % in DM (in treatments No. 2 and 6) or good supplied according to Wohltman (Škorić, 1962), to good supplied 0.155 % in DM in treatment with pure soil (table 5-1).

The lowest total carbon content was measured in trial treatment with pure soil 1.989 % in DM, and the highest was noted in trial treatment with saw dust amendment 4.936 % in DM (table 5-1).

Total sulphur mass content varied from high 0.349 % in DM up to 0.789 % DM, in trial treatment with pure ash and trial treatment with saw dust amendment, respectively (table 5-1). According to Simon-Sylvestre, 1969, TS levels depend on the OM content and on climatic conditions and for temperate regions natural concentrations in soils are in the rage of 0.005 % - 0.04 %. But according to Keefer, 1993, TS in natural soils is in the range of 0.003 % - 1 % and could be from 0.04 % to high 7.40 % in ash. Total sulphur content in ash depends on coal characteristics used as fossil fuel in power plants, because coal is a sedimentary rock composed primarily of carbon, and carbon content can vary from low 60 % in lignite to high 98 % in anthracite. The content of hydrogen in coal varies from 3 % to 6 %, the content of oxygen from 2.5 % to 35 % and of sulphur from 0.5 % to 3 % (Coal, Wikipedia, 2009). Optimal C/N ratio in soils is from 10 to 12 (SARE, 2009).

Physical and chemical analysis of coal ash taken at Jezero in 2005 was done and published by all participants of RECOAL Project (Dellantonio et al., 2008; RECOAL, D7-Annex soil, 2005).

Physical properties of Jezero ash

Mean values (n=4) for sand content (20-200 μm) of Jezero ash were 75.1 %, 19.1 % for silt content (2-20 μm) and 5.7 % for clay content (< 2 μm), and these values showed that the Jezero ash is on the border of two texture classes - loamy sand (LS) and sandy loam (SL). Mean values for two (n=2) measured samples were given for: bulk density (0.7 g/cm^3), particle density (1.9 g/cm^3), field capacity (60.9 vol. %), wilting point (8.6 vol. %), available water (52.3 vol. %), air (7.7 vol. %) and porosity (68.5 vol. %).

Chemical properties of Jezero ash

Mean values (n=5) of pH were calculated for samples measured in 1:2.5 soil suspension [*w/v*] and were 9.37 and in 1:10 water extract [*w/v*] they were 9.41. EC was measured in 1:10 water extract [*w/v*] and was 732 μS/cm.

Total content of nitrogen (n=3) was 0.05 % in DM, total content of carbon 3.23 % in DM and total sulphur content 0.41 % in DM.

Plant available phosphorus (P) expressed as P_2O_5 was also determined according to Olsen's method and mean values (n=14) were 0.0097 g/kg (0.97 mg/100g). Mean values (n=3) for cation exchange capacity (CEC) were 318 mmol$_c$/kg, for carbonates content ($CaCO_3$) mean values were 49 g/kg (or 4.9 % in DM) and for organic carbon content (C_{org}) 26.4 g/kg (or 2.6 % in DM).

Radioactivity (^{7}Be, ^{134}Cs, ^{137}Cs, ^{40}K, ^{210}Pb, ^{226}Ra, ^{228}Ra, ^{228}Th and ^{238}U) of coal ash samples (from all 5 disposal sites) was determined using Gammaspectrometry and the values were within thresholds of BiH legislation. However, values for ^{226}Ra and ^{210}Pb were above clearance levels for solids, liquids and excavated soil set by Austrian and German regulations (Dellantonio et al., 2007).

In order to quantify the **microbial activity** of ash, the CO_2 production from microbial respiration was measured (RECOAL, D8, 2005). In the experiments conducted the basal respiration (BAS), and after the addition of glucose, the substrate induced respiration (SIR) were measured for the ash material. In this analysis the Heinemeyer-method was used (Martens, 1995). The basal respiration (BAS) was measured for the period of 10 days. The substrate induced respiration (SIR) was measured for the period of 4 hours, and also for the period of ten days. Median values of BAS 10 days (n=3; -15.8 μg CO_2 g^{-1} h^{-1}), SIR 4 hours (n=3; -2.3 μg CO_2 g^{-1} h^{-1}) and SIR 10 days (n=3; -2.3 μg CO_2 g^{-1} h^{-1}) of Jezero ash (up to 10 years old ash) showed negative respiratory data under both limited and excessive C availability. These findings suggested that the recorded negative respiratory values for Jezero ash were caused by CO_2 fixation to $Ca(OH)_2$ in the ash. However, the additional observation of the amount of mineral nitrogen that was produced during the experiment suggested that microbes were present in Jezero ash, but that their CO_2 production could not be measured due to CO_2 sequestration.

Mean anions content varied for **fluorides** from 2.20 mg/kg (in treatment with 75 % of soil and 25 % of ash) up to 44.3 mg/kg (in treatment with saw dust amendment), for **chlorides** from not detectable (n.d.) content in treatment with 75 % of soil and 25 % of ash up to 22.0 mg/kg (in treatment with saw dust amendment), for **nitrites** from 2.44 mg/kg (in treatment with 75 % of soil and 25 % of ash) up to 7.91 mg/kg (in treatment with pure ash), for **bromides** content was not detectable in all treatments except for pure soil in which content was 0.21 mg/kg, for **nitrates** from 13.3 mg/kg (in treatment with 75 % of soil and 25 % of ash) up to 39.3 mg/kg (in treatment with equal quantity of soil and ash), for **sulphates** from 27.1 mg/kg (in treatment with pure soil) up to high 11 843 mg/kg (or 11.8 g/kg) (in treatment with pure ash) and for **phosphates,** that behave like bromides, the content was not detectable for all treatments except in pure soil in which it was 10.2 mg/kg, (table 5-2). According to RECOAL, D7-Annex soil, 2005 mean values of anions in ash (n=6) were: for fluorides 4.6

mg/kg, for chlorides 11.4 mg/kg, for nitrites 0.87 mg/kg, for bromides not detectable, for nitrates 19.5 mg/kg, for phosphates 1.05 mg/kg and for sulfates 10 589 mg/kg (or 10.6 g/kg).
Mean cations content varied for **lithium** from not detectable (n.d.) content (in treatments with pure soil and with 75 % of soil and 25 % of ash) up to 1.16 mg/kg (in treatment with pure ash), for **sodium** from 5.44 mg/kg (in treatment with pure soil) up to 41.3 mg/kg (in treatment with 25 % of soil and 75 % of ash), for **ammonium** from 1.34 mg/kg (in treatment with 75 % of soil and 25 % of ash) up to 8,31 mg/kg (in treatment with pure soil), for **potassium** from 8.71 mg/kg (in treatment with 75 % of soil and 25 % of ash) up to 82.1 mg/kg (in treatment with saw dust amendment), for **magnesium** from 8,60 mg/kg (in treatment with pure soil) up to 118 mg/kg (in treatment with pure ash) and for **calcium** from 224 mg/kg (in treatment with pure soil) up to 4 660 mg/kg (or 4.6 g/kg) (in treatment with pure ash)(table 5-2).

The observed results suggested that the dominant type of salt in Jezero ash was **calcium sulphate ($CaSO_4$).**

Table 5-3 shows that **total contents** of **arsenic, boron, chromium, copper** and **nickel** in treatment with pure ash were higher than normal or higher than MAC (normal and MAC values for all seventeen elements are given in table 5-5) and were 112 mg As/kg, 204 mg B/kg, 281 mg Cr/kg, 85.9 mg Cu/kg and 593 mg Ni/kg, respectively.
According to soil classification damage criteria (Bašić, 1994) there are five classes of pollution, from clean soil (1st class) to waste soil (5th class). Qualification to a certain class is calculated through substrate pollution level (PL), as mass ratio of elements present in substrate expressed in %, in accordance to MAC.
When classified according to soil classification damage criteria (table 2 in Appendix), keeping in mind that this is not soil, the ash taken from Jezero disposal site belonged to 5th class or waste soil in respect to As, Cr and Ni, to 4th class or polluted soil in respect to B and Cu, to 3rd class or high polluted soil in respect to Zn, Sr, Fe, Co and Cd, to 2nd class or low polluted soil in respect to Cs, Pb, Rb and U and to 1st class or clean soil in respect to Mo, P and Se.

It is interesting that the treatment with pure soil was also enriched with As (27.7 mg/kg; table 5-3) and Ni (96.4 mg/kg; table 5-3) and according to soil classification damage criteria (Bašić, 1994) these elements belong to 4th class or polluted soil (table 2 in Appendix). In treatments from No. 3 – 6, arsenic, boron, chromium, copper and nickel content was also higher than in natural soils (table 5-3).

Labile elements were measured in NH_4NO_3, 1:2.5, *w/v* substrate extract (table 5-4). For some of the elements there is no data in literature for their natural labile concentration in NH_4NO_3 extracts so it was compared with 1 M NH_4Ac extract (values of labile elements found in literature are given in table 5-6). Some of the elements (As, Ni, U and Zn; table 5-4) had concentrations that were higher or equal to precautionary values (Prüess, 1994, table 5-6) and in treatment with pure ash concentrations were 100 µg As/kg, 630 µg Ni/kg, 50 µg U/kg, 250 µg Zn/kg, respectively. Some of the elements (Cr and Mo) had higher concentrations than trigger values (Prüess, 1994, table 5-6) and in treatment with pure ash were (122.5 mg Mo/kg and 1 mg Cr/kg) and B and Sr (39.3 mg B/kg and 10.7 mg Sr/kg) had concentration that was higher than natural (Reimann et al., 2003 and Vago, 2006; table 5-6; in 1M NH_4Ac) in treatment with pure ash. Labile values of Mo, Sr and Zn were higher than normal in treatment with pure soil as well. Most micronutrients availability is impacted by soil pH (Lončarić et al., 2008).

After three vegetation seasons: two of winter barley (*Hordeum vulgare* L. cv. Rex) grown from 15.11.05. to 26.06.06. - barley (1), grown from 09.11.06. to 18.06.07. - barley (2) and one season of soybean (*Glycine max* L. cv. Dora) grown from 03.07.06. to 20.10.06. on the same substrate, yield components, total elements of nitrogen, sulphur, carbon and hydrogen in grain and total elements (Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Rb, Sr, U, Se, Zn, Cs, As, B and P) content in grain (in extract of HNO_3/H_2O_2=5:1, *v/v*) were determined for barley and soybean. Visible influence of the substrate on the plant quality is shown in Figure 1-4.

Yield components of barley

For barley (1) and barley (2) yield components (number of plants with ear per pot, number of plants without ear per pot, total number of plants per pot, length of stem, length of ear, number and weight of grains per pot) are shown in tables 5-7 and 5-14, respectively.

For barley (1) the worst results were noted in treatment No. 5 (treatment with saw dust amendment): minimum number of plants with ear (23), minimum average length of stem (18.6 cm), minimum average length of ear (1.4 cm), minimum number and grains (175), minimum weight of grains (3.4 g) and maximum number of plants without ear (83 sterile plants), and these values were significantly lower than in treatment with pure soil.

In second vegetation season, barley (2), the worst results were noted in treatment with pure ash: minimum number of plants with ear (28), minimum total number of plants (32), minimum average length of ear (2.3 cm), minimum number and grains (51) and minimum weight of grains (1.1 g). The number of plants with ear (28) and average length of ear (2.3 cm) in treatment with pure ash was significantly lower than in treatment with pure soil.

The best results for barley (1) were observed in treatment with pure soil: maximum average length of stem (36.9 cm), maximum average length of ear (3.5 cm), maximum weight of grains (15.5 g) and minimum number of plants without ear per pot (6 sterile plants) and for barley (2) in treatment with 75 % of soil and 25 % of ash: maximal average length of ear (3.8 cm) and maximum weight of barley grains per pot (6.3 g).

Yield components of soybean

Soybean yield components (total number of plants per pot, number of pods per stem, and weight of grains per pot) are shown in table 5-11. The worst results were in treatment with pure ash, significantly lower than in treatment with pure soil: minimal number of pods per pot (11), minimal number of pods per stem (1) and minimal weight of soybean grains (0.5 g). The best results were in treatment with pure soil: maximal number of pods per pot (33), maximal number of pods per stem (4) and maximal weight of soybean grains per pot (11.9 g).

Total nitrogen, carbon and sulphur content in barley and soybean grain

Dry plant material contains 2 - 4 % of N which appears rather low in comparison with the C content of about 40 %, and rather high in comparison with the total S content in plant tissues which **is** in the range of 0.2 - 0.5 % in DM (Mengel and Kirkby, 1987). Deloch, 1960, noted that sulphur content in grains of barley was 0.18 % DM and 0.32 % DM in soybean grain.

Table 5-8, 5-12 and 5-15 shows TN, TC and TS contents in barley and soybean grains.

Total nitrogen content was in the range of 1.16 % (in pure soil) - 2.04 % (in pure ash) in barley (1) grain and smomewhat higher, 1.82 % (in treatment with saw dust amendment) - 2.69 % (in treatment with 25 % of soil and 75 % of ash) in barley (2) grain, but lower than in soybean grain where TN content varied from relatively high 5.49 % (in treatment with pure ash) to 6.73 % (in treatment with pure soil).

Total carbon content varied in range from 42.6 % (in treatments No. 3 and 4) to 43.7 % (in pure ash) in barley (1) grain and it was somewhat lower in barley (2) grain: from 41.4 % (in treatments No. 3 and 5) to 42.7 % (in treatment with pure soil), but lower than in soybean grain where TC content varied from 50.8 % (in treatment No. 3) to 51.3 % (in treatments No. 2 and 5).

Total sulphur content was in range of 0.137 % (in pure soil) - 0.248 % (in treatment with 25 % of soil and 75 % of ash) in barley (1) grain and it varied from 0.197 % (in treatment with saw dust amendment) to 0.321 % (in pure soil) in barley (2) grain, but it was lower than in soybean grain where TS content varied from 0.327 % (in treatment with pure soil) to 0.372 % (in treatment with 75 % of soil and 25 % of ash).

Plant uptake of metals and metalloids

Mercury (Hg) and thallium (Tl) were not present in considerable concentrations in ash and were not present in considerable concentrations in barley leaf (28 µg Hg/kg; < 50 µg Tl/kg; n=1) grown at disposal site Drežnik (RECOAL, D8, 2005) which is in accordance with data given by other authors (Kabata-Pendias and Mukherjee, 2007 and Adriano, 2001) who observed normal range of Hg concentration in barley from 1.1 µg/kg up to 34 µg/kg (Wiersma et al., 1986) or gave reference content (50 µg/kg) of Tl in plants (Markert, 1992). According to (Scheffer and Schachtschabel, 1992) normal ranges of Tl and Hg for plants are 10 – 500 µg Tl/kg and 2 – 40 µg Hg/kg.

