SNOWMAN NETWORK
Knowledge for sustainable soils

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IBRACS

Integrating Bioavailability in Risk Assessment of Contaminated Soils: opportunities and feasibilities

Final Research Report

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EXECUTIVE SUMMARY

In all countries in Europe, and probably the rest of the world, soil quality criteria (SQC) are based on total concentrations of contaminants. Indeed, the total concentration is an indicator of toxicity, but vast amounts of laboratory and field studies have shown that biological effects are not directly related to the total concentration. Instead, soil organisms respond to the fraction of contaminant that is biologically available. One way to deal with bioavalability is to use biological tests directly, with the aim to cover relevant organisms and/or soil functions. Although being of high relevance, biological tests are generally costly, time consuming, and complicated to perform and evaluate, which limits their use in practical risk assessments. In this respect, chemical methods offer an alternative, having the potential to be faster, cheaper and easier to commercialise. However, before any chemical bioavailability method can be used in a risk assessment framework, a corresponding reference system based on ecotoxicity test data must have been developed. In other words, we need a framework that relates the measured bioavailable concentration to predefined ecosystem protection goals, e.g. protection of a certain fraction of species. To our knowledge, no such "official" framework including a bioavailablity methodology yet exist in any country. By introducing bioavailability in risk assessment frameworks, the accuracy of the assessment is expected to increase, which are expected to save money and result in more sustainable remediation actions.

The overall aim of IBRACS has been to provide policymakers, other authorities and service providers with guidelines on how chemical bioavailability tests and results of bioavailability-based risk assessment models can be used for risk-based management decisions on contaminated land. The specific objectives were:

1) To review existing risk assessment models for soils in Sweden, Belgium (Flanders, Wallonia) and the Netherlands with focus on bioavailablity.
2) To evaluate the ability of so-called passive samplers and established soil extracts to predict toxic responses of plants to exposures of metals (Cu, Ni, Zn) and organic contaminants (polycyclic aromatic hydrocarbons, PAHs).
3) To evaluate plant uptake models and soil tests for PAH and how to incorporate them into risk assessment models.
4) To make a cost-benefit analysis of including bioavailability tests in site specific risk assessment.
5) To give recommendations on how to integrate chemical bioavailability tests in risk assessment frameworks (Cu, Zn, Ni and PAH).

The review of risk assessment models used for deriving SQC showed that soil property corrections only are made in the Flanders (OVAM, 2008) and Dutch (VROM, 2009) models. However, the equations used for metals in the two countries are of different origin; in the Flanders model the equations are derived from ecotoxicological tests, whereas in the Dutch model the equations are based on regression analyses of observed **background concentrations** in nature area's and "unpolluted agricultural areas". For PAHs the equations used for soil property correction are similar in both countries/regions, i.e. based on the theory of equilibrium partitioning of PAHs between the water and organic matter phases.

The work on metal toxicity aimed to identify a chemical method that accounts for bioavailability and is applicable on historically contaminated soils. The method should ideally draw on existing soil limits that are based on soils spiked with metal salts. For that reason we compared the toxic response of barley in nine Zn or Cu contaminated soils and in corresponding $ZnCl_2$ or $CuCl_2$ spiked reference soils. In total, eight different soil tests were compared, including six soil extracts, diffusive gradients in thin films (DGT) and an isotopic exchange method using stable isotopes. Total metal toxicity to barley seedling grown in the field contaminated soils was up to 30 times lower than in corresponding spiked soils. Total metal (aqua regia soluble) toxicity thresholds (EC50) varied with factors up to 260 (Zn) or 6 (Cu) among soils. For Zn, variations in EC50 thresholds decreased as aqua regia > 0.43 M HNO_3 >0.05 M EDTA > 1 M NH_4NO_3 > cobaltihexamine > DGT > 0.001 M $CaCl_2$, suggesting that the latter extraction is the most robust phytotoxicity index for Zn. The EDTA extraction was the most robust for Cu contaminated soils.

Converting the limits for Zn using an intensity based soil test (e.g. 0.001 M $CaCl_2$) to obtain an estimation of bioavailable metal requires a full recalibration exercise, i.e. numerous tests (different species, endpoints, soils) with associated doses confirmed with methods such as, for instance, 0.001 M $CaCl_2$. Practically, this is a huge task. Instead, the isotopic dilution method offers a pragmatic solution. The relative metal toxicity found in barley tests (EC50 for historically contaminated soils/EC50 for spiked soils) corresponded well with the fraction of aqua regia soluble (total) metal that is isotopically exchangeable. Accordingly, the fraction of isotopically exchangeable metal can be used as a site specific measure of a "leaching/ageing factor" (L/A factor). The concept of L/A factor is presently used in a well established software for deriving soil ecotoxicological limits for metals, i.e. the soil PNEC calculator (http://www.arche-consulting.be/metal-csa-toolbox/soil-pnec-calculator/). In that software, as in the EU risk assessment, generic values for L/A factors are being used, e.g. a value of 3 has been selected for Zn contaminated soils and a factor of 2 for Cu (Smolders et al., 2009). A revised version of the soil PNEC calculator has been developed by the consulting company ARCHE, in collaboration with IBRACS, that allows for the entry of site specific L/A factors. Since an increasing proportion of laboratories have been equipped with ICP-MS, stable isotopes can now be used instead of radiosotopes, i.e. isotopic exchange methods are no longer limited to facilities with permission to use radio isotopes.

We propose the following approach to integrate different chemical measures of metal toxicity in a tierd ecological risk assessment: in tier 1 total concentrations are analysed and compared with national generic soil limits; in tier 2 soil type specific soil limits are obtained by the PNEC-calculator using total metal concentration, clay content, organic matter content and pH as input values; in site specific tier 3 risk assessments, soil and contaminant specific soil limits are obtained by applying the isotopic dilution method to obtain site specific L/A factors, which can be used as inputs to the revised version of the PNEC-calculator. Note that this approach can be used as an integrated part of a more extensive site specific ecological risk assessment procedure, also involving toxicological and ecological measures of "risks", like the Sediment Quality Triad (Chapman 2000).

The feasibility of introducing the tier 1 and 2 procedures into the soil law currently in place Wallonia was the focus of a case study investigation. Copper was chosen as the pollutant and a method inspired by the PNEC calculator was adopted to develop new soil limit values protecting ecosystems. Both local pollution and proximal atmospheric pollution taken ito consideration. The feasibility study demonstrated that

bioavailability could readily be introduced into one of the steps of the legal procedure that allows the most flexibility, namely tier 1 of the risk assessment studies. Concrete proposals have also been suggested which could allow for further developments for taking bioavailability into account at the tier 2 level.

The tier 3 procedure was used to calculated site specific guideline values for two metal contaminated sites, Björkhult in Sweden (Cu) and La Calamine in Belgium (Zn). The Björkhult site was used for impregnation of telegraph poles by so called Boucherie method using 1.5-2% copper sulphate solution as an impregnation agent. The area was severly contaminated with total Cu concentrations up to 2190 mg/kg dw. The site-specific L/A factor was 2.3, i.e. close to the default L/A factor for Cu of 2.0. The calculated site specific PNEC value for ecological risks was similar to the Swedish generic guideline value (less sensitive landuse, 200 mg/kg). As a result, no substantial changes in the final conclusions regarding the site management to the ones suggested by the previous site investigators could be made. In contrast, when applying the proposed site specific risk assessment procedure on the La Calamine site, the site specific risk limit became considerable higher than the Walloon generic trigger values for soil Zn. La Calamine is one of the two most important mines along the Geul river. A significant fraction of Zn is bound in ore minerals. The site specific L/A factor was about one order of magnitude higher than the default L/A factor (35 vs. 3), which resulted in PNEC concentrations in the range 2000-2800 mg/kg dependent on soil type. The generic trigger values for soil Zn in residential areas and industrial sites in Wallonia is 230 and 320 mg/kg, respectively. At this site it would be a considerable cost-saving if the proposed tier 3 procedure is applied in the site specific risk assessment.

Regarding PAH ecotoxicity assessment, we have evaluated the option to use a passive sampler method, in combination with the equilibrium partitioning theory, as a basis for a risk assessment framework. The equilibrium passive sampler polyoxymethylene (POM) was used to assess the bioavailability of native polycyclic aromatic hydrocarbons (PAHs) in 22 diverse historically contaminated soils (coke work, gas work and wood tar sites), alongside the lipid concentrations in exposed worms (*Enchytraeus crypticus*).

The soils studied covered a wide range in soils properties, including texture, pH and organic carbon content. Total concentrations of PAHs in soils varied considerably (0.27 - 2651 µg/g); so did the corresponding POM derived pore water concentrations (0.02 - 460 µg/). One major finding was that the TOC normalized partition coefficients for PAHs was about one order of magnitude higher than those recommended by national agencies, like the United States Environmental Protection Agency (USEPA) for sediments and the Netherlands' National Institute for Public Health and the Environment (RIVM) for soils and sediments, i.e. the sorption of PAHs was significantly stronger in the historically contaminated soils than in “spiked soils” normally used in toxicity experiments. This illustrates the need to actually measure pore water concentrations in historically contaminated soils as a first step in a site specific risk assessment that accounts for bioavailability.

Soil quality standards and critical limit values for non-polar organic compounds, like PAHs, are in most countries based on the assumption of equilibrium partitioning. According to this theory, freely dissolved PAHs in the pore water are in equilibrium with both the soil organic matter component and the lipid phase of soil organisms. Our results support that the assumption of equilibrium partitioning also holds

for diverse historically contaminated soils; i.e. we found strong correlations between pore water concentrations and lipid concentrations for the investigated PAHs.

A key issue in a risk assessment framework that uses chemical methods for assessing a "bioavailable" concentration or fraction is to develop a reference system to which this concentration or fraction can be related. In this respect we draw on a recent RIVM compilation (Verbruggen, 2012). Here, "critical lipid concentrations" for a wide range of organisms (soils, sediments and waters) were presented. The critical lipid concept is based on the assumption that toxicity of individual PAHs is similar after entering the cell membrane (narcosis model). The RIVM compilation resulted in two proposed "critical lipid concentration", corresponding to two sets of critical pore water concentrations for individual PAHs, indicating "no risk" (Maximum Permissible Concentration, MPC) or "serious risk" (Serious Risk Concentration, SRC).

We propose the following scheme to include equilibrium-based chemical bioavailability tests in site specific ecological risk assessments of PAHs contaminated soils: 1) Determine pore water concentration of freely dissolved PAHs, 2) Relate individual concentrations to risk limits (e.g. RIVM's MPC or SRC values), using the toxic unit approach, 3) Assume additive effect and calculate the toxic unit value (if > 1, risk). This procedure is in line with the one proposed by Brand et al. (2013). To facilitate the application of this procedure, we have developed the IBRACS calculator, which is available at IBRACS homepage (http://projects.swedgeo.se/ibracs/). The procedure has been applied on two Swedish PAH contaminated sites (Riksten in Botkyrka and Wermlandskajen in Karlstad) and the outcome was compared with an assessment based on the Swedish generic guideline values. The comparison showed that the number of samples indicating "no risk" (MPC) to soil organisms decreased from 80% to 20% at Riksten, and from 100% to 60% at Wermlandskajen, when applying the proposed procedure. Accordingly, the time and money invested in extra POM analyses are likely to be paid off during the remediation phase.

PAH uptake experiments with maize plants were perfomed with the same soils as the ecotoxicity experiments. In addition to pore water determinations using the POM method also a Tenax solid phase exctraction was used. The main transfer route to plants is generally supposed to be through soil solution uptake and risk assessment models rely on pore water PAH concentrations estimated from total soil concentrations using equilibrium partitioning theory. Our results lend no support to this hypothesis, because of lack of correlation between determined pore water concetrations and plant uptake. In contrast, uptake by roots was closely correlated to the total soil concentration. This would suggest a direct uptake route between roots and soil solid phase.

The most frequently applied modeling approach used is the one proposed by Briggs et al. (1982, 1983), both for roots and shoots compartments. The hypothesis supporting this model are mostly overruled in the case of PAH (log K_{ow} higher than 4), but it gave the best estimate of PAH uptake in our study. Thus, this model can be used for rough estimates of plant uptake of PAHs. However, given the great uncertainty in this modelling approach, measurements of plant root and shoot concentrations would be the superior and most accurate option in site specific rsik assessments.

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1. Background

In all countries in Europe, and probably the rest of the world, soil quality criteria (SQC) are based on total concentrations of contaminants (Carlon, 2007). Indeed, the total concentration is an indicator of toxicity, but vast amounts of laboratory and field studies have shown that biological effects are not directly related to the total concentration. Instead, soil organisms respond to the fraction of contaminant that is biologically available. To find a chemical method that measures "the bioavailable fraction" might sound easy. However, in reality there are many complicating factors that are related both to the chemical behaviour of contaminats in the soil, and to the mechanisms of interaction of contaminants with the soil organisms.

In order to get a common conceptual view of biological availability, or bioavalability as it is more commonly referred to, an international standard has been developed by ISO (ISO 17402). Bioavailablity is being defined as "the degree to which chemicals present in the soil may be taken up or metabolised by human or ecological receptors or are available to interact with biological systems". Accordingly, the bioavailablity has to be defined in relation to the organism or soil function that has to be protected. Ideally, of course, the methods or concepts proposed should be as general as possible, i.e. being applicable to a as many types of organisms as possible. One way to deal with bioavalability is to use biological tests directly, with the aim to cover relevant organisms and/or soil functions (e.g. ISO 16198 RHIZOTEST) . In biological tests, organisms are being exposed to soil materials and possible effects are being monitored. If uptake of contaminant and/or effect (e.g. mortality, growth inhibition) are being detected, it is likely that a bioavailable contaminant is present. Although being of high relevance, biological tests are generally costly, time consuming, and complicated to perform and evaluate, which limits their use in practical risk assessments. In this respect, chemical methods offer an alternative, having the potential to be faster, cheaper and easier to commercialise. However, before any chemical bioavailability method can be used in a risk assessment framework, a corresponding reference system based on ecotoxicity test data must have been developed. In other words, we need a framework that relates the measured bioavailable concentration to predefined ecosystem protection goals, e.g. protection of a certain fraction of species. To our knowledge, no such "official" framework including a bioavailablity methodology yet exist in any country.

The main driver of introducing bioavailability in risk assessment is to increase the accuracy in the risk assessment, which are expected to save money and result in more sustainable remediation actions. For example, even at strongly contaminated sites, there are normally areas that are moderately contaminated. These areas might be large, resulting in high costs if remediated. An improved risk assessment methodology, accounting for bioavailability in a proper way, could make a large difference, both in terms of treated soil masses, money and environmental impact. Furtermore, adopting the bioavailability concept in riskassessments opens up site specific management options based on immobilization of contaminants (reducing bioavailablity). As stated in the final report from the 6th International workshop on Chemical Bioavailability in the Terrestrial Environment held on 7-9 September 2011 in Adelaide, Australia "bioavailablity is a tool for smarter risk based land management" (Harmsen and Naidu, 2013).

In most countries a tiered risk assessment approach for contaminated land is being used, going from a simplistic, general level (tier 1) to more detailed levels (tier 2 and 3). A schematic presentation of the Dutch regulatory framework is shown in Figure 1.1. In tier 1 and 2, measured total concentrations are compared with postulated SQC. In a few countries, the SQC can be corrected for soil properties using simple mathematical functions based on e.g. organic matter and clay content. This is the case in the Netherlands and Belgium (Flanders), as will be discussed further in chapter 4. The most realistic level to introduce chemical bioavalability methods in a risk assessment framework is in tier 3. In IBRACS we have tested eight different soil tests to assess phytotoxiciy of metals, which is presented in chapter 5, and a passive sampler procedure to assess the bioavalability of polycyclic aromatic hydrocarbons (PAH), reported in chapter 6. Furthermore, recommendations on how to integrate the selected methods in site specific risk assessment frameworks are being discussed in chapter 9. In addition to toxic effects of PAH we have been investigated the possibility to use the passive sampler procedure for assessing uptake of PAH by plants (chapter 7).

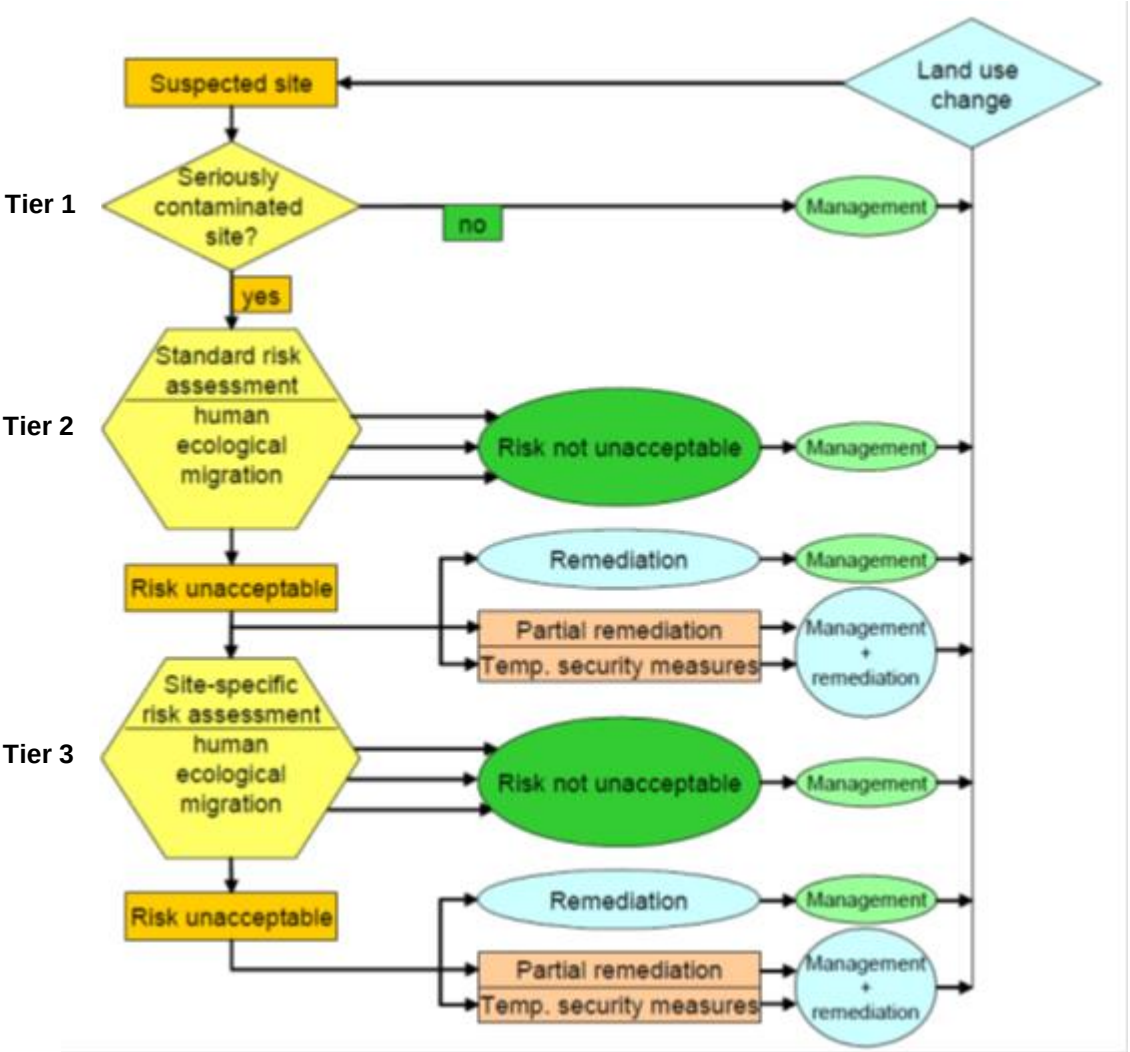


Figure 1.1 Tiers within the Dutch regulatory framework for soil contamination (VROM 2009).

2. Aims

The overall aim of IBRACS is to provide policymakers, other authorities and service providers with guidelines on how chemical bioavailability tests and results of bioavailability-based risk assessment models can be used for risk-based management decisions on contaminated land.

Experimental calibration/validation exercises have been made on soil-plant transfer and ecotoxicity of contaminants. The focus has been on selected contaminants for which either soil-plant transfer (PAH) or ecotoxicity (Cu, Zn, Ni, PAH) normally are decisive for their over-all risk assessment (e.g. Naturvårdsverket, 2009). The soil-plant transfer is the first critical step in the soil-plant-human exposure pathway.

The specific objectives were:

1) To review existing risk assessment models for soils in Belgium (Flanders, Wallonia), Sweden, and the Netherlands with focus on bioavailablity.
2) To evaluate the ability of so-called passive samplers and established soil extracts to predict toxic responses of plants to exposures of metals (Cu, Ni, Zn) and organic contaminants (PAH).
3) To evaluate plant uptake models and soil tests for PAH and how to incorporate them into risk assessment models.
4) To make a cost-benefit analysis of including chemical bioavailability tests in site specific risk assessment.
5) To give recommendations on how to integrate chemical bioavailability tests in risk assessment frameworks (Cu, Zn, Ni and PAH).

3. General description of the project

The program structure and individual work packages (WPs) are shown in Figure 3.1 and partner organisations and members in Table 3.1. The general idea with the project structure was to start with experimental work in WP4 and WP5, aiming at validating some promising chemical test methods. In WP4 we tested methods to assess phytotoxicity of metals and toxicity of PAH to *Enchytraeus crypticus*, whereas in WP5 plant uptake of PAH was in focus. The toxicity tests developed in WP4 was later applied on “real cases” in WP6 and the outcome was compared with the outcome based on national default risk limits. Parallel to this work a review of existing risk assessment models in Sweden, Belgium (Flanders, Wallonia), France and the Netherlands was made in WP3. Here, the focus was on the potential roll of bioavailability in ecological risk assessment and the soil plant transfer of pollutants.

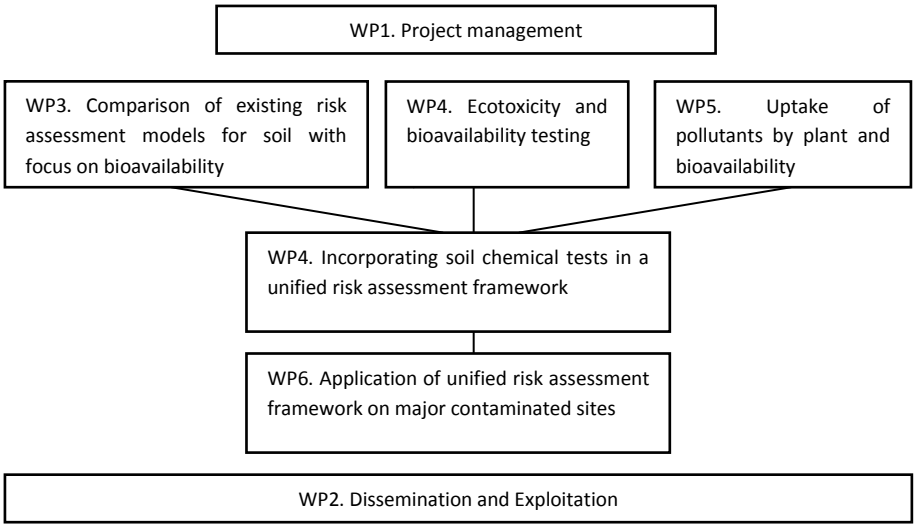


Figure 3.1 The IBRACS structure.

Table 3.1 Partners and members of the IBRACS team. Project contacs are indicated in bold.

Partners	Members	Leading
Swedish Geotechnical Institute / SGI On sub-contract: Swedish Environmental Research Institute /IVL	**Dan Berggren Kleja** Anja Enell Michael Pettersson Ann-Sofie Allard	WP1, WP2 and WP3
Stockholm University / SU On sub-contract: Norweigian Geotechnical Institute /NGI	**Gerard Cornelissen** Hans-Peter Arp	WP4 (PAH)
Luleå University of Technology/LTU	**Jurate Kumpiene**	WP6
Katholieke Universiteit Leuven /KUL	**Erik Smolders** Fanny hemmels	WP4 (metals)
Université Catholique de Louvain /UCL	**Philippe Sonnet** Henri Halen (associated) Joop Vegter (associated)	
Université de Lorraine/ UL Institut National de la Recherche Agronomique/INRA	**Thibault Sterckeman** Stéphanie Ouvrard Joan Dupuy Pierre Leglize	WP5

A report on project management and co-ordination (WP1) is given in chapter 13 and dissemination and exploitation (WP2) in chapter 14.

4. Soil property corrections in existing soil quality standards

4.1 Introduction

In this chapter we focus on soil quality standards (SQS) or soil guideline values (SGV), which can be used as reference values in tier 1 and 2 risk assessments. A fairly recent review on soil screening values in European countries, and how they have been derived, was published by EU's Joint Research Centre (Carlon, 2007). For a more detailed overview on SQS, we refer to that work. In IBRACS we have focus on the official guide line documents provided by authorities in Belgium (Flanders, Wallonia), France, Sweden and the Netherlands. In deriving SQS, relevant protection targets need to be identified, as illustrated by the Swedish model in Figure 4.1. The SQS obtained are intended to protect 1) people living on or visiting the site, 2) soil environment (ecosystem), and 3) ground and surface water (off-site effects). The final guide line value for a certain contaminant is the lowest of the values derived to protect any of these three protection targets. The protection target and exposure pathways differ for different contaminants. For example, for metals like Cd and Co, and high molecular weight PAHs human exposure via consumption of vegetables grown on the site is decisive for the guideline value (sensitive landuse scenario). As a consequence, soil factors modifying the availability of these contaminants for plants are affecting their over-all risk assessment. For other contaminants like Cu, Zn, Ni and low molecular weight PAHs, protection of the soil ecosystem is decisive for the guideline value. However, the protection targets accounted for in the risk assessment models varies between countries (Table 4.1). In all models protection of human health is being considered, whereas protection of the terrestrial ecosystem is considered in all but in the French model. Actually, in the French model only human health is being considered explicitly.

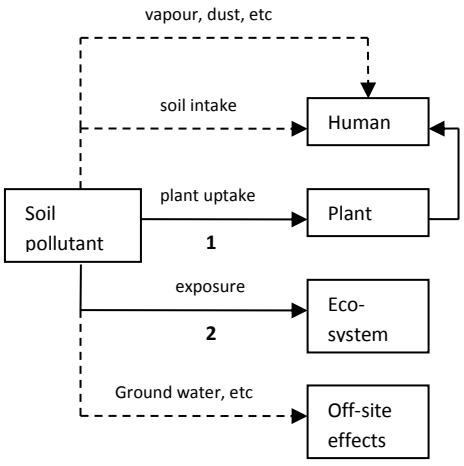


Figure 4.1 A schematic representation of risk objects and exposure pathways in the Swedish risk assessment model (Naturvårdsverket, 2009). In IBRACS we have focused on exposure pathways 1 and 2.

Table 4.1 Protection targets considered in calculating SQS in different countries (Carlon, 2007).

	Human health	Terrestrial ecosystem	Ground-water	Surface water
Belgium (Flanders)	X	X	X	
France	X			
The Netherlands	X	X	X	X
Sweden	X	X	X	X
Belgium (Waloonia)	X	X	X	

In Table 4.2, we have summarized SQS for the contaminants considered in IBRACS. As can be seen, these values are different for different landuse according to a soil multi-functionality principle. The values given are valid for a "standard soil", which are being assigned slightly different properties in different countries. In two countries/regions, the Netherlands and Belgium (Flanders), there is an obligation to make soil property corrections when applying the generic SQS for metals and PAHs, which is being discussed below. In Sweden and Belgium (Wallonia) on the other hand, such an option is not possible.

Table 4.2 Summary of generic soil quality standards (mg/kg dw) and protection target determining the soil quality standard. The values given are for standard soil conditions; 10% organic matter, 25% clay (the Netherlands, Belgium (Flanders, Wallonia)) or 2% organic matter, pH 5-7 (Sweden).

	Sweden		The Netherlands		Belgium (Flanders)		Belgium (Wallonia)	
	SQC [1)]	Protection target [2)]	SQC [3)]	Protection target [2)]	SQC [4)]	Protection target [2)]	SQC [6)]	Protection target [2)]
Cu	80/200	E/E	190	E	197/500	E/H	110/120	E/E
Ni	40/120	GW/E	100	E	95/530	E/H	150/210	E/E
Zn	250/500	E/E	720	E[6)]	333/1 250	E/H	230/320	E/E
PAH-L	3/15	E/E	-	-	1/40 [5)]	H/H	0.8/43	H/GW
PAH-M	3/20	H/H	-	-	30/270 [5)]	H/H	23/47	GW/E
PAH-H	1/10	H/E	-	-	2.9/3.6 [5)]	H/H	0.6/1.4	H/E
PAH-10	-	-	40	E	-	-	-	-

1) Naturvårdsverket (2009a). Lower value – sensitive land use, higher value – less sensitive land use
2) E = soil ecosystem, GW = groundwater, H = human health
3) Swartjes et al. (2012).
4) VLAREBO (2008). Bodemsaneringsnormen. Lower value – Type III (e.g. residential), higher value – Type V (e.g. industrial)
5) PAH-L is represented by acenaphtylene, PAH-M by fluoranthene, PAH-H by dibenz(a,h)anthracene.
6) Annex I of the Walloon Soil Decree (5/12/08) & GRER (2012) : Trigger Values ("Valeurs Seuil") ; lower value – Type III (residential), higher value – Type V (industrial). Note: natural and agricultural landuses have Trigger Values that are more restrictive than residential use.

4.2 Flanders (Belgium)

The Flemish soil decree from October 27, 2006 has been revised in October 2013. New normative values have been issued in the so-called Vlarebo, "Vlaams reglement rond bodemsanering en bodembescherming" (Flemish rules about soil remediation and soil protection) which implements the decree (http://www.ovam.be/sites/default/files/20131010_Vlarebo2008_Geconsolideerde_versie10oktober2013.pdf). The revised values take into account the concept of bioavailability. They are based on ecotoxicological data and are computed as a function of the local soil properties using formulae which take into account the bioavailability of the contaminants.

There are three types of normative values in the Flemish soil decree, increasing in values (limits) based on increased risk: “soil screening values” (streefwaarden), “soil target values” (richtwaarden) and “clean-up values” (bodem-saneringsnormen). The practical and legal consequences of exceeding the limits are beyond the scope of this study but are in principle that new contaminations should be remediated when the third limit is exceeded, that the clean-up goals are to be below the second limit and that values below the soil target values mean that there is no concern.

The “soil screening values” for metals are based on the upper percentile of natural background values derived from a geochemical survey in non-contaminated areas. They are assigned a constant value for all soils (e.g. Cd) or they are function of soil properties as derived from the survey (Table 4.3).

The “soil target values” indicate where the soil can perform all its functions without any limitation. It represents the concentration that has to be achieved in case of any soil remediation action. These values correspond to 60% of the clean up values for residential areas (type II). The “soil target values” can be adjusted to the local soil parameters (percentage of clay, organic matter content or pH-KCl value), according to Table 4.3. For ecotoxicity of metals, the target organisms used in the Flemish decree to derive the soil property corrections was only plants. From the REACH data, it was observed that for any given metal one particular soil property predominantly influenced plant toxicity. For example, Cu toxicity to plants were mainly influenced by the CEC (cation exchange capacity). Since the CEC is not among the parameters required to be analysed by the Flemish Decree, it was obtained by a formula involving pH, clay and organic matter. The equations presented in Table 4.3 were obtained from regression analyses performed on ecotoxicity data with different soil property parameters as independent variable (e.g. CEC). For arsenic, which is a metalloid, the ecotoxicity was found to be correlated with its solubility. For Cd, the main protection targed is human health, and soil parameters that were correlated with plant uptake was therefore evaluated. A comprehensive statistical analysis on a wide range of different vegetables that are grown in contaminated and non-contamined soils in Flanders and The Netherlands showed a significant correlation with soil pH. As a result, the “soil target value” for Cd is solely a function of pH (Table 4.3).

The remediation value, “clean-up value”, is the threshold value at which a significant risk of adverse effect occurs. Beyond this value, an additional investigation (tier 3) or remediation action must be

carried out. Unlike the "soil target values" they depend on the type of planned land use (natural, agricultural, residential, recreational or industrial). Values for ecosystem protection were calculated, as well as values for human health protection. The lowest value of the two is selected as the clean-up value. The "clean-up values" were also obtained using SSD curves based on ecotoxicological data. In this case, the degree of ecosystem protection was chosen to be HC_{25} or HC_{50}. For example, for Cu HC_{25} was chosen for natural and agricultural land use, whereas HC_{50} was chosen for residential land use. For Zinc, HC_{50} was chosen for all types of land use. Soil property corrections ware determined in the same way as for the "soil target values".

Table 4.3 Equations used for soil property corrections of SQS in Flanders, where x = clay content (%), y = organic matter content (%), z = pH-KCl. Index I, II and III are according to the type of use for the excavated soil, "bestemmingstype" (OVAM, 2008).

Metal	Correction equation
	Streefwaarden (soil screening values)
As	$SW = 10^{[0.764 + 0.44 \cdot \log(x)]}$
Cr	$SW = 6.911 + 60.67 \cdot \log(x) - 18.54 \cdot \log(y)$
Cu	$SW = 10^{[0.98 + 0.27 \cdot \log(x) + 0.169 \cdot \log(y)]}$
Pb	$SW = 10^{[1.231 + 0.11 \cdot \log(x) + 0.5 \cdot \log(y)]}$
Ni	$SW = 10^{[0.504 + 0.7 \cdot \log(x)]}$
Zn	$SW = 6.454 + 64.27 \cdot \log(x) + 20.85 \cdot \log(y)$
	Richtwaarden (soil target values)
As	$RW = 11.96 + 23.04 \cdot \log(x)$
Cd	$RW = 1.2 \cdot 10^{[-0.17 \cdot (5-z)]}$
Cu	$RW = 0.52696 \cdot [(38.8 + 3.5 \cdot z) \cdot x + (22.1 + 23.5 \cdot z) \cdot y]^{0.73}$
Zn	$RW = 0.098924 \cdot [(38.8 + 3.5 \cdot z) \cdot x + (22.1 + 23.5 \cdot z) \cdot y]^{1.13}$
	Bodemsaneringsnormen (soil clean-up values)
As (Type I and II)	$BSN = 19.82 + 38.18 \cdot \log(x)$
Cd (Type I and II)	$BSN = 2 \cdot 10^{[-0.17 \cdot (5-z)]}$
Cu (Type I and II)	$BSN = 0.67082 \cdot [(38.8 + 3.5 \cdot z) \cdot x + (22.1 + 23.5 \cdot z) \cdot y]^{0.77}$
Cu (Type III)	$BSN = 0.84115 \cdot [(38.8 + 3.5 \cdot z) \cdot x + (22.1 + 23.5 \cdot z) \cdot y]^{0.81}$
Zn(Type I - III)	$BSN = 0.164714 \cdot [(38.8 + 3.5 \cdot z) \cdot x + (22.1 + 23.5 \cdot z) \cdot y]^{1.13}$

For PAHs, the Flemish SQS is adjusted based on the soil content of organic matter according to:

$$SQS_{act} = SQS_{stan} \cdot \frac{OM}{10}$$

This relation was first introduced the Netherlands in 1987 (Milieuprogramma 1988-1991, Tweede Kamer, vergaderjaar 1987-1988, 20202, nrs 1-2) to allow the derivation of soil values from aquatic (and drinking water) quality criteria by equilibrium partitioning. This reflects in a way also the bioavailability of the

organic substances in question. The minimum and maximum value of organic matter in the equation above is 1 % and 10 %, respectively. For soils outside this range fixed values of 1% and 10% is used respectively. It can be noted that the same equation is used in the Netherlands for correcting intervention values for organic contaminants except PAH, but the minimum and maximum values of organic matter in the equation is 2% and 30 %.

4.3 The Netherlands

Central to the Dutch soil protection policy is the principle of soil multi-functionality, and this applies to soil remediation as well. The risk assessment procedure consists of three stages, or tiers, as indicated in Figure 1.1. The first tier is based on historical investigations and soil investigations and assessment using generic trigger values for soil and ground water. Site-specific considerations are addressed in the second and third tiers. The model tool used in the Netherlands for calculating generic trigger values is called CSOIL (VROM, 2009).

There are two types of generic soil quality criteria (or trigger values); background value/target value and intervention value[1] (Swartjes et al, 2012). Based on these values, the soil is classified as clean, slightly contaminated or seriously contaminated. *Soil background values* are estimated from sampling in top soil of undisturbed soil in agricultural areas and nature reserves over the Netherlands, and are thus not risk-based values. *Intervention values* are generally chosen as the lowest of human health and ecological risk limits, but there are exceptions. For example, for some substances policy decisions are taken that higher values should be used for socio-economic reasons.

The Dutch SQC for metals depend on the soil content of clay and organic matter. The standard soil is defined having a clay content of 25 % and organic matter of 10 %. Converting the SQC for soils with deviating content of clay and/or organic matter, the following equation is used (VROM, 2009; Swartjes *et al*, 2012):

$$SQS_{act} = SQS_{stan} \cdot \frac{X + (Y \cdot Clay) + (Z \cdot OM)}{X + (Y \cdot 25) + (Z \cdot 10)}$$

where X, Y and Z are empirical metal-dependent constants (Table 4.4). If the clay content (Clay) is less than 2 % a value of 2 % is used, and the organic content is set to 2% even if the amount of organic matter (OM) is below that value.

It should be noted that the relations between SQS for metals and soil properties were not intended to be a “bioavailability model”, as in the Flanders model. These relations were first published in a much simpler, but mathematical equivalent form in an official report of the Ministry of Environment to

[1] The Dutch framework also consists of target values and intervention values for groundwater. This is discussed in more detail in Swartjes et al (2012).

Parliament, which stated that they were based on regression analyses of observed **background concentrations** in nature area's and "unpolluted agricultural areas". “The metal dependent constants” originate directly from this regression based approach where SQC = X + Y*(Clay)+Z* (OM) introduced in 1987 (Milieuprogramma 1988-1991,Tweede Kamer, vergaderjaar 1987-1988, 20202, nrs 1-2) . Note, for oxyanions like arsenic, the positive correlation between SQS and organic matter is in contrast to what could be expected from basics in environmental chemistry.

Table 4.4 Metal-dependent soil properity correction factors in the Dutch model (VROM, 2009).

Metal	*X*	*Y*	*Z*
Arsenic	15	0.4	0.4
Barium	30	5	0
Beryllium	8	0.9	0
Cadmium	0.4	0.007	0.021
Chromium	50	2	0
Cobalt	2	0.28	0
Copper	15	0.6	0.6
Mercury	0.2	0.0034	0.0017
Lead	50	1	1
Nickel	10	1	0
Tin	4	0.6	0
Vanadium	12	1.2	0
Zinc	50	3	1.5

The so called standard soil with 10% OM and 25% clay has no special meaning other than to give an illustrative example for a soil that has a “midrange” OM content and a “midrange” clay content. However, the combination of 10% OM and 25% Clay is quite exceptional for a terrestrial soils, but more common for sediments, for which the values in the table also apply.

Soil quality standards for PAHs in the Dutch system are corrected for OM content in a similar way as in the Flemish system. The following equation is being used for calculating the intervention value (IV) for PAHs:

$$IV_{act} = 40 \cdot \frac{OM}{10}$$

where 40 is the intervention value for the standard soil (10% OM , similar to the standard soil for metals). For soils with a content of OM of up to 10 %, a fixed value of 40 mg/kg dw is used as intervention value, and a value of 120 mg/kg dw is used for soils with an OM content of 30 % or higher.

5. Validation of eight different soil tests to assess phytotoxicity of metals

5.1 Background

It is well established that not all trace metals in soil are equally accessible to organisms (Rieuwerts et al. 1998). The bioavailability of trace metals depends on the organisms exposed and the speciation of metal, that, in turn, depends on source and age of the contamination and the characteristics of the soil (mainly pH, eCEC, % OC). Soil testing for bioavailability of trace metals has been developed during more than three decades. The methods include single soil extractions, multiple extractions, kinetic fractionations, dynamic modeling or determinations of fluxes (Guptar et al. 1996; McLaughlin et al. 2000; Rao et al. 2008). Despite this development, soil screening or clean-up limits for trace metals are commonly expressed as (pseudo) total metal concentrations, e.g. aqua regia soluble metal, thereby disregarding possible differences in bioavailability among soils. Most of the soil tests for metal bioavailability have been calibrated to bioaccumulation data, but bioaccumulation data do not predict toxicity, because translocation of metals from plant root to shoot is restricted (Degryse et al. 2009b).

In previous studies the effect of soil characteristics on toxicity of single metals was tested by spiking different types of soil with metal salts. However, there was some concern about the effect of ageing on the toxicity of a contamination. Therefore experiments were set up with soils spiked with metal salts which were then exposed to rain and natural conditions for a few years. From subsequent toxicity tests on these aged soils a reduced toxicity due to leaching and ageing was detected. In the empirical toxicity models, leaching-ageing factors (L/A factor) have been implemented to account for this decreased toxicity (Smolders et al. 2009), thereby also correcting for the confounding factors of metal salt spiking (osmotic stress, acidification; (Smolders et al. 2009) and reference therein). However, only few studies determined toxicity thresholds using site specific historically contaminated soils (field-contaminated soils).

In field-contaminated soils, labile soil metal fractions are considerably smaller than in soils spiked with metal salts, even after sufficient equilibration time (Ma et al. 2013), logically because the trace metals may be present in the original unweathered minerals (Van Damme et al. 2010). However such factors were largely based on soils in which the source of metals was an added soluble salt, including Zn corrosion products. This means that the factors could overestimate toxicity in field-contaminated soils due to lower labile metal fractions. Existing soil screening limits in US, Canada, Europe and Australia have been derived using toxicity tests performed on an extensive range of organisms and soils spiked with metal salts (Checkai et al. 2014). A soil chemical test that accounts for difference in metal bioavailability between spiked and field-contaminated soils should capitalise this valuable data base.

The objective of the present study was to identify a soil test that measures the toxic dose of metal in a range of historically contaminated soils Figure 5.1. Such a method could assist site (soil) specific risk assessment and should ideally draw on existing soil limits that are based on soils spiked with metal salts. For that reason we compared the toxic response of barley in nine Zn or Cu contaminated soils with corresponding $ZnCl_2$ or $CuCl_2$ spiked reference soils. The metal salt spiked soils had not been leached or pH corrected to alleviate the confounding factors in such toxicity tests (Smolders et al. 2009) because the

soil tests developed here must translate the existing limits that are derived from tests of which the majority used mere metal salt amended soils. In total, eight different soil tests were compared, including six soil extracts, DGT and an isotopic exchange method (Hemmels et al., 2014).

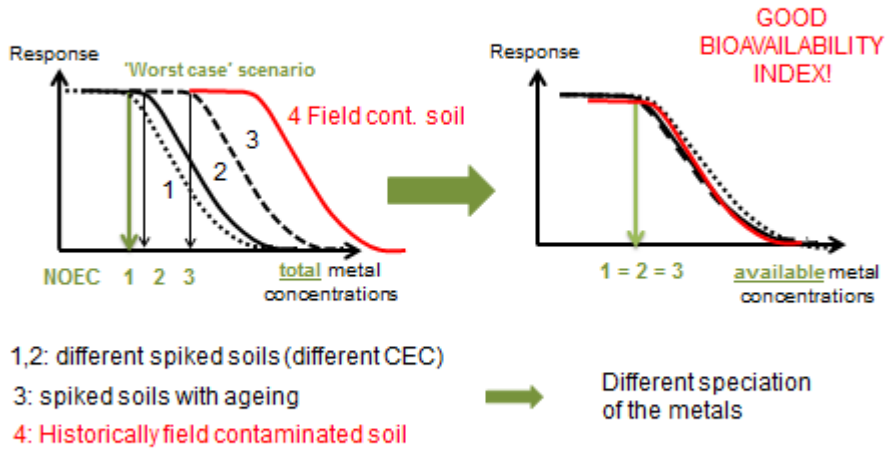


Figure 5.1 An illustration of different factors affecting the toxicity of metals and the outcome of an "ideal" chemical bioavailability method.

5.2 Description of experimental work

5.2.1 Experimental design

Barley was grown on nine contaminated soils in a 14 day pot trial. Each of the soil samples was mixed with a corresponding uncontaminated (reference) soil in different proportions. In addition, each of the reference soils was spiked with the principal metal contaminant, yielding in total 18 toxicity tests involving 9 field-contaminated and 9 spiked soils. Soil of all treatments or a selection of treatments was subjected to eight different soil tests (six chemical extractions, DGT and isotope dilution).

5.2.2 Soils

Nine contaminated soils were sampled in Belgium, France and Sweden (Table 5.1). The samples came from five Zn smelter and/or mining sites in Belgium two Zn smelter sites in France, one former wood impregnation site in Sweden and one sulphite factory site in Sweden. The field-contaminated soils were selected based on total trace metal concentration. For each field-contaminated soil, an uncontaminated control soil with similar properties was sampled. Most of the control soils were collected in the immediate vicinity of the contaminated area. These soils were air-dried and sieved to < 4 mm and thoroughly mixed to ensure homogeneity and were stored for up to one year before the pot trial started.

5.2.3 Soil characterization

Properties of the soils are presented in Table 5.1. Soil pH was determined after shaking 5 g of soil end-over-end for 2 h with 25 mL of a 0.01 M $CaCl_2$ solution. Effective cation exchange capacity (eCEC) was measured with the 0.0166 M cobaltihexamine method (Ciesielski and Sterckeman 1997). Total carbon content of the soils was determined by dry combustion using a CN analyser (VarioMax). Inorganic carbon was analysed through pressure increase after addition of a mixture of $HCl/FeSO_4$ to the soil sample in a sealed container (Sherrod et al. 2002).

Zinc was identified as the principal metal contaminant in 7 soils whereas Cu was the principal metal for the 2 other soils, despite overall mixed metal contamination. The relative contribution of each individual metal to the toxicity in the field-contaminated soil was estimated with the toxic unit approach, assuming concentration addition. The toxic units of each metal were calculated as the ratio of the total metal concentration to the corresponding EC50 (concentration yielding 50% reduction in shoot growth; total soil metal based) derived from single metal spiked soils using the same plant. For Zn and Cu, the average EC50s from the spiked soils in the present study were used, i.e. 1590 mg kg^{-1} dm for Zn and 360 mg kg^{-1} dm for Cu. No Ni, Pb or Cd spiked soils were tested here. For Pb, toxicity data for the same barley variety were available from KUL's laboratory from a range of $Pb(NO_3)_2$ spiked and leached soils (Cheyns *et al.*, KU Leuven, Heverlee, Belgium, unpublished data). The average EC50 is 13,330 mg Pb kg^{-1} dm for Pb. No toxicity data for Cd and effect on barley growth were found but a comparison of total soil Zn toxicity with total soil Cd toxicity for different plants shows that total Cd is, on average, 10 times more toxic than total Zn in the literature (Burtonet al. 1984; de Haan et al. 1985; Sikora and Wolt 1986; Dang et al. 1990; Kalyanaraman and Sivagurunathan 1993; Aery and Jagetiya 1997), yielding an estimated EC50 for barley of 160 mg Cd kg^{-1} dm. For Ni, an experiment was set up here with barley shoot growth in one soil yielding EC50 = 620 mg Ni kg^{-1} dm.

5.2.4 Soil treatment and experimental design

The pH and organic matter (OM) content of the uncontaminated reference soils were adjusted to match more exactly the properties of the corresponding contaminated soil using CaO and commercial peat. Soil properties of the reference soils after these initial amendments are given in Table 5.1. Adequate doses of CaO, peat and water was added to the soils 5 months before the start of the pot trial and were incubated for that period at room temperature to allow the soil to equilibrate. The contaminated soils were mixed with their corresponding uncontaminated control soil in different proportions (0-100% contaminated soil) to obtain five to seven different soil metal concentrations, henceforth termed doses. Deionised water was added to these mixtures to reach a constant water content across treatments. These mixtures (henceforth called 'field-contaminated soils') were incubated for 7 days at 20°C. The uncontaminated control soil was spiked with the principal contaminant of the corresponding contaminated soil (either Zn or Cu) to obtain a selected range of five to seven doses, henceforth called 'spiked soils'. Spiking was done by adding deionised water to reach constant soil water content across treatments and solutions of either $ZnCl_2$ or $CuCl_2$ to the uncontaminated soils. These spiked soils were then incubated for 7 days at 20°C. Subsequently, nutrients were added to all soil mixtures and all spiked soils by means of a KH_2PO_4 solution (50 mg P kg^{-1} dm) and a KNO_3 solution (100 mg N kg^{-1} dm). Control samples were prepared for all soils

and treated identically to the spiked soils, but without metal addition. The prepared soils were transferred to 400 mL pots (four replicates per dose per soil) on the same day after nutrient addition, with each pot filled to a set volume.

5.2.5 Toxicity test

The toxicity test was based on inhibition of barley shoot growth (International organization for standardization, 2005). Six uniform pre-germinated barley seeds were planted per pot after nutrient addition and the soil surface was covered with a thin layer of polyethylene beads to prevent excessive moisture loss. The pots were randomly placed in a growth cabinet with a 16 h/8 h day/night cycle (20 °C/16 °C) at 75 % humidity. All tests (476 pots) were performed at the same time. A constant water content of the soils was maintained during the experiment by adding water every day to reach the original weight of the pot. After three days the seedlings were thinned to three seedlings per pot. Fourteen days after planting the barley seeds, shoot biomass was harvested, dried (72 h at 70 °C) and weighed. A selection of dried barley plant material (control treatments and treatments in field and spiked soils with high metal concentration but enough plant material to digest) was crushed, digested and elemental concentrations were measured with inductively coupled plasma – mass spectrometry (ICP-MS, Agilent 7700x). After the toxicity tests, soil from planted pots was air dried pending soil extraction or soil testing

5.2.6 Soil tests for metal bioavailability

Pseudo-total metal concentrations in the soil samples were analysed with an aqua regia digestion. The digestion not only dissolves oxyhydroxides, carbonates and desorbs the metals from the soil, but it also mineralizes the organic matter (silicates not dissolved). Approximately 200 mg of ground soil was digested with 2 mL aqua regia at 140°C for 3 h in a hot block. The digests were diluted to 10 mL and elemental concentrations were measured with ICP-OES (inductively coupled plasma – optical emission spectrometry) using an Optima 3300 DV (Perkin Elmer).

A cold extraction with 0.43 M HNO_3 (Houba et al. 1989) was used to determine the so-called reactive or geochemically active metal (Pampura et al. 2007) in the samples. The extraction mechanism is based on dissolution of oxyhydroxides, carbonates and desorption of metals. Air-dry soil (2.5 g) was shaken with 25 mL of cold 0.43 M HNO_3 (1:10 soil-solution ratio) for 2 h and subsequently centrifuged for 20 min at 2200 *g*. To ensure that acid neutralised by carbonates in calcareous soils was compensated, 0.1 mL of 5 M HNO_3 was added per 10 g kg^{-1} of $CaCO_3$. The diluted supernatant was analysed with (ICP-MS, Agilent 7700x).

Table 5.1 Selected properties of contaminated soils and corresponding reference soils after correction of pH and OC.

No	Site & country	Source of contamination	pH	OC (%)	CEC (cmolc kg^{-1})	Total metal concentrations (mg kg^{-1}) Zn	Cu	Ni	Pb	Cd
1	Plombières BE	Zn-Pb mine tailings (ZnS)	6.7	2	9	6100	25	30	2900	13
		Reference	7.4	2	15	220	17	24	80	3
2	La Calamine BE	Zn-Pb mine tailings ($ZnCO_3$, $Zn_4Si_2O_7(OH)_2 \cdot (H_2O)$)	7.1	1	6	35800	16	100	900	12
		Reference	7.6	3	13	450	10	16	80	1
3	Prayon BE	Zn smelter	6.1	5	17	20000	1200	60	2900	350
		Reference	5.8	6	21	780	40	23	200	6
4	Sclaigneaux BE	Zn smelter	6.6	5	11	18000	120	50	4850	170
		Reference	6.6	5	20	75	8	10	26	1
5	Mortagne-du-Nord FR	Zn smelter	5.8	5	9	6700	270	12	3100	40
		Reference	5.7	6	26	80	7	8	30	1
6	Auby FR	Zn smelter	6.2	23	39	36700	360	14	6000	180
		Reference	6.9	17	69	260	40	13	150	1
7	Balen BE	Zn smelter	5.3	1	1	290	30	5	280	4
		Reference	4.8	2	2	6	1	11	10	1
9	Björkhult SE	Wood impregnation ($CuSO_4$)	6.1	7	9	50	4100	7	43	<0.50
		Reference	5.0	7	16	60	10	8	50	<0.50
10	Loddby SE	Sulphite factory	7.6	14	17	4600	1400	130	900	12.00
		Reference	6.5	23	29	150	40	35	18	<0.50

The cobaltihexamine (Cohex) method (Ciesielski and Sterckeman 1997) was used to determine the CEC at soil pH (effective CEC or eCEC). This extractant also allows extracting so-called ion exchangeable metals. This method is described in the standardised protocol (AFNOR 2007). Air-dry soil was weighed into 50 ml polypropylene centrifugation tubes and 30 ml of the Cohex solution (0.0166 M) was then added to the soil in centrifugation tubes. The quantity of soil used (0.5 to 5 g) depended on the expected eCEC value of the soil. The mixture was shaken for 1 h end-over-end at 20 °C and after centrifugation (20 min at 2200 *g*) the composition of the diluted supernatant was measured with (ICP-MS, Agilent 7700x). The Co content in the Cohex exchange solution was measured to confirm initial Co concentrations. The loss of Co relative to the soil weight allows calculating the eCEC.

The extraction by 1 M NH_4NO_3 was used as an alternative test based on cation exchange (Gryschko et al. 2005). Ion exchangeable metals are extracted in this soil test. Air-dry soil was shaken with 1 M NH_4NO_3 in a 1:2.5 soil-solution ratio for 2 h and subsequently centrifuged for 20 min at 2200 *g*. The diluted supernatant was analysed with (ICP-MS, Agilent 7700x).

An extraction with 0.05 M EDTA (adapted from (Chardot et al. 2007)) was used. This test is based on ligand complexation which desorbs the sorbed metal fraction from the soil. Na_2H_2EDTA was dissolved in deionised water to obtain a 0.05 M EDTA solution (pH: 4.5). Air-dry soil was shaken with 0.05 M EDTA in a 1:2.5 soil-solution ratio for 2 h and subsequently centrifuged for 20 min at 2200 *g*. The diluted supernatant was analysed with (ICP-MS, Agilent 7700x).

Diffusive gradients in thin films (DGT) measurements were performed according to the procedure described in (Degryse et al. 2003). This soil test analyses the readily available metal concentration in soil. Dried samples were reconditioned by adding deionised water to approximately 60% of field capacity and stored for 7-10 days at 8 °C. Following reconditioning, samples were brought to saturation (no free water table) by adding another portion of deionised water and stored for 24 h at 21 °C. A DGT-device (DGT Research Ltd, Lancaster) was gently pressed into soil. A deployment time of 24 h was used for all the tested soil samples. The resin was fully immersed in 0.6 mL of 1 M HNO_3 for 24 h before analysis. The time-averaged concentration at the interface of the soil and the diffusive gel (C_{DGT}, mg L^{-1}) was calculated as described elsewhere (Degryse et al. 2003) using identical elution factors and the diffusion coefficients (D) in the gel, i.e. D=5.54x10^{-6} cm s^{-1} for Zn and 5.58 x 10^{-6} cm s^{-1} for Cu.

