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Assessment of bioavailable arsenic and copper in soils and sediments from the Antofagasta region of northern Chile

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Abstract

Copper levels of nearly 500 mg l^{-1} were measured in aqueous extracts of soil and sediment samples from the lowlands of Antofagasta. Arsenic levels of up to 183 mg l^{-1} were found in river sediments, and 27.5 mg l^{-1} arsenic was found at the location of a dam where potable water is extracted. This indicates that the arsenic contamination of water supplies reported recently for the pre-Andes may be a widespread problem throughout the region. Copper contamination from smelting activities also provides cause for concern as elevated levels were found in aqueous extracts of soil up to 20 km away from a smelter. This study went beyond traditional chemical analysis by assessing the potential benefits of using microbial biosensors as an alternative to determination of chemical speciation, to provide an environmentally relevant interpretation of soil/sediment residue levels. This approach is simple to use and enables a rapid, low cost assessment of pollutant bioavailability. It may, therefore, be of use for further investigations in the region and beyond. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Arsenic; Copper; Microbial biosensors; Chile; Soil; Sediment

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1. Introduction

The Antofagasta region of northern Chile is one of the world's wealthiest mineral producing areas and is associated with elevated environmental levels of arsenic, lead, cadmium, copper and zinc (Queirolo et al., 2000a). Recent studies in the region have drawn attention to high levels of arsenic contamination in both drinking water (Queirolo et al., 2000a) and locally grown food crops (Queirolo et al., 2000b). This contamination originates in arsenic-rich ores and is liberated both by volcanic activity, which is very high in this region of the Andes, and by the mining and smelting operations which are carried out here. There is considerable concern regarding arsenic in potable groundwaters in countries such as Chile, Argentina, Bangladesh, West Bengal and China (e.g. Das et al., 1995; Hopenhayn-Rich et al., 1998; Smith et al., 2000) where vast numbers of people are exposed to elevated arsenic in their diet, and epidemiological studies reveal that these elevated levels could be having severe health effects. Thus, there is considerable impetuous in understanding the arsenic biogeochemistry of these sites.

The measurement of total arsenic is of limited value for assessing environmental samples because bioavailability and toxicity of arsenic is dependent on speciation (Pongratz, 1998). Comprehensive speciation of arsenic can only be achieved by using sophisticated and expensive coupled techniques such as HPLC-ICP-MS (Falk and Emons, 2000), which is only present in specialist laboratories. Furthermore, arsenic only very rarely occurs as a sole contaminant particularly when it is associated with mining and smelting (Hamilton, 2000), as is the case in Antofagasta where copper and arsenic are both elevated, and chemical characterisation alone cannot predict the toxicity of a single contaminant, let alone that of samples with multiple contaminants. Microbial biosensors were explored as an alternative. Bioluminescence-based biosensors offer a powerful tool for assessing pollutant bioavailability and toxicity (Paton et al., 1997) and were used here to relate water-soluble concentrations to environmental toxicity. Two types of *lux*-marked biosensors were used to show toxicity by a reduction in bioluminescence and the bioavailability of arsenite by the induction of bioluminescence in a specific biosensor. This study used 24 soil and sediment samples from a range of contaminated sites and nearby rivers in the lowlands of Antofagasta. The strength of the observed relationships suggests that such biosensors may offer regulators a cost-effective method for rapid screening of environmental samples in the region without the need for specialised chemical analysis.

2. Materials and methods

2.1. Study area and sampling

The location of the study has been described by Queirolo et al. (2000a). Samples 1, 2 and 3 were obtained from a processing plant used to remove arsenic from potable water. This plant is located approximately 20-km north east of Antofagasta. Sample 1 was the fresh sludge produced by the plant, while samples 2 and 3 represented sludge that had been aged for 1 and 3 months, respectively. Sample 4 was taken from irrigated land to which the sludge had been applied outside the processing plant.

Samples 5–11 were taken at increasing distances from a copper smelter located 30-km north east of Antofagasta, starting in the spoil on site and ending 25-km away. The smelter has concerned local environmentalists for several years on account of atmospheric emissions.

Three samples (12, 13, 14) were taken from a salt flat at Ascotan (3600 m above sea level), an area which drains into the Rio Salado.