Plant uptake from pure alkaline coal ash enriched with As, B, Cr, Cu and Ni and from its mixtures with different soil amounts was different for barley and soybean for different metals and metalloids.

Table 5-10 gives normal ranges of total element content in plants found in available literature (Scheffer and Schachtschabel, 1992; Grbeša, 2004; Kabata-Pendias and Mukherjee, 2007 and Mengel and Kirkby, 1987). Total content of Cr, Cs, Mo, Rb, Se, Sr and U was higher than normal in some treatments of barley (1) grains (table 5-9). In barley (2) grains besides above mentioned (Cs, Mo, Rb, Se, Sr and U, except Cr), B and P were higher than normal in some treatments (table 5-13) and in soybean grain B, Cd, Cr, Cs, Mo, Ni, Rb, Se, Sr and U were higher than normal in some treatments (table 5-16).

Content of As, Co, Cu, Fe, Pb and Zn in soybean and barley grains were below normal values according to the available literature in all treatments (tables 5-9, 5-13 and 5-16; Figures 1, 4, 7, 8, 12 and 17 in Appendix) although considerably high concentration of As and Cu was measured in ash (table 5-3).

Arsenic (As) concentration in barley and soybean grain was very low despite its relatively high concentration in ash (112 mg/kg; table 5-3) and its relatively high concentration in pure soil (27.7 mg/kg; table 5-3) and it varied from 0.03 mg As/kg in treatments No. 1, 3 and 4 of soybean grain and in treatments with pure soil of barley (2) grain, to 0.57 in treatment with pure ash of barley (2) grain (Figure 1 in Appendix; tables 5-9, 5-13 and 5-16). According to (Bašić et al., 1993) plant uptake of As is very low and in natural plants varies from 0.1 mg/kg to 1 mg/kg. Enrichment coefficients (table 5-17) were also very low and varied from < 0.001 (uptake lower than 0.1 %) (in treatment of soybean with equal ash and soil content) up to 0.007 (treatment of barley (1) with saw dust amendment). In barley (*Hordeum vulgare* L.) leaf, grown at disposal site Drežnik at the beginning of 2005, the measurements showed 0.15 mg/kg of total mass ratio of As (n=1, RECOAL, D8, 2005). The comparison of results for plant uptake of As and Ni and their EC was previously published (Zgorelec et al., 2008). The results of this study support the conclusion that in barley grains, as well as in soybean grains, As content was not high in any of investigated treatments (although As content in barley and soybean grain was significantly higher in treatment with pure ash compared to treatment with pure soil) and was below upper value (1 mg/kg) of normal As concentration (Scheffer and Schachtschabel, 1992), despite the fact that plants were grown on As enriched ash or on its different soil mixtures. This is in accordance with the conclusions of other authors (Adriano, 2001 and Kabata-Pendias and Mukherjee, 2007) who found that As is naturally present in plants (although there is no evidence that As is essential for plant nutrition), but As concentrations in plant tissues rarely exceed 1 mg/kg and As distribution, in general, decreases from root to stem and leaf to edible parts of plants.

Relatively high concentration of **boron (B)** in ash (204 mg/kg; table 5-3) remains a concern for barley and especially for soybean growth, although boron is an essential element for growth and development of higher plants and, according to Adriano, 2001, barley and soybean are among plants tolerant to boron. Lower concentrations were noted in barley in comparison to those noted in soybean grain. Minimal content (14.9 mg/kg) was observed in barley (1) grain grown in treatment with pure soil, and maximal content (256 mg/kg) was noted in soybean grain grown in treatment No. 3, 75 % of soil and 25 % of ash. Higher than upper value (100 mg/kg) of normal concentration in plants, according to Scheffer and Schachtschabel, 1992, was noted in two treatments of barley, those with pure soil and with pure ash of barley (2) grain, while all concentrations of B in soybean were higher than upper value of normal concentration range (Figure 2 in Appendix; tables 5-9, 5-13 and 5-16). There is a very narrow range between beneficial and toxic B concentrations in growth substrate and deficient and sufficient concentration in plants. According to Adriano, 2001, B toxicity of plants occurs if B concentration exceeds 100 mg/kg for most plants, in barley tissue > 70 mg/kg, in barley straw > 46 mg/kg and in soybean leaves > 63 mg/kg. According to Eriksson, 2001, normal B concentration in barley grains is 0.83 mg/kg. EC of B varied from not detectable (n.d.) in barley and soybean grown in treatments with pure soil, up to high 5.01 in soybean grown in treatment No. 3 (table 5-17). In barley leaf grown at disposal site Drežnik concentration of 11.02 mg/kg of total B mass ratio was noted (n=3, RECOAL, D8, 2005). Given these facts, it can be concluded that B remains a concern for barley and especially for soybean growth, even though both barley and soybean are plants tolerant to boron.

Low content of **cadmium (Cd)** in ash (0.52 mg/kg; table 5-3) resulted with low content of Cd in soybean and barley grains, which was below upper value (0.4 mg/kg; Scheffer and Schachtschabel, 1992) of normal concentration value for plants in all treatments, except for one extreme (0.90 mg/kg) noted in soybean grain grown in treatment with pure ash (Figure 3 in Appendix; tables 5-9, 5-13 and 5-16). According to Bašić et al., 1993, normal Cd concentration in plants is in range of 0.05 mg/kg - 2 mg/kg, and toxicity occurs at concentrations higher than 3 mg/kg. EC of Cd was relatively low and varied from not detectable (n.d.) in six treatments: in soybean treatments No. 1 and 3 and in barley (2) treatments from No. 3-6, to high 1.75 observed in soybean grown in treatments with pure ash (table 5-17). In barley leaf grown at disposal site Drežnik very low Cd concentration was also noted (<0.10 mg/kg; n=3, RECOAL, D8, 2005). Cadmium is not an essential element in plant nutrition. The primary factors that affect mobility and bioavailability of Cd in soils are soil pH, texture, organic matter, Cd concentration, Cd species, Zn status (Coga et al., 2007) and salinity (Ondrasek et al, 2009). Soil pH is the most important single soil property that determines Cd bioavailability to plants (Adriano, 2001). In alkaline conditions more Cd was probably adsorbed by substrate in stable $CdCO_3$, $CdSO_4$ and $CdOH^+$ forms, than it was soluble in substrate solution (in all treatments available Cd was not detectable; table 5-4). According to Kabata-Pendias and Mukherjee, 2007, biotic factors such as plant species, root activity and crop cultivation may have an impact on Cd uptake by plants, however, Cd availability to plants depends rather on soil parameters than on plants species. In this study soybean grain was more sensitive to Cd uptake than barley grain.

Cobalt (Co) concentration was low in ash (34.1 mg/kg; table 5-3) and considerably below upper value (1 mg/kg) of normal concentration range in plants, according to Scheffer and Schachtschabel, 1992, in all investigated treatments of barley and of soybean grain (Figure 4 in Appendix; tables 5-9, 5-13 and 5-16). These results are in accordance with the results of other authors who noted concentration in barley grains up to 44 µg/kg and concentration in soybean grains from 53 µg/kg to 176 µg/kg (Adriano, 2001; Kabata-Pendias and Mukherjee, 2007 and Grbeša, 2004). Above-mentioned authors also claimed that Co in nutrient or soil solution is easily accessible to plants and that essentiality of Co to microorganisms, as well as to higher plants, has not been fully established yet, although there is some evidence of favorable effects that Co has on plant growth and especially on N-fixing processes. Enrichment coefficients (table 5-17) were also very low and varied from < 0.001 (in treatments No. 3-6 of barley (2)) to 0.011 (in treatment of barley (1) with pure soil).

There is no clear evidence that **Chromium (Cr)** is essential in plant metabolisms, and thus, described positive effects of Cr on plant growth and development may be artefact. Content of Cr in plants has recently received more attention due to its importance as an essential micronutrient in human metabolic processes (Cr^{3+}), but also because of its carcinogenic effects (Cr^{6+}). Chromium is slightly available to plants and not easily translocated within them, thus it is concentrated mainly in roots (Kabata-Pendias and Mukherjee, 2007). Chromium (Cr) concentration in barley (1) and soybean grain was higher than its concentration in barley (2). Chromium concentration in pure ash was (281 mg/kg; table 5-3) considerably higher than MAC (100 mg/kg) and significantly lower (59.1 mg/kg; table 5-3) than in pure soil. Concentration of Cr in plants varied from 0.37 mg/kg in treatment with saw dust amendment (No. 5) in barley (2) grain, up to 3.73 mg/kg in treatment of barley (1) with pure soil. Extremely high concentration was noted in soybean grain grown in treatment with pure soil (17.8 mg/kg) with considerable data spread between replication (RSD = 140 %) which could be considered an exception (Figure 5 in Appendix; tables 5-9, 5-13 and 5-16). In barley leaf grown at disposal site Drežnik considerable higher concentration than in barley grain in pot experiment was noted (6.88 mg/kg; n=3, RECOAL, D8, 2005). Enrichment coefficients (table 5-17) were relatively low and varied from 0.002 in barley (2) grown in treatment with pure ash, to 0.062 in barley (1) grain grown in treatment with pure soil, with the already mentioned exception of soybean grown in treatment with pure soil where the EC value was 0.298. According to Bašić et al., 1993, normal Cr concentration in plants is in range of 0.02 mg/kg - 1 mg/kg and toxicity occurs if mass ratio is higher than 5 mg/kg. Eriksson, 2001, noted normal concentration range of Cr in Sweden barley grains of 0.01 mg/kg - 0.02 mg/kg and (Adriano, 2001) reported that phytotoxicity of plants in general appears when levels of available form in growing substrate solution are from 1 mg/kg to 5 mg/kg, for barley if available Cr^{3+} in growth media is > 8 mg/kg, if available $Cr^{6+}(CrO_4^{2-})$ in growth media is > 50 mg/kg (stunted growth) and death of plant occurs if available $Cr^{6+}(CrO_4^{2-})$ is > 500 mg/kg. According to results of alkali substrate and its different soil mixtures analisys, available Cr did not exceed 1 mg/kg (table 5-4) in neither treatment and the explanation is probably connected to pH (table 5-1), the most important factor which has the influence on Cr forms, its solubility, sorption by growing substrate and bioavailability to plants, since Cr is probably immobilized and precipitated as stable $Cr(OH)_3$ in alkali conditions.

Although **caesium (Cs)** concentration in pure ash was in the range of natural soil concentration (13.6 mg/kg; table 5-3), according to Kabata-Pendias and Mukherjee, 2007, normal range varies from 0.8 to 30 mg/kg (table 5-5), Cs concentration in edible parts of plants grown in treatment with pure ash of barley (1), barley (2) and soybean, was higher than natural (0.5 mg/kg; table 5-10) and significantly higher than in all other treatments of both species and was 0.91 mg/kg, 1.46 mg/kg and 1.65 mg/kg, respectively (Figure 6 in Appendix; tables 5-9, 5-13 and 5-16). In all other treatments Cs concentration in edible parts of plants (barley and soybean) was below 0.5 mg/kg. Enrichment coefficients (table 5-17) were very low, in accordance with concentration data, and varied from 0.002 in five treatments, No. 1, 3, 4 and 5 of barley (1) and No. 3 of barley (2) up to 0.014 in treatment No. 6 of soybean. EC in treatment with pure ash of barley (1), barley (2) and soybean were considerably higher and were 0.067, 0.108 and 0.122, respectively. Caesium is non-essential to plants, thus, its distribution in plant samples has not been intensively studied, but based on available data, normal range of Cs in various plants is estimated at < 0.1 mg/kg – 3 mg/kg (Kabata-Pendias and Mukherjee, 2007).

Copper (Cu) concentration in barley and soybean grain was very low despite its relatively high concentration in ash (85.9 mg/kg; table 5-3) and was in the range from 1.08 mg/kg in barley (1) grain grown in treatment with pure ash, up to 7.46 mg/kg in soybean grain grown in treatment with pure soil (Figure 7 in Appendix; tables 5-9, 5-13 and 5-16). Normal concentration of Cu in vegetation is in range from 2 mg/kg up to 12 mg/kg and Cu toxicity can be found at acid soils when Cu concentration is higher than 100 mg/kg (Bašić et al., 1993). In barley leaf grown at disposal site Drežnik higher concentration than in barley grain grown in pot experiment was identified (14.2 mg/kg; n=3, RECOAL, D8, 2005). In barley grains Cu concentration was in the range from 1.08 mg/kg (in barley (1) treatment with pure ash) to 5.07 mg/kg (in barley (2) treatment with 75 % of soil and 25 % of ash). Enrichment coefficients (table 5-17) varied from 0.01 (in barley (1) treatment with pure ash), up to 0.31 (in soybean treatment with pure soil). Copper is one of seven micronutrients (Zn, Cu, Mn, Fe, B, Mo and Cl) essential for normal plant nutrition. The amount of Copper required by plants is very small: 5-20 mg/kg in crop tissue is sufficient for normal growth, < 4 mg/kg is considered deficient and > 20 mg/kg is considered toxic (Adriano, 2001), while normal concentration is 7 mg/kg in barley grain and 18 mg/kg in soybean grain (Grbeša, 2004). According to Kabata-Pendias and Mukherjee, 2007, normal range of Cu concentration in cereal is from 0.3 mg/kg to 13 mg/kg, therewith the lowest concentration of Cu is identified in oats and the highest concentration in rye.

Iron (Fe) concentration in ash was in normal range for natural soils and considerably below normal concentration in barley (185 mg/kg) and soybean (250 mg/kg) grains, according to Grbeša, 2004, in all investigated treatments (Figure 8 in Appendix; tables 5-9, 5-13 and 5-16). Enrichment coefficients (table 5-17) were also very low and varied from < 0.001 in barley (1) grain (in treatment with pure ash), up to 0.005 in soybean (in treatment with pure soil). Kabata-Pendias and Mukherjee, 2007 noted that the mechanisms of Fe uptake by plants and transport within plants, as well as its metabolic functions, have been thoroughly studied because Fe is the key metal in energy transformation needed for syntheses and other life processes and cell activity. According to Romic and Romic, 2003, Fe accumulation in agricultural topsoils is not influenced by urban and industrialised (anthropogenic) environments, mainly of geogenic, i.e. pedogenic, origin.

