ISBN 978-82-575-1028-2
ISSN 1503-1667

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Trace metal availability in soils under different land uses of the Danube basin in Croatia

Tilgjengelighet av spormetaller under forskjellig arealbruk på elveslettene rundt Danube i Kroatia

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Extended summary

Acknowledgments

This research was conducted by the financial support from "Project 5: improving nutritional quality and safety of food and fodder crops in South Eastern Europe (SEE) countries", Institutional collaboration between Norwegian University of Life Sciences and Faculty of Agriculture in Osijek, Croatia. I wish to thank the project for the financial support and the IPM department for giving me the opportunity to study at UMB. I also express my gratitude to the department of forest management at "Croatian Forest Ltd." for their tremendous help with mapping of the area. I am also grateful to the Norwegian State Educational Loan Fund (Statens Lånekasse for utdanning) for providing financial assistance.

The special thanks go to my supervisors Prof. Bal Ram Singh, Dr. Åsgeir R. Almås, Prof. Trine Sogn and Prof. Zdenko Lončarić for their professional and scientific guidance throughout my entire studies. They inspired me for this work and I am sincerely grateful for that. I also express my gratitude to Irene, Grete, Karl Andreas, Jonny, Solfrid and Kurt, for their generous assistance during my lab work and to Tore, Line, Jan for all the valuable discussions that improved my work. I am thankful to Department of Agroecology at Faculty of Agriculture in Osijek and Prof. Rastija and my colleagues who have been of great help: Katica, Meri, Darko, Marija, Kruno and Brigita.

My appreciation also goes to my colleagues from UMB for their continued encouragement during my stay in Norway: Bharat, Girmay, Ambachew, Manju, Vegard, Olga, Susanne, Gudny, Shahid, Maria and Julia.

Finally, words alone can not express my gratitude to my family and my girlfriend without whose constant encouragement and support none of this would be possible.

Vladimir Ivezić Ås, October, 2011

Abstract

Soils are non-renewable resource of biological, chemical and physical properties. One of the ecological functions of the soil is its capacity to filter and buffer trace metals. Trace metals can pose a threat on the one hand, from toxicity and on the other hand, from deficiency (as some of them are essential micronutrients) point of view. Although availability of trace metals depends on soil properties, in the legislation of many countries, including Croatia, maximum permissible concentrations (MPC) of trace metals are determined based on total metal concentration. Thus, recently an initiative has been taken in considering the influence of soil properties in determination of MPC.

The objective of the present study is to evaluate the soils of Croatian main agricultural region, Osijek-Baranja County, as regards trace metal availability and soil properties controlling it. This main objective is achieved by several sub-objectives: i) Determine total and water extractable trace metal concentrations in Osijek-Baranja County (Eastern Croatia) and investigate the influence of different soil properties on solid-solution partitioning, ii) Develop regression models predicting trace metal solubility using soil properties and carry out metal speciation of soil solution using WHAM/Model VI, iii) Use the existing regression models predicting water extractable fraction of trace metals to estimate metal concentrations in wheat grain, iv) Use soil properties data to derive soil quality index (SQI) and examine the relationship between availability of trace metals and SQI.

Based on pedological map, soil samples from surface layer (0-25 cm) were collected throughout the area from 74 sites. Different land uses (forest and agriculture) were also taken into account. Samples were analysed for total (HNO_3 extractable), geochemical active (EDTA extractable) and soil solution (H_2O extractable) fraction of trace metals. Various soil properties were also determined: pH, SOC, DOC, InC, TOT N, AL-P, AL-K, CEC, BD, soil texture.

Total content of toxic and potentially toxic trace metals was below the governmental tolerance limits (MPC), and at the same time, total content of the micronutrients was above the deficiency levels. However, the results have shown that the fraction of trace metals in the soil solution, i.e. the fraction that is readily available is not dependent on the total content but rather on soil properties such as pH, DOC and CEC. Using these soil properties in regression models we were able to predict trace metal concentrations as well as free metal ion (FMI) concentrations and free ion activity (FIA) in the soil solution. Such regression models have

been widely used by many authors. However, existing models were developed mainly on polluted and slightly polluted soils. By comparing these existing well-known models with our data from unpolluted agricultural and forest soils, the results have showed good correlation. However, the models did not follow the 1:1 line, indicating that in this case, they will be overestimating the predictions. Our study points out that modelling trace metal solubility and FMI concentration is area specific. Models developed on polluted, or even what is considered slightly polluted, soils cannot be directly applied on soils with low levels of trace metals. The optimization of regression models is necessary.

In addition, we developed regression models predicting plant concentrations. Models developed by using soil properties improve prediction of plant uptake and as such are much better indicators of plant concentrations than single extraction by strong or week acid alone. Furthermore, water extraction, FMI and FIA, predicted by regression models gave us better correlation with plant concentrations than the EDTA or total extraction alone. Soil solution concentrations appeared to be good indicators of uptake. However, soil pH seems to have the key role in determining soil solution concentrations, FMI and FIA. Thus, we can conclude that pH plays an important role in the solubility of trace metals and prediction of plant concentrations. Investigated soils had low total concentrations of trace metals, which probably increased the importance of pH. Therefore, in uncontaminated soils with low concentrations of trace elements pH is the driving force influencing metal solubility and thus metal availability. By controlling the pH, we also control the solubility of trace metals in the investigated area. Thus, possible toxicity or deficiency problems can be avoided by pH control.

The results have also showed that soil properties as well as water extractable trace metal concentrations vary between land uses. Therefore, we can expect different uptake of trace metals on different land uses. Investigation of overall soil quality between forest and agricultural soils indicated no soil degradation. Nevertheless, for some soil properties we can see the degrading influence of agriculture, specifically for bulk density (BD) and organic matter (OM). In addition, from trace metal availability point of view, the trace metals will be more available in forest due to soil properties controlling its solubility (pH, DOC and CEC). The study has also showed no difference between total concentrations of trace metals between land uses. Therefore, we can say that agriculture practices did not cause excessive input of trace metals (since the total concentrations were the same in forest and agriculture), and in addition, trace metals were less available in agricultural fields than in forest (mainly as a result of the differences in soil properties between forest and agriculture).

Sammendrag

Jord er en ikke-fornybar ressurs karakterisert ved en kombinasjon av biologiske, kjemiske og fysiske egenskaper. En av jordsmonnets flere økologiske funksjoner er evnen til å bufre konsentrasjonen av spormetaller i jordvæske. Spormetaller kan forårsake giftvirkning når konsentrasjonene er særs høye, eller de av metallene som er essensielle kan forekomme i så lave konsentrasjoner at det oppstår mangel. Selv om tilgjengeligheten av metallene styres av jordas fysisk-kjemiske egenskaper, fastholder mange land, deriblant Kroatia, jordsmonnets totalkonsentrasjon av metallene for å være et mål på maksimalt tillatt konsentrasjon (MPC). På bakgrunn av dette tas det nå initiativ til å inkludere også sentrale jordkjemiske egenskaper når MPC skal bestemmes.

Målsetningen med dette studiet er å evaluere jordsmonn i et Kroatisk landbruksdistrikt, Osijek-Baranja fylke (øst Kroatia), med hensyn til hvordan jordas fysisk-kjemiske egenskaper styrer spormetallenes tilgjengelighet. Hovedmålet ble løst ved å i) bestemme total konsentrasjonene av metaller i jord og vann ekstrakter av jord samlet i Osijek-Baranja fylke, samt bestemme hvordan forskjellige jordkjemiske parametere styrer likevekten mellom fast og løst fase i jord. ii) Utvikle og optimalisere multivariate regresjonsmodeller som består av forskjellige jordkjemiske parametere for å anslå løseligheten av metaller i porevann. Metallenes tilstandsform i vannekstraktene ble bestemt ved bruk av WHAM/Model VI. iii) Deretter ble disse regresjonsmodellene brukt både direkte samt etter videre optimalisering for å estimere opptak av metaller i planter. iv) Jordsmonnets egenskaper ble til slutt brukt for å bestemme jordas kvalitetsindeks (SQI) som igjen ble korrelert med tilgjengelig metal i jord.

Med utgangspunkt i jordsmonnkart, ble 74 jordprøver samlet inn fra øvre jordlag i hele distriktet. Det ble samlet prøver fra både landbruks og skogsjord. Jordprøvene ble ekstrahert med konsentrert HNO_3, 0.01M EDTA og H_2O for å anslå henholdsvis totalkonsentrasjon, geokjemisk aktivt samt vannløselig metall. Det ble også bestemt pH, SOM, DOC, IOC, TOT-N, AL-P, AL-K, CEC, BD og tekstur.

Totalkonsentrasjonene av metaller var under MPC. Samtidig var konsentrasjonene av de essensielle metallene ikke så lave at det er fare for mangelsykdommer. Konsentrasjonen av vannløselig metal i jord styres i liten grad av totalkonsentrasjonen i jord, mens jordas pH og negativt ladde overflater karakterisert ved SOM, DOC og CEC har stor betydning. Ved å inkludere disse parameterne i multivariate regresjonsmodeller, kunne vi estimere total konsentrasjonen av metaller i vann ekstraktene samt aktiviteten av fritt metall (FIA). Det har tidligere blitt fremsatt slike modeller, men de har gjerne blitt optimalisert for forurenset jord.

Vi testet slike modeller ved å bruke våre egne målinger. Det var gode korrelasjoner mellom målt og estimert total metal og FIA i jordvann, men korrelasjonene fulgte ikke en 1:1 linje gjennom 0. De opprinnelige modellene overestimerte konsentrasjonen av total metal og FIA i jordvann, sannsynligvis fordi de er optimalisert for kraftig og moderat forurenset jord. Derfor er slike modeller sannsynligvis betinget av hvorvidt jorda er forurenset eller ikke. På grunn av dette var det nødvendig å optimalisere disse modellene på nytt basert på våre egne målinger.

Vi optimaliserte også multivariate regresjonsmodeller for å estimere metal konsentrasjonene i planter. Resultatene viste at metallkonsentrasjonene i planter var bedre korrelert med disse modellene enn med ekstraherbart metall alene. Det samme var tilfelle med FIA og FMI (konsentrasjonen av fritt metal). Metallkonsentrasjonene i planter var bedre korrelert med FIA og FMI enn med EDTA eller HNO_3 ekstrahert metall. Konsentrasjonen av metaller i vann ekstrakter var godt korrelert med innhold i planter, men siden pH virker å være nøkkelparameteren som kontrollerer konsentrasjonen av metaller i vann ekstrakter, FMI og FIA vil pH også være en nøkkelparameter for å bestemme innholdet i planter. Betydningen av pH henger sannsynligvis også sammen med at konsentrasjonen av metaller i jord er lav og at det var forholdsvis liten variasjon mellom prøvene. Disse resultatene viser at ved å kontrollere pH i jord kan mangel eller giftvirkning i planter på slik jord i stor grad styres.

Resultatene viste også at jordsmonnets fysisk-kjemiske egenskaper, inkludert konsentrasjonene av metaller i jordvann, varierer betinget av ulik bruk. Derfor forventer vi at metallopptaket fra jord også er betinget av bruk. Undersøkelsene viste at jordkvalitetsparametere i skogsjord og landbruksjord indikerte ingen forringelse av jord. Likevel viste undersøkelsene at jordbruk reduserer kvalitetsparameterne som jordtetthet (BD) og innhold organisk materiale (OM). Spormetallene var mer tilgjengelige i skogsjord særlig på grunn av lavere pH. Resultatene viser ikke at bruk av land har påvirket totalinnholdet av spormetaller i jord og derfor er det heller ingen grunn til å påstå at landbruksaktivitet har forhøyet totalkonsentrasjoner av metaller i slik jord. På grunn av at særlig pH er høyere i dyrket jord er tilgjengeligheten av metaller i slik landbruks jord lav.

List of papers:

Paper I: Ivezić, V., Singh, B.R., Almås. Å.R. & Lončarić, Z., (2011a). Water extractable concentrations of Fe, Mn, Ni, Co, Mo, Pb and Cd under different land uses of Danube basin in Croatia. *Acta Agriculturae Scandinavica Section B - Soil and Plant Science*, DOI: 10.1080/09064710.2011.557392 (published)

Paper II: Ivezić, V., Almås. Å.R., Singh, B.R., (2011b.) Predicting the solubility of Cd, Cu, Pb and Zn in uncontaminated Croatian soils under different land uses by applying established regression models. *Geoderma* (in press)

Paper III: Ivezić, V., Singh, B.R., Almås. Å.R. & Lončarić, Z., (2011c). Prediction of Cd, Cu, Fe, Mn and Zn uptake by plants from soils in Osijek-Baranja County, Croatia. *Journal of Plant Nutrition and Soil Science* (submitted)

Paper IV: Ivezić, V., Singh, B.R., Gvozdić, V. & Lončarić, Z. (2011d). Assessing soil quality in relation to trace metal availability and other soil properties under different land uses in Croatia**.** *Environmental Monitoring and Assessment* (submitted)

Extended Summary

1. Introduction

1.1. General background

Soil is a non-renewable resource with various ecological functions (biomass production; storing, filtering and transforming nutrients; maintaining biodiversity; carbon sequestration; physical and cultural environment for humans; providing raw materials; preserving geological and archeological heritage) (European Commission, 2006). Therefore, any activity affecting soil needs to be conducted with caution making sure that soil preserves its ecological function. In the past decade Joint Research Center of the European Commission recognized importance of trace metals in the soil and conducted feasibility study investigating trace metals and organic matter content of European soils (European Commission, 2006). The study included only EU countries, thus Croatia was not part of it. Since Croatia is a serious candidate for the EU and it is expected to join EU in the next few years, we investigated soils of Danube basin in Croatia with regards to water extractable and total trace metal concentrations. The investigated area is the main agricultural region of the country, Osijek-Baranja County.

The County is situated in the eastern part of Croatia. Being the main agricultural region, soil fertility and changes in soil quality are of great importance for this area. Agriculture has been practiced in this area ever since the ancient Roman times and the first settlements. Such long history and intensive agricultural practices can influence soil properties, soil quality and availability of nutrients. Macro- and micro-nutrients are constantly being monitored for potential deficiency problems or possible toxicity issues in case of excessive concentrations. Since there is no heavy industry in the area, agriculture is the main source of potentially harmful elements (Alloway, 1995). For example, applying phosphorus fertilizers contribute to the input of toxic element, such as cadmium (Singh and McLaughlin, 1999). Our study investigated total as well as available forms of trace metal concentrations in the area.

The term “trace metals” or “heavy metals” in literature usually refers to potentially toxic elements. However, some of them such as Fe, Cu, Mn, Mo, Zn and Ni are essential in small concentrations for normal growth of plants, animals and humans (Marschner, 1995). They present potential toxicity threat only in excessive concentrations. Therefore, trace metals are of great importance for agricultural practices regarding both deficiency and toxicity.

1.2. Trace metal pools in the soil

Trace metals in soil can be found in several pools. They can be (i) firmly bound to the soil solid particles, (ii) bound to the organic matter or (iii) in the soil solution where they can be in form of free ions or forming complexes (Alloway, 1995). All of these pools together represent the total trace metal content. To obtain the information on total content strong acid digestions methods are used, in general: aqua regia (HNO_3:HCl -1:3) and ultra pure HNO_3 digestion. These total concentrations are commonly used in the legislation of many countries in the determination of maximum permissible concentrations (MPC). In Croatia, for example, MPC for agricultural fields are determined by aqua regia. Such approach is somewhat misleading as the total concentrations do not represent the plant available fraction. Furthermore, most of the trace metals are strongly bound to the soil solid surface and as such are not available for plant uptake.

Trace metals in the soil solution represent the form most readily available to plants. Recent approaches to determine soil solution concentrations included methods such as water extraction, determination of pore water by installations of lysimeters in soils (Heredia and Cirelli, 2009; Moreno-Jimanez et al., 2011) or by the use of diffusive gradients in thin films (DGT) method (Almås et al., 2006; Zhang et al., 2004). The most available form of trace metals from the soil solution are the free metal ions (FMI). Soil solution speciation and FMI concentrations can be obtained by several approaches: (i) direct measurement of M^+, (ii) equilibrium and dynamic techniques (e.g. Donnan membrane technique (DMT)), (iii) Speciation modeling (e.g. WHAM Model VI, MINTEQ or NICA-Donnan model) and (iv) Computation of the equilibrium concentration of FMI by transfer functions (Peijenenburg et al., 2007).

1.3. Availability of trace metals

Since recently, it has been acknowledged that total concentration is not a good representative of trace metal availability. Many different approaches have been used to estimate the available fraction. These approaches try to mimic the plant uptake by using neutral salts, weak acids and organic extractans. Synthetic chelating agents such as DTPA and EDTA have been often used to estimate potential availability of metals. However, it has been shown that EDTA and DTPA can act more aggressively than metal chelating under natural circumstances (Meers et al. 2007a). In addition, EDTA was originally developed for acidic soils and therefore, it correlates poorly with plant uptake on neutral and alkaline soils.

On the other hand, DTPA correlates poorly for acidic soils but it can show good correlation for neutral and alkaline soils (Feng et al. 2005a; Feng et al. 2005b). Total or pseudo-total concentrations of trace elements in the soil, extracted by strong acids, are normally recognized as poor indicators of toxicity. Nevertheless, as mentioned earlier, they are still being used in the legislation of many countries.

Latest findings consider weak extraction by water, free ion approach, $CaCl_2$ or rhizosphere based method (RHIZO - method that uses organic acids) as the most appropriate methods correlating with the plant uptake (Lofts et al. 2004; Feng et al. 2005a; Feng et al. 2005b; Hough et al. 2005; Meers et al. 2007b), mainly because these methods express the concentrations of trace metals in soil solution and in addition are able to release some weakly bound trace metals ($CaCl_2$ and RHIZO method). Trace metals in soil solution are the most available fraction and, in addition, plant is capable of influencing the release of some weakly bound trace metals from the rhizosphere by excreting different exudates, mainly in the form of organic acids. The above mentioned week extraction methods practically act the same as the plant. However, it is not certain that methods used for the determination of available fraction always provide accurate estimations of bioavailability, as for bioavailability to be accurate we need to take plant species, metal and soil properties into account (Peijnenburg et al. 2007). Since several other soil properties influence availability of trace metals, regression models using extraction methods and soil properties can be a good alternative to predict the uptake (Krauss et al. 2002; Lombnæs and Singh, 2003). In the same way regression models can be used in determining FMI concentrations in soil solution (Tipping et al. 2003; Lofts et al. 2004) and, as mentioned earlier, FMI concentrations or activity can be good indicators of plant uptake (Lofts et al. 2004; Hough et al. 2005).

1.4. Solid-solution partitioning

Solid solution partitioning plays a major role on the mobility and retention of trace metals in soil system. It is usually expressed as a ratio between the total concentration of metals (in mg kg^{-1}) over the concentration of solution dissolved metals (mg L^{-1}). Therefore, the partitioning coefficient (K_d) is usually reported in L kg^{-1}:

$$K_d = \frac{M_{(tot)}}{M_{(sol)}}$$

Trace metals in soil solution are mobile and could be easily taken up by the plant roots or other soil organisms. In addition, soil solution trace metals also represent the fraction that could be easily leached out to the groundwater and surface water. As mentioned earlier bioavailability of trace metals largely depends on the concentration and chemical forms in the soil solution. It has been well acknowledged that certain soil properties such as pH and SOM control the trace metal solubility (Tipping et al., 2003; McBride et al., 1997; Sauve et al., 2000).The K_d coefficients can vary over 6 orders of magnitude mainly as a function of soil pH, total metal and soil organic matter (SOM) content (Sauve et al., 2000). Thus, if there are differences of soil properties between land uses we can expect different K_d between them as well.

2. Objectives

The main objective of this study was to determine soil properties controlling the trace metal solid-solution partitioning, develop regression models predicting solution trace metal concentrations and examine their correlation with plant uptake. The more specific objectives are:

- Determine total and water extractable trace metal concentrations in Osijek-Baranja County (Eastern Croatia) and investigate the influence of different soil properties on solid-solution partitioning.

- Develop regression models predicting trace metal solubility using soil properties and carry out metal speciation of soil solution using WHAM/Model VI.

- Use the existing regression models predicting water extractable fraction of trace metals to predict metal concentrations in wheat grain.

- Use soil properties data to derive soil quality index (SQI) and examine the relationship between availability of trace metals and SQI.

3. Materials and Methods

3.1. Study area

Osijek-Baranja County covers 4,144 km^2. It is a part of Pannonian valley that stretches through Hungary, Serbia and Croatia with Danube River as the main geographical feature. Elevation is around 90 m above the mean sea level. The climate is continental with hot and arid summers and cold winters. Average annual temperature is 11°C, while the average annual precipitation is 650 mm. The heaviest periods of precipitation are in the spring and fall (Ministry of Environmental Protection, 2010).

The county consist of 9 main soil types, i.e. Stagnosols, Dystric Cambisols, Luvisols, Haplic Gleysols, Chernozems, Fluvisols, Eutric Cambisols, Mollic Gleysols and Anthrosols (FAO, 2006). In the present study sampling sites included all of the soil types. However, since some of them cover small and insignificant area, the number of samples varied among soil types (Fig. 1). Soil samples were collected from different land uses: arable land (45), pastures (8) and forest land (21). Forest soils were mainly 80-year old oak forest with very little human activity, and thus forest was considered as natural site without human influence (i.e., fertilizers, pesticides, cultivation). In order to investigate the impact of possible metal contamination through fertilization and cultivation practices in agricultural soils, agricultural sites were compared with forest sites.

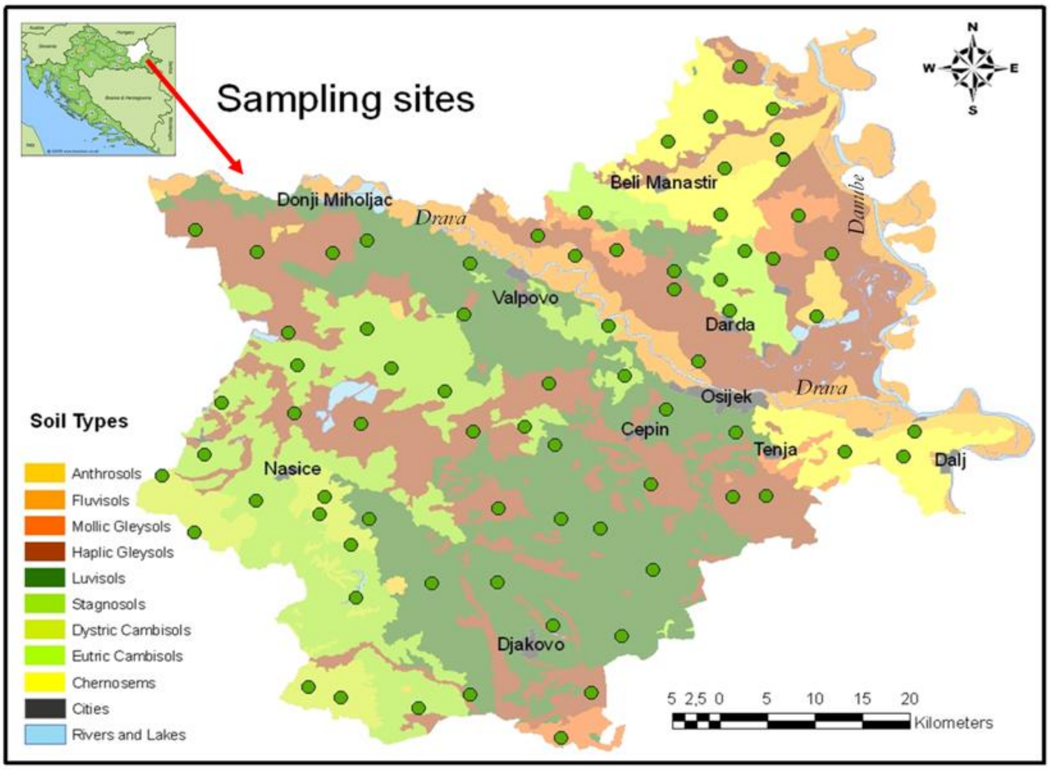

Figure 1. Sampling sites in Osijek-Baranja County

3.2. Soil sampling and analysis

Based on the pedological map, 74 sites were randomly chosen so that all soil types and different land uses were included (arable land, pasture and forest). From each site 10 subsamples from 0–25 cm depth (hereafter called surface soil) were taken within 5 m distance from each other and then combined into one sample of approximately 500 g. Samples were dried and sieved through the 2-mm sieve for the determination of soil pH, dissolved organic carbon (DOC), cation exchange capacity (CEC), ammonium –lactate extractable P (AL-P), ammonium –lactate extractable K (AL-K) and water extractable trace metals (TM). For soil organic carbon (SOC), inorganic carbon (InC), total nitrogen (TN) and total trace metals (HNO_3 extraction), samples were further ground to finer particle size using agate mortar. The concentration of SOC was determined by dry combustion method on a LECO Carbon Determinator EC12 (Nelson and Sommers, 1982), TN and InC concentrations were determined by LECO CHN-1000 Carbon and Nitrogen Analyzer. As a default we suggest SOM contains 50% carbon. Soil pH was determined in soil to water solution ratio of 1:2.5 (Mc Lean 1982) and CEC by barium chloride method with 3 g of soil and 40 mL of 0.1 M $BaCl_2$ making the soil to solution ration 1:13 (Hendershot and Duquette, 1986). The amount of dissolved organic carbon (DOC) in water was determined by the use of a Shimadzu TOC-5000 analyzer. As with SOM, we suggest dissolved organic matter (DOM) to be composed of 50% organic C. The soil particle-size distribution was measured by a pipette method (Elonen, 1971). Available P and K were determined by ammonium lactate (AL) extraction method (Egner et al., 1960.). Bulk density (BD) was determined by core method. Analysis of soil samples for pH, SOC, InC, DOC, TN, CEC, soil texture and trace metal concentrations (water extraction and HNO_3 extraction) was conducted at the Norwegian University of Life Sciences, while the determination of BD, AL-P, AL-K and EDTA extraction of trace metals was conducted at the University of J.J. Strossmayer, Faculty of Agriculture, Osijek, Croatia.

3.3. Trace metal extraction procedures

Soil samples were tested for 3 different single extraction methods: strong acid extraction (ultra pure HNO_3 extraction), EDTA extraction and extraction by water (weakest extractant). Ultra pure HNO_3 extraction and water extraction were conducted at the Norwegian University of Life Sciences and EDTA extraction at the University of J.J. Strossmayer, Faculty of Agriculture, Osijek, Croatia.

- *Ultra pure HNO_3 extraction* - Total metal concentration in soil (M_{tot}) was determined after digesting the soil in concentrated ultra pure HNO_3 (1:15 solid:solution ratio) by stepwise heating up to 250°C using a Milestone Ultra clave for 1 hr and 15 min. Trace metal concentrations were determined using a Perkin Elmer Sciex Elan Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for Cd and a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES) for Cu, Fe, Mn and Zn. Standard reference material (SRM) used was the SRM 2709 (National Institute of Standards & Technology, 2003).
- *EDTA extraction* – 10 g of air-dried soil was extracted with 20 ml EDTA (0,01 M ethylene-diaminetetraacetic acid (EDTA) and 1M $(NH_4)_2C0_3$, adjusted to pH 8,6) and shaken for 30 minutes. The soil:solution ratio was 1:2 (Trierweiler and Lindsay, 1969). Extract was filtered and concentrations of trace metals were determined by ICP-OES.
- *Water extraction* – On 4 g of air-dried soil 40 mL ultra pure water (MilliQ H_2O, electric conductivity < 18.2 MΩ cm^{-1}) was added and shaken in high density polyethylene (HDPE) centrifugation tubes for two days and centrifuged at 1400 x g for 30 min. The suspension was filled in High Density Poly Propylene (HDPP) syringes and filtered through 0.45 μm polyethersulfone membrane filters to poly propylene (PP) test tubes. The filters and the test-tubes were provided by VWR International (vwr.com). Blanks of MilliQ H_2O were stored in PP tubes for 48hr. and filtered through the same membrane filters before analysis. Water trace metal concentrations were determined using a Perkin Elmer Sciex Elan Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for Cd and a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES) for Cu, Fe, Mn and Zn. Standard reference material used for water was the SRM 1643e (National Institute of Standards & Technology, 2004).

3.4. Data analysis

Descriptive statistics, analysis of variance (ANOVA), Tukey pairwise comparison of means, simple correlation, regression analysis and principal component analysis were conducted using Minitab® Statistical Software version 15 (Minitab, 2007). In addition statistical software Statistica, version 7.0 (StatSoft, 2007) was used for 3D graphs of PCA results. Analysis of variance and Tukey pairwise comparison was done between land uses and soil types to determine soil parameters with significant differences. Multiple regression analysis was used to derive best-fit models for water extractable trace metals, free metal ion (FMI) concentrations and free ion activity (FIA).

Chemical speciation of soil solution was estimated using WHAM-VI version 6.0 for waters (Tipping, 1998). The input data was: soil solution pH, fulvic acid (FA) concentration, the solution concentration of Al, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. As a default we define 50% of DOM being chemically active FA.