The remaining samples were taken from river sediments upstream of Calama on the Rio Salado. Samples were at least 300-m upstream of any settlements and the locations were identified because of their accessibility. Sample 15 was taken at Toconce, the location at which abstraction of 500 l s^{-1} for drinking water takes place. Sample 16 was taken 6-km downstream at Quena, sample 17 a further 5-km downstream at Baden en Salado and sample 18 was taken at an unnamed bridge crossing 7-km from sample 17. Sample 19

was taken at Quinchamale, sample 21 at Lasana and sample 20 midway between the locations. Sample 22 was taken 300-m upstream of a former Borax factory while sample 23 was taken 400-m downstream. The final sample (24) was taken at the edge of Lake Chui Chui located approximately 10-km from Calama.

Samples of 50 g were taken at a depth of between 5 and 10 cm and placed in sterile USEPA glass Wheaton vials with Teflon lined caps. These were then stored at 4°C under field moisture conditions until the extraction procedure was carried out.

2.2. Extraction procedures

The samples were made up to a 1:2 soil/water mixture by adding 20 ml of deionised water to 10 g of soil/sediment (Sousa et al., 1998). The mixtures were then shaken for 2 h on an end-over-end shaker at approximately 50 rev./min. To extract the water, samples were centrifuged (Coolspin 2 RR/1061) for 10 min at $1050 \times g$ at 4°C and then filtered (Whatman qualitative filter papers No. 4) and stored at 4°C. This supernatant represented the water-soluble fraction of pollutants.

2.3. Soil water pH

The pH values of the extracts were measured using a Hanna HI8424 Microcomputer pH meter with a gel filled microelectrode.

2.4. Chemical analysis

The water-soluble arsenic fraction was determined by hydride generation (Perkin-Elmer A. Analyst 300). Samples were diluted 1:10 in 10% HCl 1% KI and left overnight at 4°C to reduce arsenic to arsenite. Sample concentrations were calibrated using a standard curve of 10, 20, 30 and 50 $\mu g\ l^{-1}$ As, made up from $NaAsO_2$ in 10% HCl 1% KI and further diluted where necessary. A solution of 1% sodium borohydride 0.1% sodium hydroxide was also used.

Copper analysis was carried out using a flame atomic absorption spectrometer (Baird AAS Alpha 4). Samples were diluted 1:5 in 2% HNO_3. Sample concentrations were calibrated using a standard curve of 2.5, 5, 7.5 and 10 mg l^{-1} Cu, prepared using a 1000-mg l^{-1} stock solution of Cu in HNO_3 diluted with 2% HNO_3. Samples were diluted with 2% HNO_3 where necessary.

2.5. Bacterial biosensors

Three different *lux*-marked bacterial biosensors were used. The two metabolic biosensors (where luminescence reflects metabolic activity which is reduced with contaminant burden) were *Escherichia coli* HB101 pUCD607 (Rattray et al., 1990) and *Pseudomonas fluorescens* 10586r pUCD607 (Amin-Hanjani et al., 1993). These sensors contain the *luxCDABE* genes isolated from *Vibrio fischeri* as a multicopy plasmid. An arsenic-specific biosensor, *E. coli* CM1166 pC200 (Corbisier et al., 1993) was also used. In this construct, the *luxAB* genes from *V. fischeri* are induced by the *ars* operon that confers resistance to arsenic (Rosen, 1986; Silver and Misra, 1988; Tsai et al., 1997; Cai et al., 1998; Ramanathan et al., 1998), so that luminescence is induced by the availability of arsenic in the form of arsenite.

Previously freeze-dried metabolic biosensors (Paton et al., 1997) were resuscitated for 1 h in 10 ml of 0.1 M KCl for *E. coli* and 10 ml LB medium (Luria-Bertani) for *P. fluorescens* at 25°C in an orbital shaker at 200 rev./min. *P. fluorescens* was washed in 0.1 M KCl before use. *E. coli* CM1166 pC200 was grown in LB medium with ampicillin 50 $\mu g\ ml^{-1}$ at 25°C.