Although **molybdenum (Mo)** concentration in pure ash was relatively low (1.68 mg/kg; table 5-3) compared to MAC (15 mg/kg; table 5-5), Mo concentration in plants grown in treatments with pure ash was above natural (1.5 mg/kg; table 5-10), in barley (1) it was 1.95 mg/kg and in barley (2) 1.83 mg/kg. Mo concentration in soybean was very high compared to natural value in all treatments and was in the range from 17.4 mg/kg in treatment with 75 % of soil and 25 % of ash (No. 3), up to 40.9 mg/kg in treatment with saw dust amendment (Figure 9 in Appendix; tables 5-9, 5-13 and 5-16). According to Bašić et al., 1993, and Kabata-Pendias and Mukherjee, 2007, natural content of Mo in plants is relatively low (< 1 mg/kg), and high concentration of Mo (> 10 mg/kg) in forage could cause disturbance in animal metabolism or so called molybdenosis (Cu deficiency). Molybdenum is an essential nutrient to all plants, but most plants require small amounts of this element. The average Mo content in barley grain varied from 0.2 mg/kg to 1.2 mg/kg (Eriksson, 2001). The uptake of Mo by plants is, in most cases, controlled by pH. Usually a positive correlation is observed between Mo content in plants and the soil pH (Kabata-Pendias and Mukherjee, 2007). EC identified in this experiment were in accordance with these results (table 5-18). Very high EC were noted for soybean, from 14.4 (in treatment with 25 % of soil and 75 % of ash) up to 60.3 (in treatment with pure soil) and high EC were noted for barley (1) and (2), particularly in first three treatments where EC were > 1, EC for barley varied from 0.16 in barley (1) treatment No. 3 up to 1.92 in barley (1) treatment with pure soil.

Nickel (Ni) concentration in soybean grain was higher than Ni concentration in barley (1) and (2) grain. Nickel concentration in pure ash was (593 mg/kg; table 5-3) considerably higher than MAC (60 mg/kg). Considerably high concentration of Ni was also noted in treatment with pure soil (96.4 mg/kg; table 5-3). Concentration of Ni in barley was below upper value (3 mg/kg) of normal concentration range for plants, according to Scheffer and Schachtschabel, 1992, in all investigated treatments and concentration of Ni in soybean was above 3 mg/kg in all investigated treatments (Figure 10 in Appendix; tables 5-9, 5-13 and 5-16). Minimal concentration of Ni in soybean grain was 16.6 mg/kg, observed in treatment No. 3 (75 % ash and 25 % soil), and maximal concentration was 54.5 mg/kg, in treatment with pure ash. According to Croatian authors (Bašić et al., 1993) normal Ni concentration in plants is from 0.5 mg/kg to 5 mg/kg. Ni enrichment coefficients were in accordance with this data (table 5-18 and Zgorelec et al., 2008). EC in barley were lower than EC in soybean and for barley (1) and (2) varied from < 0.001 (in barley (1) treatment with 75 % of soil and 25 % of ash), up to 0.009 (in barley (1) treatment with pure soil), whereas for soybean they varied from 0.06 (in treatment No. 6, 25 % soil and 75 % ash) to 0.35 (in treatment with pure soil). In barley leaf grown at disposal site Drežnik higher concentration (3.99 mg/kg; n=3) of Ni was noted in comparison with barley grain in pot experiment (RECOAL, D8, 2005). Some investigators (Eskew et al., 1983) have suggested that Ni might be essential for plants. However, there is still no evidence for metabolic role of Ni in higher plants, although reported beneficial effect of Ni on plant growth are in favor of thesis that this metal may have its function in plants. Although Ni is already considered an essential element for higher plants, its toxicity to plants has been closely surveyed in many studies. The most common symptom of Ni phytotoxicity is chlorosis followed by yellowing and necrosis of leaves. The phytotoxic

Ni concentrations vary widely among plant species and cultivars and have been reported from > 40 mg/kg (Kabata-Pendias and Mukherjee, 2007). The same author noted the range of Ni in barley grains from 0.10 mg/kg up to 0.67 mg/kg and Agarwala et al., 1977, compared the phytotoxicity of other metals with Ni in barley and the effectiveness of metals in inducting visible toxicity symptoms was in following order: Ni > Co > Cu > Mn > Zn.

Despite the fact that the supstrate had relatively low supply of phosphorus available to plant (60 mg/kg as P_2O_5 or 26.2 mg/kg as P-P_2O_5; table 5-1) or water soluble orto-phosphates (not detectable; table 5-2), total **Phosphorus (P)** content in pure ash was in normal range (217 mg/kg; table 5-3 and table 5-5). Despite high substrate pH, supposed P fixation to stable Ca-phosphates minerals and weak solubility, plant available P content was still greater than critical level (8 mg/kg; Soltanpour and Schwab, 1977) for agronomic crops. Normal concentration of total P in barley (3 800 mg/kg) and in soybean (6 700 mg/kg) grains, as given by Grbeša, 2004, are shown in table 5-10. In Barley (1) and in soybean observed concentrations of P were lower than natural in all treatments, but in barley (2) the content of P was higher (probably due to fertilization) and was above normal in all treatments except in treatment with pure soil (Figure 11 in Appendix; tables 5-9, 5-13 and 5-16). Phosphorus EC (table 5-17) were very high in barley and in soybean, in the range of 2.74 (in pure soil treatment of barley (1)), up to 23.4 (in pure ash treatment of soybean).

As already observed for As, Co, Cu and Fe, similar behaviour was noted for **lead (Pb)**. Lead content in barley and in soybean was very low (up to maximal 1.81 mg/kg in treatment No. 3 of barley (2)), considerably lower than upper value (6 mg/kg) of normal concentration range for plants according to Scheffer and Schachtschabel, 1992, in all treatments (Figure 12 in Appendix; tables 5-9, 5-13 and 5-16). According to Bašić et al., 1993, normal concentration in vegetation is in the range of 0.1 mg/kg - 5 mg/kg, although in vegetation near traffic roads concentration could vary from 5 mg/kg to 100 mg/kg. Pb is mainly accumulated in cell membrane and toxic Pb concentrations could cause inhibition of whole spectrum of enzymes in human and animal metabolism. Enrichment coefficients for Pb (table 5-17) were also very low and varied from not detectable in six treatments (No. 1, 2, 5 and 6 of barley (1) and treatments No. 5 and 6 of barley (2)), to 0.062 (in barley (2) treatment No. 3 with 75 % of soil and 25 % of ash). In barley leaf grown at disposal site Drežnik noted concentration (0.51 mg/kg; n=3) was in the range of those observed in barley grain in pot experiment (RECOAL, D8, 2005). Lead occurs naturally in plants, however it has not been shown to have an essential role in their metabolism. The absorption of Pb by root is passive and thus the rate of its uptake from soils is rather low. Pb transfer in plants is very limited and its great proportion is accumulated in roots (Kabata-Pendias and Mukherjee, 2007). Other authors also noted that the distribution of trace elements in plants can be categorized into three groups: (1) more uniformly distributed between roots and shoots (Zn, Mn, Ni and B); (2) more in roots than in shoots, with moderate to sometimes large quantities in shoots (Cu, Cd, Co and Mo) and (3) mostly in roots with very little in shoots (Pb, Sn, Ti, Ag, Cr and V)(Adriano, 2001).

Rubidium (Rb) concentration in pure ash (48.7 mg/kg; table 5-3) was in normal range for soil according to Kabata-Pendias and Mukherjee, 2007, (10 mg/kg - 140 mg/kg; table 5-5) although its concentration (total and available) in pure ash was significantly higher than in all other treatments (table 5-3 and table 5-4). Normal concentration of total Rb in barley grain (4.7 mg/kg) and in soybean seeds (220 mg/kg), as given by same authors, is shown in table 5-10. Significantly higher Rb concentration was observed in plants grown in treatment with pure ash, than in all other treatments: 54.0 mg/kg in barley (1), 70.4 mg/kg in barley (2), and 100 mg/kg in soybean (Figure 13 in Appendix; tables 5-9, 5-13 and 5-16). Still, all noted values of Rb in edible parts of plants were below normal concentration for soybean seeds (220 mg/kg) and most of them were above natural concentration of total Rb in barley grains (4.7 mg/kg), exceptions were: 1.47 mg/kg in barley (1) grain and 4.15 mg/kg in barley (2) grain grown in treatment with pure soil and 3.78 mg/kg in barley (1) grain grown in treatment No. 3. Unlike other investigated elements, completely positive correlation was noted between Rb concentration in grains for both species and the ash ratio in growing substrate. Enrichment coefficients (table 5-18) were > 1 in pure ash treatment of both species: barley (1) 1.11, soybean 2.06 and barley (2) 1.45. In all other treatments EC for all cultures varied from 0.07 (in barley (1) treatment with pure soil) up to moderately high 0.66 (in soybean treatment with 25 % soil and 75 % of ash). Rubidium, as other monovalent cations, is easily taken up by plants. The radius of Rb (298 pm) is similar to radius of K (277 pm) and therefore Rb can substitute at K sites in plants, but cannot be a substitute for K in metabolic processes. High Rb concentrations in plants might become toxic. Symptoms of its toxicity are dark green leaves, wilting and stunted foliage, and short brown roots; however in field conditions they are practically unknown. Despite the chemical similarity of Rb and K there is significant difference in uptake of these metals. The availability of Rb to several plants increases with soil acidity, while K is not easily taken up from acid soils (Kabata-Pendias and Mukherjee, 2007).

Selenium (Se) concentration in pure ash was in normal range for natural soils (0.23 mg/kg; table 5-3) according to Essington, 2004, (0.1 mg/kg - 2 mg/kg; table 5-5). Se concentration in barley (1) and (2) and in soybean grown in treatment with pure ash was higher than its natural concentration according to Grbeša, 2004, (0.10 mg/kg in barley grain and 0.20 mg/kg in soybean grain), and was significantly higher than in all other treatments: 0.79 mg/kg, 2.13 mg/kg and 0.87 mg/kg, respectively (Figure 14 in Appendix; tables 5-9, 5-13 and 5-16). In three treatments of barley Se content in grain was lower than 0.10 mg/kg, namely in: treatment with pure soil of barley (1) and treatments No. 3 and 5 of barley (2). In the same three treatments of soybean Se content in grain was lower than 0.20 mg/kg. High EC was noted in barley and especially in soybean (table 5-18). EC in soybean varied from 1.06 (in treatment with pure soil) up to 9.35 (in treatment with pure ash) and in barley (1) and (2) from 0.18 (in treatments with pure soil of barley (1) and with saw dust of barley (2)) up to 4.11 (in treatment with equal soil and ash content of barley (1)). It is known that Se is non-essential for plants growth although it is an important element in human and animal nutrition. The content of Se as nutrient and as poison to animals is in a narrow range. Selenium is toxic to plants; it can reduce germination and produce stunted and brown spotted plants, chlorotic plants, it can also decrease the yield or cause mild and severe toxicity depending on its concentration in growing substrate. The amount of 7.2 mg/kg in barley grain shows toxicity symptoms (Walsh and Fleming, 1952). Selenate (SeO_4^{2-}) is a more toxic source of Se than selenite (SeO_3^{2-}) and depends on soil pH and S concentration in medium. If S content is > 30 mg/kg, Se as SeO_4^{2-} can become more toxic (Hurd-Karrer, 1937). In this experiment with alkali conditions high content of S (water soluble SO_4^{2-} in ash was 11 843 mg/kg; or 3948 mg/kg S-SO_4^{2-}; or 0.3948 % S-SO_4^{2-} in DM) probably increased mobility and uptake of Se-SeO_4^{2-}, because the two ions are antagonists, with analogue biogeochemical reactions, and in the same active uptake processes in competition with each other.

Strontium (Sr) concentration in pure ash was in normal range for soil as given by Kabata-Pendias and Mukherjee, 2007, (table 5-5), but a significantly higher concentration was observed in pure ash (182.2 mg/kg; table 5-3) than in pure soil (87.9 mg/kg; table 5-3) and in all other treatments. The range of normal concentration for total Sr in grains (1.5 mg/kg - 2.5 mg/kg), given by the same author, is shown in table 5-10. Figure 15 in Appendix (tables 5-9, 5-13 and 5-16) shows that only two values in barley were above normal range in grains (2.66 mg/kg in barley (2) grown in treatment with pure soil and 2.71 mg/kg in barley (1) grown in treatment with saw dust amendment) and in soybean only two values were in normal range (2.16 mg/kg in treatment No. 3 and 2.21 mg/kg in treatment with saw dust amendment). Enrichment coefficients (table 5-18) were also very low and varied from 0.006 in of barley (1) treatment with pure ash up to 0.032 in soybean treatment with pure soil. These results show that more Sr was uptaken by soybean grain than by barley grain. Although Sr is apparently not a plant micronutrient, it is adsorbed owing to metabolic requirements for Ca. The interaction between Sr and Ca is complex, and although the two elements can compete with each other, Sr cannot replace Ca in biochemical functions. The increased level of Ca in growth medium may both inhibit and stimulate Sr uptake by plants, depending on several soil and plant parameters. Most often, however, the addition of Ca to soil increases its concentration in soil solution and decreases Sr phytoavailability. Main sources of Sr pollution are associated with coal combustion (Kabata-Pendias and Mukherjee, 2007).

According to Kabata-Pendias and Mukherjee, 2007, (table 5-5) **uranium (U)** concentration in pure ash (5.07 mg/kg; table 5-3) was in normal range for soils (1.2 mg/kg – 11 mg/kg) although U concentration in pure ash was significantly higher than in all other treatments. Llorens et al., 2001, estimated average content of U in coal as 6.1 mg/kg, while in fly ash its content increased above 22 mg/kg. According to Eriksson, 2001, normal concentration for total U in barley (0.2 µg/kg) grains in Sweden is shown in table 5-10. All observed concentrations for total U in barleys (1 and 2) and soybean grains were above normal (0.2 µg/kg) value for barley grains in all investigated treatments (Figure 16 in Appendix; tables 5-9, 5-13 and 5-16). Enrichment coefficients (table 5-18) were very low and varied from < 0.001 in barley (1) grown in treatment with pure ash, in barley (2) grown in treatment with 25 % of soil and 75 % of ash (No. 6), to 0.005 in barley (2) grown in treatment with pure soil. U occurs naturally in terrestrial environment and as ^{238}U isotope exists in relatively large quantities as long-lived nuclide. Geochemically, it exist in +4 and +6 oxidations states in most geological environments. Mobilized U is very likely to be quickly precipitated in hydroxides or adsorbed, and can form organic complexes and various stable compounds such as oxides, carbonates, phosphates, vanates and arsenates. Uranium exhibits affinity for absorption by organic matter and accumulation by microorganisms, and due to easy U sorption by organic matter significant accumulation in coal and peat is often reported. Little information is available about the transfer of U and transuranic radionuclides from soil to plants, although this process is very important in environmental researches (Kabata-Pendias and Mukherjee, 2007).

