Options for Assessing the Bioavailability of Metals to Soil Dwelling Organisms

Sally Gaw

Department of Chemistry, University of Canterbury, Christchurch (e-mail: *sally.gaw@canterbury.ac.nz*)

Introduction

International regulatory agencies are grappling with the appropriate way to manage contaminated land. Due to the large costs associated with remediation and disposal of soil, there is a need to find viable alternatives to *dig and dump* for managing contaminated soils that only slightly exceed soil acceptance criteria. One option is to allow marginally contaminated soil to remain in place, provided it can be demonstrated that there will be no adverse effect on terrestrial organisms or human health. Numerous studies have demonstrated that the total metal concentration(s) in soil does not always correlate with tissue metal concentrations and/or toxic effects in terrestrial organisms.[1,2] Soil acceptance criteria based on total soil metal concentrations may, for some contaminated soils, be overly conservative. Incorporating site-specific bioavailability assessments into regulatory decision making has the potential to improve the management of contaminated land. Over the last thirty years significant research effort has gone into understanding the processes that govern the bioavailability of metals in soil.[2] Herein, the processes that influence the bioavailability of metals in soils are discussed and an overview of the options currently available for assessing the bioavailability of metals in soil to terrestrial organisms is provided. A previous paper in this journal[3] outlined the options for assessing human exposure to contaminants in soil.

Defining Bioavailability

A plethora of definitions of bioavailability exist. The International Standards Organisation's (ISO) definition is: *Bioavailability is the degree to which chemicals present in the soil may be absorbed or metabolised by human or ecological receptors or are available for interaction with biological systems.*[4] The US National Research Council in their review of bioavailability, rather than defining it, referred to *bioavailability processes*. They defined these as *the individual physical, chemical and biological interactions that determine the exposure of organisms to chemicals associated with soils and sediments*.[1] The bioavailability of contaminants can vary with time as well as between species and soils. A contaminant can be considered as bioavailable when the following three criteria are met: i) a target organism is exposed to the matrix (soil) containing the contaminant, ii) a proportion of the contaminant is available for uptake, and iii) the organism is able to take up the contaminant.[5]

Effects of Metal Contamination on Ecological Receptors

Metals of concern in contaminated NZ soils include arsenic, cadmium, copper, chromium, lead, mercury, nickel and zinc.[6] Industries and activities that can be sources of these metals are listed in Table 1. Depending upon their soil concentration, metal concentrations in plant and invertebrate tissues can exceed toxicity thresholds for wildlife, and in edible crops may exceed those for food standards. Adverse effects of metal contamination in soils include reductions in crop yields, inhibited growth and reproduction in soil invertebrates, and alteration of the soil microbial community structure disrupting key soil functions, including the degradation of organic matter and nutrient cycling.[7-9]

Table 1. Metals of concern in New Zealand contaminated soils - adapted from the Hazardous Activities and Industries List, Ministry for the Environment – see ref. 6.

Activity	As	Cd	Cu	Cr	Pb	Hg	Sn	Zn
Battery recycling		x			x	x		x
Electrical transformers			x		x	x	x	
Pesticides	x	x	x		x	x		x
Fertilisers		x	x					x
Mining	x					x		
Timber treatment	x		x	x			x	
Metal works		x	x	x	x		x	
Gas works	x		x	x	x			
Firing ranges			x		x		x	x

Bioavailability of Metals in Soils

The bioavailability of metals in soils depends upon the metal species present in both the solid phase and the soil pore water, and the partitioning of the metals between these two phases. Metals interact with the soil solid phase through a variety of mechanisms including ion exchange, non specific adsorption and complexation, as well as the formation of precipitates and organometallic complexes.[2] The composition of a soil can affect the sorption and hence bioavailability of metals. Soil properties that influence metal bioavailability include cation exchange capacity, soil organic matter content, iron and aluminium oxides, clay content, moisture content, and pH.[7,9] These properties determine the soil surface charge and the number and types of sites available for sorption of metals, and hence influence the partitioning of the metals between the solid phase and the soil pore water. The composition of the soil pore water also influences partitioning as well as determining the aqueous speciation of the metals. Key soil solution properties include the ionic strength, the presence of competing ions, the concentrations of dissolved organic matter and inorganic ligands (HCO_3^-, Cl^-), and the redox conditions.[10,11]

