

Review

Microbial reporters of metal bioavailability

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Summary

When attempting to assess the extent and the implications of environmental pollution, it is often essential to quantify not only the total concentration of the studied contaminant but also its bioavailable fraction: higher bioavailability, often correlated with increased mobility, signifies enhanced risk but may also facilitate bioremediation. Genetically engineered microorganisms, tailored to respond by a quantifiable signal to the presence of the target chemical(s), may serve as powerful tools for bioavailability assessment. This review summarizes the current knowledge on such microbial bioreporters designed to assay metal bioavailability. Numerous bacterial metal-sensor strains have been developed over the past 15 years, displaying very high detection sensitivities for a broad spectrum of environmentally significant metal targets. These constructs are based on the use of a relatively small number of gene promoters as the sensing elements, and an even smaller selection of molecular reporter systems; they comprise a potentially useful panel of tools for simple and cost-effective determination of the bioavailability of heavy metals in the environment, and for the quantification of the non-bioavailable fraction of the pollutant. In spite of their inherent advantages, however, these tools have not yet been put to actual use in the evaluation of metal bioavailability in a real environmental remediation scheme. For this to happen, acceptance by regulatory authorities is essential, as is a standardization of assay conditions.

Introduction

Increasing awareness of anthropogenic environmental pollution, and of its implications for human and environmental health, has led to the continuous development of two complementary approaches for assessing the degree of contamination. Physicochemical analysis, using a wide spectrum of analytical instrumentation (Bontidean *et al.*, 2000; Köhler *et al.*, 2000), allows highly accurate and sensitive determination of sample composition. It is essential for regulatory compliance monitoring (Belkin, 2003) as well as for understanding the sources of pollution and the means for its remediation. However, the array of analytical procedures necessary for a complete analysis of environmental samples is often costly, time-consuming, highly complex and requires trained personnel. Furthermore, such analysis fails to provide information on the degree of bioavailability and/or the toxicity of the sample components (Köhler *et al.*, 2000; Tauriainen *et al.*, 2000; Flynn *et al.*, 2003).

As a partial response to these drawbacks, a complementary bioassay-based approach has also been implemented, and is continuously evolving in parallel to analytical methodologies. One such group of biological tools is toxicity bioassays. Rather than detect and quantify specific sample constituents, such assays quantify the global negative impact of the sample on a population of test organisms, with the end result 'averaging' synergistic and antagonistic effects (Belkin, 2003). The most widely used test organisms are either fish or planktonic crustaceans (*Daphnia* etc.), but numerous other test systems have been standardized, including the bacterial *Vibrio fischeri* bioluminescence test in which the decrease in light emission serves as an indication of the toxicity level (Bulich and Isenberg, 1981). A somewhat different class of bioassays aims at assessing compounds' bioavailability; such tests attempt to distinguish between the bioavailable fraction of a compound 'seen' in the bioassay, and the total concentration determined by chemical analysis. Unsurprisingly, the latter often contains biologically inert, unavailable forms of the target compound (Belkin, 2003; Ivask *et al.*, 2004; Liao *et al.*, 2006). This phenomenon, often of concern when remediation end-points have to be determined, carries particular significance for metal pollution (Kelly *et al.*, 2002).

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Bioavailability, bioaccessibility and their determination

Most commonly found definitions for the term 'bioavailability' originate from medical uses, often relating to drug absorbance in body or tissue. A somewhat broader definition (The American Heritage Dictionary of the English Language, 4th Edition, 2007) refers to the degree and rate at which a substance is absorbed into a living system or becomes available at the site of physiological activity. This definition, in essence, also applies to environmental pollutants (Impellitteri *et al.*, 2003). 'Bioavailability processes' have been defined by the US National Research Council (2003) to include the release of solid bound contaminants and their subsequent transport, direct contact, uptake by passage by a biological membrane and incorporation into a living system. For a contaminant to have a biological effect, however, the last two phases are not essential, as in principle chemicals may affect living systems also extracellularly. Another term occasionally used in the same context is bioaccessibility. In some cases it is used only in relation to human exposure, defined, for example, as 'the fraction of metals that desorbs from its matrix in the gastrointestinal tract' (Ruby *et al.*, 1996; 1999). Only after being thus absorbed the metal in question becomes bioavailable. A broader definition is provided by Semple and colleagues (2007), who regard bioaccessibility as 'that which is available to cross an organism's (cellular) membrane from the environment it inhabits, if the organism had access to it'. According to this definition, the bioavailable fraction of a pollutant may be only a fraction of the bioaccessible one. Bioaccessibility can be assessed with extraction by simulated saliva or gastrointestinal fluids (Ruby *et al.*, 1996; 1999) as well as by other media (Semple *et al.*, 2007). The latter reference provides clear schematic representations of bioavailability and bioaccessibility.