As for As, Co, Cu, Fe and Pb, similar behaviour was observed for **zinc (Zn)**. Zn content in barley and in soybean was low, lower than upper value (100 mg/kg) of normal concentration range for plants according to Scheffer and Schachtschabel, 1992, in all treatments (Figure 17 in Appendix; tables 5-9, 5-13 and 5-16) and near normal concentration in barley (32 mg/kg) and soybean (45 mg/kg) grains according to Grbeša, 2004, in all investigated treatments. According to Bašić et al., 1993, plant uptake of Zn depends on its availability in soils and on type of species (for example, normal Zn concentration in apple is 0.6 mg/kg and in hemp it is 83 mg/kg). Enrichment coefficients for Zn (table 5-18) varied from 0.10 in pure ash treatment of barley (1) up to 0.43 in pure ash treatment of barley (2). In barley leaf grown at disposal site Drežnik concentration noted was 20.21 mg/kg (n=3; RECOAL, D8, 2005), which is in the concentration range in barley grain noted in pot experiment. According to Kabata-Pendias and Mukherjee, 2007, in cereal grains grown in different countries worldwide, the mean Zn concentrations vary from 18 mg/kg to 33 mg/kg, being the lowest in rice and the highest in oats. Zinc plays essential metabolic roles in plants and it is an active component of a variety of enzymes, such as dehydrogenase, proteinases, peptidases and phosphoryrolases. It influences the permeability of membranes and stabilizes cellular components. There is evidence that Zn stimulates the resistance of a plant to dry and hot weather and also to bacterial and fungal diseases. Studies by Eisler, 1997, indicated that Zn concentrations in plant parts follow this pattern: roots > foliage > branch > trunk.

Influence of vegetation season on monitored parameters of barley

The comparison of vegetation seasons of winter barley (the first one from 15.11.05. to 26.06.06. - barley (1) and the second one from 09.11.06. to 18.06.07. - barley (2)), that grew on the same substrate in controlled conditions showed significant differences between some monitored parameters (table 5-19 and table 5-20).

In treatment with pure soil there was a significant increase of total nitrogen, total sulphur and total B, Cs, Fe, P, Pb, Rb, Sr and Zn content in grain grown in 2007 than in 2005, and a significant decrease of length of steam, number of grains, weight of grains and total content of As, Cr and Mo.

In treatment with pure ash there was a significant increase of length of stem, length of ear, total nitrogen, total sulphur, total B, Cu, Fe, P, Sr and Zn content in grain grown in in 2007 than in 2005, and a significant decrease of number of plants without ear, total number of plants, number of grains, weight of grains and total content of C and Cr.

In treatment No. 3, with 75 % of soil and 25 % of ash there was a significant increase of length of ear and total nitrogen, total sulphur, total hydrogen and total Cu, Fe, Mo, P, Rb and Zn content in grain grown in 2007 compared with 2005, and a significant decrease of number of plants with ear, total number of plants, number of grains, weight of grains and total content of C, As, Cd, Co, Se and Sr.

In treatment with equal content of soil and ash (50 %) there was a significant increase of total H, Fe, Mo, P and Zn content in grain grown in 2007 compared to 2005, and a significant decrease of number of plants with ear, total number of plants, number of grains, weight of grains and total content of N, C, S, Cd, Se and Sr.

In treatment with saw dust amendment in equal content of soil and ash (47.5 %) there was a significant increase of number of plants with ear, length of stem, length of ear and total content of N, H, Cs, Cu, Fe, Mo, P, Rb and Zn content in in grain grown in 2007 compared to 2005, and a significant decrease of total number of plants, number of grains and total content of C, As, Cd, Co, Ni, Se and Sr.

In treatment No. 6 with 25 % of soil and 75 % of ash there was a significant increase of length of ear and total content of N, H, Cu, Fe, Mo, P and Zn content in grain grown in 2007 compared to 2005, and a significant decrease of number of plants with ear, number of plants without ear, total number of plants, number of grains, weight of grains and total content of C, Cd, Co, Cr, Se and Sr.

7 CONCLUSIONS

Ash characteristics

For alkaline coal ash (8.77 in KCl; 8.75 in $CaCl_2$; 8.86 in H_2O; n=4) taken from Jezero disposal site following conclusions were made:

Electrical conductivity was 1 591 µS/cm measured in 1:5 (*w/v*) water extract or 2 380 µS/cm measured in 1:10 (*w/v*) water extract (n=4), moisture was 0.26 %, ash was low supplied with available P_2O_5 (6 mg/100g; n=4) and high supplied with available K_2O (44 mg/100 g, n=4), good supplied with TN (0.110 % in DM, n=4), total carbon content was 2.772 % in DM (n=4) and total S content was relatively low 0.349 % in DM (n=4) with C/N ratio of 25. According to the observed data, dominant type of salt in Jezero coal ash is calcium sulphate ($CaSO_4$).

Some plant available (labile) elements in ash (**As**, **B**, **Cr, Mo**, **Ni**, **Sr**, **U** and **Zn**) which were measured in 1M NH_4NO_3 1:2.5 (*w/v*) ratio (n=4) were higher than precautionary value and were respectively, 100 µg As/kg, 630 µg Ni/kg, 50 µg U/kg, 250 µg Zn/kg, some were higher than trigger value with 122.5 mg Mo/kg and 1 mg Cr/kg and other were higher than natural (in 1M NH_4Ac) with 39.3 mg B/kg (>>0.97 mg/kg) and 10.7 mg Sr/kg (>8 mg/kg).

Ash was enriched with **As, B, Cr, Cu** and **Ni** (n=4) and their mass ratios were 112 mg As/kg, 204 mg B/kg, 281 mg Cr/kg, 85.9 mg Cu/kg and 593 mg Ni/kg, analysed in aqua regia extract in 1:12 (*w/v*) ratio.

According to soil classification damage criteria, ash taken from Jezero disposal site belongs to 5th class or waste soil in respect to As, Cr and Ni; 4th class or polluted soil in respect to B and Cu; 3rd class or high polluted soil in respect to Zn, Sr, Fe, Co and Cd; 2nd class or low polluted soil in respect to Cs, Pb, Rb and U and to 1st class or clean soil in respect to Mo, P and Se, keeping in mind that this is ash not soil, but ash that is used for food and fodder production.

Pure soil was also enriched with As (27.7 mg/kg) and Ni (96.4 mg/kg) and according to soil classification damage criteria, in respect to these elements pure soil belongs to 4th class or polluted soil.

Some chemical and physical analyses of Jezero ash were done at the beginning of 2005 during preliminary investigations and the results were following: texture classes of Jezero ash were on the border of **loamy sand (LS) and sandy loam (SL)** ash [n=4; with 75.1 % of sand content (20-200 µm); 19.1 % of silt content (2-20 µm) and 5.7 % of clay content (<

2 μm)], bulk density (0.7 g/cm^3), particle density (1.9 g/cm^3), field capacity (60.9 vol. %), wilting point (8.6 vol. %), available water (52.3 vol. %), content of air (7.7 vol. %) and porosity (68.5 vol. %; n=2), cation exchange capacity (CEC) was 318 mmol$_c$/kg, carbonates content ($CaCO_3$) was 49 g/kg (or 4.9 % in DM) and content of organic carbon (C_{org}) was 26.4 g/kg (or 2.6 % in DM) (n=3).

Radioactivity (^{7}Be, ^{134}Cs, ^{137}Cs, ^{40}K, ^{210}Pb, ^{226}Ra, ^{228}Ra, ^{228}Th and ^{238}U) of coal ash samples (in ash from all 5 disposal sites) was determined using Gammaspectrometry and was within thresholds of BiH legislation, however, values for ^{226}Ra and ^{210}Pb were above clearance levels for solids, liquids and excavated soil set by Austrian and German regulations.

In order to quantify the **microbial activity** of the ash, the CO_2 production from microbial respiration was measured. Median values for basal respiration (BAS) during 10 days (n=3; -15.8 μg CO_2 g^{-1} h^{-1}), substrate induced respiration (SIR) during 4 hours (n=3; -2.3 μg CO_2 g^{-1} h^{-1}) and also during 10 days (n=3; -2.3 μg CO_2 g^{-1} h^{-1}) of Jezero ash (ash up to 10 years old) showed negative respiratory data under both limited and excessive C availability. It is assumed that the recorded negative respiratory data for Jezero ash was caused by CO_2 fixation to $Ca(OH)_2$ in the ash itself. However, additional observation of the amount of mineral nitrogen that was produced during the experiment brought the conclusion that microbes were present in Jezero ash, but that their CO_2 production could not be measured due to CO_2 sequestration.

- Influence of different ash ratios on yield components of soybean and barley

Yield components of barley

For barley (1) the worst results were obtained in treatment No. 5 (treatment with saw dust amendment) where the measurements showed the minimum number of plants with ear per pot (23), minimum average length of stem (18.6 cm), minimum average length of ear (1.4 cm), minimum number of grains (175), minimum weight of grains (3.4 g) and maximum number of plants without ear per pot (83 sterile plants), in comparison with the results of all other treatments.

One year latter the worst results for barley (2) were obtained in treatment with pure ash where the measurements showed minimum number of plants with ear (28), minimum total number of plants (32), minimum average length of ear (2.3 cm), minimum number of grains (51) and minimum weight of grains (1.1 g). Significantly lower than in treatment with pure soil was the number of plants with ear (28) and average length of ear (2.3 cm) in treatment with pure ash.

The best results for barley (1) were obtained in treatment with pure soil where the measurements showed maximum average length of stem (36.9 cm), maximum average length of ear (3.5 cm), maximum weight of grains (15.5 g) and minimum number of plants without ear per pot (6 sterile plants) and for barley (2) in treatment with 75 % of soil and 25 % of ash where the measurements showed maximal average length of ear (3.8 cm) and maximum weight of barley grains (6.3 g) per pot.

Yield components of soybean

The worst results were measured in treatment with pure ash where the measurements showed significantly lower values than in treatment with pure soil: minimal number of pods per pot (11), minimal number of pods per stem (1) and minimal weight of soybean grains (0.5 g). The best results were obtained in treatment with pure soil where the measurements showed maximal number of pods per pot (33), maximal number of pods per stem (4) and maximal weight of soybean grains (11.9 g) per pot.

- Influence of ash ratio on metals and metalloids accumulation in grains of tested plants and comparison of metals and metalloids accumulation in grains of soybean and barley grown on the same ash ratios

Mercury (Hg) and thallium (Tl) were not present in considerable concentrations in ash and were not present in considerable concentrations in barley leaf (28 µg Hg/kg; < 50 µg Tl/kg; n=1) grown at disposal site Drežnik.

Contents of As, Co, Cu, Fe, Pb and Zn in soybean and barley grains were below normal values according to available literature in all treatments, although a considerably high concentration of As and Cu was measured in ash.

Soybean grain grown on pure alkaline coal ash or on its different soil mixtures was high accumulator of B, Cd, P, Mo, Rb and Se in at least one treatment, moderate accumulator of Cr, Cs, Cu, Ni and Zn, low accumulator of Pb and Sr and non accumulator of As, Co, Fe and U. **Barley** grain grown on pure alkaline coal ash or on its different soil mixtures was high accumulator of B, P, Mo, Rb and Se in at least one treatment, moderate accumulator of Cs, Cu, Cd and Zn, low accumulator of Pb, Cr and Sr and non accumulator of As, Co, Fe, Ni and U.

The observed data showed that barley and soybean were sensitive and in their grains were high accumulators (EC > 1) of: P in all treatments of soybean and all treatments of barley; Rb only in pure ash treatments of soybean and of barley; Mo in all treatments of soybean and some treatments (No. 1, 2 and 3) of barley; Se in all treatments of soybean and all treatments of barley; B in some treatments (No. 3, 4, 5 and 6) of soybean and in one treatment (No. 3) of barley; Cd only in pure ash treatment of soybean.

Very poor yields and visible plants damage in some treatments, especially in treatment with pure ash compared to the treatment with pure soil, of both species, soybean and barley, were probably caused by toxicity or deficiency of several elements.

Regardless the macronutrients (C, N, S and P) in barley and soybean; the observed data showed that total S content was near to normal concentration in soybean (0.32 %) and in barley (0.18 %) grains in all treatments; total N content was relatively high (> 4%) in soybean grains in all treatments, while it was relatively low in barley (1) grains (<2 %) and a little above 2 % in barley (2) grains in all investigated treatments; total carbon content was near normal content in plants (40 %) in barley grains and considerably higher in soybean (~ 50 %) grains in all studied treatments; total P content, despite high substrate pH, expected P fixation to stable Ca-phosphates minerals and its weak solubility and plant availability, was still considerably higher than its critical level (8 mg/kg; or 0.8 mg/100 g) for agronomic crops, thus, P deficiency was not expected to cause concern. In barley (2) grain higher concentrations (~3 800 mg/kg) were observed than in barley (1) (<< 3 800 mg/kg) and in soybean grains the concentrations were somewhat lower than normal (~6 700 mg/kg) depending on treatment.

Soybean plants showed symptoms of B toxicity (> 100 mg/kg) in all treatments, although B is micronutrient, while for barley plants B toxicity might have occured only in two treatments of second barley vegetation. Ni toxicity remained a concern in soybean in treatment with pure ash (> 40 mg/kg), although in all other treatments Ni content in grain was higher than natural (>3 mg/kg) but lower than critical toxicity level (40 mg/kg). Ni in barley grains was not dubious since low Ni concentration (< 3 mg/kg) was observed in all treatments. Uranium concentration was above natural (0.2 µg/kg) in barley grains calculated in Sweden, in all treatments and in both species (barley and soybean), but it is necessary to keep in mind that observed U concentration was very low (2-10 µg/kg), compared to concentration of U in other plants, found in literature, as well as U enrichment coefficients, and there is still no data available in the literature on U phytotoxicity. Cu deficiency in barley grains (<4 mg/kg) was noted, especially in first barley vegetation, but also in three treatments of second barley vegetation, although, in literature, barley is known as a crop sensitive to copper deficiency. Cu deficiency can also provide explanation for relatively low content of total N in barley, since Cu is an essential element for plans nutrition and has a major role in N uptake and metabolism processes. Mo toxicity in soybean grains (>10 mg/kg) was observed in all treatments. It is very interesting that Cu in soybean and Mo in barley (except in treatment with pure ash where Mo content was somewhat higher than natural 1.5 mg/kg, but still <<10 mg/kg) were within normal natural ranges. In all treatments Cu/Mo ratios in soybean grain were lower than recommended guideline (2/1; Miltimore and Mason, 1971) and in barley they were above it; therefore these ratios should remain a concern when soybean or barley grown at disposal sites are used as forage, because grazing animals are at risk of molybdenosis (hypocuprosis).

