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Assessment of soil quality using bioaccessibility-based models and a biomarker index ☆

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ABSTRACT

Bioavailability in heterogeneous media such as soils is a multi-factorial concept which ranges from soil chemistry to toxicity. The complexity of this factor has been tackled by various studies pinpointing its relevancy for laboratory to field extrapolation of toxicity data. As contaminant bioavailability on these sites is virtually unknown, a global assessment of this issue has been conducted on soils impacted by antitank firing from a Canadian Range and Training Area (RTA) and contaminated by energetic materials (EM) and metals. Yet, the descriptive results acquired from this survey require further in-depth analysis so as to enhance understanding of soil health status. Statistical models as well as an index integrating biomarker responses were derived from this database and are proposed as diagnostic, explanatory and possibly predictive tools for soil bioavailability and quality assessment. Relationships associating bioaccessible contaminant levels to soil properties allowed to clarify contaminant behaviour in energetic material (EM)-contaminated soils. Likewise, models expressing biomarker responses as a function of bioaccessible contaminant concentrations contributed to identify the contaminants causing toxicity in earthworms and to the comprehension of those toxic effects. The index of biomarker response was adapted from similar concepts applied in the aquatic environment and is an original contribution to terrestrial sites. The biomarker index data were in agreement with soil contamination profiles and represent therefore an interesting tool for soil quality appraisal. Such tools also offer a promising potential for the management of contaminated soils.

1. Introduction

Bioavailability is a composite factor integrating both chemical, physiological and toxicological aspects (Lanno et al., 2004). This is particularly true in heterogeneous media like soils. Since bioavailability is a multi-faceted variable involving both chemical and biological aspects, the terminology in this area varies according to the authors (Semple et al., 2004). As described earlier, our contention is that bioaccessibility corresponds to the environmental availability of a contaminant and is a variable of chemical nature whereas bioavailability represents the toxicological bioavailability of a contaminant and is of biological nature in our perception (Berthelot et al., in press). Studies on contaminated soils show that issues concerning toxicity, bioavailability and ecosystem integrity are varied and complex. For instance, the work of Spurgeon and Hopkin (1995) dealt with the toxic effects on earthworm lethality, growth and reproduction of metals either separately or in mixtures, in amended artificial soil and a polluted soil around a smelting facility. They found that toxic effects of metals were less severe in field soils. It was concluded that bioavailability should explain this outcome. This case illustrates the importance of bioavailability under field conditions and the risks and limitations to extrapolate from laboratory data. Likewise, Robidoux et al. (2004a,b) assessed the toxicity to earthworms of EM-contaminated soils from a military facility by measuring standard toxic effects and a stress biomarker (damage to the lysosomal membrane). They observed that the effects were not quantitatively correlated to the EM detected in soils (1,3,5,7-tetranitro-1,3,5,7-tetrazocine or HMX), nor were they consistent with previous laboratory investigations on soils amended with the pure compound (Robidoux et al., 2001, 2002). HMX bioavailability was here also invoked as a possible cause of the lack of correlation between the recorded toxicity and HMX concentrations. However, as pointed out by Robidoux et al. (2004a), metals are likely to have contributed to the toxicity of the EM-contaminated soils as well but their bioavailability in this type of soil is unknown. One causal factor of bioavailability is the historical nature of contamination in field soils; *i.e.*, bioavailability will decrease with time (Alexander, 2000). Consequently, neglecting bioavailability can lead to an overestimation of risks which in turn may engender a waste of energy and expenses for undeserved actions (Ehlers and Luthy, 2003; Bradham et al., 2006).

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The bioavailability assessment reported earlier (Berthelot et al., in press) with soils from an antitank firing range indicated that HMX and several metals were bioaccessible and exerted toxic effects on earthworms. This survey indicated that those military soils may constitute an environmental risk and contributed to identify potentially problematic substances. However, there is a need for deeper and integrating analysis techniques in order to obtain an enhanced comprehension of the causes and consequences of bioavailability in EM-contaminated soils. Modelling can be a good tool to deal with this multi-factorial situation and for the integration of composite datasets encompassing an array of diverse variables. This procedure allows to establish links between the variables at play like those representing bioavailability and constitutes a step towards the elaboration of mechanistic models and tools for bioavailability studies as advocated by the National Research Council (2003). In the first place, relationships may be established between soluble contaminant concentrations and soil characteristics to elucidate toxicant partitioning among soil phases. This phenomenon is fundamental to the bioavailability issue. Developing such models has been a common practice for some time. For example, Janssen et al. (1997) derived equations predicting the partitioning coefficient of metals in 20 soils from major soil properties. Sauvé et al. (2000) performed the same exercise but on a wider database encompassing numerous studies. More recently, Tipping et al. (2003) refined the approach by incorporating the modeled free metallic ion activity in their analysis. All these models have an explanatory and a predictive value which are important for the understanding of contaminant availability in soils and the prediction of potential risks. In addition to these usual models, other relationships can be elaborated, *e.g.* between a biological endpoint and chemical parameters. This kind of models is more interesting from an ecotoxicological point of view. However, to date this approach is seldom adopted. Recently, Bradham et al. (2006) investigated the toxicity of 2000 mg/kg Pb added to different types of soil on the lethality, growth and reproduction of *Eisenia andrei*. They developed models expressing standard biological endpoints as a function of soil properties, thereby integrating implicitly bioavailability.