The 0.001 M $CaCl_2$ soil test, also called the leaching test according to the procedure in ISO 21268-2:2007, is a 24 h equilibration of the soil sample with 0.001 M $CaCl_2$ at a 1:10 soil-solution ratio. This soil test is a proxy for pore water metal concentrations or readily available metals. The extract was centrifuged at 2200 *g*, filtered through 0.45 µm filters and analysed by HR-ICP-MS (Thermo scientific).

The stable isotope dilution method was used to determine the so-called labile Zn and Cu in the field-contaminated soils, also denoted as isotopically exchangeable metal or E-value (Young et al. 2005) (Figure 5.2). Enriched stable isotope (^{70}Zn and ^{65}Cu) solutions with certified isotopic abundances (IA) were obtained (Isoflex). Six replicates of each soil (1 g) were weighed into centrifuge tubes and shaken end-over-end for 48 h in 30 mL of 0.01 M $Ca(NO_3)_2$ at 20°C. A volume of maximum 0.15 mL of a known concentration of enriched spike solution was added to three of the replicates. Afterwards all suspensions were shaken end-over-end for another 48 h. After centrifugation for 15 minutes at 2200 *g*, the supernatant was diluted for ICP-MS analysis. The isotopically exchangeable Zn and Cu were calculated from the isotope abundances in the enriched soil extracts, in the natural native (non-enriched) soil and in the control solutions containing enriched isotope only using the equation described in (Marzouk et al. 2013).

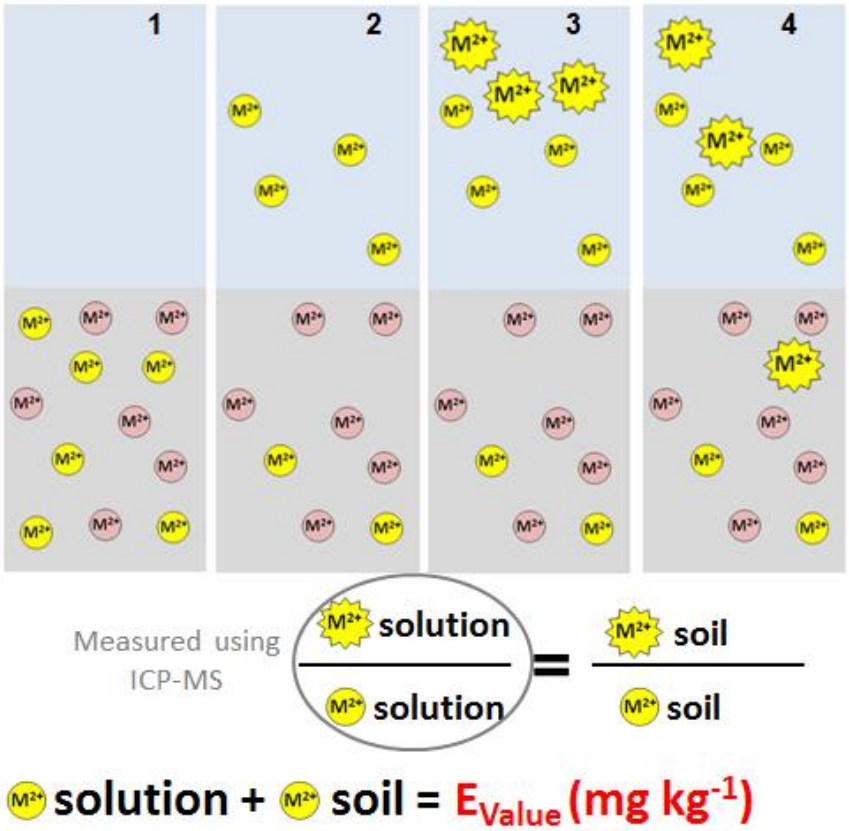


Figure 5.2 Different steps of the isotope dilution extraction. Step 1 & 2 represents the first equilibration of the soil with $Ca(NO_3)_2$ extraction solution. Step 3 is right after addition of the enriched stable isotope spike solution and step 4 is after the second equilibration with the spiked extraction solution. The isotope abundance of the metals in the extract is expected to be the same as that of the labile metal fraction in the soil. Blue: extraction solution, grey: soil. Adapted from (Garforth 2013).

Aqua regia digestions, 0.43 M HNO_3, cobaltihexamine, 1 M NH_4NO_3 and 0.05 M EDTA extractions were performed on all the field-contaminated and spiked soils (n=119, 2 replicates). The DGT test and 0.001 M $CaCl_2$ extraction were only performed at two doses bracketing the dose near 50 % inhibition of plant growth (n=36, no replicates). The isotope dilution method was only applied to the 100 % field-contaminated soils (n=9, 3 replicates).

The quality control of aqua regia soluble metals was verified by including the European soil reference materials, i.e. BCR 142 and BCR 143 with certified aqua regia soluble trace metal concentrations. For other soil properties that determine the bioavailability of metals, mainly pH, CEC and %OC, there are no officially certified reference materials, however our laboratory participate on a regular basis in ring-tests (also called proficiency testing) organized by WEPAL (NL). More information on http://www.wepal.nl/. Finally, in all analyses cited above, we included internal reference soil sample that had been measured with the WEPAL .

5.2.7 Statistical analysis

A log-logistic dose-response model (Doelman and Haanstra 1989) was used to analyse the toxicity data.

Equation 1: $y = \frac{\mathbf{100}\ \%}{\mathbf{1}+exp[b\times(ln(x)-ln(EC50))]}$

where y is the response variable (yield relative to the yield in the control soil, %), b the slope parameter, x the measured dose variable and EC50 the dose at which a 50% reduction in the response variable was observed. The dose was expressed as the measured soil metal concentration obtained using the different extraction methods (aqua regia, 0.43 M HNO_3, cobaltihexamine, 1 M NH_4NO_3 and 0.05 M EDTA). The equation was fitted with non-linear regression (Marquadt 1963) (SAS 9.3). The DGT and 0.001 M $CaCl_2$ were only tested at two doses near the EC50 level. The EC50 in terms of these soil tests was linearly interpolated using the extracted metals plotted to the aqua regia soluble metals at these two doses and the EC50 derived from Equation 1 for aqua regia soluble metals for the corresponding soil.

A field-spiked factor (FS factor) represents the difference in metal toxicity between field-contaminated soils and their corresponding spiked soils. This factor was calculated with Equation 2 or by dividing the EC50 for field-contaminated soils by the EC50 in the corresponding spiked soils (for soil tests performed only at doses bracketing the dose near 50 % inhibition of plant growth).

Equation 2: $y = \frac{100\%}{1+\exp[b\times\{\ln(x)-\ln(EC50\times(1+D\times(FS-1)))\}]}$

where *y* is the observed response (yield relative to the yield in the control soil, %), b the unknown slope parameter, *x* the applied dose, EC50 is the experimental metal toxicity thresholds (the dose at which a 50% reduction in the response was obtained), D is a dummy variable with value 1 for field soils and 0 for spiked soils and FS (field/spiked factor) is the independent variable.

5.3 Results

Selected properties of the soils, after adjustment of pH and organic matter content, are given in Table 5.1Table 5.1 Selected properties of contaminated soils and corresponding reference soils after correction of pH and OC. Field-contaminated soils 1-7 contained high total Zn concentrations (290 - 36700 mg Zn kg^{-1}dm) while the pH ranged from 5.3 to 7.6 and the organic carbon content (OC) ranged from 1% to 23%. Soils 8 and 9 contained high total Cu concentrations (1400 and 4100 mg Cu kg^{-1}dm). The pH was 6.1 and 7.6 and the OC content was 7 and 14%, respectively. Differences in soil characteristics between field-contaminated and their reference soils were reduced after addition of

lime and peat to the reference soils. It was not possible to perfectly match all characteristics at the same time and the remaining differences were still considerable. For instance, soil 6 has a high C-content (19%) due to the accumulation of organic matter caused by the metal toxicity and yielded a lower ratio of eCEC/OC than in the added peat, hence resulting in a considerably higher eCEC in the peat amended reference soil.

Shoot yield was 0.25 – 0.53 g dry weight/pot among the 9 different uncontaminated reference soils. Shoot growth was largest in the reference soil of soil 6 (highest OM content) and lowest in the reference of soil 2. Chlorosis was detected at the highest total metal doses of the mixtures of field-contaminated soils 2, 3, 4, 6, 8 and in the metal spiked referenced soils of soils 3, 6, 8 and 9. Shoot growth was affected by the metals in all toxicity tests except in field-contaminated soil 1 (Figure 5.3, Figure 5.4). Toxicity of Zn and Cu in the spiked soils was always higher than in their corresponding field-contaminated soils. This difference was generally less pronounced for soil 7 which is characterized by a low eCEC (Figure 5.4). Barley yield remained unchanged throughout the entire range of Zn doses in the field-contaminated soils of soil 1. The intersoil variation of Zn toxicity narrowed when thresholds were based on NH_4NO_3 extractable metals compared to thresholds based on aqua regia soluble Zn (Figure 5.5). Toxicity thresholds estimated with the dose-response model (Eqn. 1) for field-contaminated soils were higher (i.e. lower toxicity) than in corresponding spiked soils in all soils (Table 5.2). This change in toxicity correlated remarkably well with the labile metal fraction in the field-contaminated soil, as estimated with isotopic exchange (Figure 5.6).

The quantities of metal extracted from the soil decreased in the following order: aqua regia > 0.43 M HNO_3 > 0.05 M EDTA > E-value > Cohex > 1 M NH_4NO_3 > 0.001 M $CaCl_2$ > DGT (Table 5.2). The extracted metal fractions were larger in spiked soils than in corresponding field-contaminated soils for every soil test. Dose-response curves of the field-contaminated soils (solid lines, filled circles) and spiked soils (dotted lines, empty circles) merge when the dose is expressed as 1 M NH_4NO_3-extracted metal concentrations compared to doses expressed as total metal concentrations (aqua regia) (Figure 5.5A, Figure 5.5B). The dose-response curves for the two Cu field-contaminated soils and their corresponding spiked soils do not merge when the dose is expressed as 1 M NH_4NO_3 extracted metal compared to the total metal case (Figure 5.5C & Figure 5.5D). The coefficients of variation were calculated for the EC50 values based on the different soil tests for all the field and spiked soils (Table 5.2). For the soils with Zn as principal contaminant, the coefficient of variation of EC50 decreased in the following order: aqua regia > 0.43 M HNO_3 >> 0.05 M EDTA > 1 M NH_4NO_3 ~ Cohex > DGT ~ 0.001 M $CaCl_2$. For the soils with Cu as principal contaminant the coefficient of variation was the smallest for EC50 values estimated with the 0.05 M EDTA extraction.

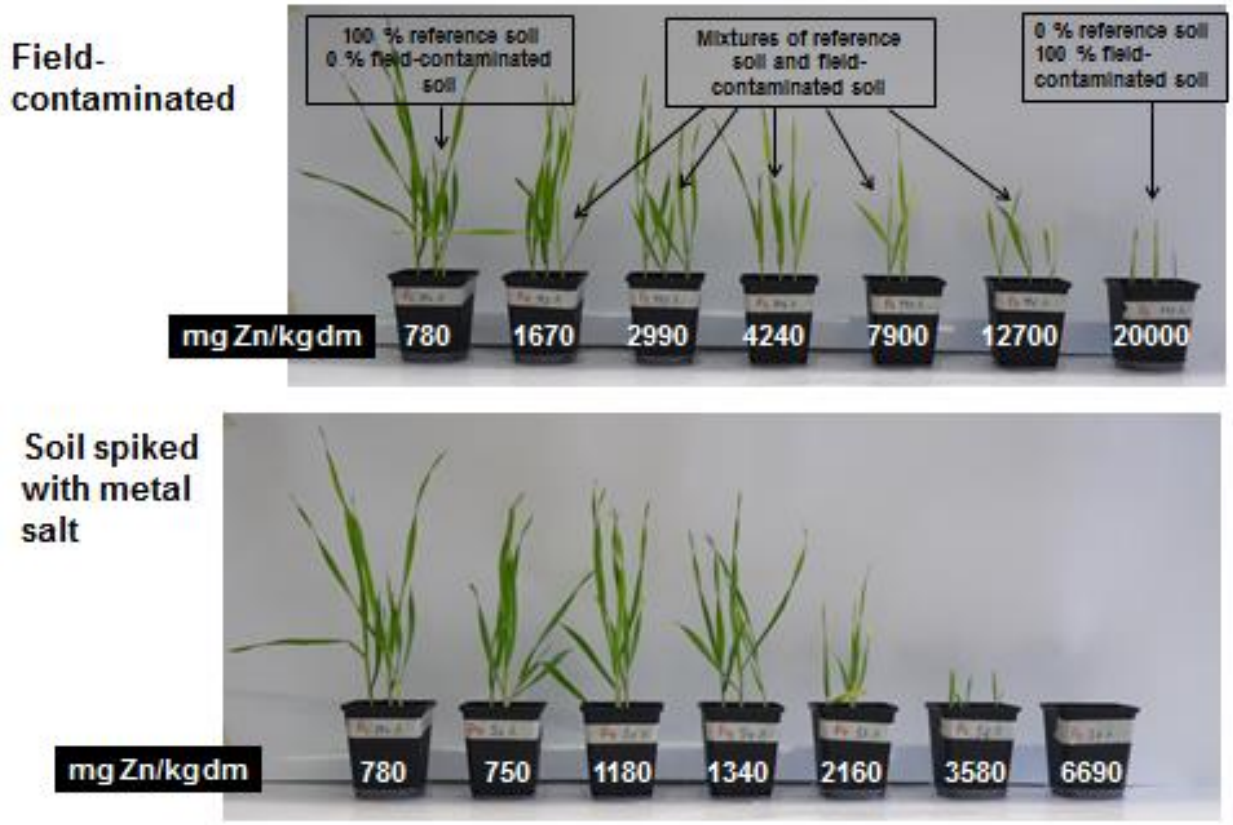


Figure 5.3 Above: barley plants grown on mixtures of a field-contaminated soil (Zn: principal metal) and its corresponding reference soil. Below: The corresponding reference soil spiked with $ZnCl_2$. There is a reduced toxicity of total Zn (aqua regia) in the field-contaminated case compared to the spiked case.

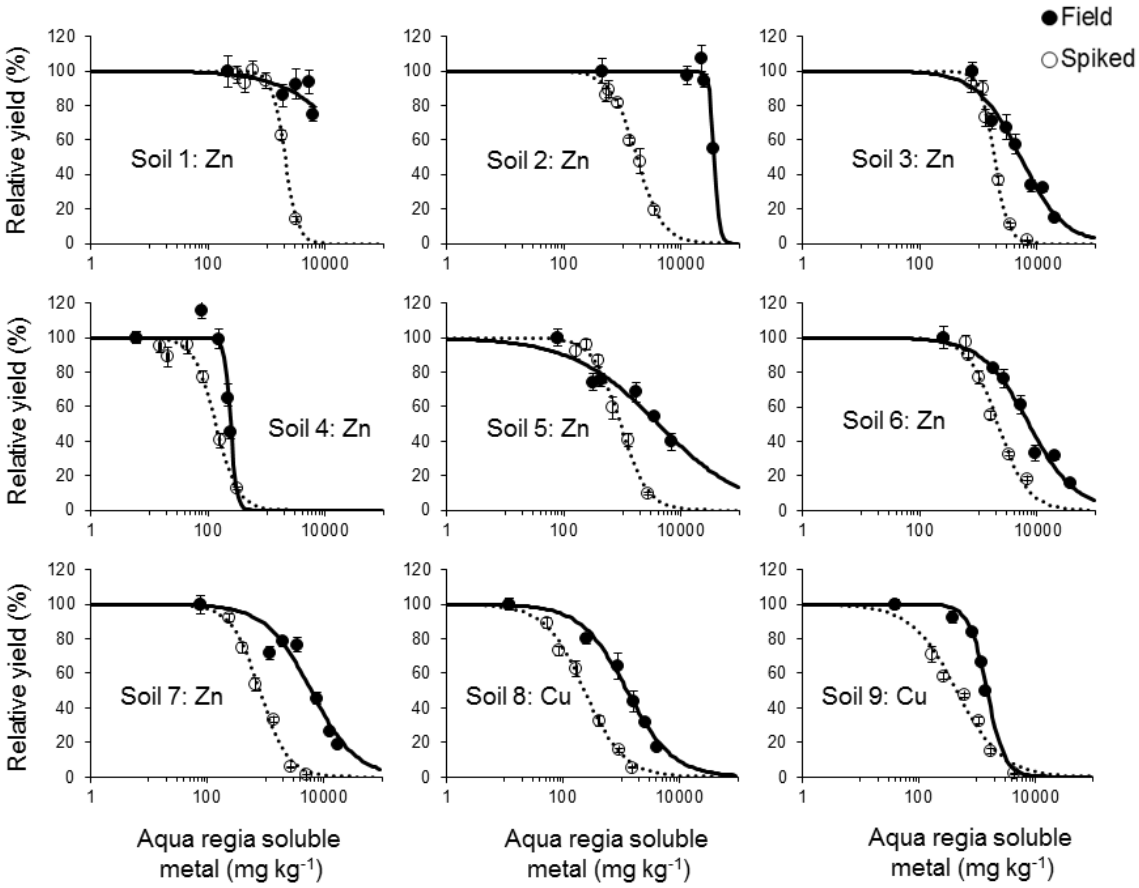


Figure 5.4 The difference in metal toxicity to barley growth between field-contaminated soils (solid circles) and their corresponding spiked soils amended with metal chloride of the principal contaminant (filled circles). Dose-response curves fitted with the log-logistic dose-response model. Standard error bars of means are represented.

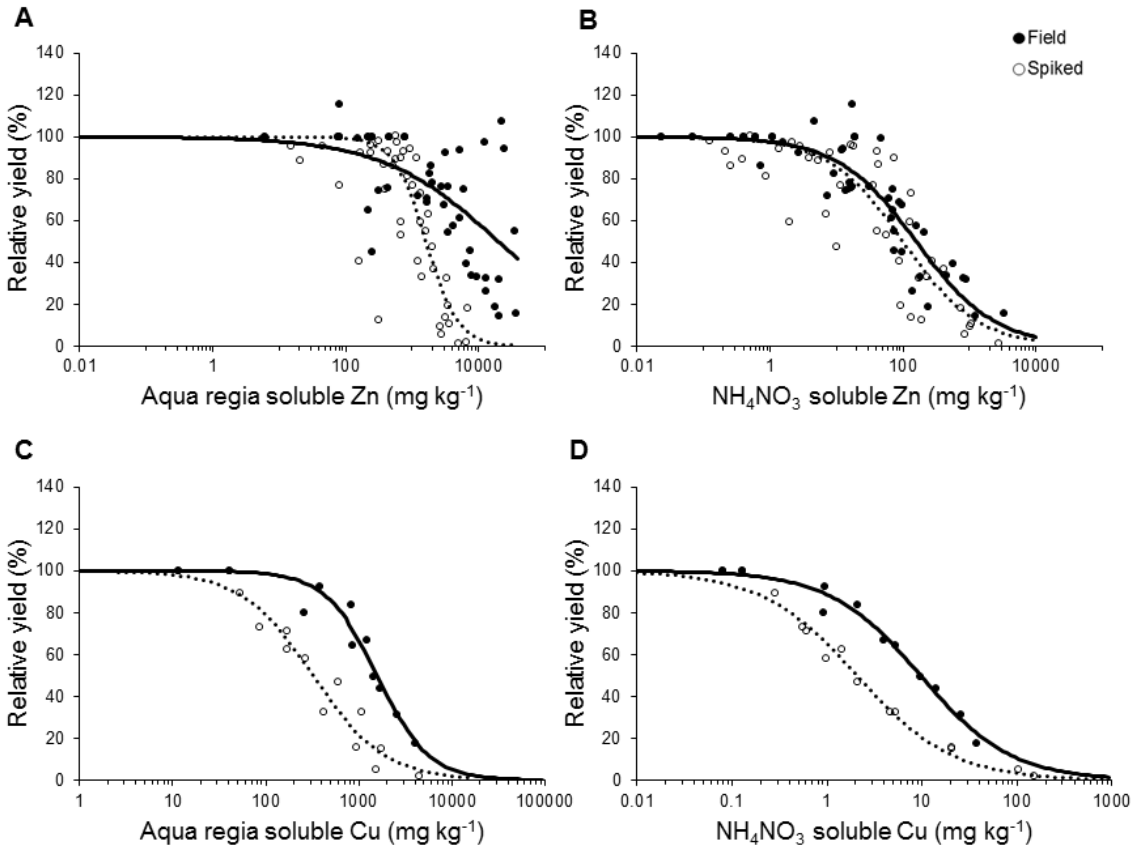


Figure 5.5 Comparison of Zn toxicity to barley growth in field-contaminated soils (solid circles) and in their corresponding reference soils spiked with metal salts (empty circles) with doses expressed as aqua regia and NH4NO3 soluble metal. A and B are data for 14 soils with Zn as principal contaminant, C and D are data for 4 soils with Cu as principal contaminant. Fitted log-logistic dose-response curves are for field soils (solid lines) and for spiked soils (dotted lines). Each symbol is the average of 4 replicates.

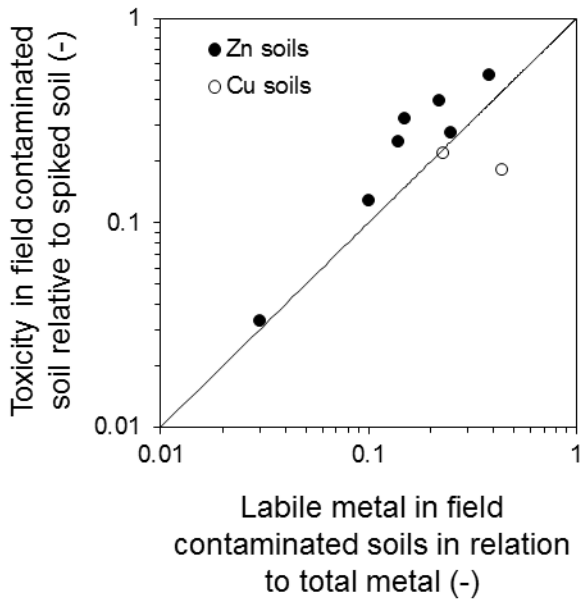


Figure 5.6 Labile metal fraction in the field-contaminated soil (fraction of aqua regia soluble metal that is isotopically exchangeable) plotted versus the relative metal toxicity (expressed as the ratio between the EC50 of the spiked soil and the EC50 field-contaminated soil). The 1:1 line is indicated, (r=0.82 on log-log plot).

Table 5.2 The extracted metal fraction using different soil tests. Data are fractions (%) of extracted metal relative to the aqua regia soluble metal and are averaged over all soils. Soil extractions/tests are sorted from most aggressive (left) to weakest extractions (right).

	Average fraction of extracted metal by different soil tests relative to aqua regia soluble metal (%)						
	0.43M HNO_3	**0.05M EDTA**	**E-value**	**Cohex**	**1M NH_4NO_3**	**0.001M $CaCl_2$**	**DGT[a]**
All Zn soils (n=14)	89	72		24	13	4.3	2.8
Field cont. Zn soils (n=7)	87	60	18	11	8	3.0	0.7
Spiked Zn soils (n=7)	91	85		37	18	5.6	4.8
All Cu soils (n=4)	87	73		3.3	0.7	0.3	0.1
Field Cu soils (n=2)	81	57	34	2.8	0.8	0.2	0.1
Spiked Cu soils (n=2)	92	89		3.9	0.7	0.4	0.2

[a] fraction calculated assuming that the DGT samples solutes from 5 mm soil adjacent to the DGT device

Table 5.3 The predictive power of soil tests for metal toxicity indicated by its potential to minimize variation in toxicity thresholds. The toxicity thresholds (50% effect, i.e. EC50) are for 14 soils with Zn as principal toxic metal and 4 soils with Cu as principal toxic metal. The variation in EC50 is expressed in different ways and lowest values among soil tests are indicated in bold. (F: field-contaminated soil series; S: spiked soil series).

		EC50 (mg kg^{-1})					EC50 (µg L^{-1})	
Principal metal	**Soil**	**aqua regia**	**0.43M HNO_3**	**Cohex**	**1M NH_4NO_3**	**0.05M EDTA**	**DGT**	**0.001M $CaCl_2$**
Zn	1 S	2040	1700	14	15	1480	3460	1820
	1 F	no fit	no fit	no fit	no fit	no fit	no fit	no fit
	2 S	1750	1100	7	7	910	940	190
	2 F	36000	29000	84	82	6860	2760	900
	3 S	1870	1350	720	280	1240	8760	6490
	3 F	5200	3680	580	230	3120	4790	3210
	4 S	140	150	110	70	130	2090	2890
	4 F	240	180	100	90	120	950	3870
	5 S	920	1010	550	16	910	5170	4490
	5 F	4080	3350	600	280	3050	5860	4010
	6 S	2170	1630	200	70	1740	1880	2170
	6 F	7300	5560	380	130	5170	1330	1560
	7 S	790	900	160	70	680	3080	1600
	7 F	6100	6530	220	60	4470	930	780
	CV	*180*	*178*	*87*	*89*	*91*	*73*	***68***
	RR	*6.80*	*6.68*	*2.49*	*2.53*	*2.93*	*2.42*	***2.41***
Cu	8 S	230	230	13	2.3	230	70	120
	8 F	1260	1220	50	8.9	930	40	390
	9 S	450	420	2	1.9	670	30	130
	9 F	1490	1000	2	9.1	550	8	15
	CV	*71*	*65*	*136*	*72*	***49***	*70*	*97*
	RR	*1.47*	*1.38*	*2.87*	*1.29*	***1.18***	*1.68*	*2.29*

CV: coefficient of variation (standard deviation/average*100), *RR*: relative range (max-min)/average*100.

5.4 Discussion and conclusions

5.4.1 Mixture toxicity

Some of the field-contaminated soils in the present study had high concentrations of two or even three different metals (Zn, Pb, Cd or Cu; Table 1). Based on results of the toxic unit approach, the relative contribution of Pb and Cd toxicity in our soils was theoretically negligible compared to Zn and Cu toxicity. The toxicity in these soils is dominated (>two third) either by Zn or Cu, with two exceptions. For soil 7, 56% of toxicity was theoretically attributed to Zn and 28 % to Cu. For soil 9, 55% of toxicity was theoretically attributed to Cu and 40% to Zn. Shoot metal concentrations can be used as a complement to the TU approach to identify the metals that explain barley growth

reductions. A selection of plants was digested and their trace metal content was verified to indicate the principal toxic metal. Data from spiked soils allowed the identification of the metal thresholds for internal shoot concentrations and comparison with internal shoot concentrations of plants grown on the corresponding field-contaminated soils. For Zn, the data obtained here suggest that 50% inhibition is found between 400 and 1800 mg Zn/kg shoot dry weight while for Cu it is 22-26 mg Cu/kg. Plants grown at the highest dose (100%) of the seven field-contaminated soils denoted as 'Zn contaminated' had Zn concentrations of 800-2400 mg Zn/kg, confirming Zn as the principal source of toxicity (data not shown). Shoot Zn concentration in the unspiked reference soil 3 was 300 mg Zn/kg suggesting that this reference soil had a Zn concentration that was phyto-toxic. In this soil, dry shoot weight was on average 0.29 g/pot and was 80 % of the mean shoot growth in other reference soils, confirming Zn stress in the 100 % reference soil. Shoot Cu of plants grown in the 100% Cu contaminated soil 9 was 29 mg Cu/kg, and shoot Zn on soil 9 was 670 mg Zn/kg, i.e. near the toxic range. Taken together, shoot analysis justifies the attribution of the most toxic effects to Zn in the Zn contaminated soils, while mixed Cu & Zn contamination could be present in soil 9, soil 9 is one of the soils where Cu is considered to be the principal toxic metal. Hence, the analysis above largely justifies to compare the soil tests for toxicity using the principal metal contaminants only.

5.4.2 Comparison of soil tests

Soil chemistry has traditionally discriminated intensity- from quantity-based soil tests. The concentration of the nutrient or contaminant in the soil solution is called the 'intensity', sometimes equated to the mobile or soluble elements. The potentially available metal or contaminant in/on the solid phase is called the 'quantity' (Frossard and Sinaj 1998; Degryse et al. 2009b). The quantity hence refers to the solid phase bound nutrient or contaminant that can replenish the solution within biologically relevant timeframes, sometimes equated to the labile quantity. Conceptually, the 0.001 M $CaCl_2$ and DGT tests best reflect the differences in metal intensity among soils while the E-value best reflects the metal quantity. The aqua regia, 0.05 M EDTA and 0.43 M HNO_3 are probably too aggressive to denote the quantity as they dissolve Fe and Al oxyhydroxides and also carbonates containing occluded metals, as indicated here by the large extracted Zn and Cu fractions (Table 5.2). The ion exchangeable metals based on Cohex and 1M NH_4NO_3 extractions may also mimic the quantity although the ion selectivity in combination with its concentration for $Co(NH_3)_6^{3+}$ or NH_4^+ extractants is probably no strong enough to extract all adsorbed Cu or Zn (Table 5.2).

The calibration of the soil tests with toxicity data allowed us to identify a suitable bioavailability index. For this purpose, we analysed the variation of Zn toxicity (EC50) among soils. According to either the coefficients of variation of EC50 or the relative range of EC50s, the most robust indices of toxicity are the 0.001 M $CaCl_2$ extraction and the DGT method (intensity based), closely followed by the ion exchangeable methods (quantity based) and well separated from the most aggressive extractions (aqua regia, 0.43M HNO_3, 0.05 M EDTA; Table 5.3). Surprisingly, this is not confirmed for Cu for which the more aggressive extractant, EDTA is a more robust index and outweighs the other extractants, especially the intensity based ones. This finding corresponds to a similar set of comparisons for predicting metal uptake in plants (Nolan et al. 2005) for which DGT was superior for Zn and, surprisingly, total soil metal was superior for Cu.

Previously eCEC and pH were thought to be the principal factors that influence toxicity of metals among soils. This may be correct for different soils spiked with metals salts. In field-contaminated soils, however, a different factor dominates the variability in toxicity compared to spiked soils. This factor is most likely the variation in the fraction of labile (isotopically exchangeable) Zn to total Zn in the soils. Indeed, in Zn spiked soils, the total Zn concentration is close to the Zn quantity as almost all added Zn can be adsorbed on the soil and remains potentially available. In contrast, several field-contaminated soils have a small and highly variable fraction of labile metals as shown in Figure 5.6. That fraction largely depends on the Zn speciation, e.g. it is only 3 % in soil 2 in which Zn is dominantly present as zinc carbonate and silicate minerals (smithsonite, willemite and hemimorphite) whereas the labile fraction is 14% in soil 1 in which Zn is dominantly present as adsorbed Zn, Zn sulphide and zinc oxide (zincite) (Van Damme et al. 2010). For Cu, the situation is somewhat different and observations suggest that intensity based methods and quantity based methods (except for NH_4NO_3) are inferior to stronger extractants (relative range in Table 5.3). This may relate to the fact that the proton-Cu^{2+} interactions at the biological membrane are particularly strong (Thakali et al. 2006), thereby strongly modifying the toxic effect of mobile Cu^{2+} ions (extracted with intensity based methods) whereas labile fractions (E-value in %) are relatively high and not very variable among soils. However, with only 4 data points, further speculation is not justified.

The fractions of labile metals (E-value in %) in the field contaminated soils are surprisingly strong predictors of the FS factor, i.e. the FS factor (in fact 1/FS) is predicted within a factor 2.2 for Zn soils (one outlier: toxicity was larger than predicted for one of the Zn-concentrates). For Cu, however, the method does not work very well and the FS factors were not too variable among soils. Further conclusions see paragraph 9.2 Metals: implementation of soil tests in risk assessment.

6. Evaluating a passive sampler method to assess bioaccumulation and ecotoxicity of PAHs in soils to worms

Author's note: the majority of text and work presented in this Chapter is published in the following article:

> Arp, H. P. H., S. Lundstedt, S. Josefsson, G. Cornelissen, A. Enell, A.-S. Allard and D. B. Kleja (2014). "Native Oxy-PAHs, N-PACs, and PAHs in Historically Contaminated Soils from Sweden, Belgium, and France: Their Soil-Porewater Partitioning Behavior, Bioaccumulation in Enchytraeus crypticus, and Bioavailability." Environmental Science & Technology. 48, 11187−11195.

The only major novel section not mentioned in the above paper is related to the extended discussion on ecotoxicity. Please refer to and cite the aforementioned paper when referring to all relevant work presented in this chapter.

6.1 Background

Soil regulatory guidelines for PAHs are generally based on benchmark total soil concentrations, C_{soil}, that are considered to represent a risk to soil-dwelling organisms or humans (Miljødirektoratet 2009, Naturvårdsverket 2009, Verbruggen 2012).However, basing soil risk assessments on C_{soil} has been criticized for nearly two decades because this does not account for the role soil properties can have on bioavailability (Belfroid et al. 1996, Brandet al. 2013). Some guidelines partially address this by normalizing C_{soil} to the mass fraction of total organic carbon, f_{TOC}, as the bioavailability of PAHs and other organic contaminants has been found to decrease with increasing f_{TOC} (Swartjes et al. 2012, Verbruggen 2012). However, even if f_{TOC} is accounted for, there remains a concern that this is not sufficient to represent bioavailability in real world, historically contaminated soils (Jager et al. 2003, Jonker et al. 2007, Kreitinger et al. 2007)

C_{soil} benchmark values for PAHs are typically derived from "laboratory-spiked" soil bioassays using pristine, reference soils, or alternatively from aquatic species bioassays (Carlon 20072012). These bioassay-systems are quite different from historically contaminated soils. Uncontaminated, reference soils do not sorb "laboratory-spiked" PAHs to the same extent as historically contaminated soils sorb their "native" PAHs. This is partly due to a lack of sufficient "aging" in laboratory systems (Alexander 2000, ter Laak et al. 2006b, Brand et al. 2013). Also, historically PAH-contaminated soils tend be near industrial areas (gasworks, coke ovens, incinerators, etc.), where the soil can contain a greater abundance of strong-sorbing carbonaceous materials, like black carbon (BC), which can lower PAH bioavailability (Cornelissen et al. 2005, Oen et al. 2006). Aquatic bioassays are problematic in terms of how they are extrapolated to soil systems. This is done by taking bioassay's water concentrations, C_w (µg/L), which would correspond to freely-dissolved porewater concentrations, C_{pw} (µg/L_{pw}) in a soil bioassay, and then convert C_w to C_{soil} (µg/$kg_{soil\ dw}$, where dw denotes "dry weight"), by use of a compound-specific TOC-water equilibrium partition coefficient, K_{TOC} (L_{pw}/kg_{TOC}), and f_{TOC} (kg_{TOC}/$kg_{soil\ dw}$):

$$K_{TOC} = C_{soil} / (C_w f_{TOC}) \qquad (6.1)$$

The theoretical K_{TOC} values used for this purpose are commonly a factor 10 to 100 lower than the highly variable, real-world K_{TOC} values measured for historically contaminated soils.(Krauss, Wilcke et al. 2000, Jager et al. 2003, ter Laak et al. 2006b, Jonker et al. 2007, Brand et al. 2013) The causes of

this are similar to the biases just described for spiked-soil bioassays. Most literature K_{TOC} values are for spiked, reference soils that do not contain strongly-sorbing BC and other carbonaceous materials, (Cornelissen et al. 2005) and which were not aged sufficiently(Alexander 2000, ter Laak et al. 2006b, Brand et al. 2013). Further, the first K_{TOC} for PAHs to appear in literature were based on *total* porewater concentrations, including the non-bioavailable PAHs bound to dissolved organic carbon and porewater colloids, biasing measured porewater concentrations to be much higher than the bioavailable, freely-dissolved C_{pw} fraction (i.e. just solvated by water) (Arp et al. 2009).

As an alternative, several researchers have explored chemical methods to directly measure bioavailability or alternatively bioaccessibility (Reichenberg and Mayer 2006, Cachada et al. 2014). Bioavailability is defined as the concentration in soil currently available for partitioning with organisms, which based on the principle of chemical activity can be quantified by the *equilibrium* freely-dissolved C_{pw} (Reichenberg and Mayer 2006). Established ways of measuring equilibrium freely-dissolved C_{pw} for PAHs include mixing soil with equilibrium passive samplers like polyoxymethylene (POM) in batch systems (Jonker and Koelmans 2001, Adams et al. 2007, Hawthorne et al. 2011b, Gomez-Eyles et al. 2012), as is used here, or by using solid-phase microextraction methods to sample flocculated and filtered porewater (Hawthorne et al. 2005, ter Laak et al. 2006a). Methods to determine bioaccessibility, which is the total concentration that will be available for partitioning with organisms over some extended time frame, include extractions with mild solvents, subcritical water, supercritical fluids, and various solubilizing agents (like hydroxypropyl-β-cyclodextrin), as well as partitioning with solid phases (like Tenax) (Cachada et al. 2014). Note also that chapter 7 of this report will include a comparison of a bioaccessibility measurement (using Tenax) with a bioavailability measurement (using POM).

A recent, comprehensive review has concluded that none of the current methods used to measure PAH bioavailability or bioaccessability can account for bioaccumulation or toxicity for all types of organisms, as different organisms can have different uptake pathways, particularly plants vs invertebrates (Cachada et al. 2014). This was also found in IBRACs, as will be evidente when comparing the bioaccummulation results with worms in this chapter, with the phytoavailability results in Chapter 8.

When just considering earthworms, however, things look more promising, particularly for bioavailability-based approaches. An increasing number of studies are reporting good correlations with bioaccumulation in worms exposed to historically contaminated soils and the freely-dissolved C_{pw} (van der Wal et al. 2004, Bergknut et al. 2007, Jonker et al. 2007, Gomez-Eyles et al. 2012, Brand et al. 2013, Cachada et al. 2014). The first such study was in 2004 for PCBs (van der Wal et al. 2004) and in 2007 for PAHs (Bergknut et al. 2007, Jonker et al. 2007). In one of the initial PAH studies, Jonker, van der Heijden et al. (2007) exposed the worm *Eisenia fetida* to 15 soils from gasworks sites, and compared measured worm lipid concentrations, C_{lipid} ($\mu g/kg_{lipid}$), with estimated values based on C_{pw} and lipid bioconcentration factors, BCF_{lipid} (L_{pw}/kg_{lipid}):

$$BCF_{lipid} = C_{lipid}/C_{pw} \qquad (6.2)$$

The study found estimated C_{lipid} agreed with measured values within a factor 10; however, use of C_{soil} and generic, conservative K_{TOC} values overestimated C_{lipid} by a factor 10 – 10 000 (for reasons stated above). Gomez-Eyles et al. (2012) did a similar screening study to Jonker et al. (2007), this time using 10 diverse historically contaminated soils, though in addition compared a broad array of non-

exhaustive extraction techniques to quantify bioaccessibility. The study found that only methods to measure the freely-dissolved C_{pw} (i.e. bioavailability) could predict worm C_{lipid} within a factor 10.

In order to go beyond these studies within IBRACS, we obtained 21 historically PAH-polluted soil samples from various locations in Sweden, Belgium and France, and measured not only the C_{soil} and passive-sampler derived C_{pw} of native PAHs but also of oxygenated-PAHs (oxy-PAHs) and nitrogen-containing heterocyclic polycyclic aromatic compounds (N-PACs). Oxy-PAHs and N-PACs are commonly present as co-pollutants or transformation products in PAH polluted soils, though only rarely considered (Lundstedt et al. 2014). These were included as part of this IBRACs study, through teaming up with the PACMAN research group, also funded through Snowman network.

Bioaccumulation and toxicological studies (mortality and reproduction) to the earthworm *Enchytraeus crypticus* were also conducted. To our knowledge, this is the first study to look at oxy-PAH and N-PAC bioavailability and partitioning in historically-contaminated soils, as well as compare freely-dissolved C_{pw} with *E. crypticus* bioaccumulation and toxicity. The results are discussed in terms of practical strategies for improving soil risk assessment of historically contaminated soils by accounting for bioavailability (Belfroid et al. 1996, Jager et al. 2003, ter Laak et al. 2006a, Arp et al. 2009).

6.2 Description of experimental and modelling work

6.2.1 Chemicals

The name of all PAHs, oxy-PAHs and N-PACs considered in this chapter, along with relevant compound properties are provided in Appendix 3, Table A3.1.

Unlabeled PAH, oxy-PAH and N-PAC standards were from LGC standards (Wesel, Germany), Sigma-Aldrich (Steinheim, Germany), Alfa Aesar (Karlsruhe, Germany), Chiron (Trondheim, Norway) and Institute for Reference Materials and Measurements (Geel, Belgium). Labelled standards were from Cambridge Isotope Laboratories (USA) and CDN Isotopes Inc. (Canada). Labelled internal standard (IS) stock solution of oxy-PAHs (24 ng/µL in toluene) contained ($^2H_8$)-9-fluorenone and ($^2H_8$)-anthracene-9,10-dione; for PAHs (35 ng/µL) ($^2H_8$)-naphthalene, ($^2H_8$)-acenaphthylene, ($^2H_{10}$)-acenaphthene, ($^2H_{10}$)-fluorene, ($^2H_{10}$)-phenanthrene, (^{2}H10)-pyrene, ($^2H_{12}$)-chrysene, and ($^2H_{12}$)-perylene, and ($^2H_{14}$)-dibenz(a,h)anthracene, and for N-PACs (23 ng/µL) ($^2H_8$)-carbazole and ($^2H_9$)-acridine. The recovery standard (RS) added to the samples before the final analysis was ($^2H_{10}$)-fluoranthene (28 ng/µL in toluene).

All solvents used (n-hexane, acetone, dichloromethane toluene and *n*-pentane) were of analytical grade quality (Suprasolv from Merck, AnalaR Normapur from VWR International, Fisher Scientific) or HPLC-grade (Rathburn).

6.2.2 Soils

21 soil samples were obtained from 6 locations in three countries: a) a former gasworks plant in Karlstad, Sweden (5 samples); b) a former wood tar production site outside Stockholm (Riksten), Sweden (10 samples); c) a coke oven plant in France (3 samples); d) a coke oven and metallurgy site in France (1 mixed sample); e) a gasworks site in France (1 mixed sample); f) a gasworks site in Belgium (1 mixed sample), g) and a wood preservation site, Holmsund, Sweden (1 mixed sample). The exact French and Belgium locations are to be kept anonymous by request.

6.2.3 Soil analysis

Particle size distribution was determined by the gravitational liquid sedimentation method (ISO 13317-2:2001), and $CaCO_3$ content by the ISO 10693:1995 method. Metal concentrations were determined by HNO_3:H_2O_2 (10:1) microwave digestion (60 min.), followed by Inductively Coupled Plasma Sector Field Mass Spectrometry. Properties of the 21 individual soils are presented in Table 6.1. The soils were predominantly sandy (>60% sand in all but two samples), with pH from 4.7 – 8.6. The Swedish Karlstad and Riksten soils were the most acidic (pH 4.7 – 6.6), having $CaCO_3$ contents below detection. Belgian, French and Holmsund samples had higher pH (7.0 – 8.6) and were more calcareous ($CaCO_3$ 13.3 – 757 g kg^{-1}).

Table 6.1 Soil sample identification, location, texture properties, pH, CaCO3 content and CEC.

Sample name	Sample code	Country	Industry	Clay (%) <0.002	silt (%) 0.002-0.05	sand (%) 0.05-2 mm	pH(H2O)	CaCO3 (g/kg)	CEC (cmol+/kg)
Karlstad 1a	K1a/SW01	Sweden	Gaswork plant	6.1	23.9	70	7.05	<1	1.97
Karlstad 2	K2/SW02	Sweden	Gaswork plant	5.1	19	75.9	8.63	8.1	2.21
Karlstad 3a	K3a/SW03	Sweden	Gaswork plant	3.6	15.9	80.5	7.75	<1	2.38
Karlstad 5	K5/SW04	Sweden	Gaswork plant	5.6	22.1	72.3	5.95	<1	1.82
Karlstad 6	K6/SW05	Sweden	Gaswork plant	8.4	29.2	62.4	7.67	<1	3.59
Riksten 1a	R1a	Sweden	Tar factory	5	35	60	4.9	<1	n.d.
Riksten 2	R2	Sweden	Tar factory	0	37	63	5.7	<1	n.d.
Riksten 3	R3	Sweden	Tar factory	1	21	78	5.9	<1	n.d.
Riksten 6a	R6a	Sweden	Tar factory	1	3	96	5.2	<1	n.d.
Riksten 6b	R6b	Sweden	Tar factory	1	4	95	5.4	<1	n.d.
Riksten 7	R7	Sweden	Tar factory	1	4	95	6.6	<1	n.d.
Riksten 8	R8	Sweden	Tar factory	1	2	97	6.2	<1	n.d.
Riksten 9	R9	Sweden	Tar factory	10	52	38	6.3	<1	n.d.
Riksten 10	R10	Sweden	Tar factory	0	35	65	5.2	<1	n.d.
Riksten 11	R11	Sweden	Tar factory	5	38	57	4.7	<1	n.d.
Belgium 1	BE01	Belgium	Gaswork plant	14.9	19.6	65.5	7.57	13.3	9.97
France 1	FR01FR01	France	Coking plant	9.8	21	69.2	8.52	357	8.64
France 2	FR02	France	Coking plant	8.6	18.4	73	8.22	175	11
France 3	FR03	France	Coking plant	11.9	26.6	61.5	8.35	217	14.9
France 4	FR04	France	Coking + metallurgical	15.5	20	64.5	7.5	20.5	9.65
France 5	FR05	France	Gas factory	6.2	21.5	72.3	8.07	71.8	7.27
Holmsund 1	H1	Sweden	Wood impregnation	6.2	21.5	72.3	8.07	71.8	0

n.d. = not determined.

6.2.4 TOC and BC Determination

The content of total organic carbon (TOC) and black carbon (BC) were measured after Gustafsson et al. (1997). Briefly, soil samples were dried overnight at 105° C, with 100 mg being weighted into ceramic weighing boats. For TOC analysis, 1 M HCl was added in 1 mL aliquots every 30 minute until bubbling stopped, minimally six times, to remove $CaCO_3$. Samples were then rinsed with 6 x 1 mL deionized water to remove chloride, dried, and analysed for C by element analysis after combustion at 1030°C (Leco EC12 Carbon Analyser, USA). For BC analysis, three silver capsules per single sample (30 mg soil per capsule) were combusted at 375 °C for 18 hours under abundant oxygen access, before adding acid as described above and analysing for C.

The content of total organic carbon (TOC) and black carbon (BC) were measured using the Chemo-Thermal Oxidation at 375°C (CTO-375) method by Gustafsson et al. (1997), as described in the SI-Section 2. Carbon concentration is reported as the mass per dry weight of soil ($kg_C/kg_{soil\ dw}$) for TOC (f_{TOC}), BC (f_{BC}), and amorphous organic carbon (AOC, f_{AOC}), where $f_{AOC} = f_{TOC} - f_{BC}$.

As can be seen from Table 6.2, the amount of total organic carbon in the soils (f_{TOC}) varied from 2.0 ± 0.3% to 49.1 ± 15.4%. Samples from Riksten and Holmsund where lowest in BC (f_{BC} < 4% of f_{TOC}). Other samples, particularly from coking sites, were BC rich, (f_{BC} comprising 26 – 95% of f_{TOC}).

Table 6.2 Carbon measurements in the soil, including total organic carbon (TOC), black carbon (BC) stable under oxidation at 375°C, amorphous organic carbon (AOC) (i.e. TOC which is not BC), and the ratio of BC to TOC.

Sample name	Short Name	f_{TOC} (%)	f_{BC} (%)	f_{AOC} (%)	f_{BC}/f_{TOC} (%)
Karlstad 1a	K1a/SW01	5.4 ± 1.6	2.7	2.7 ± 1.6	50
Karlstad 2	K2/SW02	2.0 ± 0.3	0.5	1.6 ± 0.3	22
Karlstad 3a	K3/SW03a	7.5 ± 0.2	4.9	2.5 ± 0.2	66
Karlstad 5	K5/SW04	11.8 ± 2.1	6.6	5.2 ± 2.1	56
Karlstad 6	K6/SW05	49.1 ± 15.4	12.6	36.5 ± 15.4	26
Riksten 1a	R1a	46.7 ± 0.4	0.4 ± 0.4	46.3 ± 0.6	1
Riksten 2	R2	15.2 ± 1.6	0.2 ± 0.2	15.0 ± 1.6	2
Riksten 3	R3	5.7 ± 0.4	0.1 ± 0.0	5.6 ± 0.4	1
Riksten 6a	R6a	12.9 ± 1.7	0.1 ± 0.0	12.9 ± 1.7	1
Riksten 6b	R6b	4.1 ± 1.2	0.1 ± 0.0	4.1 ± 1.2	1
Riksten 7	R7	21.5 ± 0.4	0.7 ± 0.5	20.9 ± 0.6	3
Riksten 8	R8	14.3 ± 0.5	0.3 ± 0.2	14.0 ± 0.5	2
Riksten 9	R9	3.6 ± 0.4	0.1 ± 0.0	3.5 ± 0.4	3
Riksten 10	R10	5.7 ± 0.6	0.1 ± 0.0	5.6 ± 0.6	1
Riksten 11	R11	14.1 ± 2.2	0.2 ± 0.0	13.9 ± 2.2	1
Belgium 1	BE01	2.5	1.1	1.4 ± 0.0	42
France 1	FR01FR01	12.7 ± 1.9	5.1	7.6 ± 1.9	40
France 2	FR02	17.9 ± 0.3	13.6	4.3 ± 0.3	76
France 3	FR03	20.5 ± 1.0	19.5	1.0 ± 1.0	95
France 4	FR04	7.7 ± 2.1	3.8	3.9 ± 2.1	50
France 5	FR05	33.0 ± 0.6	28.3	4.7 ± 0.6	86
Holmsund 1	H1	2.2	0.1	2.1	4

The PACs were extracted from the soil samples using pressurized liquid extraction (ASE 200, Dionex) with 30 mL hexane/acetone (1:1) at 120 °C and 14 MPa pressure. One gram of each sample was mixed with 10 grams of solvent-washed sand to fill up the extraction cell and to allow for better penetration by the solvent. Three static extraction cycles of 5 min each were used. Half of the resulting extract was then spiked with 20 µL of each IS-mixture, evaporated using reduced pressure and a gentle nitrogen stream (Turbovap), and purified on KOH-impregnated silica gel columns (5 g) eluted with 30 mL of dichloromethane. The purified samples were evaporated into 1 mL toluene, spiked with 20 µL RS, and transferred to GC-vials.

For obtaining preliminary information on the level of PAHs in the Riksten and Karlstad samples, sub samples were sent to a commercial lab to screen PAH analysis levels before the start of the experiment. These were concentrations agreed with our measurements on average by a factor 1.1, though 28% of individual PAH measurements differed by over a factor 2, and 9% by over a factor 3. This interlab discrepancy is within expectations for extracting PAHs from soot rich materials due to solvent efficiency (Jonker and Koelmans 2002), but could additionally be influenced by soil heterogeneity, as the soil samples went through extra homogenization procedures after being subsampled for analysis by the commercial lab and before being analysed as described in this study.

6.2.5 Passive Sampler analysis

Porewater concentrations, C_{pw}, were determined using polyoxymethylene (POM) passive sampler extraction (Hawthorne et al. 2011b, Josefsson et al. 2014). In brief, 76 µm thick POM from CS Hyde (Lake Villa, IL) was cut into 2 x 4 cm strips and pre-extracted with acetone:hexane followed by methanol to remove contaminants. In 40 mL amber glass jars with Teflon®-lined caps (Agilent, Santa Clara, California), approximately 10 g of homogenized soil (wet) was introduced and weighed, followed by a POM strip and approximately 35 mL of water containing 0.001 M $CaCl_2$ and 0.015 M NaN_3 (biocide), such that there was 0.5 – 1 mL headspace remaining. Vials were shaken end-over-end for 28 days (the time required to reach equilibrium (Hawthorne et al. 2011b, Josefsson et al. 2014)) in the dark. POM strips were removed with tweezers, rinsed with ultrapure water and wiped dry with a tissue before being placed in a clean 20 mL scintillation vial and frozen (-20°C) until extraction. A

The POM samples were extracted in the aluminium foil lined screw-capped scintillation vials they had been stored in, using 2 × 20 mL of n-hexane/acetone (1:1). 20 µL of each IS-mixture was added to the first aliquot of solvent. Each extraction was performed for 24 hours on a vibrating table. The two extracts were pooled, evaporated into 0.5 mL toluene, spiked with 20 µL RS and transferred to GC-vials. The extracted POM strips were air-dried overnight and weighed.

Concentrations in POM samples, C_{POM}, were only considered above the limit of quantification if they were > 3 times average blank POM levels. Freely-dissolved C_{pw} at equilibrium were calculated from C_{POM} using pre-calibrated POM-water partitioning coefficients, K_{POM}, for PAHs (Hawthorne et al. 2011b), oxy-PAHs (Josefsson et al. 2014), and Carbazole (Endo et al. 2011b), along with estimated K_{POM} for the remaining N-PACs (Endo et al. 2011b) (see Appendix 3, Table A3.1)

$$K_{POM}\ (L_{pw}/kg_{POM}) = C_{POM}/C_{pw} \qquad (6.3)$$

6.2.6 Reproduction assays

Reproduction assays with E. crypticus were carried out according to ISO 16387 (2014). The worms were cultured in-house on agar plates containing mineral salts (Westheide and Bethke-Beilfuss 1991) at 20 ± 1 °C and fed autoclaved oat flakes. Ten worms (ca. 8 days old with clearly visible clitellum) were transferred into glass containers (200 mL) with 35 g moist test soil, including one artificial reference soil, and 100 mg autoclaved crushed oat flakes as food. The containers were covered with a glass lid and incubated at 20 °C, with a 4/20 h light/dark cycle. Five replicates were used for each soil. Once a week additional food was added if needed, and water was added to correct for evaporation loss. Adult worms were removed and counted after 21 days. After another seven days, the juveniles were immobilized with ethanol, colored with Bengal red (1% solution in ethanol), and counted under a microscope.

6.2.7 Bioaccumulation assay

For the bioaccumulation assay, a similar procedure was followed to the reproduction assay, though 100 worms were used per test soil. The containers were incubated for 2 weeks and the worms were then transferred to fresh test soil. After a total of 4 weeks, incubation was terminated and the worms were transferred to agar plates with mineral salts (Westheide and Bethke-Beilfuss 1991) and kept for 2 days at 20°C to empty their digestive tracts and clean their surface. The soil-free worms were then transferred to screw-capped tubes, weighed and frozen at -20 °C until analysis.