2.6. Toxicity testing

2.6.1. Metabolic biosensors

E. coli and *P. fluorescens* were both exposed to a range of arsenic (as both arsenate and arsenite) and copper (II) concentrations to produce a dose–response curve. A 50-μl aliquot of cells was added to 450 μl of standard or environmental sample solution in a 4-ml luminometer cuvette. The cells were added at an interval of 15 s between each cuvette and were read after 15 min, on a portable Jade Luminometer (Labtech International) at ambient temperature. For the dose–response curves, copper standards were made using

anhydrous $CuSO_4$ diluted in distilled water to produce a range of concentrations from 0 to 100 mg l^{-1} at pH 5.5 (± 0.02). Arsenite and arsenate standards of 0–500 mg l^{-1} were made with $NaAsO_2$ and $Na_2HAsO_4 \cdot 7H_2O$, respectively, with distilled water also at pH 5.5. In each case, the 0-mg l^{-1} standard, distilled water at pH 5.5, was used as the control.

2.6.2. Arsenite-specific biosensor

Cultures were grown overnight to stationary phase in LB medium with ampicillin (50 μg ml^{-1}) at 25°C in an orbital shaker. Cells were washed twice in LB medium and then used to inoculate LB medium with ampicillin as before and grown to mid-exponential phase at 37°C in an orbital shaker. A 1-ml aliquot of sample was placed in a sterile universal bottle and then 9 ml of cells were added and the mixtures were incubated for a further 1 h at 37°C in an orbital shaker. A 1-ml aliquot of this mixture were then placed in a 4-ml luminometer cuvette and 5 μl of 1% v/v aldehyde in ethanol was added to each cuvette at 15-s intervals. Luminescence was read after 20 min using a Jade Luminometer. Standards of arsenite, arsenate and copper, made up as before, in a range from 0 to 100 mg l^{-1} at pH 5.5 were used to establish a dose–response curve, with 0 mg l^{-1} taken as the control luminescence value.

3. Results and discussion

3.1. Chemical analysis

The water-soluble concentration of arsenic in the samples ranged from 0.2 to 391.1 mg l^{-1}, while copper ranged from 0.4 to 499.1 mg l^{-1}

Table 1
Summary of the chemical data and bioluminescence levels (mean of three replicates, as a percentage of the control) expressed by the three biosensors for the environmental samples

Sample no.	pH	Water-soluble copper (mg l^{-1})	Water-soluble arsenic (mg l^{-1})	Metabolic *E. coli*	Metabolic *P. fluorescens*	Arsenite specific *E. coli*
1	7.75	0.6	2.3	107.1	98.3	69.4
2	8.26	0.5	0.3	85.2	85.3	63.2
3	8.40	0.4	0.3	91.1	97.8	72.1
4	8.02	0.4	0.3	132.2	83.5	74.4
5	3.59	499.1	0.2	0.7	0.6	4.7
6	7.62	0.6	3.2	58.3	96.2	127
7	5.16	409.8	0.5	0.4	1.2	157.7
8	7.30	1.2	1.0	104.7	112.5	72.2
9	7.96	1.7	0.4	102.2	114.7	114.3
10	5.02	92.2	0.6	0.3	0.9	124.5
11	4.77	6.1	0.3	29.6	15.5	112.4
12	9.29	0.5	391.1	5.6	4.2	72.8
13	9.87	0.4	33.4	4.5	3.3	238.2
14	8.32	0.9	52.5	25.0	44.8	374.9
15	8.26	1.9	27.5	10.6	21.2	198.7
16	7.92	0.4	0.8	86.2	99.9	67.3
17	7.68	0.6	0.2	151.3	98.1	84.7
18	8.42	1.4	3.6	28.8	54.8	102.5
19	7.28	0.7	183.1	16.7	63.0	298.5
20	7.84	1.5	2.9	44.2	84.3	81.9
21	8.79	0.9	3.2	57.7	76.7	85.8
22	8.46	0.4	0.6	91.5	76.3	102.8
23	8.29	0.5	2.9	57.6	97.4	105.7
24	8.17	0.4	0.3	108.7	62.8	75.2

(Table 1). The pH of the soil water ranged from 3.59 to 9.87.

The highest level of copper was found in the smelter spoil (sample 5) that also had the lowest pH value. High levels of copper also occurred at sites some distance from the smelter, most notably at a level of 92.2 mg l^{-1} water-soluble copper in soil 20 km away (sample 10). The WHO (1998) has recommended a provisional guideline limit of 2 mg l^{-1} copper in drinking water, based on acute gastrointestinal effects. This indicates that copper production may have serious repercussions for the health of the local people and the environment, particularly if the high levels measured in the aqueous extracts are leaching into water supplies, and emphasises the need for further investigations.