Weak neutral salt extraction as considered in our assessment and applied in the related study of Berthelot et al. (in press) is a relevant and common technique to determine the bioaccessible (or exchangeable) pool of metals in soils (Gupta and Aten, 1993; Gupta et al., 1996; Houba et al., 1996; Conder and Lanno, 2000; Menzies et al., 2007). In addition to that, it has been demonstrated that the soil solution/dermal route is predominant for contaminant uptake in earthworms, particularly for metals (Saxe et al., 2001; Vijver et al., 2003; Scott-Fordsmand et al., 2004) but also for organic chemicals with low lipophilicity (*i.e.*, log $K_{ow}<5$; Belfroid et al., 1995a,b, 1996; Jager et al., 2003). HMX, which has a low log K_{ow} of 0.06–0.26 falls under this latter category (Talmage et al., 1999; Monteil-Rivera et al., 2003). For example, the toxicity of Cd, Pb and Zn to earthworms was found to be better related to metal concentrations in 0.1 M $Ca(NO_3)_2$ soil extracts than to total metals levels in metal-spiked artificial soil (Conder and Lanno, 2000) as well as a non-remediated and remediated smelter soil (Conder et al., 2001). Moreover, soil water extracts have also been used to assess mobile and available HMX fraction to worms (Kuperman et al., 2003; Simini et al., 2003; Robidoux et al., 2004a).

Biomarkers are another class of biological indicators which may be representative of contaminant bioavailability (Lanno et al., 2004). While this seems an attractive perspective, biomarkers are currently not much used for this purpose. The problem is that the use of a suite of markers (*i.e.*, a multi-marker approach) is now recommended to obtain a more representative picture of the health of an organism or even a population and to prevent misinterpretations (Cajaraville et al., 2000; Dailianis et al., 2003; Handy et al., 2003). But, measuring several biomarkers generates a set of data that may be difficult to synthesize into an overall portrait of the situation. For that purpose, different more or less complex procedures were developed to compute global biomarker indexes which integrate the individual biomarkers and indicate the level of alteration of a site (Narbonne et al., 1999; Chèvre et al., 2003; Dagnino et al., 2007). Usually, index derivation requests large databases and calls for complicated statistical operations, aside from the method of Narbonne et al. (1999). The application of the multi-marker strategy has also been advocated for terrestrial ecosystems (Kammenga et al., 2000; Scott-Fordsmand and Weeks, 2000) but, like any modelling with biological parameters in soil organisms, it also is at an early stage. Further development along these lines is consequently needed to achieve a global understanding of bioavailability in soils and for soil quality assessment.

This paper presents a new analytical approach of the recently reported results (Berthelot et al., in press) in soils contaminated with EM and metals from a military Range and Training Area (RTA) and provides a biomarker-based index of soil quality. Contaminant bioaccessibility in EM-contaminated soils is analyzed with statistical models based on soil properties and the obtained relationships are compared to literature data. Potential links between toxicant bioaccessibility or tissue concentrations and biomarker responses are also investigated through analogous models. Finally, a biomarker-based index of soil quality derived using an established technique is proposed.

2. Materials and methods

2.1. Datasets

Models were developed to improve the understanding and the interpretation of strategic data for the soil quality assessment of military RTAs. These models were built upon data acquired for ecotoxicological assessment of soils from the Canadian Forces Base of Gagetown (NB, Canada) and described previously (Berthelot et al., in press). These data are summarized in Tables 1 and 2. Table 2 indicates the value ranges of the variables upon which models are built, thereby defining their validity limits. Soils were sampled along an oblique transect away from two different tanks from the same RTA: T2 (substations: T2-15; T2-28 and T2-48) and T3 (substations: T3-9; T3-19; T3-37; T3-54 and T3-86). The reference station (R) was selected outside the firing area at 55 m from the firing point. Selected variables for model derivation were: (1) soil physico-chemical properties (sand, silt and clay content, pH, Total Organic Carbon — TOC, amorphous iron and aluminium oxide contents — Fe_{ox} and Al_{ox}), (2) total soil contaminant concentrations at the beginning of the exposure to the contaminated soils — $t=0$ (metals: Bi, Cd, Cr, Cu, Ni, Pb and Zn determined upon concentrated HNO_3 digestion as well as HMX determined by an acetonitrile soil extraction), (3) bioaccessible metal and soluble HMX levels measured in aqueous weak electrolyte soil extracts at $t=0$, (4) metal concentrations in earthworm tissue after 28 days of exposure (HMX not being detected) and, (5) selected biomarkers (NRRT: Neutral Red Retention Time, CAT: Catalase, SOD: Superoxide dismutase, GST: Glutathione S-Transferase and AP: Acid Phosphatase) assessed after 2, 7 and 28 days of exposure.