The bioavailability of metals can decrease with time as

metals in soil undergo processes that inhibit their desorption from the soil solid phase to the soil pore water. These processes are referred to as *aging* and include sorption mechanisms that transform surface electrostatic interactions at ion exchange sites to more stable chemical bonds, surface precipitatation, occlusion by coatings of organic matter and amorphous iron oxides, and diffusion into the mineral matrix and organic matter.[1]

Remediation strategies for contaminated soils often rely on reducing the bioavailability of metals in soil by applying soil amendments that immobilise the metals. A wide variety of amendments have been tested and these include lime, phosphates (both soluble and rock) and organic materials (bio-solids, compost). These amendments immobilise the metals in the soil by altering the pH, increasing the ion exchange sites available to bind metals, or by forming insoluble precipitates that encapsulate the target metals.[12] In situations where the risk(s) associated with contaminated soil is managed by reducing bioavailability, landowners and regulatory agencies will need tools to monitor the effectiveness of the remediation strategy over the long-term.

Bioavailability also differs between species of organisms because exposure pathways and defence mechanisms can vary. Uptake from the soil pore water is the main pathway for plants and microorganisms. Soil invertebrates, including earthworms, may also be exposed to metals through ingestion of soil and food.[11,13] Terrestrial organisms themselves can alter the bioavailability of contaminants in soil.[11,14] Plant roots under nutrient deficient conditions excrete low molecular weight organic acids and protons in order to mobilise nutrients from the soil solid phase. Transpiration processes can reduce the concentration of metals in soil pore water inducing further desorption of metals from the solid phase. Plants may also decrease the bioavailability of metals by adding organic matter to soil thereby increasing the sites for metal sorption.[10] Microorganisms have been shown to both enhance and decrease the bioavailability of metals.[15] Microbial activity produces acids (organic, sulfuric and nitric) that alter the soil pore water pH, enhance dissolution of mineral phases, degrade organic matter and alter redox conditions. Microorganisms can decrease the bioavailability of metals by acting as a sorbent. Earthworms alter the bioavailability of metals in soil by stimulating microbial activity, altering pH through secretion of mucus, and altering the dissolved organic carbon concentration in soil pore water.[13] In addition, organisms can regulate uptake of essential metals, *e.g.* copper and zinc, that become toxic above physiologic threshholds.[7,16]

Measuring the Bioavailability of Metals in Soils

Bioassays

Bioassays can be used to determine toxicity and uptake of metals into tissues from contaminated soils, providing a true measure of bioavailability. For plants and invertebrates, the organism(s) of interest are exposed to the contaminated soil either under laboratory conditions or in the field for a set time period. The measured toxicity endpoints for plants and invertebrates include tissue accumulation, growth, reproduction, and mortality. Assays for effects on microorganisms include the measurement of enzyme activities, respiration rates and microbial biomass in the contaminated soil. Examples of available standardized methods for measuring toxicity and bioaccumulation of metals from soil are summarised in Table 2. ISO standard 17616:2008 provides guidance on the selection and evaluation of bioassays for ecotoxicity testing.[17] Landcare Research has developed protocols for soil toxicity testing of NZ soils and test organisms include kakabeak (*Clianthus puniceous*), lettuce (*Lactuca sativa*), millet (*Panicum milliaceum*), earthworms (*Aporrectodea caliginosa*), and woodlice (*Porcellio scaber*). These protocols were used to derive ecotoxicity thresholds for arsenic, copper and chromium in soil.[18] Drawbacks to the use of bioassays include the time needed to undertake them, the costs involved, and the selection of appropriate test organisms. As the toxicity and accumulation of metals may vary both between and within species, the selection of appropriate target organisms is clearly important.[19-21]