It may be generally stated that the degree by which a compound is bound to a soil or sediment particle will determine (i) the ease by which it will be washed away (such as by rainwater or irrigation) and affect, for example, water or groundwater quality, (ii) the potential facility of biological, chemical or physical remediation and (iii) the potential biological effects: although it is possible for bound contaminants to exert an effect on living systems, it is the 'free' forms that are the more bioavailable and pose the greater environmental risk. To properly assess such risks, it is essential that some quantitative measure of bioavailability can be used. Such information can be crucial for the design and cost-effective implementation of (bio)remediation schemes (Kamnev and van der Lelie, 2000; Lappalainen *et al.*, 2000; Flynn *et al.*, 2003), including the adjustments of cleanup goals (Kelly *et al.*, 2002; Turpeinen *et al.*, 2003).

In response to the need to quantify the bioavailable fraction out of the total concentration of the studied chemicals, various analytical tools have been proposed. These include physical/chemical extraction techniques, some of them attempting to mimic human exposure (Ruby *et al.*, 1996; 1999; Rodriguez *et al.*, 1999; Oomen *et al.*, 2004; Schroder *et al.*, 2004; Intawongse and Dean, 2006) or plant uptake (Zhang *et al.*, 2001; Chojnacka *et al.*, 2005; Dayton *et al.*, 2006), as well as an array of bioassays. The latter group includes methodologies based on molecular approaches, cell cultures, isolated tissues and organs, and whole-animal approaches. Among the parameters tested in the latter groups are dermal and gastrointestinal adsorption, assimilation efficiency and bioaccumulation (Sijm *et al.*, 2000; Heinz *et al.*, 2004; Darling and Vernon, 2005; Van Straalen *et al.*, 2005; Casteel *et al.*, 2006 Marschner *et al.*, 2006).

A special position among whole-organism assays is occupied by genetically engineered microorganisms, 'tailored' to respond to the presence of the target compound by a readily quantifiable signal (Daunert *et al.*, 2000; Gu *et al.*, 2004; Melten *et al.*, 2006; Sorensen *et al.*, 2006; Ron, 2007; Yagi, 2007). The present review summarizes current knowledge concerning the use of such reporter microorganisms for testing the bioavailability of heavy metals in the environment.

The use of bacteria for environmental sensing offers several advantages over higher organisms, including large and homogenous populations, short generation times, facility of maintenance and storage, low costs, and rapid responses. Furthermore, bacteria can be genetically manipulated to respond in a dose-dependant manner to specific chemicals or classes of chemicals, thus providing a true measure of bioavailability. Several extensive reviews in recent years have described the basic principles of the approach (Köhler *et al.*, 2000; Gu *et al.*, 2004; Verma and Singh, 2005; Harms *et al.*, 2006; Ron, 2007; Yagi, 2007). In all cases, the promoter of a gene or an operon that is induced by the target compound(s) is fused upstream of a reporter gene system; the fusion is harboured by the host strain either as a chromosomal integration or, in most cases, in a medium- or high-copy-number plasmid. Table 1 lists the most commonly used reporter systems and the nature of the signal they generate.

Metal resistance and bacterial metal sensors

As in the genetic construction of other bacterial reporter strains, the DNA segment acting as the sensing element is a promoter of a gene induced in the presence of the target metal(s). While in some of the original reports of metal-sensor construction the sensor elements were determined by the use of random libraries or random

Table 1. Reporter gene systems used in metals' biosensors design.