2.2. Multiple regression analysis

Linear multiple regression models were fit to relevant variables. More precisely, regressions were determined for the following pairs of explained and explanatory variables: bioaccessible contaminant concentrations in soil with soil physico-chemical properties and biomarkers against bioaccessible contaminant levels or contaminant tissue concentrations. Angular transformations were performed on variables expressed as proportions to avoid border artifacts (Zar, 1996). When the application conditions were not fulfilled (normality or homogeneity of variance), data were normalized. For that purpose, data were transformed by applying relevant mathematical functions

Table 1
Summary of biomarker data acquired upon exposure of *Eisenia andrei* to contaminated soils from the Wellington antitank firing range (WAT) on the Canadian Forces Base at Gagetown (NB, Canada)

Soil sample parameter	*R*	T2-15	T2-28	T2-48	T3-9	T3-19	T3-37	T3-54	T3-86
NRRT-28d (min)	35.8 (±3.0)	22.5 (±4.4) *	17.0 (±3.8) *	10.5 (±1.8) *	11.9 (±1.8) *	8.3 (±1.7) *	17.4 (±2.1) *	18.7 (±2.2) *	26.0 (±6.1)
SOD-2d (U)	218.7 (±32.5)	272.1 (±38.5)	249.0 (±16.6)	236.5 (±23.8)	281.4 (±14.1)	301.9 (±15.2)	333.7 (±6.8) *	275.7 (±30.6) *	262.6 (±1.9)
SOD-7d (U)	223.8 (±24.9)	288.6 (±20.0)	321.5 (±25.5) *	325.8 (±25.1) *	393.0 (±19.0) *	348.1 (±18.4) *	312.2 (±31.7) *	346.0 (±10.4) *	291.8 (±60.8) *
SOD-28d (U)	376.2 (±19.5)	478.9 (±53.6) *	543.4 (±41.2) *	459.9 (±32.8) *	509.9 (±8.7) *	543.3 (±32.5) *	508.9 (±69.1) *	449.5 (±38.0)	500.8 (±56.2) *
CAT-28d (μmol/min/mg protein)	164.9 (±5.7)	193.1 (±18.4)	198.8 (±11.6) *	163.4 (±23.0)	185.0 (±6.4)	175.5 (±19.8)	137.1 (±10.2)	173.3 (±19.5)	162.6 (±19.2)
GST-28d (nmol/min/mg protein)	359.5 (±25.9)	354.5 (±7.1)	342.9 (±21.2)	343.0 (±28.2)	374.6 (±24.8)	336.0 (±3.6)	287.3 (±15.4) *	346.5 (±36.2)	383.5 (±24.1)
AP-7d (μmol/min/mg protein)	1.04 (±0.08)	1.27 (±0.10)	1.21 (±0.09)	1.26 (±0.12)	1.16 (±0.04)	1.24 (±0.18) *	1.70 (±0.30) *	1.27 (±0.10)	1.45 (±0.19) *
AP-28d (μmol/min/mg protein)	1.02 (±0.02)	0.92 (±0.05)	0.91 (±0.09)	1.17	1.31 (±0.25)	0.85 (±0.05) *	1.05 (±0.17)	0.98 (±0.10)	1.09 (±0.15)

Data is expressed as mean±SE, $n=4$.
Note. * Significantly different from reference soil R (Dunnett's test, $p<0.05$); NRRT=Neutral Red Retention Time; SOD=Superoxide dismutase; CAT=Catalase; GST=Glutathion-S-transferase; AP=Acid Phosphatase. NRRT-28d: NRRT after 28 days of exposure; SOD-2d: SOD activity after 2 days of exposure; SOD-7d: SOD activity after 7 days of exposure; SOD-28d: SOD activity after 28 days of exposure; CAT-28d: CAT activity after 28 days of exposure; GST-28d: GST activity after 28 days of exposure; AP-7d: AP activity after 7 days of exposure; AP-28d: AP activity after 28 days of exposure.

for such type of statistical analyses (Zar, 1996). A stepwise procedure was applied to derive the significant models among those variable sets. The Jump In 4.0.4 software (SAS Institute Inc., Cary, NC, USA) was employed. Only significant models and their constitutive variables are presented.

2.3. Model sensitivity analysis

The aforementioned models were analyzed for their sensitivity towards potential explanatory variables using the Crystal Ball 4.0 software (Decisioneering, Denver, CO, USA). The following setup was applied to run the simulations: 100,000 trials, Monte-Carlo sampling method, sensitivity analysis. The model sensitivity towards each explanatory variable was expressed as the percent contribution to variance. This procedure allowed to rank the variables according to their sensitivity. Models were then simplified by eliminating minor variables. After appraisal, retained models were the best trade-off between the magnitude of R^2 and simplicity.

Table 2
Summary of the physico-chemical properties, soil contamination data and *Eisenia andrei* body residues data obtained during the exposure of earthworms to soils from the Wellington antitank firing range (WAT) on the Canadian Forces Base at Gagetown (NB, Canada)

Parameter	Range
pH	5.36–7.96
Fe_{ox} (%)	0.50–4.43
Al_{ox} (%)	0.35–1.77
TOC (%)	1.36–5.67
Clay content (%)	7.6–16.7
Total [Bi] (mg/kg)	1.2–184.8
Bioaccessible [Bi] (mg/kg)	0.06–0.97
Tissue [Bi] (mg/kg)	8.2–22.8
Tissue [Cu] (mg/kg)	15.4–204.3
Total [Ni] (mg/kg)	2.2–397.6
Bioaccessible [Ni] (mg/kg)	0.02–0.12
Tissue [Ni] (mg/kg)	0.002–10.7
Total [Zn] (mg/kg)	26.0–1334.0
Bioaccessible [Zn] (mg/kg)	0.07–7.5
Total [HMX] (mg/kg)	0.18–472.9
Soluble (aqueous KNO_3 extractable) [HMX] (mg/kg)	0.35–33.6

Data are reported as ranges (min–max). Adapted from Berthelot et al. (in press).
Note. TOC: Total Organic Carbon; Al_{ox}: amorphous aluminium oxide; Fe_{ox}: amorphous iron oxide.