Chemical Extractants

A wide variety of chemical extractions have been proposed as surrogates to bioassays in order to determine the bioavailable fraction of metals in soil. The advantages of a single extractant test compared with a bioassay to determine bioavailability include the simplicity and reproducibility, reduced costs and reduced time frames.[22] These techniques use dissolution, chelation and ion-exchange/desorption processes to extract metals from soil.[10] Several studies have reported a correlation between the extracted *bioavailable* fraction and either toxic effects on, or tissue accumulation in, terrestrial organisms.[1,2,23] The extractants can be classified into the following groups: neutral salt extractions; dilute solutions of weak or strong acids, and complexing agents (Table 3).[24] Improvements in instrumentation, including the development of commercially available ICP-MS instruments, have lowered the achievable detection limits for metals. In turn, the improved limits have enabled use of milder neutral salt extractions and the direct measurement of soil pore water to estimate the bioavailability of metals in soils.[10]

The chemical extraction methods for assessing the bioavailability of metals in soil are all operationally defined.[25] Dried soil (10-20 g) is shaken with a fixed volume of extractant solution for a set time period. The solution is then filtered and acidified with nitric acid before being analysed by ICP-OES or ICP-MS.[1] For example, the German Regulatory method DIN 19730 requires 20 g dried soil and 50 mL of 1 M NH_4NO_3 to be shaken for two hours before filtering, acidifying and analysing.[26] The experimental conditions, including the composition and concentration of the extracting fluid, soil mass to extraction volume ratio, time, and shaker speed and type, can all affect the proportion of the total metal extracted.

Complexing agents such as ethylenediamine-N,N,N,N-tetraacetic acid (EDTA) and (diethylenetriamine) pentaacetic acid (DPTA) were among the first extraction solutions to be tested as surrogate measures of bioavailability.

Table 2. Examples of standardised test methods for bioassays to assess the toxicity and bioaccumulation of metals from soils.

Organism	Target endpoint	Method
Plants	Bioaccumulation Phytotoxicity	ASTM D5435 - 03(2008) Standard Test Method for Diagnostic Soil Test for Plant Growth and Food Chain Protection
Plants	Phytotoxicity	ASTM E1963 – 09 Standard Guide for Conducting Terrestrial Plant Toxicity Tests
Plants	Phytotoxicity	OECD Guidelines for the Testing of Chemicals Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
Brassica rapa, Avena sativa	Seedling Emergence Growth	ISO 22030:2005 Soil quality – Biological methods – Chronic toxicity in higher plants
Earthworms: *Eisenia fetida/Eisenia andrei*	Reproduction	OECD Guidelines for the Testing of Chemicals Test No. 222: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*)
Earthworms: *Eisenia fetida*	Mortality Reproduction avoidance	ISO 11268 Soil quality – Effects of pollutants on earthworms (*Eisenia fetida*). Part 1: Determination of acute toxicity using artificial soil substrate. Part 2: Determination of effects on reproduction. Part 3: Guidance on the determination of effects in field situations
Juvenile snails: *Helix aspersa aspersa*	Growth	ISO 15952:2006 Soil quality – Effects of pollutants on juvenile land snails (*Helicidae*) – Determination of the effects on growth by soil contamination
Enchytraeids: Enchytraeus albidus	Reproduction	ISO 16387: 2004 Soil quality – Effects of pollutants on Enchytraeidae (*Enchytraeus sp.*) – Determination of effects on reproduction and survival
Microorganisms	Nitrification, N mineralisation (28 d incubation)	ISO 14238:1997 Soil quality – Biological methods – Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes
Microorganisms	Respiration Biomass (fumigation-extraction)	ISO 14240-1:1997 Soil quality – Determination of soil microbial biomass – Part 1: Substrate-induced respiration method – Part 2: Fumigation-extraction method
Microorganisms	Substrate induced respiration	ISO 17155:2002 Soil quality – Determination of abundance and activity of soil microflora using respiration curves

Table 3. Examples of extraction solutions used to estimate the bioavailability of metals in soil – see ref. 24.