The most elevated levels of arsenic were found on salt flats above the Alto Plano (samples 12, 13 and 14) but high levels were also found in two of the river sediment samples (15 and 19). This suggests that arsenic contamination of water supplies may be as much of a problem in the lowlands as it has been reported to be in the pre-Andes (Queirolo et al., 2000a), particularly since the sediment neighbouring a dam from which water is extracted for local drinking supplies, contained 33.4 mg l^{-1} arsenic (sample 15). Increased risks of lung and bladder cancer and of arsenic-associated skin lesions have been observed from drinking water containing less than 0.05 mg l^{-1} arsenic and the provisional guideline value for arsenic in drinking water has been set at 0.01 mg l^{-1} due to measurement limitations despite health criteria supporting a lower limit (WHO, 2001). This shows the levels found have serious implications for the health of the local people who drink the water and irrigate agricultural land with it, particularly in the light of research that indicates that arsenic and other contaminants are accumulated by food crops (Queirolo et al., 2000b).

3.2. Toxicity testing

3.2.1. Dose–response curves

The three biosensors had distinctive responses to the pollutants. Regression lines of the dose–responses are shown in Figs. 1–3 as a predicted response for comparison with the observed response for the environmental samples. Copper

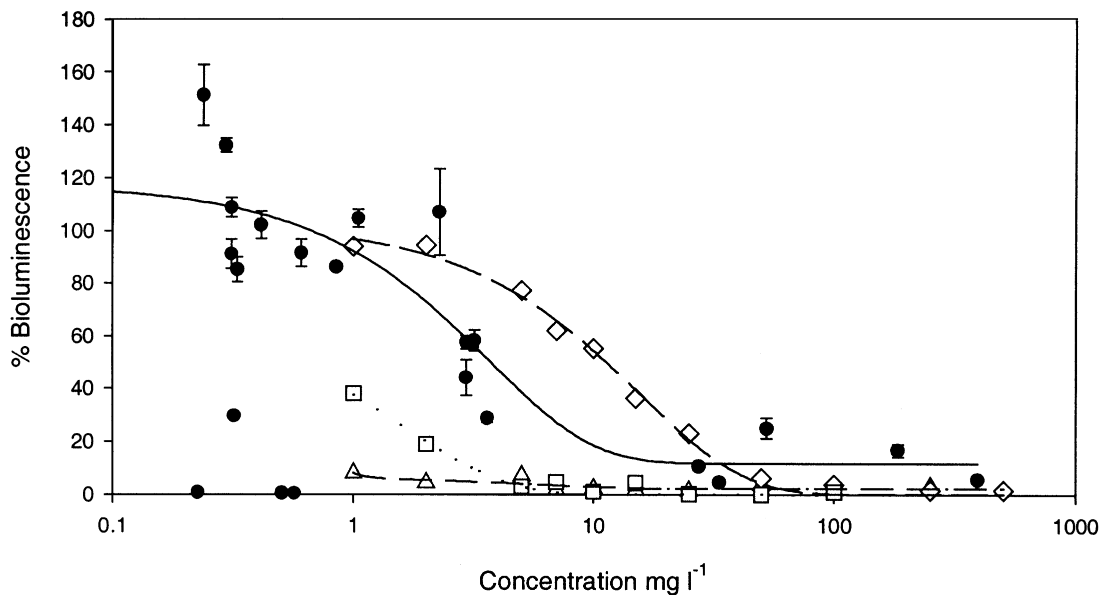

Fig. 1. Mean bioluminescence levels for *E. coli* HB101 pUCD607 expressed as a percentage of the control, for environmental samples, plotted against water-soluble arsenic concentrations (—●—), and dose–response curves for arsenite (--◇--), arsenate (--Δ- -), and copper (··· □ ···). Error bars on environmental samples represent the standard error of the mean for three replicates.