2.4. Integrated biomarker response calculation

An index of biomarker response (IBR) was determined according to the procedure described by Narbonne et al. (1999, 2001) and applied as reported in Banni et al. (2005) and Narbonne et al. (2005). This index was derived from the dataset constituted with the five biomarkers (CAT, SOD, GST, NRRT and AP) assessed after 28 days of laboratory exposure of worms to contaminated soils from the Gagetown military training area. Biomarker data were first checked for possible confounding interrelations between biomarkers used for IBR calculation and analyzed with a one-way ANOVA and Dunnett's test (Table 1). Briefly, the mean was calculated for each parameter at each station and a 95% mean confidence interval (CI) was obtained for each biomarker. Then, a response factor (RF, the ratio between the highest and the lowest mean) and a response range (RR, the arithmetic difference between the highest and the lowest mean) were determined. A discriminatory factor was calculated as DF=(RR+CI)/CI. This factor served to generate a scale indicating the theoretical number of significant differences among the soils under investigation. This DF was then rounded up to an integral number, the discriminatory level (DL), through the analysis of significant differences between means. The DL served to establish a scale used to rank each biomarker response according to the number of levels between the lower and the higher mean of the individual stations. For example, each biomarker

Table 3
Linear multiple regressions between bioaccessible/soluble contaminant concentrations and soil physico-chemical properties (TOC: Total Organic Carbon; Al_{ox}: amorphous aluminium oxide; Fe_{ox}: amorphous iron oxide)

Variable (y=)	Model ($ax+by+\ldots+c$)	R^2	p	N
Bioaccessible [Bi]=	0.78 √ total [Bi] (±0.09)+91 Arcsin (√ clay percentage/100) (±9.8)+62 Arcsin (√ Fe_{ox}/100) (±8.1)−73 Arcsin (√ Al_{ox}/100) (±11)−150 Arcsin (√ TOC/100) (±17)−14 (±1.5)	0.97	0.0132	9
Bioaccessible [Ni]=	exp[0.58 ln total [Ni] (±0.12)+8.0 Arcsin (√ clay percentage/100) (±2.6)−37 Arcsin (√ Al_{ox}/100) (±7.6)−0.42 pH (±0.12)−2.8 (±1.1)]	0.93	0.0158	9
Bioaccessible [Zn]=	[0.12 √ total [Zn] (±0.04)−20 Arcsin (√ Fe_{ox}/100) (±7.2)−pH (±0.25)+7.6 (±1.4)]^2	0.83	0.0229	9
Soluble [HMX]=	3.7 ln total [HMX] (±0.97)−89 Arcsin (√ clay percentage/100) (±50)+37 (±20)	0.84	0.0037	9

$p<0.05$ for all variables.

response must be ranked '1' or '2' when there are two levels and from '1' to '5' if there are five CI increments between lower and higher mean. The biomarker responses were subsequently normalized by deriving an individual biomarker index (BI) for each biomarker value following their rank position by means of the conversion table of Narbonne et al. (1999). The assigned indexes are arbitrary numbers proposed by Narbonne et al. (1999, 2001) to rank biomarkers into the described classification scale. Finally, an integrated biomarker response (IBR) was determined for each station by summing up the individual score of the five biomarkers. The macro developed with the Excel software (Microsoft®) by O. Brack (K.S.I.C. Society, France, olivier.brack@wanadoo.fr) was used to achieve this whole sequence of IBR computation. This index indicates the pollution status of a site according to the following ranges: 0–19 = lightly polluted; 20–29 = moderately polluted; 30–39 = highly polluted; 40–49 = severely polluted and >50 = critically polluted. The discriminating capacity between sites of the different biomarkers was also assessed by calculating the frequency (percentage) of highest individual score for each biomarker, reference soil excluded.

3. Results

3.1. Modulation of bioaccessibility by soil physico-chemical properties

Let us first note that except for HMX (R^2 = 0.76, p = 0.002), no univocal significant correlations were established between soluble contaminant levels and total soil concentrations. Equations elaborated to identify the soil factors influencing contaminant bioaccessiblity are summarized in Table 3. From these models, one can conclude that bioaccessible Bi level was correlated positively with total soil Bi concentration, clay content as well as the amount of amorphous iron oxide and inversely correlated with the amount of amorphous aluminium oxide and TOC. In terms of sensitivity analysis, these data yielded the following contribution to variance: TOC accounted for 50.9% of the variability in bioaccessible Bi concentration output, clay content for 24.9%, Bi soil concentration for 12.8%, Fe_{ox} for 7.8% and Al_{ox} for 3.6%. Bioaccessible Ni concentration was modulated positively by total Ni soil concentration plus clay content and inversely correlated with pH as well as the amount of amorphous aluminium oxide. Variation in bioaccessible Ni level was explained at 51% by Al_{ox}, 30% by soil Ni load, 10% by clay content and 9% by pH. Bioaccessible Zn concentration was influenced positively by total soil Zn content but inversely associated to pH and the amount of amorphous iron oxide. In this context, soil Zn level contributed up to 46.4% to bioaccessible Zn concentration variability, pH up to 32.5% and Fe_{ox} to 21.1%. A model was also derived for Cd but it was not consistent and is hence not presented. Finally, soluble HMX concentration resulted from a positive effect of total soil HMX concentration along with a negative effect of clay content. In this case, the clay proportion accounted for 53.3% of the variability and soil HMX content for 46.7%. The pH and total soil concentrations systematically appeared as main variables governing metal bioaccessibility. Secondary variables were soil texture properties such as TOC, clay content and the amount of amorphous iron and aluminium oxide.