Extractant Type	Simulates	Example
Water	Soil pore water concentration	Vacuum sampler Centrifugation
Neutral salt	Soil pore water concentration	0.01 M $CaCl_2$ 1 M NH_4NO_3 0.1 M $NaNO_3$
Acid extraction	Potentially soluble in water	Dilute strong acid: 0.43 M HNO_3 Weak acid: 0.43 M acetic acid
Complexing agent	Potentially soluble in water	EDTA

These extractants were originally developed for assessing nutrient levels in soil and in mimicing plant mechanisms to release essential nutrients from mineral phases in soil.[10] The complexing agents form soluble complexes with metals in solution, thus reducing their activity and causing additional metal ions to desorb from the soil. The complexing agents are aggressive and solubilize solid phase minerals giving a potential to over-estimate the bioavailable fraction of metals in soils.[27] McLaughlin *et al*.[10] suggested that complexing extractants may provide a better estimate of potential hazards from future mobilization of metals through changes to the soil, including pH and degradation of organic matter, than neutral salt extractions.

Unbuffered neutral salt solutions (0.001–1.000 M) have been used as soil extractants to predict bioavailability of metals to plants, soil invertebrates and microorganisms.[1] A wide variety of neutral salts have been trialled, with $CaCl_2$ and NH_4NO_3 being among the most commonly used.[25] These solutions target metals in soil pore water as well as metals sorbed on cation exchange sites and, depending on the salt used, they can also form complexes with metals. The overall solution pH is controlled by the soil and the extraction solution has a comparable ionic strength to the soil pore water.[1] Studies comparing results for several neutral salts indicate that, overall, these extractions provide similar information and that metals generally have the same order of extractability. Extraction with 0.01 M $CaCl_2$ is often preferred over other neutral salts because it has the lowest salt concentration, thereby reducing analytical interferences.[28] The best correlations between neutral salt extractions and organism tissue concentrations have been observed in studies where there is a wide range in soil metal concentrations.[1] A systematic review of 104 studies comparing the effectiveness of neutral salt, acid and complexing extractants found that, overall, neutral salt extractions provide the best indication of phytoavailability.[21]

Extractions with weak acids and dilute solutions of strong acids (HNO_3, HCl) target soluble and potentially soluble metals in soil.[1] Unlike total acid digests with concentrated strong acids, the metals in the mineral lattice are not removed by these less aggressive acid extractions;[29] examples of weak acid extractions include the use of acetic and citric acids.[2] Plants excrete low molecular weight

organic acids (LMWOAs) from their roots and these may then release sorbed metals from the soil matrix. Solutions of LMWOAs have been used to extract metals from soil to simulate plant uptake. For example, the *Rhizo method* uses an extraction solution containing acetic, lactic, malic and formic acids in a molar ratio of 4:2:1:1.[30]

Soil Pore Water Measurements

The advances in available analytical techniques have enabled metal concentrations to be measured directly in soil pore water. Methods for collecting soil pore water include centrifugation and the use of vacuum samplers, including rhizon samplers. After sample collection, either the free metal is measured using ion selective electrodes or anodic stripping volatammetry, or the sample is acidified and the total metal concentration is measured by ICP-MS.[1] The soil moisture content may be adjusted to a standardized water holding capacity with deionised or milli-Q water before extraction.[31] Pore water extraction methods tend to be time-consuming and the speciation can change during extraction.[10,29]

DGT Devices

Diffusive gradients in thin films (DGT) is a kinetic-based technique that simulates metal uptake by plant roots. These devices measure the soil pore water metal concentration as well as the ability of the soil solid phase to re-supply the soil pore water with metal ions. The device consists of a small round plastic deployment moulding that contains an ion exchange resin (Chelex 100) embedded in a hydro gel and covered by a protective filter. Cations in the soil pore water diffuse across the hydro gel and accumulate in the resin. The resin depletes metals from the soil pore water inducing further metal ions to desorb from the solid phase. The DGT device is deployed for a set amount of time by placing it in contact with soil. The metals are extracted from the gel by nitric acid and measured by ICP-MS and the effective concentration, C_E, calculated. It is a measure of the concentration of the metal in the soil pore water plus the metal ions re-supplied by the soil solid phase, and is determined by the physico-chemical characteristics of the soil.[2,32]