Finally, GIS technique was used to create maps of the area and to visualize the results. The maps were created in ArcGis version 9.2. (2006), software that combines table data with spatial data allowing us more comprehensive insight into a certain area (Hutchinson and David, 2000).

3.5. Soil quality index determination

Determining soil quality index consisted of three steps. First step was to choose the minimum data set (MDS) from which SQI was determined. We preformed ANOVA on all data set to examine which soil properties significantly differ among the land uses. Only the data that showed significant difference was further analyzed by standardized PCA. We assumed that PCs having high eigenvalues represent the variation in the system best. Thus, only PCs with eigenvalues higher than 1 were considered. Each variable at each PC is given a weight or a factor loading that represents variables contribution to the composition of that particular PC. The variables with highest factor loading from each PC were than chosen for the MDS. In case when one PC has two or more variables with high factor loadings, and they are uncorrelated, then both variables would be chosen. However, if the variables with high factor loading of the same PC are correlated, than the one with higher loading would be chosen for the MDS.

Once we have chosen our MDS, we transformed data values, by nonlinear scoring curves, into unit-less values (Step 2). Such scoring values can further be combined in to one single value representing SQI (Step 3).

$$\mathrm{SQI} = \sum_{i=1}^{n} \mathrm{W_i} \times \mathrm{S_i}$$

Where W is the weighting factor and S is the indicator score.

4. Results and Discussion

4.1. Soil properties

The soil samples were analyzed for the following parameters: BD, soil texture, pH, SOC, InC, DOC, N, AL-P, AL-K, CEC and trace metals (total and water extractable Cd, Co, Cu, Fe, Mn, Mo, Ni and Zn; and EDTA extractable Cd, Cu, Fe, Mn and Zn). Descriptive statistics of all soil properties is presented in (Table 1).

Table 1. Soil properties and total and extractable trace elements

		n	Mean	SD	Min.	Max.
Clay %		30	27.8	7.6	15.6	46.9
Silt %		30	69.4	7.4	49.3	80.8
Sand %		30	2.8	2.7	0.4	11.8
BD		47	1.4	0.16	0.91	1.72
pH		74	6.3	1.2	4.3	8.0
N %		74	0.17	0.09	0.04	0.56
P (mg/100g)		74	19.9	26.9	0.00	174.2
K (mg/100g)		74	20.9	11.6	7.6	84.6
SOC %		74	1.7	0.85	0.46	5.1
InC %		74	0.5	0.5	0.004	2.7
DOC (mg/l)		74	26.0	17.4	6.1	73.0
CEC (cmol/kg)		74	8.8	5.0	1.3	24.4
HNO$_3$ extractable (mg kg^{-1})	**Cd**	74	0.23	0.09	0.10	0.67
	Co	74	12.5	2.1	6.1	17.9
	Cu	74	24.3	18.4	5.8	141.9
	Fe	74	29178	4220	19642	41034
	Mn	74	660.3	233.2	218.5	1459.1
	Ni	74	33.1	8.0	13.5	54.2
	Pb	74	19.5	3.5	10.4	31.8
	Zn	74	80.2	16.7	52.3	122.4
Water extractable (mg kg^{-1})	**Cd**	74	0.0009	0.001	0.00003	0.007
	Co	74	0.016	0.024	0.0009	0.14
	Cu	74	0.15	0.09	0.05	0.5
	Fe	74	20.1	26.0	0.4	125.3
	Mn	74	1.86	3.61	0.004	20.89
	Mo	74	0.01	0.01	0.0006	0.084
	Ni	74	0.07	0.04	0.012	0.19
	Pb	74	0.01	0.01	0.0004	0.04
	Zn	74	0.24	0.25	0.03	1.05
EDTA extractable (mg kg^{-1})	**Cd**	74	0.1	0.04	0.015	0.23
	Co	74	0.25	0.17	0.05	0.92
	Cu	74	6.9	6.4	1.4	41.4
	Fe	74	205.2	341.2	13.7	1631.9
	Mn	74	54.0	33.9	7.3	131.6
	Ni	74	1.36	0.61	0.35	3.04
	Pb	74	3.2	1.1	1.1	6.0
	Zn	74	1.4	0.9	0.3	4.1

Governmental maximum permissible concentrations (MPC) of trace metals for agricultural soils are determined by *aqua regia* method (Official Gazette, 1992). In the present study total concentrations are estimated by ultra pure HNO_3 extraction. The HNO_3 extractions and the *aqua regia* extraction can both be used to estimate the total metal concentration in soil (Sabiene et al. 2004; Meers et al. 2007). Compared to governmental standards, the total trace metal contents in soils from this County are below MPC. Moreover, the total content of the essential trace metals were found to be within the average normal range for soils in Europe (Havlin et al., 2005). This suggests that, if we are considering total trace metal contents as our criteria, there is no deficiency problem either. However, soil properties such as pH, DOC, SOC and CEC and the crop grown should also be considered when making the assumption on deficiency. Water extractable fraction of trace metals was on average less than 0.5% of the total trace metal contents in the soil. In the present study water extractable fraction represents concentrations in soil solution.

4.1.2. Differences between land uses

Influence of land use on soil properties showed no significant difference between pasture and arable land, but the difference between forest and arable land and forest and pasture was significant (for BD, pH, K, N, SOC and DOC (p<0.001); for P (p<0.01); for CEC and sand (p<0.05)). Therefore, in the presentation of results, pasture and arable land were combined together into one land use, *agricultural field*. Forest soils showed much lower pH, P and K while higher SOC and DOC than the soils from agricultural fields (Paper I). Forest is a natural site with much higher organic matter input than agricultural field which explains the higher levels of SOC and DOC (Post and Kwon, 2000), while higher pH, values in agricultural fields, compared to forest soils, could be assigned to liming application as more than half of the agricultural sites had pH above 7. Higher values of P and K in agricultural fields are due to fertilization practices.

Total trace metal (HNO_3 extraction) concentrations were found not to be so dependent on land use. Land use had only significant impact on the total content of Pb ($p < 0.001$), Ni and Cu ($p < 0.05$). The higher content of Pb was found in forest soils compared to agricultural soils while Ni and Cu content was higher in agricultural soils compared to forest soil (Paper I). No significant impact of land use on total metal content of all other investigated trace elements indicates no, or insignificant, influence of agriculture.

However, when water extractable fraction was examined, land use showed significant influence on almost all investigated trace metals (except Fe and Cu). In water extracts from the forest soils, the concentrations were significantly higher compared to extracts from the agricultural fields for: Co ($p < 0.001$), Mn ($p < 0.001$), Ni ($p < 0.01$), Zn ($p < 0.001$), Cd ($p < 0.001$) and Pb ($p < 0.01$), while significantly lower for Mo ($p < 0.05$). Such behaviour of water extractable trace metals is most likely related to soil pH and DOC. As mentioned earlier, soil properties significantly differ among land uses and several authors showed that soluble concentrations of trace metals in soil solution is controlled by soil properties mainly pH, SOC and DOC (McBride et al. 1997; Tipping et al. 2003; Strobel et al. 2004).

4.1.3. Differences between soil types

There are nine soil types in the area. However, four of them cover only very small part whereas the other five cover very large areas in comparison. When examining the impact of the different soil types on the trace metal concentration, only the main five soil types were investigated; namely Stagnosols, Dystric Cambisols, Luvisols, Haplic Gleysols and Chernozems. Soil texture analysis showed that Stagnosols, Dystric Cambisols and Luvisols are silt loam soils with average 24% clay while Haplic Gleysols and Chernozems are silty clay loam soils with average 34% clay. These five soil types represent 80% of the area and account for 60 out of the 74 samples.

The pH, N, K, SOC, InC, DOC, CEC, clay and silt were significantly different between the soil types. Tukey pairwise t-test was used to partition differences between soil types for pH, SOC, DOC and CEC. The Chernozems had the highest pH (6.4–7.9 with an average of 7.2) and as such it was statistically different from all other soil types except Haplic Gleysols soils. However, all of the Chernozems sites were agricultural fields, which can explain high pH supposedly due to liming. The SOC and DOC contents were lowest in the Luvisols and Chernozems. However, these two soil types were mainly used for agricultural practices and as it was shown earlier agricultural field contain a significantly lower contents of SOC and DOC ($p<0.001$). We suggest that this is connected to the export of harvested crops. Nevertheless, the difference among other three soil types, that have sampling sites from both land uses, imply that soil type can also be indicator of difference in SOC and DOC. Cation exchange capacity (CEC) was highest for Haplic Gleysols soil which is statistically different from all other soil types except Chernozems. These two soil types are silty clay loam soils with significantly higher content of clay (34%) compared to the other three soil types (24%) which can explain the higher CEC as silty clay loam has significantly higher CEC (Paper I). There is also a visible difference between the northern and southern part of the county in the soil parameters. In the northern part of the area, soils with high pH and low organic matter content are dominating soil types (Fig. 2). These differences are related to different soil types in these parts: the northern soils are dominated by silty clay loam Chernozems and Haplic Gleysols soils while the southern soil types are dominated by silty loam Stagnosols and Luvisols soil types.

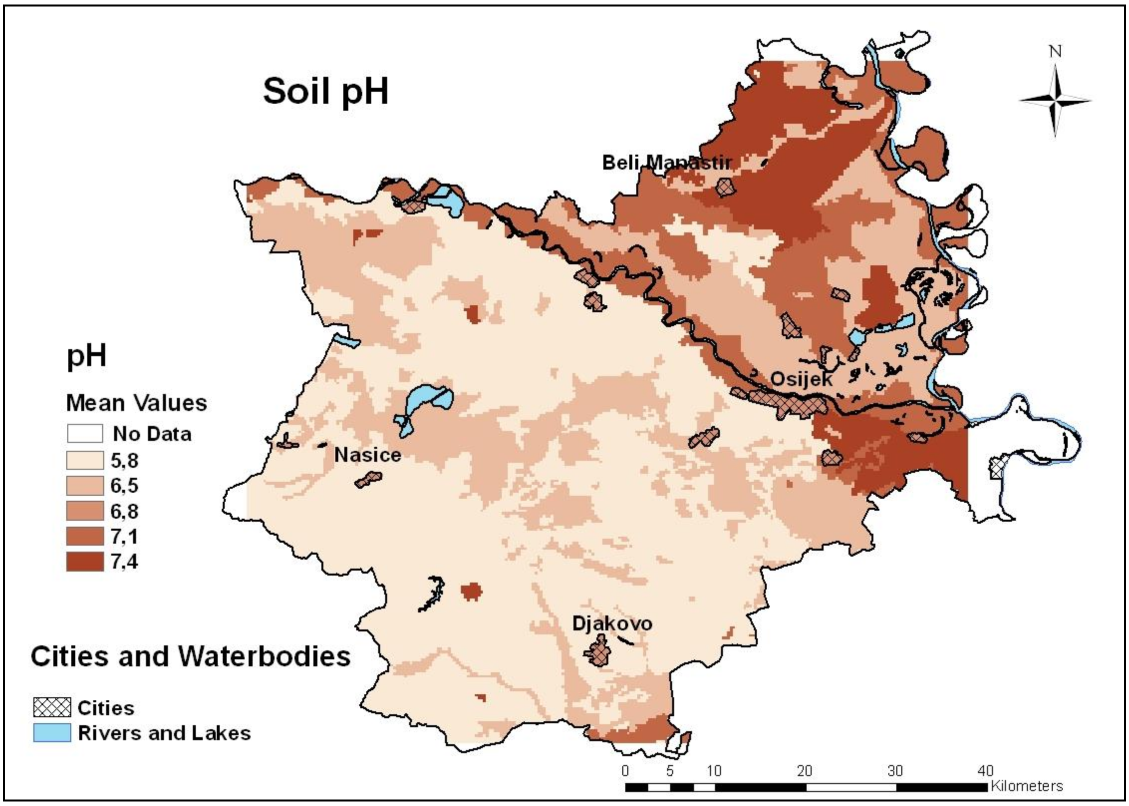

Figure 2. Soil pH map of Osijek-Baranja County

Unlike land use, soil type had significant impact on the total content of investigated trace metals. Soil types significantly differ in the content of: Cu, Fe, Co, Mn, Zn, Ni and Pb ($p < 0.001$) and Cd ($p < 0.01$). Using Tukey pairwise t-test, Dystric Cambisols generally contain the highest metal contents (Paper I). This soil type is mostly found at the very south of the county, in the hilly areas. It is the soil type most remote from the river and the parent material differs the most from other soil types. Soil texture also indicates significant influence on total metal content for several investigated elements: Fe ($p < 0.001$), Mn, Ni and Cd ($p < 0.01$). This suggests that total metal content in soil is related to parent material rather than to human activities.

On the other hand, when examining water extractable fraction, impact of soil types was only significant for water extractable: Zn ($p<0.001$), Pb ($p<0.001$), Mn ($p<0.05$), Fe ($p<0.05$) and Mo ($p<0.05$) (Paper I). For the impact of soil texture on the metal extractability, we grouped the texture classes in two: namely silt loam and silty clay loam. We found that these two texture classes have significant difference for the water extractable Fe, Mo and Pb ($p<0.001$); Mn, Ni and Cd ($p<0.01$); Co ($p<0.05$). Difference in soil texture was, however, statistically correlated with soil pH and CEC (Paper I). This shows that although the metal extractability can be connected to soil texture using soil maps, the actual difference is determined by the soil properties such as pH and CEC.

4.2. Soil properties controlling soil solution concentrations of trace metals

Soil properties play a crucial role in solubility of trace elements and therefore are important indicators of their availability. In our study pH, together with DOC, appeared to be the most significant parameter determining total solution concentration ($M_{(sol)}$) as well as free metal ion (FMI) concentrations (Paper I and II). Soil organic matter (SOM) and total trace element concentration ($M_{(tot)}$) appeared to be significant just for some metals. Low levels of $M_{(tot)}$ and small variation in concentrations among the sites was probably the reason of no significant effect of $M_{(tot)}$ on solubility of some trace metals. However, in our opinion $M_{(tot)}$ should be always considered when modelling solubility as it represents the total pool of certain trace metal, determining the concentration level of trace metals in solution.

When modelling soil solution speciation with WHAM VI model we considered fulvic acid (FA), soil solution pH, solution Ca^{2+} concentration and trace element concentrations (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) as parameters controlling the trace metal speciation in solution. Dissolved organic matter (DOM) in solution was considered to be fulvic acid (FA), which has a capability of binding and mobilizing trace elements in the soil solution. In the present study we assumed that 50% of DOM is active FA. In addition, we tested two different approaches regarding the Fe content. Some of our sites have pH > 7 and clay content > 40% and are subject of seasonal flooding, therefore we assumed the possibility of some Fe to be present as Fe^{2+}. Regression analysis was conducted assuming Fe^{3+} being 100% and 80% of total Iron. No significant differences have been observed in our results and therefore, Fe was considered to be present in the soil solution as 100% Fe^{3+}.

4.2.1. Regression modelling of trace metal solubility

Regression models have been an efficient tool in predicting metal solubility. Most of the models consist of pH, OM and $M_{(tot)}$ (Janssen et al., 1997; Meers et al., 2005; Tipping et al., 2003). However, sometimes good correlation can be already achieved by using just one parameter (Sauvé et al., 1997). The choice of parameters can vary among the metals investigated or between the types of soil (organic or mineral). In the present study, OM and $M_{(tot)}$ varied very little among the sites and therefore their influence on solubility was small, unlike the influence of pH, DOC or CEC. There is a strong correlation among pH, DOC and CEC, so it is difficult to separate the effect of one from the other. When deriving regression equations, pH and DOC seem to be the most constant parameters determining the best-fit models. In addition, CEC is significant for modelling Cd solubility (Paper II). Cation exchange capacity (CEC) is in positive correlation with pH, suggesting that CEC is pH dependent (McBride, 1994). Smolders et al. (2009) also observed positive correlation between pH and CEC in their research on toxicity of trace elements where CEC appeared to be the most significant variable explaining the toxicity.

Most of the published regression models have been derived from investigation of soils with much higher metal concentrations than in our study. Therefore, in the present study we tested three well known models (McBride et al., 1997; Meers et al., 2005; Tipping et al., 2003). The controlling variables in the investigated regression models were also significantly controlling our input data, even without optimizing the equation factors. However, the model predictions did not follow a 1:1 line through 0, and they overestimated the metal solubility (Paper II). It is our belief that the overestimation is due to the higher metal concentrations defining the premises for regression model developments in studies investigated. The higher concentration of SOM reported in Tipping et al. (2003) study may also have been important since high concentration of SOM causes higher DOC (Gandois et al., 2010). Differences among models suggest that there cannot be one universal model (Sauvé et al., 2000b). To accept a certain model, considerable changes in regression coefficients are required (Meers et al., 2005). Based on this condition, we believe that even if we had applied our data to other similar regression models, the outcome would be an overestimation as long as the equations are based on contaminated soils. It seems, therefore, of great importance to know the premises for predictive models used during risk assessments.

Free metal ion (FMI) concentrations were obtained by WHAM VI model. When our data were applied directly into unchanged regression equations from Tipping et al. (2003) and Meers et al. (2005) the controlling variables in the regression equations determined the FMI estimations very well (Paper II). But, just as with metals in solution, these studies overestimated the FMI by about 8 times (for Tipping et al., 2003) and 5 times (for Meers et al., 2005). Compared to our data, Tipping et al., (2003) study had much higher SOM content and metal concentrations, while only the metal concentrations in soils from Meers et al. (2005) diverged substantially from our findings. This may partly explain the overestimations, as overestimation occurs when modelling FMI in uncontaminated soils or if the metal extraction methods differ from the ones that were used for developing the model (Groenenberg et al., 2010). Therefore, applicability of models varies among investigating areas and optimization of models is required (Sauvé et al., 2000b). In our study, levels of $M_{(tot)}$ and $M_{(sol)}$ are much lower than in previously discussed studies, and their reported metal concentrations do not exceed the governmental maximum permissible levels. Models derived from our study can be important when assessing potential deficiency in contrast to when the issue is to assess toxicity.

4.3. Availability of trace metals in relation to soil solution concentration

Availability of trace metals depends on concentrations and chemical speciation of metals in soil solution (Allen, 1993). The concentration of water extractable metals is directly available for plant uptake and hence it is in good correlation with the FMI concentrations in the solution and with plant uptake (Almås et al. 2006; Hough et al 2005; Lofts et al. 2004; Sauvé et al. 2000b). Therefore, information on chemical speciation (i.e. FMI and FIA) and soil solution concentrations (in our study water extractable fraction) are keys to predict concentrations of metals in grain.

We tested correlation between solution concentrations and plant concentration by applying our regression models from Paper II, predicting $M_{(sol)}$, FMI and FIA concentrations, on already existing plant data. The results showed that estimated water extractable fraction, FMI and FIA of Fe, Mn, Zn and Cd were better correlated with metal concentrations in the grain than when using only EDTA extraction (Paper III). Correlation between EDTA and plant concentration was significant only for Fe. Extraction by EDTA mimics phytosiderophore release, exudates used by metal deficient plants primarily to obtain Fe (Menzies et al., 2007). We believe that this could be the reason why only Fe was correlated with EDTA while predicted solution concentrations were correlated with Fe, Mn, Zn and Cd. In addition, we developed regression models predicting plant concentrations based on soil properties as parameters. The prediction appeared to be successful for some of the investigated trace metals (Mn, Fe and Cd).

4.4. Influence of land use on soil quality and availability of trace metals

As mentioned earlier the soils samples were analyzed for vast number of soil properties. The selected ones were used to determine soil quality: BD, pH, SOC, InC, DOC, TN, AL-P, K, CEC and trace metals (total and water extractable Cu, Cd, Fe, Mn and Zn). Since soil properties differ significantly between land uses (Paper I) we wanted to use soil quality index (SQI) in order to investigate potential soil degradation in land use change (Paper IV). In addition, we were interested to see how SQI reflects trace metal availability.

4.4.1. Determination of soil quality index (SQI)

In the present study we used statistical approach using principal component analysis (PCA) to determine SQI. The first step in determining SQI is to choose indicators that are significantly different between land uses (forest and agriculture). There are various methods in using PCA or factor analysis (FA) for the selection of indicator minimum data set (MDS) (Brejda et al., 2000a, b; Fu et al., 2004; Shukla et al., 2006). In the present study we determined MDS based on Andrews et al. (2002a) approach. The PCA was performed on 10 indicators that showed significant differences between land uses. Forest soils showed significantly lower pH, BD, AL-P and K and higher SOC, DOC, TN and water extractable Cd, Mn and Zn than agricultural soils. Principal component analysis had three PCs with eigenvalues higher than 1 explaining 79.8 % of variation. Highly weighted variables with high loading factor from PC1, PC2 and PC3 were chosen as MDS. Therefore, our MDS for the determination of SQI consisted of: pH, BD, SOC and AL-P (Paper IV).

The parameters of MDS had to be transformed in to the same unit so that they can be compared. The transformation was carried out by non linear scoring as such scoring approach reduces the influence of outlier data samples. The non linear scoring system requires good knowledge of the system. However, this method is beneficial as it is more transferable to other systems. The scores were assigned in ascending or descending order. If higher values are considered as “good”, approach “more is better” is applied, if lower values are considered as “good”, the “less is better” approach is applied. In cases of values such as pH “mid-point optimum” is applied (Brejda et al., 2000a,b; Andrews et al., 2002a,b; Fu et al., 2004; Awasthi et al., 2005; Shukla et al., 2006; Imaz et al., 2010).

Finally the indicator scores were combined into SQI by simply adding the scores. However, not only the scores were considered but the weight of the indicators is taken into account as well. It is from the PCA results that we can see the weight of each variable. Each

PC explains certain percentage of the variation in the data set. This percentage, divided by the total percentage of variation from all PCs with eigenvectors > 1, determines the weighting factor for variables under particular PC (Andrews et al. 2002a,b). Knowing the indicator score and its weight factor we can calculate SQI for each observation by the following equation:

$$SQI = \sum_{i=1}^{n} W_i \times S_i \qquad (1)$$

Where W is the weighting factor and S is the indicator score. In the present study the highest weight in determining the SQI is given to pH, followed by BD and SOC, while AL-P appears to have lowest weight (Paper IV).

4.4.2. SQI of different land uses

The SQI between land uses did not differ much. Such results indicate no soil degradation due to the agricultural practices. Soil quality index for forest was 0.70 which is slightly, but not significantly, better than in agricultural field (0.67). However, PCA showed that soil properties of forest soils differ from agricultural fields. Forest soils have better BD and SOC while agricultural fields have better pH and AL-P (Paper IV). Better pH in agricultural fields is due to liming practice which helps in keeping the pH at desirable levels for agricultural production, while better AL-P is due to the P fertilization. Forest has higher SOC mainly because of constant input of organic materials such as leaves and fallen branches (forest litter). Decrease in SOC and organic matter (OM) in agricultural fields can have effect on nutrient cycling, pesticide and water retention and soil structure while degradation of BD can effect root penetration, water- and air-filled space and biological activity (Karlen et al., 1997). Therefore, even though our results indicate no overall degradation of SQ by the agricultural practices, certain soil properties has shown degradation (such as SOC content and BD). Thus, SQI can be used also if we want to evaluate the soil with regards to one particular soil property (Brejda et al., 2001). For example, property such as organic matter (OM), where parameter of OM would be determined by correlated variables of the same PC, in our case by the scores of SOC, DOC and N.

4.4.3. Availability of trace metals and SQI

In the assessments of soil quality very little attention has been given to trace metals. None of the previous work included available trace metals in the evaluation of SQI. The present study showed dependence of trace metal availability on soil properties, since SQI is determined by soil properties it was our hypothesis that SQI will also be a good indicator of trace metal availability. Therefore, we included water extractable trace metals in PCA when choosing indicators for SQI. However, we found no correlation between SQI and soil solution concentration of trace metals. Therefore, if estimating trace metal availability SQI will be misleading since it is not a good indicator of soil solution trace metals (Paper IV). Availability of trace metals is best evaluated by regression models (Paper II and III).

5. Conclusion and recommendations

Concentration of trace metals in the soils of Croatian main agricultural region has shown no reason for alarm from soil contamination point of view. Total content of toxic and potentially toxic trace metals are way below the governmental tolerance limits (MPC) at the same time total content of the micronutrients has also shown to be above the deficiency levels. However, the fraction of trace metals in the soil solution, the fraction that is readily available, has shown not to be dependent on the total content but rather on soil properties such as pH, DOC and CEC. Since soil properties differ between land uses we can also observe difference in soil solution concentrations between land uses. However, there is no difference between total concentrations of trace metals between land uses so we can say that agriculture practices did not cause excessive input of trace metals. Investigation of overall soil quality between forest and agricultural soils also indicate no soil degradation. Nevertheless, for some soil properties we can see the degrading influence of agriculture, specifically for BD and OM. In addition, from trace metal availability point of view the trace metals will be more available in forest due to soil properties controlling its solubility (pH, DOC and CEC).

The results suggest that the total content of trace metals is not a good indicator of their availability to plants. It seems that pH, DOC and CEC play a more important role in understanding the trace metals in the soil solution of Osijek-Baranja County than the total content. Regression models using these soil properties as parameters are widely used for the prediction of soil solution concentrations of trace metals. Comparing the models derived from polluted and slightly polluted soils with our data from unpolluted agricultural and forest soils the results show a good correlation. However, the models do not follow the 1:1 line, indicating, that in this case, they will be overestimating the predictions. Our study points out that modelling trace metal solubility and FMI concentration is area specific. Models developed on polluted or even what is considered slightly polluted soils cannot be directly applied on soils with low levels of trace elements. The optimization of regression models is necessary.

Regression models can also be used to predict plant uptake. Models developed using soil properties improve prediction of plant uptake and as such are much better indicators of plant uptake than single extraction by strong or week acid alone. Furthermore, water extraction, FMI and FIA, predicted by regression models give us better correlation with plant concentrations than the EDTA or total extraction. Soil solution concentrations appear to be good indicators of uptake. However, soil pH seems to have a key role in determining soil solution concentrations, FMI and FIA. Thus, we can conclude that pH plays an important role in the solubility of trace metals and prediction of plant concentrations. The soils investigated had low total concentrations of trace metals which probably increased the importance of pH. Therefore, in uncontaminated soils with low concentrations of trace elements, pH is the driving force influencing metal solubility and thus metal availability as well. By controlling the pH we also control the solubility of trace metals in the investigated area. Thus possible toxicity or deficiency problems can be avoided by pH control.

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Research Papers

Paper 1

Ivezić, V., Singh, B.R., Almås. Å.R. & Lončarić, Z., (2011a). Water extractable concentrations of Fe, Mn, Ni, Co, Mo, Pb and Cd under different land uses of Danube basin in Croatia. *Acta Agriculturae Scandinavica Section B - Soil and Plant Science*
DOI: 10.1080/09064710.2011.557392 (published)

Acta Agriculturae Scandinavica Section B – Soil and Plant Science
2011, 1–13, iFirst article

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Water extractable concentrations of Fe, Mn, Ni, Co, Mo, Pb and Cd under different land uses of Danube basin in Croatia

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Abstract

Water extraction of trace elements can simulate the concentration of elements in the soil solution from where the plant takes up the elements. The objective of this investigation was to determine the water extractable concentration of seven trace elements (Fe, Mn, Ni, Co, Mo, Pb and Cd) and to assess their relationship with soil properties of the Danube basin in Croatia. Soil samples from the surface layer (0–25 cm) of 74 sites, having different land uses (forest and agricultural land), were collected. Samples were analysed for total and water extractable trace elements as well as for pH, DOC, SOC and CEC. The concentrations of water extractable fraction of trace elements were on average: 20.14 mg kg^{-1} for Fe, 3.61 mg kg^{-1} for Mn, 0.07 mg kg^{-1} for Ni, 0.016 mg kg^{-1} for Co, 0.01 mg kg^{-1} for Mo, 0.01 mg kg^{-1} for Pb and 0.0009 mg kg^{-1} for Cd. Soil properties were in the following range: pH 4.3–8 (Avg: 6.35), DOC 6.1–73 mg l^{-1} (Avg: 26 mg l^{-1}), CEC 1.3–24 cmol kg^{-1} (Avg: 9 cmol kg^{-1}) and SOC 0.5–5% (Avg: 1.7%). The concentration of water extractable fraction of trace elements was significantly correlated with pH ($p < 0.001$), DOC ($p < 0.001 - p < 0.05$) and CEC ($p < 0.001$) but their relationship with total content of trace element and SOC was rather weak, suggesting that total metal alone cannot be an indicator of toxicity or deficiency. Results show that pH, DOC and CEC are important soil quality parameters taking part in the solubility control of trace metals in the soil rather than their total concentration. The difference between land uses has been observed as well, suggesting that a change in land use can cause a change in trace element solubility.