3.2. Relationships between biomarker responses and bioaccessibility or tissue concentrations

Models generated to examine the possible association between contaminant bioaccessiblity and the investigated biological responses are presented in Table 4. No significant relationships were found between biomarker responses and total toxicant concentrations in soil. The SOD activity after 2 days of exposure (SOD-2d) was positively correlated with soluble HMX concentration as well as bioaccessible Zn concentration. Bioaccessible Zn generated 50.6% of the variability in SOD-2d activity while accessible HMX accounted for the remaining 49.4%. After seven days of exposure, soluble HMX concentration contributed positively to the SOD activity (SOD-7d) whereas the effect of bioaccessible Ni (ln log-transformed values below 1)

Table 4
Linear multiple regressions between biomarker responses and bioaccessible/soluble or tissue contaminant concentrations

Variable (y=)	Model (ax+by+…+c)	R^2	p	N
SOD-2d=	1.9 soluble [HMX] (±0.41)+8.7 bioaccessible [Zn] (±1.8)+207 (±12)	0.86	0.0028	9
SOD-7d=	2.2 soluble [HMX] (±0.69)+47 ln bioaccessible [Ni] (±14)−71 bioaccessible [Bi] (±24)+462 (±50)	0.82	0.0266	9
SOD-28d=	2.8 soluble [HMX] (±1)+433 (±23)	0.53	0.0270	9
SOD-7d=	0.51 tissue [Cu] (±0.12)−3.0 tissue [Bi] (±0.97)+290 (±20)	0.83	0.0045	9

Refer to Table 1 for acronym definitions. p<0.05 for all variables.

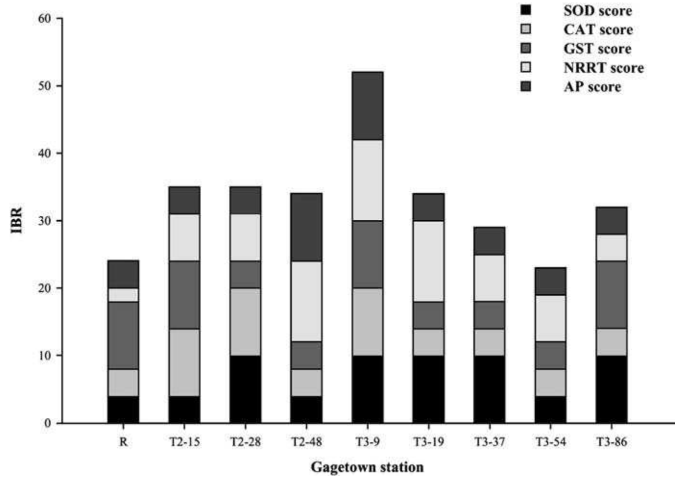

Fig. 1. Global index of biomarker response (IBR) of Gagetown soils. The IBR for each soil corresponds to the cumulative sum of the individual biomarker indexes found in the stacked bars.

and Bi concentrations was negative. In this equation, variability of SOD-7d was influenced at 41.3% by bioaccessible Ni level, 31.9% by soluble HMX concentration and 26.8% by bioaccessible Bi concentration. At 28 days of exposure, a positive correlation between SOD activity and soluble HMX concentration was evidenced. Accessible HMX concentration was consistently correlated to SOD activity levels through exposure time with similar regression coefficients. Models were also obtained for other biomarkers such as CAT activity after 28 days of exposure (CAT-28d) and AP activity after 7 days of exposure (AP-7d). Since these biomarkers were not very sensitive (Table 1) and some models were not well-substantiated, they are not reported.

The same linear regression analysis was also performed with body residues and biomarkers. The only significant model obtained is provided in Table 4. The SOD-7d was found to be positively correlated to tissue Cu content and negatively to tissue Bi concentration. In this model, tissue Cu concentration produced 67.8% of the variation in SOD-7d while the other 32.2% were provided by Bi tissue concentration. As for the importance of Bi tissue concentration, it was however higher than that of tissue Cu due to its greater coefficient in the equation. A model was also derived for the AP activity after 28 days of exposure (AP-28d). As for the bioaccessible concentrations models, it was omitted because of the poor sensitivity of AP and the inconsistency of the model.

No analogy like consistent patterns or recurrent variables was evidenced across models derived from bioaccessible concentrations or from contaminant body concentrations. It should also be specified that tissue concentrations were neither correlated to total soil toxicant contents nor to bioaccessible levels.