A variety of other techniques are being tested for their suitability as surrogate measures of bioavailability. These include isotope dilution techniques, ion exchange membranes, sequential extraction procedures, extraction solutions that mimic the digestive processes in earthworms, and solid and solution phase speciation models.[2,33,34]

Limitations of Available Methods

A key limitation to the widespread adoption of chemical extractions as surrogate measures of bioavailability is that there has only been limited validation of the available methods. Few studies have validated the results of chemical extractions with the results from bioassays over different soil types, contamination levels and organisms. There are also only a limited number of certified reference soils available to enable laboratories to validate their analytical methodologies and ensure consistency between laboratories. To date, no one extraction method has been shown to satisfactorily perform under all conditions for all organisms and/or metals.[1,25,34] The currently available extraction methods do not have a mechanistic basis and instead rely on empirical relationships. The NRC, in their review of available tools to assess bioavailability, recommended that mechanistic tools needed to be developed and that the mechanistic basis of bioavailability required further investigation.[1]

Current use of Bioavailability Testing in New Zealand

Bioavailability testing is already used in the NZ agricultural sector for assessing the nutrient status of pasture soils and essential metals (Cu, Zn). Soil tests using EDTA and Mehlich3 solutions (0.200 M CH_3CO_2H + 0.250 M NH_4NO_3 + 0.013 M HNO_3 + 0.015 M NH_4F + 0.001 M EDTA) are available through commercial analytical laboratories.[35] Extractions with neutral salt and complexing solutions have been used as research tools in studies investigating plant uptake of metals from NZ soils.[19,31,36]

Regulatory Acceptance of Bioavailability Testing

Currently bioavailability testing is not used in NZ to assess contaminated land. Only a limited number of countries

Table 4. Examples of soil acceptance criteria based on neutral salt extractable metal concentration. Units are mg/kg – see refs. 37 and 38

Country	1 M NH_4NO_3					0.1 M $NaNO_3$
	Germany		Austria		Slovakia	Switzerland
	Plant growth	*Plant quality*	*Plant growth*	*Plant quality*	*Plant quality*	*Soluble content*
As	0.4		0.6	0.1	0.4	
Cd		0.04/0.1		0.04	0.1	0.03
Cr				0.1		
Cu	1		1.5	0.8	1	0.7
Hg				0.005		
Ni	1.5		1.0		1.5	
Pb		0.1		0.3	0.1	1.0
Th		0.1				
Zn	2		4		2	0.5

currently incorporate bioavailability testing into regulatory decision-making for management of contaminated land. Countries with soil acceptance criteria for neutral salt extractions of metals include Switzerland, Germany, Austria, and Slovakia (Table 4).[37,38] The Dutch National Institute for Public Health and the Environment (RIVM) has recently selected methods for a pilot study investigating the feasibility of incorporating bioavailability tests into policy for risk evaluation for contaminated soils. The two methods selected for metals (Cu, Pb, As) are extraction with 0.01 M $CaCl_2$ to estimate actual (bioavailable) concentrations and a weak acid extraction with 0.43 M HNO_3 to estimate potential (bioavailable) concentrations.[29]

Incorporation of Bioavailability Testing into the Regulatory Framework of NZ

Before bioavailability testing can be incorporated into the NZ framework for the management of contaminated land, appropriate methods would need to be selected and validated for use in our soils. NZ soils *are* sufficiently different from many of the soils used in studies overseas, making it inadvisable to adopt bioavailability testing methodologies from overseas without appropriate validation. Overall, NZ soils tend to have a higher organic content and lower pH levels than soils in other regions of the world.[39]

The ISO has recently published a standard on method selection for assessing the bioavailability of contaminants in soils, namely *ISO 17402:2008 Soil quality -- Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials*.[40] This standard could be used to select appropriate methods for validation under NZ conditions. The recommended selection criteria include a method that does not alter the soil's physical properties, has a mechanistic/physiologic basis; has a correlation with biologically measured effects and has been validated by inter-laboratory trials.[24] RIVM also considered the acceptability of the method to policy makers, and the cost of the method, when selecting appropriate methods for their pilot study.[28]