6.2.8 Worm analysis

To measure worm PAC content, worms were homogenized with 1-2 g of anhydrous sodium sulphate and 2 mL of dichloromethane in the same test tubes they had been stored in. 20 µL of each IS-mixture was added. The slurry was left overnight to efficiently extract all PACs from the worm tissue, and was then transferred to a glass column (16 mm i.d.) with 5 g KOH-impregnated silica gel. The PACs were eluted with 30 mL dichloromethane, after which the extract was evaporated to 1 mL, reconstituted with 3 mL of toluene, and evaporated again to 0.5 mL. The samples were then spiked with 20 µL RS and transferred to GC-vials.

To measure the mass fraction of lipids in worms, 100 worms per sample were incubated in five different media (three France-coke work soils, reference soil, and agar) and frozen as in the bioaccumulation experiment. The resulting frozen worms were mixed with anhydrous Na_2SO_4, crushed by grinding in a mortar, mixed with dichloromethane and transferred to a column containing a metal filter. To eliminate traces of Na_2SO_4 the filtrate was transferred to a test tube, the solvent evaporated, and the residue dissolved in *n*-pentane. The solution was then transferred to a weighing vessel, evaporated, and the lipid residue was weighed.

6.2.9 GC/MS-analysis

Samples were analyzed by GC high-resolution mass spectrometry (HRMS) using an HP 5890 GC coupled to a Waters Autospec Ultima HRMS. The GC was equipped with a DB5-ms capillary column (60 m × 0.25 mm × 0.25 µm; J&W Scientific, Folsom, CA, USA) and the MS operated in electron ionization mode. The GC oven temperature program started at 90 °C for 1 min, increased 8 °C/min up to 320 °C, which was held for 14 minutes. Sample aliquots of 1 µL were injected in splitless mode at 290 °C. Target compounds were identified by comparing GC retention data for the molecular ions in

the samples and the reference standards. Quantification was performed using the internal standard technique. Possible losses of native compounds were thereby compensated for by the measured losses of the most similar internal standard. For quantification in this study, possible losses of native compounds were thereby compensated for by the measured losses of the most similar internal standard, i.e. ($^2H_8$)-naphthalene was used to compensate for losses of naphthalene, 1-indanone and quinoline (since the main loss of 1-indanone and quinoline was considered to be through evaporation), ($^2H_8$)-acenaphthylene for acenaphthylene, ($^2H_{10}$)-acenaphthene for acenaphthene, ($^2H_{10}$)-fluorene for fluorene, ($^2H_{10}$)-phenanthrene for phenanthrene and anthracene, (^{2}H10)-pyrene for fluoranthene and pyrene, ($^2H_{12}$)-chrysene for benz(a)anthracene and chrysene, ($^2H_{12}$)-perylene for benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene, ($^2H_{14}$)-dibenz(a,h)anthracene for indeno(1,2,3-cd)pyrene, dibenz(ah)anthracene and benzo(ghi)perylene, ($^2H_8$)-9-fluorenone for 1-Acenaphthenone, 9-Fluorenone, 4H-cyclopenta(def)phenanthrenone, benzo(a)fluorenone, 7H-benz(de)anthracen-7-one, benz(a)anthracene-7,12-dione, naphthacene-5,12-dione, and 6H-benzo(cd)pyren-6-one; ($^2H_8$)-anthracene-9,10-dione for anthracene-9,10-dione, and 2-methylanthracene-9,10-dione; ($^2H_9$)-acridine for benzo(h)quinolone, and acridine; and ($^2H_8$)-carbazole for carbazole.

6.2.10 Soil Partitioning calculations

Measured soil-porewater partitioning of PACs was fitted to both single carbon and dual-carbon models. The single carbon model assumes TOC is the principle sorbent of PACs:

$$K_D = C_{soil}/C_{pw} = K_{TOC} \cdot f_{TOC} \qquad (6.4)$$

Recommended K_{TOC} values for PAHs have been presented by the United States Environmental Protection Agency (USEPA) for sediments (USEPA 2003) and the Netherlands' National Institute for Public Health and the Environment (RIVM) for soils and sediments (Verbruggen 2012). The USEPA and RIVM recommended K_{TOC} values are based on the assumption that soil TOC sorbs similarly to octanol, and thus that K_{TOC} is similar to the octanol-water partitioning coefficient, K_{OW}. The origins of this can be traced back to initial laboratory studies from the 1970s – 1980s using laboratory-spiked soil (e.g. "log K_{TOC} = log K_{ow} – 0.21" or "log K_{TOC} = 0.98 log K_{OW}"). (Karickhoff et al. 1979, Di Toro et al. 1991). An alternative sorption proxy to octanol for historically contaminated sediments is coal tar. A 2009 study that compiled average log K_{TOC} of PAHs for over 400 pyrogenic impacted sediments concluded that these sorption data were more similar to the K_{TOC} of coal tar than that of any other tested reference carbonaceous material, including octanol or laboratory spiked soils (Arp et al., 2009; Arp et al., 2011a). As coal tar K_{TOC} are seldom measured, they can be estimated with a Raoult's Law (Chiou et al. 1979) type model for coal tar (Endo et al. 2008, Arp et al. 2009).

$$K_{TOC} = (S_L^* MW_{TOC})^{-1} \qquad (6.5)$$

Where S_L^* is the *subcooled* saturated molar water solubility (mol/L_{water}), and MW_{TOC} is the molar weight of coal tar (223 g/mol) (Endo, Xu et al. 2008, Arp et al. 2009).

The dual-carbon model considers the BC and AOC fraction as separate sorbents (Gustafsson et al. 1997, Brandli et al. 2008):

$$K_D\ (L_{pw}/kg_{soil\ dw}) = f_{AOC}K_{AOC} + f_{BC}K_{BC}C_{pw}^{n-1} \qquad (6.6)$$

where K_{AOC} (L_{pw}/kg_{AOC}) is the partitioning to amorphous organic carbon, typically assumed to be equivalent to K_{OW} (i.e. $K_{AOC} = K_{OW}$). (USEPA 2003, Verbruggen 2012) K_{BC} ($Lpw^{n(n-1)}/kg_{BC}$) is the black carbon partitioning coefficient, and *n* is the Freundlich exponent to account for sorption non-linearity to BC. Here, *n* was assumed to be 1, due to previous studies for sediments showing minor to no benefits having n < 1, and also to avoid the complexity of an extra parameter for fitting by calibration (Hawthorne et al. 2007, Arp et al. 2011a). A compilation of PAC properties, including K_{OW} and S_L^* are given in Appendix 3, Table A3.1.

6.2.11 PAH Toxicity

The Dutch National institute for Public Health and the Environment (RIVM) recently derived soil guideline values using the most up-to-date database of aquatic, soil and sediment toxicity data for PAHs (Verbruggen, 2012) after a critical evaluation of existing chronic toxicity data sets for individual PAHs, the author arrived in a final data set of **critical lipid concentrations** (no observed effect residue – **NOER**) for 54 different species, representing 13 terrestrial, 10 benthic and 31 aquatic species. The concept of critical lipid concentration is based on the assumption that toxicity of individual PAHs is similar after entering the cell membrane. Thus, each of the 54 NOER values represent a (geometric) mean of NOER values obtained in dose-response experiments with individual PAHs (note: NOER values correspond to NOEC values, but apply to lipid concentrations). Because of lack of information on analytically derived lipid concentrations in the majority of these experiments, lipid concentrations were calculated using **partition equilibrium theory**. For experiments with terrestrial and benthic species, pore water concentrations were calculated first by considering partitioning between organic matter and water. From water concentrations, the internal residues were calculated using a partition coefficient between the membrane and water.

To derive risk limits, the 54 NOER values were plotted in one species sensitivity distribution (SSD) curve Figure 6.1. As shown, the data for the different compartments (terrestrial, benthic, aquatic) follows the same SSD function. Accordingly, on basis of internal residues it appears to be no significant difference between the compartments, which confirms the assumption that indeed accumulation from (pore) water is the determining factor for toxicity. Furthermore, the evaluation supports the applicability of the equilibrium partitioning theory (at least in the case of these spiked systems).

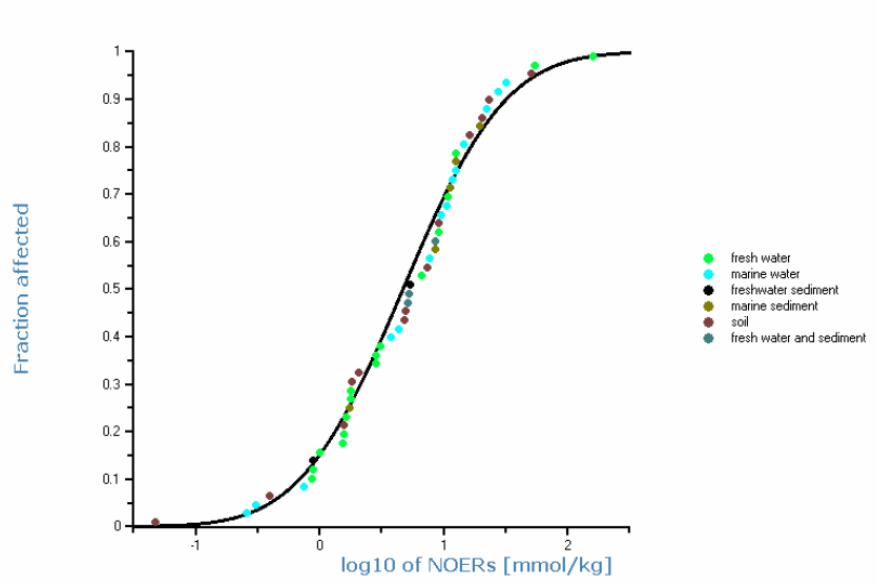


Figure 6.1 Species sensitivity distribution plot of 54 species with NOER values for various individual PAH (from Verbruggen, 2012).

Based on the SSD function, two types of guidelines were proposed, a "Maximum Permissible Concentration" (MPC) and "Serious Risk Concentration" (SRC); see Table 6.3 for an explanation. Below MPC values, the risk of PAHs to ecosystems is assumed negligible, whereas the SRC value can be used as a trigger value when remediation is needed. The MPC and SRC values for individual PAHs are listed in Table 6.4. These values were obtained from critical NOERs (0.39 and 4.7 mmol/kg lipid) divided by PAH specific membrane - water partition coefficients.

Table 6.3 Definitions of MPC and SRC (Verbruggen, 2012).

Abbreviation	Full name	How it was derived	To be used
MPC	Maximum Permissible Concentration (water, soil or sediment)	The HC5 value obtained from the SSD curve based on chronic NOERs (0.39 mmol PAH/kg lipid), divided by a safety factor 5 to give a PNEC for lipids. Critical concentrations in water, soil and sediment phases were obtained from the PNEC for lipids using equilibrium partitioning theory (HC5 = hazardous concentration to 5% of tested species).	Concentration at which no negative effect is to be expected for ecosystems. Can be used as generic environmental quality standard.
SRC	Serious Risk Concentration (water, soil or sediment)	The HC50 value obtained from the SSD curve based on chronic NOERs (4.7 mmol PAH/kg lipid). Critical concentrations in water, soil and sediment phases were obtained from the lipid HC50 using equilibrium partitioning theory (HC50 = hazardous concentration to 50% of tested species).	Concentration at which possibly serious ecotoxicological effects are to be expected. Can be used to derive intervention values for soil ecosystems. Above SRC soil is considered to be seriously contaminated.

One important practical implication of the assumption of similar internal toxicity of different PAHs, is that the **total toxic effect of a PAH mixture can be calculated as the sum of all individual PAHs in the cell lipid**, expressed on molar basis (e.g. mmol PAH per kg lipid). In other words, the toxicity is assumed to be additive, which means that the **toxic unit approach** can be used to assess toxic effects of PAH mixtures. The net-burden of all PAHs present, expressed as the sum of the relative toxicity units, $TU_{PAH\text{-}16}$, can be obtained from

$$TU_{PAH-16} = \sum_{i=1}^{16} C_{PAH-i}/Screening\ Value_{PAH-i} \qquad (6.7)$$

Here, $C_{PAH\text{-}I}$ is the measured concentration of individual PAHs in the pore water (or soil) and *Screening value* $_{PAH\text{-}i}$ is the corresponding screening values (e.g. MPC or SRC). A TU value >1 indicates a pre-selected risk situation. It should be noted here that this approach, though very practical in terms of synthesizing several data sets, is not universally accepted. In America, this approach is the status quo for PAHs, being adopted for instance by the US Environmental proection agence (USEPA 2003) for sediments. Further, there are many in the Netherlands who favour this approach. However, some regulatory bodies and scientists are criticial, as there are studies that show that in some organisms specific PAHs can present additional modes of toxic action in addition to baseline narcosis (Billiard et al. 2008).

Table 6.4 Screening values for PAHs for water and soils, and the KTOC values used as part of their derivation for soils, including the final chronic values (FCV) derived by the US Environment Protection Agency (USEPA) for sediments, Maximum Permissible Concentration (MPC) and Serious Risk Concentrations (SRC) derived by the Dutch National institute for Public Health and the Environment (RIVM). (SRC values for " All PAHs" in Table 99 of that report, using the All PAH "No Obersvable Effect Residue" (NOER) method (for 54 species)).

PAH	Screening Values in (pore)water and soil					
	USEP[a)] FCV water (µg/L)	USEPA[a)] FCV sediments ($\mu g/g_{TOC}$)	RIVM[b)] MPC_{eco} fresh water (µg/L)	RIVM[b)] SRC fresh water (µg/L)	RIVM[b)] MPC_{eco} soil ($\mu g/g_{soil,\ 10\%TOC}$)	RIVM[b)] SRC soil ($\mu g/g_{soil,\ 10\%TOC}$)
naphthalene	193.47	3059	5.4	324	0.43	26
acenaphthene	55.85	491	1.7	104	0.53	31
acenaphthylene	306.85	452	4	236	0.51	30
fluorene	39.298	540	1.1	63	0.58	35
anthracene	20.73	594	0.41	24	0.71	42
phenanthrene	19.13	597	0.58	35	0.67	40
fluoranthene	7.109	708	0.18	11	0.99	59
pyrene	10.113	698	0.27	16	0.89	53
chrysene	2.042	843	0.074	4.4	1.7	103
benz(a)anthracene	2.227	841	0.064	3.8	1.9	112
benzo(a)pyrene	0.957	964	0.053	3.2	2.6	154
benzo(b)fluoranthene	0.677	1007	0.053	3.2	2.6	153
benzo(k)fluoranthene	0.642	980	0.054	3.2	2.5	151
dibenz(ah)anthracene	0.283	1122	0.036	2.2	4.7	279
indeno(1,2,3-cd)pyrene	0.275	1115	0.035	2.1	4.9	289
benzo(ghi)perylene	0.439	1095	0.052	3.1	3.1	186

[a)]USEPA = United States Environmental Protection Agency, FCV = Final Chronic Values, [b)] RIVM = Dutch National Institute for Public Health and Safety, MPC_{eco} = Maximum Permissible Concentrations, SRC = Serious Risk Concentrations.: Ref USEPA (2003) : Ref Verbuggen (2012)

6.3 Results and Discussion

6.3.1 Soil concentrations

C_{soil} (µg/g_{dw}) ranged over four orders of magnitude , with ∑PAH-16 from 0.27 µg/g_{dw} to 2651 µg/g_{dw}, ∑oxy-PAH-11 from 0.12 µg/g_{dw} to 371 µg/g_{dw}, and ∑N-PAC-4 from 0.013 µg/g_{dw} to 22 µg/g_{dw} (see Table 6.5, and for individual compounds Tables A3.2 and A3.3 in Appendix 3). Replicate C_{soil} measurements exhibited relative standard deviation (RSD) of 4 – 15% for the PAHs and oxy-PAHs, and 10-29% for the N-PACs.

Table 6.5 Sum PAH-16, oxy-PAH and N-PAC soil concentrations (Csoil), and logCsoil, with increasing concentrations indicated by green shading.

		C_{soil} (µg g_{dw}^{-1})			
		∑PAH-16	**∑Oxy-PAH**	**∑N-PAC**	**∑All Cmpds**
Karlstad 1a-1	K1a/SW01-1	56.25	9.74	0.54	66.54
Karlstad 2	K2/SW02	56.30	12.92	0.90	70.12
Karlstad 3a	K3/SW03a	23.05	10.63	0.48	34.16
Karlstad 5	K5/SW04	21.51	6.23	0.40	28.13
Karlstad 6	K6/SW05	130.32	23.58	2.12	156.02
Riksten 1a	R1a	277.69	108.09	2.13	387.91
Riksten 2	R2	40.81	14.07	0.55	55.43
Riksten 3	R3	5.11	1.96	0.10	7.17
Riksten 6a-1	R6a-1	48.55	9.57	0.27	58.38
Riksten 6a-2	R6a-2	63.21	11.68	0.41	75.30
Riksten 6a-3	R6a-3	51.09	8.84	0.25	60.19
Riksten 6b	R6b	3.46	0.61	0.034	4.10
Riksten 7	R7	11.62	3.90	0.18	15.70
Riksten 8	R8	3.70	1.50	0.09	5.29
Riksten 9	R9	0.27	0.12	0.013	0.40
Riksten 10	R10	1.65	0.33	0.029	2.01
Riksten 11-1	R11-1	50.13	12.24	0.32	62.69
Riksten 11-2	R11-2	48.50	12.84	0.35	61.69
Riksten 11-3	R11-3	47.30	11.43	0.43	59.17
Riksten 11-4	R11-4	50.81	13.04	0.42	64.27
Riksten 11-5	R11-5	51.60	12.43	0.34	64.37
Riksten 11-6	R11-6	47.41	13.90	0.36	61.67
Belgium 1	BE01	296.88	32.81	4.77	334.47
France 1	FR01FR01	2651	371	21.61	3043
France 2	FR02	1148	204	6.84	1359
France 3	FR03	78	11	1.13	91
France 4-1	FR04-1	1084	106	9.22	1199
France 4-2	FR04-2	1260	106	9.40	1376
France 4-3	FR04-3	1129	96	7.83	1232
France 5	FR05	237	27	1.75	265
Holmsund 1-1	H1-1	2497	223	6.83	2726
Holmsund 1-2	H1-2	2320	219	5.21	2545
Holmsund 1-3	H1-3	2601	244	8.73	2854

6.3.2 Porewater concentrations

Measured C_{pw} (µg/L_{pw}) of soils also varied over 4 orders of magnitude, with ∑PAH-16 from 0.02 to 460 µg/L_{pw}, ∑oxy-PAH-11 from 0.004 to 168 µg/L_{pw} and ∑N-PAC-4 from 0.006 to 22 µg/L_{pw} (see Table 6.6 and Tables A3.4 and A3.5 in Appendix 3).

Table 6.6 Sum PAH-16, oxy-PAH and N-PAC POM concentrations in porewater, with increasing concentrations indicated with green shading.

		C_{PW} (µg mL^{-1})			
		∑PAH-16	∑Oxy-PAH	∑N-PAC	∑All Cmpds
Karlstad 1a-1	K1a/SW01-1	1.71E-03	1.63E-02	3.88E-05	1.81E-02
Karlstad 1a-2	K1a/SW01-2	2.01E-03	3.25E-02	1.40E-03	3.60E-02
Karlstad 1a-3	K1a/SW01-3	1.89E-03	9.23E-03	3.74E-05	1.12E-02
Karlstad 2	K2/SW02	2.54E-03	4.67E-03	5.22E-04	7.74E-03
Karlstad 3a	K3/SW03a	7.53E-04	4.55E-06	9.03E-06	7.66E-04
Karlstad 5	K5/SW04	8.59E-04	1.94E-06		8.61E-04
Karlstad 6	K6/SW05	5.89E-03	6.57E-05	6.85E-06	5.96E-03
Riksten 1a	R1a	4.94E-03	9.86E-03	6.00E-05	1.49E-02
Riksten 2	R2	5.77E-03	7.14E-03	1.44E-05	1.29E-02
Riksten 3	R3	1.27E-05			1.27E-05
Riksten 6a-1	R6a-1	8.15E-05	6.18E-05	6.26E-06	1.50E-04
Riksten 6b	R6b	4.75E-05	3.66E-06		5.12E-05
Riksten 7	R7	6.96E-05			6.96E-05
Riksten 8	R8	6.67E-05			6.67E-05
Riksten 9	R9	6.57E-05			6.57E-05
Riksten 10	R10	1.66E-05			1.66E-05
Riksten 11-1	R11-1	4.26E-05	6.89E-05		1.11E-04
Riksten 11-2	R11-2	3.95E-05	6.61E-05		1.06E-04
Riksten 11-3	R11-3	4.17E-05	6.15E-05		1.03E-04
Belgium 1	BE01	8.57E-02	1.68E-01	1.75E-02	2.71E-01
France 1	FR01FR01				
France 2	FR02	1.02E-02	3.32E-02	2.69E-04	4.36E-02
France 3	FR03	3.88E-04	1.74E-04	6.76E-06	5.69E-04
France 4-1	FR04-1	3.59E-02	1.11E-01	4.49E-03	1.52E-01
France 4-2	FR04-2	3.45E-02	1.14E-01	4.78E-03	1.53E-01
France 4-3	FR04-3	4.19E-02	1.28E-01	4.76E-03	1.75E-01
France 5	FR05	4.73E-04	5.40E-04	8.22E-06	1.02E-03
Holmsund 1-1	H 1-1	4.64E-01	1.67E-01	1.61E-02	6.47E-01
Holmsund 1-2	H 1-2	4.73E-01	1.42E-01	2.15E-02	6.37E-01
Holmsund 1-3	H 1-3	4.64E-01	1.56E-01	1.41E-02	6.34E-01

6.3.3 Partitioning

Measured, estimated and guideline-recommended partitioning constants are presented and compared in Figure 6.2a (raw data is presented in Arp et al. (2014)). As is evident from Figure 6.2a, the historically contaminated soil K_{TOC} values measured here for PAHs are substantially larger than USEPA recommended values (USEPA 2003) (median 13 times larger; interquartile range (IQR) 7 – 24), RIVM recommended values (Verbruggen 2012) (median 20 times larger; IQR 11 – 36), and log K_{ow} (median 13 times larger; IQR 7 – 24); however, they agree nicely with coal tar K_{TOC} (median 1.3 times larger, IQR 0.7 - 2.3). Measured K_{TOC} values for other PACs are also substantially larger than K_{OW} for oxy-PAHs (median 33 times larger; IQR 16 - 61), and N-PACs (median 18 times larger IQR 10 – 31).

Comparisons were better with the coal tar K_{TOC} (Figure 6.3) for oxy-PAHs (median 2.9 times larger; IQR 1.5 – 5.2) and N-PACs (median 2.6 times larger; IQR 1.4-4.6). This validates previous findings that the K_{TOC} of PAHs in historically contaminated soils are larger than recommended in guidelines because they were derived using reference soils with limited aging (Alexander 2000, ter Laak et al. 2006b, Brand et al. 2013), no strongly sorbing (amorphous or crystalline) carbonaceous materials present (Cornelissen et al. 2005), and based on total C_{pw} and not freely-dissolved C_{pw}.(Belfroid et al. 1996, Jager et al. 2003, ter Laak et al. 2006a, Arp et al. 2009). For the first time, this data also shows the K_{TOC} of native oxy-PAHs and N-PACs in historically contaminated soils is much better described as being similar to coal tar than octanol.

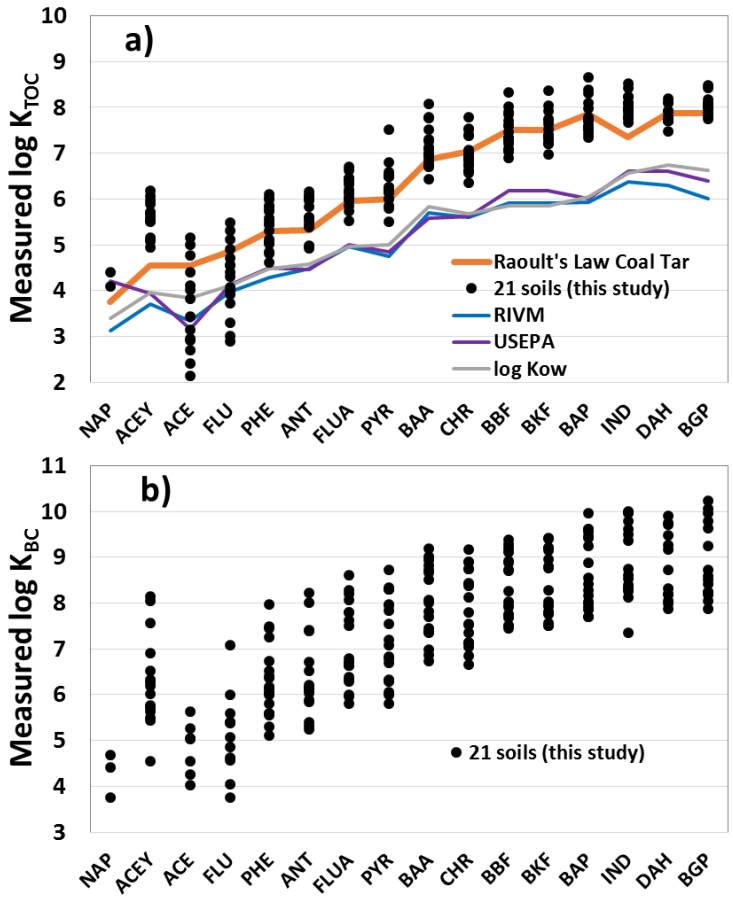


Figure 6.2 a) measured log KTOC values (L/kgTOC) for PAHs, b) measured log KBC values (L/kgBC) for PAHs. Also plotted in a) are estimated log K_{TOC} based on the Raoult's Law Coal Tar sorption model (Arp et al. 2009), recommended K_{TOC} values used by the USEPA for sediments (USEPA 2003), RIVM for soils and sediments (Verbruggen 2012), and K_{OW} values. Note the range in obtained log K_{BC} values in b) is larger than log K_{TOC} values in a), except for acenapthene but this only because there are fewer log K_{BC} values for this compound as log K_{BC} cannot be derived when log K_D < log K_{OW} (see eq 6.6).

Regarding the variability of sorption across these diverse historically contaminated soils, the standard deviations across all PACs for log K_D, log K_{TOC} and log K_{BC} were on average 0.66, 0.38 and 0.82, respectively, whereas the difference between the maximum and minimum were on average 2.2, 1.4 and 2.5, respectively. Thus, normalizing the observed K_D by f_{TOC} (eq 4) reduced the range of K-values for a given PAC; however, deriving K_{BC} values (eq 6.6) resulted in an increased range of partitioning constants. This can be seen for PAHs by comparing the increased range of compound-specific log K_{BC} values in Figure 6.2b with the narrower range of log K_{TOC} values in Figure 6.2a. Increased scatter in log K_{BC} compared to log K_{TOC} was observed previously for native PAHs and chlorinated aromatic

hydrocarbons in pyrogenic-impacted sediments (Hawthorne et al. 2007, Arp, et al. 2009, Arp, et al. 2011a, Arp, et al. 2011b, Hawthorne et al. 2011a). The central criticism of the dual-carbon (BC inclusive) model in equation 6 is that by assuming the only strong sorbing fraction is BC, it insufficiently characterizes the contribution of strong sorbing amorphous- and low-temperature burning crystalline carbonaceous phases present (Cornelissen et al. 2005, Arp, et al. 2011b), and suffers from artefacts caused by BC pores being blocked by organic matter, lipids or oil residues (Cornelissen et al. 2005, Hawthorne et al. 2007, Arp et al. 2009). As a result, the dual carbon K_{AOC}-K_{BC} model offers no advantageous over the single-carbon K_{TOC} model when considering diverse historically impacted soils, as with historically contaminated sediments (Arp et al. 2009, Hawthorne et al. 2011a).

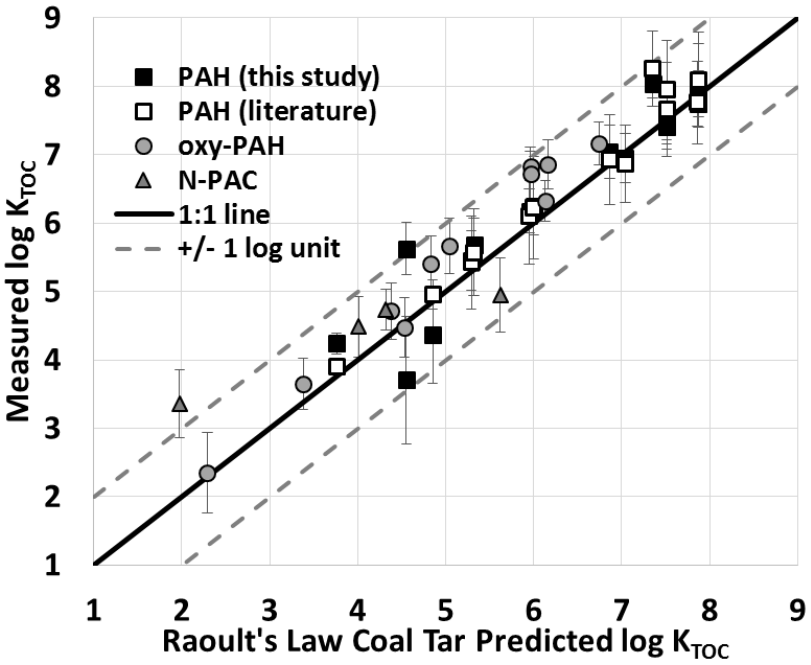


Figure 6.3 A comparison of average log KTOC values (± standard deviations in error bars) measured in this study for all PACs and in the literature for PAHs (from 24 literature soils) (ter Laak et al. 2006b, Jonker et al. 2007, Brandli et al. 2008) with those predicted using the Raoult's Law Coal Tar sorption model (Arp et al. 2009) described in eq 6.5.

Figure 6.2 compares measured log K_{TOC} values for all PACs in this study with the coal tar model (eq 6.5), and also includes all measured, literature log K_{TOC} values for PAHs in historically contaminated soils that could be found (comprising 24 soils in total) (ter Laak et al. 2006b, Jonker et al. 2007, Brandli et al. 2008). No such data for oxy-PAHs or N-PACS could be found in the literature. As is evident in Figure 6.2, not only are the literature PAH K_{TOC} values comparable with those in this study, they agree nicely with the coal tar model predictions. Measured and modelled K_{TOC} values agreed within a factor 30 (the stated accuracy of the model for historically contaminated sediments (Arp et al. 2009, Arp, et al. 2011a, Hawthorne et al. 2011a)) for 100% of the PACs and within a factor 3 for 58% of the PACs. The largest outliers included 3 PAHs (acenaphthylene, acenaphthene and indeno(cd)pyrene), 5 oxy-PAHs (cyclopentaphenanthrenone, 2-methyl anthracenedione, benzo(a)fluorenone, benz(a)anthracene-7,12-dione, naphthacene-5,12-dione), and 2 N-PACs (quinoline and carbazole). This is to some extent due to the uncertainty of S_L^* estimations (measured

values are unavailable for oxy-PAHs and N-PACs). Improvements in modelling would be enabled by the availability of experimentally determined physico-chemical data for oxy-PAHs and N-PACs. The model could also be improved through calibration, but the present accuracy is considered sufficient for general risk assessment purposes.

The success of coal tar as a sorption proxy for the 21 soils in this study and the 24 soils from the literature is largely accountable to the abundance of pyrogenic residue in these sites. Further, many of the PACs were likely introduced to the soil as a component of pyrogenic particles, and not a free phase molecule. The utility of this model at sites not contaminated by pyrogenic-residues remains untested. It could be that for such soils, an octanol-based model will be more appropriate. Unfortunately, only data from spiked tests to reference soils are available to test this hypothesis, no known studies of native PAH partitioning in reference soils, away from any contamination sources, is known. Of course, a challenge in obtaining such data is the very low concentrations at such locations.

6.3.4 Worm Bioaccumulation

Lipid concentrations are particularly important for assessing toxicity, as the the predominant mechanism for PAH toxicity in many species is accumulation in cell lipids, leading to narcosis.(Verbruggen 2012). Thus, in a PAC contaminated soil, it is of relevance to determine the net sum of all PACs in the lipids of affected organisms, $C_{lipid,total}$, (i.e. lipid bioaccumulation) as the most direct parameter to account for toxicity. At the end of the bioassay conducted here, C_{lipid} of PACs (likely at equilibrium) were determined for *Enchytraeus crypticus*.

Normalizing worm PAC concentrations, C_{worm}, to the average lipid concentration of 3.84 ± 0.77 % (average of exposed worms in five samples, including three coke soils, one reference soil and one agar plate), resulted in lipid concentrations, C_{lipid} (mmol/kg_{lipid}) ranging for ∑PAH-16 from 0.043 to 12, ∑oxy-PAH-11 from 0.010 to 0.27 and ∑N-PAC-4 from 0.00080 to 0.042. Total PAC C_{lipid} ranged from 0.051 to 12, with Swedish Riksten soils at the low end (0.051 – 1.1). Total C_{lipid} data is presented Table 6.7, with the compound specific data in Arp et al. (2014).

Table 6.7 Sum PAH-16, oxy-PAH and N-PAC lipid normalized worm (Enchytraeus crypticus)concentrations (flipid = 3.84 ± 0.77 %) expressed as internal residues (mmol kg-1lipid), with increasing concentrations indicated by green shading.

		C_{worm} (mmol kg^{-1}_{lipid})			
		∑PAH-16	∑Oxy-PAH	∑N-PAC	∑All Cmpds
Karlstad 1a-1	K1a/SW01-1	1.28	0.06	0.007	1.34
Karlstad 2	K2/SW02	3.07	0.09	0.007	3.17
Karlstad 3a	K3/SW03a				
Karlstad 5	K5/SW04	0.36	0.02	0.002	0.38
Karlstad 6	K6/SW05	0.66	0.03	0.005	0.69
Riksten 1a	R1a	0.97	0.08	0.012	1.06
Riksten 2	R2	0.31	0.03	0.004	0.34
Riksten 3	R3	0.08	0.02	0.003	0.10
Riksten 6a-1	R6a-1	0.54	0.02	0.002	0.57
Riksten 6b	R6b	0.15	0.00	0.003	0.16
Riksten 7	R7	0.04	0.01	0.002	0.05
Riksten 8	R8	0.08	0.03	0.005	0.11
Riksten 9	R9	0.04	0.02	0.002	0.05
Riksten 10	R10	0.05	0.01	0.001	0.06
Riksten 11-1	R11-1	0.54	0.04	0.003	0.58
Riksten 11-2	R11-2	0.31	0.17	0.001	0.47
Riksten 11-3	R11-3	0.26	0.02	0.001	0.28
Belgium 1	BE01	11.52	0.10	0.033	11.65
France 1	FR01FR01	3.42	0.27	0.042	3.73
France 2	FR02	2.64	0.21	0.032	2.88
France 3	FR03	0.14	0.06	0.003	0.21
France 4-1	FR04-1	10.41	0.19	0.022	10.62
France 4-2	FR04-2	10.98	0.20	0.029	11.22
France 4-3	FR04-3	8.65	0.19	0.028	8.87
France 5	FR05	0.74	0.07	0.006	0.81

Log-log correlations between C_{lipid} vs. C_{soil}, C_{lipid} vs. C_{TOC} (i.e. C_{soil}/f_{TOC}), and C_{lipid} vs. C_{pw} (i.e. C_{POM}/K_{POM}), and various other measurements, $C_{x,PAC}$, were conducted for individual and total PACs according to the following equation:

$$\log C_{lipid,PAC} = m \log C_{x,PAC}, \text{ total} + b \qquad (6.8)$$

Where *m* and b are slopes and intercepts from a linear regression, respectively. The results of these correlations are presented in Table 6.13 – 6.14 and Figure 6.4.

For PAHs, the worst log-log correlations were with C_{lipid} vs C_{soil} (r^2 from 0.67 to 0.83), and the best were with C_{lipid} vs C_{TOC} (r^2 from 0.67 to 0.92) and C_{lipid} vs C_{pw} (r^2 from 0.75 to 0.94, excluding results for the two ring PAHs which had r^2 from 0.37 to0.78). For oxy-PAHs and N-PACs, log-log correlations were poorer than with PAHs for C_{lipid} vs C_{soil} (from 0.43 to 0.82, excluding 0.06 for 1-indanone), C_{lipid} vs C_{TOC} (from 0.31 to 0.82, excluding 0.07 for 1-indanone) and C_{lipid} vs C_{pw} (from 0.39 to 0.79, excluding 0.12 for benz(a)anthracene-7,12-dione and 0.21 for naphthacene-5,12-dione). The less successful correlations of 2-ring PAHs and some oxy-PAHs and N-PACs is likely due to these compounds being more rapidly metabolized (thus causing disequilibrium between soils and lipids), as well as more volatile and soluble in the case of the 2-ring PAHs and oxy-PAHs (and thus more prone to kinetic and laboratory artefacts). For most compounds, however, the majority of log-log correlations being greater than 0.8 using C_{TOC} and C_{pw} are very encouraging, as they indicate both of these chemical measurements could give good estimations of C_{lipid}. For instance, for PAH-16, one could use the calibrated equations $\log C_{lipid,PAH\text{-}16} = 0.77\ C_{TOC,PAH\text{-}16} + 0.01$ ($r^2 = 0.92$) or $\log C_{lipid,PAH\text{-}16} = 0.72\ C_{POM,PAH\text{-}16} + 1.25$ ($r^2 = 0.94$). Similar equations (and statistics) can be found in Table 6.8 - Table 6.9.

Table 6.8 Slope and intercept values of various log-log correlation coefficients (r2) of selected lipid concentrations in Enchytraeus crypticus vs. various chemical measurements (where n > 9).

	Slope and intercepts log - log linear (y = mx + b) regressions, where y = log C_{lipid} in *Enchytraeus crypticus* and x =			
	log C_{POM}	**log $C_{POM\text{-}LIPID}$**	**log C_{soil}**	**log C_{TOC}**
Slope values (m):				
PAH-16	0.72	0.66	0.68	0.77
Oxy-PAH-11	0.29	0.29	0.49	0.54
N-PAC-4	0.31	0.31	0.52	0.55
All PAH	0.67	0.62	0.67	0.77
Intercept values (b):				
PAH-16	1.25	0.60	0.93	0.01
Oxy-PAH-11	0.96	0.87	0.44	-0.15
N-PAC-4	0.34	0.22	0.02	-0.51
All PAH	1.33	0.72	0.93	0.00

Table 6.9 log-log correlation coefficients (r^2) of lipid concentrations in Enchytraeus crypticus vs. various chemical measurements (where n > 9).

	r^2 correlations of log C_{lipid} in *Enchytraeus crypticus* vs.			
	log C_{POM} [a)]	log $C_{POM-LIPID}$ [a)]	log C_{soil}	log C_{TOC}
Naphthalene			0.78	0.67
Acenaphthylene	0.78	0.78	0.63	0.82
Acenaphthene	0.37	0.37	0.71	0.81
Fluorene	0.46	0.46	0.83	**0.89**
Phenanthrene	0.81	0.81	0.73	0.84
Anthracene	0.75	0.75	0.77	**0.89**
Fluoranthene	**0.93**	**0.93**	0.76	**0.90**
Pyrene	**0.92**	**0.92**	0.74	**0.89**
Benzo(a)anthracene	0.77	0.77	0.67	**0.86**
Chrysene	0.83	0.83	0.77	**0.92**
Benzo(b)fluoranthene	0.84	0.84	0.78	**0.92**
Benzo(k)fluoranthene	0.83	0.83	0.77	**0.91**
Benzo(a)pyrene	0.83	0.83	0.73	**0.89**
Indeno(cd)pyrene	**0.89**	**0.89**	0.74	**0.87**
Dibenz(a,h)anthracene	0.75	0.75	0.60	0.69
Benzo(g,h,i)perylene	**0.89**	**0.89**	0.77	**0.88**
1-Indanone	0.39	0.39	0.06	0.07
1-Acenaphthenone	0.79	0.79	0.73	0.76
9-Fluorenone	0.41	0.41	0.82	0.72
Anthracene-9,10-dione	0.52	0.52	0.82	0.82
Cyclopentaphenanthrenone	0.72	0.72	0.71	0.75
2-Methylanthracenedione	0.42	0.42	0.55	0.52
Benzo(a)fluorenone	0.52	0.52	0.54	0.61
7H-Benz(de)anthracen-7-one	0.73	0.73	0.53	0.73
Benz(a)anthracene-7,12-dione	0.12	0.12	0.46	0.55
Naphthacene-5,12-dione	0.21	0.21	0.40	0.42
6H-Benzo(cd)pyren-6-one	0.74	0.74	0.57	0.73
Quinoline			0.55	0.48
Benzo(h)quinoline			0.43	0.31
Acridine	0.66	0.66	0.60	0.70
Carbazole	0.54	0.54	0.70	0.71
PAH-16	**0.94**	**0.91**	0.77	**0.92**
Oxy-PAH-11	0.56	0.63	0.69	0.77
N-PAC-4	0.77	0.76	0.69	0.76
All PAH	**0.94**	**0.90**	0.76	**0.92**

a) see equation 6.9

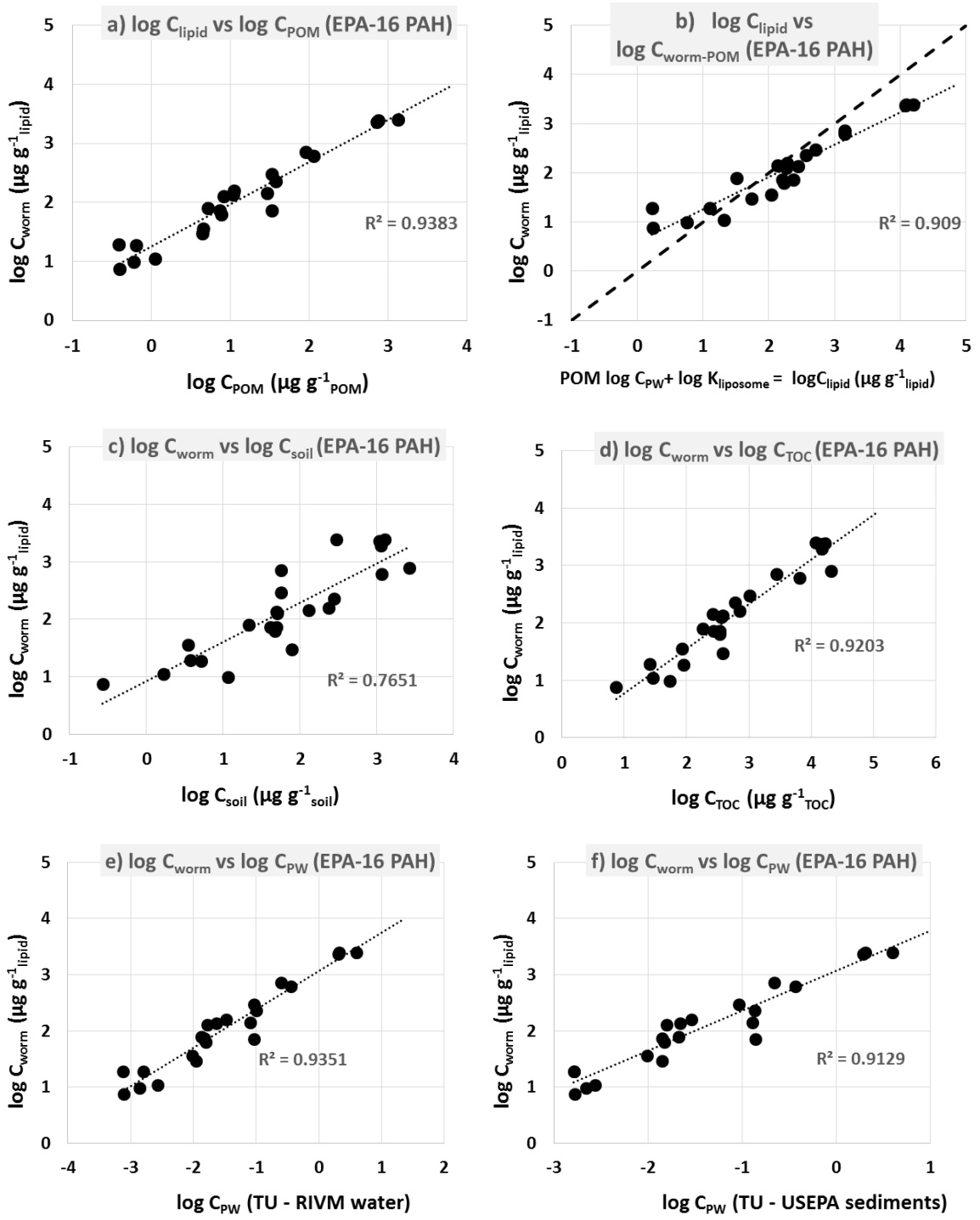


Figure 6.4 Correlations of log Cworm for EPA-16 with various chemical measurements, including a) log C_{POM}, b) estimated log C_{worm} based on C_{POM} * $K_{liposome}/K_{pom}$, c) log C_{soil}, d) log C_{TOC}, e) log C_{pw} (units TU based on SRC levels in (Verbruggen 2012), g) log C_{pw} (units TU based on FCV levels in USEPA, 2003).

As promising as these correlations are, they involve calibrations to the experimental data obtained here. A way of estimating C_{lipid} that does not involve such internal data-set calibrations is with established lipid normalized BCF values, BCF_{lipid} (eq 6.2). BCF_{lipid}, like K_{TOC}, is commonly assumed equivalent to K_{OW}. More recently, liposome-water partitioning coefficients, $K_{liposome}$, have been demonstrated as better predictors of BCF_{lipid} values than K_{OW} for a range of aquatic species (Jonker and van der Heijden 2007, van der Heijden and Jonker 2009, Endo et al. 2011a). For PAHs, $K_{liposome}$ are

generally larger than K_{OW} (Endo et al. 2011a). A comparison of measured BCF_{lipid} data for all compounds in this study with literature $K_{liposome}$ and K_{OW} values is presented in Figure 6.5 and Arp et al. (2014). Note that if experimental $K_{liposome}$ data was lacking, it was estimated using the equation log $K_{liposome}$ = 1.01 log K_{ow} + 0.12 (n = 156, r^2 = 0.948), from Endo et al.(2011a).

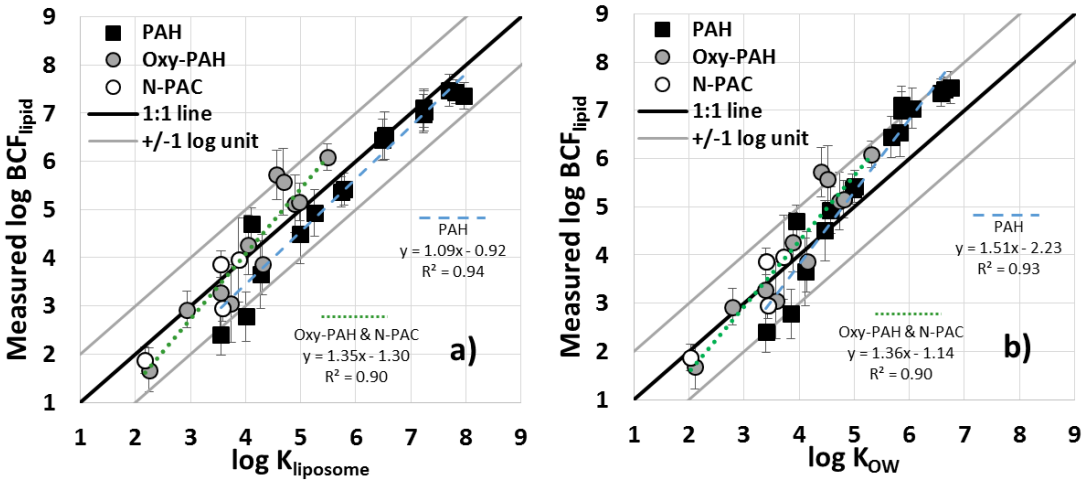


Figure 6.5 A comparison of average (± standard deviation) measured log BCFlipid values (Lpw/kglipid) for PAHs, oxy-PAHs and N-PACs for E. crypticus exposed to historically contaminated soils in this study vs. a) measured (or estimated if unavailable) log $K_{liposome}$ values and b) measured or estimated log K_{OW} values; log-log linear regression equations are presented in the figures.

As is evident by comparing Figure 6.5a and Figure 6.5b, the experimental BCF_{lipid} values in this study agree with average $K_{liposome}$ more so than K_{ow} values. A regression of log BCF_{lipid} vs log $K_{liposome}$ for PAHs is near parallel to the 1:1 line (slope = 1.09, r^2 = 0.94, Figure 6.5a), which is much better than the corresponding relationship with log K_{ow} (slope 1.51, r^2 = 0.93, Figure 6.5b). This implies that using K_{OW} as a proxy for BCF_{lipid} would underestimate C_{lipid} for larger PAHs, unlike $K_{liposome}$. Estimated log BCF_{lipid} for oxy-PAHs and N-PACs only differ from log $K_{liposome}$ by -0.05 log units on average, and from log K_{ow} by 0.21 units on average. However, there is near the same deviation from the 1:1 line for both log BCF_{lipid} vs log $K_{liposome}$ (slope = 1.35, r^2 = 0.90) and log BCF_{lipid} vs log K_{ow} (slope = 1.36, r^2 = 0.90). This is accountable to $K_{liposome}$ data for these compounds being estimated on the aforementioned log K_{ow} relationship by Endo et al. (2011a) We hypothesize that the relationship for oxy-PAHs and N-PACs in Figure 6.5a would be improved if directly measured log $K_{liposome}$ were available, which would be of interest to test in a follow up study.

No "hydrophobicity cutoff", i.e. a flattening of the log BCF_{lipid} – log K_{ow} relationship, was evident in Figure 6.5b up to a log K_{ow} of 7.5, largely because passive samplers were used and thus avoiding artefacts that can cause this cutoff (i.e. kinetic biases and measuring total porewater instead of freely-dissolved C_{pw}) (Jonker and van der Heijden 2007). Note this is does not imply that a hydrophobicity cutoff may be evident at larger K_{ow}.

Based on the good relationship between BCF_{lipid} and $K_{liposome}$ in Figure 6.5a, it was of interest to see how closely measured C_{lipid} values for individual PACs could be estimated from C_{POM}, using $K_{liposome}$ and K_{POM}:

$$C_{lipid\text{-}POM\_estimated} = C_{POM}\, K_{liposome} / K_{POM} \qquad (6.9)$$

A correlation between $C_{lipid\text{-}POM\_estimated}$ and C_{lipid} is presented in Figure 6.6 for PAHs. In general the two values agree within an order of magnitude for 85% of all PAC data, 89% of PAH data, 94% of 3-6 ring PAH data, and 78% of oxy-PAHs and N-PACs data (with Benz(a)anthracene-7,12-dione being the most deviating compound). The good agreement with 3-6 ring PAHs replicates two earlier studies using these compounds that compared measured C_{lipid} in worms with values estimated by C_{POM} or C_{pw} (Jonker et al. 2007, Gomez-Eyles et al. 2012), though these earlier studies used K_{OW} instead of $K_{liposome}$ as a BCF_{lipid} proxy (Jonker et al. 2007, Gomez-Eyles et al. 2012). The weaker results for the 2-ring PAHs, oxy-PAHs and N-PACs may be related to the aforementioned kinetic artefacts (e.g. worm metabolism, compound solubility in the case of the 2-ring PAHs.).

Sixty percent of the deviations greater than 10 between C_{lipid} and $C_{lipid\text{-}POM\_estimated}$ were for the two most clay-rich soils: the Belgian soil and a French soil (France-4). Consistently for these soils, C_{lipid} was less than estimated. This indicates that soil properties could play an influential role in bioaccumulation. Quantifying this is outside the scope of the present study, but would be of interest to pursue in a follow-up study.

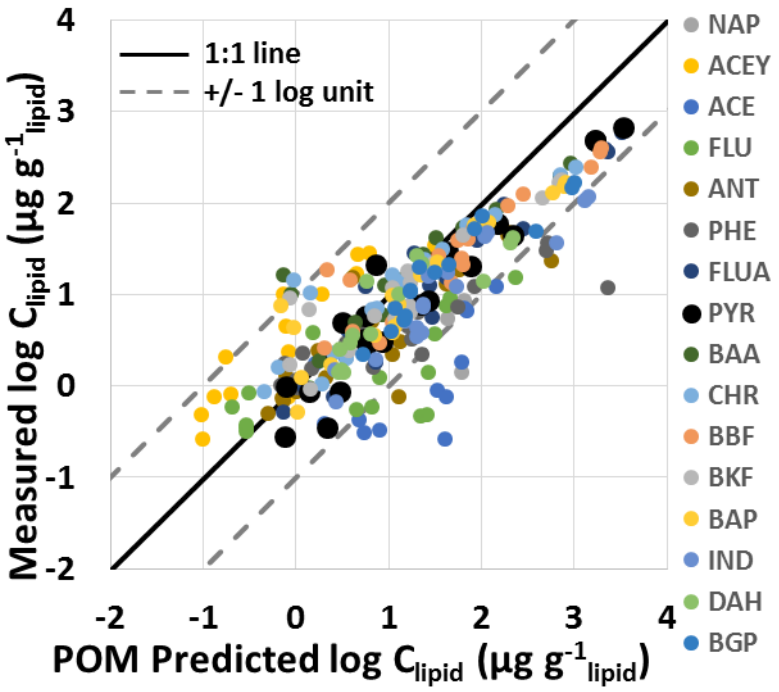


Figure 6.6 A comparison of measured Clipid values for individual PAHs with those derived from CPOM and Kliposome using Clipid = CPOM(Kliposome/KPOM).

6.3.5 Worm Toxicity

As mentioned in section 6.2.11., the predominant mechanism for PAH toxicity in earthworms is generally considered to be accumulation in cell lipids, in which all PAHs additively accumulate until narcosis occurs (USEPA 2003, Verbruggen 2012) This likely also applies to oxy-PAHs and N-PACs in many terrestrial organisms (Sverdrup et al. 2002a, Sverdrup et al. 2002b, Kobetičová et al. 2008). Though, non-additive effects have been observed for PAHs in some aquatic species (Billiard et al. 2008). Also enhanced toxicity compared to narcosis has been suggested for N-PACs like carbazole and acridine in earthworms (Sverdrup et al. 2001, Sverdrup et al. 2002b). Nevertheless, it is expected that the greater the C_{pw} and C_{lipid}, the more toxic effects would be observed. The present experimental set-up, however, did not verify if PACs were the dominant toxic agents towards mortality or reproduction in *E. crypticus* (e.g. by spiking with additional PACs to see if effect increased with dose). In addition to PACs, other factors that could be contributing to toxicity in these soils including metal contaminants (as mentioned in an earlier chapter), poor soil texture, and the presence of other organic contaminants (including other PACs not considered here, like alkyl PAHs (Hawthorne et al. 2006, Arp, et al. 2011a)). Thus, the following presentation of toxicological data is more observational than mechanistic, but is considered of interest, as it is to date the largest toxicological data set for *E. crypticus* mortality and reproducibility in diverse, historically PAC-contaminated soils.