3.3. Integration of biomarker responses

The individual biomarker responses used to derive the IBR were checked for possible interrelations and none was found. Individual biomarker scores and integrated biomarker response index (IBR) for each site are presented in Fig. 1. The global biomarker index distribution revealed that the reference soil sample R together with T3-37 and T3-54 soil samples were among the lowest IBR. These samples were followed by T2-15, T2-28, T2-48, T3-19 and T3-86 with intermediate rates and T3-9, with the highest score, was the most severely impacted soil. The most discriminating markers between sites were NRRT with 50.0% followed by SOD with 37.5%. GST and CAT came out at the last scoring position with 25.0% each and AP had a null rate.

4. Discussion

This paper exposes a synthetic analysis of data from soils sampled on an antitank training range, consisting in attempts to establish links among soil properties, contaminant bioaccessibility and body burdens, biomarker responses as well as the application of a method to integrate the biomarker responses.

4.1. Bioaccessibility as a function of soil properties

As cation exchange capacity (CEC) is a measure of the amount of available sorption sites in a soil, it encompasses all sorption components (clay, Fe_{ox} and Al_{ox} plus OM content). Due to this close connection, it was decided to exclude CEC from the model derivation process, like Janssen et al. (1997). The absence of correlation between bioaccessible metal levels and total soil concentrations illustrates the modifying effect of soil characteristics on contaminant bioavailability. This phenomenon is widely acknowledged (Peijnenburg et al., 1997; Allen, 2002; Peijnenburg and Jager, 2003). Conversely, water-extractable HMX level correlated well with total soil concentration and this may be inherent to the sorption behaviour of HMX in soils. HMX like other EMs sorbs poorly to soils and its sorption pattern is governed by the clay content (Monteil-Rivera et al., 2003; Hatzinger et al., 2004). This is a possible explanation for the concordance between soluble HMX and total soil concentrations, especially since the clay content variations were low in the tank # 2 sample series or followed the opposite trend (*i.e.*, increasing with distance) to that of total HMX in tank 3 samples (Berthelot et al., in press). Moreover, the model derived for HMX aqueous mobility in Gagetown soils with clay content as the main variable is in agreement with the previous statements concerning the factors controlling HMX solubility (Monteil-Rivera et al., 2003).

For the metals, models were issued for Bi, Ni and Zn. In the derived equations pH emerged as a consistently negative parameter, except for Bi. Our observations concord with the unanimous statement on metal partitioning and behaviour in soils that pH is negatively correlated to metal solubility (Anderson and Christensen, 1988; Janssen et al., 1997; Rieuwerts et al., 1998; Sauvé et al., 2000). Soil pH determines the number of available sorption sites for metals by affecting the soil surface charge with lower pH meaning less negative binding sites. In addition to that, low soil pH means more H^+ ions in solution which will compete with Me^+ ions for negative binding sites. Thus, the combination of those processes will render metals more soluble at low pH (Sauvé, 2002).

Bi is a rare element whose invertebrate toxicity and behaviour in soils is scarcely characterized (Hou et al., 2006). Contamination of soils by Bi arises from the move to Pb-free technologies (as Pb is a notorious toxicant) and, more specifically, from the substitution of Pb by Bi in shells of small arms ammunition and heavier military arsenal (Fahey and Tsuji, 2006; Hou et al., 2006). The Bi salts involved in the manufacture of common Bi-derived products have very low aqueous solubility (Hammond, 2007). However, the fate of Bi in soils is nearly unknown and it may undergo transformations which could render the element more soluble and available for organisms. Hou et al. (2006) actually established a regression model predicting the mobilizable Bi fraction in clean soils comparable to the equation obtained in our analysis. Certain variables and the direction of their influence were identical across the two models: clay content had a positive effect whereas organic carbon level had a negative contribution. This analogy is consistent with the identification of TOC and clay content as the major variables. The occurrence of Fe_{ox} and Al_{ox} in our equation is consistent with the presence of CEC and surface area in their. The pH did not come out in our model whereas it did in Hou et al. (2006). Nevertheless, it should be stressed that the mobilizable fraction defined by these authors encompasses more metal pools than the exchangeable metal fraction assessed in our work. Additionally, as Bi is chemically closely related to Pb, similar reactions in soil may be expected. As a matter of fact, Janssen et al. (1997) who examined the relationships between metal partition coefficients and soil characteristics determined that the Pb partition coefficient, when normalized to the soil Fe_2O_3 content, was almost insensitive to pH. This finding is further substantiated by the low R^2 and proton coefficient they obtained for the pH-regression. The observation of Janssen et al. (1997) may explain the apparent unsusceptibility of Bi bioaccessibility to pH as indicated by our model. Overall, Bi seems to be efficiently bound by common adsorbing soil phases (*e.g.* organic matter, clay, amorphous metal oxides), like Pb (Farrah and Pickering, 1977; Zimdahl and Skogerboe, 1977; Tipping et al., 2003; Rieuwerts et al., 2006). However, the positive influence of clay level here might be attributed to the fact that clays can be an important Bi reservoir but this remains to be elucidated.