Reporter gene(s)	Reporter protein	Origin	Substrate	Detection method	Comments	Reference
<i>lux</i>	Bacterial luciferase	Luminescent bacteria (<i>V. fischeri</i> , <i>V. harvey</i>)	Aldehydes (C9–C14)	Luminescence	O ₂ required	Billard and DuBow (1998); Gu <i>et al.</i> (2004)
<i>lucGR</i>	Insect luciferase	Click beetle (<i>Pyrophorus plagiophthalmus</i>)	luciferin	Luminescence	Exogenous substrate and O ₂ required	Bronstein <i>et al.</i> (1994)
<i>lucFF</i>		Firefly (<i>Photinus pyralis</i>)				Taurainen <i>et al.</i> (1997); Billard and DuBow (1998)
<i>gfp</i>	Green fluorescent protein	<i>Aequorea victoria</i>		Fluorescence	Highly stable	Billard and DuBow (1998); Hakilla <i>et al.</i> (2002)
<i>lacZ</i>	β -Galactosidase	<i>E. coli</i>	Galactopyranosides	Colorimetric Fluorescence Electrochemical	Exogenous substrate required	Biran <i>et al.</i> (2000); Gu <i>et al.</i> (2004)
<i>bla</i>	β -Lactamase	<i>E. coli</i>	Lactamides	Colorimetric		Yoon <i>et al.</i> (1991); Moore <i>et al.</i> (1997)
<i>crtA</i>	Spheroidenone	<i>Rhodovulum sulfidophilum</i>	Demethylspheroidene	Colorimetric		Yagi (2007)

promoter insertions (DuBow, 1998), many of the more recent reports describe the targeted selection of promoter elements known to take part in bacterial metal resistance or uptake mechanisms. In view of the considerable involvement of bacteria in metal transformations (Fairbrother *et al.*, 2007), there are diverse microbial biochemical reactions involving exogenous metals. Bacterial resistance may be based on extracellular precipitation, sequestration at the cell envelope, intracellular precipitation and redox transformation (Rosen *et al.*, 1999; Brunis *et al.*, 2000). Such mechanisms have been shown to be active both against toxic metals (Pb, Hg, Cd, As, Sb, Ag, Tl) and against dangerously high concentrations of essential metals (Zn, Fe, Ni, Cu, Co, Cr) (Bontidean *et al.*, 2000; 2004).

Table 2 lists genetically modified bacterial metal-sensor strains described in the scientific literature along with their sensor/reporter elements, range of concentrations detected and the induction time required prior to the accumulation of a detectable signal. It is not surprising that most target metals listed in Table 2 are heavy metals of considerable environmental significance, with Cd, Hg, As and Sb sensors comprising most of the reported constructs. All of these metals are considered 'soft', tending to form strong covalent bonds with ligand binding sites on external or internal biological surfaces (Fairbrother *et al.*, 2007). 'Hard' metals, which often act as nutrients, preferentially form ionic bonds and are generally far less toxic. The sensitivities reported are generally high, with lowest detection thresholds in the picomolar or even femtomolar range. While some of the gene promoters used as the sensing elements are highly specific, others exhibit a broader detection range. The *isiAB* genes of *Synechococcus*, *copBC* of *P. fluorescens* and *pbrR* and *chrA* of *Cupriavidus metallidurans* responding only to Fe, Cu, Pb and Cr respectively are excellent examples of the former group; the *arsR*, *cadC* and *merR* genes of *Escherichia coli* represent the latter: *arsR* responds to As, Cd and Sb, *cadC* is activated by Cd, Pb, Sb, Sn and Zn, and *zntA* is induced by Cd, Cr, Hg, Pb and Zn. Most of the assays are relatively rapid, with the results obtained within 30–180 min. In most cases, the constructs' responses were dose-dependent, and bioluminescence was the dominant reporter, using either insect *luc* or bacterial *lux*. Almost all of the promoter elements used for the construction of bioreporters drive the induction of genes involved in heavy metal resistance. Only one case, that of the iron-sensing cyanobacterial *isiAB-lux* fusion, is based on a sensing element involved in the acquisition of the metal as an essential nutrient. In principle, sensors of the latter type should exhibit enhanced sensitivity as they are normally geared for the detection and uptake of very low external metal concentrations.