No increased mortality was observed at the end of the bioaccumulation assay (Table A3.6 in Appendix 3). The control soil had an average survival rate of 94 ± 9 % (n=5), which was not significantly different from the range of survival observed (the most toxic sample had a survival rate of 78 ± 8%). 80% of samples exhibited > 90% survival on average. In the literature, increased mortality of earthworms is only very rarely observed in historically PAH contaminated soils, with no increased mortality reported in a creosote-contaminated soil (Allard et al. 2005), a cookery plant soil (Eom et al. 2007), and in only three out of 15 manufactured gas plant soils (Jonker et al. 2007).

Reported lethal concentrations for half the population (LC50 values) for *E. crypticus* in PAH spiked soil range from 520 to >58820 µg g^{-1}_{TOC} for individual PAHs (Sverdrup, et al. 2002a, Droge et al. 2006, Verbruggen 2012) (as complied in Table A3.7 in Appendix 3). To compare the multiple PAHs in this study to these single PAH toxicity studies, it is best to normalize all PAH concentrations by a toxic benchmark concentration to derive toxicity units (TU). For this purpose we chose the RIVM Maximum Permissible Concentration benchmarks for TOC, MPC_{TOC}, presented in section 6.2.11 from Verbruggen (2012) as the basis of normalization (i.e. TU = C_{TOC}/MPC_{TOC}, see eq 6.7). This leads to literature PAH LC50 of *E. crypticus* from 75 to 3767 TU_{TOC} and C_{TOC} in our study from 1 to 4264 TU_{TOC} (Table A3.7 in Appendix 3), Thus increased mortality could have been expected using this benchmark. The reason it is not observed supports the position that traditional methods to derive benchmarks like MPC_{TOC} are too conservative to account for risk in historically contaminated soils, as they do not account for bioavailability.

Unlike mortality, there were noticeable differences in *E. crypticus* reproducibility across the soils. The control soil exhibited on average 572 ± 133 juveniles, whereas the contaminated soils exhibited from 33 ± 10 up to 681 ± 147 juveniles (median 229; IQR 150-430). The chemical measures can at best offer weak correlations. For instance, plotting % reproducibility vs log C_{lipid}(∑PAC) $C_{lipid\text{-}POM\_estimated}$(∑PAC), C_{TOC}(∑PAC) and C_{pw}(∑PAC), will give r^2 values of 0.20, 0.2, 0.17 and 0.21, respectively (Table 6.10).

6.3.6 "Dose"-Response Curves

As stated above, in the experimental set-up we did not verify if PACs were the dominant toxic agents towards mortality or reproduction to *E. crypticus* in the obtained historically contaminated soils (e.g. by spiking with additional PACs to see if toxicity to *E.crypticus* increased with dose). Other toxic agents in these soils include metals,, poor soil texture and PACs not included in this soil. However, if there was a clear correlation between some measure of PAC contamination (say $C_{lipid,total\text{-}PAC}$) then perhaps an argument could be made that PAC pollution was the toxic mechanism. Here it was explored if a selection of PAC measurements could account for any of the observed responses, with the hypothesis that PACs were the dominant mechanism. This is therefore referred to a "dose"-response curve (with dose in quotations), as it is unclear if the PAC is the actual dose leading to effects in all cases.

As presented in Table A3.6 in Appendix 3 and above, the only observable toxic effect in the worm bioassays was reduced reproducibility in some of the test soils. Thus, here we correlated the relative change in reproducibility (relative to the reference OECD soil) with different measures of PAC contamination, using a simple 3-point logistic regression curve that ranges from 100% reproducibility (that of the OECD soil) to the minimum reproducibility observed in the bioassay:

$$\text{Effect (\%)} = 100\ \% / (1 + IP\ e^{-A \log C}) \qquad (6.10)$$

Where *IP* is the inflection point (i.e. the part of the logistic dose response curve where the concavity changes), *A* regulates the curvature of dose response curve, and log *C* refers to the logarithm of a concentration measurement. Regression and 95% confidence intervals were derived using the freeware PAST version 2.08b (http://folk.uio.no/ohammer/past/)(Hammer et al. 2001).

Results of the logistic regression are presented in Table 6.10, and plotted for selected measures of PAC concentration (measured C_{lipid}, estimated C_{lipid} based on POM using equation 6.9, C_{pw} normalized to the RIVM freshwater benchmark in Table S1.2 and C_{soil} normalized to f_{TOC} and the RIVM benchmark for standard soil in Table 6.4) in Figure 6.7.

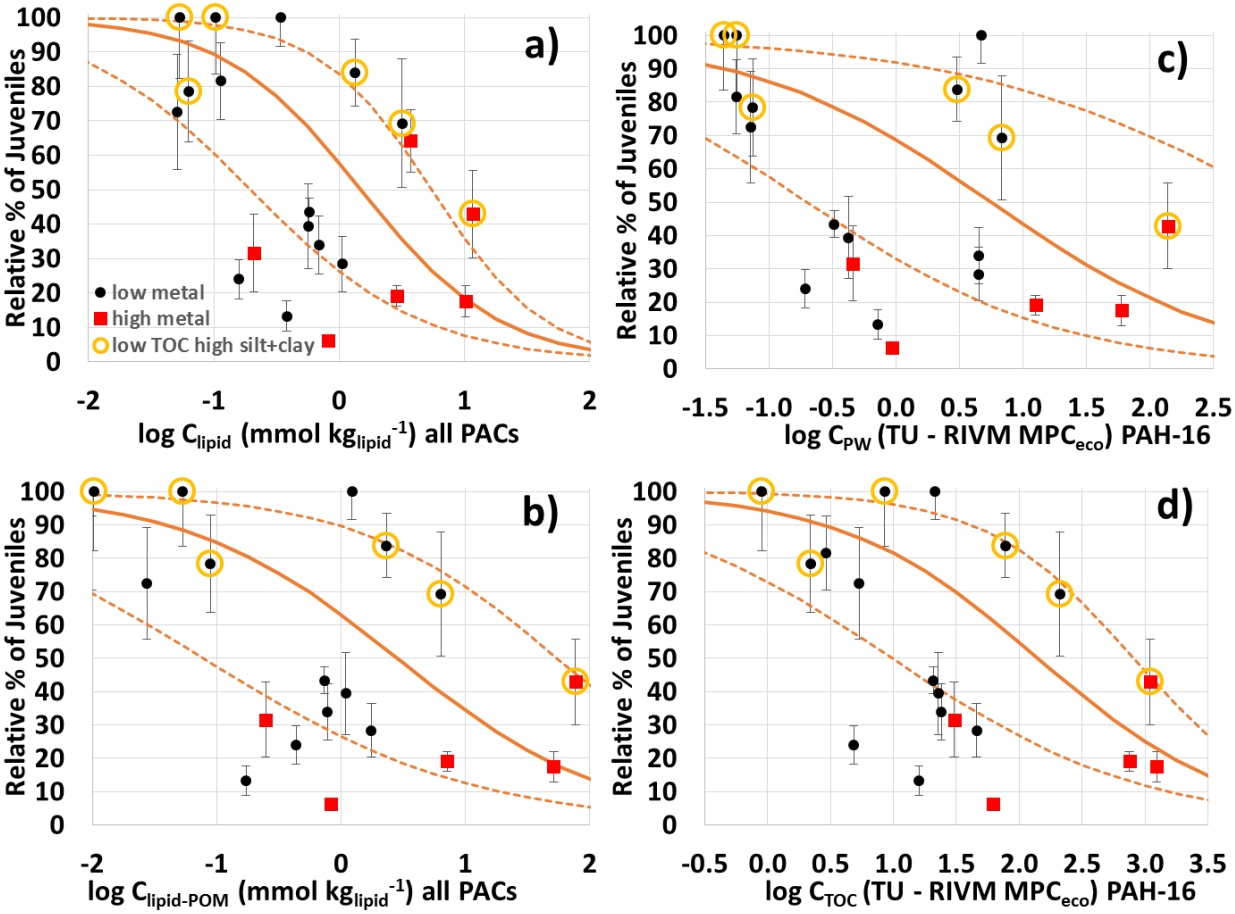


Figure 6.7 Relative % of juveniles of E crypticus in test soils vs a) measured total lipid concentrations, C_{lipid}, of all PACs analysed in this study, b) total POM estimated lipid concentrations, $C_{lipid\text{-}POM}$, of all PACs (see eq 6.9), c) C_{PW} normalized to RIVM MPC_{eco} benchmark values for PAH-16 (no benchmark values exist for N-PACs and oxy-PAHs, see Table 6.4), (Verbruggen 2012) to generate total Toxicity Units (TU), d) C_{soil} normalized to f_{TOC} and then normalized standard soil RIVM MPC_{eco} benchmarks for PAH-16 (see Table 6.4), (Verbruggen 2012) to generate total TU. Soils are differentiated by having low and high metal content (where high metal content samples are the samples from Belgium and France, indicated in red), as well as samples that contain low TOC (<6%) and high clay+silt (>24%), which are indicative of good soil texture for worms. The solid orange line is the fit from a logistic regression, and dotted lines represent the 95% confidence intervals.

Table 6.10 Derived coefficients (inflection point and slope) of a logistic regression between relative % of juveniles of Enchytraeus crypticus vs. various chemical measurements. Also presented are the range of different concentrations measured and those of literature effect concentrations (see Arp et al., 2014), and correlation coffecients for relative % juveniles vs log (Concentration).

	PAC	Units	Range	max	IP	slope	AIC	r^2	Literature[a)]			
				logistic Model: % effect = max/(1+IP*$e^{-slope*log(C)}$)				% effect vs log (C)	LC50	EC50	EC10	NOEC
C_{POM}	PAH-16	(µg g^{-1}_{POM})	0.4 - 1,336	100	0.132	-1.18	21175	0.21				
C_{POM}	All PAC	(µg g^{-1}_{POM})	0.4 - 1,478	100	0.131	-1.16	21205	0.21				
C_{PW}	PAH-16	(USEPA TU)	0.0016 - 4.00	100	2.41	-1.13	21362	0.19				
C_{PW}	PAH-16	(RIVM SRC TU)	0.0008 - 3.97	100	2.62	-1.12	21160	0.21	4 - 72	1 - 33	0.2 - 1.9	0.5 - 8.6
C_{PW}	PAH-16	(RIVC MPC TU)	0.03 - 407	100	0.321	-1.08	21197	0.21	75 - 3,676	36 - 966	11 - 58	25 - 254
C_{soil}	PAH-16	(µg g^{-1}_{dw})	0.27 - 2,651	100	0.0356	-1.55	17396	0.30				
C_{soil}	All PAC	(µg g^{-1}_{dw})	0.4 - 3,043	100	0.0306	-1.55	17514	0.29				
C_{TOC}	PAH-16	(µg g^{-1}_{TOC})	7 - 20,917	100	0.0133	-1.35	22644	0.18	520 - 58,820	520 - 17,380	400 - 1,690	170 - 4,580
C_{TOC}	All PAC	(µg g^{-1}_{TOC})	11 - 24,010	100	0.0118	-1.34	22742	0.17				
C_{TOC}	PAH-16	(USEPA TU)	0.01 - 26	100	0.670	-1.32	22651	0.18				
C_{TOC}	PAH-16	(RIVM SRC TU)	0.02 - 45	100	0.447	-1.38	22363	0.19	4 - 72	1 - 33	0.2 - 1.9	0.5 - 8.6
C_{TOC}	PAH-16	(RIVM MPC TU)	1 – 4,264	100	0.0349	-1.36	22173	0.20	75 - 3,676	36 - 966	11 - 58	25 - 254
C_{worm}	PAH-16	(ng g^{-1}_{ww})	289 - 95,697	100	0.000806	-1.75	21883	0.20				
C_{worm}	All PAC	(ng g^{-1}_{ww})	420 - 96,731	100	0.000648	-1.78	22061	0.19				
C_{lipid}	PAH-16	(µg g^{-1}_{lipid})	8 - 2,490	100	0.0130	-1.74	21881	0.20				
C_{lipid}	All PAC	(µg g^{-1}_{lipid})	11 - 2,516	100	0.0110	-1.78	22049	0.19				
POM C_{lipid}	PAH-16	(µg g^{-1}_{lipid})	2 - 15,727	100	0.0376	-1.18	20514	0.23				
POM C_{lipid}	All PAC	(µg g^{-1}_{lipid})	2 - 16,018	100	0.0376	-1.18	20504	0.23				
C_{lipid}	PAH-16	(mmol kg^{-1}_{lipid})	0.04 - 12	100	0.797	-1.77	21821	0.21				3.2 - 29
C_{lipid}	All PAC	(mmol kg^{-1}_{lipid})	0.05 - 12	100	0.729	-1.81	21974	0.20				3.2 - 29
POM C_{lipid}	PAH-16	(mmol kg^{-1}_{lipid})	0.01 - 75	100	0.590	-1.19	20723	0.22				3.2 - 29
POM C_{lipid}	All PAC	(mmol kg^{-1}_{lipid})	0.01 - 77	100	0.585	-1.18	20712	0.22				3.2 - 29

AIC = Akaike information criterion (the lower number the better the quality of the regression); a) Values are from the literature, shown in Arp et al, 2014, where EC refers to the effect of individual spiked PAHs on the number of juveniles.

As is evident from Table 6.10 and Figure 6.7, none of the PAC measurements and estimations tested described trends with a high degree of accuracy, and at best only week correlations are evident. Thus, from this data it can be concluded that PAC concentrations is at best a co-variable in PAC toxicity to *E.crypticus* reproducibility, but alongside other stresses, like metal content and soil texture. Figure 6.7 also shows that soils with high metal content (i.e. the French and Belgian soils) tended to be ones where lower reproducibility was observed, and samples with better soil texture tended to be ones where higher reproducibility was observed. It is tempting to explain these curves further, such as by considering the bioavailability of the (many) metals present, or by conducting a principle component anlaysis, but that is outside the goals of the present study.

6.4 Conclusions

Assessments based on bioavailabilty, as quantified by the freely-available pore water concentration, C_{pw}, can be made in two ways: direct measurements by passive samplers, such as POM, or through the measurements of C_{soil} combined with the coal-tar predictive model (eq 6.5) and the f_{TOC} value for the soil. For pyrogenically impacted soils, such as the one considered here, the traditional octanol based model, or partitioning K_{TOC} values based on spiked reference soils, will lead to overestimations of C_{pw}, and therefore bioavailability.

POM measured C_{pw} were found to correlate very well with lipid worm concentrations, and therefore to be good estimators of bioaccumulation. Similarly, use of the C_{soil} and predictions of C_{pw} would be expected to give similar, though not as good, predicitons of bioaccummulaiton in worms (though indeed much better than estimations based on the traditional octanol model). This was not presented explicitely in this study, but can be inferred from the overall correlations. Directly measured or estimated C_{pw} could not however, give good predictions of worm toxicity, and at best weak correlations. However, this does not diminish the utility of the technique, but merely an indication that in real world soils, organisms like invertibrates are affected by multiple stresses.

Therefore, based on this study, and from the associated review of partitioning behaviour in the literature, presented in Figure 6.2 – Figure 6.3, we strongly recommend direct measurements of C_{pw} when assessing soils contaminated by pyrogenic residues, firstly to confirm the partitioning regime is following the coal-tar paradigm, and to make more accurate assessments of bioavailability and bioaccumulation.

Recommendations of using direct measurements or estimations of C_{pw} to ensure better risk assessments is presented in section 9.3, with the cost-benefit advantage presented in section 12.

7. Evaluating soil extraction and passive sampler methods to assess plant uptake of PAHs

7.1 Background

Plant uptake of PAH from contaminated sites can follow different pathways (Figure 7.1): root uptake via pore water (1a, 1b), foliar uptake via air (2a, 2b), uptake via rain splash (3a, 3b), direct uptake (4) and atmospheric deposition from other sources (5). Uptake through root via pore water is the dominant pathway. The others are often neglected or are not directly related to soil contamination (e.g. Naturvårdsverket, 2009). Therefore plant uptake might be simplified by modelling *i)* pore water concentration as a function of soil properties (1a) and *ii)* root uptake of dissolved contaminant and further translocation to the aerial parts of the plant (1b).

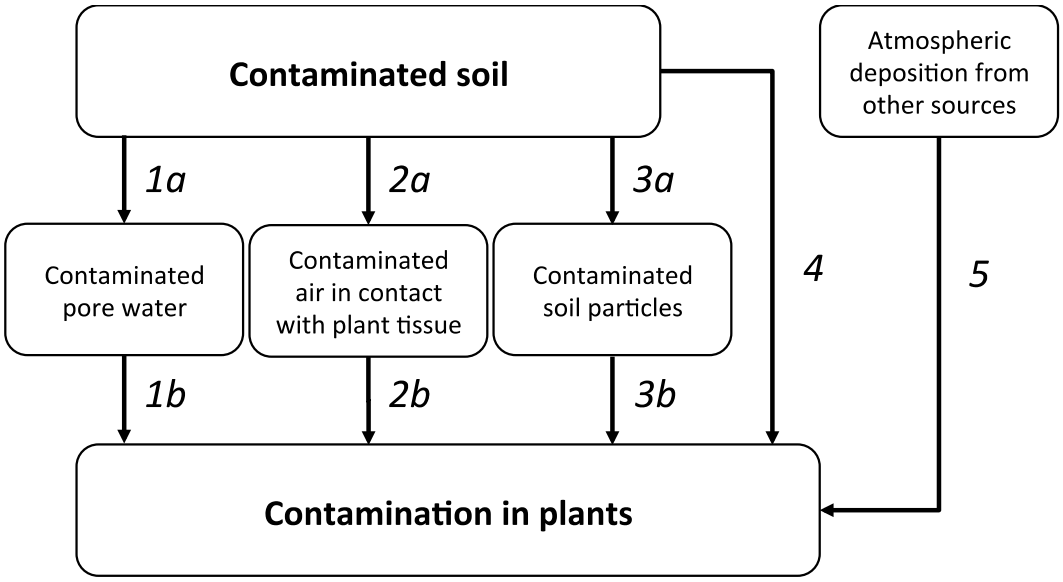


Figure 7.1 Pathways for contaminant uptake in vegetables (from Swartjes et al. 2007).

The uptake of chemicals into plants is generally described using bioconcentration factors (BCF) defined for root as the root concentration factor (RCF) or for shoots, the shoot concentration factor (SCF) (Shone and Wood 1974):

$$RCF = \frac{concentration\ in\ roots\ (mg\ g\ fresh\ weight^{-1})}{concentration\ in\ soil\ solution\ (mg\ mL^{-1})} \quad \textbf{Eq. 1}$$

$$SCF = \frac{concentration\ in\ shoots\ (mg\ g\ fresh\ weight^{-1})}{concentration\ in\ soil\ solution\ (mg\ mL^{-1})} \quad \textbf{Eq. 2}$$

The efficiency of translocation from roots to shoots is described by the transpiration stream concentration factor (TSCF)

$$TSCF = \frac{concentration\ in\ transpiration\ stream\ (mg\ mL^{-1})}{concentration\ in\ soil\ solution\ (mg\ mL^{-1})} \quad \textbf{Eq. 3}$$

Additionally, the transfer factor (TF) explains the relationship between root and shoot concentrations:

$$TF = \frac{concentration\ in\ shoots\ (mg\ g\ fresh\ weight^{-1})}{concentration\ in\ roots\ (mg\ g\ fresh\ weight^{-1})} \qquad \textbf{Eq. 4}$$

Different models have been proposed to estimate these parameters. Briggs et al. (1982) explain root uptake of organics by two processes 1) a partitioning to lipophilic root solids correlated to Log K_{ow} of the molecule and 2) a small constant uptake similar for all compounds:

$$Log(RCF - 0.82) = 0.77\ Log\ K_{ow} - 1.52 \qquad \textbf{Eq. 5}$$

For shoot the empirical relationship proposed Briggs et al. (1983) is:

$$SCF = \left[10^{(0.95\ Log\ K_{ow} - 2.05)} + 0.82\right] 0.784\ e^{-[(Log\ K_{ow} - 1.78)^2 / 2.44]} \qquad \textbf{Eq. 6}$$

These two equations have also been reported by Ryan et al. (1988) though in a slightly different form for the second.

Several modelling tools have been developed for human exposure risk assessment to contaminated soils: CSOIL2000 in The Netherlands (Brand et al. 2007), the European Union System for the Evaluation of Substances (EUSES) (Vermeire et al. 1997), the 'Umweltmedizinische Beurteilung des Exposition des Menschen durch altlastbedingte Schadstoffe' (UMS, Germany), and the Contaminated Land Exposure Assessment (CLEA) tool in the UK or CalTOX from the California Environmental Protection Agency. Most of these tools use the above-described equations for calculations of organic contaminants uptake by crops (Table 7.1).

More recently some publications have proposed alternative formulations of the initial equations proposed by Briggs and co-workers. Zhang and Zhu (2009) have shown that sorption of PAHs on roots is regulated by both lipids and carbohydrates rather than lipids alone and they propose the following equations:

$$Log\ K_{lip} = 1.23\ Log\ K_{ow} - 0.78 \qquad \textbf{Eq. 7}$$

$$Log\ K_{ch} = 1.23\ Log\ K_{ow} - 2.42 \qquad \textbf{Eq. 8}$$

$$Q = C\left(f_{lip} K_{lip} + f_{ch} K_{ch}\right) \qquad \textbf{Eq. 9}$$

where K_{lip} is the lipids-water partition coefficient, K_{ch} is the carbohydrate-water partition coefficients, Q (mg kg $^{-1}$) is the sorption amount of PAHs on root, C the PAH concentration in water at equilibrium, f_{lip} and f_{ch} are the weight fraction of lipids and carbohydrates on a fresh weight basis. Lipid and carbohydrate fractions were not determined in our experiment so lipid and carbohydrate fractions of wheat (Li et al. 2005) were used for the prediction proposed (Brennan et al. 2014).

Table 7.1: Approaches for calculating the concentration in crops used by the different models (from Rikken et al. 2001).

Model	All substances	Organic substances	Accounts for soil attached	Correction food preparation
CLEA	Paterson et al. (1994)	RCF: Briggs et al. (1982) SCF: Briggs et al. (1983) and Ryan et al. (1988)	yes	yes
CalTOX	Paterson et al. (1994)	-	yes	no
CSOIL	-	RCF: Briggs et al. (1982) SCF: Briggs et al. (1983)	yes	no
UMS	-	RCF: Briggs et al. (1982) SCF: Briggs et al. (1983) and Trapp and Matthies (1995)	no	yes, 0.5

In this modelling approach, the pore water concentration is required. At this point three options may be followed (Gomez-Eyles et al. 2012). The first is to estimate the pore water concentration (C_{pw}) from total soil concentration using a classical partitioning model. The organic carbon normalized partitioning coefficient, K_{OC}, is calculated by using a polyparameter linear free energy relationships. The relationship implemented in the CSOIL model is the one proposed by Karickhoff (1981):

$$\boldsymbol{Log\ K_{OC} = 0.989\ Log\ K_{ow} - 0.346} \qquad \textbf{Eq. 10}$$

However, more recent studies have proposed alternative formulations for PAHs like Nguyen et al. (2005):

$$\boldsymbol{Log\ K_{OC} = 1.14\ Log\ K_{ow} - 1.02} \qquad \textbf{Eq. 11}$$

The second possibility is to estimate the equilibrium pore-water concentration using direct measurement of soil solution with a solid-phase equilibrium partitioning using POM strips (Hawthorne et al. 2011b), which as described in chapter 6 is related to *bioavailability*. The third is similar to the first but supposes that only a fraction, defined as available or rapidly desorbed, governs PAH concentrations in the soil pore water. This fraction might be measured with a solid-phase extraction using Tenax® (Barnier et al. 2014), which as explained in section 6 is a measure of a type of *bioaccessiblity*. The partitioning equation is applied considering this amount of available PAH and the K_{OC} value previously given by equations 10 or 11 (Gomez-Eyles et al. 2012). The coal tar model introduced in Chapter 6 is an alternative model, but has never been investigated in the context of plant uptake.

In this study, the results given by the different approaches will be compared. Their respective benefit for PAH bioaccumulation in plant roots and shoots will be discussed.

7.2 Description of experimental work

7.2.1 Contaminated soils: origin and main properties

Fourteen soils sampled in former industrial sites were used in this study: five from Sweden (SW01-05), seven from France (FR01-07) and two from Belgium (BE01-02). Details on their main agronomic

properties are presented in the Table 7.2, which were measured by Université de Lorraine (and were quite similar to properties measured by SGI, NGI and Umeå University in Chapter 6, Tables 6.1 and 6.2).

FR01-03, FR05 and FR06 were sampled on former coking plant sites and FR06 was additionally treated in a biopile. FR04 comes from a former mixed industry site: coking and metallurgical activities. FR05, FR07, BE01 and SW01-05 come from former manufactured gas plant sites (Wermlandskajen, Karlstad, Sweden). The exact origin of the BE02 soil remains unknown but it did not appear significantly contaminated.

Table 7.2: Main properties of the industrial soils.

		FR01	FR02	FR03	FR04	FR05	FR06	FR07	BE01	BE02	SW01	SW02	SW03	SW04	SW05
Clay (<2 µm)	***g kg^{-1}***	98	86	119	155	62	96	121	149	138	61	51	36	56	84
Fine silt (2-20 µm)	***g kg^{-1}***	126	99	144	129	106	147	183	87	191	113	81	67	100	151
Coarse silt (20-50 µm)	***g kg^{-1}***	84	85	122	71	109	108	101	109	107	126	109	92	121	141
Fine sand (50-200 µm)	***g kg^{-1}***	151	153	183	87	191	201	107	387	108	238	272	141	290	161
Coarse sand (200-200 µm)	***g kg^{-1}***	541	577	432	558	532	448	488	268	456	462	487	664	433	463
pH	-	8.5	8.2	8.4	7.5	8.1	8.3	8.5	7.6	8.2	7.1	8.6	7.8	6.0	7.7
C_{org}	***g kg^{-1}***	113	177	212	91.1	335	146	28.0	48.9	18.0	65.4	22.7	75.9	103	600
N_{tot}	***g kg^{-1}***	1.9	3.2	3.3	2.5	4.2	2.3	0.7	1.4	0.64	0.8	0.3	1.0	1.3	8.9
$CaCO_3$	***g kg^{-1}***	357	175	217	20.5	71.8	210	34.9	13.3	28.0	<1	8.1	<1	<1	<1
P Olsen	***g P_2O_5 kg^{-1}***	0.06	0.03	0.04	0.06	0.08	0.09	0.04	0.05	0.04	0.01	0.02	0.01	0.01	0.01
CEC	***cmol kg^{-1}***	8.6	11.0	14.9	9.7	7.3	11.0	7.7	10.0	7.6	2.0	2.2	2.4	1.8	3.6
Cr	***mg kg^{-1}***	63.1	172	57.8	824	76.9	87.7	65.5	62.5	67.8	23.8	36.3	24.5	18.8	19.7
Cu	***mg kg^{-1}***	44.3	55.0	99.5	139	78.8	41.0	32.4	134	62.7	38.0	61.4	51.1	48.1	31.8
Ni	***mg kg^{-1}***	27.3	31.3	32.1	103	38.6	44.2	34.1	34.9	35.4	16.7	15.7	14.1	14.5	16.9
Zn	***mg kg^{-1}***	395	346	581	2680	266	765	184	328	228	171	216	250	85	38
Pb	***mg kg^{-1}***	144	329	210	711	130	197	352	652	365	85	56	68	85	77
Cd	***mg kg^{-1}***	7.3	1.2	0.7	2.5	1.3	2.9	0.4	4.4	0.4	0.8	0.3	0.4	0.2	0.1
EOM	***mg kg^{-1}***	24.7	22.2	1.4	7.1	3.4	17.5	13.8	7.2	1.9	2.3	1.8	0.8	0.7	4.6
16 PAH	***mg kg^{-1}***	1902	1230	63	973	220	641	93	145	0.2	126	54	16	8.4	162

7.2.2 Soil extraction methods

For total PAH in soil solvent extractions were performed with an automated solvent extractor (ASE 350, Dionex). Activated copper powder (1 g) and Na_2SO_4 (1 g) were added to 1 g of material in the extraction cells before the extractions in order to remove respectively the molecular sulfur and the residual water. Extractions were performed twice at 100 °C and 100 bars with HPLC grade dichloromethane (DCM) using a static time of 5 min. Organic extracts were diluted with DCM to reach 20 mL and stored in a glass vial at 4 °C before GC-MS analysis. Note that the measurements presented here were performed independently of similar measurements by the Swedish members of IBRACs in chapter 6.

The bioavailable fraction was estimated by Tenax® solid phase extraction using the protocol proposed by Barnier et al. (2014). Two grams of air-dried soil were agitated with 2 g of Tenax and 300 mL of a 0.01 M $CaCl_2$ and 200 mg L^{-1} NaN_3 solution for 30 h. The Tenax grains were removed, cleaned with water and air-dried. PAH recovery was then performed by sonication with a 1:1 mix of acetone:hexane repeated twice. The extracted PAH were quantified by GC-MS.

7.2.3 Passive sampler method

The freely dissolved concentrations of PAH at equilibrium were estimated using the POM method described by Hawthorne et al. (2011b). Five grams of soil were shaken with 100 mg of a polyoxymethylene membrane (POM) in a 30 mL solution (0.01 $CaCl_2$, 150 mg L^{-1} NaN_3) for 28 days at 20 °C. The membrane was then recovered, rinsed with deionized water and the PAH were extracted with 80:20 heptane:acetone mix. The extracted PAH were quantified by GC-MS and the PAH concentration on the POM strip (C_{POM}) was calculated. The PAH pore water concentration (C_{pw}) was further calculated using pre-determined and published K_{POM} values (Appendix 3) and the following equation: $C_{POM} = K_{POM}\ C_{pw}$. Note that the measurements presented here were performed independently of similar measurements by the Swedish members of IBRACs in chapter 6.

7.2.4 Culture experiment

Plant growth assays were conducted in four replicates for five weeks under controlled conditions in growth chamber with 16 h photoperiod, 23 °C diurnal temperature and 18 °C night temperature, 70% air humidity. Systems were filled with 1.3 kg equivalent dry weight of soil and three maize seeds were put per pot. After two days the two smallest plants were removed. Pots were watered three times a week in order to insure constant moisture content at 80% of water holding capacity. Nutrient solution of Ruakura type (Smith et al. 1983) was used once a week instead of deionized water to avoid any nutrition deficiency.

7.2.5 Plant extraction

PAH analysis in plants followed the protocol proposed by Gao and Collins (2009). Roughly 0.5 g of dry and ground biomass (either root or shoot) were sonicated for 2 h with 10 mL dichloromethane (DCM). The DCM was recovered and the extraction was repeated twice. All DCM extracts were pooled together.

For root biomass, DCM was evaporated under gentle nitrogen flux and volume adjusted to 5 mL. PAH were determined by GC-MS.

For shoot biomass, DCM was fully evaporated under gentle nitrogen flux and replaced by 5 mL acetonitrile. PAH were determined by HPLC coupled to fluorescence detection.

7.2.6 PAHs analysis

a) GC-MS analysis

The 16 US EPA PAHs were quantified using a gas chromatography-mass spectrometer (GC-MS) (GC-2010 plus, Shimadzu) equipped with a DB 5-MS column (30*0.25 mm). The GC oven temperature was programmed from 70 °C (held 2 min) to 130 °C at 15 °C min^{-1}, then from 130 °C to 315 °C (held 25 min) at 4 °C min^{-1}. An internal PAH standard mix (naphthalene-d8, acenaphtene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12) was added to the extract.

b) HPLC analysis

For shoot extracts, PAH quantification was performed using an HPLC line (9012 Solvent Delivery System and ProStar 410 Autosampler, Varian), equipped with a Pursuit 3 PAH (Varian) column and a fluorescence detector (ProStar 363 Fluorescence Detector, Varian). A water/acetonitrile mix was used for solvent at 1.3 mL min^{-1} with the following gradient conditions: initial ratio 40/60 held for 3 min, then 10/90 at 15 min held for 10 min. The detection wavelength conditions were those established by Sanz-Landaluze et al. (2006).

7.3 Results

Measured values for total PAH, Tenax extracted PAH and POM-measured PAH pore water concentrations are reported in tables in Appendix 4.

7.3.1 Pore-water concentration predictions

Individual PAH pore water concentrations calculated using Tenax and total soil concentrations with the model proposed by Nguyen and the coal-tar model are compared to POM measurements, considered here as the reference method (Figure 7.2). Values obtained with the Tenax extraction method are quite similar to those of the POM method, though higher by a factor 7 on average. Data appear quite dispersed but without any clear effect of either soil or molecule. Predictions using total soil concentration and the Nguyen relationship are dispersed as well and overestimate by almost two orders of magnitude the POM measured values (a factor 86). This is similar to the overestimation using the RIVM and USEPA methods in Figure 6.1. When using the the coal-tar model on total soil concentrations (Section 6.2.10), estimated Cpw are quite well estimated with an overestimation by a factor 13 in average. From the independent POM and soil PAH measurements in Chapter 6 by the Swedish members of IBRACs, they had an overestimation of C_{pw} for all PACs by a factor 3. The discrepancy here can be due to independent laboratory biases. Nevertheless, the stated accuracy of the coal tar TOC model is within a factor 30, and thus this model did give the best correlation with measured results. This coal tar model

approach gives similar results to the Tenax method, though does not necessitate any additional measurement than total soil concentrations.

The difference between the polyparameter linear free energy relationships of Nguyen and Karickhoff is not much significant. For the highest C_{pw} values the difference is very small. It is more important for the smallest C_{pw} values. In that case, the Nguyen relationship gives smaller values but they appear to correlate better to the experimental values measured with the POM method (Figure 7.3).

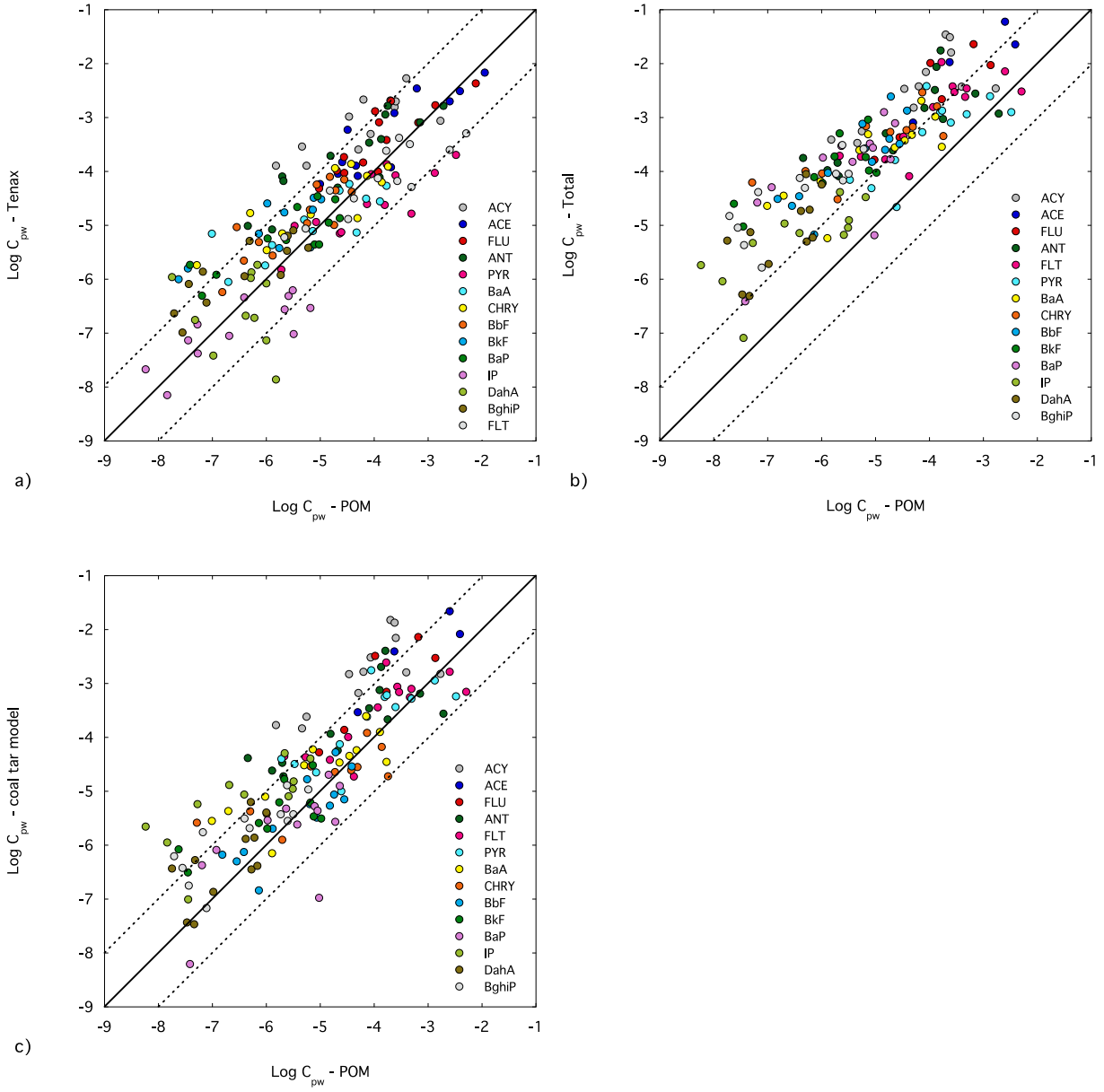


Figure 7.2 Comparison between POM measured and a) Tenax or b) total soil concentration predicted individual PAH pore water concentrations in mg/L, using the Nguyen equation and c) using the coal-tar model.

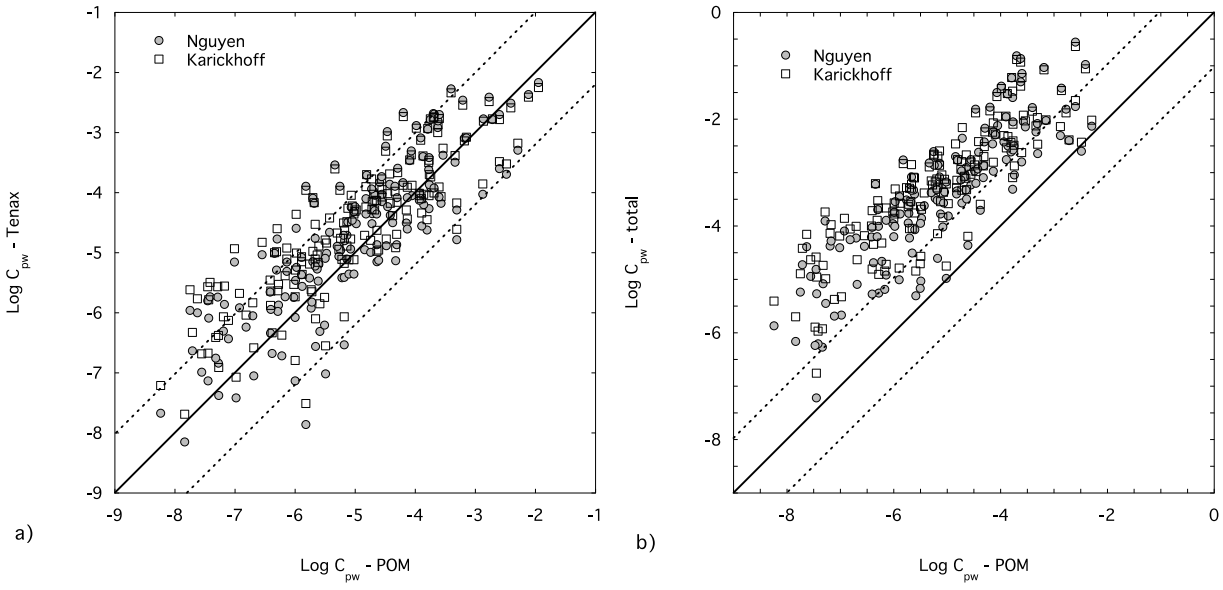


Figure 7.3 Comparison of Nguyen and Karickhoff models for pore water concentration estimates from a) Tenax extracted and b) total soil concentrations.

7.3.2 Shoot and root concentrations

Total PAH concentrations in roots and shoots are presented in the Figure 7.4. A wide variation between soils is observed. For roots, French soils 01 and 04 lead to the highest concentrations, whereas plants cultivated on the Swedish soils display much smaller root uptakes. This is partly explained by total concentration in the soils (Figure 7.5), with an R^2 value between log concentrations in roots and soils of 0.46 ($p = 0.011$). For shoots, the highest concentrations are measured on the French soils 02 and 05. Plant concentrations on the other soils are much smaller and all within the same range. In this case, shoot concentration is not correlated to total soil concentration (Figure 7.5), with a R^2 value between log concentrations in shoots and soil of 0.27 ($p = 0.081$). Tenax extractable PAH does explain root uptake better ($p = 0.00758$), but not shoot uptake ($p = 0.411$).

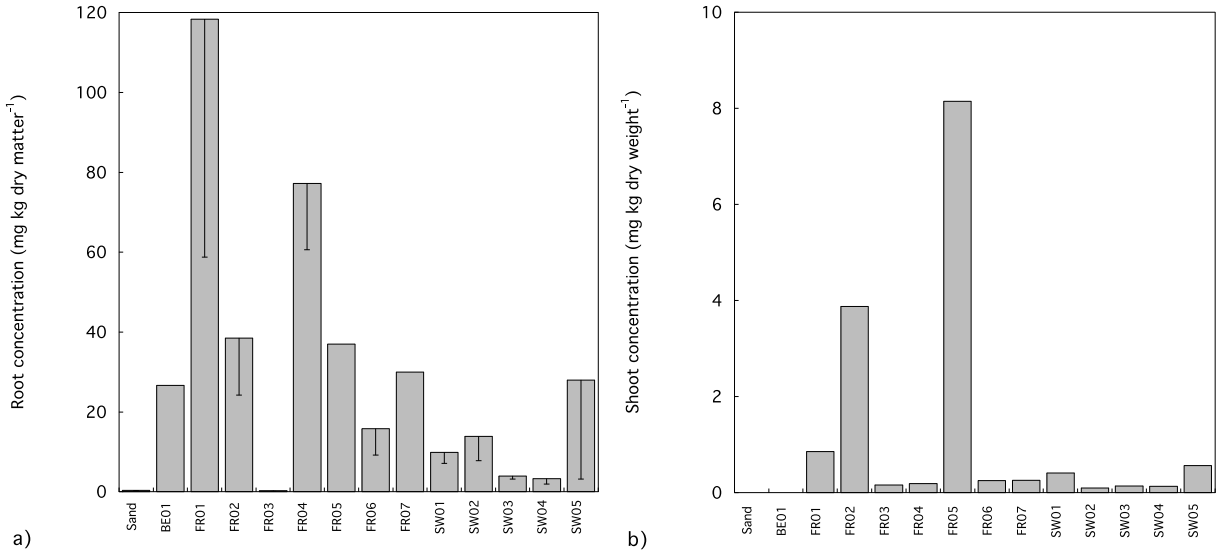


Figure 7.4 Total PAH concentrations in a) roots and b) shoots.

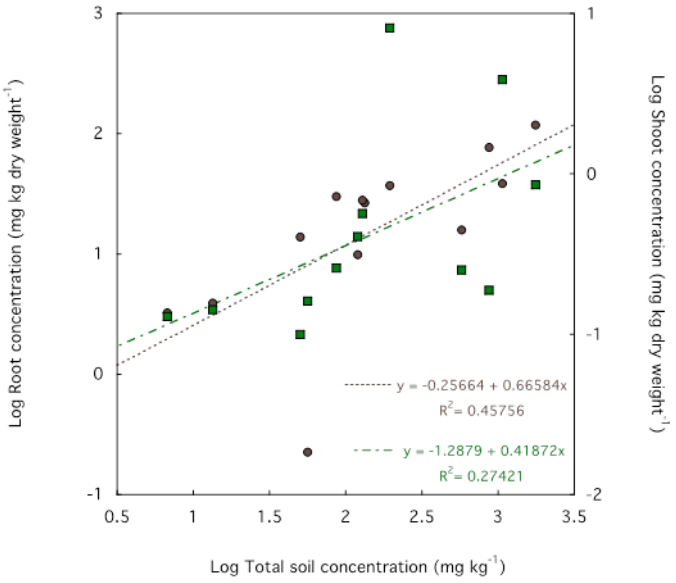


Figure 7.5 Total PAH concentration in roots (●) and in shoots (■) as a function of total soil concentration.

7.3.3 Prediction of PAH uptake

Figure 7.6 and Figure 7.7 present the results obtained with the different modelling approaches of PAH concentration in roots and shoots respectively. As previously mentioned, there is a big variability in our data, especially for shoots.

The best estimate for root concentration is obtained with the Briggs model and the pore water concentration estimated from the total soil concentration. Values calculated with the POM measured or close estimate of pore water concentration (i.e. Tenax or coal-tar model) lead to a strong (1 or 2 orders

of magnitude) under-estimate of actual root uptake, though less pronounced when the model by Zhang and Zhu is used. This model seems more relevant for PAH uptake since it has been developed for PAH and implement differentiated uptake between root compounds. With this model and for a given pore water concentration, calculated values are higher than those predicted with the Briggs model, which was developed for pesticides that can have different uptake rates and metabolization rates than PAHs. The use of pore water concentration estimated from total soil concentration even lead to an over-estimate of the root uptake with the Zhang and Zhu model by roughly one order of magnitude.

For shoots, similar trends are observed. The use of measured pore water concentrations or Tenax estimated pore water concentration under-estimate shoot uptake. When the C_{pw} value is calculated on the total soil concentration, the average uptake is better predicted. However, for some soils having the highest uptake (FR02 and FR05), the model drastically under-estimate the uptake. In these cases a possible alternative uptake pathway would have to be considered.

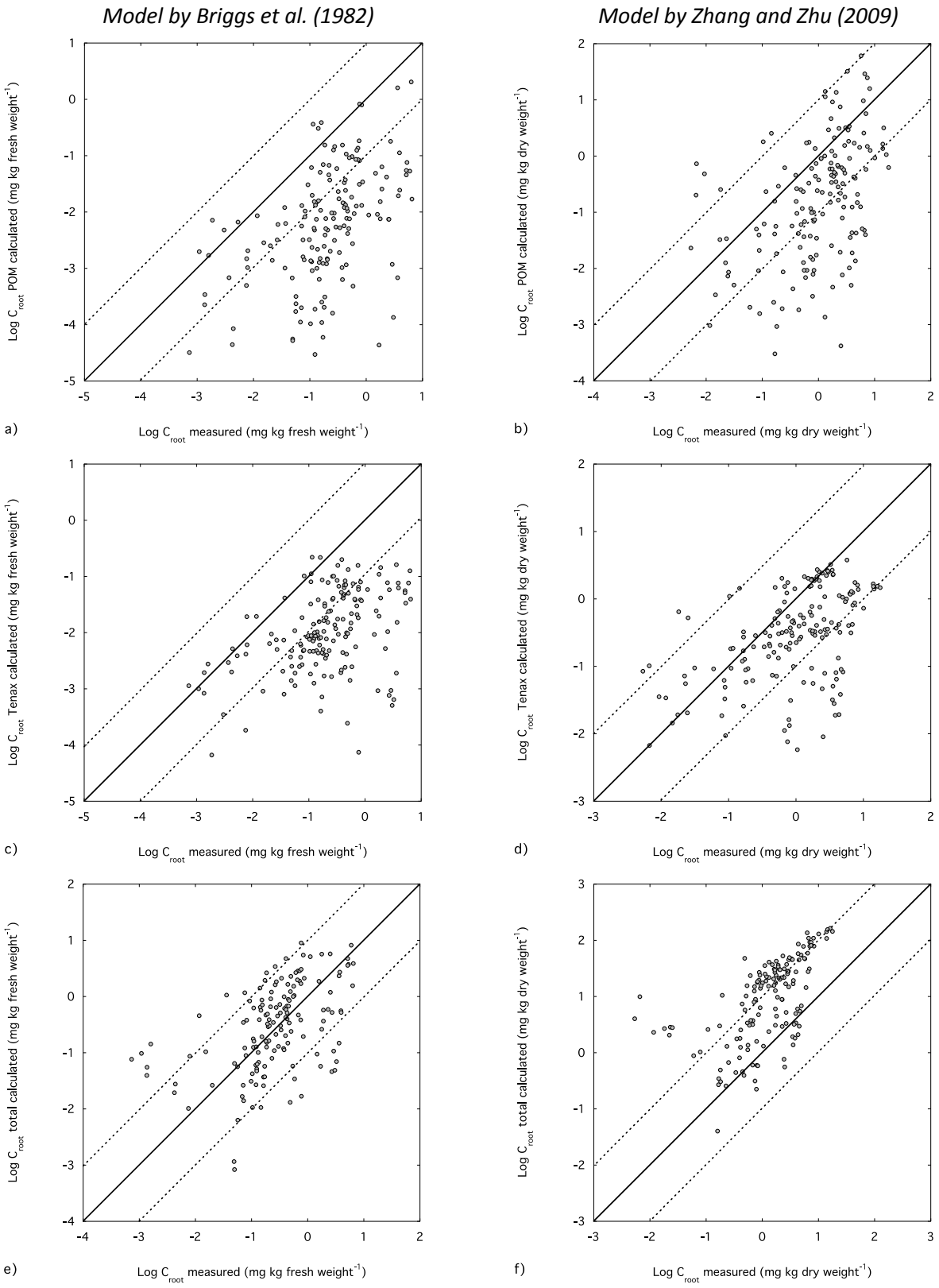


Figure 7.6 Comparison between experimental and predicted individual PAH root concentrations obtained with Briggs and Nguyen models and using pore water concentrations obtained from a) and b) POM measurements, c) and d) Tenax extracted fraction, e) and f) total soil concentrations.

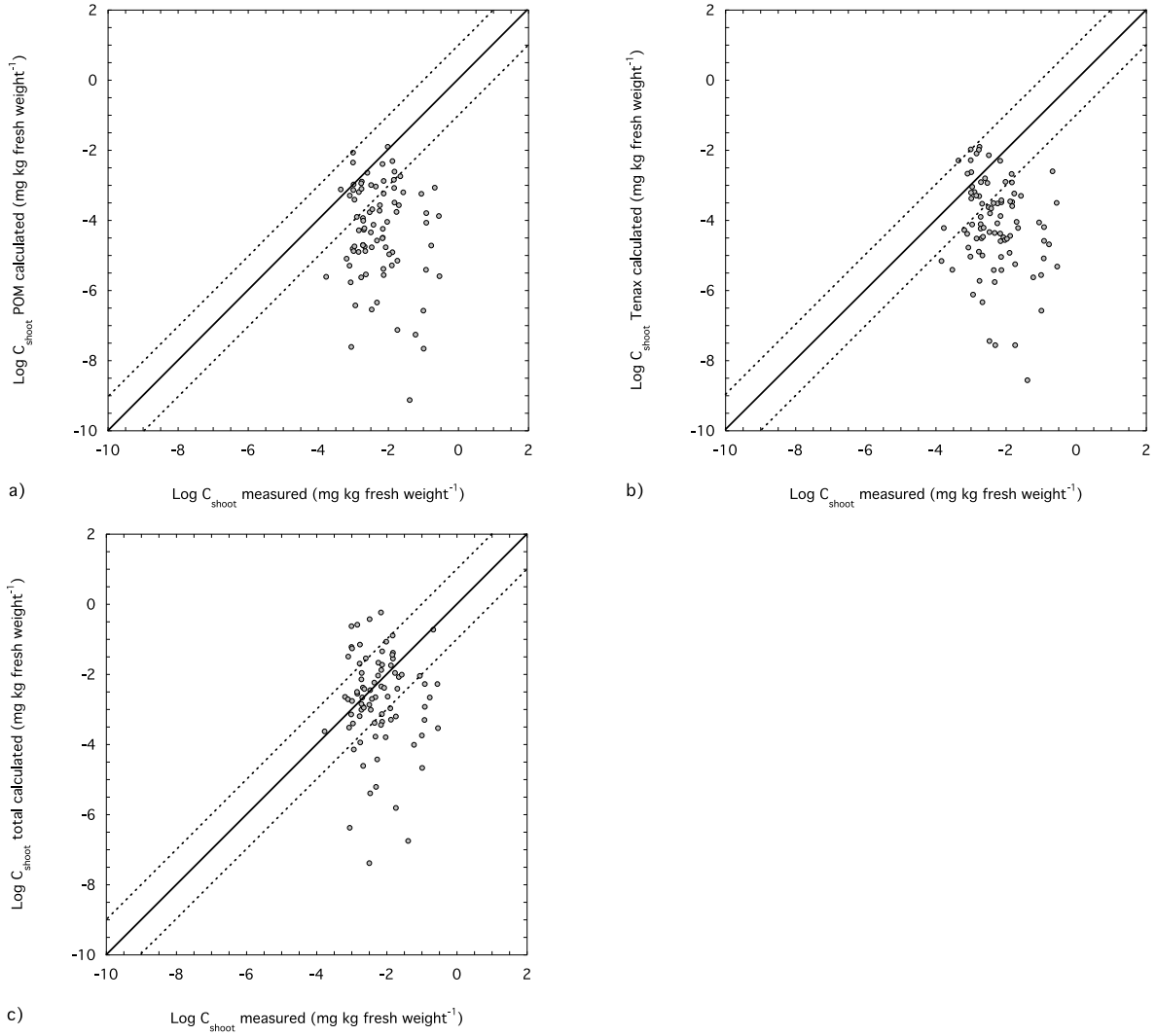


Figure 7.7 Comparison between experimental and predicted individual PAH shoot concentrations obtained with Briggs model and using pore water concentrations obtained from a) POM measurements, b) Tenax extracted fraction, c) total soil concentrations.

7.4 Discussion and conclusions

Up to now only very few studies have attempted to correlate PAH plant uptake with available fractions. Previous studies have highlighted good correlation coefficient between amounts of PAHs extracted by water, n-hexane or sequential supercritical fluid extraction and root uptakes (Tao et al. 2006; Bogolte et al. 2007). However, in these cases, no quantitative estimate of root uptake was established based on these partial extractions, supposed to mimic bioavailability.

In a more recent work, Gomez-Eyles et al. (2012) tested a wider range of extraction methods to predict plant accumulation of PAHs: exhaustive acetone/hexane extractions, mild solvent (butanol) extractions,

cyclodextrin extractions, solid phase microextraction (SPME) and polyoxymethylene solid phase extraction (POM-SPE). However, here again, none of the tested methods was able to predict PAH accumulation accurately in plants. Those results are in line with the results obtained in the present study. Similarly, exhaustive extractions, i.e. total soil concentrations, were also found to correlate more strongly with plant accumulation than bioaccessibility methods. Like for the methods tested in our study, realistic pore water concentrations estimates methods tend to under predict actual accumulated concentrations. Some explanation might be that plant root exudates act as biosurfactant and increase PAH solubility, thereby increasing their uptake. Still, a recent study by Brennan et al. (2014) show that POM extraction predicts fairly well actual root uptake. But some discrepancy is still observed and the relationship is only valid for two soils.

Based on our results and previously published data, pore water concentration (whatever the method used to obtain it, direct measurement or modeling) is not sufficient to predict PAH plant uptake. The good correlation observed between root uptake and total soil concentration tends to support the existence of an additionnal uptake pathway occurring simultaneously to the water uptake pathway and involving or more direct soil-root transfer. Additionnal research would be neeeded on this point. At this stage, the implementation of bioavailablity methods based on pore water concentration in site specific risk assessments for plant uptake estimate would lead to an underestimation of the risk.