The apparent low importance of pH in the equation obtained for Ni is at first sight surprising and may seem contradictory to what is generally reported in the literature. But, this outcome is probably the result of usual interrelations between soil parameters (Bradham et al., 2006; Rieuwerts et al., 2006). So, the effect of pH may be indirectly mediated through other variables like in Bradham et al. (2006) who established connections between Pb toxicity to earthworms and soil properties and demonstrated that the effects of certain parameters were partly indirect and mediated by other variables. Other known sorption phases also influenced Ni solubility in the present study. Aside from the classical negative effect on solubility of increasing pH and the positive one of total soil Ni, Al_{ox} content was found to reduce Ni solubility while clay content seems to increase it. Clay as well as Al and Fe oxyhydroxide in soils are known as significant binding fractions for metals like Ni (Anderson and Christensen, 1988; Alloway, 1995a,b; Yin et al., 2002). The influence of Al_{ox} is consistent with this statement (binding of metals) but the positive contribution of clay appears contradictory to its conventional metal-sequestering function as noticeable in the Ni equation by Janssen et al. (1997). This outcome may be due either to interrelation effects or the fact that the clay term in the equation represents the sorbed metal whereas the Al_{ox} term constitutes the active sorption agent since clay minerals are coated with amorphous metal oxides (Yin et al., 2002). The latter hypothesis may be supported by the relative weight of the two parameters in the model (51% for Al_{ox} versus 10% for clay).

Zn bioaccessibility was mainly driven by pH and total Zn load. The pH was an important parameter in the equation, accounting for 32.5% of the variance and up to 78.9% together with total soil Zn. It is in agreement with the behaviour of Zn in soils as reported by other authors (Janssen et al., 1997; McBride et al., 1997; Rieuwerts et al., 1998, 2006; Hobbelen et al., 2006). The last factor in importance which controlled Zn availability is Fe_{ox} and its negative contribution concurs with the sorbing action of Fe_{ox} in soils. No model could be derived for other metals (*e.g.* Cr, Cu and Pb) or was found inconsistent (Cd) and this may be attributed to insufficient data (many values below detection limits) and/or to their more complex partitioning patterns. The generated models are globally in good agreement with the literature (Janssen et al., 1997; Rieuwerts et al., 1998, 2006; Sauvé et al., 2000). However, these introductory models may be refined by extending the database, then validating them against field-collected data (*e.g.*, in mesocosms).

4.2. Relationships between biomarkers and bioaccessible or tissue concentrations

Since bioaccessible toxicant levels can be considered as better estimates of chemical availability than total soil loads as advocated in the introduction, an attempt to derive equations linking bioaccessible concentrations and biomarkers responses was made, the latter constituting a biological measure of bioavailability. SOD exhibited the most consistent pattern in time with the coherent contribution of HMX. Its toxicity mechanism in invertebrates is unknown, particularly with respect to oxidative stress and the antioxidant defence system but some insights might be found in the biodegradation pathways. In the known biodegradation pathways, HMX acts as an electron acceptor and could then be considered as an oxidant (Crocker et al., 2006). This might account for the “enhancing” effect of HMX on SOD activity. Alternatively, the HMX influence could be mediated by a degradation metabolite such as nitroso-HMX [NO-HMX] (Fournier et al., 2004). The NO-HMX derivatives are indeed oxidant agents and their formation could promote the generation of reactive oxygen species which in turn might activate or induce SOD (Crocker et al., 2006). Moreover, other organic compounds such as pesticides were also reported to affect earthworm SOD activity (Luo et al., 1999).

Metals are, for their part, recognized as oxidative stress inducers in laboratory animal and wild species (Regoli et al., 1998; Regoli, 2000; Frenzilli et al., 2004) even if it is equivocal in earthworms (Hønsi et al., 1999; Dhainaut and Scaps, 2001; Łaszczyca et al., 2004). In our assessment, the influence of metals on SOD activity was not consistent unlike that of HMX, with different metals correlating with SOD at the different exposure times and apparently no metal contribution at 28 days. This may be inherent to the difficulty to segregate the effects of a particular metal among a mixture. Nevertheless, the positive influence of Zn on SOD-2d activity might be explained by the fact that SOD seems to occur mostly as Cu,Zn-SOD in *Eisenia* sp. (Hønsi et al., 1999). Concerning SOD-7d activity, the contribution of Bi remains to be elucidated. The absence of well-substantiated models for other biomarkers, such as CAT, GST and AP may be due to the low sensitivity of their response as observed in this study. Surprisingly, no relationship was found between the sensitive NRRT and bioaccessible contaminant concentrations. This situation may result from the fact that NRRT is a generic marker which is known to react to a wide range of chemicals (Svendsen et al., 2004). The contribution of multiple contaminants and the interplay of complex patterns (like contaminant interactions) may have prevented the derivation of a straightforward model.

Internal contaminant concentrations are also regarded as a biological measure of bioavailability as they may represent the bioactive fraction of the contaminant and they may be used within the critical body residue concept (Lanno et al., 2004). In the present analysis, only one equation could be derived for contaminant tissue concentrations. This paucity of relationships may arise from the established capacity of earthworm to store metals in a non-toxic form such as mineral granules (Morgan and Morgan, 1998; Morgan et al., 1999). The positive influence of internal Cu on SOD activity may also be attributed to the prevalence of Cu,Zn-SOD.