Direct availability determination in soil and sediment samples

Practically in all of the reports summarized in Table 2, the reporter strains' responses to metal availability were characterized using standard solutions of the tested metals. As, however, the potential advantage of such tools is not in the study of laboratory solutions but rather in assessing metal availability in soils and sediments, the direct applicability of these strains to such samples is of particular interest; only a few of the quoted reports actually attempt such assays. In order to get bacterial cells in contact with soil particles, the reaction has to take place in slurry; under such circumstances, when a bacterial reporter strain responds to its target analyte, it is unclear whether its cells actually sense a bound metal atom, or whether the observed response is only to its dissolved form.

Among the few reports that address this issue, contradictory conclusions have been drawn by different researchers. Some state that soil- or sediment-bound metals become bioavailable only after dissolving into the liquid phase (Ma and Uren, 1998; Rasmussen *et al.*, 2000; Adriano, 2001). Other reports, by comparing the bacterial responses to slurries and aqueous extracts, claim that even particle-bound metals may be available (Ivask *et al.*, 2002; 2004; Rooney *et al.*, 2006). The resolution of this question is of particular practical significance for risk assessment: if bound forms of the metal are indeed bioavailable, target remediation end-points may need to be lowered and harsher cleanup measures may be required (Kelly *et al.*, 2002). It should also be considered that while metals tend to adsorb to various solid-phase fractions (Ivask *et al.*, 2002), they may be released due to the activity of microorganisms and plants roots (Kahru *et al.*, 2005).

Table 3 summarizes reported attempts to assess metal bioavailability in soils and sediments. Approximately half of the reports involve aqueous extracts, whereas in the others a direct contact assay was attempted, for either contaminated or artificially spiked samples. In almost all cases, the fraction of the total metal found to be bioavailable in the different assays was smaller than 100%. Actual values varied greatly with sample, metal and reporter strain, mostly ranging between 0.1% and 50%.

Attempts to assay microbial responses to particle-bound pollutants are also hindered by technical difficulties. Once the bacteria are mixed with a slurried soil sample, the signal emitted by the reporter strain can be distorted or optically quenched by the opaque matrix. The methodologies proposed for overcoming this problem involve the reduction of particle concentration to a minimum (Brandt *et al.*, 2006), as well as introduction of

mathematical correction factors (Lappalainen *et al.*, 2000; Hakkila *et al.*, 2004; Ivask *et al.*, 2004).

Another possible solution for this dilemma involves the separation of the reporter cells from the bulk sample after sufficient exposure has taken place. In the course of assessing Pb contamination in roadside soils, we tested the bioavailability of this element in different soil samples and subsamples using bioluminescent *Cupriavidus metallidurans* (previous names: *Alcaligenes eutrophus*, *Ralstonia eutropha* and *Ralstonia metallidurans*) CH34 reporter strain AE1433 (Corbisier *et al.*, 1999). For this purpose we have developed a methodology for efficiently separating soil-bacteria slurry by density centrifugation. This allowed quantifying the bacterial response with no physical interferences from suspended soil particles. Figure 1 presents several soil-bacteria mixtures separated in this manner on a Percoll® (Sigma) gradient, with a clear bacterial band displayed above the soil pellet. This band was later removed to assess its response to the metals it was exposed to prior to the separation. We have also observed that only dissolved ions are sensed by the reporter cells. For example, when put into direct contact with a CaCO₃ slurry (representing the carbonaceous fraction of the tested soil), the bioluminescent response of the tester strain was always equivalent to the amount of Pb re-dissolved into the reaction mixture (Fig. 2). At least for this strain/sample combination, bound Pb (which amounted to 60–90% of the total metal in the sample) had no measurable biological effect. Detailed results of this study will be published elsewhere (S. Magrisso, Y. Erel, S. Belkin, in preparation).