8. Application of phytoavailability tests for metals in national soil policy frameworks – the Swiss and German examples

In only two countries, to our knowledge, phytoavailability tests are officially used for risk assessment in the case of soil contamination by trace elements: The Swiss Confederation and the Federal Republic of Germany, where thresholds values based on extractions with saline solutions have been brought in the regulation of soil protection. We present here an analysis of the approaches used to achieve these regulatory values and how they are used.

8.1 The Swiss approach

Regulations on soil protection are based on the Swiss law for the protection of the environment enacted in 1983, and revised in 1995 (Hämmann et al. 1998; Hämmann and Gupta 1998). From this law arise ordinances on preservation of air quality, use of hazardous substances, waste management and soil pollution. The latter is regulated by the OSol Ordinance (VSBo in German) which was enacted first in 1986 (OSol 1986) and revised in 1998 (OSol 1998).

A legal distinction is made between a "contaminated soil" and "abandoned contaminated site", these two types of pollution being distinguished from each other by several criteria. The two main ones are the extent of pollution (surface layer for the contaminated soil and extension in the subsoil for contaminated site) and its impacts (on soil fertility for contaminated soil and the aquatic environment, air, with direct impact on Man and animals in the case of the contaminated site).

The preventive approach to soil protection is the fundamental principle of the Ordinance for soil pollutants (OSol 1998), which guarantees long-term soil fertility and thereby regulates

- Observation, monitoring, evaluation of chemical, biological and physical impacts on soil;
- Measures to prevent persistent compaction and erosion;
- Measures for handling soil materials;
- Additional measures that cantons can take concerning affected soils.

This concept of fertility is the central one for qualitative measures of soil protection. According to Article 2 of this Ordinance, the soil is fertile if it satisfies the following four requirements:

- It has a biologically active and diverse biocenosis, a typical structure for the station and an intact decomposition capacity;
- It allows natural or cultivated plants and plant associations to grow and develop normally and does not affect their properties;
- Forage and food plant that it supplies are of good quality and do not threaten the health of humans and animals;
- Its ingestion or inhalation poses no threat to human health and animals.

Chemical damage brought to the soil by trace elements are evaluated from “guide values”, “trigger values” and “clean-up values”. These values are concentrations in the surface layer of soil, which are either “total” (by dissolution with 2 mol L^{-1} HNO_3 or alkali fusion for fluorine) or “soluble” (by extraction with a solution of 0.1 mol L^{-1} $NaNO_3$ or with water for F). In reality, the dissolution with 2 mol L^{-1} HNO_3 is not complete and in this case, one cannot speak of total contents but rather of “pseudo-total” contents. The $NaNO_3$ extraction provides an assessment of the bioavailability of the element. It was chosen because the reagent ions poorly exchange with those the soil solid phase and because it simulates composition of the soil solution (Gupta 1991). A threshold value is considered exceeded when the total or the soluble value is exceeded.

“Guide values” (Table 8.1) deal with Cr, Ni, Cu, Zn, Mo, Cd, Hg, Pb and F are independent of land use. They are valid for all soils containing up to 15% humus. They indicate the level of contamination above which the long-term fertility of the soil and soil functions are no longer insured. However, even if the fertility of a soil is not guaranteed on the long-term, risk for human or animal is unlikely to arise for the different land uses. If exceeded, the authorities have to find the source of the pollution and to take the measures for it does not increase (Article 8). In Switzerland, many sites have pollution levels exceeding the guide values, some due to high natural content, and other because of anthropogenic inputs.

Table 8.1 Guide values for soil pollutants in soils, mg kg^{-1} soil, air-dried, for the two regulatory extraction methods according to the two successive ordinances (OSol 1986, OSol 1998).

Element	2 mol L^{-1} HNO_3		0.1 mol L^{-1} $NaNO_3$	
	1986	1998	1986	1998
Pb	50	50	1	-
Cd	0.8	0.8	0.03	0.02
Cr	75	50	-	-
Co	25	-	-	-
F	400	700	25	20
Cu	50	40	0.7	0.7
Mo	5	5	-	-
Ni	50	50	0.2	0.2
Hg	0.8	0.5	-	-
Tl	1	-	-	-
Zn	200	150	0.5	0.5

"Trigger values" (Table 8.2) indicate the level from which, for a given soil use, the health of humans, animals and plants may be threatened. In case of exceeding a trigger value, an investigation should be conducted to further confirm or refute the supposed threat. If the threat is confirmed, the land use will be restricted.

Table 8.2 Trigger values for soil pollutant contents, mg kg^{-1} dry soil, according to the ordinance OSol (1998).

Soil use	Lead		Cadmium		Copper	
	HNO_3	$NaNO_3$	HNO_3	$NaNO_3$	HNO_3	$NaNO_3$
Food crops	200	-	2	0.02	-	-
Fodder crops	200	-	2	0.02	150	0.7
Direct ingestion	300	-	10	-	-	-

If a "clean-up value" (Table 8.3) is exceeded, the cantonal authorities prohibit the concerned soil uses. In soils devoted to horticulture, agriculture or forestry, measures must be taken to bring the contamination below the clean-up value, at a level such that the intended use is possible without threatening humans, animals and plants.

Table 8.3 Clean-up values for soil pollutant contents, mg kg^{-1} dry soil, according to the ordinance OSol (1998).

Soil use	Lead		Cadmium		Copper		Zinc	
	HNO_3	$NaNO_3$	HNO_3	$NaNO_3$	HNO_3	$NaNO_3$	HNO_3	$NaNO_3$
Agriculture and horticulture	2000	-	30	0.1	1000	4	2000	5
Kitchen gardens/allotments	2000	-	20	0.1	1000	4	2000	5
Playgrounds	1000	-	20	-	-	-	-	-

8.1.1 Methods of establishing the OSol threshold values

Guide values

It seems that the guide values for "total" (i.e. 2 mol L^{-1} HNO_3) correspond mainly to the upper limit of pedo-geochemical background. For those defined with $NaNO_3$ 0.1 mol L^{-1}, experimental crops on contaminated land have been conducted (Gupta 1991). Ten soil samples were used, which came from plots affected by different sources of contamination by trace elements (metal, highway, incineration, composting, sludge). Ryegrass and radish were grown in greenhouse on these soils placed in pots. Concentrations of Cd, Cu, Ni, Pb and Zn in plants and soils were measured. Correlations between the concentrations in soils and in plants were observed. Based on these correlations, it was possible to deduce the critical concentration in the soil from a critical concentration in the plant. Critical concentrations in the plant corresponded to toxic levels for plant or for animal. The guide values of the regulation resulted from a division by two of the critical concentrations derived from these experiments, in order to introduce a safety factor.

The guide values were checked from 25 field trials (Gupta and Häni 1989). Ryegrass and lettuce were grown on plots of 1 m^2 (three replicates) throughout Switzerland for two years (1987 and 1988). The number of cases where the contents of the soils are below twice the guide values and the concentration in the plant is above the critical concentration is very low. In three cases, the soluble Cu are below the critical concentration while the growth of ryegrass seems to be affected. This is attributed to an alteration of the microflora. Ecotoxicological concentrations were also verified by measurements of microbial activity (C mineralization). In general, the guide values were validated, except that of soluble Cd. This is probably why it went from 0.03 mg kg^{-1} in the first version of the OSol (OSol 1986) to 0.02 mg kg^{-1} in the revised one (OSol 1998) (Table 8.1). Between 1986 and 1998, several guide values changed significantly. Those for Co and Tl (total and soluble) disappeared. Guide values for total Cr, Cu, Hg and Zn were lowered while that of F was revised upwards. The guide value of soluble Pb was removed, while those of Cd and F were lowered. These changes are due to changes in toxicological knowledge (Gupta, 2004, personal communication). They show the flexibility of the regulatory system that is no less rigorous.

Trigger and clean-up values

In a report published by the Swiss Agency for Environment, Forests and Landscape, (Hämmann and Gupta 1998) present the methodology used to establish the trigger and clean-up values and their related "inaccuracies". This document precisely describes how the "threat analysis" was made and how were chosen the "evaluation criteria" for this threat, concerning the exposure pathways, namely:

- Soil → Food crop → Man → (Food crop pathway)
- Soil → Fodder crop → Animal → Man (Fodder crop pathway)
- Soil → Plant (Plant growth pathway)
- Soil → Man (Direct exposure)

The "goods to protect" from threats due to soil alterations are therefore humans, other animals and plants. "Soil uses" concerned by trigger values are "food cropping", "fodder cropping" and "uses associated with possible direct human exposure". Clean-up values concern soil uses for "agriculture and horticulture", "kitchen garden and allotments" and "playground".

The choice of the "pollutants" to be considered was performed according to two main criteria: the "necessity" and the "applicability". The necessity is recognized when the element creates problematic situations, that is to say that cases of contamination exist in the Swiss Confederation and that the element is toxic to humans, animals or plants. A necessity was recognized for Pb, Cd, F, Cu, Ni and Zn. Chromium, Co, Mo, Hg and Tl were not retained because cases of contamination are rare in Switzerland. No toxicity is also recognized for Co. The applicability depends on the existence of "evaluation criteria", that is threshold values for the effect on the goods to protect (or content limits in the good to protect) and enough information on the pollutant, the dose-response and the exposure of the good to protect. On the basis of applicability, only Cd, Cu, Pb and Zn have been finally selected.

Various transfer pathways of pollutants towards goods to protect were identified, and grouped into exposure pathways. Only part of them were selected, which are presented above. Transfer from soil to plant is considered of prime importance in the exposure of goods to protect. In addition, the status of the pollutant in the soil, that is to say its availability for transfer is taken into account in the threat analysis. The authors consider three levels of availability: The mobile fraction, which is estimated from the solubility of the pollutant in 0.1 mol L^{-1} $NaNO_3$; the mobilizable fraction, which could become a concern, i.e. which could become mobile in case of modification of the physico-chemical properties of the soil; the total fraction or pseudo-total fraction, since it is estimated from an extraction in hot 2 mol L^{-1} nitric acid. Trigger and clean-up values are given for both extraction methods used in Switzerland. The development of a method for measuring the mobilizable fraction still has to be carried out.

Several methods can be used to quantify the pollutant transfer:

- A relationship between a variable representing the effect on the good to protect (generally the content in a plant) and the content in the soil. In this case, correlation is rarely usable. Fixing maximum and minimum thresholds framing the scatter plot is preferred.
- The transfer factor of the pollutant to the plant, which is the ratio of the content in the plant to that in the soil. It is considered unusable because varying with the soil concentration.

- Quantitative analysis of the exposure of humans and animals, by calculating the amount of pollutant ingested from the size and composition of the diet and/or soil ingested.
- Epidemiological studies that confirm or refute a supposed correlation between soil pollution and exposure of the organism.

The criteria of the evaluation of the threat are:

- Maximum concentrations in foodstuffs (regarding the threat to human health) or fodder (animal health) prescribed by the regulations in Switzerland or abroad (mostly from Germany and Belgium). These maximums are not always based on dose-effect relationships. They often represent the 95th percentile of the pollutant content in commercialized products.
- The protection values for external exposure. These are the amounts, which can be ingested by organisms, such as the "Provisional Tolerable Weekly Intake (PTWI)" or "Provisional Maximum Tolerable Daily Intake (PMTDI)" established by FAO and WHO for human exposure.
- The protection values for internal exposure concern human health. These threshold values were established from a medical point of view for the content levels measured in blood or organs. They are applicable once an epidemiological study established a relationship between the pollutant concentration in soil and the concentration in organs or blood.
- The maximum allowable reduction in yield or the phytotoxic concentrations, for threat to plant growth.

An evaluation of the threat for the different contamination pathways is presented in Chapter 4 of the document of Hämmann and Gupta (1998). It appears that in many cases, the methodology leads to threshold levels in soil that vary according to the exposure. For instance, exposure of humans through food plants varies with the proportion of contaminated food in the diet. Assuming that there is a relationship between the content in soil and that in contaminated food, the concentration threshold (in soil) varies according to the dietary habits of the populations concerned. This problem does not occur if the regulatory maximum concentration in food is used rather than the consumer exposure. However, in all cases, the threshold contents in soil may vary depending on the plant, since the pollutant uptake varies with the species and the cultivar.

The method for setting the trigger values attempts to reduce the variability of the exposure basing on a "realistic worst-case scenario (scenario RWC)". Here is presented, as an example, the method for determining the trigger values for the "Food crop pathway":

a) Scenario RWC
   - Select plants that have a high potential for accumulation
   - The soil-to-plant transfer of pollutant is assumed to be maximum
   - The consumption of the food crops produced on the contaminated soil is also assumed to be maximum (see also evaluation criteria just after)
b) Evaluation criteria

The evaluation criterion is the threshold prescribed by the regulation, i.e. the content in foods, completed by foreign standards.

c) Analysis of the maximum level permitted in soils

The relationship between the content in the soil and the plant is built from experimental data.

d) Verification

Literature enables to assess the validity of the determined threshold.

e) Comparison to foreign standards

Points d) and especially e) can hardly be implemented due to the diversity of the methods used in the scientific works and the lack of foreign standards.

The procedure for setting clean-up values is based on a "best-case scenario (BC)" defined for each exposure pathway, in combination with the evaluation criteria:

- Food crop pathway
  The threshold content in the plant product prescribed by the regulations in Switzerland or abroad is multiplied by three to serve as an evaluation criterion. The maximum allowed in the soil is derived by considering a minimum transfer, to allow usage restrictions (such as growing low accumulating plants) if the trigger value is exceeded.
- Fodder crop pathway
  Generally, the most frequent use is considered, i.e. grazing, essential in Switzerland. An insensible animal should be chosen, because of use restrictions are possible for the most sensitive species. The proportion of direct ingestion is assumed to be at minimum value in the "best case" scenario.
- Plant growth pathway
  The evaluation criterion is a yield reduction of 25% compared to reference plots or to 90% of the maximum yield.
- Direct exposure pathway
  BC scenario is defined according to the pollutant, as a single scenario is impossible.

The clean-up values are provided for three land uses: agriculture and horticulture, kitchen gardens and allotments, family playgrounds. The values obtained for the different pathways must therefore be aggregated. For example, the clean-up value for the use of “agriculture and horticulture” must mean that the level of soil contamination is such that the food and fodder production became impossible, whereas below clean-up value (and above the trigger value), one or the other is possible. For example, the clean-up value for “agriculture and horticulture” (VAH) is given by:

$$VAH = \max[\min(VFC ; VPG) ; \min(VFOC ; VPG)]$$

where V is the clean-up value, FC is the food crop pathway, FOC is the fodder crop pathway and et PG is the plant growth pathway. Thus the clean-up value should be achieved when it is not possible to limit the use, for example by converting food cropping into fodder cropping.

Thresholds values, which the approach resulted in are given in Table 8.2 and Table 8.3. There is no trigger value for Zn, as the element is considered as low or non-toxic. It is the same for Cu, except for fodder crops, which may be consumed by sensible animals (sheep). There is no threshold value for the concentration of soluble Pb, because the amounts extracted by $NaNO_3$ are very low, often below the quantification limit, even in highly contaminated soil. Clean-up values have been set for Cu and Zn because of the phytotoxicity of these metals.

The authors are well aware of the inaccuracy, which taints the regulatory threshold values. This is mainly due to:

- The analytical uncertainty, which is the one that can be best assessed and minimized;
- The imprecision of the evaluation criteria;
- Errors cumulated during the calculations of exposures;
- The limited number of studies in Switzerland;
- The different methodologies used in different studies, Swiss or foreign.

Extraction with 0.1 mol L^{-1} $NaNO_3$ is a major problem in the implementation of the regulation. Indeed, the sensitivity of the method is low, as the amount extracted are often below the quantification limits. The reproducibility of the method is also low. Users are therefore constrained to use thresholds values defined for the extraction with 2 mol L^{-1} HNO_3. It would be better to have a more sensitive method for assessing the phytoavailability, which produce values beyond the limits of quantification. It must be added that HNO_3 2 mol L^{-1} is a problem in its use for soil quality monitoring, as the extraction is not complete and does not allow making proper pollutant flow balances.

8.2 German approach

8.2.1 German regulation on soil protection

Two texts must be considered. The "Gezetz zum Schutz vor schädlichen Bodenveränderungen und zur sanierung für Altlasten", for which there is a translation into English (BBodSchG 1998), is the federal law on soil protection, enacted in March 1997. An ordinance was issued in July 1999 (BBodSchV 1999), which specifies a number of articles of the framework law. Overall, the German regulations are less easy to read and interpret than the Swiss regulation, which is accompanied by various explanatory documents.

The purpose of the federal law is to protect or restore soil functions in a sustainable manner. It focuses on the prevention of soil degradation, on the remediation of contaminated soil and sites, and also of water contaminated by these sites. The law sets obligations to prevent alterations of soil functions and their remediation (“sanierung”). It specifies the conditions under which risk assessment studies should be carried out, how the remediation of polluted soils or sites must be implemented, as well as their monitoring. There is also section defining good agricultural practices.

Article 8 of the law specifies three types of parameter values in soils:

- The "Vorsorgewerte" or "precautionary values" which if exceeded, indicate that a harmful change in soil composition can be suspected, the soil geochemical background and diffuse contamination being taken into account in these values.
- The "Prüfwerte", which can be translated as "trigger values", are the values that, if exceeded, indicate that a study is required on the individual case, taking into account the land use, in order to verify whether there is a harmful soil change or a contamination of the site.
- The "Maßnahmenwerte" or "action values" are the values of impact indicators or pollution, which, if exceeded, indicate the presence of a harmful soil change or site contamination and require taking protection, restriction or sanitation measures.

The various articles of the ordinance of July 1999 (BBodSchV 1999) specify the conditions of the studies and site evaluation, the obligations concerning the reduction of hazards through remediation or stabilization measures, and the establishment of the threshold values. Annex 1 of the ordinance describes the requirements for sampling, analysis and quality assurance to be applied in soil and contaminated site studies. The proposed methodology is based on numerous ISO, CEN and DIN standards. Appendix 1 gives the precautionary, trigger and action values. Precautionary values are given for the contents in Cd, Pb, Cr, Cu, Hg, Ni and Zn obtained by extraction with aqua regia (Table 8.4) and for PCBs, benzo(a)pyrene and polycyclic aromatic hydrocarbons (PAH). The precautionary values for metals mentioned above vary according to soil texture (clayey, loamy or sandy), indicating that the values are essentially the pedo-geochemical background, since it is known that it is mainly determined by the fine fraction content. This is confirmed by the values themselves, which correspond to the upper limit of pedo-geochemical background.

Table 8.4 Precautionary values, in mg kg^{-1}, for soil contents obtained after extraction with aqua regia (DIN ISO 11466).

Soil	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Clayey	1.5	100	60	1	70	100	200
Loamy	1	60	40	0.5	50	70	150
Sandy	0.4	30	20	0.1	15	40	60

Trigger and action values are defined for three exposure pathways:

- Soil → Man (Direct contact)

For this pathway, trigger values apply to playgrounds, residential areas, parks and amenity areas as well as industrial and commercial areas. They cover some organic compounds (aldrin, benzo(a)pyrene, DDT, PCBs, etc..) and six trace elements extracted by aqua regia (Table 8.5)

Table 8.5 Trigger values for the pathway Sol → Man by direct contact, in mg kg^{-1}, for soil contents obtained after extraction with aqua regia (DIN ISO 11466).

Element	Playgrounds	Residential areas	Parks and amenity areas	Industrial and commercial areas
As	25	50	125	140
Cd	10	20	50	60
Cr	200	400	1000	1000
Hg	10	20	50	80
Ni	70	140	350	900
Pb	200	400	1000	2000

Action values relate only with dioxins and furans.

- Soil → Food crop

This pathway corresponds to the land use for agriculture, horticulture and grazing. Trigger and action values regarding the impact of soil contamination on the plant quality (potentially toxic element content) are given in (Table 8.6). For As and Hg, they are given for aqua regia extraction, while for Cd, Pb and Tl values relate to the extraction with 1 mol L^{-1} NH_4NO_3. It can be noted that there is no trigger value for Cd, but two action values differentiated by the type of crop, the lowest one being applicable to wheat bread and plants with high accumulation. There is a trigger value for thallium which causes problems in Germany as it was found in large quantities in the vicinity of cement (Prüess et al. 1991).

Table 8.6 Trigger and action values, in mg kg^{-1} for the Soil → Food crop exposure pathway.

Element	Method	Trigger value	Action value
As	Eau régale	200	-
Cd	NH_4NO_3	-	0.04/0.1
Hg	Eau régale	5	-
Pb	NH_4NO_3	0.1	-
Tl	NH_4NO_3	0.1	-

For grazing, there is no trigger value, but only action values for soil content determined after aqua regia extraction (Table 8.7). This is due to the fact that the contamination of animals is mainly through soil ingestion.

Table 8.7 Action values concerning grassland, in mg kg^{-1} for soil concentrations obtained after extraction with aqua regia.

Element	Action value
As	50
Cd	20
Cu	1300/200[a]
Hg	2
Ni	1900
Pb	1200
Tl	15

[a]For sheep: 200

Regarding the risk of phytotoxicity, trigger values have been set for As, Cu, Ni and Zn concentrations measured with 1 mol L^{-1} NH_4NO_3 (Table 8.8).

Table 8.8 Trigger values for the pathway Soil → Plant and the phytotoxicity risks in mg kg^{-1}, for soil contents obtained after extraction with NH_4NO_3 in 1 mol L^{-1}.

Element	Trigger Value
As	0.4
Cu	1
Ni	1.5
Zn	2

The regulation text states that these thresholds values are set for a 30 cm surface layer, except in the case of grassland, for which they apply to a 10 cm upper layer. In case of studies on greater depths, the thresholds should be multiplied by 1.5.

- Soil → Groundwater

In this case, the thresholds values are set for the contents in groundwater or in soil leachates collected in situ or obtained by extraction or elution.

8.2.2 Method for setting the threshold values of the Federal Ordinance (BBodSchV)

a) The experience of Baden-Württemberg

The method for setting thresholds values from the extraction by 1 mol L^{-1} NH_4NO_3 have been developed by Prüess et al. (Prüess 1995a; Prüess 1995b; Prüess et al. 1991) and initially applied in the state of Baden-Württemberg. Using 400 pairs of samples of plant and associated soil and 300 soil samples from southwest Germany, Prüess defined, for the contents of many elements measured using NH_4NO_3, what

he sometimes calls the "background values", sometimes “vorsorgewerte” (precautionary values), “action values” (or "prüfwerte") or “threshold values”. These names do not necessarily correspond to those later used in the Ordinance BBodSchV

As their name suggests, the Prüess’ precautionary values are derived from background values, that is to say, the contents in the surface horizons of soil little contaminated or uncontaminated. They correspond to the 90th percentile of the content distributions. According to the principle of their establishment, they correspond, for the NH_4NO_3 extracts, to the precautionary values of the Federal Ordinance presented above.

Prüess’ “action values”, which correspond more or less to the trigger values of the Ordinance BBodSchV investigation were obtained from correlations between concentrations in plants and those in the soil. They were deduced from concentrations limits in plants set by the regulations (in the case of Cd) or levels in the plant causing a growth reduction (for As and Ni). They meet the following condition: If the content in the soil is below the “action value”, the critical concentration in the plant will be exceeded in 5% of the cases at the maximum. The “action values” have been defined in this way for As, Cd, Cu, Tl, Zn (toxicity to the consumer of the plant) and for As, Cu and Zn (yield reduction). For Cr, Ni and Co, the “action values” were estimated from observations where only one site showed critical concentrations in plants and presenting a wide range of soil content. For Ag, Be, Bi, Mo, Sb, U and V, no critical plant concentration could be found, even for soils containing high levels of these elements.

Pruess’ “threshold values”, which probably correspond to the action values of the Federal Ordinance were also derived from correlations between contents in the plants and contents in the soil (using NH_4NO_3 extraction). They are such that the critical concentrations in plants are exceeded in more than 70% of cases. The “threshold values” proposed by (Prüess 1995a) for Cd correspond precisely to the action values of the Federal Ordinance.

b) The thresholds values of the Ordinance BBodSchV

The thresholds values given in the Ordinance BBodSchV were established according to a method similar to that proposed by Prüess. The Ordinance enactment was accompanied by the publication of a voluminous document (BMU 1999), which describes the methods used to obtain the various limit values, including those for trace elements. Trigger and action values have been elaborated as follows:

1. Setting maximum allowable concentrations in plants.

In Germany there are reference values for pollutant concentrations in plant products for human consumption. These values, designated as ZEBS values (“Zentrale Erfassungs-und Bewertunstelle”, Central Agency for Inventory and Assessment), are derived from the statistical distribution of pollutant concentrations in the ‘normal’ plants, that is to say slightly contaminated. They could match (this is not explicit in the text) to the upper limit of the background levels in these plants. There is also an Ordinance (FMVO) which limits the concentrations of certain elements in the feed. Maximum allowable concentrations in plants used for the Ordinance BBodSchV correspond to twice the ZEBS and FMVO values.

2. Description of the soil-to-plant transfer of trace elements

The Federal Environment Agency has a database named "TRANSFER" which contained, at the time of the enactment of the Ordinance BBodSchV, concentrations of various substances in 320,000 pairs of soil and plant samples. These data concern 120 plant species. For 61,000 pairs, trace elements contents in soils were measured with aqua regia and for 21,000 pairs, with 1 mol L^{-1} NH_4NO_3 extraction. From these data, correlations were sought between content (for a given element) in plants and the levels in the soil. In general, these correlations and confidence intervals were constructed from log-transformed data for some species representative of the uses. They are sometimes close (case of Cd), sometimes non-significant (case of Pb).

3. Deduction of the threshold values

From the above correlations, one can derive a threshold level in the soil, above which the probability that the content in the plant exceeds the maximum allowed concentration is high. The probability level of each threshold is not clearly given in the available text (BMU 1999). It states that probabilities of 20 to 80% were used.

The method of inferring the precautionary values is not clearly explained. However, their level and their variation with soil particle size distribution suggest that they are derived from the pedo-geochemical background and correspond to its upper limit (baseline).

9. Recommendations on implementation of chemical bioavailability methods in site specific ecological risk assessment frameworks

9.1 Introduction

There is an on-going work on standardization of chemical methods for bioavailablity assessments of inorganic and organic contaminants within the ISO framework. This work has so far resulted in standard ISO 17402, where different concepts and definition are being postulated, together with a list of “available and promising chemical methods to measure bioavailabity”. Here, methods to mimic porewater concentrations as well as different extractants are listed. In a risk assessment perspective, however, it is critical to be able to relate the result obtained with the chemical method with corresponding information in a reference system based on ecotoxicity test data. The methodology proposed below for metals draw on the work performed within REACH risk assessments (Smolders et al., 2009) and the procedure for PAHs on US EPA’s work on sediment risk assessment (USEPA, 2003) and RIVM’s work on risk assessment on soils, sediments and waters (Brand et al., 2013).

At present, no “official” framework including a chemical bioavailablity methodology is available. However, RIVM’s work presented in Brand et al. (2012, 2013) is the one that comes closest. In these reports two approaches for incorporating bioavailability methods in risk assessment of PAHs are proposed; the passive sampler methodology, as discussed below, and extraction methods for assessing the “potentially bioavailable” fraction of PAHs.

The following criteria for a successful chemical method were proposed by RIVM at a workshop held with experts in the field of bioavailablity in 2008 (Brand et al., 2012):

1) wide ranging applicability, meaning
   a) the possibility to perform the technique in a standard laboratory;
   b) the possibility to assess more than one type of organisms;
   c) the possibility to assess more than one type of soil;
   d) the possibility to assess more than one type of contaminant;
2) practical use;
3) added value compared to total content;
4) validy for ecotoxicity;
5) applicability for more than ecotoxicity (e.g. leaching)

In addition to these points, we can add the need of a ecotoxicological reference framework, as mentioned above. Furthermore, we believe that a methods based on a theoretically sound concept are the ones that are easiest to accept. An example of one such concept is the theory of equilibrium partition theory for non-polar organic compounds, such as PAHs.

9.2 Metals

9.2.1 Implementation of soil tests in risk assessment

Soil limits in risk assessments are almost always expressed as total metal concentrations. The limits are derived from numerous toxicity tests for a whole range of organisms, endpoints and soils and almost all based on metal salts spiked soils (Checkai et al., 2014). Converting the limits for Zn using an intensity based soil test (e.g. DGT) to obtain a better estimation of bioavailable metal requires a full recalibration exercise, i.e. numerous tests (different species, endpoints, soils) with associated doses confirmed with methods such as, for instance, DGT. Practically, this is a huge task. The isotope dilution method (E-value) could offer a pragmatic solution (Figure 5.6 Labile metal fraction in the field-contaminated soil (fraction of aqua regia soluble metal that is isotopically exchangeable) plotted versus the relative metal toxicity). Indeed, for risk assessment, consensus values for leaching/aging factors (L/A factor) have been accepted to translate data of freshly spiked soils to the 'field' and to develop field relevant limits (Smolders et al., 2009). These L/A factors were mainly based on toxicity differences upon leaching and aging of a spiked soil. Generic leaching-aging factors were selected for the European risk assessment and were based on toxicity tests with different soils, plants, invertebrates and microbial processes. This procedure has been implemented to calculate soil type specific clean up limits in Flanders, Belgium.

The toxicity databases and the derivation of soil limits is implemented in software, (Arche, 2012), for deriving soil ecotoxicological limits for metals (see copy of software below). In that software, as in the EU risk assessment, generic values for L/A factor were used, i.e. a value of 3 was selected for Zn contaminated soils and a factor of 2 for Cu (Smolders et al., 2009). That software also derived the limits as a function of soil properties. The average field spiked factors (Figure 5.6 Labile metal fraction in the field-contaminated soil (fraction of aqua regia soluble metal that is isotopically exchangeable) plotted

versus the relative metal toxicity) for Zn contaminated soils collected here (n=7) was 8.2 (outlier La Calamine: 30; average without outlier: 3.8) and for Cu soils (n=2), it was 5.0. The average FS factors we measured are both larger than the previously reported L/A factors in Smolders et al. (Smolders et al. 2009). The fractions of labile metals (E-value in %) in the field-contaminated soil are surprisingly strong predictors of the FS factor (Figure 5.6 Labile metal fraction in the field-contaminated soil (fraction of aqua regia soluble metal that is isotopically exchangeable) plotted versus the relative metal toxicity) i.e. the FS factor (in fact 1/FS) is predicted within a factor 2.2 for Zn soils and 2.4 for Cu soils. Hence, the isotope dilution method could be used for site specific assessment: the existing thresholds based on the large range of toxicity tests spiked with metal salts can be divided by the fraction of labile metal in soil. For example, if only 20% of total soil Zn is isotopically exchangeable in a particular field-contaminated soil, then the generic L/A factor 3 of the existing soil screening limits can be replaced by FS=1/0.2=5 to determine the soil specific limit. This concept is not new since isotopically exchangeable metal fractions measured on numerous aged soils have previously corroborated the L/A factors and supported the deliberation of the finally adopted generic factors by regulators (Smolders et al., 2009). The new information here is that validation has been found for field-contaminated soils.

A proposed tierd risk assessment procedure for metals is presented in Figure 9.1. In tier 1 total concentrations are analysed and compared with national generic soil limits. In tier 2 soil specific soil limits are obtained using the PNEC-calculator using total concentration, clay content, organic matter content and pH as input values. In the site specific risk assessment made in tier 3, soil and contaminant specific soil limits are obtained applying the isotopic dilution method to obtain a site specific L/A factor, which can be used as input to the revised version of the PNEC-calculator.

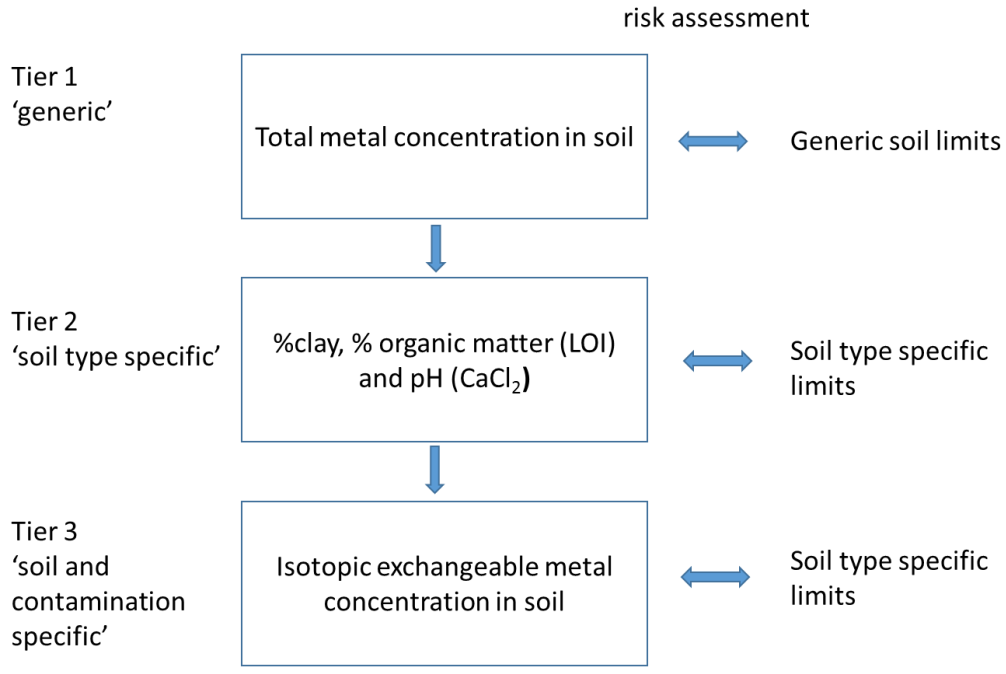
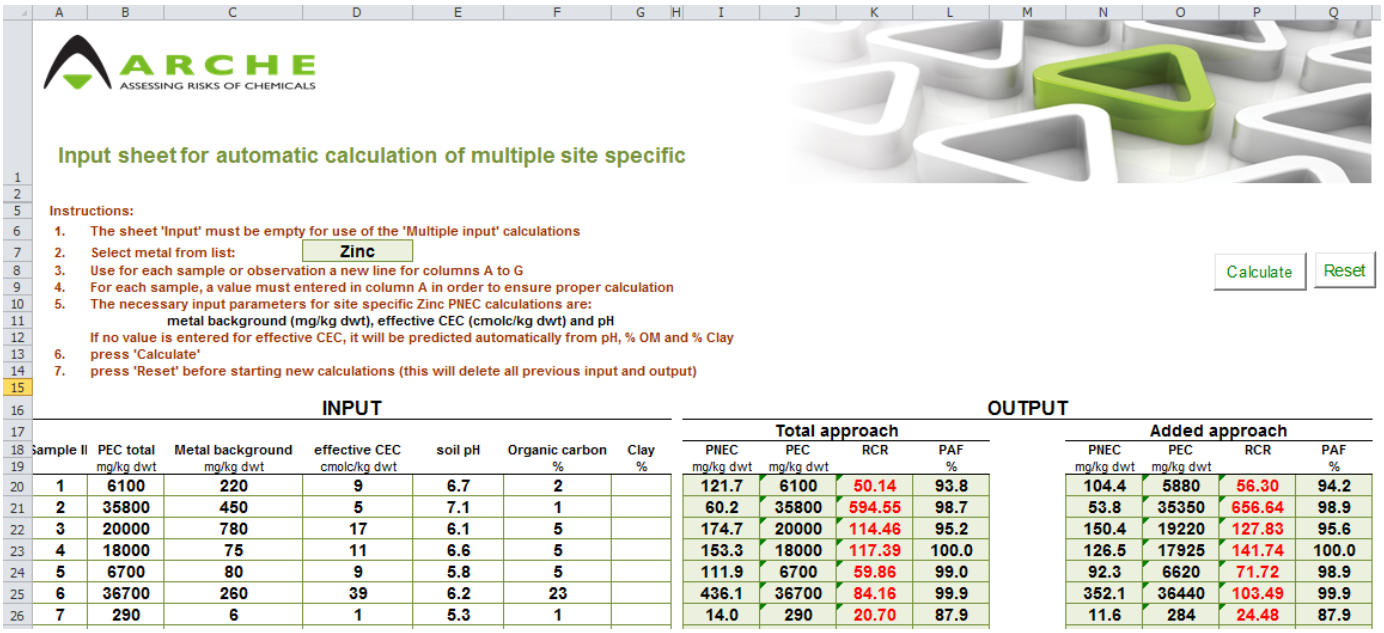


Figure 9.1 A flow chart for conducting a tiered risk assessment applying the isotope dilution method and the PNEC-calculator in tier 3.

The existing software for deriving soil ecotoxicological limits for metals allows for the entry of a specifically chosen L/A factor (ARCHE 2012) and a new version of the PNEC-calculator was developed by ARCHE to enter the site-specific L/A factor. Some additional concept validation studies in other soils with other species may strengthen the proposed methodology. Since an increasing proportion of laboratories have been equipped with ICP-MS, stable isotopes can now be used instead of radiosotopes and isotopic exchange methods are no longer limited to facilities with permission to use radio isotopes.

For risk assessment of a contaminated site (for example Mortagne-du-Nord) total metal content (aqua regia) and isotopically exchangeable metals (E-value, stable isotope dilution method) should be analysed. The E-value can predict the difference in metal toxicity between field-contaminated soils and a soil spiked with metal salt. The isotopically exchangeable Zn for the field-contaminated site Mortagne-du-Nord was 25 % of the total Zn (mg kg^{-1}). The difference in total metal toxicity thresholds or Field/spiked factor (EC50 spiked/EC50 field*100) between the spiked soils and the soils field-contaminated with metals salts was 28 %. This is a very good prediction of the toxicity difference.



INPUT

Sample II	PEC total mg/kg dwt	Metal background mg/kg dwt	effective CEC cmolc/kg dwt	soil pH	Organic carbon %	Clay %
1	6100	220	9	6.7	2	
2	35800	450	5	7.1	1	
3	20000	780	17	6.1	5	
4	18000	75	11	6.6	5	
5	6700	80	9	5.8	5	
6	36700	260	39	6.2	23	
7	290	6	1	5.3	1	

OUTPUT

Total approach				Added approach			
PNEC mg/kg dwt	PEC mg/kg dwt	RCR	PAF %	PNEC mg/kg dwt	PEC mg/kg dwt	RCR	PAF %
121.7	6100	50.14	93.8	104.4	5880	56.30	94.2
60.2	35800	594.55	98.7	53.8	35350	656.64	98.9
174.7	20000	114.46	95.2	150.4	19220	127.83	95.6
153.3	18000	117.39	100.0	126.5	17925	141.74	100.0
111.9	6700	59.86	99.0	92.3	6620	71.72	98.9
436.1	36700	84.16	99.9	352.1	36440	103.49	99.9
14.0	290	20.70	87.9	11.6	284	24.48	87.9

Figure 9.2. Screenshot of the software to calculate a soil type specific PNEC value. ***Available from:*** http://www.arche-consulting.be/metal-csa-toolbox/soil-pnec-calculator/

9.3 PAHs

The studies presented in chapters 6 and 7 regarding the correlation of bioavailability with bioaccumulation in worms and plants respectively, confirmed general findings from the literature (Gomez-Eyles et al., 2012; Cachada et al. 2014, that bioavailability may not correlate to bioaccumulation to diverse species equally well. Here, as in earlier studies, more promising correlations were found

between bioavailability and bioaccumulation in an invertebrate than in plants (Gomez-Eyles et al., 2012; Cachada et al. 2014. From a environmental risk perspective, however, the ultimate parameter to be concerned of is toxicity, and not bioaccummulation. As also shown in this study, links between the bioavailability of native concentration of PAHs and toxicity to exposed organisms are difficult to establish. The reason is, unlike in artificially spiked soils with one clear toxic agent present, organisms exposed to historically contaminated soils face many different types of stresses, with PAHs being one potential contributor, along with soil texture and other types of contaminants present. Thus, the only type of environments where clear links between bioavailability and a toxic endpoint are likely to be established are i) artificial ones in which the contaminant is spiked at different doses (such as most toxicity tests), and ii) situations in which there is only one contaminant present in an otherwise pristine, fertile soil.

Arguably, because of the complexity of multiple-stressors in real-world environments, it is quite unlikely that research will find a single parameter or approach that can anticipate toxicity to all types of species in all types of contaminated environments (Cachada et al. 2014). However, what is feasible, and what has been demonstrated by this study, is that by comparing real-world observations of bioavailability with expectations based on artificial systems, we can improve how testing in artificial systems can be applied to the real world. From this improved extrapolation, it opens the possibility of making more accurate risk assessments.

The largest confirmed discrepancy between artificial systems and real-world contaminated systems relates to partitioning, as independalty observed by the IBRACS researchers in chapter 6 and those in chapter 7, along with several other researchers mentioned earlier. The sorption of the organic content in soils can range from being stronger than coal tar to weaker than octanol, which corresponds to a variety in Koc values up to a factor 1000 between a pyrogenic impacted soil and a reference soil. This discrepancy in partitioning behavior in reference and real-worls systems is likely the largest cause of uncertainty when extrapolating an artificial system to a real-world system, and accounting for bioavailability can address this. There are various ways this can be done. Below we recommend three strategies for making boavailability based risk assessments. As will be illustrated later on in Chapter 12, application of any of these approaches would not only increase the accuracy of lower-tier risk assessments, it would reduce the frequency of expensive, higher-tier risk assessments and remediation operations.

9.3.1 Strategy 1 – Accounting for the Partitioning Regime

The first approach is to use C_{TOC} benchmarks, like MPC_{TOC} from the RIVM (Swartjes et al. 2012, Verbruggen 2012) and described in section 6.2.11, but if necessary re-derive them for historically contaminated soils, which exhibit a different type of partitioning behavior than the reference soils used to derive the C_{TOC} benchmarks (as presented in Chapter 6, and shown with the experimental data presented in chapters 6 and 7). For this re-derivation, if aquatic toxicity assays are used for the derivation of C_{TOC} benchmarks, then appropriate K_{TOC} values for contaminated sites should be used to convert C_w into C_{TOC}, such as the K_{TOC} of coal tar (eq 6.5) and not the K_{TOC} of reference soils or octanol.

When interpreting C_{TOC} benchmarks from soil toxicity assays using reference soils, results here too should be extrapolated to historically contaminated soils. One approach would be to multiply C_{TOC} in the reference soil by the ratio of K_{TOC} in historically contaminated soils to pristine soils; another would be to measure C_{pw} in the soil bioassay and multiply this by the K_{TOC} for historically contaminated soils. When conducting actual risk assessments, however, we also recommend to do some testing of samples to confirm if the on-site partitioning of the native contaminants follows a "octanol" regime, as observed in reference soils, or a "coal-tar" regime. This would indicate which set of guideline values to use. This is presented schematically in Figure 9.3.

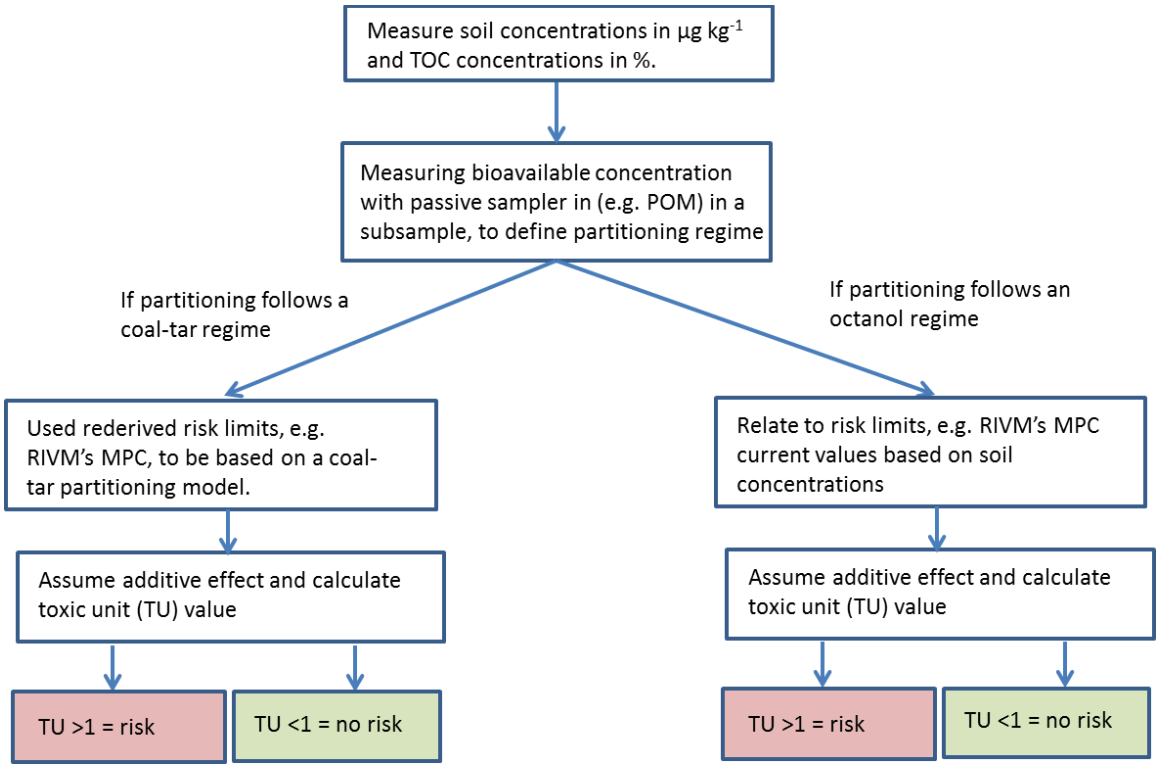


Figure 9.3 Schematic for conducting a risk assessment that accounts for difference in partitioning behavior of pyrogenically impacted sites and reference soils.

9.3.2 Strategy 2 – Porewater based risk assessments

The second approach would be to base risk assessments and soil quality guidelines directly on measured C_{pw} values. Current methods of measuring C_{pw} of PACs are more economical than C_{TOC}, such as the POM technique here or other methods(Hawthorne et al. 2005, Hawthorne et al. 2009, DiFilippo and Eganhouse 2010, Hawthorne et al. 2011b, Lohmann 2012, Ghosh et al. 2014). This would also allow for an easier extrapolation of aquatic bioassays, as no partitioning coefficients are needed. Further, it would be simpler than the above approach using C_{TOC} values, as the local partitioning regime ("octanol" vs "coal tar") would not need to be verified. Potentially, this could also allow for the harmonization of water and

soil quality benchmarks for compounds in which the predominant mode of (eco)toxic action is similar in aquatic and soil systems, like many PACs (Sverdrup et al. 2001, Sverdrup et al. 2002a, USEPA 2003, Verbruggen 2012). A way of conducting PAH risk assessment based on this approach is presented in

Figure 9.4.

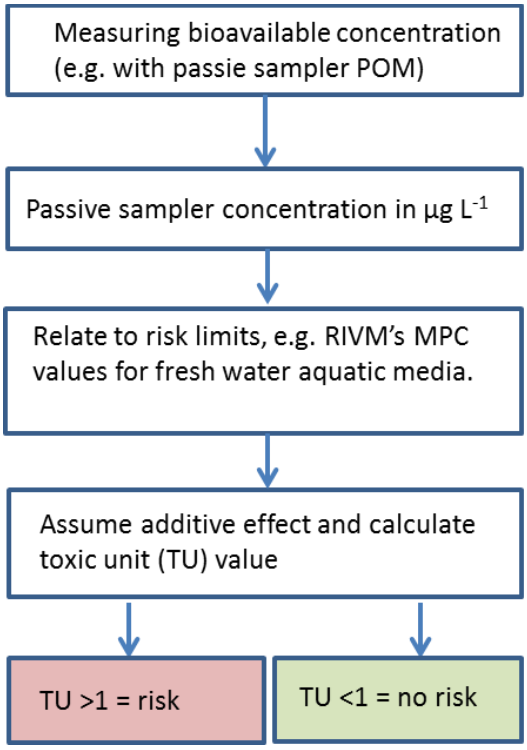


Figure 9.4 Schematic for conducting a risk assessment that is based on bioavailable porewater concentrations.

9.3.3 Startegy 3 – Lipid residue based risk assessments

Thirdly, for cases where it is clear that narcosis through lipid accumulation is the dominant mode of toxicity, like many PACs, benchmarks could also be based on estimated total C_{lipid} of all PACs present (also referred to as internal lipid residues), as has been suggested elsewhere for PAHs, including by RIVM (Verbruggen, 2012). The greatest advantage of this approach, over the previous one, is it would allow for the inclusion of diverse types of bioassays (soil, water, sediment) etc. when deriving benchmarks, as advocated by the RIVM (Verbruggen et al. 2012) and very recently forby Redman et al. (2014). These toxicity data bases of no observable effect resides (NOER, see section 6.2.11) based on large data bases of species, can be used for a diverse variety of media. Of course, they would apply best to all organisms in which lipid narcosis is the main mode of toxicity, which is likely the vast majority of sensitive low food chain numbers. Though C_{lipid} is not a measureable parameter, based on the bioaccumulation work in Chapter 6, we found it can be reasonably estimated through techniques that measure C_{pw} (e.g C_{lipid} =

$K_{liposome}$ C_{pw}), including POM, or through correlations with C_{TOC} or C_{POM} concentrations, such as the calibrations presented in Chapter 6. A schematic of this approach is presented in Figure 9.5.

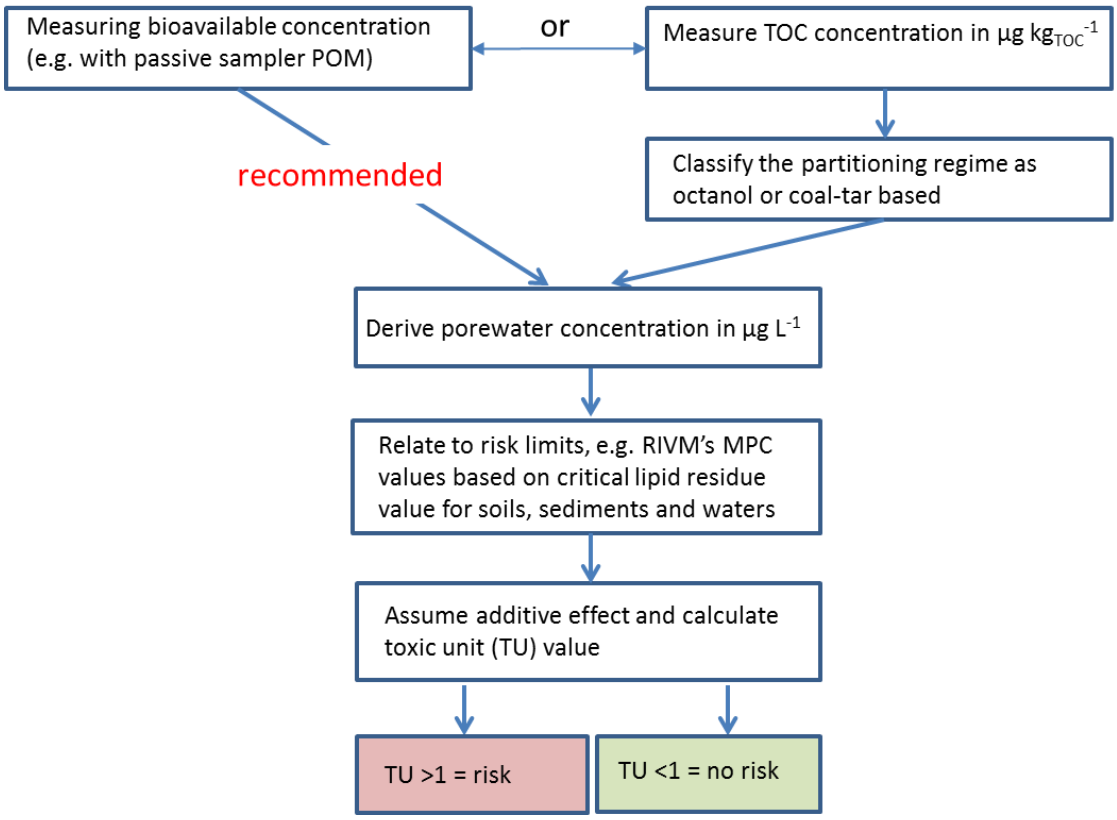


Figure 9.5 Schematic for conducting a risk assessment that is based on derived lipid concentrations, and risk guideline values developed from no observable effect residues (NOERs).

9.3.4 IBRACS calculator

To facilitate in the immediate implementation of some of these recommended approaches for risk-assessment, we have developed the IBRACS calculator. This calculator can be used to automatically calculate both USEPA (2003) and RIVM (2012) based TU values using either Csoil and fTOC as input (following Figure 9.3) or Cpw as input (following Figure 9.4). This calculator can be used for deriving TUs the traditional way, or through NOER derived guideline values (Following Figure 9.5). The IBRACS calculator is available from IBRACS homepage http://projects.swedgeo.se/ibracs/.

10. Recommendation on implementation of chemical methods assessing plant uptake of PAHs in site specific risk assessments

For plants, bioavailability testing has been proposed to improve uptake prediction of PAHs. Unfortunately, little advantage has been found in this study from either POM or TENAX methods. The main transfer route is generally supposed to be through soil solution uptake and risk assessment models rely on pore water PAH concentrations estimated from total soil concentrations using equilibrium partitioning theory. Our results (Chapter 7) lend no support to this hypothesis, because of lack of correlation between determined pore water concetrations and plant uptake. In contrast, uptake by roots was closely correlated to the total soil concentration. This would suggest a direct uptake route between roots and soil solid phase.

The most frequently applied modeling approach used is the one proposed by Briggs et al. (1982, 1983), both for roots and shoots compartments. The hypothesis supporting this model are mostly overruled in the case of PAH (log K_{ow} higher than 4), but it gave the best estimate of PAH uptake in our present study. Thus, it could still be used as a rough estimate of plant uptake using the following procedure:

1. Estimate pore water concentration using polyparameter linear free energy partitioning equation on total soil concentrations (Eq. 10);
2. Apply uptake model for roots and shoots using bioconcentration factors (BCF) given by Briggs et al. relationships (Eqs. 5 and 6).

However, given the great uncertainty in this modelling approach, measurements of plant root and shoot concentrations would be the superior and most accurate option in site specific rsik assessments. Whatever the method used to obatain plant PAH concetrations, they can then be used to calculate daily human uptake of PAHs through vegetable consumption, which in turn can be compared with the (ADI) for each molecule.

11. Using heavy metal bioavailability to derive new soil limit values protecting ecosystems in Wallonia (Belgium)

11.1 Introduction

Within the framework of the IBRACS project, a joint study was performed by the UCL, the consulting company Ram-Ses and representatives from the Walloon Soil Protection Direction (SPW/DGARNE/DSD/DPS) to examine the feasibility of introducing bioavailability into the current legislation on soils. Four working meetings were held (October 28, 2013; January 21, April 2 and April 22, 2014) and a report was delivered to the public authorities (Sonnet, Stas and Halen, Second progress report, April 2014, in French).