Summary

It is now widely accepted that measuring the soil total metal concentration may not accurately predict the risks associated with soil contamination. Over the last three decades extensive research has focussed on understanding the processes that determine the bioavailability of metals in soil. Efforts to develop tools to predict bioavailability have only had limited success. Further work will be required to develop and validate tools that enable bioavailability assessments to be incorporated into regulatory decisions for management of contaminated land.
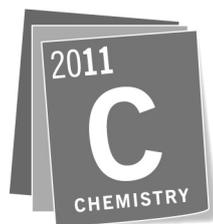
Acknowledgements

References

1. National Research Council. *Bioavailability of Contaminants in Soils and Sediments. Processes, Tools and Applications*, National Academies Press: Washington, 2003.

2. Peijnenburg, W. J. G. M.; Zablotskaja, M.; Vijver, M. G. *Ecotoxicol. Environ. Safety* **2007**, *67*, 163-179.

3. Gaw, S; Kim, N.; Northcott, G.; Wilkins, A.; Robinson, G. *Chem.in NZ* **2008**, *72*, 47-50.

4. *ISO 11074:2005 Soil quality - Vocabulary International Organisation for Standardisation*, International Organisation for Standardisation, 2005.

5. Juhasz, A.; Smith, E.; Naidu R. *Estimation of human availability of arsenic in contaminated soils*. In *Proc.5th Nat. Workshop:Assessment of Site Contam.* Adelaide, May 2002, Langley, A.; Gilbey, M.; Kennedy, B. (Eds.), NEPC: Adelaide, 2003, 183-194.

6. *Contaminated Land Management Guidelines Schedule A: Hazardous Activities and Industries List (HAIL)*. Ministry for the Environment: Wellington, 2004.

7. Kabata-Pendias, A.; Pendias, H. *Trace Elements in Soils and Plants,* CRC Press: Boca Raton, 3rd edn. 432, 2001.

8. Giller, K. E.; Witter, E.; McGrath, S. P. *Soil Biol. Biochem.* **1998**, *30*, 1389-1414.

9. Adriano, D. C. *Trace Elements in Terrestrial Environments*. In *Biogeochemistry, Bioavailability, and Risks of Metals*, 2nd edn. Springer-Verlag: New York 2001, 61-90.

10. McLaughlin, M. J.; Zarcinas, B. A.; Stevens, D. P.; Cook, N. *Comm. Soil Sci. Plant Anal.* **2000**, *31*, 1661-1700.

11. Nolan, A. L.; Lombi, E.; McLaughlin, M. J. *Aus. J. Chem.* **2003**, *56*, 77-91.

12. Bolan, N.S.; Duraisamy, V.P. *Aus. J. Soil Res.* **2003**, *41*, 533-555.

13. Sizmur, T.; Hodson, M. E. *Environ. Pollut.* **2009**, *157*, 1981-1989.

14. Peijnenburg, W. J. G. M.; Jager, T. *Ecotoxicol. Environ. Safety* **2003**, *56*, 63-77.

15. Wu, S. C.; Luo, Y. M.; Cheung, K. C.; Wong, M. H. *Environ. Pollut.* **2006**, *144*, 765-773.

16. Peijnenburg, W. J. G. M.; Baerselman, R.; de Groot, A. C.; Jager, T., *et al. Ecotoxicol. Environ. Safety* **1999**, *44*, 294-310.

17. *ISO 17616:2008 Soil quality – Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials*. International Organisation for Standardisation, 2008.