4.3. Integrated biomarker response

A multi-marker approach which consists in the application of a battery of biomarkers is now often recommended (Cajaraville et al., 2000; Kammenga et al., 2000; Scott-Fordsmand and Weeks, 2000; Dailianis et al., 2003; Handy et al., 2003) and has been used extensively in the aquatic environment (Astley et al., 1999; Blaise et al., 2002; Galloway et al., 2004) but only sparingly for terrestrial habitats (Spurgeon et al., 2005). The difficulty associated with multimarker studies resides in the huge amount of generated data which hinders global interpretation. As a result, various more or less complex techniques have been developed to integrate the biomarker responses into an index (Narbonne et al., 1999; Beliaeff and Burgeot, 2002; Chèvre et al., 2003; Dagnino et al., 2007). As a first attempt to derive such an index from a terrestrial survey, the method of Narbonne et al. (1999) was adopted due to its relative simplicity and its suitability to the dataset. This approach has been successfully applied in different contexts (Banni et al., 2005; Narbonne et al., 2005).

To our knowledge, this is the first attempt to use this kind of approach in a terrestrial context. Some of the biomarkers (NRRT, AP, SOD) included in our computation of the IBR differed from those considered in Narbonne et al. (1999) and subsequent work whereas the others, related to oxidative stress, were identical (*i.e.*, CAT and GST). The biomarkers monitored both by Narbonne et al. (1999) and in related studies (Banni et al., 2005; Narbonne et al., 2005) were GST and acetylcholinesterase activity in gills and CAT, GST and benzo(*a*) pyrene hydroxylase activity as well as lipid peroxidation level (through malonedialdehyde —MDA— formation) in the digestive gland of sentinel bivalve species. Narbonne et al. (1999) also included the CAT/MDA ratio whereas Banni et al. (2005) added the lipofuscin surface density as a biomarker.

The global index distribution reveals that T3-9 was the most impacted site whereas the reference R and T3-54 were least affected and the other stations had intermediate scores. This pattern is generally in agreement with the chemical contamination data reported earlier (Berthelot et al., in press). The calculated IBR appears therefore as a good indicator of soil quality status. Like in Astley et al. (1999) and Galloway et al. (2004), a discriminating potential between the sites was determined for the biomarkers. The most discriminating markers were NRRT and SOD whereas the least was AP and this is consistent with the response patterns and the relative sensitivity of biomarkers recorded by Berthelot et al. (in press) following exposure of earthworms to Gagetown soils. They indeed found that NRRT and SOD were the most sensitive markers. The discriminating capacity ranking of the biomarkers is also in agreement with the predominant contributions of NRRT and SOD to the IBR scores (Fig. 1). It has been demonstrated that NRRT is a workable earthworm biomarker, which responds to the presence of most contaminants, both in laboratory and field conditions (Svendsen et al., 2004; Sanchez-Hernandez, 2006) and is predictive of effects at higher levels of biological organization (Scott-Fordsmand and Weeks, 2000). Moreover, this biomarker has already been applied to soils from an RTA analogous to the area considered in this study (Robidoux et al., 2004a,b). Along with that, it should also be pointed out that the IBR distribution is comparable to that of NRRT, notably in T3 soils. The latter observation can be attributed to the sensitivity of NRRT which is endowed with a predominant share in the IBR as indicated previously. The correspondence of NRRT and IBR patterns corroborates the value of NRRT as biomarker. As for SOD, the response pattern recorded by Berthelot et al. (in press) is something new in earthworms (Hønsi et al., 1999; Łaszczyca et al., 2004), but the ranking of this biomarker is in agreement with our previously reported results. The last position of AP in the discriminating performance ranking may be explained either by the lack of sensitivity of this marker or by the protocol used in this case, which assesses whole cytosolic AP as opposed to lysosomial AP (Hønsi and Stenersen, 2000). In view of the obtained results, global biomarker indexes and similar approaches appear as promising tools to assess soil health status and for the management of soil contamination and remediation.

5. Conclusion

Relationships were established between contaminant bioaccessibility and soil properties elucidating the behaviour of HMX and metals in EM-contaminated soils. This revisiting of the data collected within the frame of the bioavailability and toxicity assessment of soils from a military RTA provided a new insight into soil quality assessment through an integrated approach. This scrutiny based on models resulted in a better understanding of processes underlying bioavailability in soils from military areas and in the development of a synthetic and intuitive tool indicating soil quality.

The role of some major factors like pH and amorphous metal oxides towards metals or clays towards HMX was substantiated for the studied soils. Other models were derived expressing biomarker responses as a function of either contaminant bioaccessibilities or tissue concentrations. The main finding was that bioaccessible HMX concentration was consistently related to SOD at the different exposure times. HMX might then be involved in oxidative stress in earthworms. This kind of models has the potential to serve as predictors of toxicant bioaccessibility in military soils or bioavailability in targeted species, particularly if expanded to other military sites.

An index of biomarker response incorporating all five biomarkers was also determined by applying a procedure used for aquatic organisms. This is the first attempt to derive such an index for contaminated soils. The index results were consistent with the contamination levels of the soil samples and with previous biomarker data, since the two most responsive biomarkers — SOD and NRRT — were also the most discriminating among soils. These results stress the relevancy of SOD and NRRT as biomarkers signalling exposure to EM-contaminated soils and of the biomarker index as a soil quality indicator. The tools developed here concerning the bioavailability issue have promising perspectives for soil quality assessment and management of contaminated sites, particularly on RTA. Since the application of these tools constitutes a first step, it holds validity only for the investigated military base. It should also be stressed that the associated tools applied in the present study to assess bioavailability (such as the biomarker index) should be used in conjunction with a suite of judiciously chosen tools. The elaboration of such tools should therefore be promoted and further work has still to be done to test, refine and validate these.

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