Summary

Using a relatively small number of gene promoters as the sensing elements, and an even smaller selection of molecular reporter systems, numerous bacterial metal-sensor strains have been developed over the past 15 years. As reviewed in this communication, these engineered microorganisms display very high detection sensitivities and cover a broad range of metal targets. Together, they comprise an impressive panel of tools for simple and cost-effective determination of the bioavailability of heavy metals in the environment, and for the quantification of the non-bioavailable fraction of the pollutant. The magnitude of this fraction should be a significant factor in the assessment of the risk posed by the polluting metal, as well as for the determination of end-point goal in any remediation scenario. We are not aware, however, of any reported case in which such bacterial sensors, their numerous advantages notwithstanding, were actually put to use for the evaluation of metal bioavailability in a real environmental remediation scheme.

To a large extent, this lack of applied implementation

Table 2. Microbial metal bioavailability assays.

Promoter ^a (origin)	Element	Reporter gene ^b	Host	Linear response (µM)	Time of induction	Reference
<i>arsB</i>	As ³⁺	<i>blaZ</i>	<i>Staphylococcus aureus</i>	1–10	60 min	Ji and Silver (1992)
	As ⁵⁺			10–100		
<i>arsR</i>	Bi	<i>luxAB</i> (<i>V. fischeri</i>) <i>lucFF</i> (Firefly) <i>luxCDABE</i> (<i>V. fischeri</i>) <i>lacZ</i> <i>lucFF</i> (Firefly)	<i>E. coli</i>	100–1000	120 min	Cai and Dubow (1997)
	Sb			0.2–1	8 h	Hakkila <i>et al.</i> (2002)
	As			0.01–1	30 min	Ramanathan <i>et al.</i> (1998)
	As			0.01–1	90 min	Tauriainen <i>et al.</i> (1999)
	As			0.1–100		
	Sb			0.005–10	60 min	Stocker <i>et al.</i> (2003)
	As ³⁺			0.033–1		
	As ⁵⁺			33–33 000	12 h	Roberto <i>et al.</i> (2002)
	Cd			10–10 000		
	Sb			0.1–100	120 min	Tauriainen <i>et al.</i> (1997)
As	0.1–0.8					
As	0.1–0.4	120 min	Liao and Ou (2005)			
As	0.13–133					
<i>arsR</i> (<i>E. coli</i>)	As	<i>lucGR</i> (<i>P. plagiophthalmus</i>)	<i>P. fluorescens</i>	0.1–10	120 min	Petänen <i>et al.</i> (2001)
<i>arsR</i>	Cd	<i>lucFF</i> (Firefly)	<i>S. aureus</i>	0.5–5	120 min	Liao and Ou (2005)
	Sb	<i>gfp</i> (<i>A. Victoria</i>)	<i>E. coli</i>	0.1–10		
<i>arsRD</i>	As ³⁺	<i>lacZ</i>	<i>E. coli</i>	0.4–25	17 h	Scott <i>et al.</i> (1997)
	As ⁵⁺			1–50		
<i>cadAC</i>	Sb	<i>blaZ</i>	<i>S. aureus</i>	0.75–8	90 min	Yoon <i>et al.</i> (1991)
	As ³⁺			0.5–100		
<i>cadC</i> (<i>S. aureus</i>)	Sb	<i>lucFF</i> (Firefly)	<i>Bacillus subtilis</i>	0.1–10	180 min	Tauriainen <i>et al.</i> (1998)
	Cd			0.5–100		
	Pb			0.003–0.1	120 min	Liao <i>et al.</i> (2006)
	Sb			1–10		
	Sn			0.033–3.3	8 h	Peitzsch <i>et al.</i> (1998)
	Zn			33–1000		
	Cd			1–33	90 min	Corbisier <i>et al.</i> (1999)
	Pb			0.0001–500		
	Sb			0.01–10	120 min	Ivask <i>et al.</i> (2002)
	Sb			0.0001–10		
<i>chrB</i>	Cr	<i>lacZ</i>	<i>C. metallidurans</i>	0.001–50	16 h	Tibazarwa <i>et al.</i> (2001)
<i>chrB</i>	Cr ³⁺	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>C. metallidurans</i>	5–80	80 min	Riether <i>et al.</i> (2001)
	Cr ⁶⁺			2.5–40		
<i>chrAB</i>	Cr ³⁺	<i>lucFF</i> (Firefly)	<i>C. metallidurans</i>	2–100	120 min	Hakkila <i>et al.</i> (2004)
	Cr ⁶⁺			0.04–1		
<i>cnr:XYH</i>	Co	<i>luxDABE</i> (<i>V. fischeri</i>)	<i>C. metallidurans</i>	9–400	16 h	Tibazarwa <i>et al.</i> (2001)
	Ni	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>E. coli</i>	0.1–60		
<i>copA</i>	Ag	<i>lucFF</i> (Firefly)	<i>E. coli</i>	0.3–3	120 min	Hakkila <i>et al.</i> (2004)
	Cu			3–30		
	Ag			0.003–0.3		
	Cu			0.3–300		