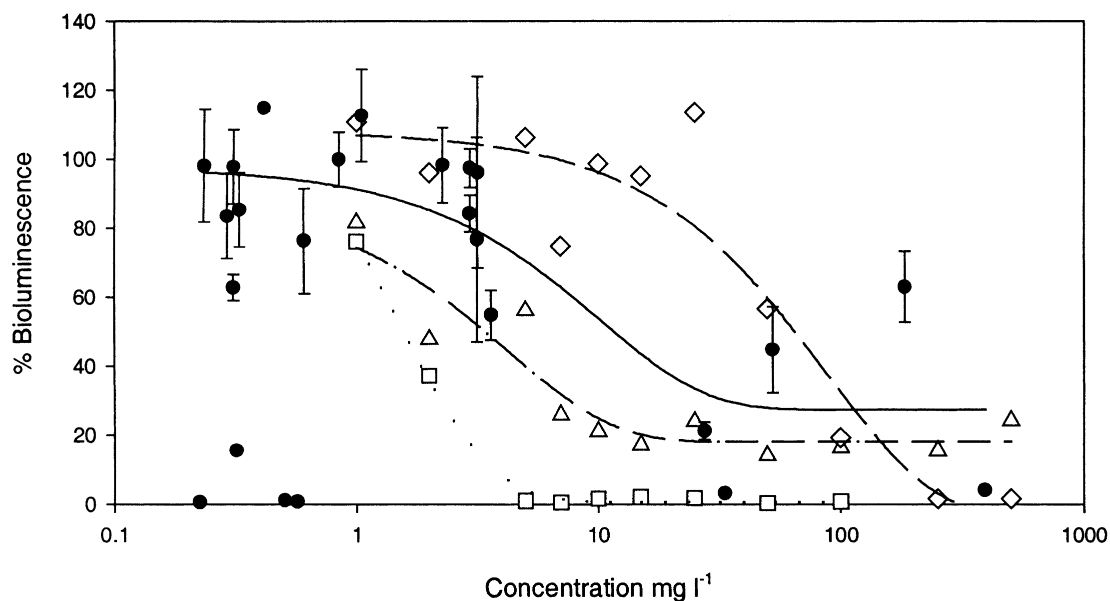

Fig. 2. Mean bioluminescence levels for *P. fluorescens* 10586r pUCD607 expressed as a percentage of the control, for environmental samples, plotted against water-soluble arsenic concentrations (—●—), and dose–response curves for arsenite (--◇--), arsenate (- -Δ- -) and copper (··· □ ···). Error bars on environmental samples represent the standard error of the mean for three replicates.