Keywords: *Metal solubility, soil properties, soil solution, soil type, water extraction.*

Introduction

Osijek-Baranja County is part of the Pannonian valley that stretches through Hungary, Serbia and Croatia with the Danube River as the main geographical feature. It is the main agricultural region of the country with food production as the most important economic sector of the county. Therefore, both soil quality and crop quality are of special interest in the area. Since there is no heavy industry or mining activity in the area, very little attention has been paid to the concentrations of trace elements in these soils. Nevertheless, due to agricultural importance of the region, information on trace elements concentration is necessary, as traffic and nonjudicious usage of fertilizers may, to some extent, contribute to elevated levels of trace elements in the soil (Alloway, 1995). So far, some preliminary studies examining micronutrients in the soil have shown no elevated concentrations of trace elements (Loncaric et al., 2008). More detailed investigations have been done in the north-west part of Croatia, near the capital city of Zagreb (Halamić et al., 2003; Romić & Romić, 2003). Romić and Romić (2003) examined the distribution of trace elements in the surface soils of agricultural fields near urban areas of Zagreb. Compared with the maximum permissible concentrations (MPC) of trace elements in the agricultural soils prescribed by the Croatian government in the Official Gazette (1992), elevated levels of trace elements were detected mainly near the airport and in the area where seasonal flooding of the river Sava occurs (Romić & Romić, 2003). These findings lead to a conclusion that most of the soil

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(Received 14 November 2010; revised 18 January 2011; accepted 19 January 2011)

ISSN 0906-4710 print/ISSN 1651-1913 online
DOI: 10.1080/09064710.2011.557392

contamination in the vicinity of Croatia's capital is caused by the fuel combustion near the airport and due to industrial spills from upstream of the capital. Similar results were obtained in the surface soils of alluvial sediments at the banks of the river Drava in the NW Croatia. Elevated concentrations of Pb, Zn and Cd, supposedly of mining and smelting origin were found (Halamić et al., 2003). Osijek-Baranja County is located east from these investigated areas. However, Drava River also runs through Osijek-Baranja County, and it is one of the main geographical features of the area. Unfortunately, samples at the banks of Drava River could not be taken due to inaccessibility of the area and thus are not part of this study.

All of the above-mentioned studies deal with the total concentrations of trace elements. However, information about solubility control and concentrations of trace elements in soil and soil solution is lacking and this information is important for assessing risk of either deficiency or toxicity to plants (Marschner, 1995). Soil physicochemical properties, plant species and particularly rhizosphere processes influence metal availability (Ehlken & Kirchner, 2002; Adams et al., 2004). Extractants such as neutral salts, weak acids and organic extractants have often been used to estimate the partitioning of trace elements in soil solution. However, the outcome of such extractions is limited as the trace element concentration in extracts is dependent on soil chemistry and thus may not provide information about solubility control or the chemical speciation. The success in correlating the extracted metal concentrations with plant uptake is thus not always satisfactory (Schnug et al., 1996; Feng et al., 2005a, 2005b; Menzies et al., 2006; Meers et al., 2007b). Water extractable fraction of trace elements is considered to be similar to concentration found in the soil solution from where trace elements are most easily available to the plant (Seguin et al., 2003, Almås et al., 2005). The solubility control of trace elements is mainly controlled by the total amount of trace elements in soil, total soil organic matter (SOM), soil pH and dissolved organic matter (DOM) (McBride et al., 1997; Tipping et al., 2003).

Trace element risk assessment around Europe is based on different extraction methods, and the legislation varies between countries (Meers et al., 2007a). In Croatia the legislation for maximum permissible concentrations (MPC) for agricultural fields is based on the total content of trace element determined by concentrated acid dissolution-aqua regia (Official Gazette, 1992).

The main objectives of this study are: (1) to create a comprehensive overview of the soils of Danube basin in Croatia, with regard to water extractable and total content of trace elements, (2) to examine the relationship between trace element solubility and soil properties such as soil pH, soil organic carbon (SOC), dissolved organic carbon (DOC), cation exchange capacity (CEC) and total content of trace elements (TOT TE) and (3) to examine the concentrations of water extractable trace elements under two different land uses (forest and agriculture). Such overview can be used as a baseline for further research of soil contamination, soil–plant relationship, influence of different fertilization methods and ultimately of soil quality. Maps will be created with the use of GIS technique to visualize the distribution of water extractable trace elements in the area.

We hypothesize that the water extractable concentrations of the investigated trace elements is controlled by soil pH, SOC, DOC, CEC and total content of trace elements (TOT TE) in the soil.

Materials and methods

Study area

Osijek-Baranja County covers 4144 km^2. It is a flat area in eastern Croatia, part of the Pannonian valley that stretches through Hungary, Serbia and Croatia. Elevation is around 90 m above mean sea level. The climate is continental with hot and arid summers and cold winters. Average annual temperature is 11 °C, while the average annual precipitation is 650 mm. The heaviest periods of precipitation are in the spring and autumn (Ministry of Environmental Protection, 2010).

The county consists of nine main soil types, i.e. Stagnosols, Dystric Cambisols, Luvisols, Haplic Gleysols, Chernozems, Fluvisols, Eutric Cambisols, Mollic Gleysols and Anthrosols (FAO, 2006). Soil samples were taken from all soil types. Since some of them cover a small and insignificant area, the number of samples varied among soil types. Soil samples were mainly collected from arable land (45), pastures (8) and forest land (21). Forest soils were mainly 80-year-old oak forest with very little human activity, so forest was considered as a natural site without human influence (i.e. fertilizers, cultivation). In order to investigate the impact of possible metal contamination through fertilization and cultivation practices in agricultural soils, agricultural sites were compared with forest sites.

Soil sampling and analysis

Based on the pedological map, 74 sites were randomly chosen so that all soil types and different land uses were included (arable land, pasture and forest). From each site 10 subsamples from 0–25 cm depth (hereafter called surface soil) were taken within 5 m distance from each other and then combined into one sample of approximately 500 g. Samples were dried and sieved through the 2-mm sieve for the determination of soil pH, DOC, CEC and water extractable Cd, Co, Mn, Mo, Ni, Pb and Fe. For soil organic carbon (SOC) and TOT TE, samples were further ground to finer particles. The concentration of SOC was determined by a dry combustion method on Leco Carbon Determinator EC12 (Nelson & Sommers, 1982). Soil pH was determined in soil to water solution ratio of 1:2.5 (Mc Lean, 1982) and CEC by barium chloride method with 3 g of soil and 40 mL of 0.1 M $BaCl_2$ making the soil to solution ration 1:13 (Hendershot & Duquette, 1986). The amount of dissolved organic carbon (in water) was determined by the use of a Shimadzu TOC-5000 analyser. The soil particle-size distribution was measured by a pipette method (Elonen, 1971).

Water extractable trace elements and total content of trace elements

Four grams of air-dry soil were weighed into 50 mL centrifuge tubes and 40 mL ultra pure water (MilliQ H_2O, electric conductivity < 18.2 MΩ cm^{-1}) was added. The tubes were then shaken on a linear shaker for 2 days and centrifuged at centrifugal force of 1409 g for 30 min. The suspension was filled in high density poly propylene (HDPP) syringes and filtered through 0.45 μm polyethersulfone membrane filters to poly propylene (PP) test tubes. One mL of ultra pure concentrated HNO_3 was added to the water extract before the analysis to prevent precipitation in test tubes. The filters and the test-tubes were provided by VWR International (www.vwr.com). These tubes are lidded when leaving the producer and not opened before filtration. Blanks of MilliQ H_2O were stored in PP tubes for 48 h and filtered through the same membrane filters before analysis. Two sets of 7 mL water-extracts were prepared, one was used for the determination of metal concentrations and another was for determination of DOC by a Shimadzu TOC-5000 analyser (Shimadzu Scientific, Colombia) and the remaining solution was used for the determination of solution pH.

Total trace element content of solid soil was determined using microwave digestion by stepwise heating and drying material with concentrated ultra pure HNO_3 up to 250 °C under high pressure using a Milestone Ultra clave for 1 h and 15 min. Approximately 0.33 g of soil was mixed with 5 mL of ultra pure concentrated HNO_3 making the soil:solution ratio 1:15.

The element concentrations in water and acid extracts were determined using two different analysers, a Perkin Elmer Sciex Elan Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was used for trace elements with low concentrations and a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES) for trace elements with higher concentrations. Blanks and reference material were used. Standard reference material (SRM) for soils was the SRM 2709 (National Institute of Standards & Technology, 2003), whereas the SRM for water analysis was the SRM 1643e (National Institute of Standards & Technology, 2004).

Data analysis

Descriptive statistics, analysis of variance (ANOVA), Tukey pairwise comparison of means, simple correlation, regression, and principal component analysis were conducted using Minitab® Statistical Software version 15 (2007). Analysis of variance and Tukey pairwise comparison was done among soil types and land uses to determine significant differences and influences of different soil types and different land uses on the water extractable trace elements and the soil properties.

Pearson correlation coefficients were determined by correlating water extractable trace elements with pH, SOC, DOC, CEC and the TOT TE. Multiple regression analysis was also used to derive best-fit models for water extractable trace elements.

Finally, GIS technique was used to create maps of the area and to visualize the results. The maps were created in ArcGis version 9.2. (2006), software that combines table data with spatial data allowing us more comprehensive insight into a certain area (Hutchinson & David, 2000).

Results and discussion

Soil properties

The soil samples were analysed for the following parameters: pH, CEC, DOC and SOC. The results were also analysed for their relations to land use and soil types. Descriptive statistics for all 74 sites showed that pH varied from 4.3–8 (average 6.3), DOC 6.1–73 mg l^{-1} (average 26 mg l^{-1}), SOC 0.46–5.08% (average 1.71%) and CEC 1.3–24 cmol kg^{-1} (average 9 cmol kg^{-1}) (Tables I and II). No significant difference was found between pasture and arable land, but the differences between forest and arable land and forest and pasture were significant (for pH, SOC and DOC $p < 0.001$ and for CEC $p < 0.05$). Therefore, in the presentation of results,

Table I. Soil pH, SOC, DOC and CEC for different land use (n = 74).

	Land use	n	Mean	S.D.	Min.	Max.
pH	Agri. field	53	6.8	1.003	4.3	8.02
	Forest	21	5.2	0.825	4.4	7.40
	All	74	6.3***	1.180	4.3	8.02
SOC (%)	Agri. field	53	1.5	0.7	0.46	4.4
	Forest	21	2.3	0.8	0.94	5.1
	All	74	1.7***	0.8	0.46	5.1
DOC ($mg\ l^{-1}$)	Agri. field	53	16.3	4.8	6.1	33.1
	Forest	21	50.5	12.9	23.8	73.0
	All	74	26.0***	17.4	6.1	73.0
CEC ($cmol\ kg^{-1}$)	Agri. field	53	9.6	0.045	2.5	21
	Forest	21	7.0	0.058	1.3	24
	All	74	9.0*	0.050	1.3	24

SOC – Soil organic carbon, DOC – Dissolved organic carbon, CEC – Cation exchange capacity.

* and *** indicate significant difference between land uses at $p < 0.05$ and $p < 0.001$, respectively.

pasture and arable land were combined together into one land use, *agricultural field*. Forest soils showed much lower pH, higher SOC and DOC and slightly lower CEC than the soils from agricultural fields (Table I). Forest is a natural site with much higher organic matter input than agricultural field which explains the higher levels of SOC and DOC (Post & Kwon, 2000), while higher pH values in agricultural fields, compared to forest soils, could be assigned to liming application.

Out of the nine soil types in the area, four of them cover only very small parts of the area whereas the other five cover very large areas in comparison. When examining the impact of the different soil types on the trace element concentration, only the main five soil types were investigated; namely Stagnosols, Dystric Cambisols, Luvisols, Haplic Gleysols and Chernozems. Soil texture analysis showed that Stagnosols, Dystric Cambisols and Luvisols are silt loam soils with average 24% clay while Haplic Gleysols and Chernozems are silty clay loam soils with average 34% clay. These five soil types represent 80% of the area and account for 60 out of the 74 samples. The pH, DOC, SOC and CEC were significantly different between the soil types. Tukey pairwise t-test was used to partition differences between soil types for each soil parameter (Table II). The Chernozems had the highest pH (6.4–7.9 with an average of 7.2) and as such was statistically different from all other soil types except Haplic Gleysols soils. However, all of the Chernozems sites were agricultural fields, which can explain high pH supposedly due to liming. The SOC and DOC contents were lowest in the Luvisols and Chernozems. However, these two soil types were mainly used for agricultural practices and as was shown earlier agricultural fields contain significantly lower contents of SOC and DOC ($p < 0.001$). We suggest that this is connected to the export of harvested crops. Nevertheless, the difference among other three soil types, that have sampling sites from both land uses, imply that soil type can also be an indicator of difference in SOC and DOC. Cation exchange capacity (CEC) was highest for Haplic Gleysols soil which is statistically different from all other soil types except Chernozems. These two soil types are silty clay loam soils with significantly higher content of clay (34%) compared with the other three soil types (24%) which can explain the higher CEC as silty clay loam has significantly higher CEC (Table III). There is also a visible difference between the northern and southern part of the county in the soil parameters. In the northern part of the area, soils with high pH and low organic matter content are dominating soil types (Figure 1). These differences are related to different soil types in these parts: the northern soils are dominated by silty clay loam rich

Table II. Soil pH, SOC, DOC and CEC for different soil types (n = 60).

	Soil type	n	Mean	S.D.	Min.	Max.
pH	DC	8	5.7^{ac}	1.2	4.4	7.6
	GLha	18	6.8^{ab}	1.0	4.9	7.8
	LV	16	5.8^{c}	0.9	4.3	7.8
	ST	12	5.4^{c}	0.9	4.3	7.4
	CH	6	7.2^{b}	0.6	6.4	7.9
	All	60	6.1^{***}	1.1	4.3	7.9
SOC (%)	DC	8	1.9^{ab}	0.76	0.76	2.87
	GLha	18	2.5^{a}	1.09	1.14	5.08
	LV	16	1.0^{c}	0.15	0.78	1.27
	ST	12	1.7^{bc}	0.55	0.88	2.52
	CH	6	1.4^{bc}	0.27	1.05	1.68
	All	60	1.7^{***}	0.89	0.76	5.08
DOC ($mg\ l^{-1}$)	DC	8	32.6^{ab}	21.3	10.5	63.9
	GLha	18	34.9^{a}	22.6	12.6	73.0
	LV	16	18.3^{b}	5.0	11.4	26.5
	ST	12	35.0^{ab}	15.8	15.1	57.3
	CH	6	13.3^{ab}	1.9	11.3	16.2
	All	60	28^{**}	18.1	10.5	73.0
CEC ($cmol\ kg^{-1}$)	DC	8	7^{ac}	0.04	2	13
	GLha	18	14^{b}	0.04	5	24
	LV	16	5^{c}	0.02	2	10
	ST	12	4^{c}	0.03	1	12
	CH	6	11^{ab}	0.02	8	13
	All	60	8^{***}	0.05	1	24

SOC – Soil organic carbon, DOC – Dissolved organic carbon, CEC – Cation exchange capacity, DC – Dystric Cambisols, LV – Luvisols, ST – Stagnosols, CH – Chernozems, GLha – Haplic Gleysols.

*, ** and *** indicate significant difference between soil types at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

a, b and c indicate groups with no significant difference among them.

Table III. pH, TOC, DOC and CEC for different soil texture (n = 60).

	Soil texture	n	Mean	S.D.	Min.	Max.
pH	Silt loam	36	5.6	0.988	4.3	7.8
	Silty clay loam	24	6.9	0.925	4.9	7.9
	All	60	6.1***	1.1	4.3	7.9
SOC (%)	Silt loam	36	1.4	0.61	0.76	2.8
	Silty clay loam	24	2.2	1.06	1.05	5.1
	All	60	1.7***	0.89	0.76	5.1
DOC (mg l^{-1})	Silt loam	36	27.02	15.62	10.5	63.9
	Silty clay loam	24	29.53	21.67	11.3	73.0
	All	60	28.0^{ns}	18.1	10.5	73.0
CEC (mol kg^{-1})	Silt loam	36	0.053	0.029	0.01	0.13
	Silty clay loam	24	0.129	0.041	0.05	0.24
	All	60	0.08***	0.05	0.01	0.24

SOC – Soil organic carbon, DOC – Dissolved organic carbon, CEC – Cation exchange capacity.

* and *** indicate significant difference between land uses at $p < 0.05$ and $p < 0.001$ respectively.

Chernozems and Haplic Gleysols soils while the southern soil types are dominated by silty loam rich Stagnosols and Luvisols soil types.

Water extractable concentrations of trace elements

On average less than 0.5% of the TOT TE in the soil were extracted by water. Water extractable concentrations of Cd, Co, Mn, Mo, Ni, Pb and Fe are presented in Tables IV and V. The two tables are designed to show the impact of different land use and soil types, respectively. Land use was found to have significant influence on the water extractability of all the examined trace elements except Fe. In water extracts from the forest soils, the concentrations were significantly higher compared with extracts from the agricultural fields: Co ($p < 0.001$), Mn ($p < 0.001$), Ni ($p < 0.01$), Cd ($p < 0.001$), Pb ($p < 0.01$), while significantly lower for Mo ($p < 0.05$). Such behaviour of water extractable trace elements is most likely related to soil pH and DOC. As mentioned earlier, soil properties significantly differ among land uses and several authors showed that soluble concentrations of trace elements are controlled by soil properties mainly pH, SOC and DOC (McBride et al., 1997; Tipping et al., 2003; Strobel et al., 2004).

On the other hand, impact of soil types was only significant for the water extractable Pb, Fe and Mo (Table V). The concentration of Pb in water extracts was significant at $p < 0.001$, whereas the concentrations of Fe and Mo were significant at $p < 0.05$ between soil types. For the impact of soil texture on the metal extractability, we grouped the texture classes in two: namely silt loam and silty clay loam. We found that these two texture classes have significant difference for the water extractable Fe, Mo and Pb ($p < 0.001$); Mn, Ni and Cd ($p < 0.01$); Co ($p < 0.05$). Difference in soil texture was, however, statistically correlated with soil pH and CEC (Table III). This shows that although the metal extractability can be connected to soil texture using soil maps, the actual difference is determined by the soil properties such as pH and CEC.

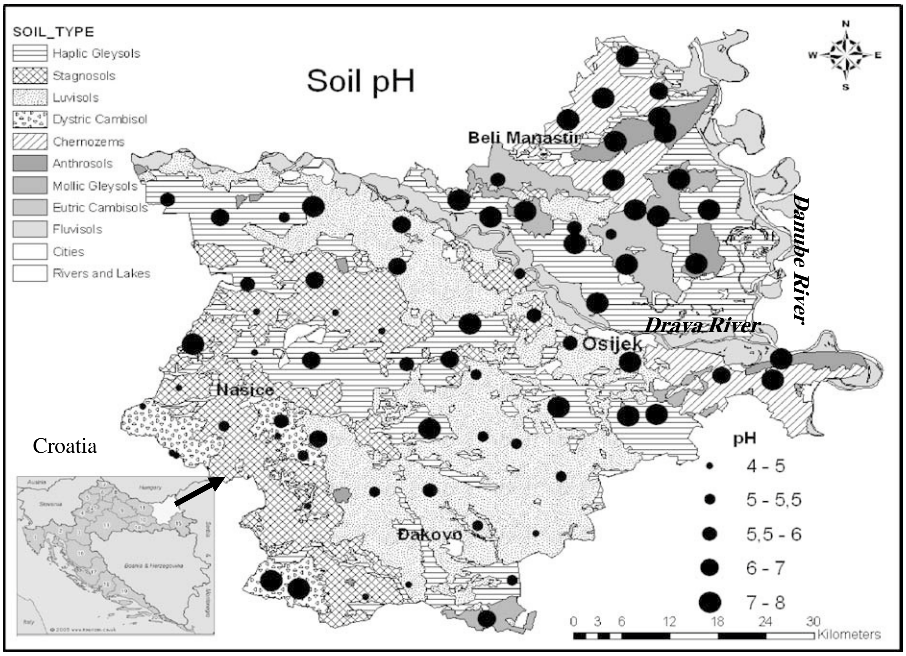

Figure 1. Map of soil pH in the Osijek-Baranja County.

Table IV. Descriptive statistics for water extractable trace elements under different land uses (n = 74).

	Land use	n	Mean	S.D.	Min.	Max.
Fe	Agri. field	53	20.13	29.29	0.36	125.3
mg kg^{-1}	Forest	21	20.17	15.37	0.61	69.4
	All	74	20.14^{ns}	26.00	0.36	125.3
Co	Agri. field	53	0.009	0.019	0.0009	0.14
mg kg^{-1}	Forest	21	0.034	0.026	0.003	0.13
	All	74	0.016^{***}	0.024	0.0009	0.14
Mn	Agri. field	53	0.9	2.9	0.004	20.89
mg kg^{-1}	Forest	21	4.3	4.1	0.096	18.15
	All	74	1.86^{***}	3.6	0.004	20.89
Ni	Agri. field	53	0.06	0.044	0.012	0.18
mg kg^{-1}	Forest	21	0.09	0.040	0.032	0.18
	All	74	0.07^{**}	0.045	0.012	0.18
Mo	Agri. field	53	0.012	0.014	0.0006	0.083
mg kg^{-1}	Forest	21	0.005	0.010	0.0008	0.048
	All	74	0.01^{*}	0.013	0.0006	0.083
Cd	Agri. field	53	0.0006	0.001	0.00003	0.007
mg kg^{-1}	Forest	21	0.0019	0.0008	0.00086	0.004
	All	74	0.0009^{***}	0.001	0.00003	0.007
Pb	Agri. field	53	0.008	0.009	0.0004	0.042
mg kg^{-1}	Forest	21	0.015	0.010	0.0010	0.038
	All	74	0.010^{**}	0.010	0.0004	0.042

Fe – Iron, Co – Cobalt, Mn – Manganese, Ni – Nickel, Mo – Molybdenum, Cd – Cadmium, Pb – Lead.
*, ** and *** indicate significant difference between land uses at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively, ns stands for not significant.

Table V. Water extractable trace elements for different soil types (n = 60).

	Soil type	n	Mean	S.D.	Min.	Max.
Fe	DC	8	22.98^{ab}	22.61	0.39	67.89
mg kg^{-1}	GLha	18	6.92^{a}	8.92	0.39	32.34
	LV	16	30.34^{b}	26.90	0.39	88.06
	ST	12	30.92^{b}	22.80	1.19	83.27
	CH	6	17.80^{ab}	28.5	0.60	69.70
	All	60	21.19^{*}	23.22	0.39	88.06
Co	DC	8	0.030	0.041	0.001	0.13
mg kg^{-1}	GLha	18	0.010	0.012	0.001	0.04
	LV	16	0.021	0.032	0.001	0.14
	ST	12	0.029	0.019	0.001	0.06
	CH	6	0.004	0.004	0.001	0.01
	All	60	0.019^{ns}	0.026	0.001	0.14
Mn	DC	8	4.82	6.52	0.010	18.15
mg kg^{-1}	GLha	18	0.55	0.75	0.006	2.28
	LV	16	2.76	5.03	0.004	20.89
	ST	12	3.19	2.39	0.018	6.34
	CH	6	0.17	0.26	0.008	0.62
	All	60	2.20^{ns}	3.89	0.004	20.89
Ni	DC	8	0.09	0.05	0.03	0.19
mg kg^{-1}	GLha	18	0.06	0.03	0.02	0.11
	LV	16	0.09	0.05	0.02	0.19
	ST	12	0.08	0.03	0.02	0.12
	CH	6	0.06	0.04	0.02	0.13
	All	60	0.07^{ns}	0.04	0.02	0.19
Mo	DC	8	0.005^{ab}	0.007	0.001	0.019
mg kg^{-1}	GLha	18	0.018^{a}	0.021	0.001	0.084
	LV	16	0.005^{b}	0.008	0.0006	0.029
	ST	12	0.002^{b}	0.002	0.0008	0.009
	CH	6	0.010^{ab}	0.007	0.002	0.019
	All	60	0.009^{*}	0.014	0.0006	0.084
Cd	DC	8	0.0015	0.0015	0.00009	0.0040
mg kg^{-1}	GLha	18	0.0006	0.0006	0.00015	0.0022
	LV	16	0.0013	0.0016	0.00009	0.0069
	ST	12	0.0014	0.0009	0.00014	0.0030
	CH	6	0.0002	0.0002	0.00003	0.0005
	All	60	0.0011	0.0012	0.00003	0.0069
Pb	DC	8	0.012^{ab}	0.008	0.0004	0.023
mg kg^{-1}	GLha	18	0.004^{a}	0.004	0.0008	0.015
	LV	16	0.012^{ab}	0.008	0.0005	0.029
	ST	12	0.018^{b}	0.010	0.0006	0.038
	CH	6	0.007^{a}	0.011	0.0006	0.026
	All	60	0.010^{***}	0.009	0.0004	0.038

Fe – Iron, Co – Cobalt, Mn – Manganese, Ni – Nickel, Mo – Molybdenum, Cd – Cadmium, Pb – Lead, DC – Dystric Cambisols, LV – Luvisols, ST – Stagnosols, CH – Chernozems, GLha – Haplic Gleysols.
*, ** and *** indicate significant difference between soil types at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively while ns stands for not significant.
a, b and c indicate groups with no significant difference among them.

Total content of trace elements

Total trace element content (TOT TE) was determined for six elements Cd, Co, Mn, Ni, Pb and Fe by ultra pure concentrated HNO_3 digestion. The determination of Molybdenum (Mo) after HNO_3 digestion is incomplete (< 15% compared with digestion of reference soils) and hence not considered in the discussion of TOT TE here. Land use and soil types were evaluated as possible external factors affecting the TOT TE content in soils over the whole sampling area. Unlike water extractable trace elements the TOT TE was found to be more dependent on soil types than land use (Tables VI and VII). Land use had only significant impact on the total content of Pb ($p < 0.001$), where the higher content was found in forest soils compared with agricultural soils while Ni content was higher ($p < 0.05$) in agricultural soils compared with forest soil (Table VI). No significant impact of land use on total metal content of all other investigated trace elements (Fe, Mn, Co and Cd) indicates no, or insignificant, influence of agriculture (Table VI). Soil type, on the other hand, had significant ($p < 0.001$) impact on the total content of all the investigated trace elements. Soil types significantly differ in the content of a certain element (Fe, Co, Mn, Ni and Pb ($p < 0.001$) and Cd ($p < 0.01$)). Using Tukey pairwise t-test, Dystric Cambisols generally contain the highest metal contents (Table VII). This soil type is mostly found at the very south of the county, in the hilly areas. It is the soil

Table VI. Descriptive statistics for total content of trace elements for different land use (n = 74).

	Land use	n	Mean	S.D.	Min.	Max.	MPC
TOT Fe	Agri. field	53	29736	3423	23909	40221	
mg kg^{-1}	Forest	21	27769	5624	19642	41034	
	All	74	29178^{ns}	4220	19642	41034	
TOT Co	Agri. field	53	12.5	1.69	9.1	16.7	50
mg kg^{-1}	Forest	21	12.3	2.90	6.1	17.9	
	All	74	12.5^{ns}	2.08	6.1	17.9	
TOT Mn	Agri. field	53	639	200.6	237.8	1144	
mg kg^{-1}	Forest	21	713	299.5	218.5	1459	
	All	74	660^{ns}	233.2	218.5	1459	
TOT Ni	Agri. field	53	34.35	6.60	21.24	54.19	50–60
mg kg^{-1}	Forest	21	29.98	10.38	13.55	52.43	
	All	74	33.11*	8.03	13.55	54.19	
TOT Cd	Agri. field	53	0.23	0.07	0.10	0.40	1–2
mg kg^{-1}	Forest	21	0.23	0.12	0.11	0.68	
	All	74	0.23^{ns}	0.09	0.10	0.68	
TOT Pb	Agri. field	53	18.25	2.54	10.39	25.94	100–150
mg kg^{-1}	Forest	21	22.85	3.41	19.26	31.77	
	All	74	19.55***	3.48	10.39	31.77	

Fe – Iron, Co – Cobalt, Mn – Manganese, Ni – Nickel, Mo – Molybdenum, Cd – Cadmium, Pb – Lead.
* and *** indicate significant difference between land uses at $p < 0.05$ and $p < 0.001$ respectively while ns stands for not significant.
MPC – maximum permissible concentrations.

type most remote from the river and the parent material differs the most from other soil types. Soil texture also indicates significant influence on total metal content for several investigated elements: Fe ($p < 0.001$), Mn, Ni and Cd ($p < 0.01$). This suggests that total metal content in soil is related to parent material rather than to human activities.

The maximum permissible concentration (MPC) for agricultural fields prescribed by the Croatian government in the Official Gazette (1992) is displayed in the last column of Tables VI and VII. Compared with these standards, the TOT TE contents in soils from this County are below MPC. Moreover, the total content of the essential trace elements Fe, Mn, Mo and Ni, were found to be within the average normal range for soils in Europe (Havlin et al., 2005). This suggests that, if we are considering TOT TE as our criteria, there is no deficiency problem either. However, soil properties such as pH, DOC, SOC and CEC and the crop grown should also be considered when making the assumption on deficiency.