<i>copBC</i>	Cu	<i>luxAB</i> (<i>V. fischeri</i>)	<i>P. fluorescens</i>	1–100	180 min	Tom-Petersen <i>et al.</i> (2001)
<i>copSRA</i>	Cu	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>C. metallidurans</i>	1–200	90 min	Corbisier <i>et al.</i> (1999)
<i>cup1</i>	Cu	<i>gfp</i> (<i>A. Victoria</i>)	<i>Saccharomyces cerevisiae</i>	0.5–5000	120 min	Shetty <i>et al.</i> (2002)
<i>isiAB</i>	Fe	<i>luxAB</i> (<i>V. fischeri</i>)	<i>Synechococcus</i>	0.001–1	12 h	Boyanapalli <i>et al.</i> (2007)
<i>flhC</i>	Al	<i>LuxAB</i> (<i>Vibrio harveyi</i>)	<i>E. coli</i>	40–400	20 min	Guzzo <i>et al.</i> (1992)
<i>katG</i>	Cd	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>E. coli</i>	2–35	90 min	Ben-Israel <i>et al.</i> (1998)
<i>merR</i> (<i>Shigella flexneri</i>)	Cd	<i>lucFF</i> (Firefly)	<i>E. coli</i>	1–100	60 min	Virta <i>et al.</i> (1995)
<i>merR</i>	Hg	<i>lucFF</i> (Firefly)	<i>E. coli</i>	10 ⁻⁹ –0.01		
	Hg	<i>gfp</i> (<i>A. Victoria</i>)	<i>E. coli</i>	0.005–0.5	8 h	Hakkila <i>et al.</i> (2002)
	Hg	<i>lucFF</i> (Firefly)		0.005–0.1		
	Hg	<i>luxCDABE</i> (<i>V. fischeri</i>)		0.0001–0.1		
	Cd	<i>lucFF</i> (Firefly)	<i>P. fluorescens</i>	1–10	120 min	Petänen <i>et al.</i> (2001)
	Hg			10 ⁻⁵ –0.1		
<i>merRB</i>	Cd	<i>lucFF</i> (Firefly)	<i>E. coli</i>	0.27–80	120 min	Ivask <i>et al.</i> (2002)
	Hg			0.1–15		
	Zn			1380–4000		
	Hg	<i>lucFF</i> (Firefly)	<i>E. coli</i>	0.0002–0.01	120 min	Ivask <i>et al.</i> (2001)
<i>merRT</i>	Hg	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>E. coli</i>	0.005–0.5	40 min	Selifonova <i>et al.</i> (1993)
	Hg	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>Vibrio anguillarum</i>	2.5 × 10 ⁻⁶ –5 × 10 ⁻⁵	80 min	Golding <i>et al.</i> (2002)
<i>merRTPA</i> (<i>Pseudomonas stutzeri</i>)	Hg	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>E. coli</i>	0.00005–0.0005		Pepi <i>et al.</i> (2006)
<i>pbrR</i>	Pb	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>C. metallidurans</i>	500–5000	180 min	Corbisier <i>et al.</i> (1999)
<i>pvd</i>	Fe	<i>gfp</i> (<i>A. Victoria</i>)	<i>Pseudomonas syringae</i>	0.1–100	Overnight	Joyner and Steven (2000)
<i>smtA</i>	Zn	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>Synechococcus</i>	0.5–4	240 min	Huckle <i>et al.</i> (1993)
<i>zntA</i>	Cd	<i>lacZ</i>	<i>E. coli</i>	0.0001–0.1	120 min	Shetty <i>et al.</i> (2003)
	Cd	Red-shifted <i>gfp</i> (<i>A. Victoria</i>)		0.1–10		
	Pb	<i>lacZ</i>		0.0001–0.1		
	Pb	Red-shifted <i>gfp</i> (<i>A. Victoria</i>)		0.1–10		
	Cd	<i>luxCDABE</i> (<i>V. fischeri</i>)	<i>E. coli</i>	0.01–0.33	80 min	Riether <i>et al.</i> (2001)
	Zn			3–30		
	Cr ⁶⁺			30–300		
	Hg			1–30		
	Pb			0.03–1		
	Cd	<i>lacZ</i>	<i>E. coli</i>	0.2–10	60 min	Biran <i>et al.</i> (2000)
<i>zntR</i>	Cd	<i>lucFF</i> (Firefly)	<i>E. coli</i>	0.05–30	120 min	Ivask <i>et al.</i> (2002)
	Hg			0.01–1		
	Zn			40–15 000		