18. Markich, S. J.; Warne, M. St. J.; Westbury, A.; Roberts, C. J. *Aust. J. Ecotoxicol.* **2002**, *8*, 1-72.

19. Gray, C. W.; McLaren, R. G.; Roberts, A. H. C.; Condron, L.M. *Aus. J. Soil Res.* **1999** *37*, 461-477.

20. Granel, T.; Robinson, B.; Mills, T.; Clothier, B., *et al. Aus. J. Soil Res.* **2002**, *40*, 1331-1337

21. Menzies, N. W.; Donn, M. J.; Kopittke, P.M. *Environ. Pollut.* **2007**, *45*, 121-130.

22. Conder, J. M.; Lanno, R. P.; Basta, N. T. *J. Environ. Qual.* **2001**, *30*, 1231-1237.

23. Catherine, M.; Daoust, C. M.; Bastien, C.; Deschênes, L. *J. Environ Qual.* **2006**, 35, 558-567.

24. Harmsen, J. *J. Environ. Qual.* **2007**, *36,* 1420-1428.

25. Rao, C. R. M.; Sahuquillo, A.; Lopez Sanchez, J. F. *Water Air Soil Pollut.* **2008**, *189,* 291-333.

26. Gryschko, R.; Kuhnle, R.; Terytze, K.; Breuer, J., *et al. J. Soils Sediments* **2005**, *5*, 101-106.

27. Singh, B. W. In *Natural Attenuation of Trace Element Availability in Soils,* Hamon, R.; Mclaughlin, M.; Lombi, E. (Eds.), Soc. Environ. Toxicol. Chem.: Pensacola, FL 2007, 1-18.

28. Pueyo, M.; Lopesz-Sanchez, J. F.; Rauret, G. *Anal. Chim. Acta* **2004**, 217-236.

29. Brand, E.; Peijnenburg, W.; Goenenberg, B.; Lizjen, J., *et al. Towards implementation of bioavailability measurements in the Dutch regulatory framework,* RIVM Report 711701084/2009 National Institute for Public Health and the Environment: Bilthoven, 2009.

30. Feng, M-H.; Shan, X-Q.; Zhang, S.; Wen, B. *Environ. Pollut.* **2005**, *137*, 231-240.

31. Speir, T. W.; Van Schaik, A. P.; Percival, H. J.; Close, M. E., *et al. Water Air Soil Pollut.* **2003**, *150*, 1573-2932.

32. Zhang, H.; Zhao, F-J.; Sun, B.; Davison, W., *et al. Environ. Sci. Technol.* **2001**, *35*, 2602-2607.

33. Ma, W. K.; Smith, B. A.; Stephenson, G. L.; Siciliano, S. D. *Environ. Toxicol. Chem.* **2009**, *28*, 1439-1446.

34. Nolan, A. L.; Zhang, H.; McLaughlin, M. J. *J. Environ. Qual.* **2005**, *34,* 496-507.

35. *Hill Laboratories Technical Note: Soil Tests and Interpretation*. R. J. Hill Laboratories Ltd., Hamilton - undated.

36. Andrewes, P.; Town, R. M.; Hedley, M. J.; Loganathan, P. *Aust. J. Soil Res.* **1996**, *34*, 441-452.

37. Carlon, C.; D'Alessandro, M.; Swartjes, F. *Derivation Methods of Soil Screening Values in Europe. A review and evaluation of national procedures towards harmonisation. JRC Scientific and Technical Reports:* European Commission 2007 – see http://ies.jrc.ec.europa.eu/uploads/fileadmin/Documentation/Reports/RWER/EUR_2006-2007/EUR22805-EN.pdf.

38. Gupta, S. K.; Vollmer, M. K.; Krebs, R. *Sci. Total Environ.* **1996**, *178*, 11-20.

39. Lowe, D. J.; Newnham, R. M.; McCraw, J. D. *Quaternary environmental change in NZ and its effects on the soil pattern*. In: *Soil 2000: New Horizons for a New Century,* Adams, J. A.; Metherell, A. K. (Eds.). Australian and New Zealand 2nd Joint Soils Conference, Lincoln University, 3-8 Dec. 2000. NZ Soc. Soil Sciences 2000, 117-118; Hewitt A. *Are New Zealand Soils Distinctive?: A Subterranean View of NZ ecosystems, NZ Soil News,* **1997**, *45,* 7-16;. Burney, B.; Rahman, A.; Oomen, G. A. C.; Whitham, J. M. *The organic matter status of some mineral soils in NZ.* In *Proc. 28th NZ Weed and Pest Control Conf.,* Hartley.M. J. (Ed.), NZ Weed and Pest Control Society: Hamilton, September 1975, 101-103.

40. ISO 17402: 2008 *Soil quality -- Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials,* International Organisation for Standardisation, 2008.