Relationship between soil properties and water extractable trace elements

Trace element solubility in soils is mostly controlled by pH, DOC, SOC, CEC and TOT TE concentration (Seguin et al., 2003; Tipping et al., 2003; Strobel et al., 2004; Almås et al., 2007). A correlation matrix (Table VIII) shows that the water extractable concentrations of the investigated trace elements were most strongly correlated with pH, DOC and CEC (Figures 2–4). No correlation was found between water extractable trace elements and the TOT TE or SOC. This could be connected to the small variation in TOT TE between sites whereas the variation in soil pH on the other hand was much larger. Hence soil pH correlated relatively much stronger with water extractable trace elements.

Principal component analysis (PCA) indicates that water extractable trace elements can be grouped in three groups. The first group would be Co, Mn and Cd, the second Ni, Fe and Pb, and the third group would be Mo alone.

- Water extractable concentrations of elements from the first group and two from second (Co, Mn, Cd, Ni and Pb) were negatively correlated with pH ($p < 0.001$) and with CEC ($p < 0.001$), but positively correlated with DOC ($p < 0.001$) (Table VIII). We can also see that not all functions are linear especially for Co, Cd, Mn and Mo with pH and CEC (Figures 2–4). None of the trace elements was correlated with SOC or TOT TE (except Mn which showed a correlation with total Mn ($p < 0.05$)). There was also significant ($p < 0.001$, $p < 0.01$) covariance among all investigated water extractable elements of first and second group (with

Table VII. Total content of trace elements for different soil types (n = 60).

	Soil type	n	Mean	S.D.	Min	Max	MPC
TOT Fe mg kg^{-1}	DC	8	29638^{ab}	3854	23801	34188	
	GLha	18	32826^{a}	4020	27901	41034	
	LV	16	27492^{b}	2573	24079	33647	
	ST	12	25843^{b}	4660	19642	35362	
	CH	6	29348^{ab}	1493	27682	31579	
	All	60	29234***	4411	19642	41034	
TOT Co mg kg^{-1}	DC	8	15.4^{a}	1.72	12.8	17.9	
	GLha	18	12.5^{b}	1.96	9.6	16.4	
	LV	16	12.4^{b}	1.59	9.9	16.0	
	ST	12	11.1^{b}	2.72	6.1	15.5	
	CH	6	12.3^{b}	0.20	12.0	12.5	
	All	60	12.6***	2.25	6.1	17.9	50
TOT Mn mg kg^{-1}	DC	8	1022^{a}	256.0	713.3	1459.1	
	GLha	18	499^{c}	170.7	218.5	809.7	
	LV	16	744^{b}	119.0	435.6	918.2	
	ST	12	622^{bc}	197.1	368.1	1056.6	
	CH	6	802^{ab}	49.0	722.4	874.0	
	All	60	689***	237.2	218.5	1459.1	
TOT Ni mg kg^{-1}	DC	8	40.0^{a}	12.10	18.68	54.20	
	GLha	18	36.3^{a}	6.11	24.73	47.11	
	LV	16	28.5^{bc}	4.14	21.61	35.04	
	ST	12	24.5^{c}	6.18	13.55	34.13	
	CH	6	35.4^{ab}	1.34	32.76	36.54	
	All	60	32.3***	8.38	13.55	54.20	50–60
TOT Cd mg kg^{-1}	DC	8	0.22^{ab}	0.09	0.10	0.34	
	GLha	18	0.27^{a}	0.12	0.13	0.68	
	LV	16	0.20^{ab}	0.06	0.12	0.34	
	ST	12	0.16^{b}	0.04	0.11	0.21	
	CH	6	0.25^{ab}	0.02	0.22	0.27	
	All	60	0.22**	0.09	0.10	0.68	1–2
TOT Pb mg kg^{-1}	DC	8	22.96^{a}	4.25	16.78	29.36	
	GLha	18	20.78^{a}	3.27	16.52	26.31	
	LV	16	17.91^{b}	1.24	15.22	20.27	
	ST	12	19.90^{ab}	1.94	17.06	23.58	
	CH	6	17.24^{b}	0.76	16.00	18.04	
	All	60	19.77***	3.10	15.22	29.36	100–150

Fe – Iron, Co – Cobalt, Mn – Manganese, Ni – Nickel, Mo – Molybdenum, Cd – Cadmium, Pb – Lead, DC –Dystric Cambisols, LV – Luvisols, ST – Stagnosols, CH – Chernozems, GLha – Haplic Gleysols, MPC – maximum permissible concentrations.

** and *** indicate significant difference between soil types at $p < 0.01$ and $p < 0.001$ respectively.

a, b and c indicate groups with no significant difference among them.

exception of Fe which had no covariance with Co, Mn and Cd) (Table VIII).

- Iron (Fe), which represents the second group of PCA, was similarly negatively correlated with pH ($p < 0.001$) and CEC ($p < 0.001$) as the other trace elements, but unlike the other trace elements it was also correlated with SOC ($p < 0.01$). We found no correlation of water extractable Fe with TOT Fe or DOC (Table VIII).
- Molybdenum, element representing the third group of PCA, was different. The anionic Mo oxide (most likely Molybdate, MoO4-) was found to be increasingly soluble with increasing pH ($p < 0.001$), CEC ($p < 0.001$) and SOC ($p < 0.001$) (Table VIII). The positive correlation between Mo and CEC is suggested to be related to pH dependent charges in soils (McBride, 1994).

The correlations between water extractable trace elements and soil chemical properties can be related to land use. As can be seen from Table IV, the concentration of water extractable trace elements was always higher in forest soils, which were acidic with higher DOC contents in water extracts. As shown earlier, DOC was positively correlated with water extractable Co, Mn, Cd, Ni, Mo and Pb. Hence it is likely that changes in soil pH or DOC will have an impact on the trace element

Table VIII. Correlation matrix of water extractable trace elements and soil properties.

	pH	SOC	DOC	CEC	Fe	Co	Mn	Ni	Mo	Cd
SOC	0.03^{ns}									
DOC	-0.63^{***}	0.56^{***}								
CEC	0.71^{***}	0.57^{***}	-0.13^{ns}							
Fe	-0.47^{***}	-0.33^{**}	0.01^{ns}	-0.48^{***}						
Co	-0.71^{***}	0.07^{ns}	0.55^{***}	-0.52^{***}	0.18^{ns}					
Mn	-0.65^{***}	0.06^{ns}	0.45^{***}	-0.49^{***}	0.05^{ns}	0.93^{***}				
Ni	-0.69^{***}	0.05^{ns}	0.43^{***}	-0.43^{***}	0.70^{***}	0.58^{***}	0.49^{***}			
Mo	0.63^{***}	0.49^{***}	-0.21^{ns}	0.69^{***}	-0.43^{***}	-0.36^{**}	-0.32^{**}	-0.35^{**}		
Cd	-0.77^{***}	0.10^{ns}	0.57^{***}	-0.53^{***}	0.13^{ns}	0.92^{***}	0.92^{***}	0.56^{***}	-0.37^{**}	
Pb	-0.66^{***}	-0.16^{ns}	0.30^{**}	-0.61^{***}	0.86^{***}	0.44^{***}	0.28^{**}	0.75^{***}	-0.48^{***}	0.37^{**}

SOC – Soil organic carbon, DOC – Dissolved organic carbon, CEC – Cation exchange capacity, Fe – Iron, Co – Cobalt, Mn – Manganese, Ni – Nickel, Mo – Molybdenum, Cd – Cadmium, Pb – Lead.
ns stands for not significant; *Correlation significant at $p < 0.05$; **Correlation significant at $p < 0.01$; ***Correlation significant at $p < 0.001$.

concentrations in soil solution. Therefore, changing the land use from agricultural land to forest land could enhance the solubility of trace elements as other investigations have shown that such changes in land use reduced the pH and increased the DOC concentrations (Strobel et al., 2004).

Multiple regression analysis

Stepwise multiple regression analysis was carried out to estimate the solubility control determined by multiple soil physicochemical properties of the investigated soils. All of the values were logarithmically transformed. The impact of soil pH, DOC, SOC, CEC and TOT TE were tested. The soil pH was the strongest predictor and by itself it gave high r^2 values of regression equation. Soil pH explains more than 80% of the extractable Cd, Co, Mn and Mo variation, but only 60% of the variation in Fe, Pb and Ni solubility. In addition to pH, DOC was used as a predictor for Cd and Co, SOC for Fe and Mo, TOT TE for Ni and Cd while CEC just for Cd. Cation exchange capacity probably did not contribute to the improvement of the regression model as it was pH dependent. Adding additional variables (DOC, CEC and TOT Cd for cadmium, DOC for cobalt, SOC for iron and molybdenum and TOT Ni for nickel) to existing pH regression equation resulted in increased r^2 from 82 to 89 for Cd, from 90 to 91 for Co, from 58 to 63 for Fe, from 81 to 88 for Mo and from 59 to 64 for Ni. Manganese and lead regression equation gave the best results with only pH.

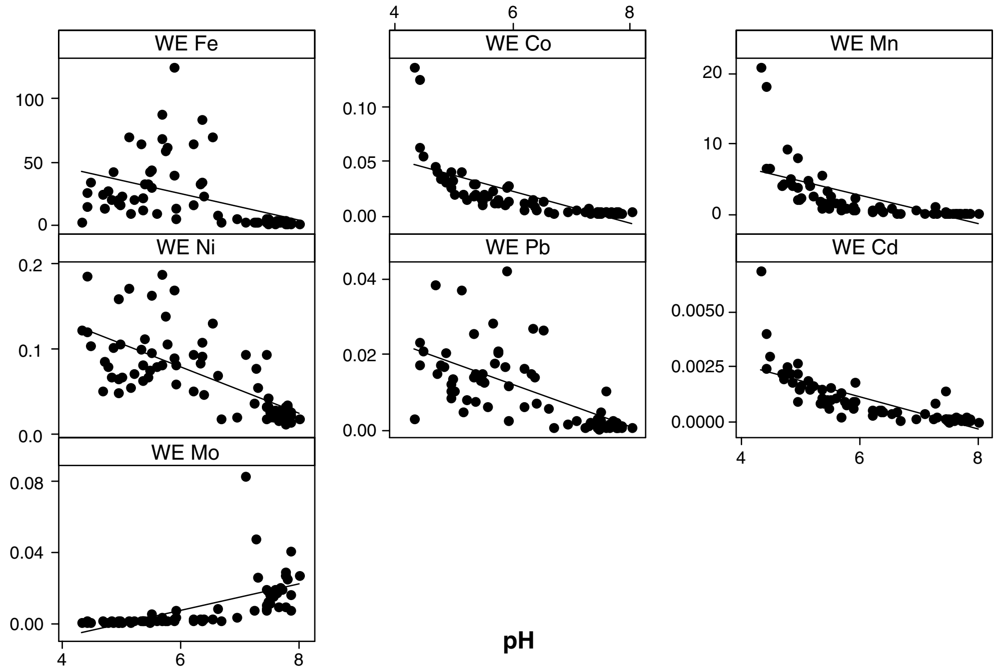

Figure 2. Relationship of soil pH and WE Fe, Co, Mn, Ni, Cd, Pb and Mo.

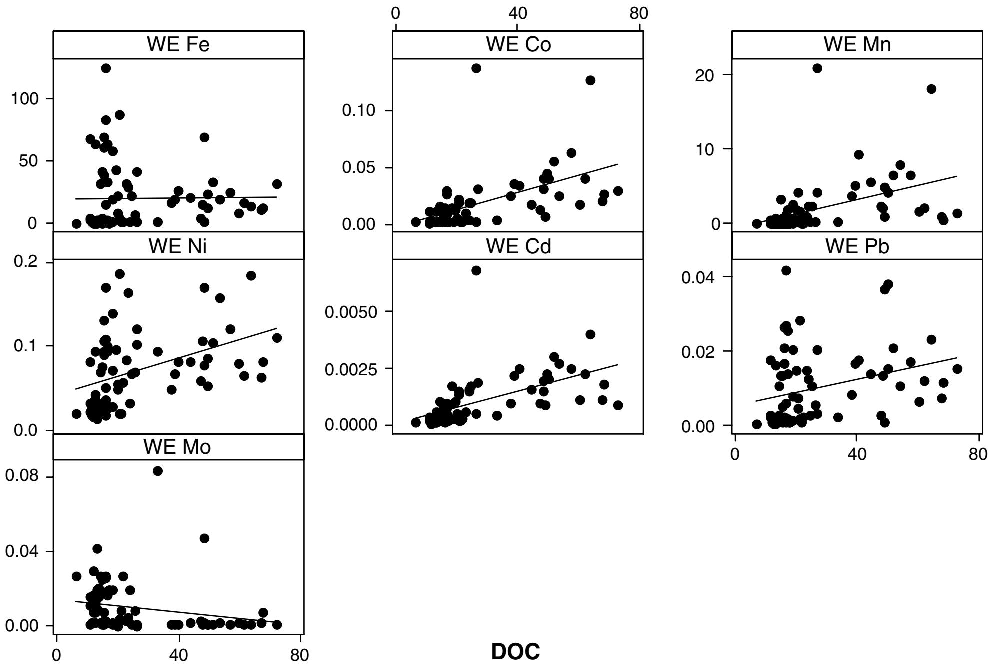

Figure 3. Relationship of DOC and WE Fe, Co, Mn, Ni, Cd, Pb and Mo.

As shown earlier, TOT TE was not significantly correlated with the water extractability of trace elements because the variation of TOT TE among sites was small, however we believe it should be included in the equations as the total amount determines the concentration level of trace elements in solution. McBride et al. (1997) found SOM, pH and TOT TE to be significant whereas Tipping et al. (2003) found loss on ignition (LOI), pH, DOC and TOT TE to be significant. Such regression equations have also been developed for describing the free metal ion concentration in soil solution, highly relevant for suggesting critical limits for trace elements in soils and soil solutions (Lofts et al., 2004; de Vries et al., 2007).

GIS mapping of the area

A comprehensive overview of the distribution of water extractable trace elements in the area is presented by maps. In the creation of these maps

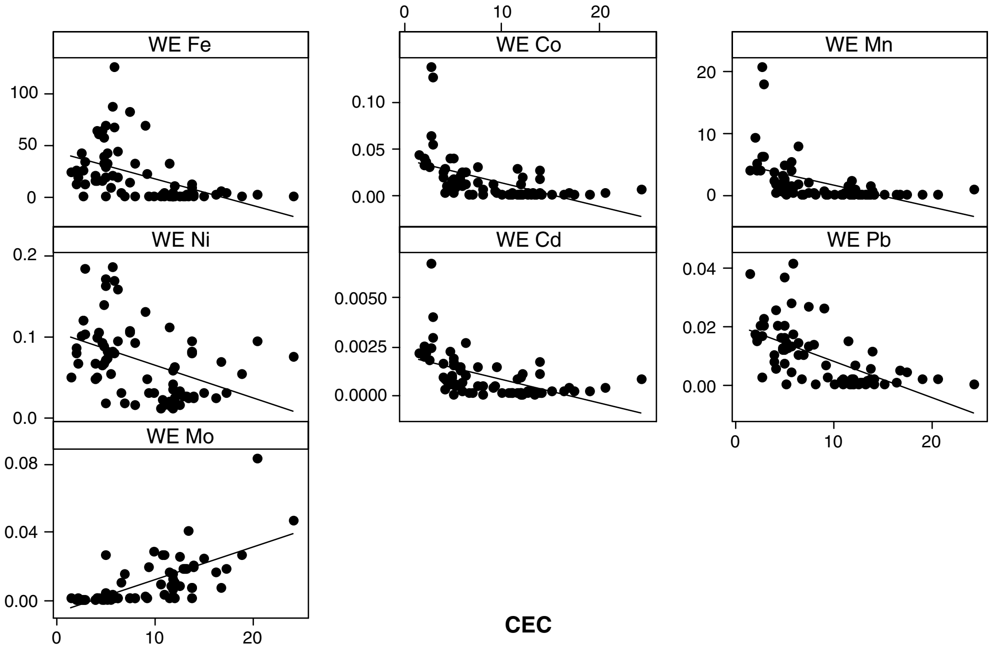

Figure 4. Relationship of CEC and WE Fe, Co, Mn, Ni, Cd, Pb and Mo.

GIS technique was used (ArcGis, 2006) so that each site is easily available for the purpose of future measurements and tracking changes. This approach will be of benefit for the future research since each sampling site was recorded by GPS, future changes in heavy metal concentrations can be monitored and source, location and extent of heavy metal impact can be identified (Bolstad, 2008). This will especially contribute when examining the changes due to the development of a new highway that will pass through the area from north to south and most likely will have an impact on agricultural fields in its vicinity.

The maps indicate the difference between the northern (above the Drava River) and southern part (below the Drava River). From the map of soil pH (Figure 1) it can be seen that the lower pH is in the southern part of the County and maps of water extractable Co, Cd and Mn are showing highest concentrations in southern part. The map also shows that the water extractability of Mo was highest in northern part, where pH was highest (Figure 5). These maps imply that higher pH causes higher concentrations of water extractable Mo while for Co, Cd and Mn the situation is opposite, lower pH causes higher water extractable concentrations which is in the accordance with the statistical analysis. Soil maps containing information about standard soil chemical parameters as soil pH, DOC, CEC etc. can be used to pinpoint areas of favourable conditions for increased metal solubility in soils. If information about TOT TE exists, the solubility of trace elements can be estimated using optimised regression models where the impact of the most important soil chemical parameters is described (Tipping et al., 2003).

According to governmental maximum permissible concentration (MPC) levels for trace elements, the soils of Croatian main agricultural region have shown no reason for alarm. Total content of toxic and potentially toxic trace elements are way below the governmental tolerance limits (MPC), at the same time total content of the micronutrients has also shown to be above the deficiency levels. However, the fraction of trace elements in the water

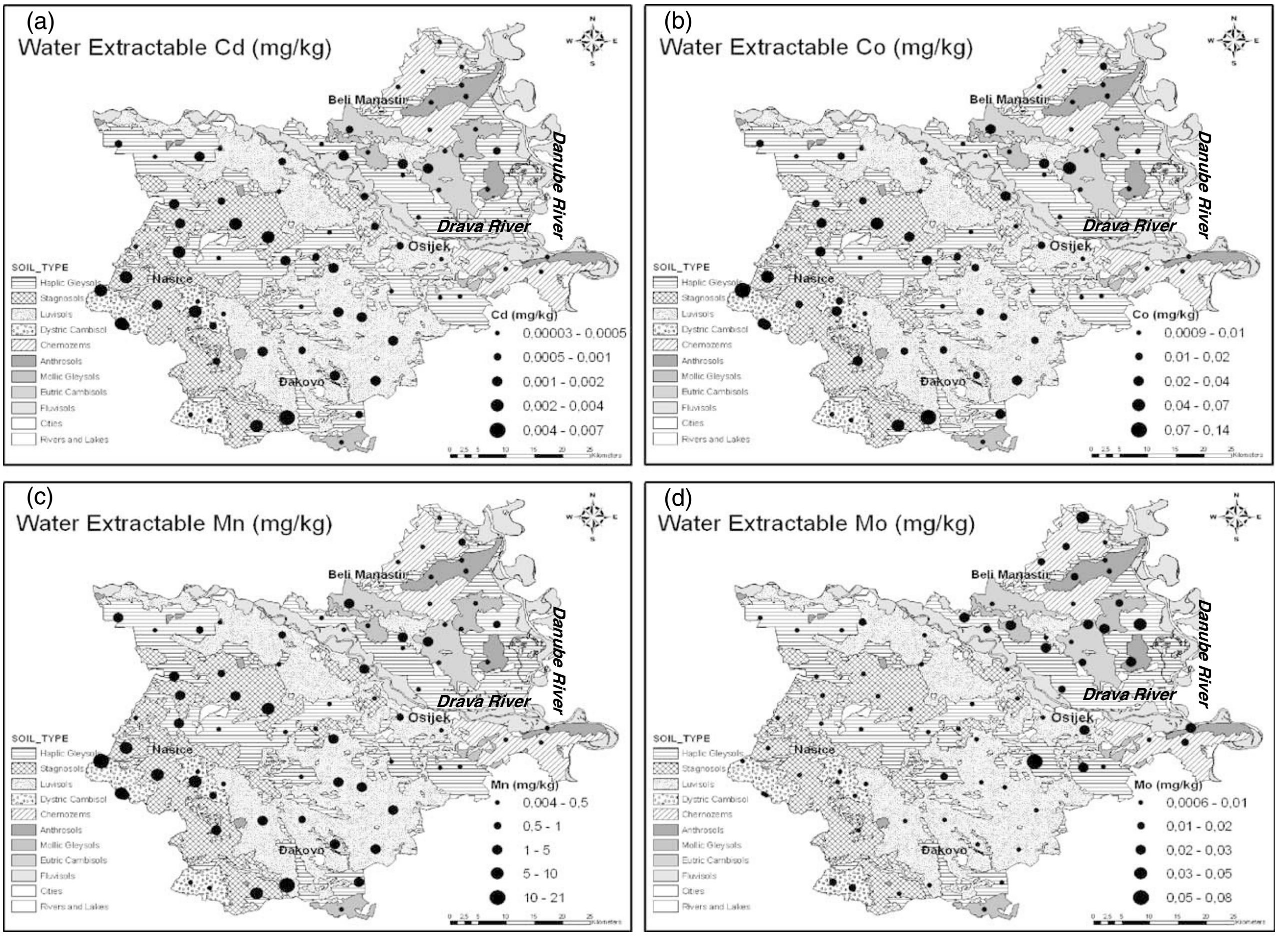

Figure 5. Maps of water extractable Cd (mg kg^{-1}) (a), Co (mg kg^{-1}) (b), Mn (mg kg^{-1}) (c) and Mo (mg kg^{-1}) (d) in Osijek-Baranja County.

solution, the fraction that is readily available, has shown not to be dependent on the total content but rather on soil properties such as pH, DOC and CEC. This suggests that the total content of trace elements is not a good indicator of their availability to plants. It seems that pH, DOC and CEC play a more important role in understanding the behaviour of trace elements in the soil solution of Danube basin in eastern Croatia than the total content. Therefore, when addressing the soil quality of the region, whether with regard to contamination by trace elements or deficiency of certain micronutrients, soil properties should be taken into consideration.

As land use had no impact on total trace element content we can conclude that trace elements in this area are not being excessively imported by agricultural activities. However, the difference of water extractable trace element concentrations between two land uses (forest and agriculture) indicate higher levels in the agriculture soils, suggesting that agricultural practices are causing higher levels of available trace elements in the soil solution than the forest soils do. Therefore, land use can be used to say something about the extent of the trace elements in the soil solution and we can assume that a land use change will result in a change in trace element solubility as well. However, this is mainly due to the fact that different land use has significant differences in the pH or DOC which are the main parameters driving the release of trace elements into the soil solution. In case of a land use change, multiple regression models can be used to estimate the solubility of certain elements and predict their availability.

Acknowledgements

This research was conducted by the financial support from 'Project 5: improving nutritional quality and safety of food and fodder crops in South Eastern Europe (SEE) countries', Institutional collaboration between Norwegian University of Life Sciences and Faculty of Agriculture in Osijek, Croatia. The authors wish to thank the project for the financial support. We also express our gratitude to the department of forest management at 'Croatian Forest Ltd.' for their tremendous help with mapping of the area. Finally, the first author is grateful to the Norwegian State Educational Loan Fund (Statens Lånekasse for utdanning) for providing financial assistance.

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Paper 2

Ivezić, V., Almås. Å.R., Singh, B.R., (2011b.) Predicting the solubility of Cd, Cu, Pb and Zn in uncontaminated Croatian soils under different land uses by applying established regression models. *Geoderma* (in press)

Predicting the solubility of Cd, Cu, Pb and Zn in uncontaminated Croatian soils under different land uses by applying established regression models.

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Abstract

Soil pH, soil organic matter (SOM), dissolved organic carbon (DOC) and total trace metal concentration ($M_{(tot)}$) control the solubility of metals in the soil. Several regression models have included these soil chemical variables for the prediction of metal solubility and free metal ion (FMI) concentrations in contaminated soils. Soils samples were collected from agricultural and forest soils located in eastern Croatia and extracted with water to determine the concentrations of Cd, Cu, Pb and Zn. We used these data to test the applicability of three regression models on existing conditions under different land uses. The same predictors issued in the three models and the same regression coefficients were utilized in the present study. The results showed a good correlation between the observed and predicted values of metal solubility. However, the models overestimate the total solution concentration ($M_{(sol)}$) and the concentrations of free metal ions (FMI) in solution, and therefore the same regression coefficients were optimized to fit our own observations. This was found to be very successful. The results showed that pH and DOC played a very important role in controlling metal solubility, while SOM and CEC were somewhat less significant. The impact of total soil concentration of metals ($M_{(tot)}$) was rather minor. However, we feel that to carry out good predictions of $M_{(sol)}$ and FMI, the $M_{(tot)}$ is needed in such regression models.

Keywords: free metal ion, modeling, solubility, trace metals

1. Introduction

Total concentrations of trace metals in soil, by themselves, are not good indicators of their bioavailability, yet they are commonly used for the determination of maximum permissible levels in the legislation of many countries. However, information about the total concentrations in solid soil together with selected controlling soil chemical parameters is used for estimating free metal ion (FMI) concentrations in the soil solution. Such use of combined soil chemical information has shown to be a good indicator of trace metal availability (Almås et al., 2005; Lofts et al., 2004; Sauvé et al., 2000b). The chemical speciation of trace metals controls their mobility and bioavailability. At the immediate interface between solid soil and soil solution, the physicochemical properties controlling cation exchange and metal binding to solid soil matter or different metal-binding ligands in solution have a dynamic impact on the fate of metal concentration and its activity in soil solution (Almås et al., 2007; Shuman, 1991; van Hullebusch et al., 2005).

Several authors have examined the controlling variables influencing metal solubility in soils and expressed the correlations by regression models (Groenenberg et al., 2009; Gandois et al., 2010; McBride et al., 1997; Sauvé et al., 2000a; Tipping et al., 2003). Such regression models usually include total concentration of trace metals ($M_{(tot)}$), soil pH and soil organic matter (SOM). Additionally, the impacts of clay content, Fe and Al oxides, DOM and CEC have also been reported as significant variables (Gandois et al., 2010; Janssen et al., 1997; Smolders et al., 2009). Recent studies have shown a good correlation between FMI concentration of trace metals in soil solution and content of trace metals taken up by the plant (Almås et al., 2005; Lofts et al., 2004). To calculate the chemical speciation of metals in solution we applied the WHAM/Model VI version 6.0 for waters (Tipping, 1998).

However, most of these studies are focused on soils containing rather high metal concentrations when predicting the solubility. Assessing the availability of trace metals is an issue, not only from a contamination point of view, but also in situations of deficiency in agricultural systems. Very few predictive regression models have used data obtained from unpolluted or slightly polluted sites (Meers et al., 2005).

Our hypothesis is that regression models developed for other terrestrial ecosystems can be applied to predict metal solubility in Croatian soils under different land uses. To test this hypothesis we have applied our data directly to some of the most commonly cited and used regression models. Thereafter, we have optimized the same model parameters to fit the observed metal concentrations in soil solution from Croatian soils to investigate possible improvements. We have applied three well-known regression models which have performed well under different soil conditions elsewhere. For example, Tipping et al. (2003) developed a model based on organic soils of Wales and England. McBride et al. (1997) developed a model for contaminated soils of Western Europe and Canada, while Meers et al. (2005) examined soil-solution speciation of Cd in unpolluted and polluted soils. Hereafter, these studies will be referred to as Study 1 (Tipping et al., 2003), Study 2 (McBride et al., 1997) and Study 3 (Meers et al., 2005). Therefore, the main objective of this study is to test the above mentioned models for their efficiency in predicting the solubility of metal in unpolluted agricultural and forest soils, and if necessary to optimize the existing models by using the soil data of the present study.

2. Materials and methods

2.1. Sample collection

Soil samples were collected from an unpolluted area of the Danube basin in eastern Croatia. All 74 samples were collected from the depth of 0-25 cm (hereafter called surface soil). Two different land uses (forest and arable land) representing 9 different soil types (Stagnosols, Dystric Cambisols, Luvisols, Haplic Gleysols, Chernozems, Fluvisols, Eutric Cambisols, Mollic Gleysols and Anthrosols) were included in the study (FAO, 2006).

From each site, 10 subsamples from surface soil were taken within a 5 m distance and then combined into one composite sample of approximately 500 g. Samples were dried and sieved through a 2-mm mesh for the determination of soil pH, dissolved organic carbon (DOC), CEC and water extractable Cd, Cu, Pb and Zn. For the determination of soil organic carbon (SOC) and total metal concentration in soil (M_{tot}), samples were further ground to finer particle size using agate mortar.