a. Unless mentioned otherwise, the source of the promoter is the host bacterium.

b. Unless mentioned otherwise, the source of the reporter gene(s) is the host bacterium.

Table 3. Bioavailability of metals in contaminated soils.

Element	Matrix	Soil sample type (No. of samples)	Time of induction	% Bioavailable	Reference
As	WE	Polluted (3)	120 min	15–35	Turpeinen <i>et al.</i> (2003)
	WE	Polluted (30)	60 min	0.2–47	Flynn <i>et al.</i> (2003)
	GW	Polluted (52)	90 min	50–110	Trang <i>et al.</i> (2005)
	GW	Polluted (2)	120 min	76–92	Liao <i>et al.</i> (2006)
Cd	SL	Spiked (1)	120 min	12	Ivask <i>et al.</i> (2002)
	WE	Spiked (1)	120 min	0.6	
	SL	Polluted (50)	120 min	0.5–50	Ivask <i>et al.</i> (2004)
	WE	Polluted (50)	120 min	0.1–0.27	
	Acetic acid extract	Polluted (40)	120 min	0.14–13.9	Kahru <i>et al.</i> (2005)
Cr ⁶⁺	SL	Polluted (5)	120 min	0–55	Liao <i>et al.</i> (2006)
	SL	Spiked (1)	120 min	46	Ivask <i>et al.</i> (2002)
Cu	SL	Spiked (1)	90 min	19–39	Brandt <i>et al.</i> (2006)
	WE	Spiked (1)	90 min	0.6–3.8	
Hg	SL	Spiked (1)	120 min	40	Ivask <i>et al.</i> (2002)
	WE	Spiked (4)	120 min	0.26–7.6	Petänen and Romantschuk (2003)
	WE	Polluted (10)	300 min	20–66	Bontidean <i>et al.</i> (2004)
	WE	Polluted (6)	120 min	0	Lappalainen <i>et al.</i> (2000)
	WE	spiked (1)	120 min	1.3	Ivask <i>et al.</i> (2002)
	WE	Polluted (10) ^a	120 min	0–0.8	Ivask <i>et al.</i> (2007)
	WE	Spiked (2)	70–90 min	0–1.6	Rasmussen <i>et al.</i> (2000)
Ni	SL	Polluted (8)	> 12 h	< DL	Everhart <i>et al.</i> (2006)
	Ca(NO ₃) ₂ extract	Polluted (8)	> 12 h	50–60	Tibazarwa <i>et al.</i> (2001)
Pb	SL	Polluted (50)	120 min	0.24–8	Ivask <i>et al.</i> (2004)
	WE	Polluted (50)	120 min	0.1–0.14	
	Acetic acid extract	Polluted (5)	120 min	0.25–0.55	Kahru <i>et al.</i> (2005)
	SL	Spiked (10)	16 h	0–12	Geebelen <i>et al.</i> (2003)
Zn	SL	Spiked (1)	120 min	2.6	Tandy <i>et al.</i> (2005)
	SL	Polluted (1)	120 min	27	Diels <i>et al.</i> (1999)

a. River sediment.