Starting with a brief presentation of the soil context in Wallonia (Belgium) including a summary of the soil legislation, its implications and the importance in distinguishing local pollution from proximal

atmospheric pollution (PAP), this chapter explains how bioavailability can be implemented to refine the soil limit values protecting ecosystems. We chose to use copper for our case study, as this metal was one of the four metals investigated in the experimental part of IBRACS on metals. We decided to avoid introducing bioavailability into the text of the legislation in a way that would entail modifying the legal numerical values used as soil quality standards. Instead, we chose to introduce it into the step of the legal procedure that allows the most flexibility, namely the risk assessment studies.

11.2 The Walloon soil context: a rapid overview

11.2.1 The Walloon soil law

Like many other countries and regions in Europe, Wallonia (southern part of Belgium) has experienced an intensive and glorious industrial past leaving behind many polluted sites. The management of these industrial sites in Wallonia is governed by the Law of the 5th of December 2008 on the management of soils (generally referred to in Wallonia as the Walloon soil decree, but will be referred to throughout this chapter as the Walloon soil law). The law specifies:

1. who is responsible for initiating action on (potentially) polluted sites;
2. which action should be taken on (potentially) polluted sites, i.e. the successive steps of the technical-legal procedure (including an orientation or simplified investigative study, an in-depth or characterization study which may include a risk assessment study, and, if required, the remediation of the site and its final assessment); and
3. how the decisions are to be made concerning the need to undertake an in-depth study, the need to remediate and/or to manage the risks through mitigation measures.

The Walloon normative system (the set of numerical standards on soil and groundwater concentrations for the different pollutants) consists of three soil quality standards. The first one is the reference value (*valeur de référence* - VR) corresponding to an indicative value of natural and normal background concentrations present in soil and groundwater. The second soil quality standard - which plays the most important role - is the trigger value (*valeur seuil* - VS). The implications of exceeding this trigger value depend on the type of pollution. In the case of historical pollution (pollution that occurred prior to 30/04/2007), exceeding the VS implies further investigative studies and a risk assessment study to establish the existence (or not) of a « serious threat » as defined in the Walloon soil decree. In the case of new pollution (subsequent to 30/04/2007) exceeding the VS implies remediation.

The third soil quality standard is the intervention value (*valeur d'intervention* - VI) which implies - when exceeded in the case of historical pollution - an action (which may take the form of remediation, safety measures and/or monitoring measures). The trigger values (VS) and intervention values (VI) for the soil are defined for five types of land uses: natural, agricultural, residential, recreational/commercial and industrial. The trigger values defined for groundwater are the same irrespective of the type of land use.

Unlike the reference values (VR), the trigger (VS) and intervention values (VI) are risk-based soil quality standards. Three kinds of risks are taken into account: risks for human health, risks for groundwater (via

leaching and dispersion) and risks for ecosystems. For each type of risk, specific values (referred to as risk limit values) are derived. The most restrictive of the three risk limit values calculated for each type of risk (VS_H for human health, VS_N for groundwater and VS_E for ecosystems) is used to define the trigger value (VS). The same is done to define the intervention value for each of three types of targets (VI_H for human health, VI_N for groundwater and VI_E, for ecosystems) corresponding to a higher risk level. These limit values (VS_H and VI_H, VS_N and VI_N, VS_E and VI_E) are used in the risk assessment procedure as explained below (see section 11.2.4).

In addition to the Walloon soil law, the Walloon Codes of Good Practice (CWBP), issued by the Walloon Soil Protection Direction, provide all the procedures (technical guidance) to be followed.

11.2.2 Importance of distinguishing local pollution from proximal atmospheric pollution (PAP)

In the "decision tree" about soil pollution introduced by the Walloon soil law, the implications of exceeding the soil quality standards also depend on the origin of the pollution. In this respect, Figure 11.1 illustrates the distinction that is made between local pollution versus proximal atmospheric pollution (PAP).

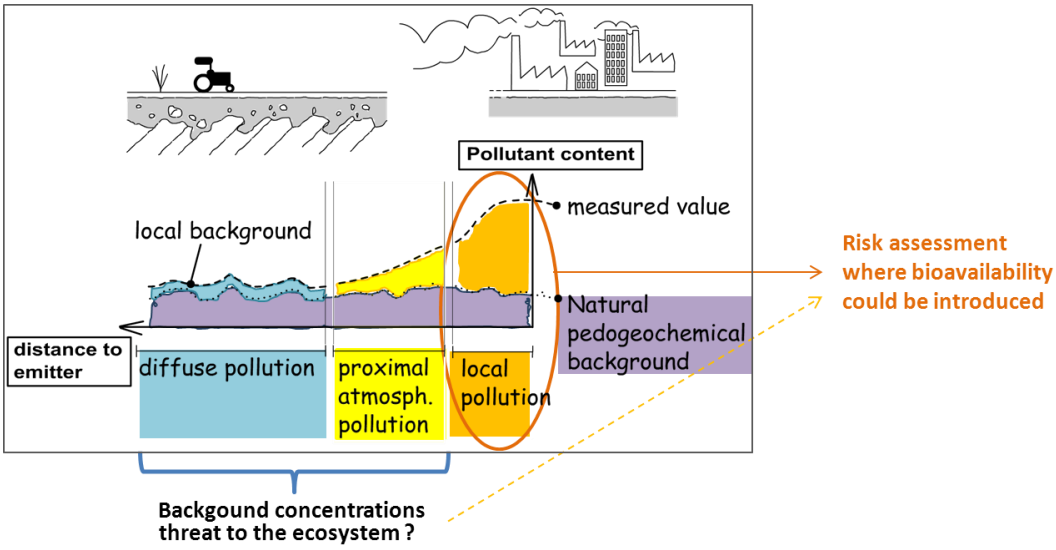


Figure 11.1 Concepts used to designate pollution in Wallonia as a function of the distance to the emitting source. The left-hand side of the diagram represents a location far away from any source of pollution. The right-hand side represents a location close to a single and well identified pollution source (local pollution). Purple: natural pedogeochemical background (originating from the parent material); blue: generalized diffuse pollution that is present even far away from any source of pollution; yellow: proximal atmospheric pollution due to historic or current sources that cannot be identified individually; orange: a spatially limited area affected by one or multiple pollution sources that are well identified individually; dashed line: where there is no local pollution, the Walloon soil legislation considers the measured pollutant concentration in the soil as the "background concentration". *Guide pour la définition des concentrations de fond en polluants dans les sols de Wallonie. Cahier de Bonnes Pratiques n°10*, Pereira, Sonnet and Capette, 2010, 84 p., SPAQuE.

The right-hand side of Figure 11.1 refers to *local pollution*, i.e. a piece of land where the source of pollution is well designated and where a simplified investigative study and (usually) an in-depth study including a risk assessment study are required by the Walloon soil law.

When no unique or clearly designated pollution source can be determined and when the levels of pollutant that are measured are in the range of concentrations generally found in the surroundings, the soil is affected by proximal atmospheric pollution (underlined by a blue bracket, Figure 11.1). In this situation, the concentrations that are generally found in the surrounding area are referred to as the background concentrations (dashed line in Figure 11.1). A problem (risk) which may arise in this situation is that, although the pollutant concentrations are in the range of the background concentrations, the level of these background concentrations is high, often well above the natural pedogeochemical background. These high levels are a consequence of industrial pollution sources that were active in the past as well as of the relatively high proportion of densely urbanised areas in Wallonia.

The extent and the significance of these high background concentrations in Wallonia will be illustrated hereunder for zinc (see Figure 11.2). This figure illustrates the background concentration for zinc for which proximal atmospheric pollution in soils occurs in many places in Wallonia. For a natural land use for example, the trigger value is determined by the protection of the ecosystem target and corresponds, according to the current values of VSE, to 117 mg/kg of zinc in the soil. The map of the zinc background concentrations for soils in Wallonia shows that this limit is exceeded in several places. The overall proportion of the area in Wallonia where this value is exceeded for zinc is estimated to be 7.7%. This proportion is significant and clearly demonstrates the importance, for the public authorities, to assess the risks for such areas where the background concentrations are above the trigger values.

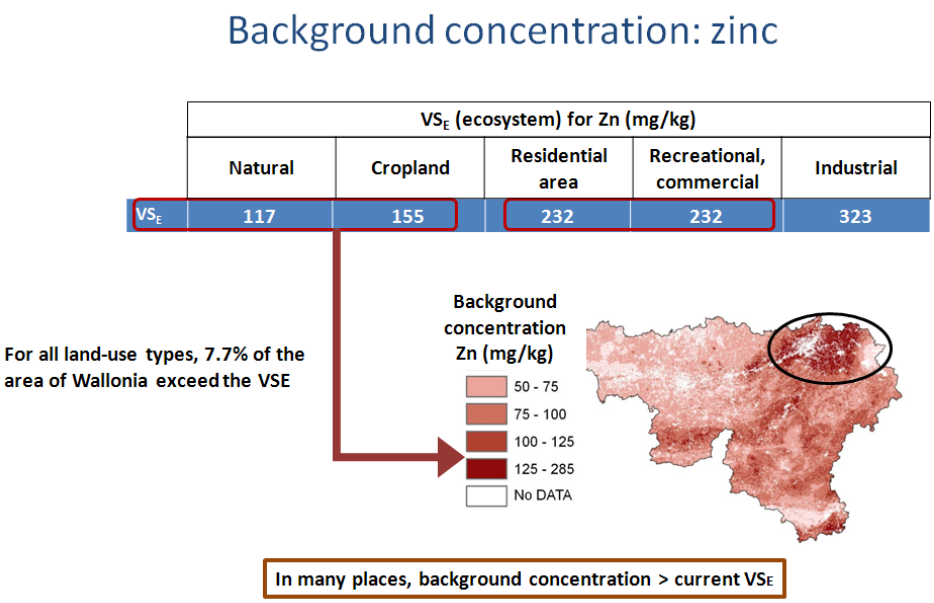


Figure 11.2 Upper table: current zinc limit values for the ecosystem (VSE). Map: zinc (Zn) background content in Wallonia. Left hand side: proportion of the overall area of Wallonia where the zinc concentration exceeds the current limit value for ecosystems (VSE).

11.2.3 Legal implications of exceeding the threshold values VS as a function of the legal status of the pollution: local pollution versus proximal atmospheric pollution (PAP)

The essential aim of the Walloon soil law is to clearly indicate in which cases a piece of land must be evaluated to determine whether or not it is polluted and who is responsible for taking action (Figure 11.3).

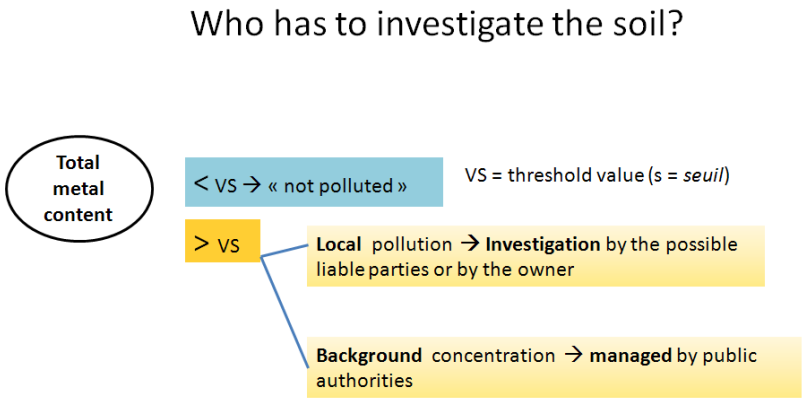


Figure 11.3 The mechanism in the Walloon soil law that designates who is responsible for taking action when the total content exceeds VS.

According to the Walloon soil law, if the measured total pollutant content (the metal content in our case study) in the soil is lower than the trigger value (in blue in Figure 11.3), the land is considered to be "not polluted" and no specific action is needed.

If the measured total pollutant content (here metal content) exceeds the trigger value (in orange in Figure 11.3), an investigation must be carried out provided that this content is due to local pollution.

If, on the contrary, the pollutant content is related to the background concentration (because of proximal atmospheric pollution, for instance), there are in principle no liable parties and the Walloon soil law also does not require any action on behalf of the landowner. However, the public authorities might want to know whether there is a risk (or not) associated to this background concentration. If there is a risk, recommendations must be given and restrictions concerning the type of land use could be imposed in order, for instance, to reduce the population exposure to soil pollutants.

A more detailed view of the lower part of the preceding figure is given in Figure 11.4. On the right-hand side of the figure, the pollutant content is considered to be due to local pollution. During the simplified site study (*étude d'orientation*), the concentrations are compared to the legal soil quality standards (VS and VI defined for a given land use). If the trigger value for a given pollutant is exceeded, a detailed site study (*étude de caractérisation*) must be carried out to determine the extent of the pollution and a risk assessment study may be requested.

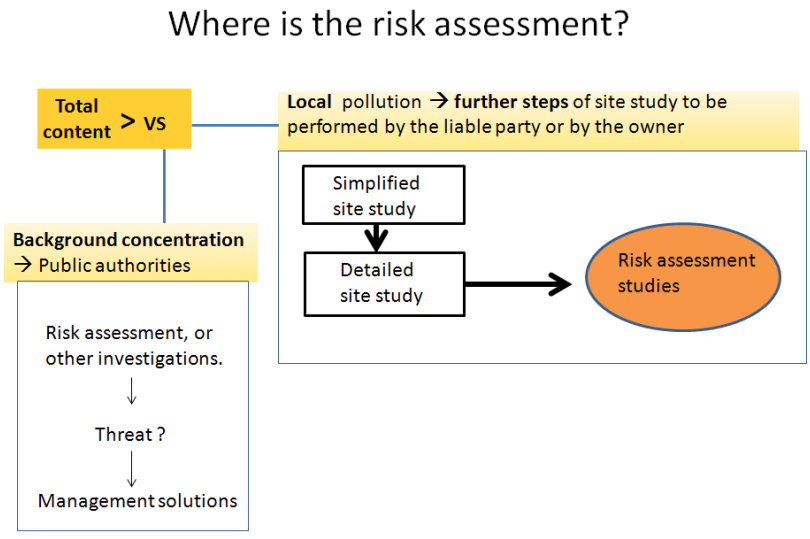


Figure 11.4 Steps in the Walloon soil law where a risk assessment studies could be performed (red ellipse or the left-hand side of the figure).

11.2.4 Risk assessment procedure: simplified (tier 1) and detailed (tier 2) risk assessment

The risk assessment procedure is detailed in the Reference Guide for Risk Assessment (GRER) issued by the Walloon Soil Protection Direction in December 2012. It provides recommendations for the assessment of risks to human health (Part B), for groundwater (leaching and dispersion risks; Part C) and for ecosystems (Part D).

The Walloon risk assessment is a two-tiered procedure (Figure 11.5). The first tier is a simplified risk assessment where the total pollutant concentrations are compared to the risk limit values defined in the GRER for each type of risk (human health, groundwater and ecosystems) and for the five pre-defined land uses. The aim of the simplified risk assessment is to evaluate if there is enough evidence to support the hypothesis of « serious threat (or a biological stress for the ecosystems) ». If this is the case, the procedure is implemented either by performing a second tier site-specific detailed risk assessment study or by directly undertaking remediation of the polluted land.

At the end of the process, a certificate of soil compliance (blue boxes in Figure 11.5) is delivered and is only valid for the intended land use.

In the case of PAP, where the soil has been affected by proximal atmospheric pollution and the pollutant content then qualifies as “background concentration”, the Walloon soil law does not require any action on behalf of the landowner. However, the public authorities can decide either to carry out a risk assessment study to evaluate the risks associated with this proximal atmospheric pollution or to take direct measures to mitigate the risks.

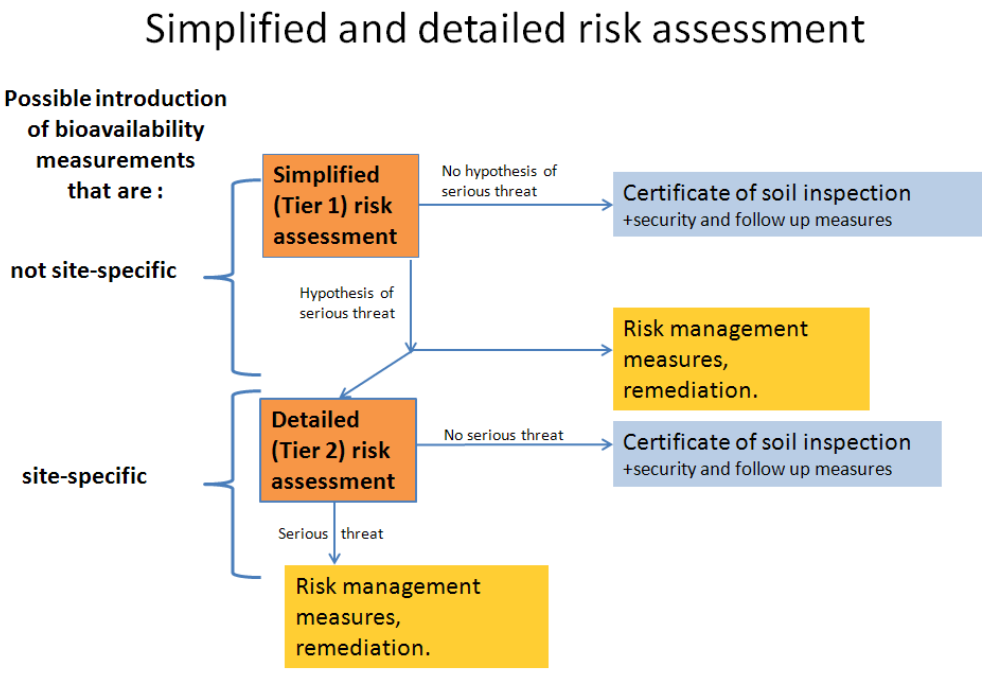


Figure 11.5 The Walloon risk assessment studies within the detailed site study. The first-tier risk assessment study (upper red rectangle) is a simplified procedure which has two possible outcomes: an hypothesis of serious threat (or biological stress for ecosystems) or an absence of serious threat. The second-tier risk assessment study (lower red rectangle) is a detailed study and has two possible outcomes: confirmation of the presence or of the absence of a serious threat.

11.3 Implementation of bioavailability in the Walloon context

Having the Walloon soil decree and its implications in mind and considering that a substantial portion of the Walloon territory is affected by proximal atmospheric pollution (PAP), we examined where and how (section 11.5) bioavailability could be introduced into the current reference tools used by the legislation. We concluded that bioavailability could be implemented at both tiers of the risk assessment procedure, i.e.:

- in the simplified risk assessment study (tier 1) by implementing bioavailability in the procedure used for calculating the risk limit values protecting ecosystems (VS_E and VI_E); this procedure is currently based on ecotoxicological limits derived from laboratory toxicity tests;
- in the detailed risk assessment study (tier 2), by measuring bioavailability on-site or/and by introducing those site-specific values in specific software that calculates risk at the ecosystem level (e.g. TerraSys^{TM} for ecosystems).

For this purpose, we proposed to the officials of the Walloon Soil Protection Direction to evaluate how bioavailability could be introduced in the definition of (new) limit values for ecosystems (more specifically the VS_E) and to perform a case study having the following characteristics:

1. land use: agricultural in Wallonia
2. trace metal element: copper
3. a risk assessment study must be performed, either because it is required by the legislation or because it is intended to provide a basis for the public authority to take action
4. target for the risk assessment study: the ecosystem

11.4 Choosing a method for correcting the limit values for ecosystems (VS_E) taking into account bioavailability

Current limit values for ecosystems (defined in Part D of the GRER) are based on the results of ecotoxicity studies performed in laboratory settings where the metal is generally introduced into the soil as a soluble salt. As a consequence, natural phenomena such as leaching and ageing do not take place, thereby resulting in an underestimation of the toxicity limits. These laboratory studies confer thus a toxicity that is higher than what is found on-site in polluted soils. This was clearly demonstrated by the IBRACS experimental work on trace metal elements (see chapter 5). What we aim to do therefore is to correct the limit values that are currently used by taking the leaching-ageing (L/A) factor into proper consideration.

Our proposal, accepted by the officials of the Walloon Soil Protection Direction, was to adopt a method inspired by the one used in the PNEC Calculator for our case study. As illustrated by Figure 11.6, bioavailability is taken into account in the PNEC Calculator through the use of the LA leaching-ageing factors (the LA factors in the PNEC Calculator correspond to the L/A field-spiking factors used in IBRACS).

Since the experimental work in IBRACS focused on L/A field-spiking factors, we used the results obtained from the Walloon soils to verify that the L/A parameters used in the Calculator were valid for the soils that are found in Wallonia. As shown in the table in Figure 11.6, the L/A factors used in the PNEC Calculator are generally lower than the L/A factors that were measured by IBRACS for the four Walloon soil samples. For zinc, the measured L/A values are higher than the L/A value of 3 used in the PNEC calculator (except for Plombières). This means that, by using the L/A value of 3, the PNEC calculator overestimates the toxicity and thus underestimates the ecotoxicity limits. For the soil of Sclaigneaux, for instance, the PNEC Calculator provides ecotoxicity limits (NOEC, No Observed Effect Concentration) that are lower than the ecotoxicity limits (EC_{10}, 10% Effect Concentration) determined by IBRACS for this soil. Aside from the soil from Plombières, using the PNEC calculator to take bioavailability into account provides therefore limit values that are safe. As shown in the lower part of the table in Figure 11.6, other results from IBRACS also support the validity of the parameters used for copper and for nickel in the PNEC Calculator.

Thus the IBRACS study demonstrates that the L/A factors used in the PNEC calculator can be considered as conservative for the soils of Wallonia[†]. Nevertheless, as mentioned in Smolders et al. (2009), even if they are derived from experimental data, the L/A values selected for the different pollutants (and used in PNEC calculator for instance) are mainly based on an expert judgment trying to balance the reality of the data (the relative dispersion of the experimental values), together with precautionary aspects and realism aspects. Consolidating the final values of L/A factors to be retained for deriving ecotoxicological limit values (VSE) and defining the principles to be adopted for using appropriate L/A factors in the framework of the first step of the ecological risk assessment process are two tasks that should be considered at the policy level (the policymaker's sphere). In this perspective, further research on assessments of parameters (a.o. soil properties) that determine the experimental values of L/A factors for different pollutants should be of real interest.

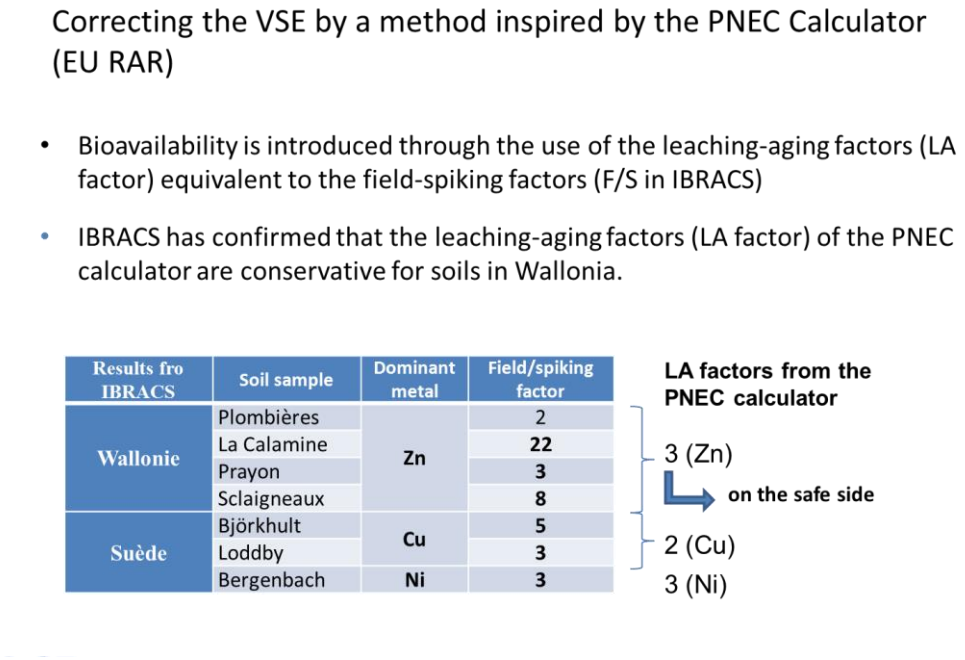


Figure 11.6 Comparison between the LA factors used in the PNEC calculator and the L/A field-spiking factors measured during the experiments performed in IBRACS on metal polluted soils.

11.5 Proposed method for introducing bioavailability into the VS_E calculation

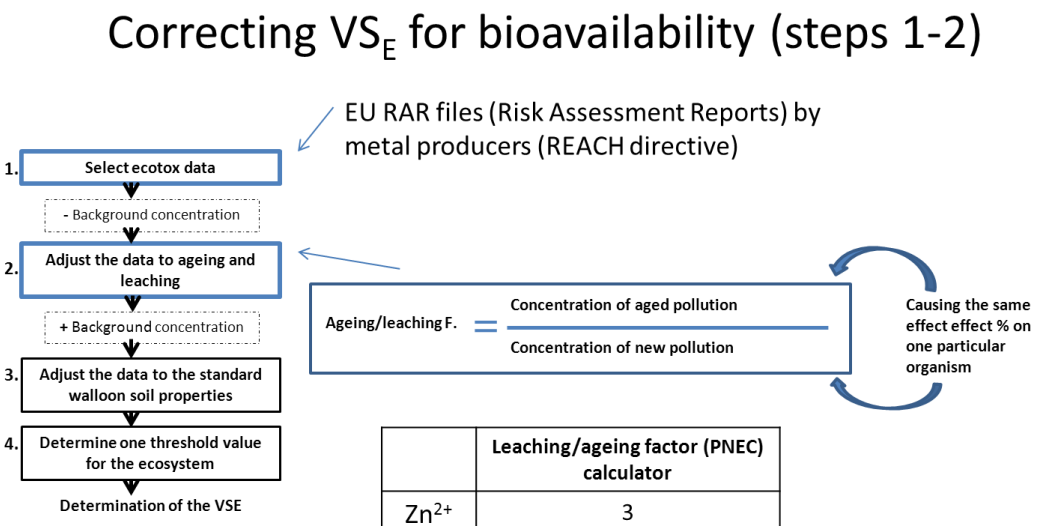
The general objective of the method is to introduce the concept of bioavailability into the calculation of the risk limit values for ecosystems (VS_E) which are currently used in the risk assessment studies. These limit values are based on laboratory ecotoxicity tests where the metal is generally added to the soil as a

[†] The L/A factors could be used in the framework of Walloon soil legislation particularly for historic pollution cases, where the soil has already undergone ageing and leaching processes.

soluble salt and therefore leaching and ageing do not take place. To correct for this absence of leaching and ageing, a method similar the one used in the PNEC Calculator has been applied. As previously explained (section 11.3), our case study investigates an agricultural type of land use with copper as the trace metal element and the ecosystem as the target for the risk assessment study.

With these goals and constraints in mind, we devised a method to introduce bioavailability into the current existing Walloon legislation that involves four steps.

The first step (Figure 11.7) consists in gathering all available ecotoxicity data (NOEC) for a wide range of living organisms (plants, invertebrates, microbes, etc.) so as to represent the spectrum of taxonomic groups that are present in the ecosystem. For our case study, we used the EU RAR files (European Union voluntary Risk Assessment Report) that have been collected by metal producers for the REACH Directive .



	Leaching/ageing factor (PNEC) calculator
Zn^{2+}	3
Cu^{2+}	2
Ni^{2+}	1-3 (depending on pH)
Co^{2+}	1,1-3,5 (depending on pH)
Pb^{2+}	4,2
Cd^{2+}	1

Figure 11.7 The first two steps of the method for introducing bioavailability into the Walloon legislation. Left-hand side: sequence of steps to apply the proposed method to any one pollutant (Cu, in our case study). The data necessary for the first two steps are examined here. For step 1, Risk Assessment Reports by metal producers have been used to provide ecotoxicity data for a range of organisms present in the ecosystem. For step 2, the definition of the leaching/ageing factors is given. The table presents the leaching/ageing factors used in the PNEC Calculator. For nickel (Ni) and cobalt (Co), the factors can be adjusted for site specific soil parameters.

The second step (Figure 11.7) consists in correcting the ecotoxicity limits (NOEC values) by multiplying them by the L/A values (developed by Smolders et al. (2009) and by analogy with the process in use in the PNEC calculator method). As a result, the ecotoxicity limits (adjusted NOEC) are no longer expressed in terms of concentration of copper introduced as soluble salt but as the total metal content of a soil that would have undergone the normal leaching and ageing processes taking place under field conditions.

The third step (Figure 11.8) consists in individually adjusting the NOEC values for each living organism to the standard Walloon soil that must be used in the risk assessment investigation depending on the type of land use. The adjustment of the NOEC value is performed by an exponential equation. The exponent (slope Figure 11.8) can be found in the EU RAR files for the soil parameter (CEC, pH, clay content or organic matter content) that influences the toxicity of the metal on the organism under consideration or its function in the ecosystem. Figure 11.8 illustrates how the copper ecotoxicity limit has been corrected to take into account that the CEC is higher in the Walloon standard soil than in the toxicity experiments found in the literature.

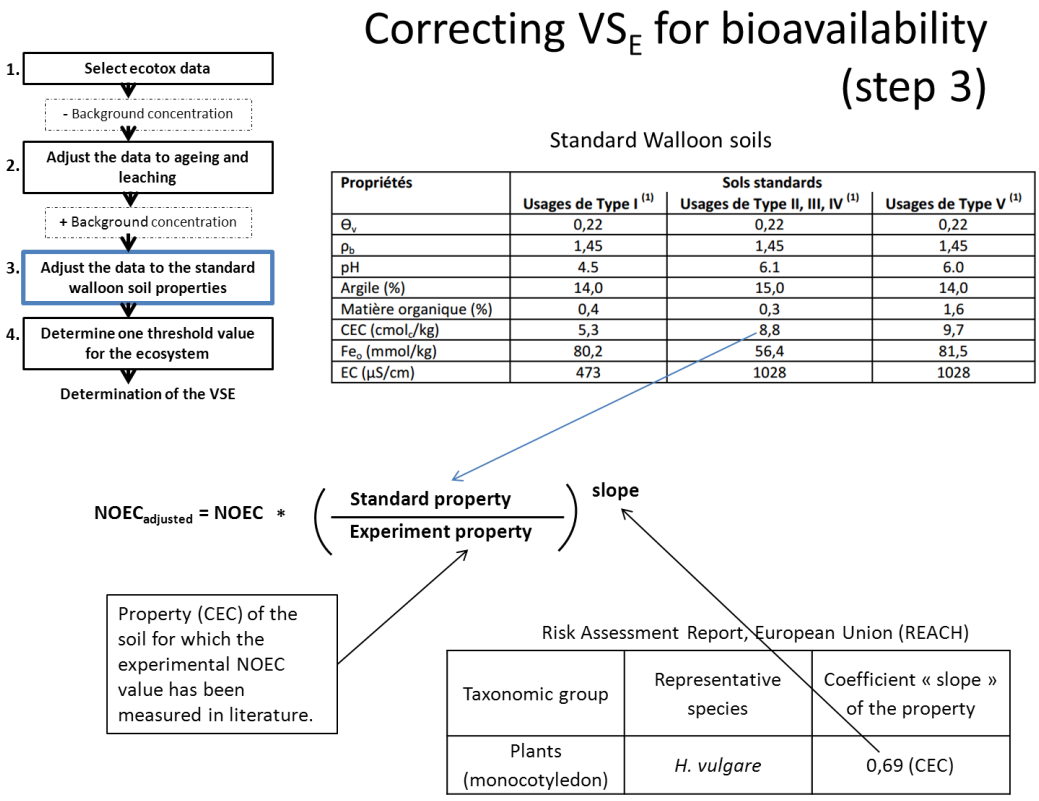
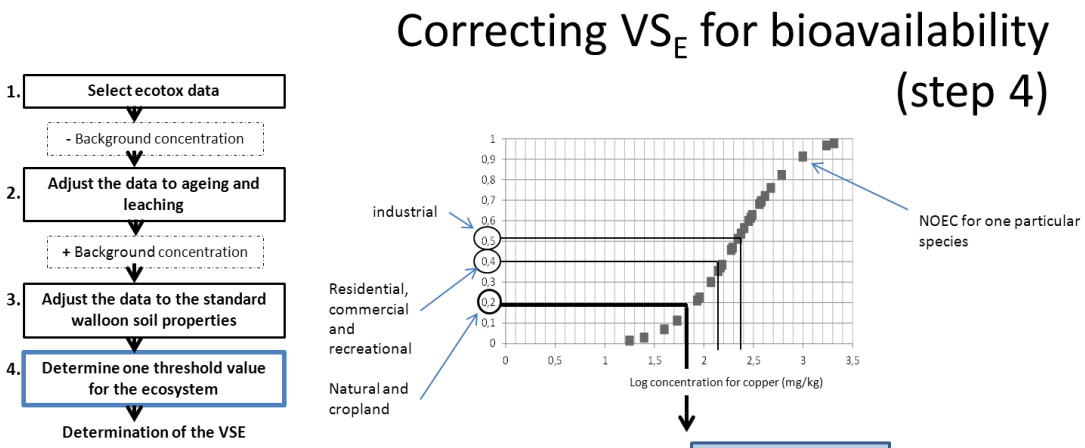


Figure 11.8 The third step of the method for introducing bioavailability into the Walloon legislation. NOEC (No Observed Effect Concentration) for each organism is adjusted to the properties of each one of the three standard Walloon soils using the given equation. The slope, obtained by regression, is found in the EU RAR files. The upper table presents the soil parameters of the three Walloon reference soils. Type I corresponds to a "natural" land use ; Types II, III and IV correspond to agricultural, residential, commercial/recreational land uses and type V to industrial land use. The lower table provides the "slope" for the effect of CEC on the bioavailability of Cu for monocotyledon plants (one of the organisms used in our case study).

Finally, the fourth step consists in obtaining the corrected limit value for ecosystem ($VS_{E\text{-corrected}}$ (Figure 11.9). To this end, all the NOEC values corrected for leaching-ageing and adapted to a given Walloon standard soil (depending on the land use) are plotted in a cumulative percentage frequency plot. Each point represents the NOEC that has been obtained for one particular species. The X-coordinate of the point is the threshold concentration that cannot be exceeded if the species is to be protected. Any horizontal shift to the right relative to the point representing the organism signifies that the organism will be adversely affected. The Y-coordinate (circled numbers in Figure 11.9) is the fraction of the organisms present in the ecosystem that is potentially affected. . These are the proportions that were chosen by the Walloon administration in order to establish the current VS_E values. In our case study considering an agricultural land use, this fraction is 20 %. In other words the proportion of the organisms present in the ecosystem that must be protected is equal to 80% (100% – 20%).

From the curve of the cumulated proportion, it can be observed that the corrected limit value for the ecosystem ($VS_{E\text{-corrected}}$) that affects less than 20% of the organisms is 83 mg/kg Cu. This new VS_E value now takes bioavailability into account. In Figure 11.9, the new VSE value is compared with the current risk limit value, which is 46 mg/kg. Correcting the VS_E for bioavailability therefore leads to higher values which make it possible to carry out more realistic risk assessment studies.



Copper: VSE corrected for bioavailability (mg/kg, total metal content)

Land use	Natural land	Cropland	Residential	Commercial	Industrial
Current VSE	40	46	110	110	117
VSE corrected for bioavailability	68	83	162	162	214

Figure 11.9 The fourth step of the method for introducing bioavailability into the Walloon legislation. Graph X-axis: logarithm of the total concentration of copper (Cu) corrected for leaching/aging and for the properties of the Walloon standard soil; Y-axis: cumulative proportion of organisms that are affected, expressed as a fraction of 1. Each point represents one particular species of the ecosystem for which a NOEC value has been obtained by following the first three steps. Circled numbers along the Y-axis are the proportions of the organisms present in the ecosystem that must be protected. The table compares the VSE values currently used in the simplified risk assessment procedure to the new values corrected to account for bioavailability.

11.6 Future work

Future collaborative studies with the representatives of the Walloon Soil Protection Direction could focus on two other procedures that are recommended by the GRER for the most advanced investigations in tier 2. These advanced investigations correspond to what many countries generally refer to as tier 3 risk assessment.

The first procedure is the Sediment Quality Triad (SQT) approach (Chapman 2000), which could also benefit from introducting of the concept of bioavailability. This approach involves a chemical component (total analyses, chemical extractions), a toxicological component (biotests performed on the soil) and an ecological component (the observed ecosystem is compared to that of a reference site). Indexes are attributed to each component on a 0-1 scale and are integrated using a weight of evidence method.

The second procedure is the TerraSys software[‡], which provides an integrated approach (chemical, toxicological, ecological, risks on target organisms, responses of various trophic levels). The software computes risk indexes which take into account site-specific bioavailablity for target organisms. Each risk index is computed as a ratio between the exposure dose and a reference value. The software could benefit from more accurate estimations of the dose and reference values made possible by improving the way bioavailability is taken into account.

The approach presented in chapter 9.2, based on the isotope dilution method and the PNEC-calculator, can be used as a chemical assay in these procedures.

12. Cost-benefit analysis of applying bioavailability in site specific ecological risk assessment – some case studies

Due to on-going soil investigations and remediation actions, contaminated soil is becoming a common waste at landfills. In Sweden, and in many other countries, the majority of site remediation cases, contaminated soil materials are handled as wastes, i.e. excavated and disposed of. As a consequence, contaminated soil has become the largest contributor of hazardous waste to the Swedish landfills. About 850 000 tons of contaminated soil was destined for disposal in 2012 (SEPA, 2014), which composed ca 1/3 of the total amount of hazardous waste generated in Sweden.

Guideline values for soil contaminants comprise their total concentrations in soil. Site specific conditions such as soil organic matter (OM), cation exchange capacity (CEC) and pH can affect contaminant mobility, bioavailability and toxicity. Including these factors into risk assessment might show a different risk level for particular sites, which may help to reduce the need for relocation of large volumes of soil. Such risk assessment, however, is often associated with increased time and costs of investigation, although these costs may be compensated by avoiding over-remediation and generation of unnecessary waste and emissions. It is therefore highly relevant to develop tools allowing for a more precise assessment of risks at contaminated sites and in a more cost efficient way.

In this chapter we apply the methodologies proposed in chapter 9 for metals and PAHs on two metal contaminated sites and two PAH contaminated sites. The aim is to see to what extent the risk assessment for soil protection will be changed, compared to an assessment based on conventional national risk limits, and estimate to what extent this will effect the cost of remediation (assuming a the same remediation technique).

12.1 PAH sites

Two Swedish sites were chosen for the evaluation, Wermlandskajen in Karlstad and Riksten in Botkyrka (close to Stockholm). At both these sites several samples were taken, which is contrast to the sites in

[‡] Terrasys, created by SANEXEN, is a professional software for ecotoxicological risk assessment of contaminated sites. http://sanexen.com/en/terrasys/terrasys-in-brief/what-is-terrasys/

France and Belgium where sampling was focused on hotspots at individual sites. In the evaluation we used the POM method and RIVM's screening values based on critical lipid residues (NOER values), as shown in Figure 12.1. As a comparison we also used US EPA sediment benchmarks as screening value. The calculations were made using the the IBRACS TU calculator (http://projects.swedgeo.se/ibracs/).

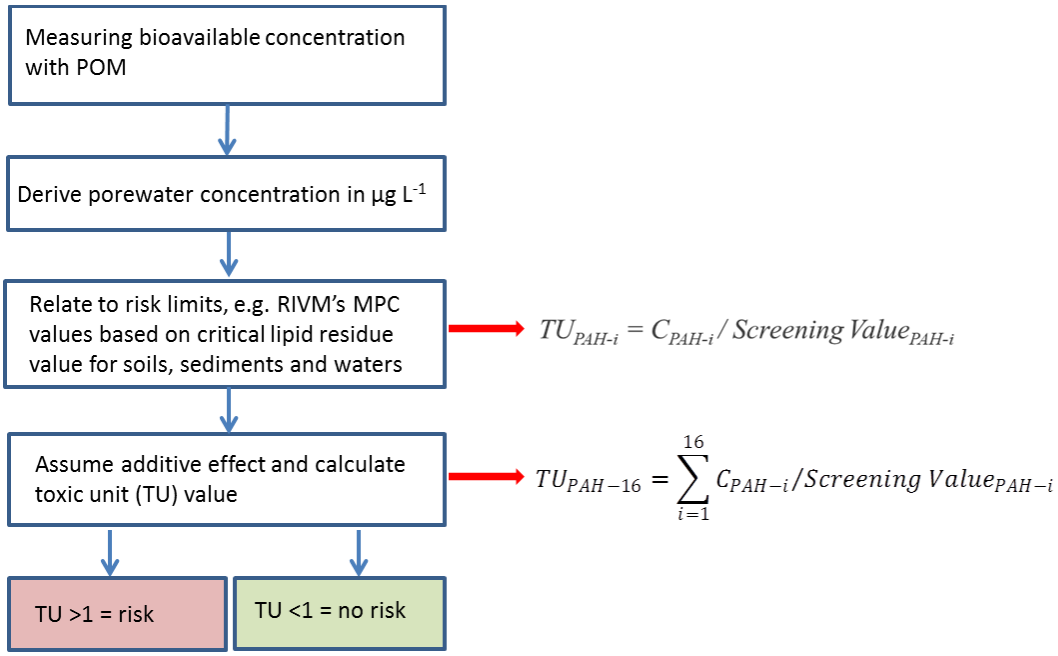


Figure 12.1 The procedure used to derive site specific "Serious risk concentration" (RIVM SRC) and "Maximum permissible concentration serious risk concentrations (RVIM MPC (SRC). The procedure is identical to the one described in Figure 9.5 using the POM approach.

12.2 Case study 1: Riksten

12.2.1 Background

All following information of this chapter is obtained from a MIFO (Methods for Inventories of Contaminated Sites) form filled by Erik Blomqvist (MIFO report, 2012). The MIFO form contains information on the investigated site providing answers to a number of questions related to the present conditions at and around the site. Associated risks to humans and the environment are then defined considering the following aspects: hazard assessment, contamination level, migration potential and sensitivity/protection value. In the final step, the four aspects are weighed together in a comprehensive assessment, which is used to assign the site to one of the four risk classes: class 1: Class 1: Very high risk; Class 2: High risk; Class 3: Moderate risk; Class 4: Low risk.

The investigated site was formerly used by Rikstens Kol- och Tjärfabrik in Botkyrka municipality for production of coal and petroleum products from wood, including coal, tar, turpentine and coal-tar oils. The factory was shut down over 60 years ago.

It was estimated that the size of the area that may have been affected by the manufacturing activities is about 30-50 m wide and stretching out towards the Lake Bysjön (ca 25-30 m). The amount of contaminants was estimated to be very high, as the entire surface of the area is covered with coal as well as in several spots with an unidentified solidified product. Tar was detected in a number of places.

Contaminant hazard assessment: According to the Swedish EPA's classification of industrial contaminants, the hazard of the identified substances is considered as very high.

Migration potential: The soil in the area largely consists of sand, therefore the migration potential of the contaminants through the soil and groundwater was assessed to be very rapid. The migration potential to surface water was also considered to be very high due to the steep slope from the former factory towards the lake, which creates conditions favouring the transport of pollutants towards the lake.

The sensitivity of the site was assessed as very high considering the surface water was assumed to feed Lake Bysjön with contaminants. The lake is classified as a secondary protection zone for one of Botkyrka municipality's groundwater sources. Considering soil, the sensitivity is assessed as high since the area is not enclosed and is accessible to the public.

The protective value was judged to be very high regarding the soil and water since the area is classified as of national interest for outdoor recreation (Ågesta-Lida).

In the comprehensive assessment, the entire investigated area (from the coast in the west to the mill in the north) was taken into consideration, including waste, debris, rusted containers, etc. According to the investigators, the area is heavily polluted.

On the basis of the above arguments, the object was assessed as Class 1: Very high risk to human health and the environment.

12.2.2 Site specific ecological risk assessment

PAH analyses were performed on the samples collected in a close vicinity to the former factory (Figure 12.2). Soil PAH concentrations exceeded SEPA guideline values for soil with sensitive use (7 mg/kg dw) in five out of ten samples. Based on the site investigation according to the MIFO methodology, the site was assigned to the risk class 1, meaning that it has a high priority to be remediated.

Taking PAHs as the main contaminants at the site, the calculated toxicity units based on porewater concentration using US EPA sediment benchmarks and RIVM "Serious risk concentration" (SRC) as references, indicate that all the measured porewater concentrations might not cause any considerable risks for soil ecosystem (Table 12.1). Only when using the most conservative RIVM "Maximum permissible concentration" (MPC) as reference, the calculated TU predicts a risk of chronic exposure to organisms at points 1 and 2. The discrepancy here between the risk derived from soil and porewater

measurements originates from the PAHs present being tightly bound to coals, tars and other pyrogenic residues in the soil, and not easily being released into porewater or organisms. Hence, if the site specific ecological risk assessment was based on PAH concentrations determined by freely dissolved porewater (using the POM method), no soil excavation might be advocated. In such case, the measurement and calculations of TU can substantially modify the prioritization of financial means allocated for the remediation of the site.

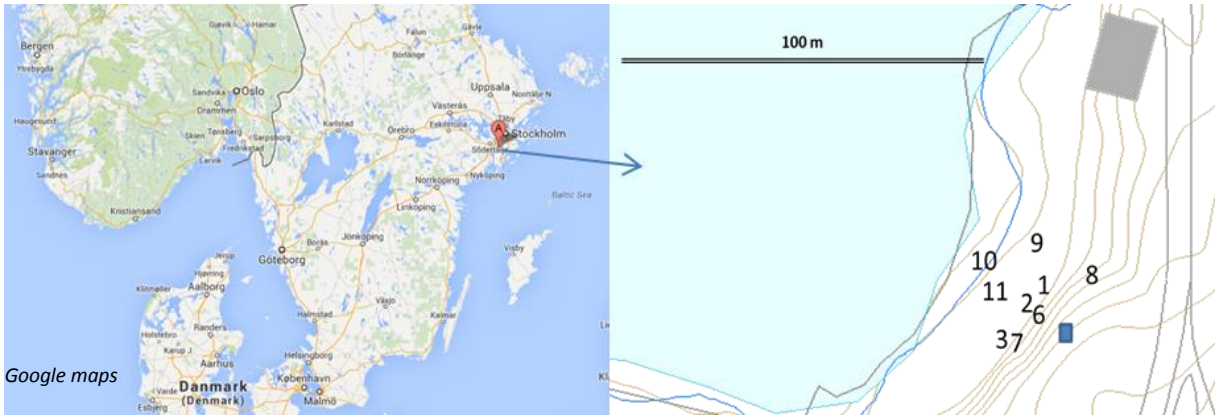


Figure 12.2 The scheme of sampling points at Riksten, Sweden.

Table 12.1 Log Cpw TU for soil from Riksten site calculated using US EPA sediment benchmarks (US EPA), RIVM "Serious risk concentration" (RIVM SRC) and RIVM "Maximum permissible concentration" (RVIM MPC) as references. The values marked orange exceed the Swedish generic guideline values for the protection of soil environment for soil with sensitive use and those in red exceed the values for soil with less sensitive use.

Sampling point	Depth (cm)	C soil (mg/kg dw)				Log C_{pw} TU		
		PAH-16	PAH-L*	PAH-M*	PAH-H*	US EPA	RIVM SRC	RIVM MPC
1	0-30	277.7	23.5	100.4	153.7	-0.87	-1.12	**0.65**
2	0-20	40.8	5.6	13.6	21.7	-0.86	-1.11	**0.67**
3	0-20	5.1	1.4	1.3	2.4	-2.79	-3.14	-1.36
6A	0-30	48.5	4.0	10.5	39.8	-1.65	-2.15	-0.37
6B	30-70	3.5	0.3	0.5	2.7	-2.01	-2.49	-0.71
7	0-20	11.6	3.5	3.1	5.0	-2.65	-2.92	-1.14
8	0-20	3.7	1.5	1.0	1.2	-2.78	-3.03	-1.26
9	0-15	0.3	0.1	0.1	0.1	-2.78	-3.03	-1.25
10	0-25	1.6	0.2	0.4	1.0	-2.56	-2.91	-1.13
11	0-35	49.9	3.7	6.6	40.5	-1.83	-2.28	-0.51

*The Swedish generic guideline values for PAH accounting for the protection of soil environment for soil with sensitive use (KM): PAH-L=3, PAH-M=10, PAH-H=1.1, and for soil with less sensitive use (MKM): PAH-L=15, PAH-M=40, PAH-H=10. PAH-L = naphthalene + acenaphtene + acenaphthylene; PAH-M = fluorene + phenantrene + anthracene + pyrene + fluoranthene; PAH-H = benzo[a]anthracene + chrysene + benzo[k]fluoranthene + benzo[b]fluoranthene + benzo[a]pyrene + dibenz[a,h]anthracene + indeno[1,2,3-cd]pyrene + benzo[g,h,i]perylene.

12.2.3 Cost-benefit-analysis

Assuming that only a limited area is affected by PAHs (ca a half of a 30 m x 30 m area down to 25 cm depth towards the lake from the former factory) to the point where remediation is necessary in order to reach the generic guideline values for soil with sensitive use, the costs of excavation and landfilling might not be high (Table 12.2). However, the question would be, whether it is necessary to excavate the site or not in the first place. In this case, invasive remediation through excavation may cause additional environmental risks (e.g. slope slides, dusting, diffuse pollution through transportation, emission of greenhouse gases, etc.).

Serious ecotoxicological risks may occur in areas where log C_{pw} TU values, calculated using RIVM SRC as the reference, exceed 1. None of the analysed samples at Riksten had such TU values (Table 12.1). Nevertheless, it would be advisable to monitor PAH levels in lake to confirm that no runoff of particle-bound PAH occurs. If this is the case, such spots might need remediation through *e.g.* excavation and treatment *ex situ*.

In summary, for this site where the total PAH concentrations in soil are moderately high (exceeding the guideline values by up to 6 times), determination of the actual ecological risks based on bioavailable PAH concentrations and calculated toxicity units (TU) can give a more precise base for decision making on the site management. Based on the calculated C_{pw} TU using the US EPA and RIVM SRC as references, no significant risks might be expected for soil ecosystem on the site. The time needed to implement POM measurements (one month) is clearly justified. This would add ca 50% to the costs of analysis of the total PAH concentrations in soil (ca 700 SEK/sample + costs of PAH analyses). But it would allow for saving hundreds of thousands SEK needed for excavation and treatment/landfilling of the soil, which might be decided based on the total PAH concentrations in soil (Table 12.2).

Table 12.2 Cost estimates of soil remediation through excavation and landfilling (in Swedish crowns (SEK), 1 EURO = 8-9 SEK).

	General cost estimates		Costs for remediation of Riksten site[2]	
	MIN	**MAX**	**MIN**	**MAX**
Excavator (SEK/day)	4 800	10 000	4 800	10 000
Refill masses (SEK/m^3)	45	70	5 000	7 000
Handling of contaminated masses[1] (SEK/ton)	600	1 200	108 000	216 000
Total:			**118 000**	**233 000**

[1] Includes transportation to approved facility and the disposal of the contaminated masses

[2] Assuming the size of the impacted area is a half of 30 m x 30 m plot, 900/2 = 450 m^2 x 0.25 m depth = 112.5 m^3 x 1.6 kg/m^3 soil density = 180 t soil.

12.3 Case study 2: Karlstad (Wermlandskajen)

12.3.1 Background

On behalf of the municipality of Karlstad, Sweco consultants have performed several investigations of the contamination situation in the area around the former gas works at Wermlandskajen (Sweco 2012). Besides gas works carried out in the factory, the area was used for storing and managing coal and coke. During the investigations, PAHs were identified as the main soil pollutants at the site. Taking all the investigated area into consideration, the soil contains elevated concentrations of PAHs, reaching on average 140 mg/kg dw of PAH-M and 220 mg/kg dw PAH-H calculated as $UCLM_{95}$. If only samples with mixed filling material that are suspected to contain PAHs (presence of soot, coal, odor, etc.) are taken into consideration, the average concentrations of PAH-M were 237 mg/kg dw and of PAH-H 230 mg/kg dw calculated as $UCLM_{95}$. No extensive analysis of groundwater was performed, but the study indicates that the spread of PAHs to groundwater is limited due to the prevailing soil conditions at the site (high density and low permeability). The PAH values in soil exceed the Swedish guideline values for soil with less sensitive use.

A larger part of the area is covered with an asphalt layer. In the open-surface areas the elevated concentrations of contaminants were mainly found at 0.3-1.5 m depth. Due to the limited exposure, the health risks were assessed as minor for people that might be present at the site. However, the site use might change, which would lead to removal of the asphalt layer. In such case, the health risks might increase.

In order to meet the Swedish guideline values for PAH in soil with sensitive use, the site might need to be remediated. To remove soil contaminants in the area, traditional excavation and disposal/treatment *ex situ* was suggested as the most viable method. Soil conditions at the site are such that no *in situ* method was judged to be applicable or would function optimally.

12.3.2 Site specific ecological risk assessment using POM method

Five soil samples were collected in the gas works area for the determination of PAH in porewater using POM method (Figure 12.3, Table 12.3). Total soil PAH concentrations in nearly all the samples exceeded the generic guideline values for protection of soil environment for soil with sensitive use (KM), while PAH-H were above the guideline values for soil with less sensitive use (MKM). In sample No 6, all the PAH groups were above MKM.

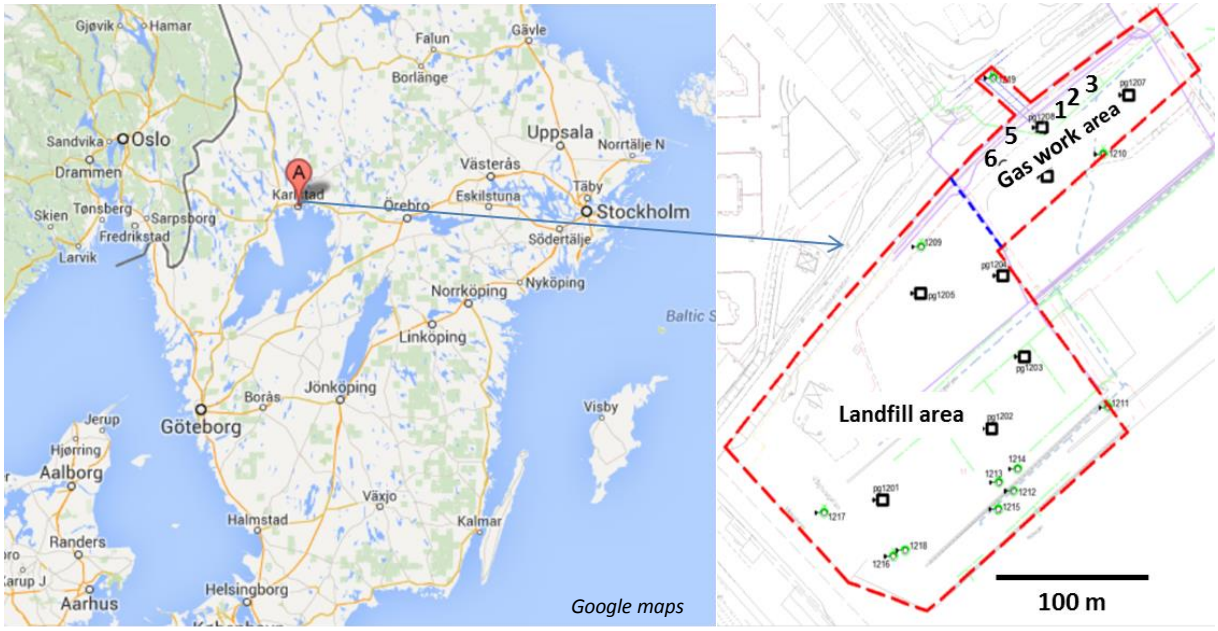


Figure 12.3 The scheme of sampling points at Wermlandskajen.