2.2. Soil analysis

The concentration of SOC was determined by a dry combustion method on a Leco Carbon Determinator EC12 (Nelson and Sommers, 1982). Soil organic matter (SOM) is suggested to contain 50% carbon. Soil pH was determined in soil to water solution ratio of

1:2.5 (Mc Lean, 1982) and CEC by the barium chloride method where 3 g soil was added to 40 mL of 0.1 M $BaCl_2$ making the soil to solution ration 1:13 (Hendershot and Duquette, 1986). The amount of dissolved organic carbon (DOC) in water was determined using a Shimadzu TOC-5000 analyzer (Shimadzu Scientific, Colombia). As with SOM, we suggest dissolved organic matter (DOM) is composed of 50% organic C. The soil particle-size distribution was determined by the pipette method (Elonen, 1971).

2.3. Total and water extractable metals in soils

Total metal concentration in soil (M_{tot}) was determined after digesting the soil in concentrated ultra pure HNO_3 (1:15 solid:solution ratio) by stepwise heating up to 250°C using a Milestone Ultra CLAVE for 1 hr and 15 min.

Four grams air-dried soil was added to 40 mL ultra pure water (MilliQ H_2O, electric conductivity < 18.2 $M\Omega\ cm^{-1}$), shaken in high density polyethylene (HDPE) centrifugation tubes for two days and then centrifuged at 1400 x g for 30 min. The suspension was filled in high density polypropylene (HDPP) syringes and filtered through 0.45 μm polyethersulfone membrane filters to polypropylene (PP) test tubes. The filters and the test tubes were provided by VWR International (vwr.com). Blanks of MilliQ H_2O were stored in PP tubes for 48 hr and filtered through the same membrane filters before analysis. Two sets of 7 mL water-extracts were prepared, one was used for the determination of trace metal concentrations and another 7 mL aliquot was prepared for determination of DOC by the Shimadzu TOC-5000 analyzer and the remaining solution for the determination of solution pH. There was no significant difference between soil pH measured before the treatment by shaker and centrifugation and the pH from the solution after the treatment.

The concentrations of metals either in acid digestion or in extractable solution were determined using a Perkin Elmer Sciex Elan Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for Cd and Pb and a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES) for Zn and Cu. Blanks and reference material were used for both determinations. Standard reference material (SRM) for soils was the SRM 2709 (National Institute of Standards & Technology, 2003), whereas the SRM for water analysis was the SRM 1643e (National Institute of Standards & Technology, 2004).

2.4. Chemical speciation of soil solution

Chemical speciation of soil solution was estimated using WHAM-VI version 6.0 for waters (Tipping, 1998). The input data was: soil solution pH, fulvic acid (FA) concentration, the solution concentration of Al, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. Intitially we tested whether the oxidation state of dissolved Fe had an effect on metal binding. Since soils taken from some sites have pH > 7, clay content > 40 % and periodically are subject to seasonal flooding, a fraction of total Fe in solution was defined as Fe(II). The metal binding to FA was not increased when 20 % of Fe was defined as Fe(II). Obviously the effect of Fe(II) or Fe(III) partitioning did not affect the regression model optimizations. We also experimented with the fraction of activity of metal binding acids on DOM. As a default it is recommended to define 50 % of the organic acids being active in protonation and metal binding. We defined the metal binding activity being 50 %, 65 % or 80 % of total, but found no effect. Thus we tested the regression model parameter correlations with our data, estimating FMI when 50 % of DOM was chemically active FA.

2.5. Statistical analysis

Descriptive statistics, simple correlation analysis and regression analysis were performed using Minitab® Statistical Software version 15 (2007). Analysis of variance and Tukey pairwise comparison were performed between the land uses to determine significant differences among soil properties under different land uses.

Pearson correlation coefficients were determined by correlating water extractable trace metals and free metal ion concentrations (FMI) with pH, SOM, DOC, CEC and the $M_{(tot)}$. Multiple regression analysis was also used to derive best-fit models and compare it with existing models for the concentration of elements in the soil solution and free metal ion concentrations.

3. Results

3.1. Soil properties (pH, DOC, SOM, CEC, $M_{(tot)}$)

Soil samples were collected from agricultural fields and forest land. We obtained information about soil pH, DOC, SOM, CEC and total concentrations of Cd, Cu, Pb and Zn in soil and soil water extracts of 74 different soil samples. The solubility of Cd, Cu, Pb and Zn were also investigated in three comparable studies (McBride et al., 1997; Meers et al., 2005; Tipping et al., 2003). Soil pH in our samples varied from 4.3–8.1 (average 6.3), DOC 6.1 – 73 mg L^{-1} (average 26 mg L^{-1}), SOM 0.9 – 10.2 % (average 3.4 %) and CEC 1.3–24 cmol kg^{-1} (average 9 cmol kg^{-1}). Most of the acidic samples were from the forest soils. The forest soils contained significantly higher SOM and DOC than agricultural soils ($p < 0.001$). Soil pH was strongly correlated with DOC and CEC, but the relationship was negative with DOC ($r^2 = 0.63$) and positive with CEC ($r^2 = 0.71$). By comparing the findings of this study with the three other studies, we see that soils in Study 1 have much higher organic matter content, Study 2 does not provide such data, while Study 3 included CEC in the modeling (unlike Studies 1 and 2) (Table 1). Study 1 measured geochemically active metal concentration by 0.43 mol l^{-1} HNO_3 extraction, Study 2 used acid digestion for total metal concentrations, but it does not specify with what acid, while Study 3 measured total metal concentration in soil by *Aqua regia* digestion. All three studies had significantly higher total, or geochemically active, concentrations of Cd, Cu, Pb and Zn compared to the present study (extracted with ultra pure HNO_3). Even the soils in Study 3 referred to as unpolluted were considerably higher in total metals than the soils in the present study (Table 1).

3.2. Water extractable concentrations

The range of water extractable metal concentration was: 0,003 – 0,69 µg L^{-1} (Avg. 0,09 µg L^{-1}) for Cd; 4,9 – 49,9 µg L^{-1} (Avg. 15,4 µg L^{-1}) for Cu; 0,05 – 4,2 µg L^{-1} (Avg. 0,99 µg L^{-1}) for Pb and 4,1 – 104,9 µg L^{-1} for Zn (Avg. 23,9 µg L^{-1}). The concentrations of Zn were below the detection limit in 32 out of 74 samples (43 %). An average value of detection limit was used for these samples (dl = 8,2 µg L^{-1}). The extracts with concentrations below the detection limit were mainly from calcareous soils with pH > 7. In comparison with Study 1, our samples contained much lower concentrations of metals (Studies 2 and 3 had no data available). In Study 1, Tipping et al. (2003) analyzed pore water for metal concentration. The concentrations from Tipping et al. (2003) are visual estimations from graphic displays, and hence the data are regarded as approximations (Table 1).

Table 1. Soil properties, total metal concentrations and soil solution concentrations of metals in present study and three comparing studies

	Our Study	**Study 1** (Tipping et al. 2003)	**Study 2** (McBride et al. 1997)	**Study3** (Meers et al. 2004)
pH	4.3 – 8.1	3.4 – 8.2	n/a	5.6 – 8.2
SOM (LOI) %	0.9 – 10.2	10 – 90 (LOI)	n/a	0.9 – 12.8
DOC (mg L^{-1})	6 – 73	5 – 500	n/a	20 – 538
CEC *(cmol kg^{-1})*	1.3 - 24	-	n/a	3.7 – 39.2
TOT Cd (mg kg^{-1})	0.1 – 0.68	~ 0.1 – 50	0.1 – 90	0.2 – 41.4
TOT Zn (mg kg^{-1})	52.3 – 122.4	~ 3 – 3500	9 – 2400	18 – 2422
TOT Cu (mg kg^{-1})	5.7 – 141.8	~ 3 – 190	7 – 1000	0 – 231
TOT Pb (mg kg^{-1})	10.4 – 31.7	~ 15 – 2000 *(Max. Outlier ~12000)*	6 – 1100	6 - 655
Solution Cd (μg L^{-1})	0.003 – 0.69	~ 0.1 – 45	-	-
Solution Zn (μg L^{-1})	4.1 – 104.9	~ 6 – 700	-	-
Solution Cu (μg L^{-1})	4.9 – 49.9	~ 4 – 500	-	-
Solution Pb (μg L^{-1})	0.05 – 4.2	~ 0.2 – 1400 *(Max. Outlier ~12000)*	-	-

SOM – Soil organic matter, LOI – Los on ignition, DOC – Dissolved organic carbon, CEC – Cation exchange capaciy

In the present study, water extractable Cd, Pb and Zn were correlated with soil pH, DOC and CEC (p < 0,001), with the exception of Pb with DOC (p < 0,01). Soil pH was negatively correlated and it was the strongest correlated parameter for dissolved Cd, Pb, and Zn: Cd (r^2 = 0.77), Pb (r^2 = 0.66) and Zn (r^2 = 0.80). Unlike Cd, Pb and Zn, Cu showed no correlation with any of the investigated soil parameters. The tested parameters affected the solubility of Cd and Zn more than Pb, and particularly Cu. Soil pH has less effect on Cu partitioning because Cu generally has a strong affinity for soil organic matter, as well as for carbonates in alkaline soils and iron oxides in acid soils (Shuman, 1991).

3.3. Soil-solution partitioning

In addition to simple correlation between trace metals and soil properties, we calculated the soil-solution partitioning coefficient (K_D):

$$K_D = \frac{M_{(tot)}}{M_{(sol)}} \qquad \text{(eq. 1)}$$

Where $M_{(tot)}$ is the total metal concentration in soils and the $M_{(sol)}$ is the water extractable metal concentration or concentration in the soil solution. Partitioning coefficients of all four metals, including Cu, were strongly correlated with soil pH, DOC and CEC ($p < 0.001$). The following equations were obtained from our study:

$$\log K_D\ (Cd) = 2.58 + 0.325\ \text{pH} - 0.431\ \log(\text{DOC}) + 0.383\ \log(\text{CEC}) \qquad (r^2 = 0.91)\ \text{(eq. 2)}$$

$$\log K_D\ (Cu) = 3.98 - 0.596\ \log(\text{DOC}) + 0.205\ \log(\text{CEC}) + 0.483\ \log(\text{SOM}) \quad (r^2 = 0.50)\ \text{(eq. 3)}$$

$$\log K_D\ (Pb) = 1.92 + 0.378\ \text{pH} + 0.561\ \log(\text{SOM}) \qquad (r^2 = 0.63)\ \text{(eq. 4)}$$

$$\log K_D\ (Zn) = 0.349 + 0.541\ \text{pH} + 0.209\ \log(\text{SOM}) \qquad (r^2 = 0.87)\ \text{(eq. 5)}$$

Partitioning of Pb and Zn are best predicted with pH and SOM only (eq. 4 and 5) while the partitioning of Cd is best predicted with pH, DOC and CEC (eq. 2) and Cu with DOC, CEC and SOM (eq. 3). We tested correlation between CEC and SOM and they are highly correlated ($r^2 = 0.54$; $p < 0.001$). The different metals affinities for solid phases affect the development of K_D. For instance, high concentrations of Pb in solution will promote its adsorption and increase the K_D, while for Zn it is opposite (Sauvé et al., 2000b). High Zn concentrations in solution will decrease the K_D since solid phases have lower affinities for Zn. Therefore, it is not recommended to use K_D values obtained from data with high metal concentrations and apply them in modeling of low level conditions (Sauvé et al., 2000b).

3.4. Regression modeling of solution concentration

Most of the published regression equation models have been derived from investigation of soils containing much higher metal concentrations than in our soil samples. We have compared the regression equations from Studies 1, 2 and 3 using the data of the present study.

Soils from Study 1 (Tipping et al., 2003) contained much higher organic matter contents and somewhat higher concentrations of metals (Table 1). Tipping et al. (2003) used SOM, pH, $M_{(tot)}$ and DOC as controlling variables for all four Cd, Cu, Pb and Zn. We applied our data using their model:

$$\log M_{(sol)} = a \log(\mathrm{SOM}) + b\, \mathrm{pH} + c \log M_{(tot)} + d \log(\mathrm{DOC}) + e \quad \text{(eq. 6)}$$

The model was significant for all four metals (p=0,001) (Table 2a). However, the predictions did not follow a 1:1 line, suggesting that the regression model overestimates the trace metal concentrations in soil solution (Figure 1a). After optimizing the variables based on our findings, the regression line followed a 1:1 line, through 0 (Figure 1b). The r^2 values were also improved (Table 2b).

Table 2a and b: Regression model parameters from: a) Tipping et al. eq. and b) optimized parameters for Tipping et al. eq.

2a

	a	***b***	***c***	***d***	***e***	r^2
Cu	-0.30	-0.03	0.38	0.51	-4.50	0.22^{***}
Zn	-0.54	-0.18	0.60	0.39	-1.11	0.80^{***}
Cd	-0.61	-0.20	0.78	0.28	-0.31	0.77^{***}
Pb	-0.47	-0.20	0.89	0.79	-1.23	0.53^{***}

2b

	a	***b***	***c***	***d***	***e***	r^2
Cu	-0.53	-0.02	0.80	0.54	-0.24	0.42^{***}
Zn	-0.48	-0.30	0.04	0.51	2.53	0.83^{***}
Cd	-0.04	-0.38	0.68	0.37	1.13	0.88^{***}
Pb	-0.04	-0.42	-0.70	-0.12	3.48	0.65^{***}

*** indicates significant difference (p<0.001)

In Study 2 (McBride et al., 1997), the predictors were not fixed for all four elements. The authors used those variables that best explained the trace metal concentrations in the solution (Table 3a). General form of the equation was:

$\log M_{(sol)} = a + b\,\mathrm{pH} + c \log M_{(tot)} + d \log(\mathrm{SOM})$ (eq. 7)

We found that this model was also significant for all four metals ($p = 0.001$) (Table 3a). Once again, the predictions did not follow the 1:1 line, resulting in overestimation of metal solubility (Figure 1c). Not all soil chemical parameters are equally represented in all four equations, indicating the solubility of the different metals. For Cu solubility, only the total soil concentration of Cu is included in the model, while for Pb both pH and OM were included. We did not, however, find total Pb as being significant in predicting $Pb_{(sol)}$ (Table 3a). Even so, we believe that the $M_{(tot)}$ is needed for the prediction of $M_{(sol)}$ in other soil systems as reference for metal load in soil. Thus we have included $M_{(tot)}$ as a part of the optimizations using the model from Study 2 for our data. In addition to pH, SOM and $M_{(tot)}$, we also used DOC and CEC, and thus the general equation looks like this:

$\log M_{(sol)} = a + b\,\mathrm{pH} + c \log M_{(tot)} + d \log(\mathrm{SOM}) + e \log(\mathrm{DOC}) + f \log(\mathrm{CEC})$ (eq. 8)

As in Study 2, different variables were significant for predicting the solution concentrations of the four metals (Figure 1d). Soil pH was a significant predictor for Cd, Zn and Pb, SOM for Zn and Cu, DOC for Cd, Zn and Cu, and CEC only for Cd (Table 3b). The best fit for Pb solubility was obtained when only pH and $M_{(tot)}$ were included. For the prediction of Cd solubility, pH, DOC, $M_{(tot)}$ and CEC were significant.

Table 3 a and b: Regression model parameters from: a) McBide et al. eq and b) Best fit model. eq parameters

3a

	a	*b*	*c*	*d*	r^2
Cu	-0.05	-	0.76	-	0.17^{***}
Zn	4.44	-0,71	0.68	-	0.82^{***}
Cd	3.62	-0,50	0.96	-0.45	0.82^{***}
Pb	-0.34	-0,15	-	0.61	0.36^{***}

3b

	a	*b*	*c*	*d*	*e*	*f*	r^2
Cu	-0.45	-	0.77	-0.62	0.65	-	0.42^{***}
Zn	2.53	-0.30	0.04	-0.48	0.51	-	0.83^{***}
Cd	0.97	-0.32	0.84	-	0.46	-0.33	0.89^{***}
Pb	3.56	-0.41	-0.98	-	-	-	0.65^{***}

*** indicates significant difference ($p<0.001$)

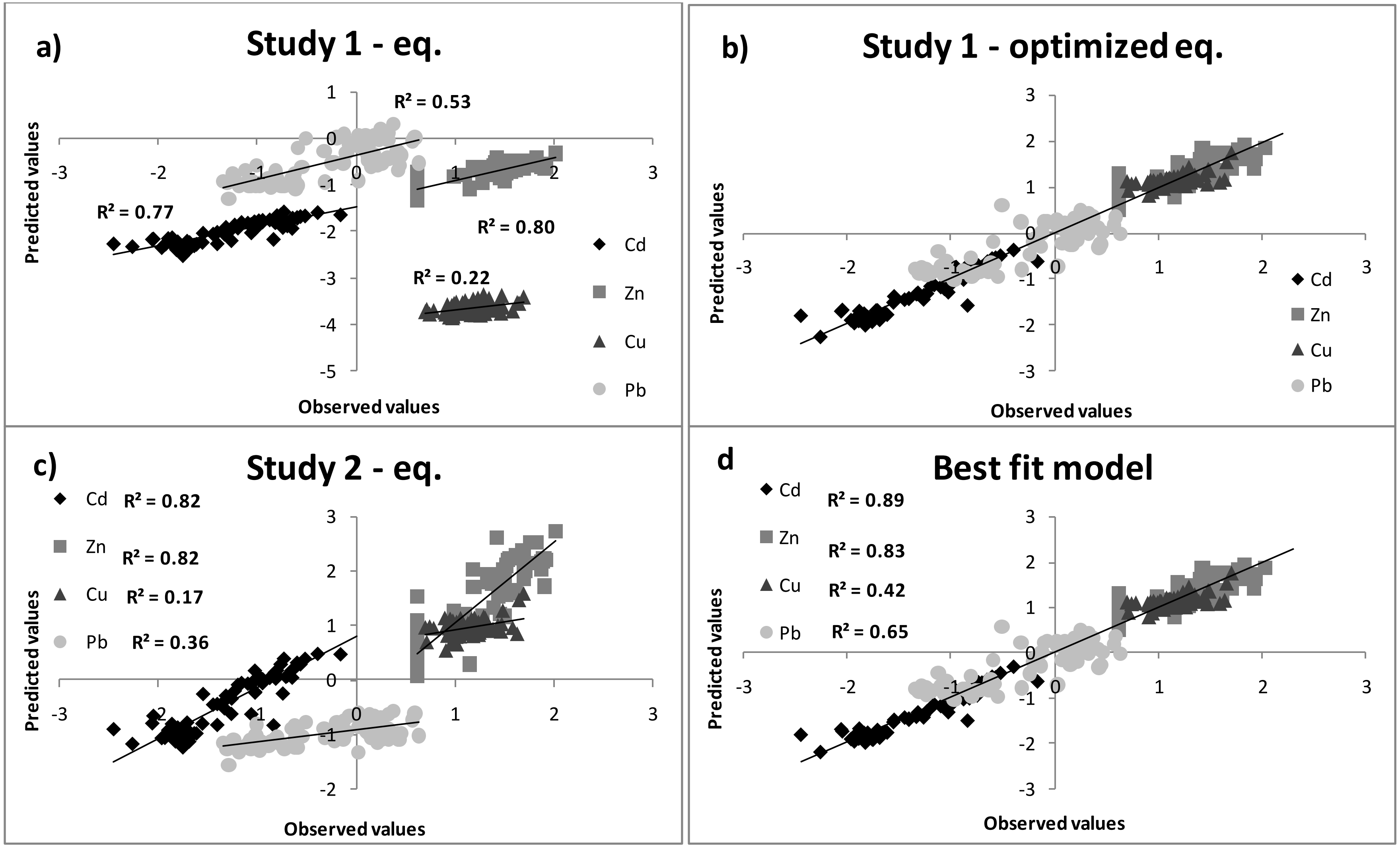

Fig. 1 (a, b, c and d)- Regression line graph of predicted and observed values of trace metals in solution, using parameters from Study 1 (a), optimizing parameters from Study 1 (b), using parameters from Study 2 (c) and using best fit model parameters (d)

Study 3 predicts only the solubility of Cd. Meers et al. (2005) used various parameters in several equations, however, we focused only on the equation which included CEC (together with pH and $M_{(tot)}$) because CEC showed to be a significant variable for Cd solubility in our model. The equation from Study 3 turned out to be:

$$\log M_{(sol)} = a + b \log M_{(tot)} + c\, \mathrm{pH} + d \log(\mathrm{CEC}) \qquad \text{(eq. 9)}$$

Applying our data directly in the original equation overestimated the Cd concentration in soil solution, although the prediction was highly significant ($p = 0.001$) (Table 4, Figure 2). After optimization of the model to our data set, the Cd solubility estimation and r^2 were improved slightly (Table 4).

Table 4: Regression model parameters from Meers et al. and optimized parameters for Meers et al. eq.

	a	***b***	***c***	***d***	r^2
Cd (Study 3)	3.79	0.82	-0.42	-0.47	0.85^{***}
Cd (optimized eq.)	2.23	0.99	-0.43	-0.14	0.87^{***}

*** indicates significant difference (p<0.001)

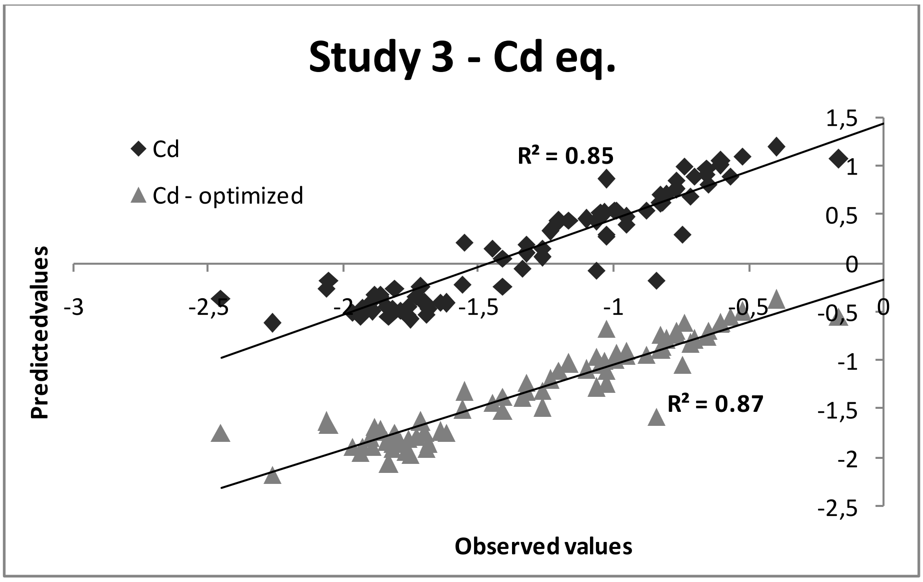

Fig. 2 - Regression line graph of predicted and observed values of Cd in solution using parameters from Study 3 and using optimized model parameters

3.5. Regression modeling of free metal ion concentrations (FMI)

Free metal ion (FMI) concentrations in soil solution are key parameters for assessing metal uptake in living organisms. Even the biological ligand model (BLM) leans on information about the FMI of the metal of interest. Many authors have reported a good correlation between FMI concentration in solution and plant uptake (Sauvé et al., 2000b; Lofts et al., 2004; Almås et al., 2005). In Studies 1 and 3, solution speciation of trace metals were conducted using WHAM Model VI (Tipping, 1998), while Study 2 used ion selective electrodes to determine the FMI concentrations. In the present study we used WHAM Model VI as in Studies 1 and 3. We have compared regression models for predicting FMI concentrations by applying our data in suitable equations, as reported in Studies 1 and 3. No such information was available in Study 2. In Study 1, the same three parameters: SOM (in their case loss on ignition (LOI)), pH and $M_{(tot)}$, were initially tested for predicting FMI concentrations for all four metals. Dissolved organic carbon (DOC) was included in the WHAM VI model so it was omitted from the regression equation:

$$\log (M^{2+}) = a \log (SOM) + b \text{ pH} + c \log M_{(tot)} + d \quad \text{(eq. 10)}$$

In our data set, pH was significantly important for all four metals, SOM for Cu and Pb, and $M_{(TOT)}$ for Cd and Cu. The r^2 values were highly significant (Cd = 0,79; Cu = 0,80; Pb = 0,81; Zn = 0,80). However, just as in the case of solution concentration, the default regression equation overestimated the FMI concentrations (Figure 3a). After optimization, the FMI predictions and WHAM calculated FMI followed a 1:1 line through 0 and the r^2 increased slightly (Figure 3b, Table 5).

Study 3 examined only FMI concentrations of Cd and found the same predictors as in Study 1:

$$\log (Cd^{2+}) = -\ 1.32 \log (SOM) – 0.99 \text{ pH} + 1.24 \log Cd_{(tot)} + 4.03 \quad \text{(eq. 11)}$$

By applying our data to the default equation from Study 3, the predicted values were still overestimated (about 5 times higher than the WHAM modeled FMI concentrations), but not as much as in Study 1 (Figure 4).

Table 5: Regression model parameters for FMI concentrations from Tipping et al. eq. (A) and optimized parameters for Tipping et al. eq. (B)

A)	*a*	*b*	*c*	*d*	r^2	**B)**	*a*	*b*	*c*	*d*	r^2
Cu	-0.40	-1.15	0.54	1.34	0.80^{***}	**Cu**	-1.45	-0.91	0.90	-3.04	0.85^{***}
Zn	-0.61	-0.36	0.73	1.19	0.80^{***}	**Zn**	-0.15	-0.42	0.06	-4.19	0.84^{***}
Cd	-0.69	-0.42	0.97	2.63	0.79^{***}	**Cd**	0.18	-0.46	0.64	-6.10	0.88^{***}
Pb	-0.53	-1.14	0.87	3.51	0.80^{***}	**Pb**	-1.13	0.88	-2.08	0.88	0.85^{***}

*** indicates significant difference ($p<0.001$)

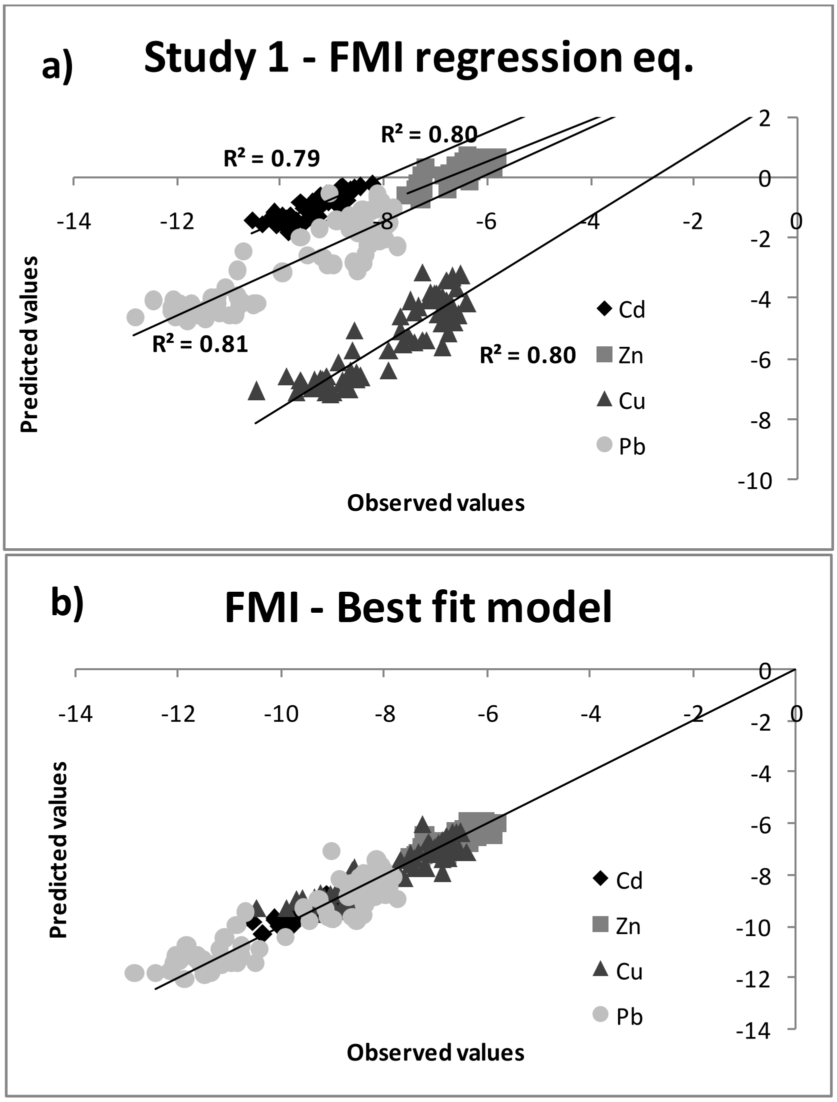

Fig. 3 a and b - Regression line graph of predicted and observed values of FMI concentrations, a) using parameters from Study 1 and b) optimizing parameters from Study 1

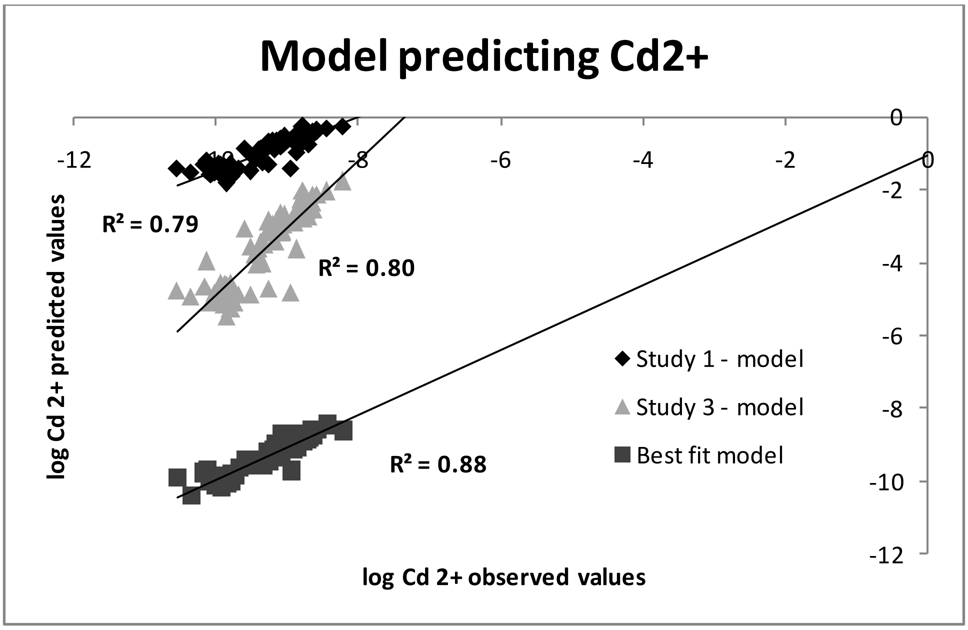

Fig. 4 - Regression line graph of predicted and observed values of Cd^{2+} concentrations using parameters from Study 1, Study 3 and best fit model

4. Discussion

Availability of trace metals depends on concentrations and chemical speciation of metals in soil solution (Allen, 1993). The physicochemical properties of soils control the metal solubility and FMI concentration. In our study, pH and DOC were the most significant parameters determining total solution concentrations and the FMI concentrations of the metals investigated. Soil pH is highly important as it has major impact on most of the chemical species in soil solution (McLaughlin et al., 1998; Rieuwerts et al., 2005; Shuman, 1991). Dissolved organic carbon (DOC) binds dissolved metal in soil solution and by that increases the metal solubility (Sauvé et al., 2000b). In our study, we found that FA-bound Cd and Zn were insignificant as only 9 % of Cd and 7 % of Zn out of total dissolved Cd and Zn was associated with FA. This is not surprising as Cd and Zn are less prone to bind strongly to FA. On the other hand, we found that Cu and Pb were both 66 % bound to FA in soil water extracts. Soil pH and DOC were highly correlated. The more acid soils contained higher DOC concentrations. Thus, it is difficult to separate the influence of pH from DOC on the observed metal solubility. Cation exchange capacity (CEC), soil organic matter (SOM) and $M_{(tot)}$ were statistically significant for regression equations of only some of the metals. However, most of the existing regression models include $M_{(tot)}$ (McBride et al., 1997; Sauvé et al., 2000a; Tipping et al., 2003). In the present study, $M_{(tot)}$ concentrations were very low and variations among sites were small, and therefore $M_{(tot)}$ concentrations were not statistically significant for the model as was also reported by Watmough et al. (2004). Despite this anomaly, $M_{(tot)}$ is needed if such models are to be used for the prediction of $M_{(sol)}$ in soils.