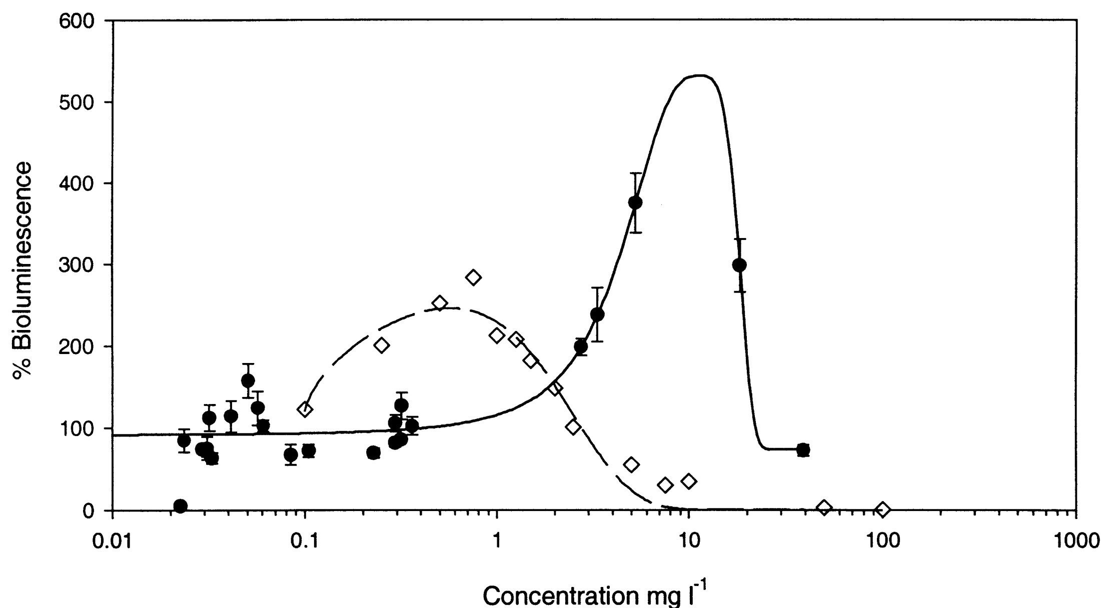

Fig. 3. Mean bioluminescence levels for *E. coli* CM1166 pC200 expressed as a percentage of the control, for environmental samples, plotted against water-soluble arsenic concentrations in the bioassay, diluted by the addition of cells (—●—), and the dose–response curve for arsenite (--◇--). Error bars on environmental samples represent the standard error of the mean for three replicates.

was highly toxic to both metabolic biosensors with values of 0.7 mg l^{-1} for *E. coli* and 1.6 mg l^{-1} for *P. fluorescens* causing a 50% decrease in luminescence of the control. Of the two arsenic species, arsenate was the more toxic to the metabolic biosensors at lower concentrations. The EC_{50} values for arsenate were 0.5 and 2 mg l^{-1} compared with 11 and 56 mg l^{-1} for arsenite. *P. fluorescens* was the less sensitive of the two metabolic biosensors for all the pollutants and this was most likely due to more pronounced levels of extra cellular polysaccharide associated with the cells.

There is a general perception that arsenite is more toxic than arsenate, however, toxicity is dependent on both target organism and conditions of exposure. This perception is largely based on clinical toxicology studies on mammals, which show that ingested arsenate is excreted from the body at significantly higher rates than arsenite and is less likely to be absorbed across the gut wall (Zielhuis and Wibowo, 1984). The ecotoxicology of arsenic in soils is more complex as factors such as bioavailability are crucial. Studies conducted on environmental exposure to arsenic in soil using organisms such as fungi and plants (e.g. Sharples et al., 1999, 2000) support the results presented here for bacteria, illustrating that higher toxicity of arsenate is probably due to uptake by the phosphate transport system as arsenate is known to act as a structural analogue of phosphate (Willsky and Malamy, 1980).

The arsenic-specific biosensor showed a very different response. This bioassay is carried out in a rich carbon medium, which causes the inorganic analytes to be more readily complexed and hence rendered less bioavailable. This resulted in neither copper nor arsenate inducing a toxic response in this biosensor. Arsenite induced a strong increase in luminescence at a range of concentrations less than 1 mg l^{-1} and then exerted a toxic effect at concentrations above 2.5 mg l^{-1}. This means that the performance of the sensor is restricted to a limited range of arsenite concentrations and cannot be used to reflect actual toxicity of the samples, instead its usefulness lies in adding an extra layer of information to the metabolic biosensor responses, helping to distinguish the contribution of arsenic to the observed toxicity of the samples.

3.2.2. Environmental samples

As shown by Figs. 1 and 2, the metabolic biosensors responded to the concentration of water-soluble arsenic in the samples, although the response for *P. fluorescens* did not correlate as well as for *E. coli*. *P. fluorescens* has a higher level of extra cellular polysaccharide associated with it and therefore, this response may have been due to an effect on free ion movement of the target analyte. There are only four outlying samples (5, 7, 10, 11) that show significantly greater toxicity than predicted by the dose–response curves for arsenic. By referring to the chemical results these smelter samples have elevated copper concentrations, above 90 mg l^{-1} for the three with no bioluminescence (samples 5, 7 and 10) and 6.1 mg l^{-1} for the sample imposing less toxicity on the sensors (11). Hence, the response relates to copper, rather than arsenic concentrations for these four samples. The less toxic of these outlying samples (11) illustrates the advantage of using two metabolic biosensors with different sensitivity levels. The lower sensitivity of *P. fluorescens* to arsenate illustrates that the toxicity of this sample is not due to arsenate, which cannot be seen from Fig. 1 because it is outside the range of the dose–response curve. These responses should not be influenced by the different pH values of the extracts because the cells have a linear performance against the pH range tested and are also tolerant of salinity (Paton et al., 1995).