The calculated porewater TU using US EPA and RIVM SRC as references indicate no risk for ecosystem at the measured concentrations. Using the most restrictive reference values (RIVM MPC), samples No 1, 2 and 6 can be considered as potentially causing chronic risks to soil ecosystem (Table 12.3). If using less conservative values, the site could be considered as not causing any acute risks to soil ecosystem despite the exceedance of the generic guideline values for PAH in soil. Hence, soil excavation might not be necessary.

Table 12.3 Log C_{pw} TU for soil from Wermlandskajen site calculated using US EPA sediment benchmarks (US EPA), RIVM "Serious risk concentration" (RIVM SRC) and RIVM "Maximum permissible concentration" (RVIM MPC) as references. The values marked orange exceed the Swedish generic guideline values for the protection of soil environment for soil with sensitive use and those in red exceed the values for soil with less sensitive use.

Sampling point	Depth (cm)	C soil (mg/kg dw)				Log C_{pw} TU		
		PAH-16	PAH-L*	PAH-M*	PAH-H*	US EPA	RIVM SRC	RIVM MPC
1	20-50	56.3	2.4	20.2	33.7	-0.94	-1.20	**0.57**
2	20-50	56.3	1.9	21.7	32.6	-0.66	-0.94	**0.84**
3	20-30	23.1	1.4	8.6	13.0	-1.66	-1.92	-0.14
5		21.5	1.6	8.8	11.1	-1.68	-1.92	-0.14
6		130.3	10.6	57.7	62.0	-0.88	-1.12	**0.65**

*The Swedish generic guideline values for PAH accounting for the protection of soil environment for soil with sensitive use (KM): PAH-L=3, PAH-M=10, PAH-H=1.1, and for soil with less sensitive use (MKM): PAH-L=15, PAH-M=40, PAH-H=10.

12.3.3 Cost-benefit-analysis

It is difficult to judge the size of the affected area as no demarcation of contaminant distribution on the site was performed. We will assume here that soil from half of the gas works area contains elevated concentrations of PAHs in the soil layer up to 1 m, ca 200 m x 50 m / 2 = 5 000 m^3 x 1.6 kg/m^3 = 8 000 tons soil. If the only feasible method for site remediation is excavation, the estimated costs would be between 5 and 10 million SEK for the area of former gas works (Table 12.4), which is ca 1/3 of the landfill area at the site (Figure 12.3). It is therefore reasonable to assume that at least as much of soil might be exceeding the generic guideline values at the landfill area. Hence, the total costs of the site remediation by excavation and transportation to a landfill could be as high as 20 million SEK.

None of the calculated log C_{pw} TU (SRC) values exceeded 1 (Table 11), meaning that even if all the collected samples exceeded generic guideline values, the soil contamination level is not expected to cause serious ecological risks.

Table 12.4 Cost estimates of soil remediation through excavation and landfilling (in Swedish crowns (SEK), 1 EURO = 8-9 SEK).

	General cost estimates		Costs for remediation of Wermlandskajen site	
	MIN	**MAX**	**MIN**	**MAX**
Excavator (kr/day)	4 800	10 000	14 400[1]	30 000[1]
Refill masses (kr/m^3)	45	70	360 000[3]	560 000[3]
Handling of contaminated masses[2] (kr/ton)	600	1 200	4 800 000[3]	9 600 000[3]
Total:			**5 174 400**	**10 190 000**

[1] Assuming that the excavation of the site would take 3 days.
[2] Includes transportation to approved facility and the disposal of the contaminated masses
[3] Assuming the size of the impacted area is a half of the gas works area, i.e. 200 m x 50 m plot /2 = 5 000 m^2 x 1 m depth = 5 000 m^3 x 1.6 kg/m^3 soil density = 8 000 t soil.

It should be noted that only a limited number of soil samples were used for POM measurements in this study. The results should be considered as indicative regarding the further decision on risk management actions. However, the results suggest that it is highly advisable to perform site specific risk assessment to define ecotoxicity-based site specific guideline values. A more extensive POM sampling campaign could help to define the sub-areas that might need remediation. Only then a more reliable decision on the site management could be made. Using the same cost estimates as in the above example, i.e. ca 700 kr/sample + costs of PAH analyses, ≈2-3 kSEK/sample in total, even one hundred samples collected at the site would not lead to substancial costs (200-300 kSEK) compared with the expences for excavation and landfilling (10-20 million SEK).

12.4 Metal contaminated sites

The modified Soil PNEC calculator was used to calculated site specific guideline values for two metal contaminated sites in Sweden (Björkhult) and Belgium (La Calamine) according to the procedure described above in chapter 9.2. The definition of PNEC, i.e. the "Predicted No-Effect Concentrations" and other concepts related to the Soil PNEC calculator are summarized below.

Definitions:
PNEC: Predicted No Effect Concentration of the metal, concentration below which exposure to the metal is not expected to cause an adverse effect **PEC**: Predicted (or in this case measured) Environmental Concentration of the metal of interest in the soil **RCR**: Risk Characterisation Ratio – the PEC divided by the PNEC **PAF**: Potentially Affected Fraction, the fraction of terrestrial species predicted to be affected at the metal concentration (PEC) entered **Added approach:** calculations are made taking into account corrections for background metal concentrations **Total approach:** calculations are made without corrections for background concentrations

In the proposed procedure, measured (pseudo)-total metal concentrations are corrected for leaching-ageing phenomena and site specific soil properties. In previous applications of the Soil PNEC calculator, default L/A factors have been applied (Table 12.5). Here, we compare the outcome using default REACH values of L/A factors with the results using site specific L/A factors. Site specific L/A factors were calculated from EC50 field/EC50 spiked soil measured using i) barley plants (biological test) and ii) isotopic dilution method (chemical test). Furthermore, the site specific PNEC values were compared with the generic and site specific guideline values (where available) for the metals that would be considered the main drivers of potential site remediation.

Table 12.5 Default leaching-ageing factors in REACH dossier used in PNEC and RCR calculations.

Element	Leaching-Ageing factor
Cadmium (Cd)	-
Cobalt (Co)	1.2-3.5 (increases with increasing pH)
Copper (Cu)	2.0
Lead (Pb)	4.2
Molybdenum (Mo)	2.0
Nickel (Ni)	1.0-4.0 (increases with increasing pH)
Zinc (Zn)	3.0

12.5 Case Study 1: Björkhult

12.5.1 Background

The background information is obtained from a site investigation report performed by WSP consultancy (WSP, 2011).

The Televerk site in Björkhult, Kinda Municipality (Figure 12.4), was used between 1916 and 1944 for impregnation of telegraph poles by so called Boucherie method using 1.5-2% copper sulphate solution as an impregnation agent. Investigation of 7 300 m^2 of the site showed a significant soil contamination with copper in the entire area. The representative Cu concentration in soil at the site was calculated as UCLM 95 (upper confidence limit of the mean) to 828 mg/kg dw. The value expresses the average Cu concentration at the site with a 95% confidence level, which means that there is a 5% probability that the average concentration is higher than the calculated value. Concentration of Cu in an esker material, 12.4 mg/kg dw, sampled in the vicinity of the investigated area was used as a natural background level of Cu. According to a report from the Swedish Environmental Protection Agency (SEPA, 1997), the 90'th-percentile concentration of Cu in an unaffected moraine is approximately 26 mg/kg dw. All the analysed samples exceeded the natural background levels of Cu from 3 to 800 times. The highest Cu concentration in moraine at the site was 2190 mg/kg dw.

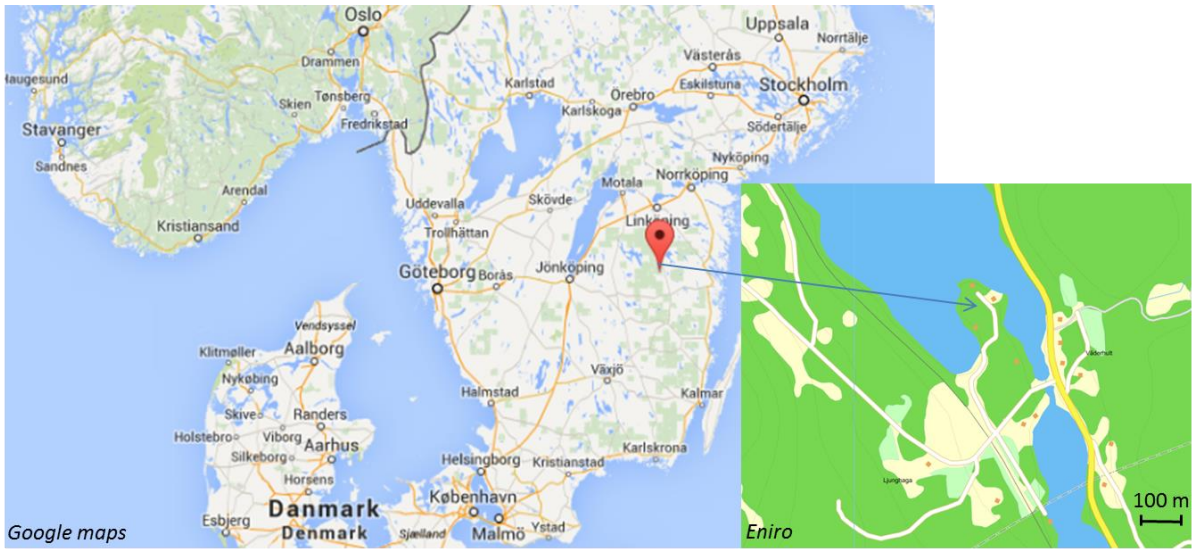


Figure 12.4 Location of Televerk site in Björkhult, Kinda Municipality, Sweden.

Closest to the former impregnation plant (area of ca 4 200 m^2) elevated Cu levels in soil were found down to four meters (307-486 mg/kg dw Cu). Surrounding areas and timber storage sites (area of ca 3 100 m^2) were less contaminated. The total amount of copper in the area has been estimated at about 25 tonnes. Ca 0.5 m layer of highly Cu contaminated bark (the determined representative concentration in bark was 43 845 mg/kg dw) overlays the majority of the site surface. Moraine, which is the main soil type at the site and composed of stony gravelly sand, contained the largest quantities of copper, while the highest concentrations have been found in the bark.

The representative Cu concentration in soil was stated to pose no health risks to humans. Health risk assessment identified the ingestion of berries and mushrooms in the most polluted area as the main path of Cu intake by humans.

After closing down the industrial activities, the use of area is limited. The area at the lake Verveln is used for recreation, swimming and fishing. There are no known plans to change the current land use or to increase urbanisation.

The generic guideline values for Cu in contaminated soil in Sweden are 80 mg/kg for soil with sensitive land use (residential and agricultural areas, KM) and 200 mg/kg for soil with less sensitive land use (industrial and commercial sites, MKM). The site-specific guideline values for protection of human health were calculated during the investigation and were 95 mg/kg dw soil for 0-1 m layer and 190 mg/kg dw for >1m depth. According to site investigators, comprehensive measures are needed to reach Cu concentrations in soil that are below or at the level of estimated site-specific guidelines in more than 7 000 m^2 area. The main risk reduction actions should be focused on the re-establishment of the natural biotope, so that vegetation and wildlife can thrive. However, these remediation objectives were assessed to be difficult to meet without performing an extensive site clean-up by means of excavation. Therefore the municipality and the County administration have formulated new so-called alternative overall remediation objectives that exclude soil excavation.

12.5.2 Site specific PNEC

The site specific PNEC values were first calculated using the default L/A factors entered into the Excel-based PNEC calculator (listed in Table 12.5). The soil properties, such as the total Cu concentration, the background Cu concentration, pH and organic carbon (OC) content were taken from the site investigation report (WSP, 2011). Gravelly sandy moraine (till) prevails on the site with some local lenses of clay and silt. The amount of clay was assumed to 10%, which is the top limit for soil classified as sandy. The effective cation exchange capacity (eCEC) was calculated by the program from pH, clay and OC content using the following equation (Helling et al., 1964):

$$eCEC\ (cmol_c/kg) = (30 + 4.4\ pH) \times \%\ clay\ /100 + (-59 + 51pH) \times \%\ OC/100 \qquad \text{(Eq. 1)}$$

The information needed for PNEC calculations was available only for soil samples from five sampling points. No information is provided in what medium the pH values were measured, but most common way of doing so is in water suspensions and hence this was assumed here. The pH measured in $CaCl_2$ suspensions is usually slightly lower than in water (by approximately half a pH unit), therefore the calculated PNEC values might be slightly higher than if pH_{CaCl2} values were used. Hence, the PNEC values calculated with pH_{CaCl2} results would be more conservative, which is recommended when using predictive estimators of risks.

All calculated PNEC values using the default L/A factor of 2.0 were below the Swedish guideline values for sensitive soils (i.e. <80 mg/kg) (Table 12.6). The PNEC values are also lower than the site-specific guideline value (95 mg/kg dw for the upper soil layer) calculated during the site investigation (WSP,

2011). It should be noted that the generic guideline value for soil with sensitive land use in Sweden are assumed to protect up to 75% of soil species in (based on NOEC data) and the value for soil with less sensitive land use represents protection of 50% of soil species. The PNEC values, on the other hand, represent "no effect" concentrations, hence these can be considered as the most conservative values.

The predicted Potentially Affected Fraction (PAF) of terrestrial species at the measured total Cu concentration (PEC) on the Björkhult site, with or without correction for the background Cu concentration, was about 100% in all samples except one (GP5). In this sample the Cu concentration was by an order of magnitude lower than in the remaining four samples, which resulted in about one third of the terrestrial species that could potentially be negatively affected by Cu. This means that to fully restore the ecosystem at the site, reduction of the total Cu concentrations might be needed. Using a default L/A factor of 2, the site specific PNEC values are similar to the generic guideline values for sensitive land use (80 mg/kg), suggesting that extensive remediation measures would be needed in order to restore soil functions at the Björkhult site. Slightly lower PNEC values can be obtained if the actually measured background concentration (12.4 mg/kg) is entered into the calculation sheet.

Table 12.6 Site specific PNEC and PAF values calculated with the default L/A factor of 2.0 for five sampling points of Björkhult Cu-contaminated site.

	Sampling point				
	PG3	**PG5**	**PG9**	**PG12**	**PG15**
Depth, m	0.35-0.85	0.8-1.4	0.3-0.8	0.3-0.7	0.4-0.55
Soil texture	Moraine (gravely, sandy)	Moraine (with glaciofluvial deposits)	Sandy stony esker	Moraine (sandy, gravely)	Moraine (bark layer at 0.50 -0.55 m depth), (sandy, gravely, stony)
Cu, mg/kg dw	2070	132	1350	2190	1770
pH_{H2O}	5.6	7	6.1	5.8	7.1
Organic carbon, %	0.8	1.2	3.4	1.3	1.9
Clay (arbitrary), %	10	10	10	10	10
eCEC, $cmol_c$/mg dw (estimated from pH, Clay and OC content)	7.28	9.66	14.26	8.63	11.88
Total approach					
PNEC, mg/kg	**48.5**	**57.8**	**89.8**	**59.0**	**69.2**
Total conc., mg/kg	2070	132	1350	2190	1770
RCR	42.64	2.28	15.03	37.10	25.59
PAF, %	100	30.2	98.4	100	99.7

Using the L/A factor of 6, derived from the measured EC50 values for barley plants grown on soil collected from Björkhult site (see chapter 5, above), the calculated PNEC values were ca 2 times higher (Table 12.7) than when the default L/A factor of two was used (Table 12.6).

Now, the PNEC values Table 12.7) are in between the Swedish generic guideline values for sensitive and less sensitive land use (80 and 200 mg/kg, respectively) and in four out of five samples the values are above the site specific guideline value calculated by investigators (WSP, 2011) (i.e. >95 mg/kg). It should be noted that the latter value was derived considering human health risks, which might be questioned based on the relatively low toxicity of Cu to humans.

The newly calculated PAF values are slightly lower for the samples PG3, 12 and 15, and considerably lower for PG5 and PG9, i.e. in the samples having the lowers Cu concentration and the highest OC content, respectively. This demonstrates, once again, the importance of OC for mitigating Cu toxicity in soil. Except for the sample PG5, the fraction of terrestrial species that can be affected by the measured soil Cu concentrations is substantial (Table 12.7).

Table 12.7 Site specific PNEC and PAF values calculated with the site specific L/A factor of 6.0 for five sampling points of Björkhult Cu-contaminated site. The L/A factor was calculated from EC50 field/EC50 spiked soil measured using barley plants.

	Sampling point				
	PG3	**PG5**	**PG9**	**PG12**	**PG15**
Total approach					
PNEC, mg/kg	**106.2**	**118.3**	**193.4**	**128.3**	**141.5**
Total conc., mg/kg	2070	132	1350	2190	1770
RCR	19.49	1.12	6.98	17.07	12.51
PAF, %	97.4	6.5	77.9	97.0	90.5

*corrected for background concentration, which is 12.4 mg/kg for Björkhult site.

As a final exercise we calculated the PNEC values using the L/A factor obtained with the isotopic dilution method (2.3), which is similar to the default value (2). As expected there was only a minor effect on the PNEC values obtained with the two values (Table 12.8).

Table 12.8 Site specific PNEC and PAF values calculated with the site specific L/A factor of 2.3 for five sampling points of Björkhult Cu-contaminated site. The L/A factor was measured with a soil test, i.e. using isotopic dilution method.

	Sampling point				
	PG3	**PG5**	**PG9**	**PG12**	**PG15**
Total approach					
PNEC, mg/kg	**54.0**	**63.8**	**99.7**	**65.6**	**76.2**
Total conc., mg/kg	2070	132	1350	2190	1770
RCR	38.33	2.07	13.54	33.37	23.22
PAF, %	99.9	25.3	97.6	99.9	99.5

*corrected for background concentration, which is 12.4 mg/kg for Björkhult site.

To be able to directly compare the results obtained with the PNEC calculator with the Swedish generic guideline values, assuming the same fraction of protected species, the values for 50% and 75% species protection levels are given in Table 12.9 with the site specific L/A factor of 2.3.

Table 12.9 Calculated total soil Cu concentrations at which 50% (land with less sensitive use) and 75% (land with sensitive used) species are protected assuming the same site conditions as in Table 12.6 and L/S factor of 2.3.

	Sampling point [mg/kg]					Average
	PG3	PG5	PG9	PG12	PG15	
PAF 50% - land with less sensitive use	167.7	193.0	285.0	182.5	228.0	211
PAF 75% - land with sensitive used	102.0	117.5	172.5	118.0	138.0	130
Measured concentration in field	2070	132	1350	2190	1770	

One can conclude that the variation between soil samples due to variation in soil properties is within a factor of 1.7, and that the risk limits obtained are slightly higher than those indicated by the Swedish generic guideline values. However, the general picture of an urgent need to remediate the site in order to restore the soil function does not differ between the two approaches.

The average of the calculated PAF values for land with less sensitive use (PAF 50%) is 211 mg/kg Cu. Assuming that the future site use remains as it is today, areas with concentration <211 mg/kg Cu could be considered to be left on site. Concentration of Cu in soil samples taken during the detailed site investigation (WSP, 2011) are summarized in Table 12.10. The Cu concentrations below the calculated site-specific guideline value were found for ca 20% of the total analysed samples (Table 12.10; Figure 12.5). Only the samples that have Cu concentration below the calculated value through the entire profile are considered. If an assumption is made that this distribution can be extrapolated to the entire site, the remediation measures could be reduced by one fifth.

Table 12.10 Copper concentration in soil samples collected in pits and boreholes at Björkhult (WSP, 2011). Samples with Cu concentration <211 mg/kg, corresponding the calculated site specific guideline value for land with less sensitive use, are marked in green.

Sampling pit	Depth, m	Cu concentration, mg/kg DW	Borehole	Depth, m	Cu concentration, mg/kg DW
PG1	0.3-0.65	736	KB1	2.2	1270
PG3	0.0-0.35	2070	KB1	3.2	1790
PG4	0.35-0.85	1750	KB1	3.8	40.9
PG5	0.25-0.45	132	KB2	2.2	296
PG8	0.35-0.80	73.1	KB2	3.8	486
PG9	0.8-1.4	1350	KB3	3.8	307
PG12	0.3-0.8	2190	KB4	2.2	57.8
PG15	0.0-0.3	1770	KB4	3.2	240
PG16	0.3-0.8	1340	KB5	1.2	347
			KB5	3.5	486
			KB6	2.5	199
			KB7	1.2	295
			KB7	1.8	127
			KB7	2.2	162
			KB7	3	143
			KB8	1.2	144
			KB8	2	333
			KB9	1.1	166
			KB9	1.7	135
			KB10	1	153
			KB10	1.5	104

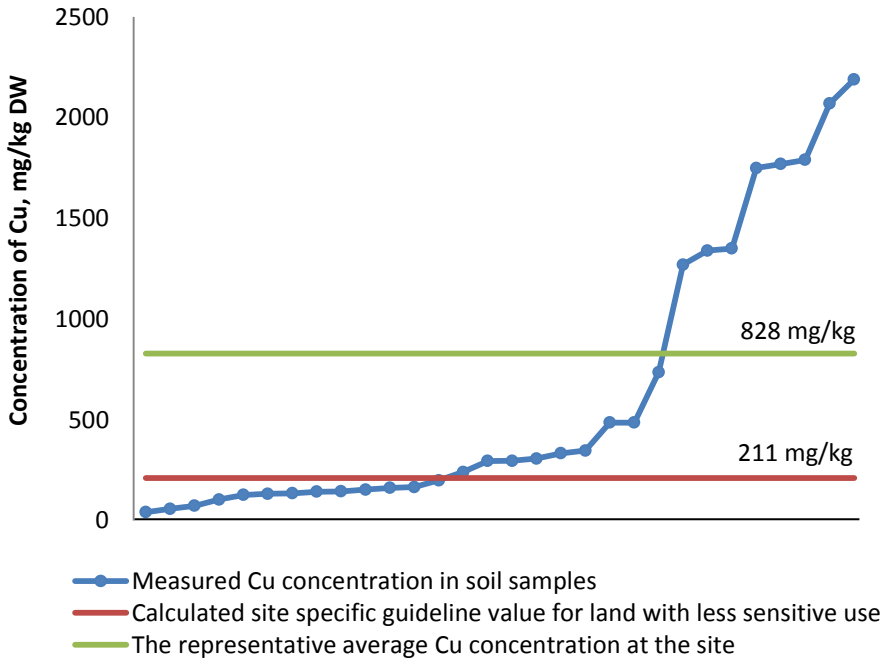


Figure 12.5 Distribution of samples based on the measured Cu concentration at Björkhult site and calculated site specific guideline value for soil with less sensitive use (211 mg/kg DW).

In summary, the calculated site specific PNEC values for ecological risks are similar to the Swedish generic guideline values and are quite far from the representative average Cu concentration at the site (828 mg/kg). This means that if the aim of the risk management is to restore soil ecosystem at the site, no substantial changes in the final conclusions regarding the site management to the ones suggested by the site investigators (WSP, 2011) could be made. However, if the site is not intended to be used for residential areas or agriculture, the site specific guideline value that was calculated considering protection of 50% of soil ecosystem and applying L/A factor calculated using the isotopic dilution method might be considered. In such case, a part of the site (e.g. up to 20%) could have acceptable levels of Cu in soil. Nevertheless, the consequences of accepting the protection level of 50% of soil organisms are yet to be investigated. That is, it is not quite clear at which PAF level impacts on soil functions can be acceptable.

12.6 Case study 2: La Calamine

12.6.1 Background

The background information is obtained from a site investigation and studies reported by Van Damme et al. (2010). La Calamine (named Kelmis in German) is one of the two most important mines along the Geul river (Figure 12.6). Pre-industrial mining was carried out already in the Middle Ages. Mining and smelting activities reached their peak in the middle of the 19th century, and ceased in 1884. Smelting of imported ores and mining at other locations continued in the first decades of the 20th century. Even today, metals are still introduced into the river system through the weathering and erosion of waste dumps (Kucha et al., 1996) and remobilization of contaminated overbank sediments. The ore minerals at La Calamine are composed mainly of a mixture of oxidized minerals, such as smithsonite ($ZnCO_3$), hemimorphite ($Zn_4Si_2O_7(OH)_2H_2O$) and willemite (Zn_2SiO_4). Investigations of the overbank sediments from the Geul river in Belgium shows highly elevated Zn concentrations reaching 10,000-69,000 mg/kg resulting from mining and smelting activities.

Figure 12.6 Location of the La Calamine mine (Van Damme et al., 2010).

12.6.2 Site specific PNEC

The calculated PNEC values for La Calamine site are summarised in

Table 12.11. The following data was taken from Van Damme et al. (2010) and used in the calculations: total Zn concentration 10 000–69 000 mg/kg dw, pH=7.2–7.8, Organic carbon 0.6–4.4%, soil texture - Loam (sandy loam) and Brown (brownish black), which was assumed to be a peaty soil. Furthermore, three values of L/A factor were used: i) default (from REACH dossier), calculated from EC50 field/EC50 spiked soil measured using either ii) barley plants or iii) isotopic dilution method. Since no exact data was available, the lower pH value and the higher organic carbon content were assigned for the brown soil and vice versa for the loam soil. The clay content was selected arbitrarily as 10% for the brown soil and 20% for the loam. The total Zn concentration was taken the same in all calculations, i.e. 10 000 mg/kg (

Table 12.11).

The calculated PNEC values using the site specific L/A factors were by an order of magnitude higher than the ones calculated with the default L/A factor. Comparing with the Dutch threshold values for Zn in contaminated soil (the target value 140 mg/kg and the intervention value 720 mg/kg), the site specific PNEC values allow for having considerably higher Zn concentrations in soil with expected "no effects" on the soil ecosystem. This is also supported by the plant toxicity experiment made with IBRACS (Figure 5.3).

Table 12.11 Site specific PNEC and PAF values calculated with the default and site specific L/A factors for two samples of Zn-contaminated site at La Calamine.

	Sample					
	Loam (sandy loam)			Brown (brownish black)*		
Default L/A factor (REACH)	3			3		
Site specific L/A factor		21.8[1]	34.8[2]		21.8[1]	34.8[2]
Zn, mg/kg dw	10 000	10 000	10 000	10 000	10 000	10 000
pH_{H2O}	7.8	7.8	7.8	7.2	7.2	7.2
Organic carbon %	0.6	0.6	0.6	4.4	4.4	4.4
Clay (arbitrary), %	20	20	20	10	10	10
eCEC , $cmol_c$/mg dw (estimated from pH, Clay and OC content)	14.90	14.90	14.90	19.73	19.73	19.73
Background[4]	54	54	54	191	191	191
Total approach						
PNEC, mg/kg	**212.1**	**1289.2**	**2032.2**	**287.5**	**1778.6**	**2807.3**
PEC, mg/kg	10 000	10 000	10 000	10 000	10 000	10 000
RCR	47.15	7.76	4.92	24.79	5.62	3.56
PAF, %	99.9	76.8	57.7	98.4	54.5	36.1

* In the PNEC calculator selected as peaty soil

[1] L/A factor calculated from EC50 field/EC50 spiked soil measured using barley plants

[2] L/A factor calculated from EC50 field/EC50 spiked soil measured using isotopic dilution method

Assuming 50% protection level of soil organisms, the calculated threshold concentration is 8490 mg/kg in loam and 14 180 mg/kg in peaty soil (Table 12.12). This means, that the calculated site specific intervention values might be higher than the actual total soil Zn concentrations in parts of the site when bioavailbility in included in the risk assessment, not when the corrections are not used. They are also considerably higher than the generic trigger values for soil Zn in residential areas (230 mg/kg) and industrial sites (320 mg/kg) in Wallonia (Walloon Soil Decree, 5/12/08). A detailed site assessment is need in order to have a broader coverage of soil conditions (pH, OC, clay content), which can then be used to calculate the site specific threshold values for different parts of the site.

Table 12.12 Zinc concentrations at which 50% and 75% soil organisms are protected assuming the same site conditions as in Table 12.11 and the L/S factor of 34.8.

	Loam (sandy loam)	Peaty soil
	Zn concentration [mg/kg]	
PAF 50% - land with less sensitive use	8 490	14 180
PAF 75% - land with sensitive used	4 740	7 400
Measured concentration in field	10 000-69 000	10 000-69 000

Determination of a site specific L/A-factor could be the limiting step. The isotopic dilution method might be more reliable than one biological test (plant growth), which usually provide more variable results than chemical tests. The cost of the isotopic dilution method is estimated to ca 100 Euro (ca 1000 SEK per sample). Assuming that there are accredited laboratories that offer such method, it would be a considerable cost-saving if this method along with the PNEC calculation is applied in a site specific risk assessment. This is especially recommended where contaminants are expected to be found predominantly in the form of sparingly soluble minerals.

13. Project management and co-ordination

Major activities are listed below:

Project kick-off meeting in Stockholm 19-20 October 2011. Planning of project.

All-projects kick off meeting Meeting in Paris 8-9 November, 2011. Presentation of project (available on http://www.snowmannetwork.com/upload/documents/call3/IBRACS%20danbk_SGI_final.pdf)

Meeting with Chair Person of Project Board, Griet van Gestel in Paris on 9 November, 2011.

A video conference was held 2 October 2012, including all IBRACS researchers. Meeting was held at one location per country; UCL (Flanders, Wallonia), UL/INRA (France) and SGI (Stockholm, Sweden).

All-projects mid-term meeting in Paris 19 November 2013. Presentation of project (available on http://www.snowmannetwork.com/upload/documents/call3/IBRACS%20danbk_SGI_midterm_Paris%20nov%202013_final.pdf).

A project meeting in Nancy, France 12-13 June 2013. Discussion on experimental results, publication plan, and implementing of results in existing risk assessment models.

Final project meeting in Leuven, Belgium 21-22 May 2014. Final discussions on implementation of results, dissemination of results and final report.

Planned participitation in all-projects final meeting in Paris 25-26 March, 2015.

14. Dissemination and exploitation

14.1 Stakeholder interactions

Presentations to the Direction de la protection des sols of Wallonia (contact: Philippe Sonnet, UCL)
presentation_experimentation_19_6_2012.pdf
presentation_workpackages_19_6_2012.pdf
presentation_ WP3 _Walloon Region _RAM-SES.pdf
presentation_experimentation_23-10-2013.pdf
presentation_regulatory_2_4_2014.pdf
presentation_experimentation_22_4_2014.pdf
presentation_regulatory_22_4_2014.pdf

Presentation of IBRACS in Formas magazine "Sustainability", September 2013. Available on http://sustainability.formas.se/en/Issues/Issue-3-September-2013/Content/Focus-The-threat-from-underneath/The-most-toxic-soil-is-not-necessarily-the-most-dangerous/

Swedish national seminar on soil protection 9 October 2014 in Visby, Sweden. Joint arrangement by network "Clean Soil Network" and IBRACS. Two presentations from IBRACS:
"*Implementering av en procedur för platsspecifik riskbedömning som rekommenderas av RIVM/IBRACS*" (Dan Berggren Kleja)
"*SOIL PNEC calculator – ett excelbaserat program för beräkning av platsspecifika riktvärden för metaller*" (Jurate Kumpiene)
Presentations available on http://wp.renaremark.se/2014/06/seminarium-med-temat-skydd-av-markmiljo/

14.2 National and international workshops and conferences

Kleja D.B. et al. *A presentation of the SNOWMAN project "IBRACS" (Integrating Bioavailability in Risk Assessment of Contaminated Soils: opportunities and feasibilities)*. AquaConSoil, Barcelona 16-19 April 2013. Poster.

Hamels, F., Sonnet, P., Kleja, D.B. and Smolders, E. *Phytotoxicity of Trace Metals in Field Contaminated Soils: Linking Soil Extractable Metals with Toxicity*. 12th International Conference on the Biogeochemistry of Trace Elements (ICOBTE), Athens, Georgia, June 16-20, 2013. Oral presentation.

Kleja et al. *Results from the IBRACS project – integrating bioavailability in ecological risk assessment of PAH contaminated soils*. NORDROCS, Stockholm 16-17 Sepember 2014. Poster.

Enell, A., Lundstedt, S., Arp, H.P.A., Josefsson, S., Kleja, D.B. Assessment of soil-water partitioning PACs using passive samplers and leaching tests. NORDROCS, Stockholm 16-17 Sepember 2014. Oral presentation.

DUPUY J., OUVRARD S., LEGLIZE P., STERCKEMAN T. Evaluation of availability tools for PAH plant uptake prediction. *10th SETAC Europe Special Science Symposium "Bioavailability of organic chemicals: Linking science to risk assessment and regulation", Brussels, 14 – 15 October 2014.* Poster.

DUPUY J., OUVRARD S., LEGLIZE P., STERCKEMAN T. Intégration de la biodisponibilité dans l'évaluation du transfert sol/plante des hydrocarbures aromatiques polycycliques. *3èmes Rencontres Nationales de la Recherche sur les Sites et Sols Pollués, Paris, 18-19 November 2014.* Oral presentation

Arp, H. P. H., S. Lundstedt, S. Josefsson, G. Cornelissen, A. Enell, A.-S. Allard and D. B. Kleja (2014). "Native Oxy-PAHs, N-PACs, and PAHs in Historically Contaminated Soils from Sweden, Belgium, and France: Their Soil-Porewater Partitioning Behavior, Bioaccumulation in Enchytraeus crypticus, and Bioavailability." SETAC Vancouver, 9 – 13 november 2014. Poster.

14.3 Reports

Progress reports to the Service Public de Wallonie, Direction Générale Agriculture, Ressources Naturelles et Environnement, Département du sol et des déchets, Direction de la protection des sols :

« IBRACS Intégration de la biodisponibilité dans l'évaluation des risques des sols pollués : opportunités et faisabilités ». First progress report, October 2013, Sonnet Ph., Hamels F. (UCL Earth and Life Institute), Halen H., Vegter J. (RAM-SES), 65 pages, in French.

« IBRACS Intégration de la biodisponibilité dans l'évaluation des risques des sols pollués : opportunités et faisabilités ». Second progress report, April 2014, Sonnet Ph., Stas M., (UCL Earth and Life Institute), Halen H. (RAM-SES), 31 pages, in French.

14.4 Scientific publications

Dupuy, J., S. Ouvrard, P. Leglize and T. Sterckeman. Morphological and physiological responses of maize (*Zea mays*) exposed to phenanthrene. Chemosphere, *in press*.

Dupuy, J., S. Ouvrard, P. Leglize and T. Sterckeman. Prediction of PAH Plant Uptake from Historically Contaminated Industrial Soils: Comparison of Passive Sampler and Tenax Extraction Methods. *in preparation.*

Arp, H. P. H., S. Lundstedt, S. Josefsson, G. Cornelissen, A. Enell, A.-S. Allard and D. B. Kleja (2014). "Native Oxy-PAHs, N-PACs, and PAHs in Historically Contaminated Soils from Sweden, Belgium, and France: Their Soil-Porewater Partitioning Behavior, Bioaccumulation in Enchytraeus crypticus, and Bioavailability." Environmental Science & Technology. 48, 11187−11195.

Josefsson, S., H. P. H. Arp, D. Berggren Kleja, A. Enell and S. Lundstedt. 2015. "Determination of POM-water partition coefficients for oxy-PAHs and PAHs." Chemosphere 119, 1268–1274.

Enell, A., Lundstedt, S., Arp, H.P.A., Josefsson, S., Cornelissen, G., Kleja, D.B. Comparison of a Column Leaching Test and a Passive Sampler Test for Assessment of Mobility and Partitioning of Native Oxy-PAHs, N-PACs and PAHs in Historically Contaminated Soils. *in preparation.*

Hamels F., J. Malevé, P. Sonnet, D. Berggren Kleja and E. Smolders 2014. "Phytotoxicity of trace metals in spiked and field-contaminated soils: linking soil-extractable metals with toxicity." Environmental Toxicology and Chemistry 33, 2479-2487.

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Arp, H. P. H., N. A. Azzolina, G. Cornelissen and S. B. Hawthorne (2011a). "Predicting pore water EPA-34 PAH concentrations and toxicity in pyrogenic-impacted sediments using pyrene content." Environmental Science and Technology **45**(12): 5139-5146.

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Arp, H. P. H, S. Lundstedt, S. Josefsson, G. Cornelissen, A. Enell, A.-S. Allard and D. Berggren Kleja (2014). "Native Oxy-PAHs, N-PACs and PAHs in historically contaminated soils from Sweden, Belgium and France: their soil-porewater partitioning behavior, bioaccumulation to *Enchytraeus crypticus*, and bioavailability." Environmental Science and Technology **48**(19): 11187−11195.

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16. List of abbreviations

ADI	acceptable daily intake
AOC	amorphous organic carbon
BC	black carbon
BCF	bioconcentration factors
BC-secario	best-case scenario
CEC	cation exchange capacity
Cohex	cobaltihexamine
C_{org}	organic carbon
CWBP	the Walloon Codes of Good Practice
D	diffusion coefficients
DCM	dichloromethane
DGT	diffusive gradients in thin films
EC50	50%-effect concentration
eCEC	effective cation exchange capacity
E-value	fraction of labile metals (isotopically exchangeable metal)
FCV	final chronic values
FS-factor	field-spiking factor
GC-MS	gas chromatography coupled to mass spectrometry
GRER	the Reference Guide for Risk Assessment (GRER) , published in December 2012, and issued by the Walloon Soil Protection Direction
HC25	hazardous concentration to 25% of population
HC50	hazardous concentration to 50% of population
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
ICP-MS	inductively coupled plasma – mass spectrometry
ICP-OES	inductively coupled plasma – optical emission spectrometry
IQR	interquartile range
IS	internal standard
IV	intervention value
KM	Swedish generic guideline values for protection of soil environment for soil with sensitive use
L/A factor	leaching/ageing factor
LC50	lethal concentration for half the population
MIFO	Methods for Inventories of Contaminated Sites
MKM	Swedish generic guideline values for protection of soil environment for soil with less sensitive use
MPC	maximum permissible concentration
MPCeco	maximum permissible concentration for ecosystem
MPCtoc	maximum Permissible Concentration benchmarks for PAHs normalized by TOC
NOEC	no observed effect concentration
NOER	no observed effect residue
N-PACs	nitrogen-containing heterocyclic polycyclic aromatic compounds

Ntot	total nitrogen
OC	organic carbon
OM	organic matter
oxy-PAH	oxygenated-PAHs
PACs	heterocyclic polycyclic aromatic compounds
PAF	potentially affected fraction
PAH	polycyclic aromatic hydrocarbons
PAP	proximal atmospheric pollution
PEC	predicted environmental concentration
PMTDI	provisional maximum tolerable daily intake
PNEC	predicted no effect concentration
POM	Polyoxymethylene
POM-SPE	polyoxymethylene solid phase extraction
PTWI	provisional tolerable weekly intake
RCF	the root concentration factor
RCR	risk characterisation ratio – the PEC divided by the PNEC
RS	recovery standard
SCF	the shoot concentration factor
senario RWC	realistic worst-case scenario
SGV	soil guideline values
SPME	solid phase microextraction
SQC	soil quality criteria
SQS	soil quality standards
SQT	Sediment Quality Triad
SRC	Serious Risk Concentration
SSD	species sensitive distribution
TF	transfer factor
TOC	total organic carbon
TSCF	the transpiration stream concentration factor
TU	toxicity units
UCLM95	upper confidence limit of the mean
VI	Walloon intervention value
WP	work package
VR	Walloon reference values
VS	Walloon trigger values
ZEBS	German reference values for pollutant concentrations in plant products for human consumption (Zentrale Erfassungs-und Bewertunstelle)

IBRACS

Appendicies with supplementary information

A1 Appendix Stable Isotope dilution method

A2 Appendix POM extraction method for soils

A3 Appendix Supporting information to Chapter 6: Evaluating a passive sampler method to assess bioaccumulation and ecotoxicity of PAHs in soils to worms

A4 Appendix Supporting information to Chapter 7: Evaluating a soil extraction and passive sampler methods to assess plant uptake of PAHs

Division Soil and Water Management[†]

Stable Isotope dilution method

(labile metal pool or E-value)

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1. Principle

This method was developed to measure the labile metal pool (Zn, Cu, Pb, Ni and Cd) in contaminated soils (field contaminated soils and spiked soils). Although we assume that freshly spiked soils have a metal pool which is 100% available. Compared to other soil extractions, which try to determine the labile metal pool (e.g. extractions with 0.05 M EDTA or with 0.43 M HNO_3), the isotope dilution method is conceptually more attractive because it minimizes the change of chemistry in the extraction (extractant is 0.01 M $Ca(NO_3)_2$ which is an ionic strength comparable to that of soil solution). A good review of possible obstacles of the isotope dilution method a described in Hamon (2008) [1].
The principle is explained in Figure A1.1 and is based on the fact that the isotopic abundance of metals in an extract (after equilibration with soil) is expected to be the same as that of the labile metal pool in the soil. The fraction of metals that is isotopically exchangeable relative to the total metal concentration (E-value, %) can be calculated from the isotopic abundances of reference and enriched isotopes in the extracts which are measured with ICP-MS.

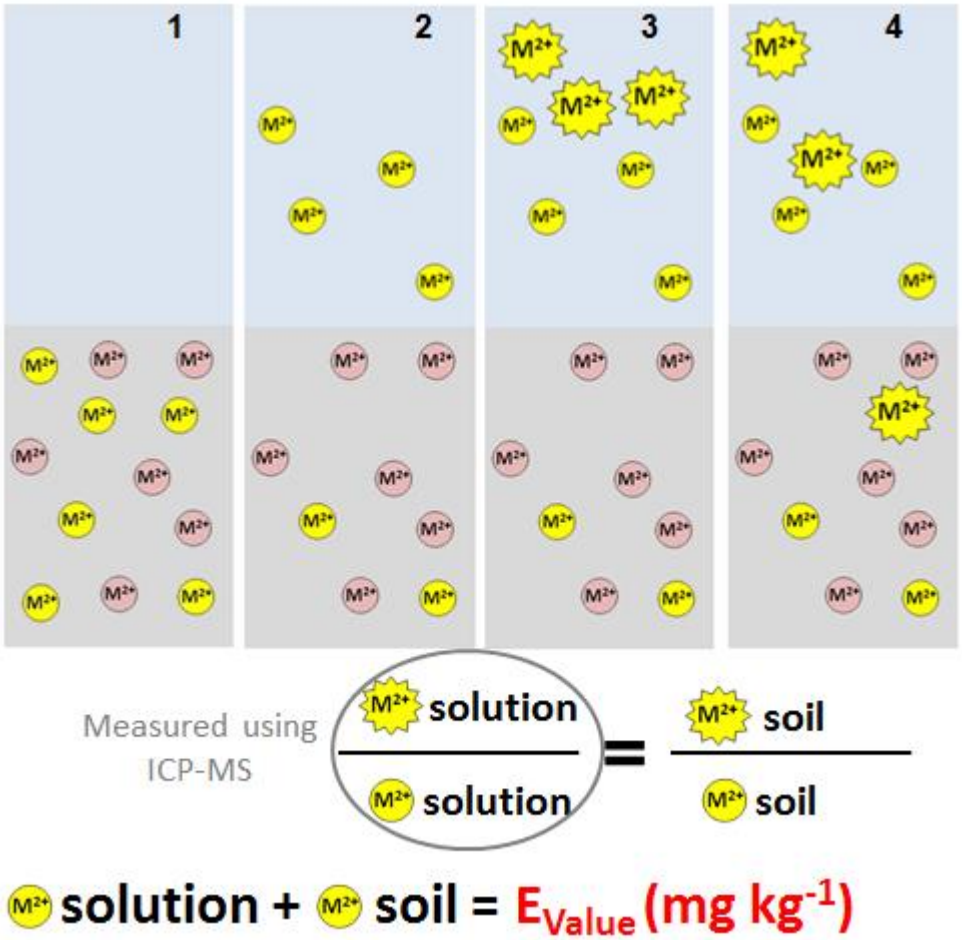

Figure A1.1 Different steps of the isotope dilution extraction. Step 1 & 2 represents the first equilibration of the soil with Ca(NO3)2 extraction solution. Step 3 is right after addition of the enriched stable isotope spike solution and step 4 is after the second equilibration with the spiked extraction solution. The isotope abundance of the metals in the extract is expected to be the same as that of the labile metal fraction in the soil. Blue: extraction solution, grey: soil. Adapted from [2].

2. Apparatus

All recipients and volumetric flasks should be acid washed and rinced with mQ (very clean!)

- Centrifuge tubes (Oak Ridge Centrifuge Tube, PPCO, 50ml), acid washed
- End-over-end shaker
- Precision balance (0.0001g accuracy)
- Centrifuge
- Sarstedt tubes (15 ml) for storage of supernatant and dilutions
- 1000ml or 2000ml volumetric flask, brown bottles for storage
- Dispenser for 30 mL
- pipettes
- ICP-MS

3. Reagents

- Calcium nitrate ($Ca(NO_3)_2.4H_2O$) 0.01 M (Chem-Lab NV)
- Enriched stable isotopes with certified isotopic abundances (IA), were obtained as metal foils from Isoflex (USA) ^{62}Ni (IA = 97.0 %), ^{65}Cu (IA = 99.2 %), ^{108}Cd (IA = 70.3 %) and ^{204}Pb (IA = 99.4 %) and dissolved in 5 % TAG HNO_3. ^{70}Zn (IA = 95.4 %) was obtained from Trace Sciences International Corp. (Canada) and was dissolved in 10% HNO_3.

4. Protocol

Weigh four replicates of a soil (each 1 g of air dry 2 mm sieved soil) into centrifuge tubes and keep two tubes for $Ca(NO_3)_2$ solution + enriched spike (six tubes for one soil type, Figure A1.2). Also include at least 3 centrifuge tubes for blanks (only $Ca(NO_3)_2$ solution). The first and second step (Figure A1.1) is an equilibration of the soil (1 g) with 30 mL of a 0.01 M $Ca(NO_3)_2$ solution for 72 h in the end-over-end shaker. Dilute isotope stock solutions of ^{62}Ni, ^{65}Cu, ^{70}Zn, ^{108}Cd and ^{204}Pb are combined to create bespoke mixed-isotope spikes (cocktails) for each soil-extractant combination. The concentrations used are intended to cause an increase of approximately 20 % of the natural abundance of the spike isotopes in the soil, based on a spike volume of 0.5 mL. Mixed-isotope spikes (0.5 mL) is added to four of the centrifuge tubes (two tubes without soil, and two with soil) (step 3, Figure A1.1, Figure A1.2) before all tubes are shaken end-over-end for a further 72 h. After centrifugation for 15 minutes at 2500 RCF (3500 rpm with rotordiameter 182 mm), the supernatant is diluted for ICP-MS analysis. The isotopically exchangeable metals are calculated from the isotopic abundances in the enriched soil extracts, in the natural native (non-enriched) soil and in the control solutions containing enriched isotope only, using equation 1 also described in [3].

ICP measurements:

The extracts are diluted 50x in HNO_3 (1%, ultra pure). Make sure that the ICP-MS measurement happens without Ge (Germanium) internal standard, because this element has the same atomic mass as ^{70}Zn and this would cause a wrong measurement.

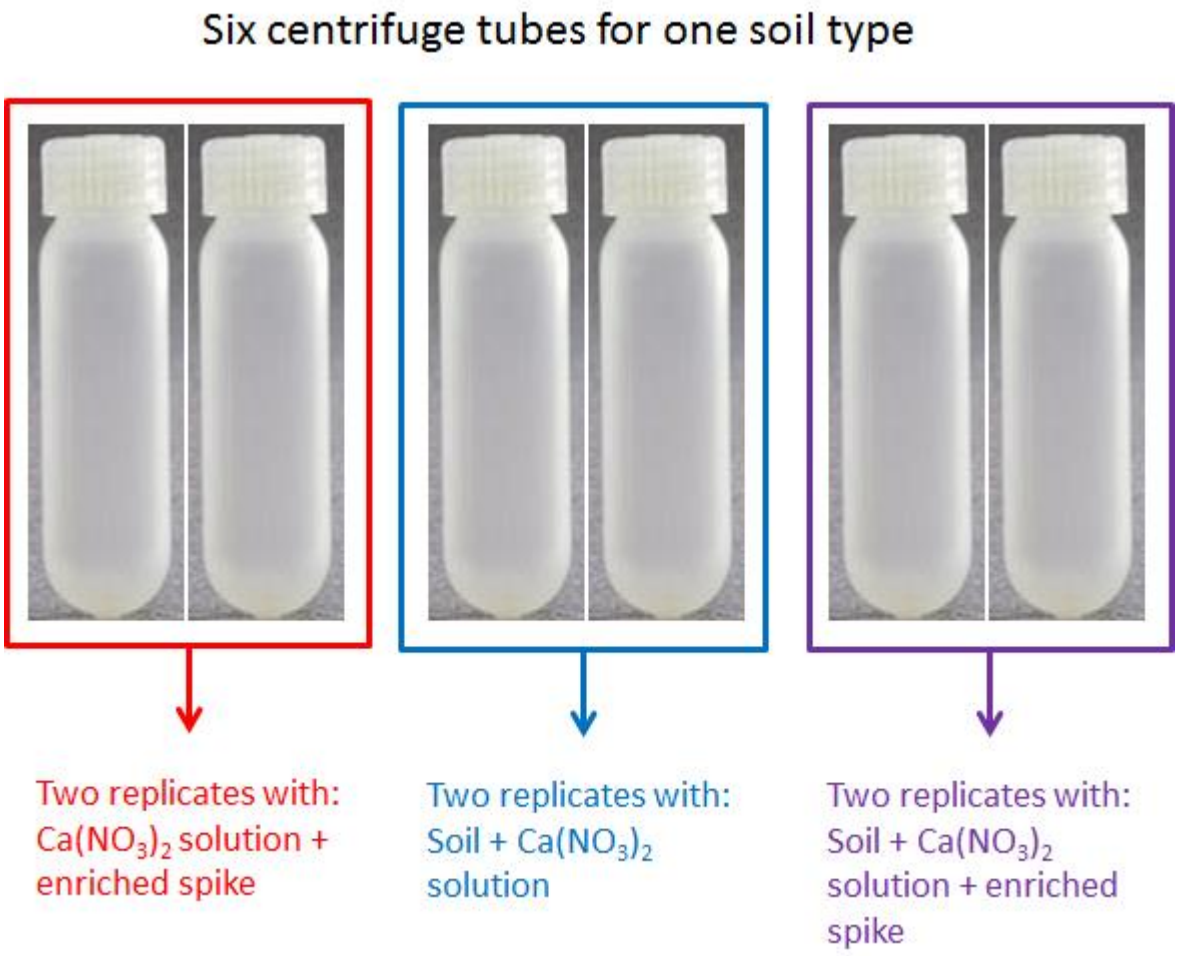

Figure A1.2. The content of the different centrifuge tubes per soil type.

5. Calculations

The isotopically exchangeable metal concentrations (mg kg^{-1}) in soil suspended in 0.01 M $Ca(NO_3)_2$ (E_{Value}) is determined from the isotopic abundance (IA) of the spike isotope (^{s}IA), and a reference isotope (^{r}IA), measured in three solutions: the spike solution (spike), the spiked soil-solution (sp-soil) and the un-spiked soil-solution (natural native, control). For a given metal this is calculated from Equation 1, where $AM_{control}$ and AM_{spike} are the average atomic masses of the metal in the unspiked soil and the spike respectively, C_{spike} and V_{spike} are the concentration (mg L^{-1}) and volume (L) of the spike respectively, and W is the mass of oven dry soil (kg). So moisture content should be considered for the calculations.

$$E_{Value} \text{ or } E_{Ext} = \left(\frac{AM_{control}}{W}\right)\left(\frac{C_{spike}\,V_{spike}}{AM_{spike}}\right)\left(\frac{{}^{s}IA_{spike} - \left(\frac{{}^{s}IA_{sp\text{-}soil}}{{}^{r}IA_{sp\text{-}soil}}\right){}^{r}IA_{spike}}{\left(\frac{{}^{s}IA_{sp\text{-}soil}}{{}^{r}IA_{sp\text{-}soil}}\right){}^{r}IA_{control} - {}^{s}IA_{control}}\right) \quad (1)$$

6. Safety and chemical waste

Potential hazards: Toxic metals when you work with contaminated soils.

Disposal of chemical waste: All solutions containing toxic metals have to be collected into waste container **Category 5**, soil sediments to be collected into a blue barrel (waste container) (**Category 6** contaminated soil).

7. Literature

[1] Hamon RE, Parker DR, Lombi E. 2008. ADVANCES INISOTOPIC DILUTION TECHNIQUES IN TRACE ELEMENT RESEARCH: A REVIEW OF METHODOLOGIES, BENEFITS, AND LIMITATIONS. In Sparks DL, ed, *Advances in Agronomy, Vol 99*. Vol 99-Advances in Agronomy, pp 289-343.

[2] Garforth J. 2013. Measuring labile metal in soils. *ICOBTE Conference, presentation*.

[3] Marzouk ER, Chenery SR, Young SD. 2013. Predicting the solubility and lability of Zn, Cd, and Pb in soils from a minespoil-contaminated catchment by stable isotopic exchange. *Geochimica Et Cosmochimica Acta* 123:1-16.

POM extraction method for soils

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Contact: hpa@ngi.no

Chemicals:
Methanol
Acetone
Hexane
Deionized water
Sodium Azide
$CaCl_2$
Labelled internal and reference standards (depending on analyte)

Materials
POM-76
Scissors
Glass vials with non-sorbing lid (PTFE or all glass)
Shaker (end over end)
Box (to pack glass vials in)
Rotovap or upconcentrator (to upconcentrate solvent extracts)
GC/MS and vials

1 Preparation of Materials

1.1 Preparation of POM strips for use

Cut POM strips (76 µm thickness) into 100 mg coupons (approximately 2 x 4 cm) with acetone rinsed scissors. Precleaning is done by placing all strips into a glass bottle, adding different solvents and overnight shaking as follows:

- Rinse by shaking for 1 day in hexane
- Remove hexane, replace with methanol, and rinse by shaking for 1 day, replace with new methanol and repeat one more time.
- Remove methanol, and rinse deionized / Millipore water, replacing water each day for up to three days.
- Storage in deionized / Millipore water.

1.2 Preparation of water solution

Per batch test, 40 mL of the following solution is needed:

0.001 M $CaCl_2$,0.015 M NaN_3

It is recommended to prepare several liters in advance, depending on the amount required.

1.3 Preparation of glassware

Rinse out all glassware with acetone prior to use, and let dry.