Regression models have proved to be an efficient tool in predicting metal solubility. Most of the models have pH, OM and $M_{(tot)}$ as a core of the equation. In some cases, using just one parameter already gives us a good correlation of metal solubility (Sauvé et al., 1997), while in others, several parameters seem to be applicable (Janssen et al., 1997; Meers et al., 2005; Tipping et al., 2003) and as such provide good results. The choice of parameter to use can vary among the metals investigated or between the types of soil (organic or mineral). In the present study, OM and $M_{(tot)}$ varied very little among the sites and therefore their influence on solubility was small, unlike the influence of pH, DOC or CEC. There is a strong correlation among pH, DOC and CEC, so it is difficult to separate the effect of one from the other. When deriving regression equations, pH and DOC seem to be the most constant parameters determining the best-fit model. In addition, CEC is significant for modeling Cd solubility. Cation exchange capacity (CEC) is in positive correlation with pH, suggesting that CEC is pH dependent (McBride, 1994). Smolders et al. (2009) also observed positive correlation between pH and CEC in their research on toxicity of trace elements where CEC appeared to be the most significant variable explaining the toxicity.

The controlling variables in the three investigated regression models were also significantly controlling our input data, even without optimizing the equation factors. However, the model predictions did not follow a 1:1 line through 0, and they overestimated the metal solubility. We believe the overestimation is likely explained by the higher metal concentrations defining the premises for regression model developments in Studies 1, 2 and 3. The higher concentration of SOM reported in Study 1 may also have been important since high concentration of SOM causes higher DOC (Gandois et al., 2010). The same results, when comparing models from two different studies, have been observed by others as well (Meers et al., 2005; Meers et al., 2006; Sauvé et al., 2000b; Tipping et al., 2003). Variations among models suggest that there cannot be one universal model (Sauvé et al., 2000b). To adopt a certain model, considerable changes in regression coefficients are required (Meers et al., 2005). Based on this condition, we believe that even if we had applied our data to other similar regression models, the outcome would be an overestimation as long as the equations are based on contaminated soils. It seems, therefore, of great importance to know the premises for predictive models used during risk assessments.

Free metal ion (FMI) concentrations were obtained by WHAM VI model. When our data were applied directly into unchanged regression equations, the controlling variables in the regression equations determined the FMI estimations very well. But, just as with total dissolved metals in solution, Study 1 overestimated the FMI by about 8 times and the equation from Study 3 overestimated FMI by about 5 times (Figure 4). Compared to our data, Study 1 had much higher SOM content and metal concentrations, while only the metal concentrations in soils from Study 3 diverged substantially from our findings. This may partly explain the overestimations, as overestimation occurs when modeling FMI in uncontaminated soils or if the metal extraction methods differ from the ones that were used for developing the model (Groenenberg et al., 2010). Prediction of FMI concentrations is often used for the risk assessment and prediction of critical loads. Estimating risk by FMI concentrations showed better results than making risk assessment from total metal concentration alone (Lofts et al., 2004; Lofts et al., 2005; de Vries et al., 2007). However, FMI concentrations just indicate the readily available species of trace metals but it does not necessarily indicate toxicity (Smolders et al., 2009). Under alkaline soil conditions FMI can underestimate metal toxicity for soil organisms (Gandois, 2010). Therefore, applicability of models varies among investigating areas and optimization of models is required (Sauvé et al., 2000b). In our study, levels of $M_{(tot)}$ and $M_{(sol)}$ are much lower than in previously discussed studies, and their reported metal concentrations do not exceed the governmental maximum permissible levels. Models derived from our study can be important when assessing potential deficiency in contrast to when the issue is to assess toxicity.

5. Conclusion

The present study points out that modeling trace element solubility and FMI concentrations is area specific. Models developed on polluted or even what is considered slightly polluted soils may not be straightforwardly applied to soils with low concentrations of trace metals.

However, some soil parameters appear to be significant, with pH and DOC as the most significant. Therefore, by optimizing the parameters, these models become appropriate and they give satisfactory results when predicting water extractable concentrations and FMI concentrations. Total trace metal concentrations do not play a significant role in unpolluted soils; nevertheless, information on total content is necessary. Modeling availability of trace elements does not have to be applied only in estimating risk assessment of contaminated soils but also in evaluating possible deficiencies. Further research on correlating these prediction models in unpolluted soils with plant uptake would be beneficial.

Acknowledgements

This research was conducted by the financial support from "Project 5: improving nutritional quality and safety of food and fodder crops in South Eastern Europe (SEE) countries", Institutional collaboration between Norwegian University of Life Sciences and Faculty of Agriculture in Osijek, Croatia. The authors wish to thank the project for the financial support. Finally, the first author is grateful to the Norwegian State Educational Loan Fund (Statens Lånekasse for utdanning) for providing financial assistance.

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Paper 3

Ivezić, V., Almås., Å.R., Singh, B.R & Lončarić, Z., (2011c). Prediction of Cd, Cu, Fe, Mn and Zn uptake by plants from soils in Osijek-Baranja County, Croatia. *Journal of Plant Nutrition and Soil Science* (submitted)

Prediction of Cd, Cu, Fe, Mn and Zn uptake by plants from soils in Osijek-Baranja County, Croatia

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Abstract

The chemical speciation of trace metals in soil solution using thermodynamic equilibrium modeling or various analytical methods is demanding on a large scale. Hence chemical simplifications and statistical correlations are often developed to aid reasonable predictions of metal solubility ($M_{(sol)}$) and free ion activity (aM^{Z+}) in terrestrial ecosystems. In the present study we wanted to test if optimized model prediction of $M_{(sol)}$ and aM^{Z+} in soil can estimate concentrations in plants. We have previously optimized a model for the prediction of free metal ion (M^{Z+}) concentrations in soils from 74 sites in the Danube basin of Eastern Croatia using information about total metal concentration (concentrated HNO_3), soil pH, soil organic matter (SOM) and dissolved organic carbon (DOC) for the $M_{(sol)}$ and M^{Z+} concentration modeling (Ivezić et al., 2011b). In this study we report soil chemical characteristics from the same 74 sites and results reported by Lončarić et al. (2010) after an investigation of plant uptake from soils taken from a smaller part of the same sampling area. The soil metal concentrations in this study were obtained using concentrated HNO_3, 0.01 M EDTA or water. Several soil chemical quality parameters and metal extraction combinations were tested for the prediction of $M_{(sol)}$ and aM^{Z+}. The regression models were then correlated with plant concentration data from the Lončarić et al. (2010) dataset. Using the model from Ivezić et al. (2011b) based on 74 samples (forest and agriculture), and optimizing the same model (taking into account only agricultural fields), the predicted $M_{(sol)}$, aM^{Z+} and M^{Z+} concentrations correlated well with Fe, Mn, Zn and Cd concentrations in the wheat grain, but the different extraction methods and parameter optimizations, affected the significance of correlations differently. Based on these tests, we believe that regression models can be used to predict metal concentrations in plants grown on the same soil types fairly well when they are optimized using a limited number of soil chemical parameters for predicting $M_{(sol)}$ and aM^{Z+} in soils of a specific area.

Keywords: availability, modeling, plant uptake, trace metals

1. Introduction

The utilization and management of natural soil resources requires regional and local planning assisted by information about the physicochemical characteristics of the different soil types. Such information is normally limited to soil classification related variables or basic soil analysis required for optimizing fertilization in agriculture. Only occasionally, or from studies of specific sites, has detailed soil chemical information been provided. For assessing risk and bioavailability of trace metals in the aquatic, soil and biological compartments of the ecosystem, chemical speciation of metals is of great importance. The chemical speciation is controlled by soil pH, the reservoir of metals in soils (often referred to as total concentration in soil), the amount and quality of negative surface charges present in the solid soil (organic matter, clay and oxides) and soil solution phases (organic and inorganic anions) (Gandois et al., 2010; Groenenberg et al., 2010; Sauvé et al., 2000a; Tipping et al., 2003). The different biological, hydrological and physical characteristics also affect the metal chemistry in soil to a great extent. The metal bioavailability is most often chemically estimated using dilute neutral salts, water or complexing agents like EDTA and DTPA (Feng et al., 2005a; Manouchehri et al., 2006; Meers et al., 2007; Menzies et al., 2007). A more advanced method is the deployment of Diffusive Gradients in Thin films (DGT), or equally, for estimating the integrated bioavailable metal concentration in humid soils (Almås et al., 2006; Jansen et al., 2003; Zhang et al., 2004). Also the installations of lysimeters in soils, like the Rhizons, have been investigated for evacuating small volumes of pore water *in situ* (Heredia and Cirelli, 2009; Moreno-Jimanez et al., 2011). The chemical analysis of pore water or dilute soil extracts is often completed by chemical speciation of soil solution using thermodynamic equilibrium modeling or various analytical methods. This is a demanding job to obtain on a large scale, and hence chemical simplifications and statistical multivariate analysis are often developed to aid reasonable predictions in metal solubility and speciation in terrestrial ecosystems (Almås et al., 2007; Groenenberg et al., 2010; Sauvé et al., 2000b; Tipping et al., 2003).

We have earlier optimized the Tipping et al. (2003) functions for the prediction of metal solubility and free metal ion activity in soil water extracts of 74 Croatian soils (Ivezić et al., 2011b). The same model parameters as published in Tipping et al. (2003) were used, but the parameter constants had to be optimized as the Tipping et al. (2003) function constants were developed for a different soil system. The optimized regression functions predicted the analyzed total metal concentrations in soil water extracts and the WHAM/Model VI estimated free metal ion (M^{Z+}) concentrations very well. Since intensively used agriculture crops cover the investigated area, the applicability of these soils in providing trace metals in sufficient concentrations is of great practical importance. Therefore, Ivezić et al. (2011b) investigated in more detail the soil chemical parameters for the Danube basin of Eastern Croatia.

In the present study we used metal concentration in wheat grain ($M_{(ObsG)}$) from Lončarić et al. (2010) to optimize multivariate regression models for predicting wheat grain-metal concentration ($M_{(GRAIN)}$). The different optimized models were then tested on samples from the present study, representing a larger area. In addition, we wanted to test how the Ivezić et al. (2011b) model, predicting water extractable fraction ($M_{(sol)}$) and free metal ion concentration (M^{Z+}), correlated with $M_{(ObsG)}$. Alternatively we wanted to test if an optimization of the Ivezić et al. (2011b) model constants would improve the correlation between aM^{Z+} (free ion activity) in soil solution with $M_{(ObsG)}$. Finally, we wanted to test how much a best-fit model, containing other combinations of model parameters, would improve the correlations. Our hypothesis is that multivariate regression models predicting aM^{Z+} of metals in soils, can provide reasonably good estimates of plant uptake of metals on large regional scales. More specifically, we hypothesize that multivariate regression models estimating $M_{(sol)}$, M^{Z+} concentrations or aM^{Z+} will correlate better with $M_{(ObsG)}$ than EDTA extraction alone.

To test this hypothesis we extracted trace metals from 74 soil samples collected from a large area, using concentrated HNO_3, 0.01 M EDTA and ultra pure H_2O. Thereafter, we applied regression models predicting plant concentrations for the whole area, developed from Lončarić et al. (2010) data, and tested for differences between predicted values and observed values. Moreover, we tested the Ivezić et al. (2011b) model and other combinations of model parameters on the correlation with plant concentration dataset reported by Lončarić et al. (2010). Lončarić et al. (2010) investigated the correlation between EDTA extractable metals and wheat grain concentrations in 10 samples taken from a smaller part of the same area as the present study.

2. **Materials and methods**

2.1. Study area

The investigation was conducted in Danube basin area of eastern Croatia, Osijek-Baranja County. The county covers 4,144 km^2. It is a flat area part of the Pannonian valley that stretches through Hungary, Serbia and Croatia. Elevation is about 90 m above the mean sea level. The climate is continental with hot and arid summers and cold winters. Average annual temperature is 11°C, while the average annual precipitation is 650 mm. The highest periods of precipitation are in the spring and fall (Ministry of Environmental Protection, 2010).

2.2. Soil sampling and analysis

Seventy-four soil samples were collected from the depth of 0-25 cm (hereafter called surface soil). Two different land uses (forest and arable land) were included in the study representing 9 different soil types (Stagnosols, Dystric Cambisols, Luvisols, Haplic Gleysols, Chernozems, Fluvisols, Eutric Cambisols, Mollic Gleysols and Anthrosols) (FAO, 2006). Forty-five of these 74 samples were from agricultural fields, representing 8 soil types (without Fluvisols). At each site, 10 surface soil samples were taken within 5 m distance and combined into one composite sample of about 500 g. They were dried and sifted through a 2-mm mesh prior to the determination of soil pH, dissolved organic carbon (DOC) and trace metal extractions. Samples were further ground to finer particle size using agate mortar, prior to determination of total soil organic carbon (SOC) and total metal extraction by ultra pure HNO_3.

The concentration of SOC was determined by a dry combustion method on Leco Carbon Determinator EC12 (Nelson and Sommers, 1982). As a default we suggest SOM contains 50 % carbon. Soil pH was determined in soil-to-water solution ratio of 1:2.5 (Mc Lean, 1982). The amount of dissolved organic carbon (DOC) in water was determined using a Shimadzu TOC-5000 analyzer (Shimadzu Scientific, Colombia). As with SOM, we suggest dissolved organic matter (DOM) to be composed of 50 % organic C.

2.3. Extraction procedures

Soil samples were treated with 3 different single extraction methods: strong acid extraction (ultra pure HNO_3 extraction), EDTA extraction and extraction by water (weakest extractant). Ultra pure HNO_3 extraction and water extraction were conducted at the Norwegian University of Life Sciences and EDTA extraction at the University of J.J. Strossmayer, Faculty of Agriculture, Osijek, Croatia.

- *Ultra pure HNO_3 extraction* - Total metal concentration in soil (M_{tot}) was determined after digesting the soil in concentrated ultra pure HNO_3 (1:15 solid:solution ratio) by stepwise heating up to 250 C using a Milestone Ultra clave for 1 hr and 15 min. Trace metal concentrations were determined using a Perkin Elmer Sciex Elan Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for Cd and a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES) for Cu, Fe, Mn and Zn. Standard reference material (SRM) used was the SRM 2709 (National Institute of Standards & Technology, 2003).
- *EDTA extraction* – 10 g of air-dried soil was digested with 20 ml EDTA extract (0.01 M ethylene-diaminetetraacetic acid (EDTA) and 1M $(NH_4)_2C0_3$, adjusted to pH 8,6) and shaken for 30 minutes. The soil:solution ratio was 1:2 (Trierweiler and Lindsay, 1969). Extract was filtered and concentrations of trace metals were determined by ICP-OES.
- *Water extraction* – On 4 g of air-dried soil 40 mL ultra pure water (MilliQ H_2O, electric conductivity < 18.2 MΩ cm^{-1}) was added and shaken in high-density polyethylene (HDPE) centrifugation tubes for two days and centrifuged at 1400 x g for 30 min. The suspension was filled in High-Density Polypropylene (HDPP) syringes and filtered through 0.45 μm polyethersulfone membrane filters to polypropylene (PP) test tubes. The filters and the test tubes were provided by VWR International (vwr.com). Blanks of MilliQ H_2O were stored in PP tubes for 48 hr and filtered through the same membrane filters before analysis. Water trace metal concentrations were determined using a Perkin Elmer Sciex Elan Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for Cd and a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES) for Cu, Fe, Mn and Zn. Standard reference material used for water was the SRM 1643e (National Institute of Standards & Technology, 2004).

2.4. Chemical speciation of soil solution

Chemical speciation of soil solution was estimated using WHAM-VI version 6.0 for waters (Tipping, 1998). The input data was: soil solution pH, fulvic acid (FA) concentration, the solution concentration of Al, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. As a default we define 50 % of DOM being chemically active FA.

2.4. Plant data (wheat grain concentration) from Lončarić et al. (2010)

Lončarić et al. (2010) conducted their study in as small a part of the same area as the present study. The Lončarić et al. (2010) study does, however, present both soil and plant data, which is of interest since we have no information about plant uptake of metals in our own study. Here we use Lončarić et al. (2010) data in statistical comparisons between our soil data with information on wheat grain metal concentration of *Srpanjka* genotype from Lončarić et al. (2010). The soil metal concentrations in their study were provided by extracting soils with *aqua regia* (as a total extraction) and EDTA (Table 1).

Table 1. Soil and plant data from Lončarić et al. (2010) study

		n	Average	SD	Min.	Max.
	pH	10	7.5	0.48	6.9	8.1
	SOM	10	2.6	0.52	1.7	3.3
Fe	*Aqua Regia* (mg kg^{-1})	10	25820	656	25100	27400
	EDTA (mg kg^{-1})		32.4	21.2	10.2	58.8
	Grain conc. (ppm)		31.3	3.6	26.1	35.9
Mn	*Aqua Regia* (mg kg^{-1})	10	761.2	67.1	673.2	914.9
	EDTA (mg kg^{-1})		37.3	9.2	30.7	62.8
	Grain conc. (ppm)		35.0	5.5	26.6	44.5
Zn	*Aqua Regia* (mg kg^{-1})	10	76.3	2.8	72.7	81.2
	EDTA (mg kg^{-1})		1.6	0.31	0.8	2.0
	Grain conc. (ppm)		14.9	2.08	12.4	18.2
Cu	*Aqua Regia* (mg kg^{-1})	10	24.5	2.12	21.2	26.9
	EDTA (mg kg^{-1})		5.1	0.57	4.2	5.9
	Grain conc. (ppm)		2.2	0.23	1.9	2.7
Cd	*Aqua Regia* (mg kg^{-1})	10	0.36	0.043	0.28	0.42
	EDTA (mg kg^{-1})		0.032	0.016	0.012	0.061
	Grain conc. (ppb)		30.9	24.03	13.42	96.81

Lončarić et al. (2010)

We used our data to develop models predicting water extractable ($M_{(sol)}$) concentration, free metal ion concentration (M^{Z+}) and free ion activity (aM^{Z+}). Furthermore, we applied these models to Lončarić et al. (2010) data and tested the correlation between $M_{(sol)}$, M^{Z+} and aM^{Z+} with observed wheat grain concentrations ($M_{(ObsG)}$). Finally, the Lončarić et al. (2010) set of data was tested using the optimized model versions of Ivezić et al. (2011b) that test how models developed for predicting speciation in soil solution correlate with $M_{(ObsG)}$.

2.5. Statistical analysis

Descriptive statistics, simple correlation analysis, two sample t-test and regression analysis were performed using Minitab® Statistical Software version 15 (2007). Multiple regression analysis was used to derive regression models predicting plant concentrations, $M_{(sol)}$, M^{Z+} and aM^{Z+}. Pearson correlation coefficients were determined by correlating different extraction methods and concentrations in wheat grain ($M_{(ObsG)}$) with $M_{(sol)}$, M^{Z+}, aM^{Z+} and EDTA extractable concentrations.

3. Results

3.1. Soil properties

The surface soil was sampled from 74 sites, out of which 45 were from cereal crop production agricultural fields, 21 from forest and 8 from pasture and fallow land. Soil pH in all 74 samples varied from 4.3 – 8 (average 6.3), DOC 6.1 – 73 mg l^{-1} (average 26 mg l^{-1}), SOM 0.9 – 10.1 % (average 3.4 %) (Table 2). Soil pH was negatively correlated with DOC, while DOC was positively correlated with SOM (Ivezić et al., 2011a). Soil pH in the 45 samples taken from agricultural fields varied from 5.2 – 8.0 (average 6.9) (except one site having pH 4.3). More than half of the 45 samples had pH above 7. Dissolved organic carbon was in the range: 6.1 – 33.1 mg l^{-1} (average 15.8 mg l^{-1}) and SOM 0.9 – 8.8 % (average 2.9 %) (Table 2). Soil pH was negatively correlated with DOC ($p < 0.01$) and SOM ($p < 0.05$), while DOC was in positive correlation with SOM ($p < 0.001$). The northern part of the investigated area was dominated by high pH soils (Figure 1).

Table 2. Soil properties

	Forest, Agriculture and Pasture (n=74)				**Agriculture (n=45)**			
	Avg.	**SD**	**Min.**	**Max.**	**Avg.**	**SD**	**Min.**	**Max.**
pH	6.3	1.2	4.3	8.0	6.8	1.0	4.3	8.0
DOC (mg L^{-1})	26	17.4	6.1	73	15.8	4.9	6.1	33.1
SOM (%)	3.4	1.7	0.9	10.1	2.9	1.5	0.9	8.8

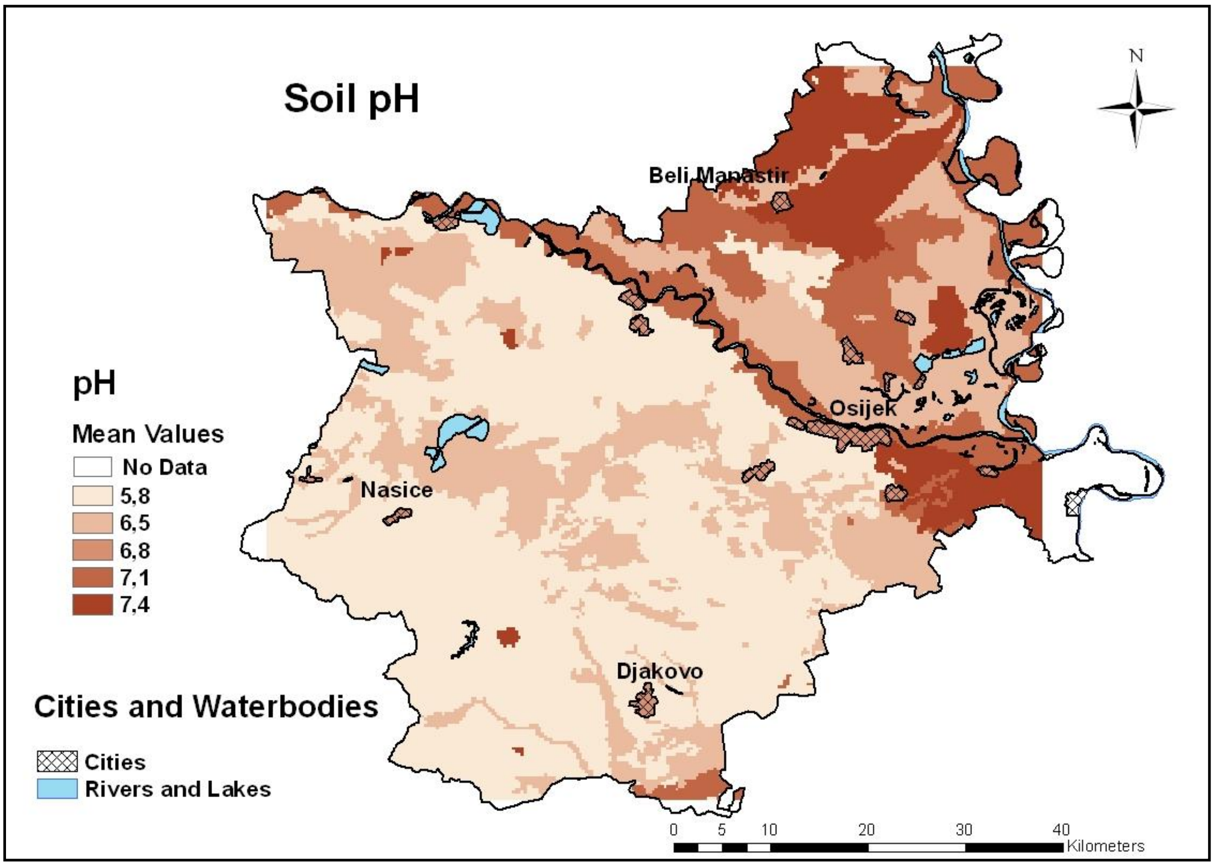

Figure 1. Soil pH of Osijek-Baranja County

Governmental maximum permissible concentrations (MPC) of Zn and Cd, for agricultural soils, are determined by *aqua regia* extraction (Official Gazette, 1992). In the present study total concentrations are estimated by ultra pure HNO_3 extraction (Table 3). The HNO_3 extractions and the *aqua regia* extraction reported in Lončarić et al. (2010) can both be used to estimate the total metal concentration in soil (Sabiene et al., 2004; Meers et al., 2007). The total concentrations of Zn and Cd did not exceed the MPC limit for agricultural fields in Croatia (Table 3). The $M_{(sol)}$ concentrations of Zn were below the detection limit in 32 out of 74 samples (43 %), therefore we used the median value of the detection limit for those samples. The Zn concentration in water extracts of soils with pH > 7 were the ones below detection limit, which indicates a strong influence of pH on Zn solubility (Shuman, 1991; Alloway, 1995).

The M^{Z+} and aM^{Z+} in the soil solution of the investigated metals were determined using WHAM Model VI (Tipping, 1998), where the $M_{(sol)}$, the soil solution pH and the DOC concentrations (as fulvic acid, FA, in WHAM) were applied to the input file. The $M_{(sol)}$ was highly correlated with M^{Z+}. The FA-bound metal fraction was small for all metals, except for Cu (Fe = 7 %; Zn =7 %; Cd = 9 %; Mn = 10 % and Cu = 66 %).