E. coli CM1166 pC200 also showed a clear response (Fig. 3) with peak luminescence levels shifted to the right to reflect the mixture of arsenic species present in the environmental samples. Only one sample (5) showed toxicity not predicted by the dose–response curve for that concentration of arsenic. The dose–responses illustrate that copper complexes with the growth medium and, therefore, in this sample there were high levels of other bioavailable contaminants. This sample was taken from the spoil heap at the smelter plant and is likely to contain elevated

levels of many other toxic elements including lead and zinc. The low pH value of this sample was not the cause of the observed toxicity, as the bioassay procedures, particularly those for *E. coli* CM1166 pC200, will have moderated the environmental physicochemical values.

A summary of the responses of the three biosensors for each sample is given in Table 1. There is a clear advantage in combining metabolic and the arsenite-specific biosensors. The two metabolic biosensors show overall toxicity of all the bioavailable contaminants in combination while the specific biosensor indicates the bioavailability of a specific element or form. Four out of the five samples with high levels of arsenic were indicated by significant bioluminescence induction for the specific biosensor. Only the salt flat sample with 391.1 mg l^{-1} arsenic (12) could not be clearly identified as toxic due to arsenic from bioluminescence levels alone, without dilution, because the level was so high that it gave a toxic response even for the specific biosensor. This toxic response did provide useful information, however. It confirmed that the toxicity was not due to copper or arsenate because these contaminants do not induce toxicity in *E. coli* CM1166 pC200. The specific biosensor responses also indicated that the smelter spoil (sample 5) contained high levels of bioavailable contaminants while the toxicity of the samples taken at 5, 20 and 25 km from the smelter (7, 10 and 11) was due to contaminants that are not bioavailable in growth medium. Using the chemical results, it is known that the smelter spoil was not high in arsenic and that the toxicity of the other three samples was most probably due to copper, but combining the biosensor responses allows a degree of interpretation without this knowledge. Arsenic toxicity is indicated by a toxic response from the metabolic biosensors and significant induction of bioluminescence in the arsenite-specific sensor. Where the arsenic is present as arsenite and at sufficient levels to give a toxic response for the arsenite-specific sensor, the extract can be diluted to give an induction of bioluminescence. A toxic response from all three biosensors which does not produce an induction of bioluminescence in the arsenite-specific biosensor upon dilution, indicates toxic levels of other bioavailable pollutants, which do not become complexed in carbon rich media, while a toxic response from the metabolic biosensors combined with a control level of bioluminescence from the arsenite-specific biosensor, which remains so even when diluted, indicates that toxicity is caused by pollutants which become complexed.

4. Conclusions

Microbial biosensors have been demonstrated to offer a potential complimentary, and in some cases alternative, technique to chemically determining speciation for giving an environmentally relevant interpretation of samples. Metabolic sensors enable low cost, rapid screening of samples for toxicity, which could be carried out in the field to identify sites where chemical characterisation may be necessary. Combining biosensors which are specific to certain pollutants with the more commonly used metabolic sensors greatly increases the degree of interpretation possible without chemical analysis and indicates both bioavailability and toxicity, measures that can only be inferred from chemical data. This technique would be of great use to regulators in areas such as northern Chile where the soil type ensures there is a minimum of interference from factors such as high organic matter to complicate interpretation of results, and also has the potential to aid the development of remediation strategies by indicating whether pollutants can be complexed by organic carbon or made unavailable to the biota by a change in pH.

Acknowledgements

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References

Amin-Hanjani S, Meikle A, Glover LA, Prosser JI, Killham K. Plasmid and chromosomally encoded luminescence marker systems for detection of *Pseudomonas fluorescens* in soil. Mol Ecol 1993;2:47–54.

Cai J, Salmon K, DuBow MS. A chromosomal *ars* operon homologue of *Pseudomonas aeruginosa* confers increased resistance to arsenic and antimony in *Escherichia coli*. Microbiology 1998;144:2705–2713.

Corbisier P, Ji G, Nuyts G, Mergeay M, Silver S. *LuxAB* gene fusions with the arsenic and cadmium resistance operons of *Staphylococcus aureus* plasmid pI258. FEMS Microbiol Lett 1993;110:231–238.

Das D, Chatterjee A, Mandal BK, Samanta G, Chakraborti D, Chanda B. Arsenic in groundwater in six districts of West Bengal, India; the biggest arsenic calamity in the world. Part 2. Arsenic concentration in drinking water, hair, nails, urine, skin-scale and liver tissue (biopsy) of the affected people. Analyst 1995;120:917–924.