A piece of POM is being added to a glass vial in SGI's laboratory

2 Method: Laboratory shaking tests with soils, sediments or mixed media

2.1 General good laboratory practice

- Passive samplers should be handled while wearing gloves

- Clean apparatus should be used. This is especially important when working with PAHs as background levels of these compounds can affect results. All glassware should be rinsed with acetone before use.
- Extended exposure to laboratory air must be avoided, particularly for PAH analysis.

2.2 Batch experiments

Aim: to determine the freely-dissolved organic pollutant concentration (or chemical activity) in water that is in contact with sediment, soil or mixed media.
Time scale of experiment: Approximately 4 weeks

- Add approximately 5 g of soil or sediment to a 40 mL glass flask. This will vary depending on the level of pollutants present. For very polluted soils, less can be used, and for pristine soils, more soil can be used. *Take weight of the soil added*

- add 39.5 mL of water spiked with 0.001 M $CaCl_2$ and 0.015 M sodium azide (to leave some headspace).

- Dry (using a tissue) and preweigh the 0.1 g POM 76 coupon

- Close vials with appropriate lid, then secure the lid with clips or parafilm. Pack all vials in a box to avoid exposure to light, use plenty of packing material to avoid breakage.

- Shake the closed box for 28 days end-over-end at 7 – 10 rpm (ideally 10 rpm).

- Following this period, open the vials and remove POM strips with clean (acetone rinsed) tweezers. Remove all particles from the POM surface by first rinsing them in Millipore water and then drying them with a clean paper towel. Perform this step as efficiently and as quickly as possible, until no more particles are visibly present on the POM. Ideally this should take less than 1 minutes of exposure to laboratory air per POM.

2.3 POM extraction

- Place the clean POM strip in a vial for solvent extraction (best is a 50 mL glass flask with glass stopper). Close the vial.

- The remaining sediment and water can be analysed for remaining soil PAH concentration (e.g. determining the soil for the purpose of partitioning experiments, or to test for PAH depletion by the POM strip). However, this can also be determined with a separate soil sample, if the sample is homogenized prior to sampling.

- To the vials containing POM, add 20 mL of an 50:50 mixture of hexane:acetone for extracting them.

- Add an appropriate Internal Standard to check method recovery. Typically this 0.1 – 1 µg of mass-labelled internal standard. The exact amount should be decided on depending on likely concentrations in the sample and detection limits of the GCMS method. Refer to

- Pack in a box, to prevent light exposure, and shake the box for 2 days, either end over end at 10 rpm or on an orbital shaker at 100 rpm.

- Remove POM from the solvent and discard (all PAHs are now in the solvent). Reduce the volume of solvent with evaporation until approximately 1 mL.

2.4 **Clean-up and analysis**

We note that this section is for the NGI method for PAHs; other labs can use their own method for PAH clean-up and analysis.

2.4.1 *Preparation of silica gel for use*

Note – this is needed for PAH quantification/ different labs may use a different material

Silica gel should be prepared according to the following method:
- Weigh a known mass of silica gel
- Heat to 350 °C for 5 hours
- Leave to cool and add 10 % by silica gel mass, Millipore water
- Mix over night using an end over end shaker
- Add heptane to make a slurry and store until use

2.4.2 *Silica gel clean up (modified Silica gel cleanup method 3630C [1])*

Aim: separation of analytes from interfering compounds of a different chemical polarity.

Procedure

- The column (small Pasteur pipette) is packed with a small amount of glass wool in its base. Push it in to place using the tip of a long Pasteur glass pipette
- Mark a 3 cm level on the side of the pipette containing the glass wool, measuring from the place where the pipette bottom begins to widen
- Add silica gel to the line from the mixture of silica gel and heptane. Allow heptane to drain through and add more silica gel until the required height is reached. Tap the column to ensure a tight packing as any air bubbles or loose packing will cause preferential flow paths of the eluate
- Add 3 small spatula tips of anhydrous sodium sulphate
- Clean the column by eluting at least 5 mL of heptane and discard this to solvent waste
- Pipette the sample to be analysed on to the top of the column

NOTE: It is critical that before and after the sample is added that the top of the column must never be dry, so always stop the flow when the meniscus reaches the top of the sodium sulphate adsorbent, or add more solvent.

- After the sample has completely entered the silica column, elute the column with 10 mL heptane.
- Rinse the glass vial containing the sample with a few mL of heptane in order that the entire sample is transferred to the column.
- Once the 10 mL of heptanes has completely passed through the column, the vial containing the solvent can be closed until the next step.

2.4.3 *Prepare for GCMS*

- Reduce the volume of solvent from the silica gel clean up to around 0.7 mL
- Transfer the solvent to a GCMS vial and add a known amount of reference standard (RS) to each sample. PCB 77 or ($_2H^{10}$)-fluorantheneis most often used.
- Analyse for PAHs or GCMS in order to obtain the weigh in the POM (W_{POM}).
- Use this and the weight of the POM to calculate the concentration in C_{POM}

2.4.4 *Determine C_W concentrations from C_{POM} according to ($K_{POM} = C_{POM}/C_{PW}$)*

- The calculation of pore water concentrations can be carried out with the use of the excel spreadsheet that contains K_{POM} values and the mathematical method. Available from IBRACS homepage http://projects.swedgeo.se/ibracs/
- Constants ($K_{POM)}$ used in IBRACS are reported in Josefsson et al. (2015).

References

Josefsson, S., Arp, H.P., Kleja, D.B., Enell, A., Lundstedt , S. 2015. Determination of polyoxymethylene (POM) – water partition coefficients for oxy-PAHs and PAHs. *Chemosphere* 119, 1268–1274.

Supporting information to Chapter 6: Evaluating a passive sampler method to assess bioaccumulation and ecotoxicity of PAHs in soils to worms

List of tables

Table A2.1 The molecular properties of polycyclic aromatic hydrocarbons (PAHs), oxygenated-PAHs (oxy-PAHs) and nitrogen containing polycyclic aromatic compounds (N-PACs) considered in this study, including logarithms of POM-water (KPOM), octanol-water partitioning coefficients (K_{OW}), liposome-water partitioning coefficients ($K_{liposome}$) and subcooled liquid solubility (S^*_L).

Class	Name	ID	CAS	MW (g/mol)	log K_{POM} (L/kg)	log K_{OW} (L/L)	log S^*_L (mol/L)	log $K_{liposome}$ (L/kg)
PAH-16	Naphthalene	NAP	91-20-3	128.2	3.05[a]	3.40[d]	-3.11[f]	3.55[h]
	Acenaphthylene	ACEY	83-32-9	154.2	3.78[a]	3.85[d]	-3.90[f]	4.11[h]
	Acenaphthene	ACE	208-96-8	152.2	3.50[a]	3.95[d]	-3.90[f]	4.01[h]
	Fluorine	FLU	86-73-7	166.2	3.83[a]	4.11[d]	-4.20[f]	4.27[h]
	Phenanthrene	PHE	85-01-8	178.2	4.20[a]	4.47[d]	-4.64[f]	4.99[i,j,k]
	Anthracene	ANT	120-12-7	178.2	4.30[a]	4.57[d]	-4.67[f]	5.25[i,j]
	Fluoranthene	FLUA	206-44-0	202.3	4.56[a]	4.97[d]	-5.30[f]	5.74[i,j,k]
	Pyrene	PYR	129-00-0	202.3	4.57[a]	5.01[d]	-5.35[f]	5.79[i,j]
	benz(a)anthracene	BAA	56-55-3	228.3	5.46[a]	5.83[d]	-6.21[f]	6.53[i,j]
	Chrysene	CHR	218-01-9	228.3	5.43[a]	5.67[d]	-6.39[f]	6.49[i,j]
	benzo(b)fluoranthene	BBF	205-99-2	252.3	5.80[a]	5.86[d]	-6.86[f]	7.23[i,j]
	benzo(k)fluoranthene	BKF	207-08-9	252.3	5.97[a]	5.86[d]	-6.86[f]	7.24[i,j]
	benzo(a)pyrene	BAP	50-32-8	252.3	5.96[a]	6.05[d]	-7.21[f]	7.24[i,j,k]
	indeno(1,2,3-cd)pyrene	IND	193-39-5	276.3	6.26[a]	6.57[d]	-6.70[f]	7.97[i,j]
	dibenz(ah)anthracene	DAH	53-70-3	278.4	6.30[a]	6.75[d]	-7.22[g]	7.69[i,j,k]
	benzo(ghi)perylene	BGP	191-24-2	276.3	6.09[a]	6.63[d]	-7.22[f]	7.81[i,j,k]
Oxy-PAH	1-Indanone	IndO	83-33-0	132.2	0.96[b]	2.11[e]	-1.64[g]	2.25[h]
	1-Acenaphthenone	AceO	2235-15-6	168.2	2.36[b]	2.79[e]	-2.73[g]	2.94[h]
	9-Fluorenone	FluO	486-25-9	180.2	3.08[b]	3.58[e]	-3.88[g]	3.74[h]
	Anthracene-9,10-dione	AQ	84-65-1	208.2	3.29[b]	3.39[e]	-3.73[g]	3.54[h]
	4H-Cyclopenta(def) Phenanthrenone	Cyclo	5737-13-3	204.2	4.02[b]	4.14[e]	-4.39[g]	4.30[h]
	2-Methylanthracene -9,10-dione	MeAQ	84-54-8	222.2	3.86[b]	3.89[e]	-4.18[g]	4.05[h]
	Benzo(a)fluorenone	BFluO	479-79-8	230.3	5.11[b]	4.73[e]	-5.51[g]	4.90[h]
	7H-Benz(de)anthracen-7-one	BAO	82-05-3	230.3	4.52[b]	4.81[e]	-5.48[g]	4.98[h]
	Benz(a)anthracene-7,12-dione	BaQ	2498-66-0	258.3	5.28[b]	4.40[e]	-5.31[g]	4.56[h]
	Naphthacene-5,12-dione	NaQ	1090-13-7	258.3	5.05[b]	4.52[e]	-5.31[g]	4.69[h]
	6H-Benzo(cd)pyren-6-one	BPO	3074-00-8	254.3	5.16[b]	5.31[e]	-6.09[g]	5.48[h]
N-PAC	Quinoline	QUIN	91-22-5	129.2	1.36[c]	2.03[e]	-1.33[e]	2.17[h]
	Benzo(h)quinoline	BhQUIN	230-27-3	179.2	3.12[c]	3.43[e]	-3.36[e]	3.58[h]
	Acridine	ACR	260-94-6	179.2	3.09[c]	3.40[e]	-3.67[e]	3.55[h]
	Carbazole	CBZ	86-74-8	167.2	3.49[c]	3.72[e]	-4.97[e]	3.88[h]

a) Hawthorne et al.(2011b)(; b) Josefsson et al. (2014); c) Endo et al. (2011b),(with experimental value for carbazole, but other values estimated using the PP-LFER model derived within; d) Ma et al. (2010) e) Database from EPISuite v4.1 (note this database does not clarify if solubility is subcooled), www.epa.gov/opptintr/exposure/pubs/episuite.htm;
f) van Noort (2009), with value for DAH based on BGP; g) estimated with the SPARC online calculator May 2013 (http://www.archemcalc.com/sparc.html); h) Endo et al. (2011a)(, using either experimental values and if not present they were calculated as $K_{liposome}$ = 1.01 log K_{ow} + 0.12, i) Jonker (2007) averaged with literature $K_{liposome}$ values, j) van der Heiden and Jonker (2009), averaged with literature $K_{liposome}$ values; k) Kwon et al. (2009) averaged with literature $K_{liposome}$ values.

Table A2.2 Soil concentrations (µg/gdw) of individual PAH-16.[a]

C_{soil}	(µg g_{dw}^{-1})	NAP	ACEY	ACE	FLU	PHE	ANT	FLUA	PYR	BAA	CHR	BBF	BKF	BAP	IND	DAH	BGP
Karlstad 1a-1	K1a/SW01-1	0.66	1.70	0.03	0.23	2.90	1.37	8.74	6.93	6.04	4.50	7.37	3.20	5.06	3.07	1.18	3.27
Karlstad 2	K2/SW02	0.46	1.42	0.07	0.32	3.21	1.53	9.47	7.19	5.96	4.85	7.01	2.74	4.81	3.03	1.17	3.09
Karlstad 3a	K3/SW03a	0.95	0.41	0.03	0.12	1.96	0.95	3.25	2.35	1.89	2.59	3.04	1.06	1.55	1.20	0.47	1.23
Karlstad 5	K5/SW04	1.08	0.51	0.03	0.15	2.43	0.46	3.38	2.37	1.81	1.91	2.76	1.04	1.31	0.94	0.37	0.95
Karlstad 6	K6/SW05	7.61	2.72	0.28	1.38	11.86	3.39	22.95	18.08	12.86	9.15	13.13	5.67	8.87	5.08	2.05	5.23
Riksten 1a	R1a	0.51	9.43	2.10	0.44	3.47	7.56	39.33	61.11	31.61	26.84	32.67	9.75	21.93	11.88	2.80	16.25
Riksten 2	R2	0.76	1.26	0.15	0.22	2.25	0.92	5.83	7.77	2.71	2.81	5.57	1.55	3.48	2.39	0.47	2.68
Riksten 3	R3	0.40	0.12		0.04	0.76	0.10	0.60	0.66	0.24	0.35	0.67	0.19	0.37	0.25	0.06	0.31
Riksten 6a-1	R6a-1	0.51	1.06	0.05	0.12	1.08	0.70	3.30	5.59	3.04	2.84	10.08	3.16	5.49	4.41	0.84	6.28
Riksten 6a-2	R6a-2	0.74	1.34	0.06	0.15	1.55	1.15	5.37	7.95	4.15	4.07	12.14	3.85	6.89	5.90	0.93	6.95
Riksten 6a-3	R6a-3	0.45	1.03	0.04	0.11	1.24	0.66	3.58	5.73	2.96	3.12	11.22	3.11	5.69	5.03	0.80	6.33
Riksten 6b	R6b	0.07	0.09			0.05	0.04	0.20	0.27	0.16	0.19	0.93	0.27	0.28	0.41	0.07	0.43
Riksten 7	R7	0.93	0.15	0.02	0.07	2.14	0.21	1.47	1.62	0.65	0.80	1.31	0.33	0.75	0.46	0.09	0.61
Riksten 8	R8	0.43	0.02		0.02	0.95	0.05	0.55	0.48	0.17	0.25	0.32	0.10	0.17	0.08	0.02	0.09
Riksten 9	R9	0.07				0.04		0.04	0.02		0.03	0.04	0.01		0.01		0.01
Riksten 10	R10	0.06	0.03			0.11	0.02	0.24	0.20	0.10	0.15	0.29	0.10	0.13	0.10	0.02	0.09
Riksten 11-1	R11-1	0.34	1.59	0.21	0.07	0.51	0.87	2.41	3.94	5.41	4.96	10.93	2.82	5.80	4.62	0.92	4.73
Riksten 11-2	R11-2	0.37	1.40	0.05	0.08	0.61	0.84	2.30	4.02	5.26	4.81	10.11	2.64	5.46	4.34	0.98	5.24
Riksten 11-3	R11-3	0.45	1.43	0.05	0.08	0.73	0.82	2.26	3.74	4.49	4.26	10.16	3.12	5.86	4.13	0.85	4.89
Riksten 11-4	R11-4	0.41	1.73	0.05	0.10	0.53	0.92	2.60	4.04	5.57	4.87	10.64	2.86	6.00	4.36	0.96	5.20
Riksten 11-5	R11-5	0.37	1.52	0.06	0.09	0.51	0.87	2.21	4.09	5.27	4.52	11.33	3.18	6.25	4.91	1.03	5.38
Riksten 11-6	R11-6	0.33	1.41	0.07	0.08	0.61	0.86	1.89	3.39	5.05	4.21	10.41	2.81	5.65	4.53	0.91	5.20
Belgium 1	BE01	5.25	7.27	4.57	7.83	42.49	7.64	49.74	44.43	18.41	18.93	27.97	10.45	18.63	13.64	2.65	17.00
France 1	FR01FR01	31.14	68.34	15.10	27.92	150.14	111.66	474.51	351.84	267.82	189.36	326.80	120.64	208.04	143.04	41.36	123.51
France 2	FR02	8.66	72.82	153.22	89.72	165.97	63.12	146.84	100.60	61.65	42.46	79.54	28.24	53.38	37.68	11.41	33.17
France 3	FR03	3.05	1.25	0.43	0.54	5.32	2.88	11.37	8.56	6.82	5.46	10.44	3.66	7.00	5.23	1.50	4.98
France 4	FR04																
France 4-1	FR04-1	22.99	26.24	28.57	22.60	80.09	32.79	163.70	127.34	82.67	64.36	140.05	50.55	89.39	72.80	16.28	63.39
France 4-2	FR04-2	23.37	29.91	31.25	23.16	86.80	34.30	192.41	146.37	102.48	82.33	167.92	55.59	106.26	79.23	20.27	78.84
France 4-3	FR04-3	21.67	27.59	30.16	24.42	80.67	33.56	168.84	128.38	82.97	64.65	147.74	55.52	94.43	75.98	17.80	74.68
France 5	FR05	5.86	9.48	5.16	5.41	20.96	7.15	34.46	25.82	18.41	13.99	28.72	10.90	19.47	14.16	3.59	13.21
Holmsund 1-1	H1-1	1.67	10.15	110.94	132.84	164.81	126.73	1138.82	492.56	106.64	93.87	60.25	20.42	21.02	7.49	2.52	6.24
Holmsund 1-2	H1-2	1.71	10.62	111.53	132.05	161.85	100.60	1027.41	461.67	105.23	89.36	60.22	20.21	20.71	7.81	2.35	6.51
Holmsund 1-3	H1-3	1.80	11.25	124.23	152.32	181.62	137.32	1159.85	505.73	116.21	88.97	60.34	23.86	21.61	7.73	2.49	6.13

[a)]From replicate samples, rsd for PAH-16 ranged from 4 – 15% withing a single lab, larger rsd resulted in using different methods from different labs

Table A2.3 Soil concentrations (µg/gdw) of oxy-PAHs and N-PACs.

C_{soil}	(µg g_{dw}^{-1})	IndO	AceO	FluO	AQ	Cyclo	MeAQ	BFluO	BAO	BaQ	NaQ	BPO	QUIN	BhQUIN	ACR	CBZ
Karlstad 1a-1	K1a/SW01-1	0.04	0.07	0.52	0.69	0.88	0.37	1.96	2.27	0.68	0.70	1.58	0.04	0.04	0.15	0.32
Karlstad 2	K2/SW02	0.06	0.07	0.69	1.00	1.08	0.55	2.24	2.36	0.65	2.85	1.35	0.04	0.06	0.17	0.63
Karlstad 3a	K3/SW03a	0.03	0.02	0.59	0.66	0.40	0.27	0.90	0.68	0.65	5.83	0.60	0.04	0.02	0.07	0.36
Karlstad 5	K5/SW04	0.02	0.02	0.75	0.72	0.47	0.36	1.22	0.68	0.88	0.70	0.42	0.03	0.04	0.05	0.27
Karlstad 6	K6/SW05	0.12	0.06	2.85	1.92	2.27	1.01	4.16	5.86	1.13	1.09	3.10	0.23	0.16	0.35	1.38
Riksten 1a	R1a	11.50	0.38	1.00	4.62	15.51	6.02	15.12	17.95	6.20	2.74	27.04	0.46	0.39	0.20	1.09
Riksten 2	R2	1.36	0.06	0.57	1.10	1.17	0.75	1.29	2.32	1.10	0.39	3.97	0.17	0.08	0.05	0.24
Riksten 3	R3	0.13	0.01	0.23	0.34	0.14	0.17	0.15	0.23	0.20	0.05	0.31	0.03	0.01	0.01	0.05
Riksten 6a-1	R6a-1	0.47	0.08	0.21	0.52	0.86	0.44	1.27	1.31	0.75	0.36	3.30	0.06	0.04	0.04	0.13
Riksten 6a-2	R6a-2	0.65	0.09	0.29	0.71	0.97	0.43	1.68	1.64	0.83	0.43	3.96	0.05	0.04	0.05	0.27
Riksten 6a-3	R6a-3	0.45	0.08	0.21	0.60	0.80	0.41	1.17	1.15	0.59	0.31	3.08	0.05	0.03	0.04	0.14
Riksten 6b	R6b	0.06	0.01		0.04	0.04	0.02	0.06	0.07	0.07	0.04	0.23	0.01	0.00	0.00	0.01
Riksten 7	R7	0.15	0.01	0.64	0.62	0.26	0.34	0.42	0.47	0.37	0.11	0.51	0.06	0.02	0.01	0.10
Riksten 8	R8	0.03	0.00	0.39	0.39	0.11	0.16	0.13	0.09	0.11	0.03	0.06	0.03	0.01	0.00	0.04
Riksten 9	R9			0.02	0.03	0.01	0.01	0.01	0.01	0.01		0.01	0.00	0.00	0.00	0.01
Riksten 10	R10		0.00		0.05	0.02	0.03	0.03	0.05	0.04	0.02	0.08	0.01	0.00	0.00	0.02
Riksten 11-1	R11-1	0.30	0.08	0.20	0.42	1.07	0.44	1.05	1.51	1.50	0.49	5.17	0.07	0.03	0.04	0.19
Riksten 11-2	R11-2	0.32	0.07	0.20	0.41	1.07	0.51	1.11	1.55	1.59	0.53	5.49	0.09	0.03	0.04	0.19
Riksten 11-3	R11-3	0.31	0.07	0.23	0.46	1.08	0.53	1.01	1.38	1.40	0.38	4.58	0.07	0.03	0.05	0.28
Riksten 11-4	R11-4	0.32	0.08	0.20	0.40	1.23	0.54	1.24	1.75	1.57	0.57	5.13	0.09	0.03	0.05	0.25
Riksten 11-5	R11-5	0.31	0.07	0.21	0.39	1.13	0.43	1.03	1.51	1.64	0.49	5.23	0.07	0.02	0.03	0.21
Riksten 11-6	R11-6	0.33	0.08	0.20	0.44	1.20	0.47	1.07	1.65	1.71	0.59	6.18	0.09	0.03	0.04	0.21
Belgium 1	BE01	0.28	0.23	2.18	8.40	3.33	3.02	3.99	4.23	2.03	1.88	3.25	0.12	0.44	1.07	3.14
France 1	FR01FR01	1.53	2.80	34.63	21.19	46.42	8.77	67.07	98.28	10.75	21.59	57.47	0.94	1.20	4.24	15.23
France 2	FR02	1.71	8.28	108.97	18.38	15.11	3.67	11.80	15.55	3.36	4.22	12.85	2.13	1.05	0.72	2.94
France 3	FR03	0.17	0.07	1.38	0.97	0.71	0.31	1.34	2.40	0.90	0.73	1.97	0.47	0.04	0.12	0.50
France 4-1	FR04-1	0.67	1.85	20.12	13.55	13.37	2.24	13.24	13.63	3.98	5.85	17.56	0.18	0.42	1.77	6.85
France 4-2	FR04-2	0.71	1.74	18.06	13.24	14.10	2.13	13.79	14.07	4.26	5.91	18.06	0.22	0.32	1.52	7.34
France 4-3	FR04-3	0.67	1.19	16.07	12.68	12.30	2.00	13.10	12.05	3.76	4.86	16.87	0.27	0.32	1.49	5.75
France 5	FR05	0.38	0.51	9.16	2.95	2.15	0.56	2.91	3.05	1.04	1.24	2.91	0.37	0.12	0.29	0.97
Holmsund 1-1	H1-1	0.34	0.41	3.84	11.25	103.79	8.18	59.47	5.27	13.99	15.13	0.86	0.27	0.39	1.56	4.62
Holmsund 1-2	H1-2	0.22	0.39	3.68	11.98	99.30	9.41	59.30	5.04	13.27	16.13	0.78	0.32	0.29	1.52	3.08
Holmsund 1-3	H1-3	0.35	0.39	4.10	11.93	110.04	10.45	64.83	5.34	15.57	19.96	0.75	0.21	0.27	1.59	6.65

Table A2.4 Freely-dissolved porewater concentrations (Cpw, µg/mL) of individual PAH-16. [a)]

C_{PW}	µg/mL	NAP	ACEY	ACE	FLU	PHE	ANT	FLUA	PYR	BAA	CHR	BBF	BKF	BAP	IND	DAH	BGP
Karlstad 1a-1	K1a/SW01-1	<5.9E-03	6.0E-05	7.8E-04	3.5E-04	1.6E-04	3.7E-05	1.6E-04	1.2E-04	9.6E-06	1.2E-05	4.8E-06	1.8E-06	1.9E-06	4.6E-07	2.6E-07	6.7E-07
Karlstad 1a-2	K1a/SW01-2	<4.2E-03	7.0E-05	7.9E-04	3.7E-04	2.1E-04	4.6E-05	2.8E-04	2.0E-04	1.7E-05	2.0E-05	7.6E-06	3.0E-06	3.1E-06	6.5E-07	2.8E-07	8.0E-07
Karlstad 1a-3	K1a/SW01-3	<6.3E-03	7.7E-05	7.9E-04	3.6E-04	1.7E-04	4.4E-05	2.3E-04	1.7E-04	1.5E-05	1.7E-05	6.3E-06	2.5E-06	2.5E-06	5.2E-07	2.2E-07	6.1E-07
Karlstad 2	K2/SW02	<LOD	1.5E-04	4.8E-04	4.3E-04	5.7E-04	2.3E-04	3.2E-04	2.5E-04	4.4E-05	4.5E-05	1.7E-05	5.5E-06	7.0E-06	1.2E-06	4.7E-07	1.3E-06
Karlstad 3a	K3/SW03a	<3.9E-03	1.6E-05	4.6E-04	2.0E-04	3.7E-05	1.2E-05	1.5E-05	9.4E-06	7.7E-07	1.5E-06	5.7E-07	2.5E-07	1.7E-07	6.0E-08	<2.6E-08	6.3E-08
Karlstad 5	K5/SW04	<3.8E-03	1.3E-05	5.4E-04	2.4E-04	3.6E-05	9.8E-06	1.0E-05	5.2E-06	2.7E-07	4.7E-07	2.2E-07	8.1E-08	5.5E-08	<1.8E-08	<LOD	<1.8E-08
Karlstad 6	K6/SW05	<4.1E-03	6.4E-05	4.1E-03	1.4E-03	2.1E-04	7.5E-05	3.4E-05	1.2E-05	2.2E-07	3.1E-07	1.3E-07	5.0E-08	4.0E-08	<1.5E-08	<LOD	<2.0E-08
Riksten 1a	R1a	<3.5E-03	6.2E-05	3.3E-03	1.2E-03	1.8E-04	6.2E-05	7.7E-05	7.1E-05	7.1E-06	7.9E-06	3.3E-06	9.4E-07	1.3E-06	2.9E-07	1.2E-07	3.3E-07
Riksten 2	R2	<4.7E-03	6.5E-05	3.9E-03	1.4E-03	2.3E-04	7.1E-05	5.3E-05	3.6E-05	1.3E-06	2.1E-06	1.7E-06	4.1E-07	4.1E-07	1.7E-07	5.9E-08	1.8E-07
Riksten 3	R3	<LOD	<3.1E-06	<2.1E-05	<8.5E-06	7.6E-06	<1.3E-06	2.2E-06	2.2E-06	<1.2E-07	2.1E-07	2.4E-07	5.0E-08	6.7E-08	2.8E-08	<1.3E-08	<3.4E-08
Riksten 6a-1	R6a-1	<LOD	1.0E-05	<2.4E-05	1.1E-05	1.5E-05	5.6E-06	1.1E-05	1.4E-05	2.5E-06	2.4E-06	6.6E-06	1.6E-06	7.9E-07	5.9E-07	1.3E-07	4.8E-07
Riksten 6b	R6b	<4.4E-03	<4.4E-06	<3.8E-05	1.7E-05	1.2E-05	2.8E-06	5.0E-06	4.8E-06	7.7E-07	1.1E-06	2.8E-06	6.7E-07	1.8E-07	2.2E-07	5.8E-08	1.6E-07
Riksten 7	R7	<4.1E-03	<2.3E-06	4.1E-05	1.6E-05	1.0E-05	<1.4E-06	1.3E-06	1.2E-06	<6.5E-08	1.5E-07	1.2E-07	<2.5E-08	<2.7E-08	<1.5E-08	<LOD	<1.8E-08
Riksten 8	R8	<4.6E-03	<LOD	4.1E-05	1.6E-05	9.9E-06	<1.2E-06	<6.7E-07	<3.9E-07	<LOD	<LOD	<2.8E-08	<LOD	<LOD	<LOD	<LOD	<LOD
Riksten 9	R9	<4.4E-03	<LOD	3.9E-05	1.5E-05	1.1E-05	<1.5E-06	<1.0E-06	<4.9E-07	<LOD	<6.9E-08	<4.3E-08	<LOD	<LOD	<LOD	<LOD	<LOD
Riksten 10	R10	<4.8E-03	<LOD	<1.6E-05	<4.7E-06	7.1E-06	<1.1E-06	4.6E-06	3.5E-06	2.3E-07	6.2E-07	4.7E-07	8.2E-08	5.9E-08	2.9E-08	<LOD	<2.5E-08
Riksten 11-1	R11-1	<3.9E-03	7.5E-06	<1.5E-05	<4.5E-06	<4.0E-06	4.3E-06	6.5E-06	9.2E-06	4.0E-06	4.5E-06	4.2E-06	9.8E-07	7.4E-07	2.6E-07	7.9E-08	2.7E-07
Riksten 11-2	R11-2	<LOD	7.3E-06	<LOD	<3.9E-06	<3.6E-06	4.0E-06	6.2E-06	8.8E-06	3.5E-06	3.9E-06	3.6E-06	9.6E-07	6.7E-07	2.2E-07	6.3E-08	2.3E-07
Riksten 11-3	R11-3	<4.2E-03	7.7E-06	<1.6E-05	<4.1E-06	<4.0E-06	4.2E-06	6.3E-06	9.3E-06	3.7E-06	4.6E-06	3.8E-06	9.8E-07	6.4E-07	2.4E-07	6.6E-08	2.3E-07
Belgium 1	BE01	1.7E-02	2.5E-03	1.4E-02	1.2E-02	2.4E-02	3.2E-03	6.1E-03	5.5E-03	2.7E-04	3.4E-04	9.0E-05	2.6E-05	3.5E-05	6.9E-06	2.1E-06	6.0E-06
France 1	FR01FR01																
France 2	FR02	<3.9E-03	3.5E-04	6.0E-03	1.7E-03	8.6E-04	3.2E-04	5.1E-04	3.5E-04	1.9E-05	2.2E-05	1.1E-05	3.7E-06	4.9E-06	1.3E-06	4.1E-07	1.6E-06
France 3	FR03	<4.7E-03	1.5E-05	2.0E-04	7.4E-05	7.0E-05	1.2E-05	1.3E-05	1.3E-06	5.4E-07	8.3E-07	6.5E-07	2.1E-07	1.4E-07	7.8E-08	<2.6E-08	8.0E-08
France 4-1	FR04-1	1.4E-02	3.7E-04	6.4E-03	2.4E-03	5.2E-03	1.2E-03	3.7E-03	2.4E-03	1.9E-04	2.2E-04	1.1E-04	3.3E-05	4.2E-05	1.3E-05	3.9E-06	1.4E-05
France 4-2	FR04-2	1.2E-02	3.7E-04	6.4E-03	2.2E-03	5.1E-03	1.1E-03	3.9E-03	2.7E-03	2.1E-04	2.3E-04	1.1E-04	4.0E-05	4.5E-05	1.4E-05	4.1E-06	1.5E-05
France 4-3	FR04-3	1.8E-02	4.8E-04	6.9E-03	2.5E-03	5.3E-03	1.1E-03	4.2E-03	2.8E-03	2.1E-04	2.3E-04	1.2E-04	4.2E-05	4.6E-05	1.5E-05	4.4E-06	1.6E-05
France 5	FR05	<5.2E-03	5.7E-05	1.6E-04	8.1E-05	5.0E-05	2.3E-05	5.1E-05	4.3E-05	2.7E-06	3.6E-06	2.1E-06	6.4E-07	6.3E-07	2.5E-07	8.2E-08	2.6E-07
Holmsund 1-1	H1-1	<5.5E-03	3.2E-03	2.0E-01	1.2E-01	5.7E-02	2.3E-02	3.8E-02	3.1E-02	9.0E-04	7.3E-04	1.3E-04	3.8E-05	3.0E-05	3.4E-06	1.7E-06	3.7E-06
Holmsund 1-2	H1-2	<5.7E-03	3.2E-03	2.0E-01	1.1E-01	5.6E-02	2.2E-02	4.2E-02	3.2E-02	9.5E-04	6.7E-04	1.5E-04	4.3E-05	3.3E-05	2.8E-06	1.1E-06	2.4E-06
Holmsund 1-3	H1-3	<5.8E-03	3.3E-03	2.0E-01	1.2E-01	5.3E-02	2.2E-02	3.6E-02	2.8E-02	1.0E-03	7.3E-04	1.8E-04	5.0E-05	3.8E-05	2.8E-06	1.2E-06	2.6E-06

a) LOD = limit of detection, defined as the average conversation in blank POM samples (n=3), samples < 3* LOD are considered below the limit of quantification, indicated by "<" and are not considered in this study for analysis of bioaccumulation or toxicity.

Table A2.5 Freely-dissolved porewater concentrations (Cpw, µg/mL) of oxy-PAHs and N-PACs.

C_{PW}	µg/mL	IndO	AceO	FluO	AQ	Cyclo	MeAQ	BFluO	BAO	BaQ	NaQ	BPO	QUIN	BhQUIN	ACR	CBZ
Karlstad 1a-1	K1a/SW01-1	1.56E-02	3.34E-04	1.73E-04	1.22E-04	3.91E-05	2.43E-05	4.41E-06	1.44E-05	2.20E-06	3.07E-06	4.08E-06	<5.0E-04	<LOD	<1.5E-05	3.88E-05
Karlstad 1a-2	K1a/SW01-2	3.20E-02	2.21E-04	1.31E-04	1.46E-04	4.37E-05	2.18E-05	5.19E-06	1.16E-05	1.56E-06	1.72E-06	1.87E-06	1.36E-03	<1.0E-05	<1.2E-05	4.84E-05
Karlstad 1a-3	K1a/SW01-3	8.66E-03	2.57E-04	1.13E-04	1.08E-04	4.63E-05	1.94E-05	5.80E-06	1.25E-05	1.67E-06	1.65E-06	1.64E-06	<7.6E-04	<LOD	<8.4E-06	3.74E-05
Karlstad 2	K2/SW02	3.37E-03	1.87E-04	4.93E-04	2.41E-04	2.37E-04	3.48E-05	2.36E-05	5.68E-05	2.83E-06	2.38E-05	3.69E-06	<LOD	6.87E-05	6.64E-05	3.87E-04
Karlstad 3a	K3/SW03a	<1.1E-03	<3.4E-05	<LOD	<LOD	2.99E-06	<LOD	3.21E-07	<LOD	<1.6E-07	1.24E-06	<1.2E-07	<LOD	<LOD	<LOD	9.03E-06
Karlstad 5	K5/SW04	<1.1E-03	<2.2E-05	<LOD	<LOD	1.94E-06	<LOD	<1.8E-07	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<4.4E-06
Karlstad 6	K6/SW05	<1.1E-03	6.14E-05	<3.7E-05	<LOD	4.30E-06	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.85E-06
Riksten 1a	R1a	9.16E-03	3.50E-04	9.86E-05	1.31E-04	6.12E-05	4.21E-05	3.21E-06	7.23E-06	1.47E-06	1.77E-06	2.16E-06	<7.0E-04	<1.6E-05	2.95E-05	3.05E-05
Riksten 2	R2	6.79E-03	1.51E-04	9.03E-05	7.00E-05	1.54E-05	1.33E-05	9.02E-07	2.89E-06	6.23E-07	<5.6E-07	9.88E-07	<3.8E-04	<LOD	<LOD	1.44E-05
Riksten 3	R3	<8.3E-04	<1.8E-05	<LOD	<LOD	<1.3E-06	<LOD	<1.3E-07	<LOD	<LOD	<LOD	<2.2E-07	<LOD	<LOD	<LOD	<3.0E-06
Riksten 6a-1	R6a-1	<1.5E-03	4.93E-05	<LOD	<LOD	9.11E-06	<2.1E-06	9.59E-07	<1.6E-06	8.89E-07	<7.3E-07	1.57E-06	<LOD	<LOD	<LOD	6.26E-06
Riksten 6b	R6b	<1.6E-03	<2.7E-05	<LOD	<LOD	2.47E-06	<LOD	2.52E-07	<LOD	5.37E-07	<3.4E-07	4.03E-07	<LOD	<LOD	<LOD	<3.6E-06
Riksten 7	R7	<1.1E-03	<1.5E-05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Riksten 8	R8	<1.1E-03	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Riksten 9	R9	<8.8E-04	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Riksten 10	R10	<1.4E-03	<1.5E-05	<LOD	<LOD	<1.0E-06	<LOD	<1.3E-07	<LOD	<2.0E-07	<LOD	<1.7E-07	<LOD	<LOD	<LOD	<3.6E-06
Riksten 11-1	R11-1	<1.5E-03	5.23E-05	<LOD	<2.1E-05	7.60E-06	6.25E-06	5.29E-07	<1.5E-06	1.14E-06	<7.2E-07	1.12E-06	<LOD	<LOD	<LOD	<5.0E-06
Riksten 11-2	R11-2	<1.3E-03	5.09E-05	<LOD	<1.8E-05	7.64E-06	4.85E-06	5.07E-07	<1.2E-06	1.09E-06	<5.7E-07	1.08E-06	<LOD	<LOD	<LOD	<3.6E-06
Riksten 11-3	R11-3	<1.5E-03	4.47E-05	<LOD	<2.0E-05	8.19E-06	5.60E-06	4.91E-07	<1.3E-06	1.20E-06	<5.9E-07	1.30E-06	<LOD	<LOD	<LOD	<4.1E-06
Belgium 1	BE01	1.34E-01	5.07E-03	6.87E-03	1.76E-02	2.03E-03	2.11E-03	9.31E-05	2.18E-04	2.30E-05	2.24E-05	7.82E-06	4.35E-03	2.74E-03	1.45E-03	8.94E-03
France 1	FR01FR01															
France 2	FR02	1.34E-02	6.74E-03	1.23E-02	5.83E-04	1.66E-04	2.28E-05	5.55E-06	1.82E-05	1.09E-06	2.10E-06	2.63E-06	<6.8E-04	2.55E-05	3.03E-05	2.13E-04
France 3	FR03	<1.9E-03	6.68E-05	1.05E-04	<1.7E-05	2.29E-06	<LOD	<1.4E-07	<LOD	<LOD	<LOD	<1.1E-07	<LOD	<LOD	<LOD	6.76E-06
France 4-1	FR04-1	4.63E-02	5.01E-03	4.92E-02	8.79E-03	1.37E-03	3.17E-04	8.14E-05	1.37E-04	1.56E-05	2.28E-05	2.36E-05	1.28E-03	2.44E-04	3.81E-04	2.59E-03
France 4-2	FR04-2	4.52E-02	5.39E-03	5.16E-02	9.26E-03	1.51E-03	3.11E-04	7.96E-05	1.45E-04	1.59E-05	2.38E-05	2.46E-05	1.52E-03	2.15E-04	3.78E-04	2.67E-03
France 4-3	FR04-3	5.65E-02	5.72E-03	5.43E-02	9.50E-03	1.69E-03	3.00E-04	8.35E-05	1.63E-04	1.90E-05	2.43E-05	2.54E-05	1.39E-03	2.43E-04	4.15E-04	2.70E-03
France 5	FR05	<1.9E-03	2.71E-04	2.63E-04	<2.5E-05	6.34E-06	<LOD	4.36E-07	<8.7E-07	<1.8E-07	<LOD	<2.0E-07	<LOD	<LOD	<LOD	8.22E-06
Holmsund 1-1	H1-1	8.54E-02	1.72E-02	9.61E-03	2.34E-02	2.56E-02	4.05E-03	8.63E-04	2.30E-04	1.01E-04	2.44E-04	2.16E-05	1.49E-03	4.53E-04	1.16E-03	1.30E-02
Holmsund 1-2	H1-2	5.85E-02	1.61E-02	9.81E-03	2.46E-02	2.70E-02	4.12E-03	8.35E-04	1.90E-04	9.87E-05	2.63E-04	5.18E-06	<8.9E-04	3.43E-04	1.42E-03	1.97E-02
Holmsund 1-3	H1-3	6.87E-02	1.61E-02	1.01E-02	2.64E-02	2.85E-02	4.52E-03	8.81E-04	1.94E-04	1.08E-04	2.70E-04	3.50E-06	9.85E-04	6.54E-04	1.01E-03	1.15E-02

a) LOD = limit of detection, defined as the average conversation in blank POM samples (n=3), samples < 3* LOD are considered below the limit of quantification, indicated by "<" and are not considered in this study for analysis of bioaccumulation or toxicity.

Table A2.6 Results of the survival and reproducibility study of Enchytraeus crypticus exposed to the historically contaminated soils considered in this study. For reference, the soils with high metal content are indicated, as well as those with low TOC (<6%) and high clay/silt content (>24%).

Sample		% survival	s.d. (n=5)	No. Of Juveniles	s.d. (n=5)	Relative % Jurveniles[a)]	s.d. (n=5)	High Metal[b)]	Low TOC & High Clay/Silt[b)]
Karlstad 1a	K1a/SW01	100		442	58	84	11		Y
Karlstad 2	K2/SW02	95	10	365	134	69	25		Y
Karlstad 5	K5/SW04	98	5	70	35	13	7		
Karlstad 6	K6/SW05	98	5	179	59	34	11		
Riksten 1a	R1a	78	8	150	59	28	11		
Riksten 2	R2	96	5	653	59	100	9		
Riksten 3	R3	96	9	640	125	100	20		Y
Riksten 6a	R6a	90	17	208	95	39	18		
Riksten 6b	R6b	84	11	127	40	24	8		
Riksten 7	R7	86	13	382	115	73	22		
Riksten 8	R8	92	4	430	68	82	13		
Riksten 9	R9	98	4	681	147	100	22		Y
Riksten 10	R10	96	9	413	94	78	18		Y
Riksten 11	R11	92	12	229	23	43	4		
Belgium 1	BE01	94	9	226	96	43	18	Y	Y
France 1	FR01FR01	96	5	338	56	64	11	Y	
France 2	FR02	96	5	101	19	19	4	Y	
France 3	FR03	96	5	166	92	32	18	Y	
France 4	FR04	84	5	92	32	18	6	Y	
France 5	FR05	96	9	33	10	6	2	Y	
OECD		94	9	527	133	100	25		

a) No. Of Jueveniles divided by results for OECD soil. The value is given as 100% if the result is > 100%.

b) French and Belgian soils had remarkably higher metal contents than the Karlstad and Riksten soil content

c) low TOC & High Clay/Silt is here defined relatively, based on an TOC < 6% and clay/silt content > 24 %.

Table A2.7 Literature no observable effect concentrations (NOEC), 10th and 50th percentile effect concentration (EC10, EC50) on reproduction (number of juveniles) as well as Lethal Concentration (LC) for Enchytraeus crypticus exposed to soils spiked with individual PAHs. The concentrations are also expressed as toxicity units (TU) based on the RIVM system of Maximum Permissible Concentration (MPC) and Serious Risk Concentration benchmarks.(Verbruggen 2012) Note that the literature values were used as part of the database to establish the RIVM values.

E(L)/C	PAH	Value standard soil (10% TOC) ($\mu g\ g^{-1}_{dw}$)		RIVM MPC_{eco} TU	RIVM SRC TU
NOEC	Naphthalene	72	b,d)	167.4	2.8
NOEC	Naphthalene	17	b,d)	39.5	0.7
NOEC	Fluorene	99	a)	170.7	2.8
NOEC	Phenanthrene	125	a)	186.6	3.1
NOEC	Phenanthrene	<251	d)	<375	<6
NOEC	Phenanthrene	<129	d)	<193	<3
NOEC	Anthracene	≥2302	d)	>3242	>55
NOEC	Pyrene	458	d)	514.6	8.6
NOEC	Pyrene	66	a)	74.2	1.2
NOEC	Fluoranthene	140	a)	141.4	2.4
NOEC	Benz(a)anthracene	>2379	d,b)	>1252	>21
NOEC	Benzo(a)pyrene	≥2379	d,b)	>915	>15
NOEC	Benzo(a)pyrene	≥3382	d,b)	>1301	>22
NOEC	***Range***	***17 - 458***		***40 - 515***	***0.7 - 8.6***
EC10	Fluorene	92	a)	158.6	2.6
EC10	Phenanthrene	147	a)	219.4	3.7
EC10	Phenanthrene	169	b)	252.2	4.2
EC10	Phenanthrene	87	b)	129.9	2.2
EC10	Pyrene	40	a)	44.9	0.8
EC10	Fluoranthene	55	a)	55.6	0.9
EC24	Benzo(a)pyrene	26	c)	10.0	0.2
EC 10	***Range***	***40 - 169***		***45 - 252***	***0.8 - 4.2***
EC50	Naphthalene	220	b,d)	511.6	8.5
EC50	Naphthalene	52	b,d)	120.9	2.0
EC50	Fluorene	202	a)	348.3	5.8
EC50	Phenanthrene	320	a)	477.6	8.0
EC50	Phenanthrene	255	b,d)	380.6	6.4
EC50	Phenanthrene	131	b,d)	195.5	3.3
EC50	Anthracene	>2302	b,d)	>3242	>55
EC50	Pyrene	1738	b,d)	1952.8	32.8
EC50	Pyrene	154	a)	173.0	2.9
EC50	Fluoranthene	224	a)	226.3	3.8
EC50	Benz(a)anthracene	>2379	b,d)	>1252	>21
EC50	Benzo(a)pyrene	>2379	b,d)	>915	>15
EC 50	***Range***	***52 - 1,738***		***121 - 1,953***	***2 - 33***
LC10	Phenanthrene	454	b)	677.6	11.4
LC10	Phenanthrene	234	b)	349.3	5.9
LC50	Naphthalene	220	b,d)	511.6	8.5
LC50	Naphthalene	52	b,d)	120.9	2.0
LC50	Fluorene	5882	a)	10141.4	168.1
LC50	Phenanthrene	>7400	a)	>11045	>185
LC50	Phenanthrene	912	d)	1361.2	22.8
LC50	Phenanthrene	961	b)	1434.3	24.0
LC50	Phenanthrene	470	d)	701.5	11.8
LC50	Phenanthrene	496	b)	740.3	12.4
LC50	Anthracene	>2302	d,b)	>3242	>55
LC50	Pyrene	>2174	d,b)	>2443	>41
LC50	Pyrene	>8456	a)	>9501	>160
LC50	Fluoranthene	>9191	a)	>9284	>156
LC50	Benz(a)anthracene	>2379	b,d)	>1252	>21
LC50	Benzo(a)pyrene	>2379	b,d)	>915	>15
LC 50	***Range***	***52 - 5,882***		***121 - 10,141***	***2 - 168***

RIVM guideline, all references cited within. a) Sverdrup et al. (2002a),(b) Droge et al. (2006), c) Achazi et al.(1995) as cited in Verbruggen (2012), d) Bleeker (2003) as cited in Verbruggen (2012).

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Supporting information to Chapter 7: Evaluating a soil extraction and passive sampler methods to assess plant uptake of PAHs

Table A2.8 Total PAH concentrations in mg kg^{-1}

	FR01	FR02	FR03	FR04	FR05	FR06	FR07	BE01	BE02	SW01	SW02	SW03	SW04	SW05
NAP	28.1	9.6	2.1	28.4	5.4	20.6	0.0	1.9	0.0	0.1	0.0	0.0	0.0	6.9
ACY	60.6	84.8	1.8	22.7	7.9	15.9	1.6	2.6	0.2	3.5	1.3	0.5	0.5	3.3
ACE	15.9	138.9	0.0	27.0	3.5	8.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FLU	26.3	91.9	0.0	19.4	3.3	7.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3
PHE	109.1	153.5	4.6	71.7	19.9	44.5	6.6	9.5	0.0	5.5	3.9	2.7	1.7	26.7
ANT	96.2	75.5	1.1	12.4	4.0	23.2	1.3	2.8	0.0	1.6	1.7	0.5	0.4	5.2
FLT	248.4	137.7	8.2	134.4	30.6	103.6	17.2	30.7	0.0	21.2	11.3	2.6	1.7	24.0
PYR	200.1	100.4	6.8	104.1	25.1	77.0	17.2	28.4	0.0	16.0	8.4	1.7	1.0	23.9
BaA	204.7	77.0	6.6	83.5	19.4	61.8	9.2	12.4	0.0	14.4	5.6	1.6	0.5	13.4
CHRY	151.6	56.2	6.1	67.1	14.1	45.5	7.5	10.2	0.0	12.5	5.7	3.5	1.4	15.6
BbF	194.7	95.5	5.2	85.4	22.0	41.6	5.0	11.2	0.0	12.3	2.4	1.2	0.5	13.0
BkF	111.2	36.1	2.2	49.8	9.1	29.2	3.1	5.0	0.0	5.5	1.5	0.0	0.0	4.1
BaP	166.0	61.6	6.6	83.7	19.9	56.1	8.9	9.7	0.0	13.7	4.0	0.6	0.0	7.4
IP	128.6	52.3	5.4	83.0	16.7	49.9	7.0	8.9	0.0	8.5	4.4	0.2	0.0	7.0
DahA	52.8	17.2	0.5	27.3	3.4	15.0	0.9	1.3	0.0	2.6	0.6	0.0	0.0	1.7
BghiP	108.4	41.4	6.0	73.5	15.5	40.6	7.8	10.3	0.0	8.4	3.5	1.0	0.5	8.1
16 PAH	1902	1230	63	973	220	641	93	145	0.2	126	54	16	8.4	162

Table A2.9 Tenax extractable PAH in mg kg^{-1}

	FR01	FR02	FR03	FR04	FR05	FR06	FR07	BE01	BE02	SW01	SW02	SW03	SW04	SW05
NAP	0.58	0.42	0.45	0.68	0.30	0.40	0.42	0.57	0.16	0.26	0.25	0.35	0.29	0.23
ACY	0.82	0.97	0.09	0.63	0.29	0.25	0.52	0.66	0.00	0.23	0.17	0.03	0.10	0.08
ACE	0.38	0.99	0.09	0.80	0.08	0.24	0.27	0.94	0.00	0.02	0.04	0.03	0.03	0.10
FLU	0.83	0.79	0.12	0.86	0.17	0.31	0.31	1.17	0.00	0.07	0.10	0.06	0.06	0.16
PHE	6.05	5.09	1.23	1.77	1.23	0.58	0.62	2.22	0.33	0.53	1.10	0.35	0.32	0.31
ANT	1.85	1.01	0.03	1.07	0.24	0.16	0.66	1.16	0.00	0.18	0.11	0.09	0.10	0.08
FLT	2.34	1.00	0.16	1.96	0.37	0.64	1.00	2.13	0.06	0.42	0.62	0.29	0.28	0.31
PYR	2.34	0.98	0.48	1.98	0.55	0.56	0.88	2.30	0.04	0.37	0.44	0.20	0.18	0.21
BaA	1.85	0.72	0.10	1.32	0.32	0.56	0.86	1.38	0.00	0.44	0.53	0.28	0.23	0.22
CHRY	2.16	0.65	0.09	1.34	0.27	0.46	0.89	1.39	0.01	0.43	0.60	0.30	0.17	0.14
BbF	1.97	0.75	0.18	1.49	0.36	0.58	0.87	1.36	0.00	0.50	0.51	0.28	0.20	0.13
BkF	1.45	0.45	0.22	1.05	0.22	0.38	0.60	0.98	0.01	0.30	0.39	0.15	0.11	0.08
BaP	0.94	0.69	0.08	0.91	0.30	0.48	0.71	1.10	0.00	0.28	0.36	0.25	0.14	0.00
IP	1.14	0.58	0.06	0.99	0.26	0.52	0.64	0.87	0.00	0.35	0.38	0.21	0.16	0.00
DahA	0.56	0.18	0.00	0.36	0.06	0.13	0.25	0.31	0.00	0.05	0.12	0.03	0.01	0.00
BghiP	0.94	0.50	0.05	0.85	0.19	0.43	0.55	0.77	0.00	0.22	0.29	0.15	0.09	0.04
16 PAH	26.20	15.77	3.43	18.05	5.22	6.67	10.05	19.30	0.61	4.65	6.00	3.03	2.47	2.08

Table A2.10 Pore water concentrations measured with the POM method in µg L^{-1}

	FR01	FR02	FR03	FR04	FR05	FR06	FR07	BE01	BE02	SW01	SW02	SW03	SW04	SW05
NAP	0.948	1.148	1.095	6.812	1.214	0.686	0.925	1.002	1.366	0.955	0.924	0.707	1.345	0.734
ACY	0.200	0.239	0.006	0.252	0.051	0.086	0.394	1.695	0.000	0.034	0.064	0.002	0.005	0.000
ACE	0.236	2.523	0.026	3.881	0.050	-0.001	0.615	11.234	-0.470	0.021	0.032	0.046	0.204	0.010
FLU	0.104	0.654	0.020	1.369	0.028	0.170	0.205	7.625	0.010	0.027	0.123	0.063	0.119	0.010
PHE	0.117	0.374	0.080	3.131	0.058	0.593	0.498	13.527	0.408	0.097	0.295	0.110	0.176	0.024
ANT	0.161	0.134	0.001	0.706	0.021	0.126	0.179	1.914	-0.021	0.016	0.080	0.002	0.002	0.000
FLT	0.168	0.269	0.005	2.503	0.033	0.490	0.290	5.081	0.000	0.116	0.461	0.015	0.042	0.002
PYR	0.088	0.156	0.003	1.327	0.023	0.493	0.172	3.276	-0.002	0.073	0.249	0.008	0.025	0.002
BaA	0.071	0.007	0.000	0.127	0.001	0.047	0.035	0.168	0.000	0.005	0.023	0.000	0.001	0.000
CHRY	0.073	0.007	0.000	0.138	0.001	0.049	0.037	0.181	-0.002	0.000	0.019	0.001	0.002	0.000
BbF	0.019	0.006	0.000	0.039	0.001	0.018	0.015	0.028	0.000	0.006	0.009	0.000	0.001	0.000
BkF	0.007	0.002	0.000	0.002	0.000	0.007	0.008	0.010	0.000	0.001	0.001	0.000	0.000	0.000
BaP	0.014	0.002	0.000	0.023	0.000	0.008	0.009	0.019	0.000	0.001	0.004	0.010	0.000	0.000
IP	0.002	0.000	0.000	0.007	0.000	0.003	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.000
DahA	0.001	0.000	0.000	0.001	0.000	0.001	0.001	0.001	0.000	0.000	0.000	0.001	0.002	0.000
BghiP	0.002	0.000	0.000	0.006	0.000	0.002	0.003	0.002	0.000	0.000	0.000	0.000	0.000	0.000
16 PAH	2.211	5.521	1.237	20.323	1.480	2.778	3.389	45.767	1.290	1.354	2.283	0.965	1.922	0.783