Table 3. Trace metal concentrations (n = 45)

		Cd	**Cu**	**Fe**	**Mn**	**Zn**	**MPC**	
							Cd	**Zn**
HNO$_3$ Extraction (mg kg^{-1})	Avg.	0.23	25.7	29915	635	80.4	1-2	200–300
	SD	0.07	13.4	3476	195	13.2		
	Min.	0.1	13.3	24318	238	58.5		
	Max.	0.4	98.7	40221	1038	119.5		
EDTA Extraction (mg kg^{-1})	Avg.	0.1	6.8	205.2	54	1.4		
	SD	0.04	6.4	341	33.9	0.9		
	Min.	0.015	1.4	13.7	7.4	0.3		
	Max.	0.23	41.4	1631.9	131.6	4.1		
Water extraction (mg kg^{-1})	Avg.	0.0006	0.15	20.5	0.89	0.14		
	SD	0.001	0.08	30.5	3.2	0.17		
	Min.	0.00003	0.05	0.4	0.004	0.03^{*}		
	Max.	0.007	0.45	125.3	20.9	0.82		

* indicates values that had some observations bellow detection limit; MPC-maximum permissible concentrations

3.3. Extraction methods

In the present study, EDTA extraction was well correlated ($p < 0.001$) with ultra pure HNO_3 extracted Cd, Cu and Mn ($r = 0.87$; $r = 0.95$ and $r = 0.62$, respectively) (Table 4), but not with Fe and Zn. Water extracted metals were best correlated with EDTA-extractable Cu ($r = 0.60$), Fe ($r = 0.38$) and Mn ($r = 0.47$) and with HNO_3-extractable Cu and Zn ($r = 0.53$; $r = -0.30$).

Table 4. Correlation analysis of extraction methods (n = 45)

	Cd	**Cu**	**Fe**	**Mn**	**Zn**
HNO$_3$ – EDTA	0.87^{***}	0.95^{***}	ns	0.62^{***}	ns
EDTA – WE	ns	0.60^{***}	0.38^{*}	0.47^{***}	ns
HNO$_3$ – WE	ns	0.53^{***}	ns	ns	-0.30^{*}

* and *** indicate significant difference at p<0.05 and p<0.001 respectively, ns - not significant

3.2. Regression model prediction of plant metal concentrations, $M_{(GRAIN)}$

Firstly, we used Lončarić et al. (2010) data to develop best-fit multiple regression models using soil pH, SOM and *aqua regia* (AR) extractable metal concentration ($M_{(AR)}$) as model parameters when predicting metal concentration in grains of wheat, $M_{(GRAIN)}$. Thereafter, $M_{(AR)}$ was replaced with $M_{(EDTA)}$ (EDTA-extractable metal). The first model containing $M_{(AR)}$ was only statistically significant for Mn ($p < 0.01$). After replacing $M_{(AR)}$

with $M_{(EDTA)}$, also Fe ($p < 0.05$) and Cd ($p < 0.05$) were significantly predicting the $M_{(GRAIN)}$, in addition to Mn ($p < 0.01$). The model parameters and constants were:

$$\log Mn_{(GRAIN)} = - 0.22 + 0.13\ pH + 0.28 \log Mn_{(AR)} \qquad \text{eq. 1.}$$

$$\log Mn_{(GRAIN)} = 0.78 + 0.14\ pH - 0.21 \log Mn_{EDTA} \qquad \text{eq. 2.}$$

$$\log Fe_{(GRAIN)} = - 0.10 + 0.15\ pH + 0.32 \log Fe_{EDTA} \qquad \text{eq. 3.}$$

$$\log Cd_{(GRAIN)} = 3.44 - 2.42 \log SOM + 0.76 \log Cd_{EDTA} \qquad \text{eq. 4.}$$

Therefore, the best-fit model predicting $Mn_{(GRAIN)}$ and $Fe_{(GRAIN)}$ contained only soil pH and soil metal concentration ($M_{(EDTA)}$ and $M_{(AR)}$) as significant model parameters, as shown above. Soil organic matter (SOM) was only a significant model parameter for Cd.

Secondly, equations 1-4 were then tested on the 74 sample data set covering the whole Osijek–Baranja County (Ivezić et al., 2011a,b). These $M_{(GRAIN)}$ predictions were then compared with the observed metal concentrations in grains (from now on referred to as $M_{(ObsG)}$ to distinguish observed concentrations from the predicted concentrations referred to as $M_{(GRAIN)}$) in Lončarić et al. (2010) using two sample t-test. In other words, we wanted to test if the models shown in eqs. 1-4 can predict $M_{(GRAIN)}$ using a different set of data from the same region. Two sample t-tests showed that there was no significant difference (Mn and Cd ($p< 0.001$) and Fe ($p < 0.01$)).

3.4. Regression modeling of trace metals in soil solution and free ion activity

In the foregoing tests, the Lončarić et al. (2010) soil and plant data were used to optimize multiple regression models for the prediction of $M_{(GRAIN)}$. Those regression models were then tested against a different dataset presented in Ivezić (2011a,b). Next, we wanted to test if $M_{(sol)}$ and M^{z+} prediction in a study like the Ivezić et al. (2011b), containing no plant uptake data, correlated with metal concentrations in the wheat grain samples using soil and plant data from Lončarić et al. (2010). The reason for this operation was to test if model predictions, as in Ivezić et al. (2011b) and for instance Tipping et al. (2003), provided bio-available relevant information. Ivezić et al. (2011b) presented two model optimizations based on Tipping et al. (2003) for the prediction of $M_{(sol)}$ and M^{z+}. Here we were only testing how the Ivezić et al. (2011b) model predictions of $M_{(sol)}$ and M^{z+} correlated with observed $M_{(ObsG)}$ for Zn, Cu and Cd given in Lončarić et al. (2010). The Ivezić et al. (2011b) model is:

$\log M_{(sol)} = a \log(\mathrm{SOM}) + b\,\mathrm{pH} + c \log M_{(tot)} + d \log(\mathrm{DOC})$ eq. 5 (Ivezić et al. 2011b)

$\log M^{Z+} = a \log(\mathrm{SOM}) + b\,\mathrm{pH} + c \log M_{(tot)}$ eq. 6 (Ivezić et al. 2011b)

Only Zn^{2+} from eq. 6 was significantly correlated with $Zn_{(ObsG)}$ ($r^2 = 0.71$) from Lončarić et al. (2010). The Ivezić et al. (2011b) model optimization included soil chemical parameters from 74 sampling sites, withdrawn from agricultural soils as well as forest soils. However, since we are investigating concentrations of trace metals in wheat, we wanted to test eqs. 5 and 6 by using only samples from the agricultural fields (45 samples). Therefore, we had to optimize model coefficients for these models. In Ivezić et al. (2011b), the model was optimized for the free metal ion (M^{Z+}) concentration prediction. However, since free ion activity (aM^{Z+}) might be more important for the plant uptake, eq. 6. was re-optimized for the prediction of aM^{Z+}, shown as eq. 8. The model parameters were unchanged (eqs. 7 and 8), but the parameter constants were different (Table 6). Moreover, Lončarić et al. (2010) provide no information of DOC, and hence eq. 5 was re-optimized without DOC, as eq. 7.

$\log M_{(sol)} = a + b\,\mathrm{pH} + c \log M_{tot} + d \log(\mathrm{SOM})$ eq. 7

$\log aM^{Z+} = a + b\,\mathrm{pH} + c \log M_{tot} + d \log(\mathrm{SOM})$ eq. 8

Soil pH was the most dominant parameter controlling the metal solubility of all the metals investigated while SOM and $M_{(tot)}$ were significant just for some of them.

The predicted $M_{(sol)}$ and aM^{2+} from eqs. 7 and 8, respectively, were all significant even after reducing the input data from 74 to 45. Moreover, the predicted $M_{(sol)}$ and aM^{2+} for Fe, Zn and Mn were generally well correlated with $M_{(ObsG)}$, which is an improvement from correlating only with observed values of EDTA extraction (Table 5), as only EDTA-Fe was significantly correlated with $M_{(ObsG)}$ (Lončarić et al., 2010).

Since SOM was significant only in predicting the aM^{2+} of Cu and Zn, the model was simplified even more by omitting SOM:

$\log M_{(sol)} = a + b\,\mathrm{pH} + c \log M_{(tot)}$ eq. 9

$\log aM^{Z+} = a + b\,\mathrm{pH} + c \log M_{(tot)}$ eq. 10

The models were still significant for all five metals. The model simplification did not lower the correlation with $M_{(ObsG)}$ for Fe, Mn and Zn (Table 5).

Table 5. Plant uptake - Correlations between models predicting water extractable fraction, M^{Z+} and aM^{Z+} and trace metal grain concentrations reported in Lončarić et al. (2010).

	Grain concentrations (*Lončarić et al. 2010*)				
	Fe	**Mn**	**Zn**	**Cu**	**Cd**
EDTA (*Lončarić et al. 2010*)	**0.68***	ns	ns	ns	ns
M^{Z+} (predicted by Eq. 6 (n=74)) (*Ivezić et al., 2011b*)	**N/A**	**N/A**	**0.71***	ns	ns
$M_{(sol)}$ (predicted by Eq. 7 (n=45))	**0.64***	**-0.86*****	**0.73***	ns	ns
aM^{Z+} (predicted by Eq. 8 (n=45))	**0.62***	**-0.87*****	**0.73***	ns	ns
$M_{(sol)}$ (predicted by Eq. 9 (n=45))	**0.63***	**-0.86*****	**0.70***	ns	ns
aM^{Z+} (predicted by Eq. 10 (n=45))	ns	**-0.86*****	**0.70***	ns	ns
$M_{(sol)}$ (predicted by Eq. 11 (n=45))	**0.64***	**-0.88*****	**0.70***	ns	**0.70***
aM^{Z+} (predicted by Eq. 12 (n=45))	ns	**-0.87*****	**0.70***	ns	**0.69***

* and *** indicate significant difference at $p<0.05$ and $p<0.001$ respectively. $M_{(sol)}$ - water extractable trace metal fraction, M^{Z+} - Free metal ion concentration, aM^{Z+} - free ion activity.

Eq6: FMI concentrations predicted by pH, SOM and HNO_3 as predictors. Eq7: water extractable fraction predicted by pH, SOM and HNO_3 as predictors; Eq8: FIA predicted by pH, SOM and HNO_3 as predictors; Eq9: water extractable fraction predicted by pH and HNO_3 as predictors; Eq10: FIA predicted by using pH and HNO_3; Eq11: water extractable fraction predicted by using pH and EDTA; Eq8: FIA predicted by using pH and EDTA

Finally, we tested the effect of replacing the $M_{(tot)}$ with $M_{(EDTA)}$ extractable metals. That is because EDTA is a chelating agent capable of chelating even organically bound metals, and partially metals in oxides and secondary clay minerals (Bermond et al., 1998; Feng et al., 2005a). Hence, we would test the effect of using a more geochemically active pool of metals in the regression estimates of bioavailable metals instead of the “total” concentration that also includes the inert metal fractions. Originally, the EDTA metal extraction was developed for acidic soils and hence EDTA-extractable metals correlate well with plant uptake from acidic soils and not with plant uptake from alkaline and neutral soils (Feng et al., 2005a). The models changed to:

$$\log M_{(sol)} = a + b\,\mathrm{pH} + c \log M_{(EDTA)} \qquad \text{eq. 11}$$

$$\log aM^{Z+} = a + b\,\mathrm{pH} + c \log M_{(EDTA)} \qquad \text{eq. 12}$$

The model predictions were still significant for Cd, Cu, Fe, Mn and Zn, but the r^2 was only slightly improved (Table 6). The correlations between the model prediction and the $M_{(ObsG)}$ was, however, positively improved. In addition to Fe, Mn and Zn (as after testing eqs.

7-10), the predicted $M_{(sol)}$ of Cd was also correlated with $M_{(Obs)}$ after this optimization (Table 5).

Table 6: Multiple regression parameters for eq.7-12

Soil solution conc. ($M_{(sol)}$)						**Free ion activity (aM^{Z+})**					
Eq 7.	***a***	***b***	***c***	***d***	r^2	Eq 8.	***a***	***b***	***c***	***d***	r^2
Fe	-5.39	-0.59	2.62	-0.80	0.58^{***}	**Fe**	-1.20	-2.46	2.54	-0.84	0.93^{***}
Mn	5.00	-0.95	0.22	0.007	0.93^{***}	**Mn**	5.29	-1.01	0.20	-0.10	0.93^{***}
Zn	0.96	-0.33	0.29	-0.41	0.70^{***}	**Zn**	1.29	-0.41	0.30	-0.47	0.77^{***}
Cu	-1.16	-0.08	0.82	-0.21	0.25^{**}	**Cu**	3.27	-1.02	1.38	-1.10	0.84^{***}
Cd	-0.22	-0.46	0.48	0.33	0.85^{***}	**Cd**	0.02	-0.52	0.51	0.22	0.88^{***}
Eq 9.	***a***	***b***	***c***		r^2	Eq 10.	***a***	***b***	***c***		r^2
Fe	-0.78	-0.65	1.59		0.56^{***}	**Fe**	3.59	-2.52	1.47		0.93^{***}
Mn	5.01	-0.95	0.21		0.93^{***}	**Mn**	5.14	-1.01	0.25		0.93^{***}
Zn	1.67	-0.34	0.12		0.67^{***}	**Zn**	2.10	-0.43	-0.17		0.75^{***}
Cu	-1.07	-0.09	0.73		0.23^{**}	**Cu**	3.74	-1.07	0.94		0.81^{***}
Cd	0.04	-0.46	0.74		0.84^{***}	**Cd**	0.02	-0.52	0.69		0.88^{***}
Eq 11.	***a***	***b***	***c***		r^2	Eq 12.	***a***	***b***	***c***		r^2
Fe	4.90	-0.51	0.25		0.56^{***}	**Fe**	7.45	-2.26	0.50		0.93^{***}
Mn	4.84	-0.90	0.27		0.93^{***}	**Mn**	5.00	-0.96	0.29		0.94^{***}
Zn	1.45	-0.34	0.05		0.67^{***}	**Zn**	1.81	-0.43	0.04		0.75^{***}
Cu	-0.50	-0.08	0.47		0.30^{***}	**Cu**	4.47	-1.05	0.59		0.81^{***}
Cd	0.04	-0.45	0.56		0.86^{***}	**Cd**	0.18	-0.50	0.50		0.89^{***}

** and *** indicate significant difference at $p<0.01$ and $p<0.001$ respectively

4. Discussion

Multivariate regression modeling is used to predict $M_{(sol)}$ and M^{Z+} (Gandois et al., 2010; Groenenberg et al., 2010; Tipping et al., 2003) as well as plant uptake (Krauss et al., 2002; Lombnæs and Singh, 2003). In the present study, we optimized multivariate regression models for the prediction of $M_{(GRAIN)}$ (eqs. 1-4), based on data from a small area (Lončarić et al., 2010). The prediction of $M_{(GRAIN)}$ was then tested using soil chemical data from Ivezić et al. (2011a), who investigated a much wider area in the same region. We found no statistical difference between $M_{(GRAIN)}$ predictions using the large dataset compared to the $M_{(GRAIN)}$ prediction using the smaller Lončarić et al. (2010) dataset. Since there was no significant difference between the two $M_{(GRAIN)}$ predictions, we tested if the optimized Ivezić et al. (2011b) model developed for the prediction of $M_{(sol)}$ and aM^{Z+} (optimized for the prediction of aM^{Z+} in place of M^{Z+}, eq 8) was significantly correlated with $M_{(ObsG)}$ using Lončarić et al. (2010) soil data. Although the biotic ligand model (BLM) describes the importance of metal speciation in soil solution more substantially than the FIA model due to the inclusion of competitive ions during uptake, the FIA provides good estimates of bioavailability of metals in soil solution (Almås et al., 2006; Hough et al., 2005; Lofts et al., 2004; Sauvé et al., 2000b). We found that the $M_{(sol)}$ and aM^{Z+} (eqs. 7 and 8) were correlating well with $M_{(ObsG)}$, which is an interesting finding. In fact, the more radical simplification of eqs. 7 and 8 containing only soil pH and $M_{(tot)}$ provided significant correlations between $M_{(sol)}$ and aM^{Z+} and $M_{(ObsG)}$. Importance of soil pH has been reported earlier by Adams et. al. (2004). They reported that plant metal uptake was successfully predicted using only $M_{(tot)}$ and pH as model parameters. That confirms the importance of soil pH in controlling metal solubility and speciation in soil solution, at least when the variation in SOM and $M_{(tot)}$ content is small. When predicting trace metal uptake, soil pH is always an important parameter (Lombnæs and Singh, 2003; Adams et al., 2004). Since model simplifications do not reduce the correlation between predicted solution concentrations ($M_{(sol)}$ and aM^{Z+}) and $M_{(ObsG)}$, we believe even the simplest models containing only pH and total (or geochemically active) metal concentrations as model parameters can provide reasonable estimates of metal bioavailability.

The Lončarić et al. (2010) study showed that only EDTA extractable Fe is significantly correlated with grain concentrations. Extraction by EDTA mimics phytosiderophore release, where exudates are excreted from metal deficient plant roots primarily to obtain Fe (Menzies et al., 2007). We believe that this partly explains why only Fe was correlated with EDTA. In addition, soils from Lončarić et al. (2010) had a quite high pH (pH was in the range 6.9 - 8.1), which reduces EDTA ability to predict plant uptake of metals with less affinity for EDTA than Fe(III) (*log* K M-EDTA; Fe(III): 27.97, Cd: 17.36, Cu: 19.7, Mn: 14.81, Zn: 17.44 (Lindsay, 1979)). The EDTA method was initially developed for acidic soils and the best correlations with uptake was reported from soils with low pH (Feng et al., 2005a,b).

Copper was the only trace metal that showed no correlation either with EDTA or with predicted $M_{(sol)}$ and aM^{Z+} obtained from either equation. However, Cu is an element which is known to accumulate in the roots (Menzies et al., 2007). Therefore, having no correlation is probably due to weak translocation of Cu from the roots to the upper parts of the plant. Root accumulating elements such as Cu and Pb often poorly reflect the toxicity (Brun et al., 1998; Faust and Christians, 1999; Menzies et al., 2007).

Comparing regression models, we can see that metal concentrations calculated by equations 11 and 12, where $M_{(EDTA)}$ is used as a parameter, correlated better with metal concentrations in grain compared to equations 6-10 where $M_{(tot)}$ is used (Table 5).

When predicting $M_{(sol)}$ and aM^{Z+} of Mn, we found a negative correlation with $M_{(ObsG)}$ of Mn. This basically indicates that when there is more Mn in the soil solution, less of it will be in the grain (Table 5). This is an unexpected observation. Such results may be related to the soil pH as soil pH and $Mn_{(sol)}$ data were *negatively* correlated, meaning lower pH corresponds to higher concentration of Mn in soil solution (which is as expected). However, in the Lončarić et al. (2010) study, the soil pH and Mn concentrations in grain were *positively* correlated, meaning a higher soil pH corresponds to higher Mn concentration in grain. This is an unexpected finding as increasing soil pH usually means less available Mn (Alloway, 1995; Marschner, 1995; Havlin et al., 2005). However, soil pH in Lončarić et al. (2010) did not differ much (pH range: 6.9 – 8.1). Basically five sites had pH near 7 and five sites near 8. The examination of the other four metals showed no significant difference in grain concentrations of Cu and Cd between these sites. The Fe and Zn concentrations were higher in grain from plants grown on soils with lower pH (pH = 7). We expected the same for Mn, but the situation was opposite. The soils investigated in Lončarić et al. (2010) had pH between 7 and 8, and hence these soils are rather calcareous. According to Marschner (1995), Mn availability on calcareous soils is mainly determined by soil structure and aeration. We tested the correlation of Mn in water extraction on both plant concentration and plant uptake, but in both cases we got the same results. Another explanation could be in the translocation of Mn from the roots to the grains as distribution via phloem depends on the development stage of the plant (Herren and Feller, 1994). According to Page et al. (2006) root-to-shoot transfer of Mn is rapid, so if there was a time difference in sample collection from two sites it could explain the difference in grain concentration. Nevertheless, we have no data on the root concentrations and hence we can only speculate on this matter.

5. Conclusion

Total or pseudo-total concentrations of trace elements in the soil, extracted by strong acids, are recognized as poor indicators of toxicity. Nevertheless, they are still being used in the legislation of many countries for the determination of trace metal maximum permissible concentrations in the soil. The results from the present study show that regression models developed using soil properties improve prediction of plant uptake. Furthermore, $M_{(sol)}$ and aM^{Z+} predicted by regression models, give us better correlation with plant concentrations than the EDTA or total (*aqua regia*) extraction alone. Water extraction represents soil solution concentrations ($M_{(sol)}$ and aM^{Z+}) of trace metals, and even though it is not the total concentration taken up by the plant, it is a good indicator of uptake. Soil pH has a key role in determining $M_{(sol)}$ and aM^{Z+}, and for predicting plant concentrations as well. The soils investigated had low concentrations of $M_{(tot)}$ and little variation in SOM. Tillage of agricultural soils blurs the vertical and horizontal physical and chemical heterogeneity of the upper soil layers. When, in addition, the soil types and vegetation cover do not vary to a great extent, the control of soil pH is a key in controlling the metal solubility, and consequently, the metal availability to plants. Thus, metal toxicity or deficiency can be controlled by managing the soil pH.

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Paper 4

Ivezić, V., Singh, B.R., Gvozdić, V. & Lončarić, Z. (2011d). Assessing soil quality in relation to trace metal availability and other soil properties under different land uses in Croatia. *Environmental Monitoring and Assessment* (submitted)

Assessing soil quality in relation to trace metal availability and other soil properties under different land uses in Croatia

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Abstract

Osijek-Baranja County situated in the southern part of Croatia has been a well-known agricultural area for centuries. Intensive agriculture throughout such a long period is likely to cause soil degradation. Therefore, the present study investigates potential soil degradation by assessing soil quality based on trace element solubility and other major soil properties. Although various methods are currently used to determine overall soil quality, in the present study we used a statistical approach by principal component analysis to determine soil indicators, assigned them weights and scores and combined them into soil quality index (SQI). Out of 19 soil properties tested, only 10 showed significant differences between land uses. Out of these 10, 4 soil properties i.e. pH, SOC, BD and AL-P, were chosen as indicators of soil quality. We found no statistical differences in SQI between land uses, indicating that agriculture practices did not cause any soil degradation. However, we observed differences in single soil properties such as SOC and BD, suggesting degradation of these soil properties due to agriculture practices. Trace metal availability and solubility showed no significant correlation with SQI, implying that SQI is not a good indicator of trace metal availability and solubility.

Keywords: principal component analysis, soil quality, soil properties, trace metals

Introduction

With increasing demand for food, public attention on environmental protection and decreasing supplies of nonrenewable energy and mineral resources, the concept of soil quality has become increasingly important (Karlen et al., 1997). Soil quality is most commonly defined as: "the capacity of the soil to function" (Doran and Parkin, 1994). The soil quality can have many functions such as (i) biomass production, (ii) storing, filtering and transforming nutrients, (iii) maintaining biodiversity, (iv) carbon sequestration, (v) physical and cultural environment for humans, (vi) providing raw materials and (vii) preserving geological and archeological heritage (European Commission, 2006). Main threats to soil and its functions have been recognized by the European commission in the proposal for the soil directive published in 2006 (European Commission, 2006). In the present study we focus on soil quality with respect to soil's function for biomass production (both agriculture and forestry) and on its connection to trace element concentration in soils and other soil properties.

Soil is a dynamic system and the complexity of soil quality cannot be explained by individual soil parameter. Overall soil quality should be expressed as a function of several measurable soil attributes (Larson and Pierce, 1994). In the past two decades soil quality index (SQI) has been used as a tool for evaluating and understanding the effects of different land uses and different management practices on soil resources and soil resilience. Most common approach in determining SQI is the use of principal component analysis (PCA) (Brejda et al., 2000a,b; Andrews et al., 2002a,b; Shukla et al., 2006; Imaz et al., 2010). The PCA approach takes into account evaluation of biological, chemical, and physical indicators to determine how well the soil is functioning (Karlen et. al., 2003). It consists of three step framework that was first developed by Andrews et al. (2002a) and later used by many authors (Fu et al., 2004; Awasthi et al., 2005; Shukla et al., 2006; Imaz et al., 2010). It has become an internationally accepted science based tool for the assessment, education and understanding of soil resource and its resilience.

The framework consists of three main steps: 1) selection of minimum data set (MDS) of indicators that will best represent the soil function; 2) transformation of indicator values into the scores, so that all indicators have the same unit; and 3) combining the indicator scores into the index (Figure 1) (Andrews et al., 2004).

Many soil attributes are correlated through a multivariate statistical analysis, such as PCA, which provides a technique for simultaneous analysis of correlated variables (Brejda et al., 2000a,b; Andrews et al., 2002a,b; Shukla et al., 2006) and only uncorrelated variables are chosen for the MDS. However, the PCA method is site specific. Therefore, if conditions change, indicators might change as well. The process needs to be repeated every time a different management practice or land use need to be evaluated (Andrews et al., 2002a; Andrews et al., 2004).

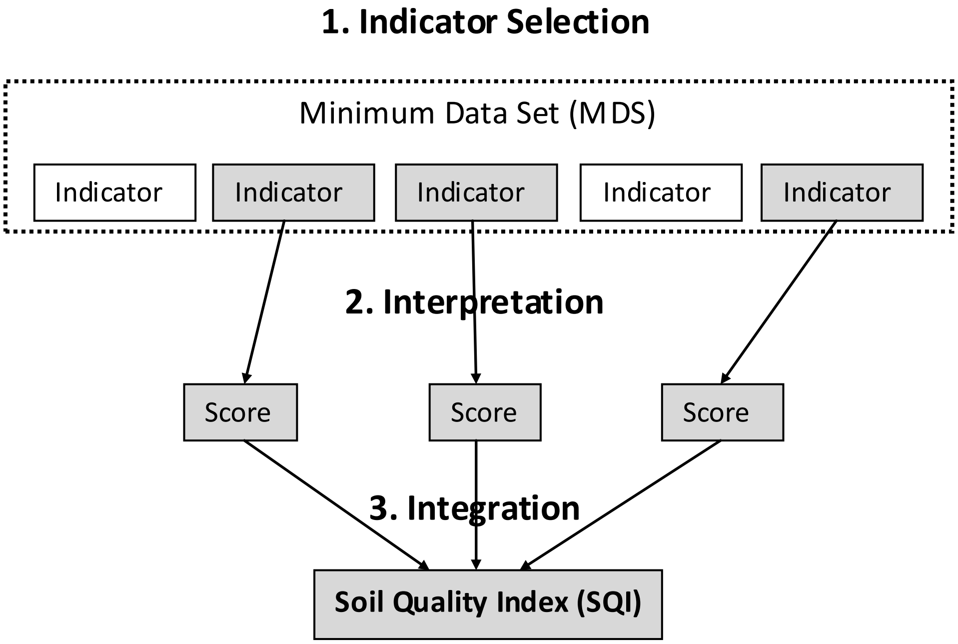

Figure 1. Framework for the determination of SQI (after Andrews et al., 2004)

Once the MDS has been determined, scoring of indicators is required so that all indicators have the same unit. It can be carried out in two ways: linear and non linear scoring. Both approaches assign scores in ascending or descending order. If higher values are considered as "good", approach "more is better" is applied, if lower values are considered as "good", the "less is better" approach is applied. For values such as pH "mid-point optimum" is applied

(Brejda et al., 2000a,b; Andrews et al., 2002a,b; Fu et al., 2004; Awasthi et al., 2005; Shukla et al., 2006; Imaz et al., 2010). Andrews et al. (2002a) compared linear and non linear scoring approach and found the non linear approach more appropriate as it reduces the influence of outlier data samples. The non linear scoring system is therefore better but more difficult to determine since it requires better knowledge of the system. However, this method may be more transferable to other systems. Final step of integration of indicator scores into indexes can be achieved in several ways: (i) simply by adding the scores, (ii) by taking the weight of each indicator into account and (iii) by decision support system. Andrews et al. (2002a) found no significant differences in these approaches thus suggesting the simplest approach, i.e. adding the scores, to be used.

In the assessment of soil quality very little attention has been given to trace metals. Trace metals affect soil and crop productivity on one hand, and lead to soil contamination and degradation on the other. However, none of the previous work included available trace metals in the evaluation of SQI. Since solubility of trace metals is controlled by soil properties (Ivezić et al. 2011a,b) and at the same time SQI is determined by the use of soil properties it is our hypothesis that SQI will be a good indicator of trace metal availability. In addition we believe that the difference in soil properties between land uses will also result with a difference in SQI between them. Therefore, the objectives of the present study are to: (i) determine the SQI for two different land uses (forest and agriculture) in the Danube basin of eastern Croatia, (ii) evaluate the magnitude of potential soil degradation due to agricultural practices and (iii) examine if the availability of trace metals is good attribute for assessing SQI.

Materials and methods

2.1. Study area

Study area is a flat area in the eastern Croatia, a part of the Pannonian Valley that stretches through Hungary, Serbia and Croatia. More detailed description of the area is provided elsewhere (Ivezić et al., 2011a) but a brief description is provided here.

The area consists of 9 main soil types, i.e. Stagnosols, Dystric Cambisols, Luvisols, Haplic Gleysols, Chernozems, Fluvisols, Eutric Cambisols, Mollic Gleysols and Anthrosols

(FAO, 2006). Soil samples were taken from all soil types. Since some of them cover small and insignificant area, the number of samples varied among soil types. Soil samples were mainly collected from arable land (30), pastures (5) and forest land (12). Forest soils were mainly 80-year old oak forest with very little human activity, therefore forest was considered as natural site without human influence (i.e., fertilizers, pesticides, cultivation).