Falk K, Emons H. Speciation of arsenic compounds by ion-exchange HPLC-ICP-MS with different nebulizers. J Anal Atom Spectrom 2000;15:643–649.

Hamilton EI. Environmental variables in a holistic evaluation of land contaminated by historic mine wastes: a study of multi-element mine wastes in West Devon, England using arsenic as an element of potential concern to human health. Sci Total Environ 2000;249:171–221.

Hopenhayn-Rich C, Biggs ML, Smith AH. Lung and kidney cancer mortality associated with arsenic in drinking water in Cordoba, Argentina. Int J Epidemiol 1998;27:561–569.

Paton GI, Campbell CD, Glover LA, Killham K. Assessment of the bioavailability of heavy metals using *lux* modified constructs of *Pseudomonas fluorescens*. FEMS Lett Appl Microbiol 1995;20:52–58.

Paton GI, Rattray EAS, Campbell CD, Meussen H, Cresser MS, Glover LA, Killham K. Use of genetically modified microbial biosensors for soil ecotoxicity testing. In: Pankhurst CS, Doube B, Gupta V, editors. Bioindicators of soil health. Wallingford: CAB International, 1997:397–418.

Pongratz R. Arsenic speciation in environmental samples of contaminated soil. Sci Total Environ 1998;224:133–141.

Queirolo F, Stegen S, Mondaca J, Cortes R, Rojas R, Contreras C, Munoz L, Schwuger MJ, Ostapczuk P. Total arsenic, lead, cadmium, copper, and zinc in some salt rivers in northern Andes of Antofagasta, Chile. Sci Total Environ 2000a;255:85–95.

Queirolo F, Stegen S, Restovic M, Paz M, Ostapczuk P, Schwuger MJ, Munoz L. Total arsenic, lead, and cadmium levels in vegetables cultivated at the Andean villages of northern Chile. Sci Total Environ 2000b;255:75–84.

Ramanathan S, Shi W, Rosen BP, Daunert S. Bacteria-based chemiluminescence sensing system using β-galactosidase under the control of the ArsR regulatory protein of the *ars* operon. Anal Chim Acta 1998;369:189–195.

Rattray EAS, Prosser JI, Killham K, Glover LA. Luminescence-based non-extractive technique for in situ detection of *Escherichia coli* in soil. Appl Environ Microbiol 1990; 56:3368–3374.

Rosen BP. Recent advances in bacterial ion transport. Ann Rev Microbiol 1986;40:263–286.

Sharples JM, Meharg AA, Chamber SM, Cairney JWG. Arsenate sensitivity in ericoid and ectomycorrhizal fungi. Environ Toxicol Chem 1999;18:1848–1855.

Sharples JM, Meharg AA, Chamber SM, Cairney JWG. Mechanisms of arsenate resistance in the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. Plant Physiol 2000;124:1327–1334.

Silver S, Misra TK. Plasmid-mediated heavy metal resistances. Ann Rev Microbiol 1988;42:717–743.

Smith AH, Lingas EO, Rahman M. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. Bull World Health Organ 2000;78:1093–1103.

Sousa S, Duffy C, Weitz H, Glover LA, Bar E, Henkler R, Killham K. Use of a *lux*-modified biosensor to identify constraints to bioremediation of BTEX-contaminated sites. Environ Toxicol Chem 1998;17:1039–1045.

Tsai K, Hsu C, Rosen BP. Efflux mechanisms of resistance to cadmium, arsenic and antimony in prokaryotes and eukaryotes. Zool Studies 1997;36:1–16.

WHO, World Health Organisation, Guidelines for drinking-water quality, 2nd Edition, volume 2, Health criteria and other supporting information. World Health Organisation, Geneva, 1996, pp. 940-949. Addendum to Vol. 2. 1998, pp. 281–283.

WHO, World Health Organisation, Arsenic in drinking water. Fact sheet No. 210. World Health Organisation, Geneva, 2001.

Willsky GR, Malamy MH. Effect of arsenate on inorganic phosphate transport in *Escherichia coli*. J Bacteriol 1980;144:366–374.

Zielhuis RL, Wibowo AAE. Standard setting and metal speciation: arsenic. In: Nriagu JO, editor. Changing metal cycles and human health. Berlin: Springer, 1984:323–344.