Very similar behaviour was observed for micronutrients Zn and Fe. In all investigated treatments Zn and Fe concentations in barley and in soybean were within normal natural range (15-100 mg/kg for Zn; or 32 mg Zn/kg in barley grains and 45 mg Zn/kg in soybean grains), not exceeding natural 185 mg Fe/kg in barley grains and 250 mg Fe/kg in soybean grains, although reduced leaf size suggested Zn and Fe deficiency especially in first barley vegetation in all treatments and in soybean in some treatments where concentration <20 mg Zn/kg was observed, which is a critical Zn deficiency level. Observed <<185 mg Fe/kg in both vegetations of barley and << 250 mg/kg in soybean might also be critical. Such Fe and Zn behaviour can probably be explained by high pH and high content of S in growing substrate (water soluble SO_4^{2-} in ash was 11 843 mg/kg; or 3948 mg/kg S-SO_4^{2-}; or 0.3948 % S-SO_4^{2-} in DM) and its influence on mobility and uptake of Fe and Zn which were possibly reduced, since these elements are more likely to be in substrate in stable $Zn(OH)_2$, $Fe(OH)_2$ or Zn and Fe carbonates and sulphates forms.

For Cd and Cs, as elements which are non-essential to plants, concentrations higher than normal (0.4 mg Cd/kg and 0.5 mg Cs/kg) were observed only in plants grown in treatments with pure ash, namely, Cd in soybean and Cs in both species, but concentrations were below toxic levels (<3 mg/kg).

Cr, Se, Sr and Rb might require continued monitoring.

Chromium concentrations, as a non-essential plant element, were higher than normal (1 mg/kg) in barley (1) grains in 5 treatments and in soybean grains in 4 treatments. In barley (2) grains noted Cr concentrations were in the normal range in all treatments. All observed Cr concentrations in all treatments of both species were below the level at which toxicity can occur (< 5 mg/kg). At the same time, all observed Cr concentrations in all treatments of both species were (>>0.02 mg/kg), which is normal barley grain content. Non-essential element for plants but essential for animals and humans is selenium, whose concentrations in both species in treatment with pure ash were significantly higher than in all other treatments; and only in 3 treatments in barley grains and in 3 treatments in soybean grains, concentrations lower than normal were noted (0.10 mg/kg for barley and 0.20 mg/kg for soybean). In all other treatments concentrations above normal were observed, but toxic levels (> 7.2 mg/kg) were not observed in any of the treatments. For strontium, which is not one of the micronutrients of plants, the investigation showed that more Sr was taken up by soybean than by barley grains; only in 2 treatments of barley higher concentrations than normal were noted in grains (2.5 mg/kg), and for soybean lower than normal values were noted only in 2 treatments. Rubidium, as well as other monovalent cations, is easily taken up by plants and at high concentrations in plants it can become toxic. Unlike other investigated elements, Rb showed completely positive correlation between concentration in grains for both species and the ash ratio in growing substrate. Opposite to Sr behaviour, measurements of Rb concentrations showed that more Rb was taken up by barley than by soybean grains, compared to normal concentrations found in literature (4.7 mg/kg in barley grain and 220 mg/kg in soybean seeds); significantly higher Rb concentrations were observed in both species grown in treatment with pure ash than in all other treatments. All noted values in edible parts of soybean were below natural (220 mg/kg) value, but 9 noted values in barley grains were above natural (4.7 mg/kg).

In all treatments of both species other investigated non-essential trace elements (As, Co and Pb) were within normal concentration ranges and far below critical levels (mg As/kg, 1 mg Co/kg and 6 mg Pb/kg).

- Influence of vegetation season on metals and metalloids accumulation in grains of barley

The comparison of vegetation seasons of winter barley, the first from 15.11.05. to 26.06.06. - barley (1) and the second from 09.11.06. to 18.06.07. - barley (2), that grew on the same substrate in controlled conditions, showed significant differences between some monitored parameters.

Treatment with pure soil showed a significant increase of total nitrogen, total sulphur and total B, Cs, Fe, P, Pb, Rb, Sr and Zn content in grain grown in 2007 than in 2005, and a significant decrease of length of steam, number of grains, weight of grains and total content of As, Cr and Mo.

Treatment with pure ash showed a significant increase of length of stem, length of ear, total nitrogen, total sulphur, total B, Cu, Fe, P, Sr and Zn content in grain grown in 2007 than in 2005, and a significant decrease of number of plants without ear, total number of plants, number of grains, weight of grains and total content of C and Cr.

Treatment No. 3 with 75 % of soil and 25 % of ash showed a significant increase of length of ear and total nitrogen, total sulphur, total hydrogen and total Cu, Fe, Mo, P, Rb and Zn content in grain grown in 2007 compared to grain grown in 2005, and a significant decrease of number of plants with ear, total number of plants, number of grains, weight of grains and total content of C, As, Cd, Co, Se and Sr.

Treatment with equal content of soil and ash (50 %) showed a significant increase of total H, Fe, Mo, P and Zn content in grain grown in 2007 compared to grain grown in 2005, and a significant decrease of number of plants with ear, total number of plants, number of grains, weight of grains and total content of N, C, S, Cd, Se and Sr.

Treatment with saw dust amendment in equal content of soil and ash (47.5 %) showed a significant increase of number of plants with ear, length of stem, length of ear and total content of N, H, Cs, Cu, Fe, Mo, P, Rb and Zn content in grain grown in 2007 compared to grain grown in 2005, and a significant decrease of total number of plants, number of grains and total content of C, As, Cd, Co, Ni, Se and Sr.

Treatment No. 6 with 25 % of soil and 75 % of ash showed a significant increase of length of ear and total content of N, H, Cu, Fe, Mo, P and Zn content in grain grown in 2007 compared to grain grown in 2005, and a significant decrease of number of plants with ear, number of plants without ear, total number of plants, number of grains, weight of grains and total content of C, Cd, Co, Cr, Se and Sr.

- General investigation outputs

In general, observed negative environmental impacts of coal ash disposal include the destruction of landscapes and contamination of soil, air and water:

- changes in the hydrological system, both in terms of redefinition of the underground flows and their chemical composition
    - pollution of associated surface waters
    - potential contamination of underground waters, particularly fresh water springs
- air-pollution and deposition of ash particles (wind erosion)
- introduction of pollutants such as heavy metals into the ecosystems and potential distribution of these pollutants into the food chain.

In terms of land use, tests on soils and different land cover options showed that agricultural and pastoral use of the coal ash disposal sites, at least in the first 20 or so years, could pose significant risks to transferring pollutants into the human food chain.

An improvement in the regional economic situation and employment opportunities in the last 10 years could render cultivation of the sites unnecessary. Aside from demands for agricultural land, there is a more general demand for flat areas suitable for creating a healthy environment and expanding the productive area of the city (recreational green space).

But if this coal ash disposal sites are to be used for agricultural purposes, for risk reduction (RECOAL, Handbook, 2008) following measures should be considered:

- establishment of safe soil cover (expensive)
  - reasonable depth of good quality available surrounding topsoil
- potential use of ash amendment (unpolluted organic materials)
  - different combinations of amendments as compost, produced from locally available municipal and industrial organic residues as an amendment to ash to improve substrate fertility (pH ↓, N and P ↑)
- possibility of using cultivar alternatives
  - each crop and cultivar tends to respond to pollution differently, tolerating and taking up different amounts and kinds of pollutants present in the air, growing substrate and water. Results confirm the well-known observation that grassy species (Monocots, graminaceous plants, e.g. barley) are known to tolerate higher metal levels in soil and take up less amounts of disadvantageous trace elements than herbal plants (Dicots e.g. soybean). Our investigation suggested that in term of trace element uptake is safer to

grow barley than soybean but at the same time economic analysis showed that agricultural crops such as winter wheat, barley and oat were not profitable but could form a part of subsistence farming

- safe cultivation and crop rotation system

  - two key points will contribute to a safe cultivation: (1) Prevent tillage on the disposal sites and (2) Ensure that the surface is covered during the warm period of the year, thus preventing dust dispersion. Only crops that do not require tillage or crops that provide thick coverage and do not require tilling or deep ploughing are recommended. For example, two rotation alternatives that could be employed, from spring to winter, are: (1) Alfalfa – Alfalfa – Alfalfa – Winter wheat and (2) Red Clover – Red Clover – Winter Barley – Oat (which requires the Oat to be planted with the Red Clover in spring). It is not recommended to cultivate more labour-intensive crops such as corn or potato, due to the working/disturbing of the soil, the amount of exposed soil, and the crops/cultivars’ uptake of pollutants. Focusing on grassland may also help

- compulsory monitoring system and long-term tests

  - interdisciplinary monitoring system, the influence on soil, plant, water, forest, animal, human

REFERENCE

Adriano D.C., Page A.L., Elseewwi A.A., Chang A.C. and Straughan I. (1980). Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems: a review. Journal of Environmental Quality 9: 333-334

Adriano D.C. (2001). Trace Elements in Terrestrial Environments: biogeochemistry, bioavailability, and risks of metals. 2nd Edition, Springer Science

Agarwala S.C., Bisht S.S. and Sharma C.P. (1977). Canadian Journal of Botany 55: 1299-1307

Alloway B.J. (1995). Heavy Metals in Soils. 2nd Edition, Blackie Academic & Professional

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

Bašić F., Butorac A., Vidaček Ž., Racz Z., Ostojić Z. i Bertić B. (1993). Program zaštite tala Hrvatske: Inventarizacija stanja - Trajno motrenje - Informacijski sustav. Fond stručne dokumentacije Zavoda za OPB Agronomskog fakulteta Sveučilišta u Zagrebu

Bašić F. (1994). Klasifikacija oštećenja tala Hrvatske. Agronomski glasnik 3-4: 291-310

Bašić F. (2009). Oštećenje i zaštita tla, drugo izdanje, pisana predavanja, Agronomski fakultet Sveučilišta u Zagrebu

Bohn H.L., McNeal B.L. and O'Connor G.A. (2001). Soil Chemistry. 3rd Edition, John Wiley&Sons, inc.

Borowska K. (1998). Distribution of total and the DTPA-extractable selenium in soil profiles from Kujawy Upland (Poland). In: proceedings of the 16th World Congress of Soil Science, 20th – 25th August 1998, Montpellier, France. Available [online] on: http://natres.psu.ac.th/Link/SoilCongress/bdd/symp6/1338-r.pdf [18.05.2009.]

Brown S.L., Chaney R.L., Angle J.S. and Baker A.J.M. (1995). Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris* grown on sludge-amended soils. Environmental Science & Technology 29: 1581–1585

Chesworth W. (2008). Encyclopedia of Soil Science. Springer Science

Coga L., Herak Custic M., Cosic T., Poljak M. and Petek M. (2007) Distribution of cadmium and zinc in different fractions of gley-amphigley soil. Cereal Research Communications. 35: 2: 317-320

Cummings D. (1999). What is soil degradation? Landcare Notes, State of Victoria, Department of Sustainability and Environment, Melburne

Custovic H. (2005). An overview of general land and soil water conditions in Bosnia and Herzegovina. In: Soil Resources of Europe. Jones R.J.A., Houšková B., Bullock P. and Montanarella L (ed). 2nd Edition, Office for Official Publications of the European Communities, Luxembourg: 73–82

Dellantonio A., Fitz W.J., Custovic H., Grünewald H., Repmann F., Schneider B.U., Edwards D., Zgorelec Z., Markovic M. and Wenzel W.W. (2007). Trace Element Uptake in Agricultural and Wild Plants Grown on Alkaline Coal Ash Disposal Sites in Tuzla (Bosnia and Herzegovina). In: proceedings of the 9th International Conference on the Biogeochemistry of Trace Elements (9th ICOBTE), 15th – 19th July 2007, Beijing, China: 356-357

Dellantonio A., Fitz W.J., Custovic H., Repmann F., Schneider B.U., Grunewald H., Gruber V., Zgorelec Z., Zerem N., Carter C., Markovic M., Puschenreiter M. and Wenzel W.W. (2008). Environmental risks of farmed and barren alkaline coal ash landfills in Tuzla, Bosnia and Herzegovina. Environmental Pollution 153 (3): 677-686

Deloch W.H. (1960). Analytical determination of sulphur in biochemical materials and the uptake of sulphur by crops in relation to fertilizer application. Diss. Landw. Fakultät Giessen

De Vos W. and Tarvainen T. (Chief-ed) (2006). Geochemical Atlas of Europe: Part 2: Interpretation of geochemical maps, additional tables, figures, maps and related publications. Geological survey of Finland, Espoo

Dobrin R.I., Dulama C.N. and Toma A.L. (2004). Soil – Plant Experimental Radionuclide Transfer Factors. Romanian Journal of Physics 51 (1–2): 73–76

Dos Santos Utmazian M.N. and Wenzel W.W. (2007). Cadmium and zinc accumulation in willow and poplar species grown on polluted soils. Journal of Plant Nutrition and Soil Science 170 (2): 265-272

Eisler R. (1997). Zinc hazards to plants and animals with emphasis on fishery and wildlife resources. In: Cheremisinoff P.N. (ed) Ecological issues and environmental impact assessment. Advance in environmental control technology series. Gulf Publ Comp, Houston, TX, 443-537

Elektroprivreda BiH, d.d. Sarajevo (2009). Available [online] on: http://www.elektroprivreda.ba/np/ep/epp [10.03.2009.]

Eriksson J.E. (2001). Concentrations of 61 trace elements in sewadge sludge, farmyard manure, mineral fertilizers, precipitation and in oil and crops. Swedish EPA Rep 5159, Stockholm

Eskew D.L., Welch R.M. and Cary E.E. (1983). Nickel: an essential micronutrient for legumes and possible all higher plants. Science 222: 621-623

Essington M.E. (2004). Soil and Water Chemistry: An Integrative Approach. CRC Press, Florida, 7-8

EU Directive (COM 232), proposal. (2006). Framework for Soil Protection, Brussels

EUROSTAT, Statistical Office of the European Communities. (1999). Agriculture, environment, rural development: facts and figures – a challenge for agriculture

FAO, Food and agriculture organization of the United Nations. (2000). ESCAP, United Nations Economic and Social Commission for Asia and the Pacific, Chapter Biodiversity & Land degradation: Soil Degradation

FAO, Food and agriculture organization of the United Nations. (2006). World reference base (WRB) for soil resources, A framework for international classification, correlation and communication, Rome

Fismes J., Echevarria G., Leclerc-Cessac E. and Morel J.L. (2005). Uptake and transport of radioactive nickel and cadmium into three vegetables after wet aerial contamination. Journal of Environmental Quality 34: 1497-1507

Fitz W.J., Wenzel W.W. (2002). Arsenic transformations in the soil-rhizosphere-plant system: fundamentals and potential application to phytoremediation. Journal of Biotechnology 99 (3): 259-278

Ghodrati M., Sims J.T. and Vasilas B.L. (1995). Evaluation of fly ash as a soil amendment for the Atlantic coastal plain: Soil hydraulic properties and elemental leaching. Water, Air & Soil Pollution 81 (3/4): 349-361

Grbeša D. (2004). Metode procjene i tablice kemijskog sastava i hranjive vrijednosti krepkih krmiva. Hrvatsko agronomsko društvo, Zagreb

Holdgate M.W. (1979). A Perspective of environmental pollution. University Press, Cambridge

http://cs-caesium.info/ . Available [online, 27.04.2009.]

http://en.wikipedia.org/wiki/Coal. Available [online, 25.05.2009.]