WE, water extract; GW, ground water; SL, soil water slurry; DL, detection limit.

of a useful set of tools is due to the fact that they have yet to be recognized by national and international regulatory agencies, and be adopted as legitimate members of the bioavailability assays arsenal. While a set of *C. metallidurans*-based assays is available as a kit for

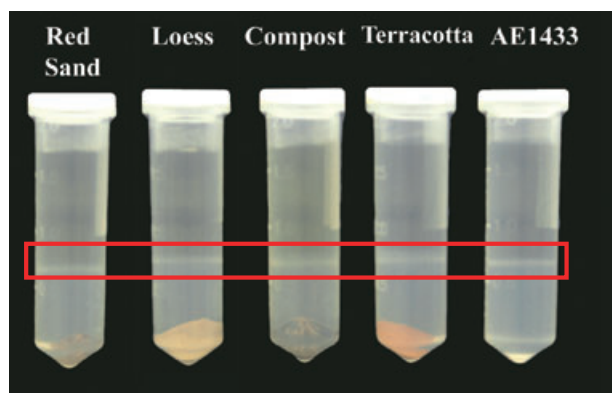


Fig. 1. Separation of *C. metallidurans* AE1433 bioreporter cells (frame) from four different soils by density gradient centrifugation (80% Percoll, 12 000 r.p.m., 2 min) following a 3 h direct slurry exposure in continuous agitation. The test tube on the right contains bacterial cells that have undergone a similar treatment in the absence of a soil sample.

general use (BIOMET®; Corbisier *et al.* 1998; 1999) and is routinely used in Belgian laboratories, it has not yet been recognized by any regulatory authority as an official

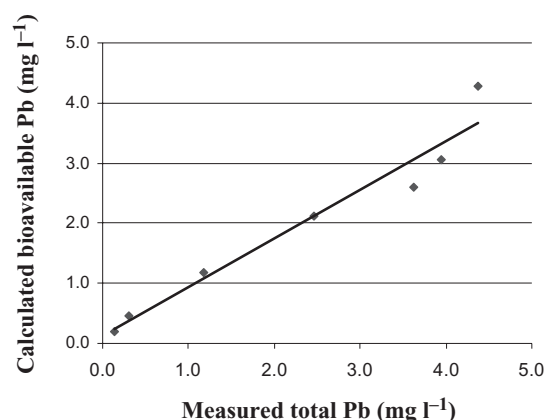


Fig. 2. Correlation between measured (Perkin-Elmer 5100PC Atomic Absorption Flame Spectrometer) total Pb concentrations and calculated bioavailable Pb using *C. metallidurans* AE1433. CaCO₃ was spiked with Pb at six different concentrations, and Pb bioavailability was determined by direct exposure of strain AE1433 to 15 mg of each of the samples. Luminescence was transformed to 'bioavailable Pb' using a calibration curve (bioluminescence as a function of Pb concentration in standard solutions).

measure of metal bioavailability. Neither are such assays specifically mentioned in the US EPA Framework for Metals Risk Assessment (Fairbrother *et al.*, 2007). Another acute need is standardization and verification: only standardized and fully verified assays will allow meaningful risk assessment as well as the comparison of data from different sites. In addition, it would be advantageous if the detection spectrum could be expanded to cover additional metals. As indicated above, only a limited number of molecular sensing and reporting elements have been taken advantage of to date; our continuously increasing understanding of the molecular basis of both metal resistance and metal acquisition pathways should provide an almost unlimited selection of additional avenues for metal bioavailability sensor development.

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