2.2. Soil sampling and analysis

Based on the pedological map, 47 sites were randomly chosen so that all soil types and different land uses were covered in the sample area (arable land, pasture and forest). From each sampling site, 10 subsamples from 0–25 cm depth (hereafter called surface soil) were taken within 5 m distance from each other and then combined into one sample of approximately 500 g. Samples were dried and sieved through the 2-mm sieve for the determination of soil pH, DOC, CEC, AL-P, K and water extractable trace metals (TM) Cd, Cu, Fe, Mn and Zn. For soil organic carbon (SOC), inorganic carbon (InC), total nitrogen (TN) and TOT TM, samples were further ground to finer particle size using agate mortar. The concentration of SOC was determined by dry combustion method on a LECO Carbon Determinator EC12 (Nelson and Sommers, 1982), TN and InC concentrations were determined by LECO CHN-1000 Carbon and Nitrogen Analyzer. Soil pH was determined in soil to water solution ratio of 1:2.5 (Mc Lean, 1982) and CEC by the barium chloride method, where 3 g soil was added to 40 mL of 0.1 M $BaCl_2$ making the soil to solution ration 1:13 (Hendershot and Duquette, 1986). The amount of dissolved organic carbon (DOC) in water was determined by using a Shimadzu TOC-5000 analyzer (Shimadzu Scientific, Colombia). Available P and K were determined by ammonium lactate (AL) extraction method (Egner et al., 1960.). Bulk density (BD) was determined by core method. Analysis of soil samples for pH, SOC, InC, DOC, TN, CEC and trace metal concentrations was conducted at the Norwegian University of Life Sciences, while the determination of BD, AL-P and K at the University of J.J. Strossmayer, Faculty of Agriculture, Osijek, Croatia.

2.3. Water extractable and total content of trace metals in soil

Total metal concentration in soil (M_{tot}) was determined after digesting the soil in concentrated ultra pure HNO_3 (1:15 solid:solution ratio) by stepwise heating up to 250°C using a Milestone Ultra CLAVE for 1 hr and 15 min.

For water extraction, 4 grams of air-dried soil was added to 40 mL ultra pure water (MilliQ H_2O, electric conductivity < 18.2 MΩ cm^{-1}), shaken in high density polyethylene (HDPE) centrifugation tubes for two days and then centrifuged at 1400 x g for 30 min. The suspension was filled in high density polypropylene (HDPP) syringes and filtered through 0.45 µm polyethersulfone membrane filters to polypropylene (PP) test tubes. The filters and the test tubes were provided by VWR International (vwr.com). Blanks of MilliQ H_2O were stored in PP tubes for 48 hr and filtered through the same membrane filters before analysis. Two sets of 7 mL water-extracts were prepared, one was used for the determination of trace metal concentrations and another 7 mL aliquot was prepared for the determination of DOC by the Shimadzu TOC-5000 analyzer and the remaining solution for the determination of solution pH.

The concentrations of metals either in acid digestion or in water extractable solution were determined by using a Perkin Elmer Sciex Elan Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for Cd and a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES) for Cu, Fe, Mn and Zn. Blanks and reference material were used for both determinations. Standard reference material (SRM) for soils was the SRM 2709 (National Institute of Standards & Technology, 2003), whereas the SRM for water analysis was the SRM 1643e (National Institute of Standards & Technology, 2004).

2.4. Soil quality index determination

Determining soil quality index consisted of three steps. The first step was to choose the minimum data set (MDS) from which SQI was determined. We preformed ANOVA on all data set to examine which soil properties significantly differ among the land uses. Only the data that showed significant difference was further analyzed by standardized PCA. We assumed that PCs having high eigenvalues best represent the variation in the system. Thus, only PCs with eigenvalues higher than 1 were considered. Each variable at each PC was given a weight or a factor loading that represents variables contribution to the composition of that particular PC. The variables with the highest factor loading from each PC were then chosen for the MDS. In cases when one PC had two or more variables with high factor loadings which were uncorrelated, then both variables were chosen. However, when the variables with high factor loading of the same PC were correlated, then the one with the higher loading was chosen for the MDS.

Once the MDS was chosen, data values were transformed into unit-less values by nonlinear scoring curves (Step 2). Such scoring values can further be combined into one single value representing SQI (Step 3).

2.5. Statistical analysis

Descriptive statistics, analysis of variance (ANOVA), Tukey pairwise comparison of means, simple correlation and principal component analysis were conducted using Minitab® Statistical Software version 15 (Minitab, 2007). In addition statistical software Statistica, version 7.0 (StatSoft, 2007) was used for 3D graphs of PCA results. Analysis of variance and Tukey pairwise comparison was done between land uses to determine soil parameters with significant differences.

Pearson correlation coefficients were determined when correlating investigated soil properties, while PCA was used to choose the minimum data set (MDS) for the determination of SQI. Finally, GIS technique was used to create maps of the area and to visualize the results. The maps were created in ArcGis version 9.2. (ArcGis 9.2., 2006), a software that combines table data with spatial data, allowing us more comprehensive insight into a particular area of interest (Hutchinson and David, 2000).

Results and discussion

3.1. Soil properties and land use

The soils were analyzed for the following parameters: BD, pH, SOC, InC, DOC, TN, AL-P, K, CEC and trace metals (total and water extractable Cu. Cd, Fe, Mn and Zn). Soil properties were also analyzed for their relations to land use. No significant difference was found between pasture and arable land for any of the parameters. The difference was found between forest and arable land as well as between forest and pasture. Therefore, in the present study pasture and arable land were combined together into one land use, i.e. *agricultural field*. Descriptive statistics of soil properties and significant differences between land uses are shown in Table 1. Significant differences of water extractable trace metals between land uses are related to soil properties as soil properties control their solubility (Ivezić et al. 2011a). Forest soils showed significantly lower pH, BD, AL-P and K and higher SOC, DOC, TN and water extractable Cd, Mn and Zn (Table 1) than agricultural soils. Forest is a natural site with much higher organic matter input than agricultural field which explains the higher levels of SOC and DOC (Post and Kwon, 2000), while higher pH values in agricultural fields, compared to forest soils, could be assigned to liming application. Higher concentrations of AL-P and K in agricultural fields are caused by fertilization practices.

Table 1. Effect of land use on soil properties

	Forest	Agriculture	*p*
n	12	35	
BD	1.27	1.47	0.001
pH	5.4	6.7	0.001
SOC %	2.4	1.6	0.01
In C %	0.6	0.5	NS
DOC (mgL^{-1})	49.4	17.3	0.001
TN %	0.25	0.16	0.01
AL-P_2O_5 (mgkg^{-1})	62.4	260.8	0.05
K (mgL^{-1})	14.9	23.7	0.05
CEC	8.11	10.01	NS
TOT Cu (mgkg^{-1})	18.6	28.0	NS
TOT Cd (mgkg^{-1})	0.26	0.23	NS
TOT Fe (mgkg^{-1})	28834	29743	NS
TOT Mn (mgkg^{-1})	760	624	NS
TOT Zn (mgkg^{-1})	81.7	81.5	NS
WE Cu (µgL^{-1})	16.9	15.3	NS
WE Cd (µgL^{-1})	0.17	0.06	0.01
WE Fe(µgL^{-1})	2311	1986	NS
WE Mn (µgL^{-1})	355	114	0.05
WE Zn (µgL^{-1})	39.9	15.8	0.001

3.2. Soil quality assessment

When addressing soil quality, it is essential to take into account that soil quality is related to particular soil function (Karlen et al., 2003). For the SQI that will accurately represent soil investigated we first need to know which soil function we are evaluating. For example, if we are evaluating different management practices or land uses we need to focus our choice of indicators on the soil properties that are sensitive to management or land use change (Andrews et al., 2002a; Karlen et al., 2003).

The assessment of soil quality requires as many soil properties as possible. Usually biological, physical and chemical parameters are represented (Brejda et al., 2000a, b; Andrews et al., 2002a, b; Shukla et al., 2006; Imaz et al., 2010). However, in our data set we had no information on biological data; physical data was represented only by BD while all other parameters were chemical properties of the soil. Nevertheless, we believe that our data set was large enough to be able to evaluate soil quality for agricultural and forest biomass production function.

3.2.1. Indicator selection

Choice of indicators can be based on expert opinion or on statistical approach (Andrews et al., 2002a). In the present study we use statistical approach by using principal component analysis (PCA). There are various methods in using PCA or factor analysis (FA) for the selection of data set (Brejda et al., 2000a, b; Fu et al., 2004; Shukla et al., 2006). In the present study we determined MDS based on Andrews et al. (2002a) approach. The PCA was performed on 10 indicators that showed significant differences between land uses (Table 1). Three PCs had eigenvalues higher than 1 explaining 79.8 % of variation (Table 2). Highly weighted variables with high loading factor under PC1 were pH, WE-Cd, WE-Zn and WE-Mn. Since all four variables of PC1 were correlated, we chose pH as the most highly weighted variable for the MDS. In PC2, BD, DOC, SOC and TN had high loading factor. They were all correlated so we chose SOC to represent the PC2 in MDS. However, we decided to keep the BD as well, since it is our only physical parameter and it was negatively correlated with SOC, DOC and TN. In PC3 AL-P and K were the ones with high factor loadings. They were also correlated so we kept only AL-P as a variable for MDS. Therefore, our MDS for the determination of SQI consisted of: pH, BD, SOC and AL-P (Table 2).

Table 2.

Principal components	PC1	PC2	*PC3*
Eigenvalue	3.81	2.65	1.51
Percent	38.1	26.5	15.1
Cumulative percent	38.1	64.7	79.8
Variables[a,b]			
BD	-0.164	**0.394**	-0.020
pH	**-0.452**	-0.149	0.106
DOC	0.340	**-0.321**	-0.123
SOC	0.016	**-0.588**	-0.046
N	0.006	**-0.582**	-0.073
K	-0.289	0.031	**-0.632**
P	-0.296	-0.011	**-0.632**
WE Cd	**0.432**	0.085	-0.252
WE Zn	**0.371**	0.119	-0.196
WE Mn	**0.397**	0.112	-0.254

[a] Bold factor loadings are considered highly weighted

[b] Bold-underlined factor loadings correspond to indicators included in the MDS

3.2.2. Indicator Interpretation (scoring)

Bulk density varied from 0.91 – 1.71 g cm^{-3} (average: 1.42 g cm^{-3}), pH from 4.3 – 7.9 (average: 6.4), SOC from 0.76 – 5.87% (average: 1.81%) and AL-P from 0.0 – 174.2 mg 100g^{-1} (average: 21.01 mg 100g^{-1}). In order to have all indicators in the same unit, indicator values were transformed into scores. Nonlinear scores were derived by the use of distribution percentiles (Brejda et al., 2001). For each observation it was determined as to which percentage of data set it belongs to. Scores were assigned in percentages (from 0.0 to 1.0). In case of P and SOC "more is better" approach was considered, meaning that values in the upper part of the distribution were assigned higher scores (with 1 being the highest). Bulk density was assigned scores by "less is better" method, where lower values of BD were given a higher score. Soil pH was scored based on "mid-point optimum" approach, where each score was assigned to a pH interval of 0.4 starting from pH between 6.4 and 6.8 as the best pH (it was assigned score 1), pH between 6.2 - 6.4 and 6.8 – 7.0 had score 0.9, pH between 6.0 - 6.2 and 7.0 – 7.2 were given score 0.8, pH between 5.8 - 6.0 and 7.2 – 7.4 were given score 0.7. Assigning scores in such an interval continued to pH ≤ 4.8 which was assigned score 0.0 (Figure 2).

Such determination of scores based on non linear curves shows better results than linear scoring method. Results of linear scoring are highly dependent on the variance of each indicator as each observation is the proportion of the highest or the lowest indicator observation (Andrews et al., 2002a).

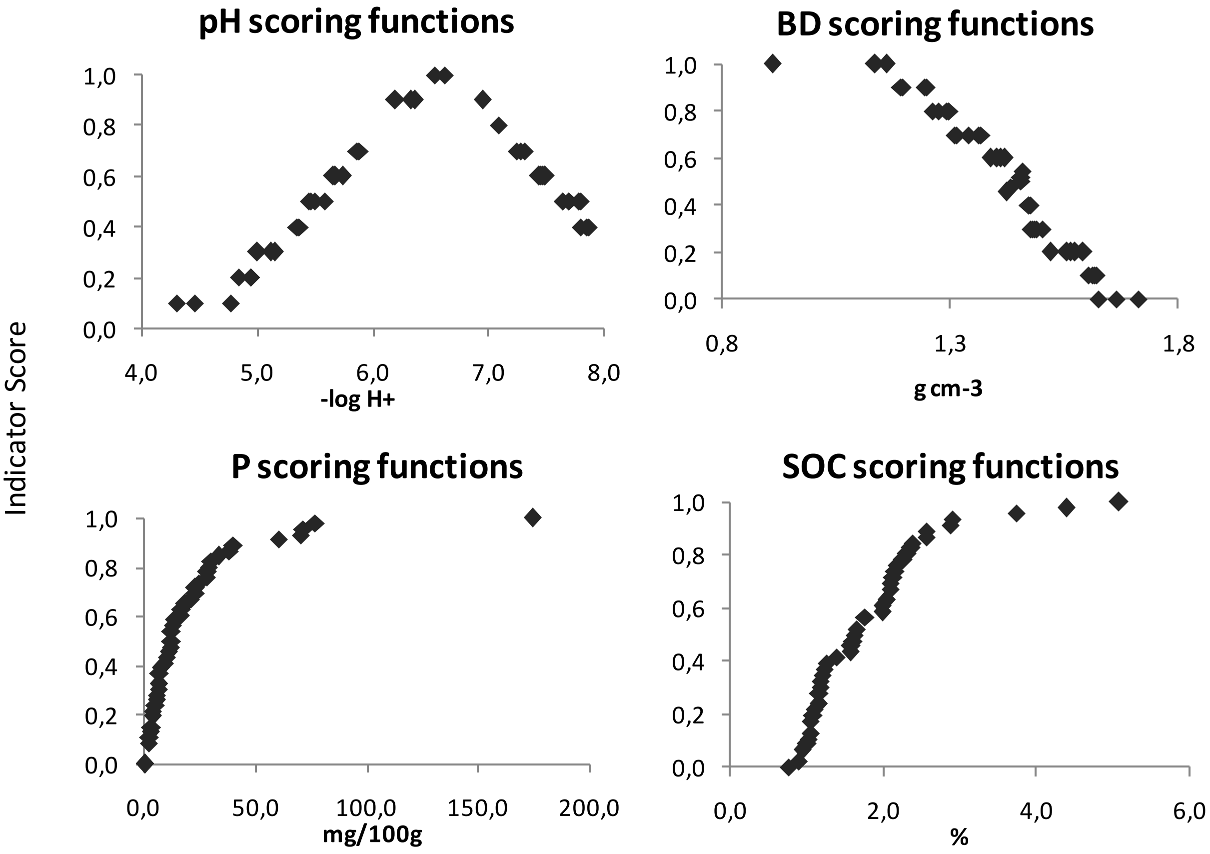

Figure 2. Soil property scores

3.2.3. *Soil quality index*

When calculating the overall soil quality index, not only the scores are considered but the weight of the indicators is taken into account as well. It is from the PCA results that we can see the weight of each variable. Each PC explains certain percentage of the variation in the data set. This percentage, divided by the total percentage of variation from all PCs with eigenvectors > 1, determines the weighting factor for variables under particular PC (Andrews et al. 2002a,b). Knowing the indicator score and its weight factor, we can calculate SQI for each observation by the following equation:

$$\mathrm{SQI} = \sum_{i=1}^{n} W_i \times S_i \qquad (1)$$

where W is the weighting factor and S is the indicator score. The weighting factor can be calculated from the Table 2. Therefore, according to our data, the equation for overall SQI would be:

$$\mathrm{SQI} = \sum_{i=1}^{n} 0.48 \times S_{pH} + 0.33 \times S_{BD} + 0.33 \times S_{SOC} + 0.19 \times S_{P} \qquad (2)$$

From the above equation we can see that the highest weight in determining the SQI is given to pH, followed by BD and SOC, while AL-P appears to have lowest weight.

3.3. SQI of different land uses

Overall SQI does not vary much between investigated land uses. Soil quality index for forest was 0.70, which is slightly, but not significantly, better than in agricultural field (0.67) (Figure 3). Therefore, such results indicate no degradation of soil due to agricultural practices. However, PCA showed that soil properties of forest soils differ from agricultural fields (Figure 4). Forest soils have better BD and SOC while agricultural fields have better pH and AL-P (Figure 3).

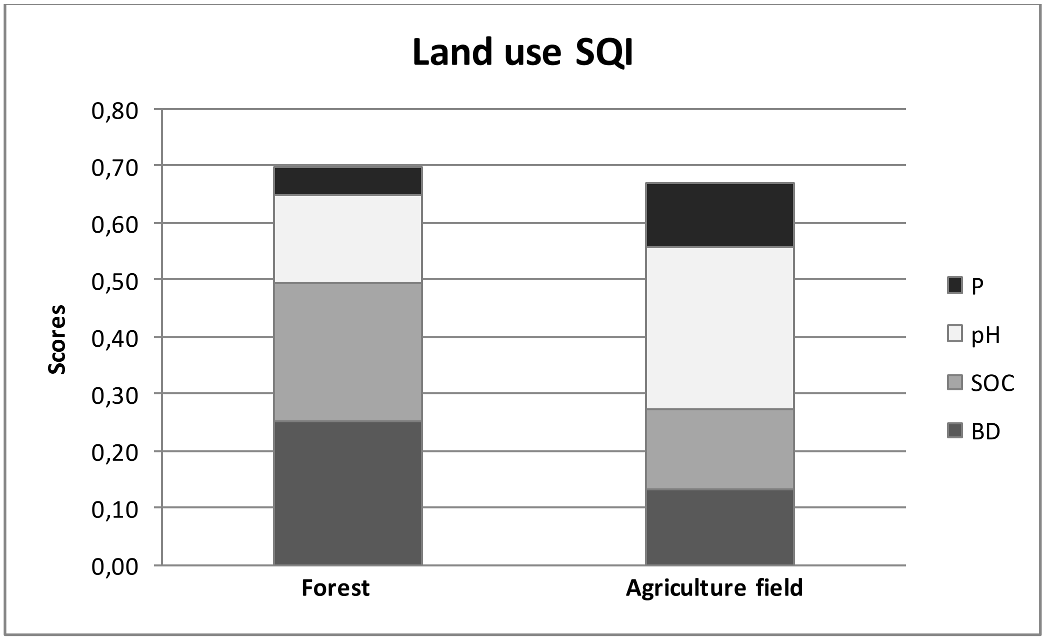

Figure 3. Soil quality index (SQI) of different land uses

Better pH in agricultural fields is a result of liming practice which helps in keeping the pH at desirable levels for agricultural production, while better AL-P is due to the P fertilization. Forest has higher SOC mainly owning to constant input of organic materials such as leaves and fallen branches (forest litter). Decrease in SOC and organic matter (OM) in agricultural fields can affect nutrient cycling, pesticide and water retention as well as soil structure, while degradation of BD can effect root penetration, water- and air-filled space and biological activity (Karlen et al., 1997). Therefore, even though our results indicate no overall degradation of SQ by agricultural practices, certain soil properties have shown degradation (such as SOC content and BD). Thus, SQI can also be used if we want to evaluate the soil in regard to one particular soil property (Brejda et al., 2001). For example, property such as organic matter (OM), where parameter of OM would be determined by correlated variables of the same PC, in our case by the scores of SOC, DOC and N.

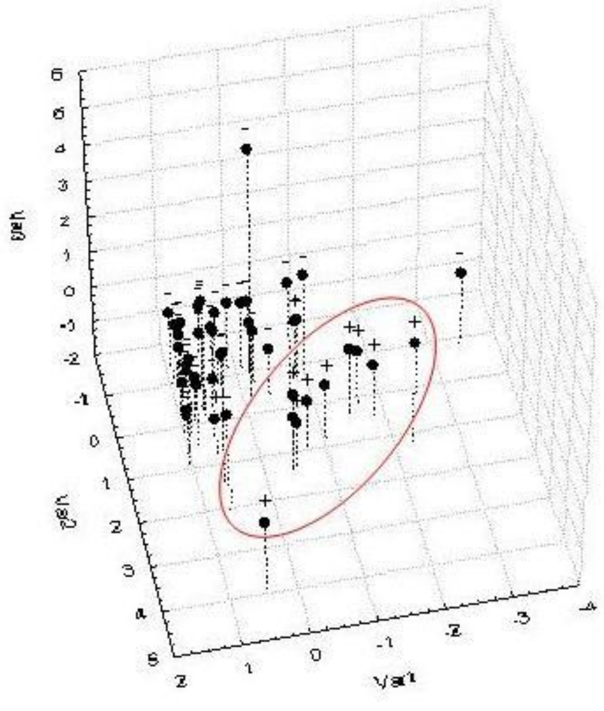

Figure 4. Three dimensional plot of PCA results (+ Forest samples, - Agriculture field samples)

3.4. Relationship between availability of trace metals and SQI

Even though water extractable fraction of Cd, Zn and Mn were included in the PCA when choosing the MDS, and they were correlated with pH, they showed no correlation with an overall SQI. Such results imply that SQI is not a good indicator of trace metal availability for the present study. Solubility of trace metals is controlled by soil properties (Groenenberg et al. 2009; Sauvé et al. 2000; Tipping et al., 2003). In the present study main soil property controlling their solubility was pH. As the PCA results show, soil pH, WE-Cd, WE-Mn and WE-Zn were correlated variables of the PC1 (Table 2). Predicting trace metal availability is best achieved by regression models, using relevant soil properties (Gandois et al. 2010; Ivezić et al. 2011b; Tipping et al. 2003). However, SQI determined with the use of soil properties does not provide us with the adequate information on trace metal availability. Therefore, SQI is a good estimate of soil with regards to specific variables that are being evaluated. In the present study, these variables were pH, SOC, BD and P. However, for information on the trace metal availability some other approaches like regression models should be used.

When discussing the concept of soil quality, it is important to keep in mind what the goal of the investigation is, and what function of soil or what management practice or land use is being evaluated, as soil quality largely depends on the particular use of that soil (agriculture production, remediation of waste, urban, forest, recreation) (Karlen 1997). Therefore, SQI has to be taken with caution. Firstly, the function of particular soil has to be identified, and secondly, variables that are to be used for the determination of SQI have to be chosen. (Doran and Parkin, 1994).

To make the issue of soil quality more understandable to non-experts and policymakers, mapping of soil quality can provide simple visual insight in the current situation of soil quality. Mapping is a useful approach in describing spatial and temporal dynamics of SQ (Cambardella and Karlen, 1999; Wanyama et al., 2005). Considering above mentioned results, GIS technique of mapping SQ is a reliable tool for spatially presenting SQ and potential changes in SQ through time and land use changes. In addition, it can be used to visualize our results with regards to micronutrient availability and SQ. For example, the availability of Mn is not represented by the SQI as well as by pH alone (Figure 5a,b).

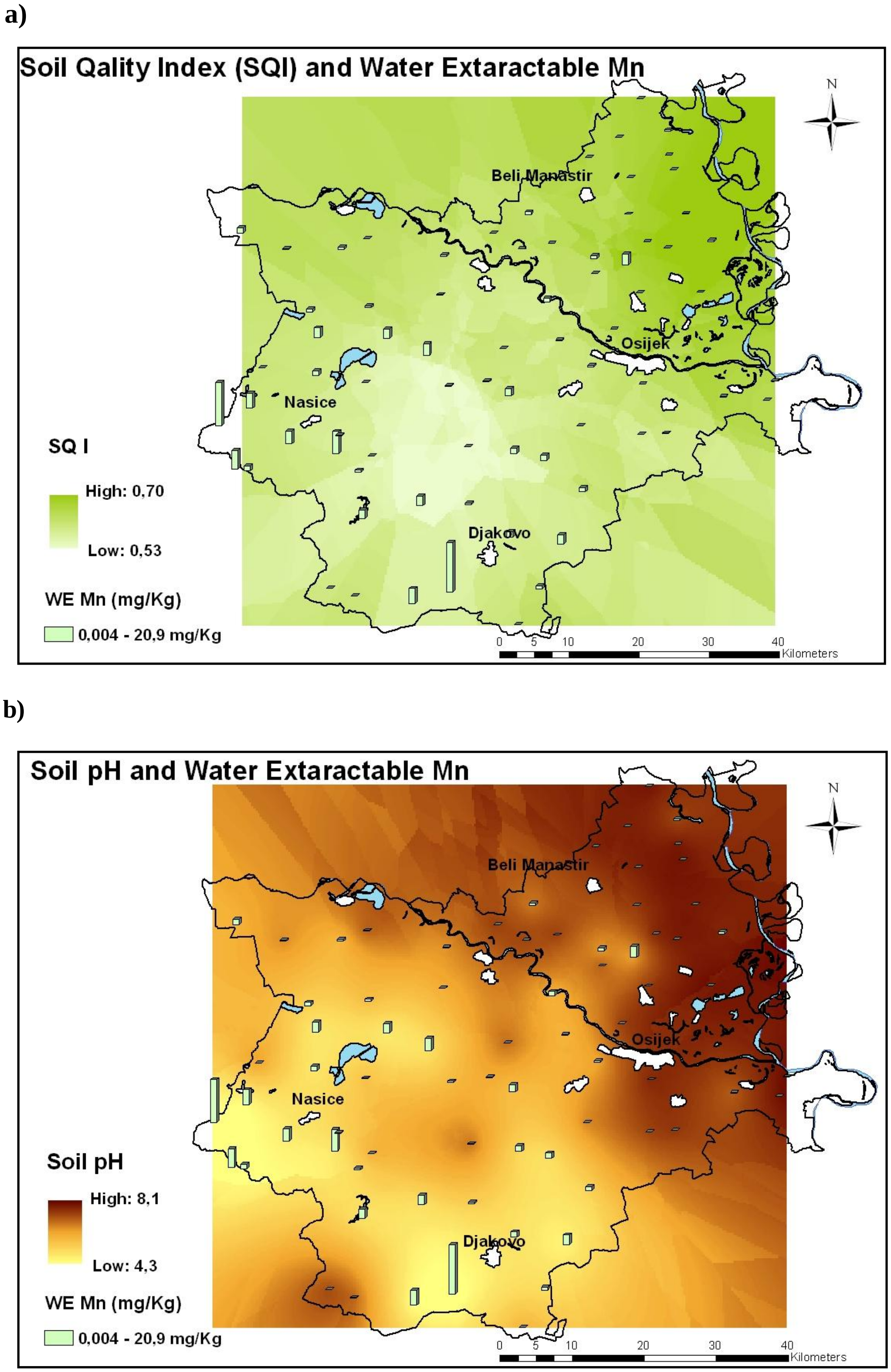

Figure 5 – Water extractable Mn in relation to SQI **(a)** and pH **(b)**

It can be observed from the maps showing dependence of water extractable Mn on SQI and water extractable Mn dependence on soil pH that areas with lower pH cause increase of water extractable Mn. However areas with higher SQI do not indicate higher soil water extractable Mn concentrations, confirming the failure of our hypothesis that SQI could be the indicator of solubility and availability of micronutrients such as Mn. In the present study and in the area investigated pH alone is a good indicator of micronutrient solubility, rather than the SQI.

Conclusion

In the present study, indicators for the determination of SQI were: pH, BD, SOC and AL-P. The use of SQI, as a tool for evaluation of soil, can be beneficial when presenting the soil degradation problems to policymakers. It is a simple indicator of soil degradation that is understandable to the non-experts. However, we have to be careful when deriving the SQI as we first need to know the function of particular soil. It is necessary to emphasize that SQI of particular soil refers to a particular function. In addition, SQI is site specific, so different indicators will have different scores in different regions. If determined properly, SQI can have a practical use in decision making.

There was no significant difference in SQI between forest and agriculture, which indicates that long term agriculture did not result in soil degradation of the area investigated. However, it is evident from the results that some soil properties, such as bulk density (BD) and soil organic carbon (SOC) have degraded over time due to agricultural practices.

In addition, our study showed no correlation between SQI and solubility and availability of trace metals. Information on availability of trace metals cannot be attained from overall SQI. Even though solubility and availability of trace metals is controlled by soil properties, and SQI is determined by the use of main soil properties overall SQI is not a good indicator of trace metal availability. Therefore, availability and solubility of trace metals should be predicted only by specific models that include variables controlling its solubility.

Acknowledgements

This research was conducted with the financial support from “Project 5: improving nutritional quality and safety of food and fodder crops in South Eastern Europe (SEE) countries”, institutional collaboration between Norwegian University of Life Sciences and Faculty of Agriculture in Osijek, Croatia. The authors wish to thank the project for financial support. Finally, the first author is grateful to the Norwegian State Educational Loan Fund (Statens Lånekasse for utdanning) for providing financial assistance.

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