Huang P.M. and Gobran G.R. (2005). Biogeochemistry of Trace Elements in the Rhizosphere. Elsevier Science

Hurd-Karrer A.M. (1937). American Journal of Botany 24:720-728

IUPAC, International Union of Pure and Applied Chemistry. (2009). Available [online] on: http://www.iupac.org/ [13.07.2009.]

Jala S. and Goyal D. (2006). Fly ash as a soil ameliorant for improving crop production – a review. Bioresource Technology 97: 1136-1147

Kabata-Pendias A. and Mukherjee A.B. (2007). Trace Elements from Soil to Human. Springer Science

Kabata-Pendias A. (2000).Trace Elements in Soils and Plants. 3rd Ed. CRC Press

Keefer R.F. (1993). Coal ashes - Industrial wastes or beneficial by-products? In: Trace Elements In Coal and Coal Combustion Residues. Lewis Publishers. 3-9

Keefer R.F. and Sajwan K.S. (2000). Trace Elements in Coal and Coal Combustion Residues. Lewis Publishers.

Khan A.G. (2001). Relationships between chromium biomagnification ratio, accumulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil. Journal of Environment International 26: 417-423

Khan K.S., Heinze S. and Joergensen R.G. (2009). Simultaneous measurement of S, macronutrients, and heavy metals in the soil microbial biomass with $CHCl_3$ fumigation and NH_4NO_3 extraction. Soil Biology & Biochemistry 41: 309-314

Kisic I., Basic F., Mesic M., Veronek B., Vadic Z. and Mesic S. (2005). Changes in soil and crop yield caused by oil incidents. Cereal Research Communications 33 (1): 243-246

Kukier U. and Summer M.E. (1996). Boron availability to plants from coal combustion by-products. Water, Air and Soil Pollution 87 (1/4): 93-110

Latkoczy C., Prohaska T., Stingeder G. and Wenzel W.W. (2000). Simultaneous multi-element analysis of trace elements in soil samples by means of high-resolution inductively coupled plasma sector field mass spectrometry (SF-ICP-MS). Analytical and Bioanalytical Chemistry 368 (2-3): 256-262

Liu X., Gao Y., Sardar K., Duan G., Chen A., Ling L., Zhao L., Liu Z. and Wu X. (2008). Accumulation of Pb, Cu and Zn in native plants growing on contaminated sites and their potential accumulation capacity in Heqing, Yunnan. Journal of Environmental Science 20: 1469-1474

Llorens J.F., Fernandez-Turiel J.L. and Querol X. (2001). The fate of trace elements in a large coal-fire power plant. Environmental Geology 40 (4-5): 409-416

Lončarić Z., Karalić K., Popović B., Rastija D. and Vukobratović M. (2008). Total and plant available micronutrients in acidic and calcareous soils in Croatia. Cereal Research Communications 36 (1-S5): 331-334

Malayeri B.E., Chehregani A., Yousefi N. and Lorestani B. (2008). Identification of the hyper accumulator plants in copper and iron mine in Iran. Pakistan Journal of Biological Science 11 (3): 490-492

Markert B. (1992). Multy-element analysis in plant materials – analytical tools and biological questions. In: Adriano D.C. (ed) Biogeochemistry of trace elements Lewis Publ, Boca Raton, FL, 401-428

Martens R. (1995). Methoden zur quantitativen Bestimmung und Charakterisierung der mikrobiellen Biomasse des Bodens. Bundesforschungsanstalt für Landwirtschaft, Landbauforschung Völkenrode

Mengel K. and Kirkby E.A. (1987). Principles of plant nutrition. 4th Edition. International Potash Institute, Worblaufen-Bern, Switzerland

Menon M.P., Sajwan K.S., Ghuman G.S., James J. and Chandra K. (1993). Elements in coal and coal ash residues and their potential for agricultural crops. In: Trace Elements In Coal and Coal Combustion Residues. Lewis Publishers. 259-285

Mesić H., Bakšić D., Bašić F., Čidić A., Durn G., Husnjak S., Kisić I., Klaić D., Komesarović B., Mesić M., Miko S., Mileusnić M., Nakić Z., Novak T., Pernar N., Pilaš I., Romić D., Vrbek B. i Zgorelec Ž. (2006). Priručnik za trajno motrenje tala hrvatske. Prvo izdanje-radna verzija, AZO, Zagreb. Available [online] on: http://www.azo.hr/Default.aspx?art=1123&sec=497 [12.05.2009.]

Miltimore J.E. and Mason J.L. (1971). Copper to molybdenum ratio and molybdenum and copper concentrations in ruminant feeds. Canadian Journal of Animal Science 51:193-200

NN, Narodne novine. (2005). Pravilnik o toksinima, metalima, metaloidima, te drugim štetnim tvarima koji se mogu nalaziti u hrani. Zagreb, HR NN 16/05, Available [online] on: http://narodne-novine.nn.hr/clanci/sluzbeni/2005_02_16_283.html [11.11.2009.]

NN, Narodne novine. (1992). Pravilnik o zaštiti poljoprivrednog zemljišta od onečišćenja štetnim tvarima. Zagreb, HR NN 15/92, Available [online] on: http://narodne-novine.nn.hr/clanci/sluzbeni/255828.html [27.04.2009.]

Ondrasek G., Romic D., Rengel Z., Romic M. and Zovko M. (2009). Cadmium accumulation by muskmelon under salt stress in contaminated organic soil. Science of the total environment 407: 7: 2175-2182

Prüess A. (1994). Einstufung mobiler Spurenelemente in Böden. In: Rosenkranz, D., G. Einsele & H.-M. Harress (Hrsg.): Bodenschutz. 3600, 1-59, Berlin

Puschenreiter M., Schnepf A., Millán I.M., Fitz W.J., Horak O., Klepp J., Schrefl T., Lombi E. and Wenzel W.W. (2005). Changes of Ni biogeochemistry in the rhizosphere of the hyperaccumulator *Thlaspi goesingense*. Plant and Soil 271 (1-2): 205-218

RECOAL, Reintegration of coal ash disposal sites and mittigation of pollution in the West Balkan area (2005). Deliverables 7 (D7): Physical and chemical analyzes of water and ashes and registered hydrodynamic relations in deposit site: Annex II-Soil-Result of physical-chemical analysis of ash/soil. Available [online] on: http://www.rhizo.at/download.asp?id=827 [12.05.2009.]

RECOAL, Reintegration of coal ash disposal sites and mittigation of pollution in the West Balkan area (2005). Deliverables 8 (D8): Risk Assessment: Evaluation of potential risks of pollutant transfer and toxicity to ecosystem and human food chain. Available [online] on: http://www.rhizo.at/download.asp?id=828 [21.07.2009.]

RECOAL, Reintegration of coal ash disposal sites and mittigation of pollution in the West Balkan area (2008). Handbook on management of coal ash sites. Available [online] on: http://www.rhizo.at/download.asp?id=1313 [10.03.2009.]

Reimann C., Siewers U., Tarvainen T., Bityukova L., Eriksson J., Gilucis A., Gregorauskiene V., Lukashev V.K., Matinian N.N. and Pasieczna A. (2003). Agricultural soils in Northen Europe: A Geochemical Atlas, Hannover

Romic M. and Romic D. (2003). Heavy metals distribution in agricultural topsoils in urban area. Environmental Geology 43: 795-805

Salminen R. (Chief-ed) (2005). Geochemical atlas of Europe, Part 1: Background Informations, methodology and maps, geological survey of Finland, Espoo

Sanborn J.R. and Brodberg R.K. (2006). Evaluation of bioaccumulation factors and translators for methylmercury. Available [online] on: http://oehha.ca.gov/fish/special_reports/pdf/BAF020907.pdf [13.07.2009.]

Sandelin K. and Backman R. (2001). Trace elements in two pulverized coal-fired power stations. Environmental Science & Technology 35 (5): 826-834

Sandhu S.S., Mills G.L. and Sajwan K.S. (1993). Leachability of Ni, Cd, Cr and As from coal ash impoundments of different ages on the Savannah River Site. In: Trace Elements In Coal and Coal Combustion Residues. Lewis Publishers, 165-182

SARE, Sustainable Agriculture Research and Education (2009). Building Soils for Better Crops, 2nd Edition. Available [online] on: http://www.sare.org/publications/bsbc/chap8.htm#top [22.05.2009.]

Scheffer F. and Schachtschabel P. (1992). Lehrbuck der Bodenkunde, Enke-Verlag, Stuttgart

Schwab A.P. (1993). Extractable and plant concentrations of metals in amended coal ash. In: Trace Elements In Coal and Coal Combustion Residues. Lewis Publishers, 185-209

Scherwood Lollar B. (2005). Environmental Geochemistry, Treatise on Geochemistry, Vol 9. Elsevier

Simon-Sylvestre G. (1969). First results of a survey on the total sulphur content of arable soils in France. Annales agron. 20, 609-625

Sinha B.K. and Singh N.T. (1976). Root uptake coefficient for C1 ions in corn as affected by transpiration rates and solution concentration. Plant and Soil 44: 521-525

Soltanpour P.N. and Schwab A.P. (1977). A new soil test for the extraction of micro and macronutrients in alkaline soils. Communications in Soil Science and Plant Analysis 8:195

Stout W.L., Sharpley A.N. and Landa J. (2000). Effectiveness of coal combustion by-products in controlling phophorus export from soils. Journal of Environmental Quality 29 (4): 1239-1244

Škorić A. (1966). Priručnik za ispitivanje zemljišta, Knjiga I: Hemijske metode ispitivanja zemljišta, JDPZ, Beograd

Škorić A. (1982). Priručnik za pedološka istraživanja, Liber, Zagreb

Unterbrunner R., Wieshammer G., Hollender U., Felderer B., Wieshammer-Zivkovic M., Puschenreiter M. and Wenzel W.W. (2007). Plant and fertiliser effects on rhizodegradation of crude oil in two soils with different nutrient status. Plant and Soil 300 (1-2): 117-126

Vasilj Đ. (2000). Biometrika i eksperimentiranje u bilinogojstvu. Hrvatsko agronomsko društvo, Zagreb, Hrvatska

Vego G., Kucar-Dragicevic S., Koprivanac N. (2008). Application of multi-criteria decision-making on strategic municipal solid waste management in Dalmatia, Croatia. Waste Management 28 (11): 2192-2201

Vego I. (2006). Comparasion of some soil extractants for determination of the plant available boron content. In: proceedings of the 18th World Congress of Soil Science, 9th – 15th July 2006, Philadelphia, Pennsylvania, USA

Vukadinovic V., Bertic B., Kovacevic V., Vukadinovic V. and Loncaric Z. (2006). Potassium fixation in heavy soils of East Croatia. Cereal Research Communications 34 (1): 351-354

Walsh T. and Fleming G.A. (1952). Selenium levels in rocks, soils and herbage from a high selenium locality in Ireland. Int Soc Soil Sci Trans (Comm. II and IV) 2:178-183

Weinstein L.H., Arthur M.A., Schneider R.E., Woodbury P.B., Laurence J.A., Beers A.O and Rubin G. (1993). Uptake of chemical elements by terrestrial plants growing on a coal ash landfill. In: Trace Elements In Coal and Coal Combustion Residues. Lewis Publishers, 213-236

Wenzel W.W., Adriano D.C., Salt D. and Smith R. (1999). Phytoremediation: a plant–microbe-based remediation system. Agronomy Monograph 37, Madison, USA, 457–508

Wenzel W.W., Lombi E. and Adriano D.C. (2004) Root and rhizosphere processes in metal hyperaccumulation and phytoremediation technology. Heavy metals in plants: from biomolecules to ecosystems. Springer, Berlin; ISBN 3-540-40131-8, 313–344

Wenzel W.W. (2009). Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils: review article. Plant and Soil 321 (1-2): 385-408

Wiersma D., van Goor B.J. and van der Veen N.G. (1986). Cadmium, lead, mercury and arsenic concentration in crops and corresponding soils in the Netherlands. Journal of Agricultural and Food Chemistry 34: 1067-1074

Woodbury P.B., Arthur M.A., Rubin G., Weinstein L.H. and McCune D.C. (1999). Gypsum application reduces selenium uptake by vegetation on a coal ash landfill. Water, Air and Soil Pollution 110 (3/4): 421-432

Zgorelec Z., Basic F., Kisic I., Wenzel W.W. and Custovic H. (2008). Arsenic and nickel enrichment coefficients for crops growing on coal ash. Cereal Research Communications 36 (Suppl. 5 Part 2): 1219-1222

Zhao F.J., Lombi E. and McGrath S.P. (2003). Assessing the potential for zinc and cadmium hytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant and Soil 249: 37–43

Zu Y.Q., Li Y., Chen J.J., Chen H.Y., Qin L. and Christian S. (2005). Hyperaccumulation of Pb, Zn and Cd in herbaceous grown on lead-zinc mining area in Yunnan, China. *Environ Int*. 31: 755–762

Zurayk R., Sukkariyah B., Baalbaki R. and Ghanem D.A. (2002). Ni phytoaccumulation in *Mentha aquatica* L. and *Mentha sylvestris* L. Water, Air, and Soil Pollution 139 (1-4): 355-364

APPENDIX

Table 1 Correlation coefficients for substrate pH and EC values measured in three different media

Correlation coefficient	**pH, 1 M KCl, 1:2.5**	**pH, 0.01 M $CaCl_2$, 1:2.5**	**pH, H_2O, 1:2.5**	**EC, H_2O, 1:5, µS/cm**	**EC, H_2O, 1:10, µS/cm**
pH, 1 M KCl, 1:2.5	1				
pH, 0.01 M $CaCl_2$, 1:2.5	0.9949	1			
pH, H_2O, 1:2.5	0.9950	0.9954	1		
EC, H_2O, 1:5, µS/cm				1	
EC, H_2O, 1:10, µS/cm				0.9970	1

Table 2 Substrate Pollution levels of investigated elements according to soil damage classification (Bašić, 1994)

Substrate pollution level (PL) in %						
treatment/element	1	2	3	4	5	6
As, 75	**138**	558	243	348	338	453
B, 10	0	**152**	38	76	66	**114**
Cd, 111	29	52	35	40	37	46
Co, 59	32	68	41	50	46	59
Cr, 53	**100**	468	**192**	284	255	376
Cs, 133	9	45	18	27	22	36
Cu, 63	40	**143**	66	92	82	**117**
Fe, 57	65	96	73	80	73	88
Mo, 95	4	17	7	11	11	14
Ni, 62	**193**	1185	441	689	617	937
P, 31	5	1	4	3	3	2
Pb, 208	29	29	29	29	27	29
Rb, 85	16	35	20	25	21	30
Se, 82	3	11	5	7	5	9
Sr, 88	42	87	53	64	57	76
U, 238	15	46	23	31	27	38
Zn, 68	30	51	36	41	37	46

1st class	0-25 %	Clean soil
2nd class	25-50 %	Low polluted soil
3rd class	50-100 %	High polluted soil
4th class	**100-200 %**	Polluted soil
5th class	> 200 %	Waste soil

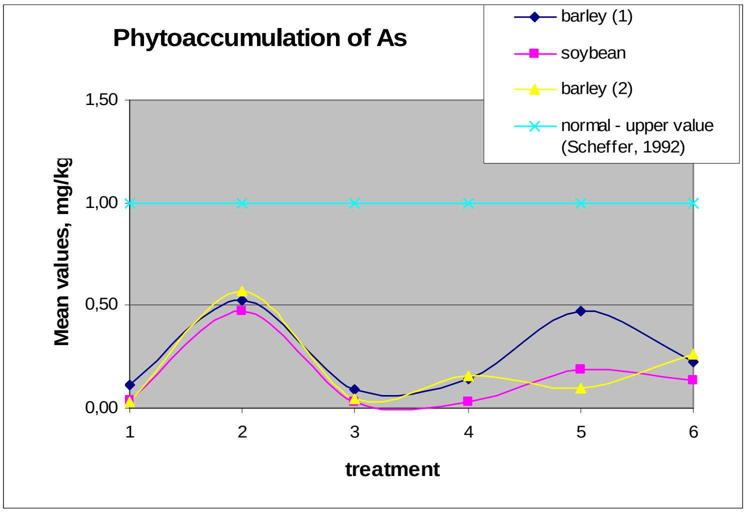

Figure 1 Phytoaccumulation of As in barley and soybean grain

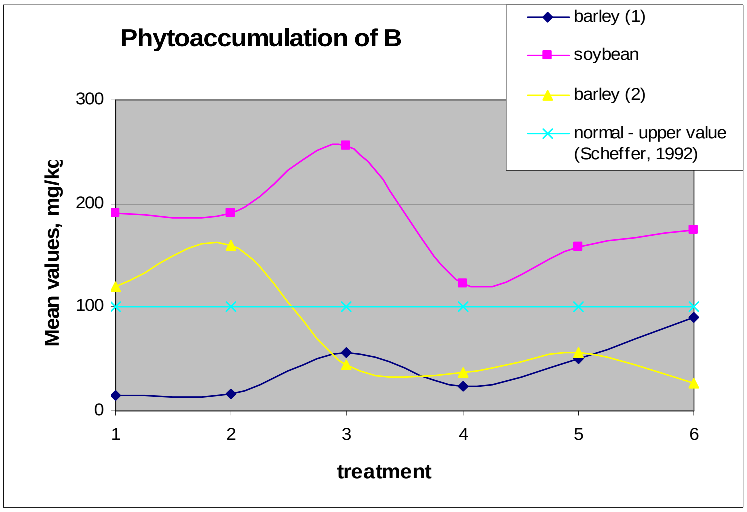

Figure 2 Phytoaccumulation of B in barley and soybean grain

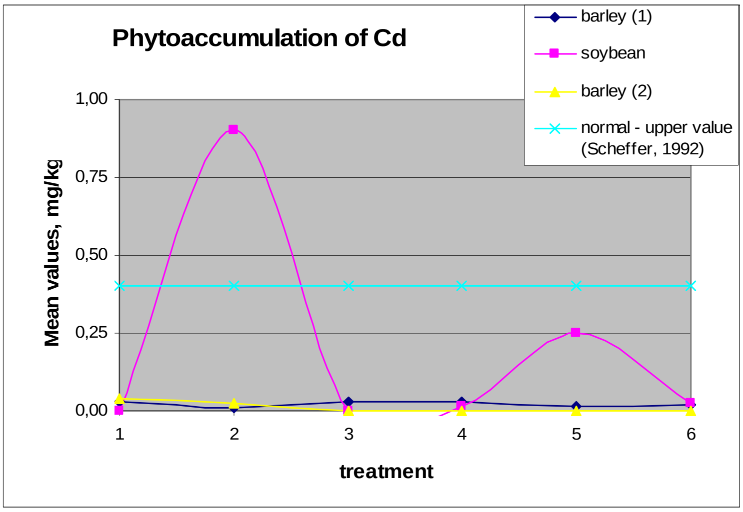

Figure 3 Phytoaccumulation of Cd in barley and soybean grain

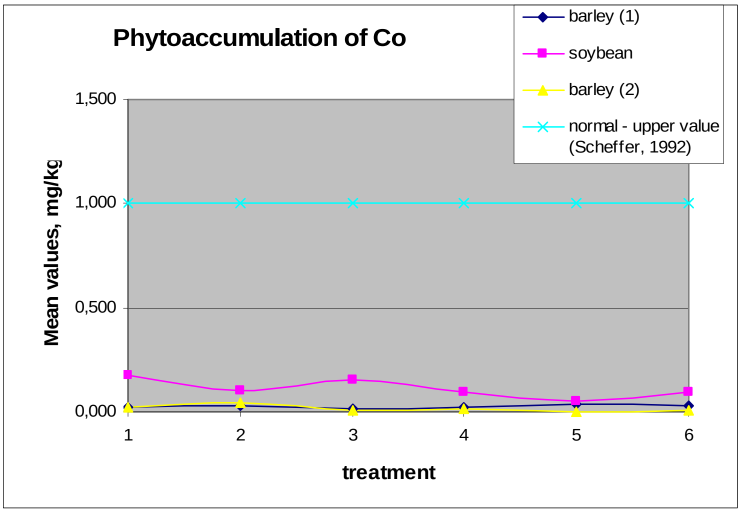

Figure 4 Phytoaccumulation of Co in barley and soybean grain

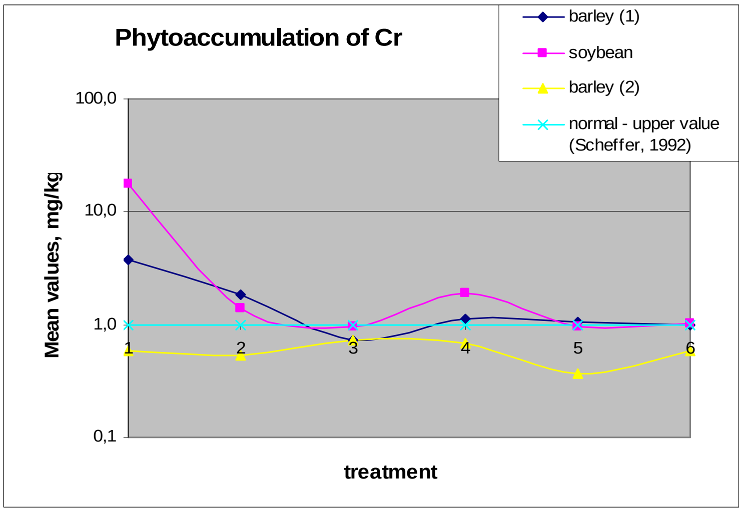

Figure 5 Phytoaccumulation of Cr in barley and soybean grain

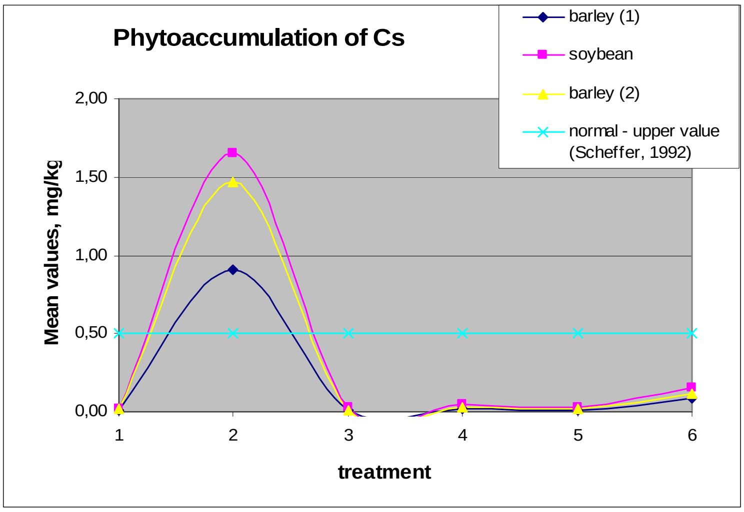

Figure 6 Phytoaccumulation of Cs in barley and soybean grain

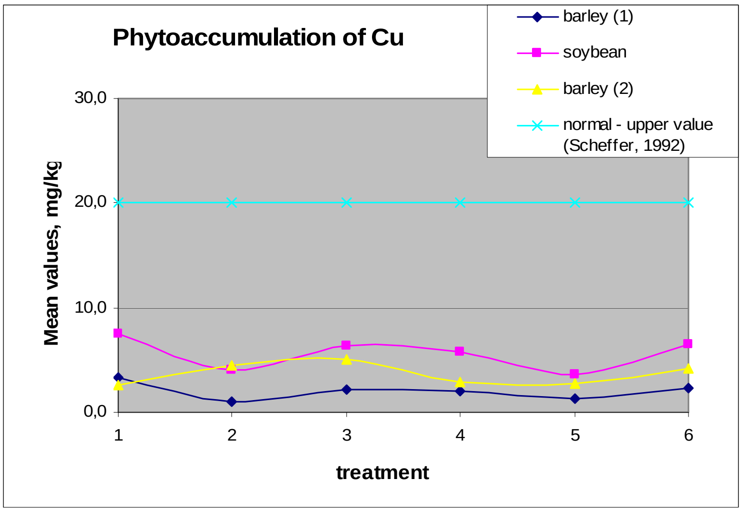

Figure 7 Phytoaccumulation of Cu in barley and soybean grain

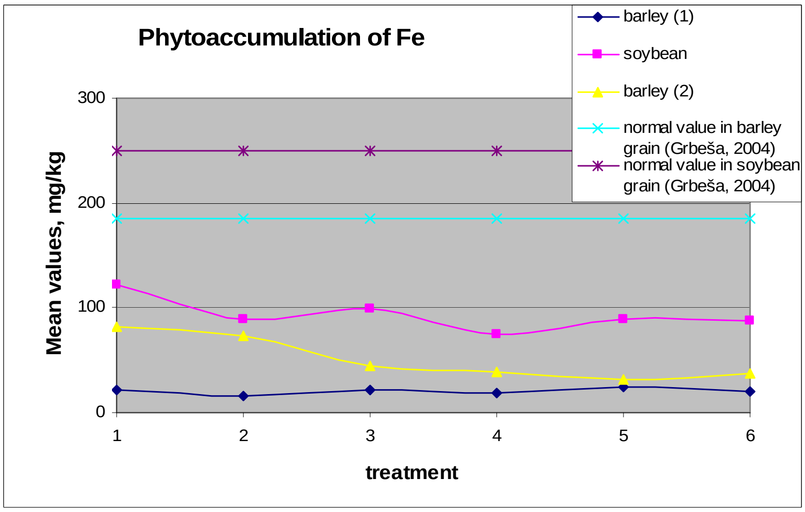

Figure 8 Phytoaccumulation of Fe in barley and soybean grain

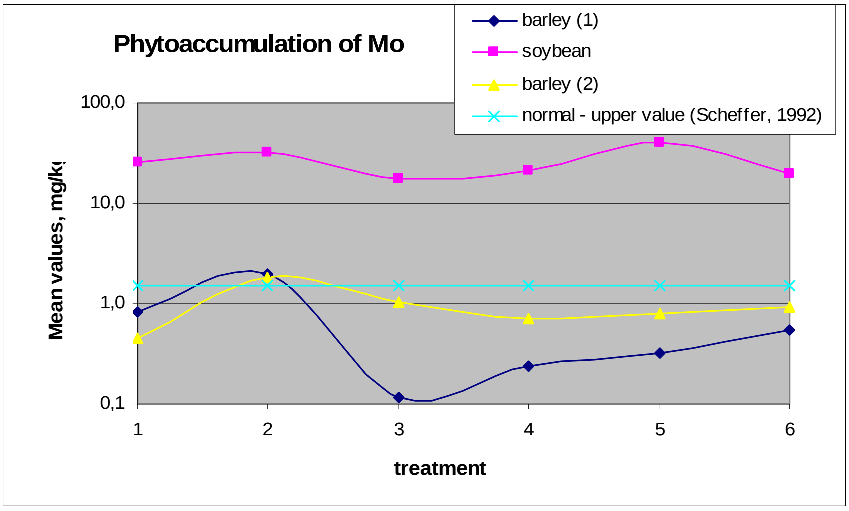

Figure 9 Phytoaccumulation of Mo in barley and soybean grain

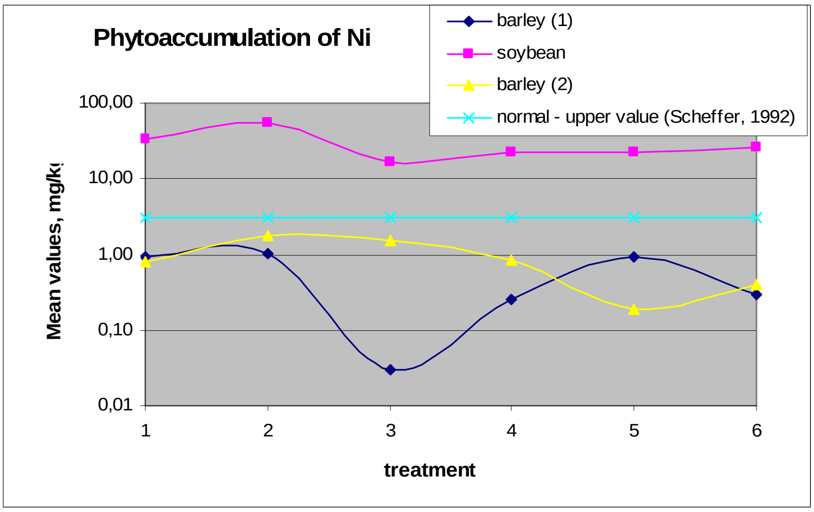

Figure 10 Phytoaccumulation of Ni in barley and soybean grain

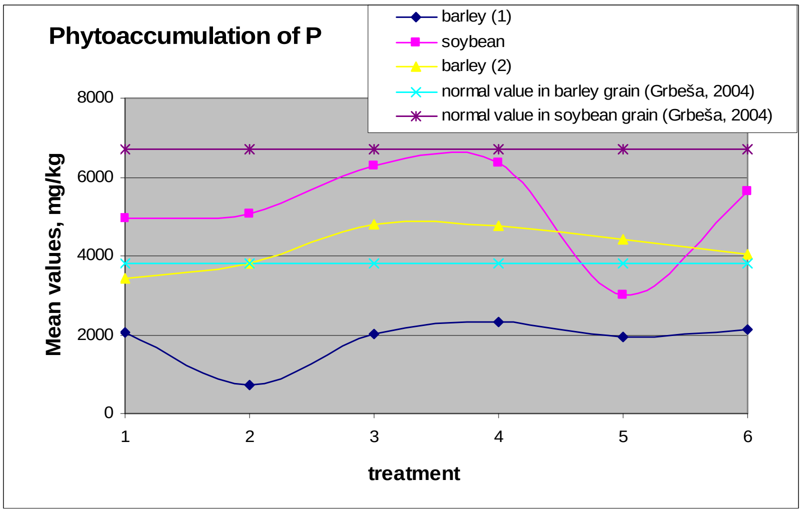

Figure 11 Phytoaccumulation of P in barley and soybean grain

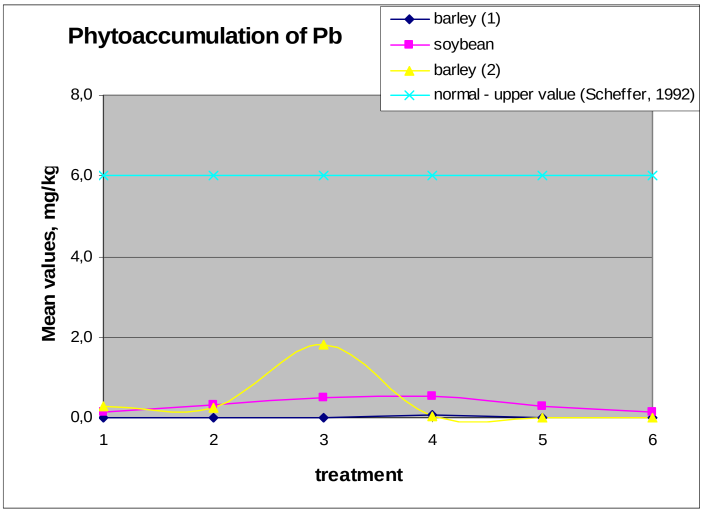

Figure 12 Phytoaccumulation of Pb in barley and soybean grain

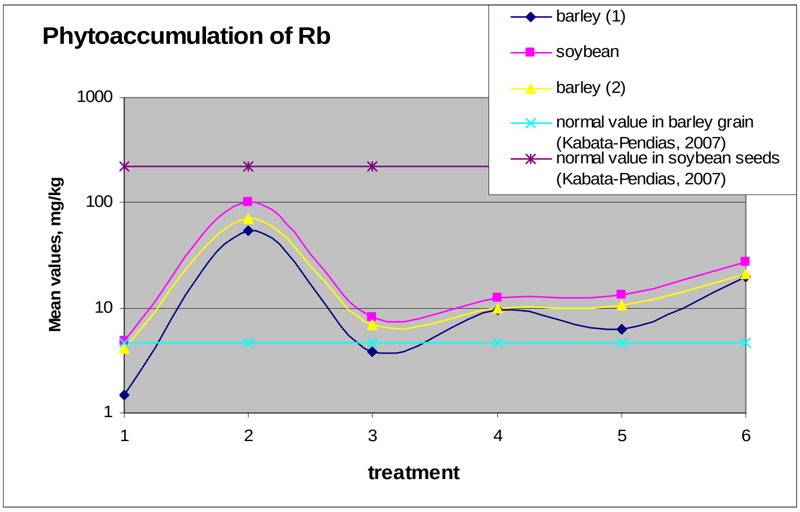

Figure 13 Phytoaccumulation of Rb in barley and soybean grain

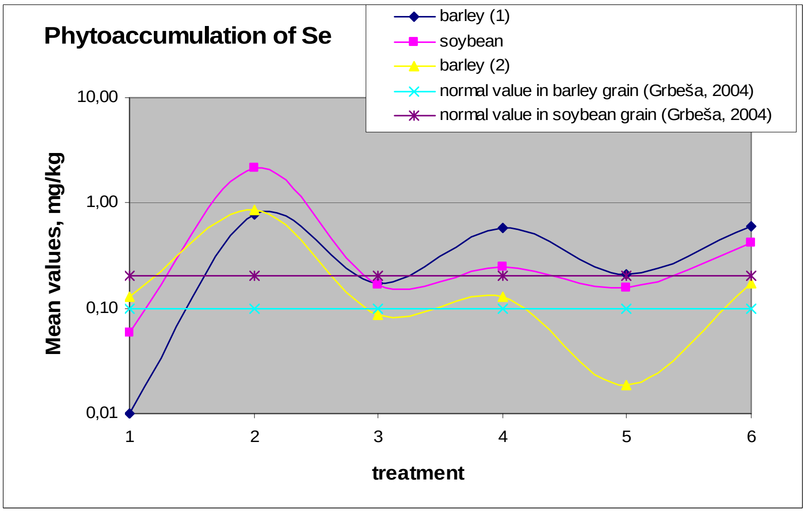

Figure 14 Phytoaccumulation of Se in barley and soybean grain

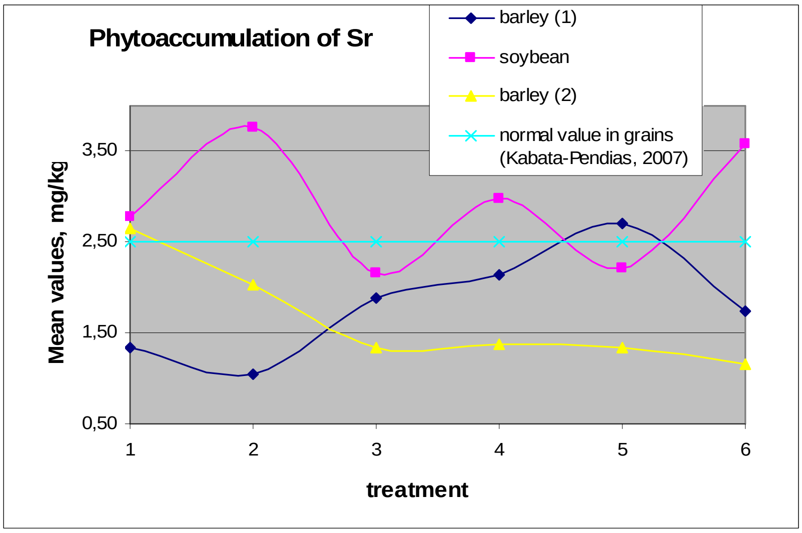

Figure 15 Phytoaccumulation of Sr in barley and soybean grain

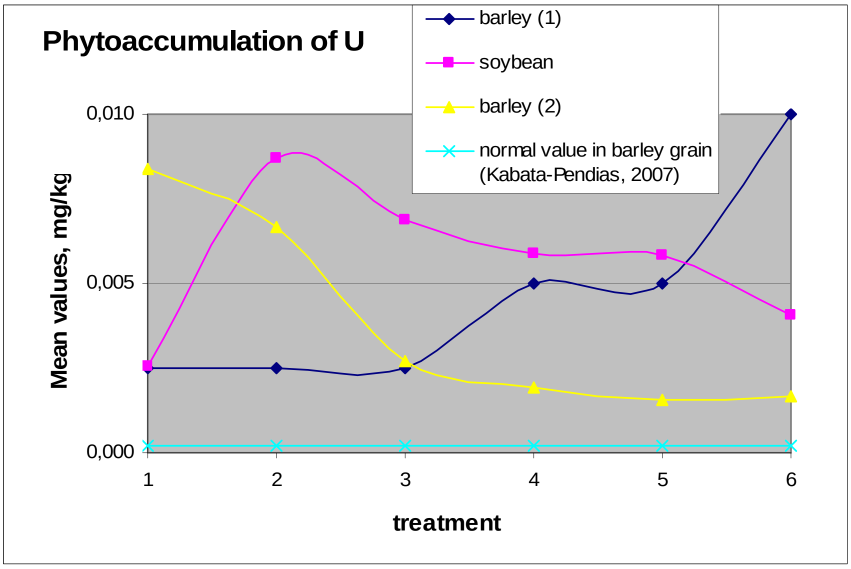

Figure 16 Phytoaccumulation of U in barley and soybean grain

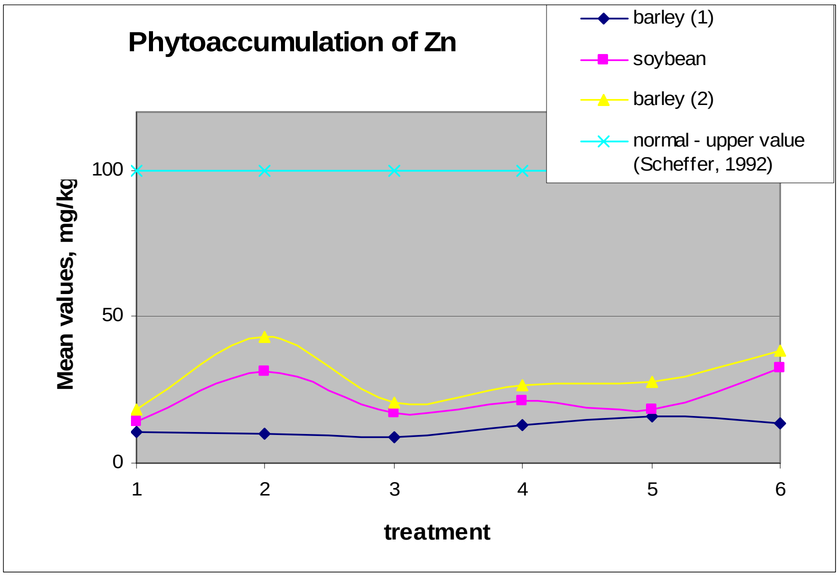

Figure 17 Phytoaccumulation of Zn in barley and soybean grain

INDEX

ANOVA	- **AN**alysis **O**f **VA**riance
BAC	- **B**iological **A**bsorption **C**oefficient
BiH	- cro. **B**osna **I** **H**ercegovina or eng. Bosnia and Herzegovina
BOKU	- germ. Universität für **BO**den**KU**ltur Wien or eng. University of Natural Resources and Applied Life Sciences, Vienna
BP	- **B**ase **P**arcel
BTUC	- **B**randenburg **U**niversity of **T**echnology **C**ottbus, Germany
CC	- **C**orrelation **C**oefficient
CEC	- **C**ation **E**xchange **C**apacity
C_{org}	- **ORG**anic **C**arbon Content
CRM	- **C**ertified **R**eference **M**aterial
DM	- **D**ry **M**atter
EC	- **E**nrichment **C**oefficient
FAZ	- cro. Agronomski fakultet Sveučilišta u Zagrebu or eng. University of **Z**agreb, **F**aculty for **A**griculture
GLP	- **G**ood **L**aboratory **P**ractice
HEIS	- **H**ydro **E**ngineering **I**nstitute **S**arajevo, BiH
HM	- **H**eavy **M**etals
IC	- **I**on **C**hromatography
ICP-MS	- **I**nductively **C**oupled **P**lasma **M**ass **S**pectrometry
IFA	- Proficiency Testing Scheme for Water Analysis organized by University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria, Department for Agrobiotechnology, **IFA**-Tulln, Canter for Analytical Chemistry
IPE	- **I**nternational **P**lant-analytical **E**xchange Programme organized by WEPAL
ISE	- **I**nternational **S**oil-analytical **E**xchange Programme organized by WEPAL
JRC, IRMM	- **J**oint **R**esearch **C**entre, **I**nstitute for **R**eference **M**aterials and **M**easurements
LOD	- **L**imit **O**f **D**etection
LS	- **L**oamy **S**and soil texture
LSD	- **L**east **S**ignificant **D**ifference
MAC	- **M**aximal **A**cceptable **C**oncentrations

MOEL	- Soil ring test organized by National Office for Agriculture (germ. Thüringer Landesanstalt für Landwirtschaft) from Jena, Germany
n.d.	- **N**ot **D**etected (concentration below LOD)
NPK	- **N**itrogen - **P**hosphorus – potassium (**K**) – fertilizer
OPB	- cro. Zavod za **O**pću **P**roizvodnju **B**ilja or eng. Department of General Agronomy
OPBLab	- cro. Analitički **LAB**oratorij Zavoda za **O**pću **P**roizvodnju **B**ilja or eng. Analytical Laboratory of Department of General Agronomy
PL	- **P**ollution **L**evel
PT	- **P**roficiency **T**esting
RECOAL	- **RE**integration of **COAL** Ash Disposal Sites and Mitigation of Pollution in the West Balkan Area
RM	- **R**eference **M**aterial
RSD	- **R**elative **S**tandard **D**eviation
SEE	- **S**outh **E**astern **E**urope
SL	- **S**andy **L**oam soil texture
SOP	- **S**tandard **O**perating **P**rocedures
TEP	- **T**hermo **E**lectric **P**ower **P**lant Tuzla
TC	- **T**otal **C**arbon content in % DM
TCD	- **T**hermal **C**onductivity **D**etector
TH	- **T**otal **H**ydrogen content in % DM
TN	- **T**otal **N**itrogen content in % DM
TS	- **T**otal **S**ulphur content in % DM
QM	- **Q**uality **M**anagement
QC	- **Q**uality **C**ontrol
WEPAL	- **W**ageningen **E**valuating **P**rograms for **A**nalytical **L**aboratories, organized by Soil Chemistry and Chemical Soil Quality group, Department of Soil Quality of Wageningen University (WU), Netherlands.

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